Posters

Susceptibility testing and detection of resistance

P573 Comparison of ceftriaxone susceptibility rates of Enterobacteriaceae in the TEST programme using EUCAST vs. 2009 and 2010 CLSI breakpoints

R. Badal, S. Bouchillon, B. Johnson, M. Hackel, M. Dowzicky (Schaumburg, Collegeville, US)*

Objective: EUCAST and CLSI have different breakpoints (bp) for many drugs, including cephalosporins, complicating the process of evaluating global data from surveillance studies. In 2010, CLSI will lower susceptibility bp for several cephalosporins and aztreonam vs. Enterobacteriaceae by as much as 2 doubling dilutions. The TEST program has been monitoring susceptibility levels of tigecycline and other drugs since 2004. This report evaluates the impact of the CLSI bp change on reported susceptibility of Enterobacteriaceae to ceftriaxone (Cax), both in Europe and globally.
Methods: 59,644 isolates of Enterobacteriaceae were tested from 2004–2009 using broth microdilution following CLSI guidelines. Susceptibility to Cax was compared using 2009 and 2010 CLSI (<8mcg/ml and <1mcg/ml, respectively) and EUCAST (<1) bp.
Results: See the table.

Isolate Source	BP Used	% Susceptible						
		Enterob.	*E. coli*		*K. pneumoniae*		*K. oxytoca*	
			ESBL+	ESBL−	ESBL+	ESBL−	ESBL+	ESBL−
Europe	EUCAST/CLSI 2010	72	2	93	1	89	3	82
	CLSI 2009	79	8	96	11	93	17	90
Rest of World	EUCAST/CLSI 2010	75	2	91	2	88	6	88
	CLSI 2009	82	9	94	13	92	39	94

ESBL = extended spectrum beta-lactamase.

Conclusions: Although the convergence of the EUCAST and CLSI Cax bp in 2010 will eliminate discrepancies in susceptibility levels due to different bp used in Europe and rest of world, analyses using CLSI will see some large declines in %S values in some organism types (e.g., ESBL+ *K. oxytoca)*. The new bp does a good job of classifying most ESBL+ isolates as non-susceptible to Cax.

P574 Belgium goes EUCAST: critical aspects in a national move from CLSI to EUCAST breakpoints

J. Van Eldere, P. Melin, J. Verhaegen, K. Magerman for the BVIKM-SBIMC EUCAST working party*

Objectives: In 2008 – at a national meeting of the Belgian society of clinical microbiology and infectious diseases (BVIKM-SBIMC) – the members voted to switch from CLSI to EUCAST breakpoints from the 1st of January 2010. Belgium till now had no national antibiogram committee and almost all labs used CLSI breakpoints and methodology. Here we report on some of the critical aspects of a wholesale nationwide shift towards EUCAST breakpoints.
Method: the BVIKM-SBIMC decided to install a EUCAST working party to promote and facilitate the transition from CLSI to EUCAST. Members from the working party have had numerous meetings and interviews with clinical microbiologists but also with antimicrobial susceptibility testing (AST) manufacturers, drug manufacturers, official organizations involved in clinical microbiology and also with clinicians.
Results:

1. A local committee to take the lead in the shift towards EUCAST breakpoints is absolutely necessary. Although most microbiologists – thanks to several national and local meetings – are by now aware of EUCAST, most take a 'wait and see' attitude.
2. A 'translation' of EUCAST guidelines into 'national' EUCAST guidelines is needed. The EUCAST website is not user-friendly and most labs expect clear and practical advice on how to implement EUCAST. In addition, specific national peculiarities still need a local input (eg use of temocillin in Belgium for which no EUCAST breakpoints are available). AST manufacturers and drug manufacturers also have expressed the need for national guidance on matters related to AST.
3. Microbiologists want advice on practical issues related to implementation of EUCAST breakpoints and in particular issues related to quality control. In this context it is important for national committees to seek collaboration and endorsement of all stakeholders including the manufacturers but also national quality control and/or accreditation agencies, reference labs, professional organizations etc.

Conclusion: Successful implementation of EUCAST breakpoints necessitates an concerted effort. We believe that it takes a sufficient time (2 years) to prepare this shift and a substantial and sustained communication effort to all stakeholders. To achieve this, the institution of a national antibiogram committee is felt to be vital.

P575 EUCAST breakpoints in automated susceptibility testing: evaluation of Vitek® 2

R. Smyth, L. Bjelkenbrant, G. Kahlmeter (Växjö, SE)*

Objectives: To evaluate the performance of VITEK® 2 with software version 04.02 for susceptibility testing and SIR-category interpretation for clinical isolates using the breakpoints developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).
Methods: 211 clinical isolates of known identities and susceptibility patterns were tested in VITEK® 2 using cards AST-GP68 for *S. pneumoniae* and AST-P592 for staphylococci and enterococci. The isolates were stored strains comprising 131 staphylococci, 44 enterococci and 37 *S. pneumoniae*.
SIR-category for all strains had previously been established using the Swedish Reference Group for Antibiotics (SRGA) disc diffusion method, calibrated to EUCAST breakpoints. Antibiotics evaluated were cefoxitin, oxacillin, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, linezolid, moxifloxacin, penicillin, tetracycline and vancomycin for the staphylococci. Ampicillin, gentamicin, imipenem, linezolid, teicoplanin and vancomycin were evaluated for the enterococci and cefotaxime, erythromycin, moxifloxacin, penicillin and tetracycline for the *S. pneumoniae*.
Reference strains *Staph. aureus* ATCC 29213, ATCC BAA-1026, ATCC BAA-976, ATCC BAA-977, *E. faecalis* ATCC 29212, ATCC 51299 and *S. pneumoniae* ATCC 49619 were included as appropriate on each day of testing. Discordant results were resolved using Etest.
Results: Overall category agreement was obtained in 98.4% of tests. Minor errors (mE) for 7 strains of CNS to tetracycline, three *S. pneumoniae* to cefotaxime and one *S. pneumoniae* to moxifloxacin. One VME for *S. pneumoniae* to erythromycin. One strain of *E. faecium* was falsely reported to be HLGR and one CNS strain falsely negative in the test for inducible clindamycin resistance. Two strains of *S. pneumoniae* failed to grow in AST-GP68 and are excluded from the results table.
Conclusion: This first evaluation of EUCAST breakpoints in VITEK® 2 indicates it to be a reliable tool for susceptibility testing of common Gram positive organisms.

Bacterial group/characteristic	Total number of strains (n)	Total number of tests (n)	Category agreement (CA)	CA %
MSSA	64	768	768	100
MRSA	30	360	360	100
CNS	28	336	329	97.9
Staph. lugdunensis	9	108	108	100
ICR	10	10	9	90
E. faecalis	15	90	90	100
E. faecium	10	60	59	98.3
HLGR	9	9	9	100
VRE	10	10	10	100
Str. pneumoniae	37	148	143	96.6
PNSSP	21	21	21	100

P576 Evaluation of new ATB EU (08) panels compliant with EUCAST guidelines

R. Martelin, G. Zambardi, M. Albertini-Balland, E. Pillon, M. Pompilio, J. Oliger, N. Carboni, P. Dufour (La Balme-les-Grottes, FR)*

Objectives: In 2009, EUCAST breakpoints and expert rules will be implemented through Europe to reach the unique goal of European harmonization. As a consequence, bioMérieux has updated both the reagents and software of its semi-automated antimicrobial susceptibility testing system for being compliant with EUCAST 2008 recommendations. The aim of this study was to determine the performances of these new panels.
Methods: To evaluate the accuracy, each of the 8 panels was tested with a set of 64 to 120 strains. ATB G- EU (08), ATB UR EU (08) and rapid ATB UR EU (08) were tested with Enterobacteriaceae, ATB PSE EU (08) with non-fermentative Gram-negative bacilli, ATB STAPH EU (08) with Staphylococci, ATB STREP EU (08) with Enterococci, Pneumococci, β-haemolytic and other Streptococci, ATB HAEMO EU (08) with *Haemophilus* and *Moraxella catarrhalis*, and ATB ANA EU (08) with Anaerobes. Category agreements and error rates were determined in comparison to the reference agar dilution method (or Etest for Anaerobes). To evaluate the reproducibility, a minimum of 10 strains were tested 3 times on each reference and the rates of identical interpretations were calculated.
Results: Average, minimum and maximum agreement and error percentages respectively were as follows:
- category agreements: 93.2, 89.4 (ATB PSE EU 08) and 96.0 (ATB STREP EU 08)
- major errors: 2.4, 1.2 (ATB HAEMO EU 08) and 3.1 (ATB UR EU 08)
- very major errors: 2.2, 0.5 (ATB STREP EU 08) and 4.6 (ATB PSE EU 08)

The average reproducibility was 96.2%, with a minimum of 93.7% (ATB PSE EU 08) and a maximum of 97.6% (ATB STAPH EU 08).
Conclusions: Overall performances of the new ATB EU panels are satisfactory and are not negatively impacted by the use of the breakpoints defined by EUCAST, with few exceptions for non-fermentative species and for *Haemophilus*. These new ATB panels and software will help hospital and private laboratories to safety switch from CLSI or CA-SFM recommendations to EUCAST.

P577 Assessment of the Phoenix system and EUCAST breakpoints for antimicrobial susceptibility testing against contemporary isolates expressing relevant resistance mechanisms

M.I. Morosini, M. García-Castillo, R. Cantón (Madrid, ES)*

Objective: To evaluate the accuracy of the PHOENIX system (BD, USA) in combination with the EUCAST breakpoints for antimicrobial susceptibility testing (AST) against contemporary clinical isolates expressing relevant resistance mechanisms.
Methods: A total of 393 isolates were included: i) 200 fresh consecutively recovered isolates (51 Enterobacteriaceae, EB; 40 *P. aeruginosa*, PA; 30 *S. aureus*, SA; and 29 *Enterococcus* spp., E) and ii) 193 stored (−80ºC) isolates with characterized resistance mechanism [96 extended-spectrum-(ESBL) and 50 metalo-(MBL) β-lactamase producing EB, 10 MBL-producing PA, 20 methicillin-resistant SA, MRSA, and 17 vancomycin-resistant E, VRE]. Comparator AST results were either routine or historical data obtained following CLSI guidelines (broth microdilution). Discrepancies were resolved using Etest. Quality control ATCC strains were used to assure MIC reproducibility. MBL- and ESBL-coding genes and mecA and van determinants were confirmed by PCR and sequencing. PHOENIX performance was assessed based on MICs concordances (essential agreement, EA, ±1 log2 dilution) and percentage of interpretive category error rates (ER) (minor, mi; major, M; very major, VM).
Results: An overall EA of 96% (3,283 organism-antimicrobial combinations) and global 2.4% mi, 1.2% M and 1.1% VM errors were observed. Among fresh isolates, EA was: 97% (97% EB; 94% PA; 98% SA; and 98% E). Global ER in these isolates were: 0.8% mi; 0.6% M; and 2.3% VM, being this latter value mainly due to discrepancies in the combination gentamicin-PA. In isolates with characterized resistance mechanisms, EA was: 95% (99% ESBL-EB; 94% MBL-EB; 100% MBL-PA; 97% SARM; and 91% EVR) Overall ER in this subset of isolates were: 3.5% mi; 2.0% M; and 0.8% VM. In these isolates, mi errors were mainly due to discrepancies in ciprofloxacin and ESBL-EB as well as in imipenem, meropenem and ciprofloxacin and MBL-EB whereas M errors were mainly attributed to amoxicillin–clavulanic acid-ESBL-EB discrepancies.
Conclusion: This study indicates reliable AST results of PHOENIX system and accurate interpretive categorization when using EUCAST breakpoints in contemporary isolates expressing relevant resistance mechanisms. Interpretive category discrepancies were mainly due to particular organism-antimicrobial combinations due to specific breakpoint definition such as gentamicin and PA or heterogeneous expression of resistance mechanisms such MBL affecting carbapenems in EB.

P578 EUCAST disc diffusion antimicrobial susceptibility testing method

E. Matuschek, R. Smyth, D. Brown, G. Kahlmeter (Växjö, SE; Peterborough, UK)*

Objective: The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was recently tasked with developing a European disk diffusion method calibrated to the harmonized European MIC breakpoints. Method descriptions, quality control tables and zone diameter breakpoints are to be developed by the end of 2009.
Methods: The method is based on Mueller-Hinton agar without supplements (MH) for Enterobacteriaceae, *Pseudomonas*, *Stenotrophomonas*, staphylococci and enterococci, and Mueller-Hinton agar with 5% horse blood and 20 mg/L β-NAD (MH-F) for *Haemophilus*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, α- and β-haemolytic streptococci and other fastidious organisms. The inoculum is McFarland 0.5 and the plates are incubated at 35ºC for 16–20 h. MH plates are incubated in air and MH-F in 5% CO2. Data for quality control strains and clinical isolates was gathered during 2009 at the Swedish External Reference Laboratory for Antimicrobial Susceptibility Testing, Växjö Central Hospital.
Results: For MH, the disk diffusion method was, when appropriate, calibrated to quality control acceptable ranges established by Clinical and Laboratory Standards Institute (CLSI). Repeated testing of a number of antimicrobial agents resulted in reproducible results; with 64/75 mean values targeting the mean of the CLSI range with a maximum of 1 mm difference for *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. There were no mean values differing more than 2 mm from the target. For reference strains and antibiotics/disks not represented in CLSI tables, as well as for all data on MH-F, quality control ranges were calculated from repeated testing by EUCAST on Mueller-Hinton agar from several manufacturers. Clinical zone diameter breakpoints were established by inhibition zone histogram analysis and by correlation of inhibition zone diameters with corresponding MIC values.

Conclusions: The main part of the EUCAST disk diffusion antimicrobial susceptibility testing method is now developed and published on the EUCAST website www.eucast.org where method descriptions, quality control tables and clinical breakpoints are now freely available.

P579 EUCAST: validation of Mueller-Hinton agar with 5% horse blood and 20 mg/L β-NAD (MH-F) for gradient tests on fastidious micro-organisms

C. Karlsson, E. Matuschek, G. Kahlmeter (Växjö, SE)*

Objectives: The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is currently developing a European standard method for antimicrobial susceptibility testing by disk diffusion on Mueller-Hinton agar (MH). The medium for fastidious organisms is MH supplemented with 5% horse blood and 20 mg/L β-NAD (MH-F). The objective of this study was to validate MH-F for MIC determination using gradient tests (Etest® and M.I.C.Evaluator™) for *Haemophilus influenzae* (HI), *Streptococcus pneumoniae* (PN) and *Streptococcus pyogenes* (GAS).
Methods: MIC values were determined using Etest® (bioMérieux) and M.I.C.Evaluator™ (Oxoid) on MH-F and on media recommended by the manufacturers; i.e. *Haemophilus* test medium (HTM) for HI and MH with 5% sheep blood (MHstr) for PN and GAS. MIC determination was repeated ten times for reference strains and five times for clinical isolates. MIC values were read after 20 hours incubation at 35–36ºC in 5% CO2. Susceptibility to amoxicillin–clavulanic acid, ampicillin, ciprofloxacin, tetracycline and trimethoprim-sulfamethoxazole (SXT) (Etest only) was determined for HI ATCC 49247 and HI NCTC 8468 and benzylpenicillin, erythromycin, moxifloxacin (E-test only), tetracycline and SXT (Etest only) for PN ATCC 49619 and GAS CCUG 25571. Additionally, for HI and PN, three clinical isolates with known resistance and borderline MIC values for β-lactam antibiotics were tested. Etest and M.I.C.Evaluator were validated separately.
Results: On MH-F and respective reference media for β-lactams, fluoroquinolones, erythromycin and tetracycline, identical MIC values were obtained for 69% of Etest readings and 72% of M.I.C.Evaluator readings, with another 31% (Etest) and 27% (M.I.C.Evaluator) readings±one dilution respectively. Corresponding numbers for SXT were 30% identical MIC values, 44% of readings±one dilution and 25% differing by two dilutions. Results were easily read on both media.
Conclusion: MH-F showed excellent agreement with reference media (HTM and MHstr) for gradient test MIC determination of HI, PN and GAS. We recommend that the manufacturers further validate the use of MH-F as an alternative to the currently recommended media. This will allow the use of MH-F for both disk diffusion and gradient tests. For SXT, further investigation is needed.

P580 MH-F: a common medium for fastidious organisms in the EUCAST disk diffusion antimicrobial susceptibility testing method

E. Matuschek, D. Brown, G. Kahlmeter (Växjö, SE; Peterborough, UK)*

Objective: The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was recently tasked with developing a European disk diffusion method. One important part of the method was to develop a common medium for fastidious organisms, with focus on *Haemophilus influenzae* (HI), *Streptococcus pneumoniae* (SP) and other streptococci.
Methods: Mueller-Hinton agar (MH) with different supplements was investigated as the medium for testing HI and SP with a McFarland 0.5 inoculum and incubation at 35ºC and 5% CO2 for 16–20 h. The proposed medium, MH with 5% horse blood and 20 mg/L β-NAD (MH-F), was then evaluated for growth of a number of fastidious organisms under the described conditions.
Results: Supplementation of MH with 20 mg/L β-NAD and 5% horse blood resulted in good growth of SP and HI. When MH was supplemented with sheep blood instead of horse blood, much more β-NAD (>100 mg/L) was needed for growth of HI. MH plates with 5% horse blood were further evaluated with β-NAD (purity ranging from 95 to >99%) from seven different manufacturers. With 20 mg/L β-NAD, all products tested gave similar growth of both HI and SP. MH-F supported good growth of a number of additional fastidious organisms including streptococcus groups A, B, C and G, α-haemolytic streptococci *(S. anginosus, S. bovis, S. constellatus, S. gordonii, S. intermedius, S. mitis, S. mutans, S. oralis, S. salivarius, S. sanguinis, S. vestibularis)*, *Moraxella catarrhalis*, *Haemophilus parainfluenzae*, Aggregatibacter aphrophilus, Pasteurella spp., *Corynebacterium* spp., *Listeria monocytogenes*, *Arcanobacterium haemolyticum*, *Burkholderia cepacia*, *Eikenella corrodens*, *Kingella kingae* and *Neisseria meningitidis*.
Conclusions: Mueller-Hinton agar with 5% horse blood and 20 mg/L β-NAD (Mueller Hinton-Fastidious, MH-F) incubated in 5% CO2 was chosen as the medium and atmosphere for fastidious organisms in the EUCAST disk diffusion antimicrobial susceptibility test. Good growth on MH-F was shown for a number of fastidious organisms. Anaerobes and *Neisseria gonorrhoeae* will be further investigated.

P581 Screening for high-level aminoglycoside resistance in enterococci. The EUCAST standard disc diffusion method

E. Matuschek, J. Åhman, C. Claesson, L. Nilsson, G. Kahlmeter (Växjö, Linköping, SE)*

Objective: The presence of high-level aminoglycoside resistance (HLAR) in enterococci continues to increase and it is important to differentiate strains without acquired resistance mechanisms from those with HLAR. EUCAST recommends screening with gentamicin for detection of HLAR *in vitro*. The objective of this study was to investigate which gentamicin disk strength most reliably predicts HLAR, using EUCAST breakpoints and standard disk diffusion method on Mueller-Hinton agar.
Methods: A total of 125 clinical isolates of *Enterococcus faecalis* (107) and *Enterococcus faecium* (18) were selected from Växjö Central Hospital (n = 82) and Linköping University Hospital (n = 43). All isolates had gentamicin MIC values ranging from 16–1024 mg/L, confirmed with Etest. Disk diffusion was performed on Mueller-Hinton agar without supplements according to the EUCAST disk diffusion method, using disks containing 10, 30 and 120 ug gentamicin. The inoculum suspension was equivalent to McFarland 0.5 and plates were incubated at 35ºC for 18±2 h.
Results: HLAR was detected with gentamicin Etest (MIC > 128 mg/L, as defined by EUCAST) in 50/102 *E. faecalis* and 18/18 *E. faecium*. For *E. faecalis*, HLAR strains were clearly separated from those without acquired resistance mechanisms and with gentamicin 10- and 30-ug disks inhibition zones were absent (= 6 mm) for all HLAR strains. For gentamicin 120-ug, three HLAR strains had inhibition zones of 7–10 mm. Separation of HLAR strains was less distinct for *E. faecium*, when gentamicin 120 ug was used; five HLAR strains had inhibition zones of 8–15 mm. When comparing these results with data on *E. faecalis* from routine laboratory work in Växjö, the gentamicin 30-ug disk (n = 686) shows a clear separation, whereas discrimination may become a problem with the gentamicin 10-ug disk (n = 237).
Conclusions: All gentamicin disks predicted high-level aminoglycoside resistance in the *E. faecalis* investigated, whereas the results were less conclusive for *E. faecium*. The results of this study suggest that gentamicin 30-ug disks are more reliable than gentamicin 10- and 120-ug disks for predicting HLAR in enterococci, using EUCAST breakpoints and methodology.

P582 Amoxicillin–clavulanate and piperacillin–tazobactam: comparison of different susceptibility testing methods

S. Persson, C.G. Giske, V. Özenci (Stockholm, SE)*

Objectives: To analyse the susceptibility of clinical *Escherichia coli* isolates against amoxicillin–clavulanate and piperacillin–tazobactam and compare the performance of three susceptibility testing methods.

Methods: A total of 179 clinical *Escherichia coli* isolates were included. Species identification was performed with Vitek2, and susceptibility testing was performed with disc diffusion, Etest and Vitek2. Etest-results were considered the gold standard. EUCAST breakpoints were used for both antibiotics when analyzing the MIC and Vitek2 data. Disc diffusion breakpoints for piperacillin–tazobactam were derived from the Swedish Reference Group for Antibiotics. The results from the three methods were compared using Pearson correlation coefficient (r). Discrepancies between the methods were describes as minor, major and very major.
Results: The correlation for MICs for amoxicillin–clavulanate by Etest and Vitek2 was relatively high (r = 0.82). Seventeen major (9.49%) and one very major (0.56%) errors were observed with Vitek2. In contrast there was poor correlation for the MICs for piperacillin/tazobactam by Etest and Vitek2 (r = 0.37). As many as forty-seven (26.26%) major and seven (3.91%) minor discrepancies were observed between the two methods. In all major discrepancies observed in testing both antibiotics, Vitek2 result was resistant whereas the Etest result was susceptible. When disc diffusion method is considered, the breakpoint for amoxicillin–clavulanate was estimated at 18 mm in the wild type *E. coli* population studied. Results from disc diffusion tests were significantly correlated with Etest for amoxicillin–clavulanate (r = 0.79). There were twenty-three (12.85%) major discrepancies between the two methods. In contrast, relatively poor correlation between Etest and disc diffusion results was observed when piperacillin/tazobactam tested (r = 0.49). There were twenty-eight (15.64%) minor discrepancies between the two methods.
Conclusion: The susceptibility testing results for amoxicillin–clavulanate obtained by three methods were highly correlated. However there was poor correlation in piperacillin/tazobactam testing in the studied material. The discrepancies observed for susceptibility testing of piperacillin/tazobactam might be clinically important. Especially the major errors observed with Vitek2 are of potential great concern.

P583 Evaluation of E-test to determine tigecycline MICs in *Enterobacter* sp. from blood culture isolates

J.W. Cohen Stuart, J.W. Mouton, N. Al Naiemi, A.C. Fluit, N. Arents, B.M. Diederen, S. Thijsen, B. Vlamincx, M. Leverstein-Van Hall (Utrecht, Nijmegen, Amsterdam, Veldhoven, Haarlem, Nieuwegein, NL)*

Introduction: Tigecycline is often one of the last therapeutic options for infections by multi-drug resistant Gram-negative bacteria. Therefore, accurate MIC testing is critical. Previous studies have however shown that tigecycline MICs determined by Etest may be influenced by the brand of Muller-Hinton (MH) agar, hypothetically due to variations in concentrations of manganese. Furthermore, for *Acinetobacter* spp. with MICs ⩾2 mg/L by Etest, the MIC was at least two doubling-dilutions lower using broth micro-dilution (BMD). The aim of this study was to evaluate the Etest as a method to determine tigecycline MICs for *Enterobacter* spp.
Methods: Tigecycline MICs were determined in 271 *Enterobacter* bloodculture isolates from 12 laboratories throughout the Netherlands, using Becton Dickinson (BD) MH II medium for BMD (Merlin, Germany) according to the ISO-guideline 20776–1 (reference method EUCAST and CLSI). The Etest (Biomerieux, France) was performed using the same MH medium as well as an Iso-sensitest agar (Oxoid, UK). In each experiment, *E. coli* ATCC 25922 was used as control strain. EUCAST clinical breakpoints were used (susceptible(S)⩽1 mg/L), as well as FDA breakpoints (S⩽2 mg/L).
Results: Compared to BMD, MICs determined by Etest were significantly higher using either MH agar or Iso-sensitest agar (see Table). In 84% of the isolates, tigecycline MICs were 2 or more doubling dilutions higher by Etest on MH agar compared to BMD. MICs determined by Etest on MH agar were significantly higher than on Iso-sensitest agar. Susceptibility rates were significantly higher when using the BMD method compared to Etest, using either EUCAST or FDA breakpoints (see Table). When EUCAST breakpoints were used, 97% of isolates were S in the BMD method, whereas only 30% were S with Etest on MH agar. Almost all isolates (96%) that tested non-S (EUCAST) by Etest on MH agar, were S in the BMD method.
The MICs of the *E. coli* control strain were within the specified range (0.03 mg/L-0.25 mg/L) for all three methods, although in the BMD method the value was 0.0325 mg/L and using Etest on MH agar, the value was 0.25 mg/L..
Conclusion: In *Enterobacter* spp., tigecycline MICs determined by Etest are significantly higher than MICs determined by BMD. Therefore, Etest should not be used for tigecycline susceptibility testing in *Enterobacter* spp. The misclassification as tigeclycline non-susceptible may incorrectly deny patients an important antibiotic option.

Method	MIC (mg/L)			% S	
	MIC_{50}	MIC_{90}	Geometric mean ±SD	EUCAST (S⩽1)	FDA (S⩽2)
BMD	0.25	1	0.42±0.41*	97%*	100%
Etest					
BD MH agar	1.5	3	1.94±1.6*	30%*	82%*
Iso-sensitest Agar	0.5	1.5	0.88±1.2*	88%*	94%*

*$p < 0.001$ for comparisons between each method using t-test (comparisons of means) or Fisher-Exact test (comparisons of susceptibility rates).

P584 A simple colourimetric method for antimicrobial susceptibility testing of bacteria in biofilms

S. Kim, H.Y. Kang, S.M. Kim, D.T. Cho, J. Kim (Daegu, KR)*

Objective: Bacteria in biofilms are protected from antibiotics. For some antibiotics, the concentration required to kill biofilm cells may be greater than a thousand times that required to kill planktonic cells of exactly the same strain. Therefore, standard antimicrobial susceptibility test has limited relevance for determining antimicrobial susceptibility of bacteria in biofilms. We here developed a simple colorimetric method for antimicrobial susceptibility testing of bacteria in biofilms using trimethyl tetrazolium chloride (TTC) as an indicator of viable bacteria in biofilms.
Method: Biofilms were formed on 96-well polystyrene microtiter plate for 1 day, treated with antibiotics for additional 1 day and reacted with 0.02% TTC for 1 hr. The optical density at 540nm (OD540) was measured on a microtiter plate colorimeter. The OD540 value of biofilms after treatment of antibiotics was compared with that of biofilms before treatment of antibiotics. Results were expressed as reduction (%) of OD540 value in respect to controls. Using this new method, biofilms formed by *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* pneumonia were tested for their susceptibility to several antibiotics. Minimum inhibitory concentration (MIC) was determined by the standardized CLSI method.

		Ampicillin/ Sulbactam	Cefotaxime	Amikacin	Ciprofloxacin
E. coli ATCC 25922	MIC	4	0.5	0.5	0.0078
	MBIC	4	0.5	1	0.0078
	MBEC50	16	8	8	1
	MBEC90	>512	>512	>512	>8
K. pneumoniae B170	MIC	2	0.5	0.5	0.0625
	MBIC	2	0.5	0.5	0.0156
	MBEC50	64	16	2	1
	MBEC90	256	128	>512	>8
K. pneumoniae 172	MIC	16	0.5	0.5	0.125
	MBIC	64	16	8	0.25
	MBEC50	128	64	>512	>8
	MBEC90	256	>512	>512	>8
		Methicillin	Vancomycin	Teicoplanin	Azithromycin
S. aureus ATCC 25913	MIC	4	1	1	2
	MBIC	0.5	4	0.5	0.5
	MBEC50	8	8	8	>512
	MBEC90	>512	>512	>256	>512
S. aureus WS-3	MIC	64	2	2	4
	MBIC	4	4	0.25	1
	MBEC50	>512	8	8	>512
	MBEC90	>512	>512	>256	>512
S. aureus WS-8	MIC	8	1	2	4
	MBIC	2	4	1	2
	MBEC50	>512	16	16	>512
	MBEC90	>512	>512	>256	>512
S. aureus WS-22	MIC	8	2	1	4
	MBIC	0.5	1	0.5	0.5
	MBEC50	4	8	8	32
	MBEC90	>512	>512	>256	>512

Results: Reduction of TTC by viable bacteria produced red formazan that could be measured quantitatively by colorimetric absorbance at 540 nm. The minimum biofilm inhibitory concentration (MBIC) was determined as the concentration at which the % of OD540 value is 100%. Minimum biofilm eradication concentration (MBEC) was assessed such as MBEC50 and MBEC90, which was termed as the concentration of drugs that kill 50% and 90% of bacteria in pre-formed biofilms, respectively. MBEC50 and MBEC90 were determined as the concentration at which the % of OD540 value is 50% and 10%, respectively. The results were summarized in Table 1. Estimation of the efficacy of antibiotics for bacterial biofilms was enabled by comparing MBIC, MBEC50 and MBEC90 of drugs. In addition, prominent differences in susceptibility for drugs between biofilm cells and their planktonic counterparts of the same strain (MBIC versus MIC) were observed.
Conclusion: We developed a new simple colorimetric antimicrobial susceptibility testing of bacteria in biofilms using TTC as a viable cell indicator. This method may be useful to screen the effectiveness of antibiotics or biocides at eradicating bacterial biofilms.

P585 Evaluation of performances of the Phoenix automated system in the antimicrobial susceptibility testing using EUCAST breakpoints

T. Giani, V. Conte, M.M. D'Andrea, G.M. Rossolini (Siena, IT)*

Objectives: The objective of this work was to evaluate the performances of the PHOENIX (PHX) system for antimicrobial susceptibility testing (AST) and detection of clinically relevant resistance mechanisms following the EUCAST guidelines.
Methods: 362 isolates, including routine clinical and collection isolates with known MICs data, were used in this study. Gram-negatives (69%) were represented by Enterobacteriaceae (including KPC and ESBL producers), *A. baumannii*/calcoaceticus complex (IMP producers and representative of the major nosocomial European clones), *P. aeruginosa* (including metallo-carbapenemase producers) and *S. maltophilia.* Gram-positives (31%) were *Enterococcus* spp (including VRE and high-level aminoglycoside resistant strains), *Staphylococcus* spp (including MRSA) and *Streptococcus* spp (including penicillin- and macrolide-resistant strains). ASTs were performed using PHX using 3 ad hoc panels able to determine the appropriate MICs in line with the EUCAST breakpoints. Experimental Concordance (EC), defined as MIC agreement ± 1 log2 dilution and Category Concordance (CC) to determine Very Major Error (VME), Major Error (ME) and minor Error (mE), were used to analyze raw data obtained with PHX. Occurrences of discrepancies were resolved first by retesting and ultimately by Etest and/or micro-dilution method.
Results: Overall EC was 99.1% and CC was 96.2%. In total VME accounted for 1.3%, ME 0.8% and mE 2.8%. For Enterobacteriaceae no ME were seen, VME were 0.4% and mE 2.6%. All ESBL and KPC producers were correctly identified as resistant isolates. Less agreement was found for non-fermenting isolates. For this group VME were 2.6%, ME 2.3% and mE 5%. The majority of the errors were observed for *P. aeruginosa* and gentamicin (n = 6 VME), meropenem (n = 14 mE) and piperacillin/tazobactam (n = 3 VME, n = 5 ME). For Staphylococci VME were 1.7%, ME 1.8% and mE 1.6%. All MRSA were correctly identified as such. No ME or VME were seen for *Streptococcus* and *Enterococcus* spp and mE 1.7% and 1.3% respectively.
Conclusions: PHX showed excellent performances in the assessment of MICs of clinical isolates using EUCAST compliant panels, as demonstrated by the very high EC and CC. Accurate interpretative categorization and detection of main resistance mechanisms was also obtained using EUCAST breakpoints. Discrepancies observed for specific species/antibiotic combinations will be further investigated.

P586 Ability of ciprofloxacin 1μg discs and British Society for Antimicrobial Chemotherapy (BSAC) zone diameter breakpoints to detect plasmid-mediated quinolone resistance determinants in urinary enterobacterial isolates

A. Amin, D. Wareham (London, UK)*

Objectives: Plasmid-mediated quinolone resistance (PMQR) genes have been increasingly reported amongst members of the Enterobacteriaceae worldwide. In London we recently reported an incidence of 53% in a set of ciprofloxacin resistant urinary isolates collected at our centre. As PMQR genes confer only low-level resistance it has been suggested that modification of screening procedures and breakpoints may be required in order to optimize their detection (Cavaco & Aarustrup, JCM 2009). The aim of this study was therefore to determine any correlation between carriage of PMQR genes and zone of inhibition to 1μg ciprofloxacin discs using BSAC susceptibility testing methodology and breakpoints.
Methods: Consecutive non-duplicate enterobacterial isolates obtained from nosocomial and community-acquired urinary tract infections were screened for PMQR genes using primers specific for qnrA,B and S, qepA, and aac(6′)-Ib-cr and oqxA,B genes. Antimicrobial susceptibility testing was performed for using the BSAC method with a zone size of $\leqslant$16 mm considered resistant.
Results: 167 isolates were included, of which 76.0% were identified as *Escherichia coli*. PMQR genes were found in 37.1% of isolates. qnrS was present in 13.2% of isolates, qnrA in 0.6%, qepA in 2.4%, and aac(6′)-Ib-cr in 32.9%. The oqxAB genes were detected in 18 isolates, all of which were identified as *K. pneumoniae*, in which these genes are located chromosomally Of the isolates harbouring PMQR genes, 98.4% had a zone diameter of $\leqslant$16 mm to 1μg ciprofloxacin discs.
Conclusions: These results suggest that the BSAC zone diameter breakpoint for 1μg ciprofloxacin discs is adequate to detect isolates with PMQR genes, although a very small proportion of such isolates may be missed.

P587 Comparison of disc diffusion, E-test and Vitek2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with various breakpoint systems

M. Vading, Ø. Samuelsen, B. Haldorsen, A. Sundsfjord, C.G. Giske (Stockholm, SE; Tromsø, NO)*

Objectives: Carbapenem resistance among *Klebsiella pneumoniae* has increased over the last years and their detection is challenging. The aim of this study was to compare detection of carbapenemase producing strains with the current CLSI and EUCAST breakpoints.
Methods: *K. pneumoniae* (n = 51) with known carbapenemases (KPC n = 31, VIM n = 20) were collected from laboratories in Sweden, USA, Greece and Norway. Disk diffusion (DD; Oxoid) and Etest (bioMérieux) with imipenem (IPM), meropenem (MEM), and ertapenem (ERT) were performed. Further, all strains were tested with the VITEK2-system with 5 different antibiotic cards containing either IPM or MEM or ERT, and various combinations of these. ESBL-testing was performed with VITEK2, ESBL combination disks (Becton Dickinson) and ESBL Etest.
Results: With current CLSI and EUCAST clinical MIC breakpoints, respectively 11 and 7 of the strains were susceptible to IPM and MEM, but none to ERT. Using the EUCAST epidemiological cut-off value (ECOFF) all strains were resistant to both MEM and ERT, but 5 strains were susceptible to IPM. All carbapenemase-producing strains were detected with DD when using a cut-off value of 22 mm for IPM, MEM and ERT. The VITEK2 expert system failed in detecting 3 carbapenemase producers when using a card with IPM as the only carbapenem. One failure was observed with ERT as the only carbapenem, and one failure when using a card containing both IPM and MEM. Antibiotic cards containing MEM or IPM plus ERT recognized all strains as carbapenemase-producing. Many of the strains were ESBL-positive both in VITEK2 and with combination disks (CDT), but not with ESBL Etest.

Conclusion: Both MEM and ERT performed better than IPM in separating between wild-type isolates and carbapenemase-producers. For MEM it was possible to detect all carbapenemases in *K. pneumoniae* with the EUCAST ECOFF, but not with clinical breakpoints. All carbapenemase producers were identified with the ERT clinical breakpoints. Tentative disk diffusion cut-offs could be defined, but should be compared to EUCAST disk diffusion ECOFFs when these become available. Strains with positive ESBL tests according to CDT method or VITEK2 can still be carbapenemase producers, and this should be tested if one or several carbapenem MICs are above the ECOFFs. To detect carbapenemases with the VITEK2 system one should use antibiotic cards containing more than one carbapenem, and always ERT.

P588 Two-centre evaluation of a microarray for rapid detection of antibiotic resistance genes in Gram-negative species that cause community and healthcare-associated infections

J-C. Zhang (London, UK)*

Objectives: To evaluate a DNA extraction and microarray procedure for rapid and accurate detection of resistance genes in Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp.
Methods: The panel of 32 test strains comprised *A. baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, *Hafnia alvei*, *P. aeruginosa*, and *Salmonella*; all had partially-defined resistance genotypes, as determined previously by PCR analysis. All strains were tested in two centres. DNA was extracted using a simple lysis method; resistance genes were amplified in a multiplex labelling reaction and hybridized against a Clondiag strip-format array chip. The array comprised 159 probes in triplicate, representing genes encoding: aminoglycoside-modifying enzymes; acquired AmpC b-lactamases, ESBLs, and carbapenemases (the latter including metallo-enzymes, OXA-48, KPC, and SME enzymes); plasmidic fluoroquinolone resistances; and resistance to chloramphenicol, macrolides, rifampicin, sulphonamides, tetracyclines and trimethoprim. Results were analysed with an Arraymate reader and IconoClust software. PCR and DNA sequencing were used to verify results and to establish criteria for interpreting array data.
Results: The test strains allowed 113/159 (70%) probes to be validated; 46 probes did not hybridize with test DNA. The simple extraction protocol yielded DNA suitable for analysis from diverse species, including multiple genera of Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*. Generally there was good agreement between the genotypes assigned at the two centres and, using a threshold of >0.4, 92% of array 'hits' were confirmed by PCR. The benefit was illustrated by detection by array of rmtC and armA genes, subsequently confirmed by PCR and sequencing, in several clinical isolates that produced NDM-1 metallo-carbapenemase.
Conclusion: This simple array technology is able to detect resistance genes in a broader range of host species than previously studied, and shows good agreement with PCR-based detection strategies. The current array needs further validation against a larger panel of strains, and refinement to replace non-specific probes. However, this initial work clearly shows the potential of arrays as reference microbiology tools and highlights the benefits of the approach to detect antibiotic resistance genes.

P589 Development of a real-time multiplex PCR assay for the detection of CTX-M type ESBLs directly from blood cultures

G.L. Vanstone, L. Wilkie, B.M. Charalambous, I. Balakrishnan (London, UK)*

The rapid detection of Extended-spectrum β-Lactamase (ESBL) producing Enterobacteriaceae, particularly the CTX-M types, is critical to implementing optimal antibiotic therapy and appropriate infection control procedures. Currently, detection is based on phenotypic methods that can take 48 hours to complete.

Objectives: To evaluate the novel combination of a rapid DNA extraction method directly from blood cultures with a real-time multiplex PCR (RT-PCR) that can detect the five-phylogenetic groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M25) of CTX-M type ESBLs (Birkett et al. 2007).
Methods: Five control organisms representing the CTX-M groups were spiked into negative blood cultures to give concentrations of between 10^3 to 10^7 bacteria/ml. A bead-beating DNA extraction method (BD Diagnostics) and RT-PCR was performed on all of the blood cultures. The RT-PCR was performed as described previously (Birkett et al. 2007) on a RotorGene 6000 (Qiagen) using a commercially available master mix (Lightcycler 480 Probes Master, Roche), following the manufacturer's instructions.
Results: All 5 strains were detected directly from blood cultures in 3.5 hours, with a detection limit of 10^4 bacteria/ml. This is significantly lower than the average number of bacteria found in blood cultures when they flag positive ($>10^7$ bacteria/ml) on our currently used, automated blood culture detection system (Bactec FX, BD Diagnostics).
Conclusion: The detection time of the 5 CTX-M ESBL groups directly from blood cultures was reduced by >10 fold compared with standard methods. This rapid methodology is currently being evaluated on actual blood cultures from patients. This simple and rapid technique has the potential to dramatically improve patient outcome and facilitate rationalisation of both antibiotic use and infection control practices much earlier than currently possible.

P590 Pre-real-time PCR steps standardization for appropriate interpretation of mexA and mexX gene expression by mex Q-Test in *P. aeruginosa*

L. Avrain, D. Hocquet, T. Laurent, T. Leclipteux, P. Mertens (Gembloux, BE; Besançon, FR)*

Background: Absolute and relative gene expression analysis by Real-Time PCR may be dependent on pre-Real-Time PCR steps, including bacteria culture and cDNA synthesis. The successful completion of these preliminary steps may affect the final interpretation of the Real-Time PCR results. The aim of the present study was to examine the impact of culture phase, reverse transcription kits and Sybr Green mix kits on the absolute and relative gene expression of mexAB-oprM and mexXY-oprM pumps in *P. aeruginosa*.
Methods: Bacterial pellets were obtained by harvesting growing bacteria at OD from 0.5 to 1.25 at 620 nm, for control strains (PAO1, PT629 and mutGR1 respectively for wild type, mexAB and mexXY over-expressing strains) and for 9 clinical strains overproducing one or both efflux systems. Five reverse transcription kits and five Sybr Green mix kits were compared on purified total RNA. The mexA and mexX genes absolute and relative expressions were detected by mex Q-Test Real-Time PCR kit (Coris BioConcept) with cut-off of significant over-expression at 2 and 5 for mexA and mexX genes (relative to PAO1), respectively.
Results: Analysis of mexA and mexX genes expression during bacterial growth revealed an increase in relative expression of mexA at higher OD particularly for strains over-expressing mexA. The best discrimination between wild-type and mexA or mexX overexpressing strains was obtained at OD 1.0. Analysis of the five reverse transcription kits showed important variations (more than 2 orders of magnitude) in absolute expression of mexA and mexX. Some Reverse transcriptases showed higher transcription of mRNA in high copies as for mexA while others perform better on the mRNA with lower copy numbers like mexX. Table 1 lists the reverse transcriptase kits to the best (rank 1) to least efficient (rank 5). This observation is less perceptible when working in relative expression. No major differences were observed with the Sybr Green mix kits.
Conclusions: For robust and reproducible mex expression analysis with the mex Q-TesT Real-Time PCR (Coris BioConcept), we recommend to harvest bacterial pellets at an OD of 1 ± 0.1 at 620 nm and to choose the best Reverse Transcription kit. Though the list of tested kits is not exhaustive, we recommend suppliers able to identify both genes in low copies number (as the basal level of mexX in wild type strains) and

genes with a low ratio of over-expression compared to PAO1, as strains overexpressing mexA.

Table: Efficacy rank of Reverse Transcription Kits for *mexA* and *mexX* genes

Reverse Transcription Kits	*mexA*	*mexX*
First Strand cDNA Synthesis Kit (Roche Diagnostics)	1	3
Superscript III Reverse Transcriptase (Invitrogen)	2	2
Multiscribe Reverse transcriptase (Applied Biosystems)	3	4
RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas)	4	1
Reverse Transcription System (Promega)	5	5

P591 Comparative evaluation of disc synergy tests with EDTA and dipicolinic acid for detection of MBL production in *Pseudomonas aeruginosa*

E. Skleenova, J. D'Souza, N. Ivanchik, M. Edelstein, R. Kozlov (Smolensk, RU)*

Objectives: Production of acquired metallo-β-lactamases (MBLs) is one of the most clinically and epidemiologically significant mechanisms of resistance to carbapenems in *P. aeruginosa*. The aim of our study was to compare the effectiveness of the double-disk synergy tests (DDSTs) using two different inhibitors: EDTA and dipicolinic acid (DPA) for detection of MBLs in *P. aeruginosa*.
Materials and Methods: Ten reference strains producing the known MBLs: IMP-1, IMP-2, IMP-4, IMP-12, IMP-13, VIM-1, VIM-2, VIM-7, VIM-10, SPM-1, and 480 clinical *P. aeruginosa* isolates non-susceptible to imipenem and/or meropenem (MICs >4 mg/L) were used in this study. The clinical isolates were collected from 36 hospitals of diverse geographic locations. DDSTs were performed by placing the disks containing inhibitors (5 mkl of 0.5M EDTA or 5 mkl of 0.5M DPA) 15 mm (center-to-center) from disks with imipenem. A clearly visible extension of the inhibition zone of any β-lactam disk towards the inhibitor disk was interpreted as positive result for MBL production. PCR for the most common VIM- and IMP-type MBL genes was used as a reference method and imipenem-hydrolysis assay was used as additional method to resolve discrepant results of phenotypic and genotypic tests.
Results: Both the DDST-EDTA and DDST-DPA correctly detected the presence of MBLs in all the control strains and all clinical isolates (n = 159) that demonstrated positive PCR results for MBL genes. True negative results were obtained by both assays for 306 isolates; false positive results – for 15 isolates, of which 5 revealed extension of the inhibition zones with both inhibitors, 5 with EDTA only, and 5 with DPA only.
Conclusion: In our study, DDSTs with DPA and EDTA demonstrated equal performance with 100% sensitivity, 96.9% specificity, 100% NPV, and 94.1% PPV for detection of MBLs in *P. aeruginosa*. The specificity of phenotypic MBL detection could be increased to 98.4% if positive results are confirmed by both assays.

P592 A novel screening agar for the detection of vancomycin non-susceptible *Staphylococcus aureus*

C.D. Burnham, K. Anderson, C.J. Weber, W.M. Dunne Jr. (Saint Louis, Atlanta, US)*

Objectives: The ability to accurately identify isolates of *Staphylococcus aureus* with reduced susceptibility to vancomycin is of critical clinical importance. Guidance for susceptibility testing for *S. aureus* has recently been in flux. In January 2006, CLSI updated breakpoints for vancomycin susceptibility testing for *S. aureus* such that a minimum inhibitory concentration (MIC) greater than 2 mg/L was considered to be non-susceptible to vancomycin. In 2009, the CLSI deemed that disk diffusion was no longer an acceptable means for susceptibility testing for vancomycin in staphylococci. The objective of this study was to design a cost-effective medium to detect vancomycin non-susceptible *S. aureus*.
Methods: A medium consisting of brain heart infusion agar with 3 mg/L vancomycin (BHI-V3) was created to screen for isolates of *S. aureus* with reduced susceptibility to vancomycin. The agar was inoculated with 10 uL of a 0.5 McFarland standard suspension of organism and incubated at 35 C for 24 h. Any growth on BHI-V3 was interpreted as positive. This agar was validated using a collection of 100 *S. aureus* strains previously characterized by the CDC using broth microdilution. This collection included 55 vancomycin susceptible isolates and 45 vancomycin intermediate (VISA) isolates. Once validated, BHI-V3 was incorporated into routine use in the Barnes-Jewish Hospital Microbiology Laboratory. The vancomycin MIC of all isolates growing on BHI-V3 was also determined using multiple methods.
Results: All of the VISA isolates (45) and 19 of the vancomycin susceptible isolates in the challenge set grew on BHI-V3, for 100% sensitivity and 65% specificity. In the first 60 days the agar was implemented in clinical practice, we identified 17 potential VISA isolates out of 421 *S. aureus* strains tested. Thirteen of these isolates were confirmed as VISA. The MIC of the confirmed VISA isolates was determined using four different methods. The Microscan Pos MIC Panel 26 had the highest sensitivity of VISA detection (92%), followed by Etest (85%), and Sensititre GPALL (54%). Vitek2 GP67 was the least sensitive, detecting only 1 of the 13 VISA isolates.
Conclusions: BHI-V3 has excellent sensitivity for detection of VISA. We recommend that clinical laboratories use this media to screen all isolates of *S. aureus* for reduced susceptibility to vancomycin. Isolates that do not grow on the agar can be considered vancomycin susceptible.

P593 Wild type MIC distribution and epidemiological cut-off values in clinical *Legionella pneumophila* serogroup 1 isolates

J.P. Bruin, E. Ijzerman, J.W. den Boer, J.W. Mouton, B.M. Diederen (Haarlem, Nijmegen, NL)*

Objectives: Surveillance studies on the *in vitro* activity of antibiotics used for Legionnaires' disease are necessary to identify changes in susceptibility rates. Wild type distribution and epidemiological cut off values (ECOFF) used to detect resistance have not been described for *Legionella pneumophila*. The goal of this study was to determine MICs in clinical *L. pneumophila* isolates collected from patient materials and to determine ECOFF.
Methods: 187 clinical *L. pneumophila* serogroup 1 isolates collected from unique patients were tested. All patients were included as part of a national outbreak detection programme. All strains were typed using AFLP typing and/ or SBT according to the European Working Group on *Legionella* Infections (EWGLI) guidelines. E-tests for ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, azithromycin, clarithromycin, cefotaxim, rifampicin, tigecycline, and doxycycline (AB Biodisk, Solna, Sweden) were performed. Buffered yeast extract agar supplemented with α-ketoglutarate (BCYE-a; Oxoid, Basingstoke, UK) was used in the testing procedure. The MICs were read after culture at 35ºC after 2 days of incubation. ECOFF were determined according to EUCAST criteria and expressed as WT $\leqslant$ X mg/L.
Results: The MIC90 and wild-type cut-off values were determined at 0.50 (range 0.5–2) and 1.0 mg/l for ciprofloxacin, 0.25 (range 0.064–1.0) and 0.50 mg/l for levofloxacin, 0.50 (range 0.25–1.0) and 1.0 mg/l for moxifloxacin, 0.25 (range 0.032–2) and 1.0 mg/l for erythromycin, 0.25 (range 0.032–8) and 1.0 mg/l for azithromycin, 0.25 (range 0.064–1) and 0.50 mg/l for clarithromycin, 0.50 (range 0.016–1.0) and 1.0 mg/l for cefotaxim, 0.032 (range 0.016–0.032) and 0.032 mg/l for rifampicin, 8 (range 2–16) and 16 mg/l for tigecycline, 8 (range 1–8) and 16 mg/l for doxycycline.
Conclusion: All isolates were inhibited by low concentrations of the quinolones and macrolides tested, with somewhat higher MICs for the fluoroquinolones. Rifampicin was found to be the most active against *L. pneumophila* isolates. Only one isolate (0.5%) showed a MIC outside the WT distribution for the fluorochinolones and macrolide antibiotics (MIC 2 mg/l for ciprofloxacin and 6 mg/l for azithromycin). These data can be used as a reference for the detection of resistance in clinical *L. pneumophila* isolates.

P594 Correlation of MICs and diskzone diameters in clinical *Legionella pneumophila* serogroup 1 isolates

J.P. Bruin, E.P. Yzerman, B.M. Diederen, J.W. Den Boer, J.W. Mouton (Haarlem, Nijmegen, NL)*

Objectives: Routine use of disk diffusion tests for detecting antibiotic susceptibility for *Legionella pneumophila* in relation with MICs have not been described. The goal of this study was to determine the correlation of MICs and zone diameter (MDcorr) in clinical *L. pneumophila* isolates collected from patient materials for 10 antibiotics. We used BCYE agar because this is most commonly used for culturing legionella.
Methods: 194 *L. pneumophila* serogroup 1 isolates collected from unique patients were tested. All patients were included as part of a national outbreak detection program. All strains were typed using AFLP typing and/ or SBT according to the European Working Group on *Legionella* Infections (EWGLI) guidelines. E-tests (AB Biodisk, Solna, Sweden) and disk diffusion tests (Becton, Dickinson and Company, USA) for ciprofloxacin (Cip), levofloxacin (Lev), moxifloxacin (Mox), erythromycin (Ery), azithromycin (Azi), clarithromycin (Cla), cefotaxim (Cef), rifampicin (Rif) and doxycycline (Dox) were performed. Buffered yeast extract agar supplemented with α-ketoglutarate (BCYE-a; Oxoid, Basingstoke, UK) was used in the testing procedure. The MICs and zone diameter were read after culture at 35ºC and 2 days of incubation.
Results: Of the three fluoroquinolones Cip showed the best MDcorr and no major or minor errors were observed in differentiating susceptible and resistant strains. All three macrolides Ery, Azi and Cla showed a wide MIC distribution and good MDcorr. MICs also correlated well with each other with $R > 0.7$. The disk zones were relatively wide, 22–56 mm for Azi and Ery, and less for Cla (35–55 mm). Resistance could be differentiated from susceptible for Azi and Ery, but not for Cla. Depending on the diskzone criterium there was one minor or one major error for Cla. The MDcorr for cefotaxim was good, while for rifampicin, doxycyclin and tigecyclin they were not (<0.4). However, as there were no clearly resistant strains these were difficult to interpret.
Conclusion: Good MIC-diskzone size correlates were found for the quinolones, macrolides and cefotaxim. For quinolones the ciprofloxacin and for macrolides either azithromycin or erythromycin could be used as an indicator for class resistance.

Susceptibility testing with Gram-positives

P595 Inducible clindamycin resistance should not be neglected in erythromycin intermediate *Staphylococcus aureus* blood isolates

D. Gülmez, B. Sener, G. Hasçelik (Ankara, TR)*

Objectives: Detecting inducible clindamycin resistance is a challenge for laboratories which use automated systems for antibiotic suscebtibility testing. BD Phoenix system is used for antimicrobial testing of *Staphylococcus aureus* isolates and BDXpert system is used for reporting the susceptibility results in our laboratory. BDXpert System warns the laboratory to perform a D-test for all erythromycin resistant isolates (Rule 335) and does not report clindamycin susceptibility results. Clindamycin susceptibility results of erythromycin intermediate isolates are reported according to the detected minimum inhibitory concentration. Since the panels to detect inducible clindamycin resistance are not yet available in Turkey, this study was conducted to determine inducible clindamycin resistance in erythromycin resistant and intermediate *S. aureus* isolates.
Methods: *S. aureus* strains isolated from blood from January 2004 to October 2009 were included. Phoenix system detected 67 erythromycin resistant and 9 intermediate isolates. D-test was performed for all isolates and compared with Phoenix results.
Results: Distribution of *S. aureus* isolates according to D-test results and erythromycin susceptibility is given in the Table. Of the 67 erythromycin resistant isolates, 8 (11.9) were D-zone negative and all were clindamycin susceptible by the Phoenix system. Of the 9 erythromycin intermediate isolates, 3 (33.3%) were D-zone positive and falsely reported as clindamycin susceptible.
Conclusions: BDXpert system does not report clindamycin susceptibility results for erythromycin resistant *S. aureus* isolates, however inducible clindamycin resistance in erythromycin intermediate isolates may be overlooked. Therefore all erythromycin intermediate *S. aureus* strains should also be tested for inducible clindamycin resistance.

Table. Distribution of *S. aureus* isolates according to D-test results and erythromycin susceptibility

	Erythromycin R, n (%)	Erythromycin I, n (%)	Total, n (%)
D-zone positive	59 (88.1)	3 (33.3)	62 (81.6)
D-zone negative	8 (11.9)	6 (66.7)	14 (18.4)
Total	67 (100)	9 (100)	76 (100)

P596 Evaluation of spiral gradient endpoint technique for rapid detection of vancomycin intermediate-resistant *Staphylococcus aureus*

V. Doddangoudar, M. O'Donoghue, M. Boost, P. Appelbaum (Kowloon, HK; Hershey, US)*

Objectives: Emergence of *Staphylococcus aureus* strains which are non-susceptible to vancomycin has made treatment of infections by these strains more difficult. Their non-susceptibility cannot be easily and rapidly determined by current methods as they lack sensitivity and reproducibility or are slow and/or tedious to perform, thereby delaying appropriate therapy for the patient. This study evaluated the spiral gradient endpoint (SGE) technique for determination of vancomycin MIC, aiming to provide an economical, rapid, and simple method for the detection of vancomycin non-susceptible *S. aureus*.
Method: Using a spiral plater, vancomycin stock solution (2076 mg/L) was deposited on an agar plate in a spiral pattern to produce a concentration gradient. Three agar media were evaluated: Mueller Hinton (MH), Brain heart infusion (BH) and 5% Glucose Brain heart infusion (GBH). Three isolates were inoculated in duplicate on each plate across the spiral to expose them to the exponential concentration gradient and then incubated at 37ºC for 24 h. SGE was determined by measuring the distance from the centre of the plate to the endpoint of growth. The antibiotic concentration at this point, which represents the MIC, was calculated using SGE software. Results for 30 isolates were compared to standard agar dilution method (SAD).
Results: The intra-batch, inter-batch and inter-observer reproducibilities of SGE method were excellent for all media with best results being obtained for BH. The ranges of standard deviation and coefficients of variation for BH were: 0.35–0.83 & 6.89–12.14% for intra-batch; 0.35–0.53 & 5.77–9.62% for inter-batch, and 0.58 & 8.66–13.3% for inter-observer. There was excellent correlation between MICs generated by the SGE method using BH and SAD (r2 = 0.961) over the range 0.5–20μg/ml.
Conclusion: In the era of increasing antibiotic resistance, early detection of non-susceptibility can lead to improved therapy of multidrug resistant infections and shorten expensive hospitalization stays. This study has shown SGE technique to be a simple, rapid, and cost-effective alternative for the routine laboratory. The cost of consumables is low when compared to other recommended methods. Although there is an initial outlay for purchase of a spiral plater (US$12,500), this investment can be rapidly recovered by savings in consumables and labour, as well as possible reduced hospitalisation costs as a result of rapid susceptibility reporting made possible by SGE.

P597 Methicillin-resistance detection in 5 minutes: novel rapid immunochromatographic kit directly from *Staphylococcus aureus* primary isolates

J-P. Rasigade, O. Raulin, A.M. Freydiere, L. Parmeland, C. Roure-Sobas, H. Salord, J. Etienne, S. Tigaud, F. Laurent (Lyon, FR)*

Objective: Rapid detection of methicillin resistance in *Staphylococcus* spp., especially *S. aureus*, is crucial in patient management. The availability of fast molecular diagnostics has strengthened infection control strategies by providing results in hours rather than days, as the time required for culture-based methods. However, costs and equipment requirements have limited the widespread use of such molecular methods. In this context, we evaluated a novel rapid detection kit (Dipstick PLP2a, Inverness Medical) using immunochromatography for sensitive and specific detection of the PLP2A protein causing methicillin resistance. This method is performed in 5 minutes directly from primary isolates, and is similar to the strep throat test routinely used for diagnosing streptococcal sore throat.
Methods: A total of 222 strains of *Staphylococcus* spp. were grown for 24 h on Columbia blood agar and trypticase soy blood agar plates (bioMérieux, Lyon, France): 153 *S. aureus* (102 methicillin resistant and 51 methicillin susceptible *S. aureus*) and 69 coagulase-negative staphylococci (CoNS) (50 methicillin resistant and 19 methicillin susceptible CoNS). Each strain was tested for methicillin resistance using Dipstick PLP2A assay. The assay was also performed after PLP2A induction in colonies harvested near the inhibition zone of oxacillin and cefoxitin discs on Mueller-Hinton agar plates (bioMérieux, Lyon, France).
Results: Methicillin resistance detection in *S. aureus* had 96% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 98% negative predictive value (NPV). The same detection in CoNS had 86% sensitivity, 100% specificity, 100% PPV and 73% NPV. In both *S. aureus* and CoNS, sensitivity and specificity reached 100% after PLP2A induction or an additional 24 h incubation.
Conclusions: Our findings demonstrate that the Dipstick PLP2A rapid immunochromatographic kit allows sensitive and specific detection of methicillin resistance directly from primary isolates of *S. aureus* and CoNS. In the light of the importance of early adaptation of antibiotic therapy in patients with staphylococcal infections, this rapid diagnostic kit could soon play a routine part in microbiology laboratory testing.

P598 Performance of a new MicroScan WalkAway panel for detection of oxacillin resistance in a French nationwide set of *Staphylococcus aureus* isolated from community-acquired skin and soft tissue infections

O. Gallon, P. Pina, F. Laurent, B. Lamy, X. Palette, F. Doucet-Populaire, J.W. Decousser on behalf of the College de Bactériologie Virologie Hygiène Study Group*

Objectives: Detection of Oxacillin resistance (OR) in *Staphylococcus aureus* is a daily challenge for clinical laboratories. Cefoxitin testing is now currently recommended and used for this purpose. We evaluated the performance of a new MicroScan WalkAway panel (Siemens, Sacramento) including this compound for the detection of OR.
Methods: During 2006 203 *S. aureus* isolates were collected from a nationwide study through the French College de Bacteriologie Virologie Hygiene network interesting in community-acquired skin and soft tissue infections. OR was detected by a mecA PCR, cefoxitin (FOXdm) and moxalactam (MOXdm) disk diffusion method according to French criteria (susceptible to oxacillin if diameter >26 and 23 mm, respectively), and the new MicroScan WalkAway panel PC30. This panel contains oxacillin in doubling dilution from 0.25 to 2 μg/mL and an additional cefoxitin test (4 μg/mL); each strain was categorized as oxacillin resistant if its oxacillin MIC was superior to 2 μg/mL or if the cefoxitin test was positive (i.e. yielded a growth). The panels was automatically run according to the manufacturer's instructions. ATCC25923 and 43300 were used. In addition the Panton-Valentine leukocidin coding gene (pvl) was detected by PCR. Very Major (VMe, false susceptible) and Major errors (Me, false resistance) were reported.
Results: OR was detected in 15.3% of isolates (31/203) by PCR. Considering the mecA PCR as the gold standard, the sensibility, specificity, Positive Predictive and Negative Predictive Value were respectively 96.8%, 100%, 100% and 99.4% for the FOXdm, 93.5%, 100%, 100% and 98.8% for the MOXdm, 100%, 99.4%, 96.9% and 100% for the PC30 panel. VMe and Me were identified for respectively 1 and 0 isolate for the FOXdm, 2 and 0 isolates for the MOXdm and 0 and 1 isolates for the PC30 panel. Comparing to the oxacillin MIC determination, the cefoxitin test detected true OR in two additional isolates (mecA PCR positive). Among the 18 pvl PCR positive isolates, only the PC30 panel accurately identified the oxacillin susceptibility for the totality of this subgroup (17 oxacillin-susceptible and 1 oxacillin-resistant isolates), the FOX and MOX diffusion method misidentifying the OR in the only one mecA and pvl PCR positive isolate.
Conclusions: Both FOXdm or MOXdm and the PC30 panel are highly accurate methods for OR detection in clinical relevant *S. aureus*, the later method belonging to an automated antimicrobial susceptibility testing system.

P599 Resistance to macrolides and licosamides. Comparison of ICR test from Vitek-2C AST-577 card to D-test

R. Soloaga, N. Carrion, M. Vazquez, A. Procopio, P. Amaya, M. Giovanakis, A. Sujemecki, A. Rossetti, D. Lanzetta, S. Guaycochea, L. Biblieri, J. Pares, S. Scorza, R. Pereda, A. Fernandez, A. Meo, L. Derdoy, L. Guelfand (Buenos Aires, AR)*

Objectives: Resistance to macrolides and lincosamides in *Staphylococcus* strains isolated in Argentina is mainly due to efflux mechanisms (E) or to the modified target (ribosome23s); the latter could occur by induction (MLSi) or constitutive resistance (MLSc). Clindamycin may select intratreatment resistance if MLSi is present. The objective was to compare the ICR test performance (inducible clyndamycin resistance) of the Vitek 2C AST-P577 card (bioMérieux, Marcy l'Etoile, France) to the D-test suggested by the CLSI (Clinical and Laboratory Standards Institute) to differenciate E from MLSi..
Materials: 372 isolated *Staphylococcus* strains were studied at 8 hospitals in Buenos Aires, Argentina. They correspond to *S. aureus* (n:197), *S. epidermidis* (n: 86); *S. hominis* (n:30); *S. haemolyticus* (n:21), *S. capitis* (n:6), *S. saprophyticus* (n:4), *S. simulans* (n:4), *S. cohnii* (n:4), *S. warneri* (n:3), *S. lugdunensis* (n:3), *S. auricularis* (n:1), *S. xylosus* (n:1), *Staphylococcus* spp (n:12).
The strains were identified using the Vitek 2C GPI card.
Resistance to macrolides and lincosamides, and the ICR test were determined using the Vitek 2C AST-P577 card, considering breakpoints and D-test methods suggested by the CLSI (CLSI 2009, M2 and M7, Table 2C).
Results: 172 strains (46.3%) were erythromycin and clindamycin susceptible, 97 (26%) were only resistant to erythromycin and 103 (27.7%) were resistant to both antibiotics.
According to the erythromycin and clindamycin MIC result, to the AES Vitek 2C suggestions, to the IRC test and D-test, 23% of the erythromycin resistant strains displayed efflux compatible mechanism, 51.5% MLSc and 25.5% MLSi.
Susceptibility, specificity, positive predictive value, negative predictive value and essential correlation of the IRC test as regards the D-test were 100%.
Conclusions: The IRC test together with the Vitek 2 Advanced Expert System (AES) offer an excellent correlation with the D-test to differenciate MLSi from E.

P600 Comparative evaluation of BD Phoenix system and conventional agar dilution method for detection of oxacillin resistance in *Staphylococcus* spp.

A. Nikulin, M. Sukhorukova, M. Edelstein, R. Kozlov, A. Dekhnich (Smolensk, RU)*

Objectives: To evaluate the performance of the BD Phoenix system and conventional phenotypic methods for the detection of oxacillin resistance in clinical isolates of *Staphylococcus* spp.
Methods: In total, 299 clinical non-duplicate isolates of *Staphylococcus* spp. collected from patients with different types of invasive infections in different regions of Russia were included in the study.
Minimum inhibitory concentrations (MICs) of oxacillin were determined using the agar dilution method on Mueller-Hinton agar (Becton Dickinson). *Staphylococcus aureus* strain ATCC® 29213 and strains carrying the SCCmec cassettes of types I-IV were used for quality control. The results of agar dilution susceptibility testing were interpreted according to the EUCAST clinical breakpoints. The combination panels PMIC/ID-60 were used with the BD Phoenix system. Real-time PCR (RT-PCR) assay for the mecA and internal 23S rRNA staphylococcal gene sequences was used as a reference method to which the phenotypic methods were compared.
Results: The results of oxacillin susceptibility testing by agar dilution method and BD Phoenix system compared to those of RT-PCR are presented in the table. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for both methods were calculated. In three series of repetitive tests, the BD Phoenix system did not yield any susceptibility results for 11 isolates of coagulase-negative staphylococci (CNS) which were therefore excluded from analysis. Most of the isolates that showed discrepant results of oxacillin susceptibility testing and mecA status had the oxacillin MICs differing by no more than one two-fold dilution from the breakpoint value.
Conclusion: Using the molecular test for mecA detection as a reference method, the BD Phoenix system demonstrated higher sensitivity and specificity than the agar dilution method for detection of oxacillin resistance in *S. aureus* and CNS.

	Number of isolates				Percent			
	True positive	False positive	True negative	False negative	Sensitivity	Specificity	PPV	NPV
S. aureus (n = 200)								
RT-PCR	78		122					
Phoenix	72	2	120	6	92.3	98.4	97.3	95.2
Agar dilution	66	30	92	12	84.6	75.4	68.8	88.5
CNS (n = 99)								
RT-PCR	43 (35*)		56 (53*)					
Phoenix*	33	15	38	2	94.3	71.7	68.8	95
Agar dilution	41	17	39	2	95.3	69.6	70.7	95.1

*Excluding isolates which did not yield any susceptibility result with BD Phoenix.

P601 Detection of inducible macrolide-lincosamide-streptogramin B resistance in *Staphylococcus* species: Vitek2 Advanced Expert system versus double disc method (D-zone test)

I. Geerts, A. Smismans, J. Frans (Bonheiden, BE)*

Objective: The double disk induction test (CLSI, M100-S19) is recommended to determine inducible macrolide-lincosamide-streptogramin B (iMLSB) resistance in *Staphylococcus* species. The Advanced Expert System (AES) of Vitek 2 (bioMérieux) claims to detect this resistance mechanism as well. To evaluate the reliability of the AES, we compared Vitek 2 with the double disk method.
Methods: On 125 consecutive Staphylococci spp. (52 *Staphylococcus aureus* en 73 coagulase negative Staphylococci), collected in Imelda Hospital Bonheiden (27/10/2008–28/07/2009), we retested erythromycin-resistant Staphylococci on Vitek card AST-P549 (bioMérieux) for which the AES reported a "resistant" clindamycin result (Vitek expert rule: 'Therapeutic interpretation suggests corrections to clindamycin'), applying the CLSI double disk induction protocol. *Staphylococcus* strains were suspended to a 0.5 McFarland turbidity before inoculation on a Mueller-Hinton agar (Biorad). Subsequently, the erythromycin and clindamycin disks (Biorad) were placed exactly 15 mm edge to edge and the plates were incubated for 16 to 18 h at 35±2ºC. Flattening of the zone of inhibition adjacent to the erythromycin disk confirmed the presence of iMLSB.
Results: Each isolate resistant for erythromycin and susceptible to clindamycin was inferred by the AES to harbour an iMLSB phenotype. These data are inconsistent with surveillance data of methicillin susceptible *S. aureus* strains obtained by our reference center in Belgium (2007) showing a resistance for clindamycin and erythromycin of respectively 26% and 7%. In the same survey methicillin resistant *S. aureus* expressed 62% resistance to erythromycin and 43% to clindamycin.
We obtained equal results between the AES and the double disk induction in only 58.4% of our isolates. The results were discordant for 21.1% of the *S. aureus* strains and 56.1% of the coagulase negative Staphylococci. During our evaluation, Vitek 2 reported two major errors for clindamycin and erythromycin susceptibility in two strains: one with confirmed constitutive MLSB for clindamycin (0.8%) and another found susceptible for erythromycin (0.8%).
Conclusion: The high false positive rate excludes the application of the AES for the reliable detection of iMLSB in *Staphylococcus* species. The company announced to provide an improved iMLSB detection method in the near future.

P602 Evaluation of MicroScan WalkAway for identification and antimicrobial susceptibility testing of Gram-positive cocci

H. Poupet, J.M. Adam, N. Dmytruk, A. Billoet, C. Poyart (Paris, FR)*

Objectives: The aim of this study was to evaluate MicroScan WalkAway (Siemens) automated system for the identification and antimicrobial susceptibility testing (AST) of Gram-positive cocci. A total of 335 non-redundant clinical isolates including 100 *Staphylococcus aureus* (SA), 50 coagulase-negative staphylococci (CNS), 55 *Streptococcus agalactiae* (GBS), 71 *Enterococcus* sp (En), 20 β-hemolytic streptococci (BHS), 40 α-hemolytic streptococci (AHS) were tested.
Methods: MicroScan identifications were compared to: (i) phenotypic routine tests for SA, GBS, and BHS; (ii) Vitek2 system (bioMérieux) for CNS and En; and (iii) sodA sequencing for AHS. AST results were compared to the agar dilution method according to the 2007 CA-SFM recommendations.
Results: MicroScan correctly identified: 98% of SA, 98% of CNS, 94.5% of GBS, 96% of En, and 100% of BHS (identified as first choice >85% probability: 96% of SA, 71% of CNS, 84% of GBS, 89% of En, and 90% of BHS, identified with low-level discrimination <85%: 2% SA, 27% SCN, 8% SGB, 7% En, and 10% BHS). Nine strains were misidentified: SA (2%), CNS (2%), GBS (9%), En (4%). Using sodA sequence determination as the gold standard, 72% of AHS were correctly identified by MicroScan among which 15% were identified with low-level discrimination. However 28% AHS were misidentified.
AST results were as follows:

i. SA: methicillin resistance was detected in 98% of the strains. Two strains expressing low level methicillin resistance were not detected. Very Major Error (VME) (susceptible (S) for resistant (R)) were observed for penicillin G (2.5%), quinolones (1.2%), and trimethoprim/sulfamethoxazol (1.2%).
ii. CNS: methicillin resistance was detected in 98% (1 major error R for S). 2% VME were observed for quinolones, tobramycin, and trimethoprim/sulfamethoxazol; 14% VME for fucidic acid; and 10% for fosfomycin.
iii. Streptococci: 100% agreement was observed between the two methods concerning β-lactams. Among GBS, 28 strains were resistant to erythromycin (Ery) and/or clindamycin (Cli). Ery and Cli resistance was not detected by MicroScan in 18% and 4%, respectively.
iv. En: all strains resistant to glycopeptides were detected by MicroScan. 1 VME was observed for kanamycin.

Conclusion: This study shows that the MicroScan system is an acceptable system for identification and AST of Gram-positive cocci in routine laboratory. However, macrolide resistance detection in streptococcal species remains to be improved.

P603 Multicentre evaluation of the MicroScan Dried Overnight cefoxitin screen test for coagulase-negative staphylococci

J. Hindler, K. Ward, D. Bruckner, K. Sei, M. Bacsafra, H. Bains, L. Mann, B. Zimmer (Los Angeles, West Sacramento, US)*

Objectives: Accurate detection of methicillin resistance in clinically significant coagulase-negative staphylococci (CNS) is essential. CLSI recently introduced disk diffusion testing with cefoxitin (FOX) as an alternate method to determine mecA-mediated methicillin resistance in CNS. Interpretive criteria using FOX broth microdilution have not yet been established for CNS. A multicenter study was performed to evaluate the MicroScan broth microdilution Cefoxitin Screen (CfxS) Test used in combination with the 18 hour oxacillin MIC. Results obtained were compared with mecA or FOX disk results.
Methods: MicroScan panels were evaluated at three sites with 184 efficacy and 32 challenge isolates (114 *S. epidermidis* and 102 other CNS including 16 *S. lugdunensis)*. Isolates were tested on a MicroScan Dried Overnight Gram Positive Panel containing a single well with 4 ug/ml cefoxitin and oxacillin concentrations of 0.12 to 8 ug/ml. MicroScan results were compared to FOX disk and to mecA PCR. Panels were inoculated using the turbidity standard method and read visually at 18 h. FOX disk diffusion was performed as described and interpreted by CLSI. MecA PCR was performed using primers described by Murakami et al. (J. Clin. Microbiol. 29: 2240–2244).
Results: Sensitivity and specificity of the MicroScan panel using CfxS and the 18 h OX MIC for detection of OX resistance compared to mecA PCR were 99.2% and 95.9%. The MicroScan panel had 0.8% VME (1/118) and 4.1% ME (4/98). Two of the 4 ME triggered a software Alert rule indicating that CNS with OX MICs of 1 or 2 and negative CfxS results may still be mecA negative and that additional testing might be performed to resolve the mecA status. Sensitivity and specificity for CfxS and 18 h OX MIC compared to FOX disk were 96.6% and 92.8%. Sensitivity and specificity of the FOX disk results compared to mecA PCR were 95.8% and 93.9%.
Conclusion: This multicenter study showed the MicroScan Dried Overnight panel Cefoxitin Screen (CfxS) results correlate well with mecA PCR testing for CNS.

Diagnosis and susceptibility testing of *Staphylococcus* and *Enterococcus*

P604 Validation of the MicroScan WalkAway®-96 for the species identification and susceptibility testing of clinically significant coagulase-negative staphylococci

L. Patteet, L. Peeters, H. Goossens, M. Ieven (Antwerp, BE)*

Objectives: Because of their increasing importance Coagulase Negative Staphylococci(CNS)should be identified to the species level and susceptibility testing be performed in clinically significant specimens by a reliable method. Whereas *S. epidermidis* is usually easily identified with a high accuracy, some less prevalent species among the CNS are identified with much lower accuracy by some commercial systems. The aim of this study was to validate an automatic system, MicroScan WalkAway®-96 in conjugation with the MicroScan®–Pos BP Combo Panel Type 28 (Siemens Healthcare Diagnostics, Sacramento CA) for the identification and methicillin susceptibility of CNS.
Methods: The MicroScan system was evaluated on a collection of 428 CNS isolated in the University Hospital Antwerp. Identification to the same species level by a validated in-house method (J. Clin. Microbiol. 1995; 33: 1060), 2 commercial methods (API ID 32 Staph® and Staph-zym®) and the biochemical reference method was considered the gold standard. Methicillin susceptibility was compared to MICs for oxacillin combined with PCR for the mecA gene.
Results: 413/428 (96.5%) of isolates could be identified correctly by the MicroScan WalkAway®-96 system: *S. epidermidis* 292/296 (99%), *S. haemolyticus* 48/50 (96%), *S. hominis* 33/34 (97%), *S. capitis* 13/14 (93%), *S. warneri* 9/14 (64%), *S. lugdunensis* 4/5 (80%), *S. schleiferi* 6/6, *S. cohnii* 2/2, *S. saprophyticus* 2/2, *S. hyicus* 1/1, *S. intermedius* 1/1, *S. sciuri* 1/1, *S. xylosis* 1/1, *S. lentus* 0/1.
Of the 15/428 (3.5%) strains not correctly identified, 5 were *S. warneri*: 3 were identified at the genus level, 2 were misidentified; the 12 other species were misidentified as other CNS.
The MicroScan system determines the methicillin susceptibility by interpreting the susceptibility for oxacillin and cefoxitin, both integrated in the Combo Pos panel. The correlation between the oxacillin and cefoxitin susceptibility results was 98%. When the methicillin susceptibility of the CNS determined by MicroScan was compared with the MecA PCR method, the correlation was 97.6% both with oxacillin and cefoxitin: 10/406 (2.5%) isolates showed a discrepant result, 8 were reported false resistant in mecA negative strains, 2 were reported false susceptible in MecA positive strains.
Conclusion: From this study, we concluded that, except for *S. warneri,* the MicroScan WalkAway®-96 system is a simple and reliable method for the identification and methicillin susceptibility testing of CNS.

P605 Rapid detection of *Staphylococcus aureus* strains with reduced susceptibility to vancomycin by isothermal microcalorimetry

A. Bizzini, A. Trampuz (Lausanne, CH)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing cause of morbidity and mortality. Vancomycin (VAN) is the first-line antibiotic for serious and invasive MRSA infections. However, MRSA can exhibit heterogeneous (hVISA) or homogeneous (VISA) intermediate susceptibility to VAN, which has been associated with treatment failure. Therefore, the detection of VAN intermediate resistance is of high importance in the clinical setting. Current microbiological techniques, such as population analysis profile (PAP), are laborious and time-consuming. We investigated the potential of microcalorimetry as a rapid and simple method to detect subpopulations of reduced susceptibility to VAN by detection of growth-related heat production in the presence of VAN.
Methods: CLIN, a clinical MRSA isolate (VAN MIC 1 mg/L) was used as a VAN susceptible control (VSSA). *S. aureus* Mu3 (VAN MIC 2 mg/L) was the representative strain for hVISA and *S. aureus* Mu50 (VAN MIC 8 mg/L) for VISA. PAP was performed according to standard techniques. Microcalorimetry was performed by inoculating 10^7 CFU of overnight cultures into vials containing 2 ml of brain heart infusion broth with serial dilutions of VAN (from 0 to 8 mg/L). Heat production was measured at 37ºC under static conditions for 48 hours. Detection of heat (representing bacterial growth) was set at 20 microWatts, and the time to detection (TTD, in hours) was determined.
Results: Table 1 shows the calorimetric TTD of the tested *S. aureus* strains at various VAN concentrations. No heat production was detected in the VSSA MRSA isolate at VAN concentration $\geqslant$1 mg/L during the 48 hours of incubation. The Mu3 (hVISA) showed heat production with a dose-proportional delay to 3.5 h (VAN 2 mg/L) and 28.5 h (VAN 4 mg/L). The Mu50 (VISA) produced heat up to VAN 8 mg/L.
Conclusions: Microcalorimetry was able to detect the presence of the VAN resistant subpopulations in hVISA and VISA within 4 h. Measuring of bacterial heat production may represent a simple and rapid method for detection of various heteroresistant bacterial phenotypes. The accuracy and reproducibility need to be tested with clinical strains and validated with PAP.

Table 1

VAN concentration (mg/L)	TTD		
	VSSA (CLIN)	hVISA (Mu3)	VISA (Mu50)
0	0.8	0.1	0.1
1	–	0.1	0.1
2	–	3.5	0.1
4	–	28.5	7.1
8	–	–	37.5

P606 Gram staining and recovery capacity of methicillin-resistant *Staphylococcus aureus* by the Copan Eswab™ system versus the Copan dry swab system

V. Saegeman, J. Flamaing, J. Muller, W. Peetermans, J. Verhaegen (Leuven, BE)*

Objectives: The Copan flocked swab system supplied with liquid Amies medium (Eswab™) is getting established in a growing number of hospitals. The aim of this study was to compare the performance of the methicillin resistant *Staphylococcus aureus* (MRSA) Eswab™ with that of the traditional Copan dry swab in terms of Gram staining, total recovery capacity and MRSA detection.
Methods: Swabs were taken from 70 patients hospitalised in the University Hospitals Leuven (geriatric medicine, internal medicine, intensive care unit burn wounds). Nose and perineum were sampled both with two conventional dry swabs and with the MRSA Eswab™. The Eswab™ and the dry swab were alternate used first.
Gram stains were performed by rolling the dry swabs over a slide and by placing a drop of the vortexed Amies medium of the Eswab™on a slide. Gram staining was performed with a Mirastainer® (Merck KGaA).
Recovery capacity was evaluated based on the recommendations of CLSI document M40-A. More specifically, the swab elution method was used to provide quantitative comparisons. The dry swabs were vortexed in 1 mL sterile physiologic saline. Three serial 10-fold dilutions were prepared from each swab medium (saline/Amies). From each dilution, 100 µl was plated with the spiral plater (Spiral Biotech, autoplate 4000) on mannitol-salt agar and MRSA chromogenic agar plate. Bacterial counts were performed by means of the IUL Countermat Flash 4.2 automatic reader system.
Statistics: paired t-test and McNemar χ^2.
Results: Gram stains from the Eswab™ had a better quality than those from the dry swab: more bacteria per high-power field (67% vs 7%), more bacterial species (51% vs 9%), and more epithelial cells (17% vs 13%) were noticed on the Eswab™ slide.
The total recovery capacity (CFU/mL) on average was 0.35 log higher with the Eswab™. This was significantly higher than the recovery with the dry swab (paired t-test of log-transformed data, $p < 0.01$).
MRSA was detected only with Eswab™ in 3/70 samples. In 1/70 samples, MRSA was detected only with the dry swab. This difference was statistically significant (McNemar χ^2, $p < 0.01$).
Conclusion: This study showed that the higher recovery capacity of the Eswab™ system also entails a higher chance of detecting MRSA. A more extensive study should confirm this hypothesis. Implementation of this Eswab™ will require a cost-benefit analysis. The extra costs of the Eswab™ should be compared with the savings of detecting more MRSA carriers.

P607 Isolation of MRSA: a comparison of direct plating on Brilliance™ MRSA agar and broth enrichment on Chromagar MRSA agar

S. Jobbitt, E. Scopes (Dunedin, NZ; Basingstoke, UK)*

Objective: Standard laboratory procedures at Southern Community Laboratories, NZ, for MRSA isolation currently involve broth enrichment prior to plating onto CHROMagar MRSA Agar (BD). This study compares the standard laboratory procedures to direct inoculation onto Brilliance™ MRSA Agar (Oxoid).
Method: 362 swabs were collected from patients undergoing screening for MRSA. Swabs were directly inoculated onto Brilliance MRSA Agar and plates were incubated at 37ºC for 24 h. The swabs were then tested according to standard laboratory procedures. Procedures involve swab inoculation into an MRSA enrichment broth and incubation at 37ºC for 24 h. Post-enrichment the broth was subcultured onto CHROMagar MRSA Agar and plates were incubated at 37ºC for 24 h. Growth of presumptive MRSA on Brilliance MRSA Agar and CHROMagar MRSA Agar was confirmed by Gram stain, coagulase test, latex agglutination, and cefoxitin-resistance testing. This confirmation algorithm was considered to be the gold standard.
Results: Eight of the 362 swabs were confirmed as MRSA positive (2% prevalence rate). The direct plating method on Brilliance MRSA Agar gave a sensitivity and negative predictive value (NPV) of 100% and specificity of 99%. The broth enrichment method on CHROMagar MRSA Agar gave a sensitivity and NPV of 100% and specificity of 98%. Direct inoculation of Brilliance MRSA Agar yielded fewer false positive results than broth enrichment onto CHROMagar MRSA Agar.
Conclusions: Direct inoculation of Brilliance™ MRSA Agar is a highly effective technique for the screening of patients for MRSA, showing high sensitivity, specificity and NPV. Although the number of positives was low, the study indicates that MRSA can be isolated on Brilliance MRSA Agar without the need for additional, time-consuming and costly steps such as broth enrichment. Brilliance MRSA Agar also successfully inhibits the growth of most non-MRSA organisms, reducing the need for confirmation procedures. Early presumptive MRSA identification by Brilliance MRSA Agar allows appropriate treatment and infection control procedures to be adopted earlier, improving both treatment outcomes and the effectiveness of infection control measures.

Table 1. Performance of direct plating and broth enrichment methods for detection of MRSA

Performance	Direct plating onto *Brilliance* MRSA Agar	Broth enrichment onto CHROMagar MRSA Agar
Sensitivity (%)	100	100
Specificity (%)	99	98
NPV (%)	100	100
Time to result (h)	18–24	42–48

P608 Optical mapping reveals a genetic insertion in colonizing *Staphylococcus aureus* isolated from diabetic foot ulcers

A. Sotto, G. Lina, J.L. Richard, G. Skorski, C.W. Dykes, J. Etienne, J-P. Lavigne (Nîmes, Lyon, Bernis, FR; Gaithersburg, US)*

Objective: Recently we highlighted the coexistence of two *Staphylococcus aureus* populations in diabetic foot ulcers (DFU): one colonizing with low virulence potential and one infecting with high virulence potential. The purpose of this study was to assess the epidemiological links and genomic rearrangements between these 2 *S. aureus* populations with the goal of developing a tool to discriminate non-infected from infected DFU.
Methods: Diabetic patients hospitalized in a diabetic foot department with a foot ulcer were prospectively enrolled if they had been free of antibiotic treatment over the previous 6 months. At admission, ulcers were classified as infected or noninfected on the basis of clinical examination, according to the International Working Group on the Diabetic Foot system. Only patients carrying *S. aureus* as the sole pathogen were included. Twenty-two patients harboring DFU (8 Grade 1, 4 Grade 2, 7 Grade 3 and 3 Grade 4) were enrolled. Strains were subjected to analysis by Pulsed Field Gel Electrophoresis (BioRad), using restriction endonuclease SmaI, and by Optical Mapping (OpGen Inc., Gaithersburg, USA) to examine their genomic organization and to search for shared features. Optical Maps were grouped according to similarity using the UPGMA method.
Results: Using PFGE, no clonal link was detected between the different strains, notably those isolated from Grade 1 ulcers (~70% homology). Using Optical Maps, we identified that colonizing *S. aureus* (Grade 1) belonged to a clonal group (CI) near to USA300 and infecting *S. aureus* (Grade 2–4) to three clonal groups (CI, CII near to USA800, CIII near to USA200). Four infecting strains also belonged to the same CI clone as the colonizing strains, with more than 90% homology between these strains. Optical Mapping identified the presence, and approximate location, of an inserted DNA element in colonizing strains that was not present in infecting strains. This insertion was exclusively present in all

Grade 1 strains and served to distinguish between the 2 populations. The possibility that the insertion disrupts a gene related to virulence is under investigation.
Conclusion: Optical Mapping provides considerably greater resolving power than PFGE for distinguishing between colonizing and infecting *S. aureus* in DFU and could contribute to more appropriate use of antibiotics in these patients.

P609 Evaluation of chromogenic and selective media for the detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in respiratory samples from cystic fibrosis patients

E. Andre, P. Lebecque, A. Simon, T.D. Huang (Brussels, BE)*

Objectives: Early detection of *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) infection in cystic fibrosis (CF) patients remains a challenge for the microbiology lab. The aim of this study was to compare commercial selective media to standard culture media for the recovery and the presumptive identification of PA and of SA directly from sputum collected in these patients.
Methods: 67 sputa from 67 ambulatory CF patients were cultured onto 6 selective media including MacConkey agar (MC; home-made), *Pseudomonas* Isolation Agar (PABD; Becton-Dickinson), ChromID PA agar (PABM; bioMérieux), a colistin aztreonam blood agar plate (CAP, Oxoid), CHROMagar SA medium (SABD; BD) and ChromID SA agar (SABM; bioMérieux). After up to 7 days of incubation at 35ºC, colonies suggestive of PA or SA according to the plates reading guidelines provided by the manufacturers were selected for phenotypic identification. Isolates showing atypical testing results were further submitted for 16S rRNA sequencing.
Results: PA isolates were found in 14 samples (21%) growing at least on one of the three selective media for PA. No significant difference could be observed between the three media in terms of the median time of growth which ranged from 48 to 72 hours for both PA and SA. For PA, the sensitivities were 71% (n = 10), 93% (n = 13) and 78% (n = 11) for MC, PABD and for PABM respectively. The specificities of the two selective media calculated on the basis of interpretative criteria provided by the manufacturers were 69% for PABD and 85% for PABM. 29 patients (43%) were found positive for SA and SABM showed significantly higher sensitivity (96%; n = 28; $p < 0.05$) compared to those obtained for CAP (52%; n = 15) and SABD (79%; n = 23). The specificities of the two chromogenic media were 58% for SABD and 72% for PABM according to the recommended interpretative criteria. 46% (n = 13) of the total SA isolates recovered by this method displayed small colony variants phenotype.
Conclusion: The use of specific selective media could increase the recovery of PA and SA isolates in CF respiratory samples and could lead to an earlier management and antimicrobial therapy for these colonized or infected patients. However, the identification of the isolates should be confirmed as suggested by the low specificity of these media.

P610 Rapid detection of *Staphylococcus aureus* from blood culture with SaSelect or MRSASelect

A. Decoster, E. Dehecq (Lomme, FR)*

Objectives: Only few hours incubation time are necessary to identify *Staphylococcus aureus* and differentiate methicillin resistant (MRSA) from blood culture by using SaSelect and MRSASelect.
Methods: After Gram staining, all blood cultures containing Gram positive cocci in clusters were subcultured on SaSelect and MRSASelect (Bio-Rad). In parallel, 10 blood culture bottles were spiked with 8 MRSA strains and 2 methicillin resistant coagulase negative staphylococci. The readings were performed from 6 h until 32 h of incubation (in general, the readings were performed at 6, 8, 24 and 32 h at 35–37ºC). The identification of *S. aureus* was confirmed by Pastorex Staph (Bio-Rad).
Results: 103 blood cultures positive to Gram positive cocci in clusters were collected from February to June 2009, 44 of them containing *Staphylococcus aureus* (17 MRSA and 27 MSSA), 58 holding coagulase negative staphylococci, in one culture was identified a Micrococcus. All the *S. aureus* have grown on SaSelect, all the MRSA have grown on MRSASelect. The percentage of detection of *S. aureus* is described in the following table according to the incubation time.
Even though about 13% of them were slightly pinkish, no coagulase negative staphylococci showed the typical coloration of *S. aureus* even after 32 h incubation time. The spiked specimens have confirmed the prospective data.
Conclusion: From 6 to 32 h incubation time, as soon as pink colonies are detected on SaSelect or MRSASelect, the microbiologist can report the presence of a *S. aureus* or more precisely of a MRSA.

Time after incubation	Percentage of detection of *S. aureus*	
	Sa Select	MRSA Select
6 h	43%	8%
8 h	93%	27%
10 h	98%	75%

P611 An evaluation of different methods to recover methicillin-resistant *Staphylococcus aureus* from hospital environmental surfaces

A. Dolan, M. Bartlett, B. McEntee, E. Creamer, O. Sherlock, H. Humphreys (Dublin, IE)*

Objective: Due to the increased costs and clinical importance of healthcare-associated infections (HCAI), there is a need for evidence-based approaches to environmental sampling and its evaluation. We compared the recovery of MRSA from two hospital surfaces using different sampling methods, with a wild-type patient strain of methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: One-hundred cm^2 sections of mattress cover and laboratory bench surface were contaminated with known inocula of MRSA. Bacteria were recovered at three different time intervals (0 h, 24 h and 72 h) using either saline moistened cotton swabs, neutralising buffer swabs (Technical Service Consultants, U.K.), eSwabs (Copan, Italy), or direct contact plates (MRSA Brilliance, Oxoid, U.K.). Swabs were enriched in tryptone soya broth (Oxoid), nutrient broth (Oxoid), Amies solution (Copan) or brain heart infusion (Oxoid) at 37ºC. Ten microlitres of enrichment media were transferred to MRSASelect chromogenic agar plates (BIO-RAD, France) after 18 h and incubated along with the contact plates for 24 h at 37ºC. The sensitivity of each method cm^{-2} was determined for all time intervals and surfaces.
Results: The most sensitive method was eSwabs enriched in Amies solution, which required 1.1 bacteria cm^{-2} to generate a positive result. The least sensitive method was saline moistened cotton swabs, which required 8.0×10^4 bacteria cm^{-2}. MRSA Brilliance contact plates were the quickest (24 h for a presumptive positive) and least labour intensive method, and were highly sensitive, requiring 2.4 bacteria cm^{-2} to generate a positive result. All sampling methods required less bacteria cm^{-2} from the laboratory bench surface (flat) than the mattress cover surface (highly undulated) to generate a positive result. All sampling methods showed a significant reduction in sensitivity at the 72 h time interval.
Conclusions: The recovery of bacteria from environmental samples varies with the swabs, methodology used and the surface. Negative culture results may not exclude a pathogen-free environment. Greater standardisation in environmental sampling is required to facilitate the assessment of and monitor improvements in the cleanliness of healthcare institutions.

P612 How many lumens should be cultured in the conservative diagnosis of catheter-related bloodstream infections?

M. Guembe, M. Rodríguez-Créixems, C. Sánchez-Carrillo, A. Pérez-Parra, P. Martín-Rabadán, E. Bouza (Madrid, ES)*

Objectives: Recent practice guidelines for the diagnosis of catheter-related bloodstream infection (CRBSI) describe as an "unresolved issue" the number of lumens from which blood cultures should be drawn to make a conservative diagnosis of CRBSI. Our objective was to determine how many CRBSI episodes would be missed if not all catheter lumens were sampled.
Methods: We performed a retrospective study in patients with microbiologically proven CRBSI in which all available catheter lumens were used to drawn blood cultures. We artificially and randomly eliminated the information and laboratory results of 1 or more lumens in order to recalculate the number of episodes that would have been missed in double and triple-lumen catheters.
Results: We collected 171 episodes of proven CRBSI corresponding to 154 patients. Overall, if 1 lumen culture had been eliminated in both double-lumen and triple-lumen catheters, we would have missed 37.5% and 25.4% episodes of CRBSI, respectively. If we had eliminated 2 cultures in triple-lumen catheters, 47.5% episodes would have been missed.
Conclusion: Samples for blood culture should be obtained through all catheter lumens in order to establish a diagnosis of CRBSI.

P614 Comparison of two chromogenic media for detection of vancomycin-resistant enterococci from South Australian patients

E. Scopes, H. Henry (Basingstoke, UK; Adelaide, AU)*

Objective: To compare the performance of two chromogenic media for detecting vancomycin-resistant enterococci (VRE) from faecal samples and rectal swabs sourced from patients undergoing VRE-screening at a South Australian hospital.
Method: 114 VRE isolates previously isolated from clinical samples were streaked onto Brilliance VRE Agar (Oxoid) and chromID™ VRE Agar (bioMérieux). 222 faecal samples and 370 rectal swabs were inoculated onto Brilliance VRE Agar and chromID VRE Agar. Plates were incubated at 35ºC and read at 20–24 h and 48 h. Presumptive VRE colonies on Brilliance VRE Agar and chromID VRE Agar were identified by Gram stain or biochemical identification and susceptibility testing. Isolates identified biochemically and phenotypically as VRE were confirmed by RT-PCR. The gold standard was deemed to be the identification of VRE by RT-PCR.
Results: 108 and 105 VRE isolates grew on Brilliance VRE Agar and chromID VRE Agar respectively at 24 h. At 48 h, both agars showed growth of all 114 isolates. For clinical samples, Brilliance VRE Agar showed sensitivity and specificity comparable to or exceding that of chromID VRE Agar at 20–24 h and 48 h incubation, although both agars showed a reduction in specificity at 48 h. Both agars showed consistently high NPV at both 20–24 h and 48 h.

Table 1. Performance of *Brillance* VRE Agar and chromID VRE Agar for detection of VRE

Product	Incubation time (h)	Sensitivity (%)	Specificity (%)	NPV (%)
Brilliance VRE Agar	20–24	96.2 (95% CI 97.4–97.7)	96.5 (95% CI 95.0–98.0)	99.8 (95% CI 99.5–100)
chromiD VRE Agar	20–24	75.0 (95% CI 71.5–78.5)	94.8 (95% CI 93.0–96.6)	99.5 (95% CI 98.3–100)
Brilliance VRE Agar	48	100 (95% CI 100)	86.2 (95% CI 83.4–89)	100 (95% CI 100)
chromID VRE Agar	48	97.9 (95% CI 96.7–99.1)	67.1 (95% CI 63.3–70.9)	99.7 (95% CI 98.8–100)

Conclusions: Brilliance™ VRE Agar showed exceptionally better sensitivity at 24 h than chromID™ VRE Agar and has proven to be a highly sensitive and specific medium for the presumptive detection of VRE from clinical samples. Brilliance VRE Agar produced significantly fewer false positive results (non-VRE) than chromID VRE Agar, thus reducing the number of unnecessary further confirmation procedures. Reliable and accurate results were available within 24 h when using Brilliance VRE Agar, allowing rapid initiation of infection control measures and patient treatment.

P615 Evaluation of Brilliance™ VRE Agar for detection of vancomycin-resistant enterococci from four geographically different hospitals in the United States

E. Scopes, M.A. Silvius (Basingstoke, UK; Lenexa, US)*

Objective: To evaluate the performance of Brilliance VRE Agar for detecting vancomycin-resistant enterococci (VRE) from stool samples and rectal swabs sourced from four geographically different hospitals in the United States.
Method: 398 stool samples and 250 rectal swabs were collected from asymptomatic patients undergoing screening for VRE colonization. Stools/swabs were streaked onto Brilliance VRE Agar and Bile Aesculin Azide Agar containing 6μg vancomycin (BAAV). Plates were incubated at 35±2ºC; Brilliance VRE Agar was read at 24 h and BAAV was read at 24 h and 48 h. Presumptive VRE colonies on Brilliance VRE Agar and BEAV were identified to species level and their antibiotic susceptibility determined using laboratory standard methods. The gold standard was deemed to be identification of an enterococcus with a vancomycin MIC > 6μg/ml.
Results: 221 vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (>256 μg/ml) were recovered from 208 specimens (34% prevalence). Overall performance (sensitivity, specificity and negative predictive value (NPV)) of Brilliance VRE Agar was greater than BAAV. Performance of Brilliance VRE Agar was equivalent to the gold standard (McNemar's χ^2 test $P = 0.6171$) whereas performance of BAAV was significantly lower ($P \leqslant 0.0001$).
Conclusions: Brilliance™ VRE Agar is a highly effective product for the screening of gastrointestinal colonization of VRE. Brilliance VRE Agar detects the most clinically relevant enterococci, allowing clear differentiation of *E. faecalis* and *E. faecium* while inhibiting growth of intrinsically resistant *E. gallinarum* and *E. casseliflavus,* whereas BEAV is unable to distinguish between *Enterococcus* species. Early presumptive identification of *E. faecium* and *E. faecalis* allows appropriate treatment and infection control procedures to be adopted earlier, improving treatment outcomes and the effectiveness of infection control measures.

Table 1. Performance of *Brilliance* VRE Agar and BAAV for detection of VRE

Performance	*Brilliance* VRE Agar	BAAV
True positive	218	238
True negative	435	336
False positive	1	73
False negative	3	10
Sensitivity (%)	98.6 (95% CI 97.4–99.8)	96.0 (95% CI 93.9–98.1)
Specificity (%)	99.8 (95% CI 99.3–100)	82.2 (95% CI 78.2–86.2)
NPV (%)	99.3 (95% CI 98.4–100)	97.1 (95% CI 95.3–98.9)

P616 CAMP-inhibition-like phenomenon: a novel character for identification of *Enterococcus faecalis*

W. Laolert, P. Santanirand (Bangkok, TH)*

Objectives: Various bacteria such as *Streptococcus agalactiae*, *Listeria monocytogenes* are known to produce CAMP factor which shows synergism with *Staphylococcus* hemolysin to enhance hemolysis on sheep blood agar. On the other hand, organisms like *Arcanobacterium haemolyticum* and some *Corynebacterium* spp. produce factor which inhibits CAMP-reaction (so called reverse CAMP). These characters have also been used as parts of bacterial identification key. This study

was aimed to establish the CAMP and inhibition of CAMP phenomenon among *Enterococcus* spp.
Method: Four hundred and twenty three isolates of *Enterococcus* species including *E. faecalis* (n = 225), *E. faecium* (n = 182), *E. casseliflavus* (n = 8), *E. gallinarum* (n = 6) and *E. raffinosus* (n = 2) were tested for CAMP and CAMP-inhibition factors with *S. aureus* ATCC 25923 on sheep versus human blood agar. Group B streptococcus and *A. haemolyticum* were used as positive control for CAMP and CAMP-inhibition test, respectively.
Result: Interestingly, among tested organisms, one hundred and fifty three isolates (36.17%) were found to produce CAMP-inhibition-like factor. This inhibition of CAMP phenomenon was observed only when tested on human blood agar but not on sheep blood agar. Furthermore, all CAMP-inhibition-like positive isolates were *E. faecalis* (68% of tested *E. faecalis* isolates). Incubation conditions such as temperature (room temperature versus 37ºC) or atmosphere (ambient air versus 5% CO2) showed minimal effect on this phenomenon.
Conclusion: To our knowledge, this is the first report of CAMP-inhibition-like phenomenon in *Enterococcus faecalis*. Since the test is easily performed and can distinguished between the most two common clinical isolated *Enterococcus* spp. (*E. faecalis* and *E. faecium*), therefore, it would be worth adding this test as a part of identification key for *Enterococcus* species.

P617 Comparative study of selective chromogenic (chromID VRE) and bile esculine agar for isolation and identification of vanA-containing glycopeptide-resistant enterococci using a mice model

G. Birgand, F. Wallet, M. Simonet, R.J. Courcol, R. Leclercq, B. Grandbastien (Lille, Caen, FR)*

Objectives: Since the early 90's, several French hospitals are affected by the emergence of *Enterococcus faecium* resistant to Glycopeptides (GRE). This study compared qualities of microbiological techniques in order to set up the most efficient screening method for GRE control.
Methods: This experimental study was performed with the inoculation of various quantities (10^2 to10^8 CFU/L) of 2 GRE strains with vanA gene (vancomycin MIC=32 mg/L and >256 mg/L) to 8 groups of 4 CF1 mice. Drinking water was constituted of suspensions of vancomycin. Techniques compared were bile esculine agar containing 6 mg/liter vancomycin (BEV), BEV after enrichment (ECE) and the chromogenic medium chromID VRE® (CID; bioMérieux®). A quantification of the colonization was assessed after dilutions of feces in sterile water. Mice feces were collected at J0, J6 and J9 after colonization and inoculated on each medium. Qualitative and quantitative microbiological data were collected after 24 to 72 hours of incubation. Data analysis was performed with epidemiological tools in reference to a Gold standard (GS).
Results: Before inoculation, none mouse was colonized with GRE. The number of CFU of GRE in mice feces was varying from 0 to 9.5 10^6/ml according to the technique and delays of incubation and colonization. The rate of GRE isolated varied from 15 to 21/29 mice at J6 and from 5 to 23/32 mice at J9. Overall, at J6, the interpretation was easier with the CID than with the BEV or ECE. This difference between medium was reduced at J9. The isolation and the interpretation were easier in feces from mice colonized with GRE owning the high MIC to vancomycin. Sensitivity was varying from 16.7 for the BEV to 87.5% for the CID and the specificity from 57.1 for the ECE to 100% in several techniques according to the length of incubation and the delay after colonization. The rate LR+/LR– varied from 1.14 for BEV to plus infinity. The Youden's index varied from 0.5 for BEV to 0.96 for CID.
Conclusion: The CID after 48 h of incubation appeared as the best technique in term of quality and delay of analysis. During an outbreak of GRE carriage, the inclusion of the CID in first intention in a screening strategy could allow getting a quick view of the situation. During this study, the enrichment didn't increase the sensibility of BEV. These techniques will be compared with molecular methods based on simplex and multiplex PCR.

Diagnosis and susceptibility testing of Gram-negative and other bacteria

P618 Evaluation of four fully-automated immunoassays for diagnosis of syphilis

T.D. Ly, C. Marcenaro, M. Dautigny (Ivry-sur-Seine, FR)*

Background: Serology is central to the screening and diagnosis of syphilis infection. The serologic diagnosis of syphilis has for many years been carried out mainly with Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR) test, Treponemal Haemagglutination Assay (TPHA) or Fluorescent Treponemal Antibody Absorption test (FTA-ABS). Since there were limitations of these tests (i.e. their lack of sensitivity in early dark-field positive primary cases and the relatively high incidence of false-positive reactions), the quest for a specific serologic test for syphilis began many years ago and still continues. In recent years, immunoassays using purified recombinant proteins have been developed to this aim.
Objectives: In this study, the performance of four automated tests: Architect Syphilis TP (Abbott), BioPlex 2200 Syphilis IgM, IgG (Bio-Rad), Immulite 2000 Syphilis Screen (Siemens) and Liaison Treponema Screen (DiaSorin), using recombinant proteins, was compared to that of TPHA (Syphagen TPHA, Biokit Barcelona, Spain).
Methods: 392 unselected sera and seven selected samples from patients with primary syphilis were tested by TPHA as well as these four assays. Whenever one assay scored reactive, IgG and IgM Line Immunoblot (Virotech) was used for confirmation.
Results: The Architect, BioPlex, Immulite and Liaison tests had an overall agreement of 96.7%, 96.4%, 96.9% and 96.4% with 95.2%, 94.3%, 94.4% and 94.4% negative agreement, and 97.4%, 97.4%, 98.1% and 97.4% positive agreement respectively versus TPHA (equivocal results were excluded). Immunoblot IgG confirmed positive 3/12, 3/13, 3/14 and 3/14 discrepant Immulite, Architect, BioPlex and Liaison results versus TPHA respectively. In addition, seven primary syphilis specimens (i.e., TPHA-negative) scored positive by Architect, Immulite, Liaison and BioPlex IgM. By contrast two patients scored negative by Bioplex IgG.
Conclusion: Architect Syphilis TP, Liaison Treponema Screen and Immulite 2000 Syphilis screen with their suitability of automation are an ideal screening test. However, the BioPlex IgM helps to differentiate past infections from recent as demonstrated from the negative BioPlex IgG results for 2 of the 7 primary samples.

P619 Diagnostic specificity of rTPN17 chemiluminescence immunoassay or CLIA (Liaison®) in comparison with TPN15-TPN45 Elisa on true and false-positive syphilis tests

A. Rossi, A. Tamborini, L. Pinese, E. Canali, A. Milano, A. Berrone, A. Bassani, D. Rossi, A. Toniolo (Varese, IT)*

Objectives: The aim of this study was to compare the diagnostic performance of rTPN17 chemiluminescence assay LIAISON® Treponema screen (DiaSorin, Saluggia, Italy) with TpN17-TpN45 ELISA routinely performed on clinical patients and on blood donors syphilis screening. Positive tests were analyzed with both methods and with rapid plasma reagin (RPR), with hemoagglutination treponemal tests (TPPA) and immunoblot.
Methods: The routinely performed ELISA test (ETI-Treponema Plus, DiaSorin) was an automated indirect immunoassay based on TpN17 and TpN45 antigens and detection time was 3 hours.
The chemiluminesce assay (LIAISON® Treponema screen, DiaSorin) was an automated immunoassay based on recombinant specific *Treponema pallidum* antigen rTpN17, which was used for coating magnetic particles (solid phase) and the same antigen was linked to an isoluminol derivative (isoluminol-antigen conjugate). Detection time of CLIA syphilis test was 1 hour. Between October 2007 and May 2008 syphilis screenings were done on 18533 clinical specimens.

Results: The positive syphilis ELISA tests were 2.6% (N = 481) and of these 0.29% (N = 53) were "possible false positive"; the tests of these patients were repeated 3 weeks and 3 months later with ELISA, RPR, TPPA and immunoblot and no "relevant" syphilis clinical signs emerged. All the positive syphilis ELISA tests were also analyzed with rTPN17 chemiluminescence assay and "true positive" were confirmed.
But of the 53 "false positive" 35 became "true negative" and of the 26 ELISA "false positive" tests with low index values (OD/CO < 2.0) only 2 remained positives; thus CLIA diagnostic test performance was more significant [p < 0.0001] with low index values. Diagnostic specificity of ELISA and of CLIA syphilis tests were 99.71 and 99.90%.
Conclusions:
1. ELISA and CLIA syphilis tests were highly specific (CLIA was only significantly more specific for "false positives" with low index values)
2. CLIA syphilis test had a shorter time of detection: it might be usefull for solid organ/tissue donor tests.
3. The lower number of "false positive" tests performed with CLIA might be usefull in blood donors screening.

P620 Evaluation of indeterminate or low-level positive results with the LIASON chemiluminescence immunoassay for laboratory diagnosis of syphilis

M. Gozalo, R. Balbas, M.A. Martinez-Bernal, M.A. Rodriguez, L. Martinez-Martinez (Santander, ES)*

Objective: Positive and negative results for laboratory diagnosis of syphilis with the LIASON Treponema Screen (Diasorin, Italy) chemiluminescence immunoassay (CLIA) are considered for index values of >1.1 and <0.9, respectively, with indeterminate results for index values of 0.9 to 1.1. Additionally, low-level positive results (index value <10) are occasionally obtained. The objective of this study was to compare indeterminate and low-level positive results obtained with the LIASON assay with those obtained with a second CLIA, with reagin test and with two treponemal tests.
Methods: Thirty-eight sera obtained from different patients in the period February to October 2008 for which index values of [>0.9 to 1.1] (n = 6) or [>1.1 and <10] (n = 32) were obtained with LIASON were also tested with the CLIA Architect Syphilis TP (Abbott), a reagin test (RPR, Spinreact), indirect hemagglutination (TPHA, Spinreact) and a line immunoassay (Inno-LIA Syphilis Score, Innogenetics). The assays and interpretation of results were done according to the corresponding manufacturer's instructions. Clinical charts of the patients were also reviewed.
Results: Thirteen out the 38 tested sera were from pregnant women without any clinical suspicion of syphilis, while the remaining 25 samples corresponded to patients in whom a serologic diagnosis of syphilis was requested. Positive/indeterminate/negative results were as follows: 16/0/22 (Architect), 1/0/37 (RPR), 8/0/30 (TPHA) and 11/4/23 (Inno-LIA). The RPR assay was positive in only 1/38 (2.6%) samples, which was also positive for all other tests. Both treponemal tests TPHA and Inno-LIA were positive in 8 (21.0%) samples. The 3 additional positive and 3 out the 4 indeterminate results with Inno-LIA corresponded to patients without any clinical evidence of syphilis (one of them was HIV-positive), while the remaining indeterminate case was in patient with a suspicion of syphilitic chancre. Overall, 23 (60.5%) samples positive with LIASON were negative with both the reagin test and the two treponemic assays.
Conclusion: Indeterminate and positive low index values (>1.1 and <10) obtained with the LIASON Treponema Screen assay should be confirmed with a treponemal test for avoiding false serologic diagnosis of syphilis.

P621 Evaluation of nine *Mycoplasma pneumoniae* ELISAs using latent class analysis: circumventing the lack of a gold standard

C.W. Ang, D.I. Van 't Oever, N. Al Naiemi, J.A. Bogaards (Amsterdam, NL)*

Objectives: To compare nine commercially available assays for detection of IgM and IgG antibodies against *Mycoplasma pneumoniae.*
Methods: 92 serum samples from clinically well defined patients (including healthy controls and cross-reactivity controls) were tested in nine ELISA systems (Fujirebio, Anilabsystems, Biorad, Diacheck, Medac, Novagnost, Savyon, Serion, Viro-Immun) for either total Ig or IgM and IgG antibodies. As there is no gold standard for detecting recent *M. pneumoniae* infections, we used a latent-class model with three classes (uninfected, recently infected, past infection). Latent class analysis (LCA) involves fitting a statistical model using all available diagnostic tests to define an internal reference standard. This is the first report of the use of a latent-class model with three classes in the evaluation of serological tests.
Results: Using the cut-offs of the manufacturers, LCA resulted in a probability for each serum sample to belong to one of the three classes (uninfected, recently infected, past infection). 9/92 samples had a probability of >0.95, only 1/92 samples had a probability of 0.69 and the other 82 samples had a probability of <0.05 to be in the "recently infected" class. 19/92 had a probability of >0.95 to be in the "past infection" class, and only 4/92 had a probability between 0.05–0.95 to be in the "past infection" class. Based on the probabilities for each serum sample, sensitivity and specificity for each ELISA could be determined. The sensitivities of the IgM ELISA's for detecting a recent infection ranged from 61% (Novagnost) to 96% (Anilabsystems). The specificities ranged from 60% (Fujirebio) to 99% (Viro-Immun).
Conclusion: LCA using three classes allows to categorize samples based on their infection status and can be widely applied in serological studies. LCA allows comparing sensitivity and specificity of diagnostic tests even without a gold standard.

P622 Direct latex agglutination for group B streptococcus carriage detection

M. Martínez, P. Ferrer, M.R. Vicente, E. Riquelme, M. Pariente, L. Moreno, M.D. Crespo (Albacete, ES)*

Objectives: The purpose of this study was to compare direct latex agglutination on incubated selective broth with the CDC recommended method for group B streptococcus (GBS) detection in pregnant women.
Methods: 242 vaginorectal swabs submitted to our laboratory during 1 month were tested by both methods. The swabs were immersed in SBM broth (Todd-Hewitt with gentamicin, nalidixic acid, 5% blood; Biomedics®) and incubated at 35ºC in an ambient atmosphere. After overnight incubation they were subcultured onto 5% blood agar and incubated for 24 h at 35ºC with 5% CO2. In addition, group B latex testing (Slidex® Strepto Plus, bioMérieux®) was performed on all SBM adapting the protocol to broth. Subcultures were examined and GBS colonies were confirmed by catalase reaction, Gram stain and latex assay (also hippurate hydrolysis test when colonies were non-hemolytic). Negative plates were reincubated and examined on the following day. Subcultures were repeated when we obtained discrepant results.
Results: The results are shown in Table 1. Only in 1 of the 16 repeated subcultures (positive latex samples with negative culture) GBS colonies were recovered. All of them revealed heavy growth of *Enterococcus* spp., which can mask the presence of GBS in subcultures, especially when the initial load is low. In 8 of them we also detected polyagglutinating colonies (usually non-hemolytic streptococci) that could have given false-positive latex results. Despite this limitation, there is a possibility that these specimens could be in fact true positive ones according to previous studies with molecular methods. Unfortunately, we cannot confirm this. The method had a sensitivity and specificity of 94.3% and 92.8%, respectively; and the predictive values were 99% for a negative test and 68.8% for a positive one. Specificity and positive predictive value could improve if some of the latex-positive culture-negative samples were confirmed as true positive. Turnaround time in days was significantly lower for the latex method (0.94±0.03) compared to the standard one (4.71±1.05).
Conclusion: Our results show that direct latex agglutination on overnight incubated selective broth is at least as sensitive as the CDC recommended method and it decreases the turnaround time and the laboratory workload. Although further investigation is needed to confirm its real sensitivity,

these data suggest that it is a useful method to give reliable negative results in less than one day.

Table 1. Results of CDC culture and GBS antigen detection methods

Subculture	Latex	No. of specimens
+	+	32
+	–	2
–	+	16
–	–	192
34 (14.1%)	**48 (19.8%)**	**242**

P623 Evaluation of two chromogenic media for screening of group B *Streptococcus* in pregnant women

L. Book, G. Al-Rawahi, E. Thomas (Vancouver, CA)*

Objectives: To compare the performance of StrepB Select (SBB) (BioRad) and chromID™ Strepto B agar (STRB) (bioMérieux) media to that of our routine method for group B *Streptococcus* (GBS) detection in women at 35 to 37 weeks gestation.
Methods: Five hundred consecutive vaginal/anal swabs were submitted directly to our microbiology laboratory for routine GBS screen between January and July 2009. Swabs were immediately emulsified in 0.5 mL of sterile saline and processed as follows:

1. Routine: Blood agar plates (BAP) (PML) + *Streptococcus* Selective broth, Group B (GBS broth) (PML). The BAP:s and the GBS broths were inoculated, then incubated at 35ºC. BAP:s were examined for colonies with typical GBS morphology. Negative BAP:s were re-incubated for an additional 24 hrs and the corresponding GBS broths were sub-cultured to a BAP. All suspect colonies were confirmed by Prolex™ Streptococcal grouping latex reagents (Group B) (Pro-Lab Diagnostics).
2. 2. Study: SBB plates + STRB plates + GBS broth. The SBB, STRB plates and GBS broths were incubated for 24 hours at 35ºC, then processed as follows: all plates were examined for the presence of GBS (SBB: blue colonies; STRB: pale pink/red colonies) followed by group B Strep latex agglutination on all suspicious colonies. Negative SSB/STRB plates were reincubated for an additional 24 hours and the GBS broth was subcultured to SBB and STRB. Confirmation by latex agglutination was considered the gold standard.

Results: A total of 109/500 samples (22%) were positive for GBS as follows: Our routine method detected 88/109 (80%); SBB detected 90/109 (83%) at 24 hrs, 99/109 (91%) at 48 hrs, 107/109 (98%) after selective broth culture; STRB detected 85/109 (78%) at 24 hrs, 95/109 (87%) at 48 hrs, 104/109 (95%) after selective broth culture.
Conclusion: Both chromogenic media were more sensitive than our routine method for detection of GBS. The SBB was easier to read and more sensitive whilst the STRB was more specific after direct reading. However, the specificity becomes less of an issue if all suspect colonies are confirmed by latex agglutination.

Media	Direct plate at 24 hrs		Direct plate at 48 hours		Broth enrichment	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
BioRad	83%	95%	91%	89%	98%	91%
bioMérieux	78%	98%	87%	91%	95%	95%

P624 Evaluation of the performance of chromID™ Strepto B, a new chromogenic medium, to search for *Streptococcus agalactiae* in pregnant women

R. Soloaga, S. Montibello, L. Guelfand, M. Machain, N. Carrion, D. Ferreira, J. Pidone, E. Ceregido, S. Kaufman (Buenos Aires, AR)*

Objectives: *Streptococcus agalactiae* (EGB) may colonize the perineal area and genital tract. This is important in pregnant women since the transmission rate to newly born children is 50%, from which 1–2% of colonized ones develop clinical infection with a 10% mortality rate. The Center for Disease Control and Prevention (CDC) suggests rectal and vaginal exudate culture be performed within 35–37 weeks of pregnancy and prophylaxis intrapartum be provided to all EGB carriers in order to reduce incidence of neonatal disease. The objective was to determine the usefulness of bioMérieux chromogenic medium chromID Strepto B (CR) for detecting *Streptococcus agalactiae* in pregnant women from the Todd Hewitt broth (THB) relating to the methods proposed by the CDC.
Methods: 1924 swabs were analyzed, 962 from vaginal introitus (VI) and 962 rectal (RS) belonging to 962 women within 35–37 weeks. Both samples were referred to the laboratory in Stuart medium. VI and RS swabs were directly seeded in CR and incubated at 37ºC in aerobiosis for 48hs. Both swabs were later placed in THB with 15 ug/ml supplement of nalidixic acid and 10 ug/ml of colistin. After 24hs incubation, subcultures in CR medium and agar were performed with 5% sheep blood (ASO). Suspicious colonies, red in CR and gray±β hemolysis in ASO were identifiedd by conventional test such as Gram stain, catalase, bile-aesculin, CAMP, hippurate hydrolysis and group B serology.
Results: EGB was isolated in 168 patients, with a prevalence of 17.4%. Sensitivity, specificity, positive and negative predictive value of THB subcultures with CR supplement and 48 hs incubation was 98.8, 100, 100 and 99.7% respectively. The corresponding values of direct harvest of the sample were 57.8, 100, 100 and 90% respectively. Sensitivity of THB in ASO was 85%.
Conclusions: THB subculture performance in CR was outstanding as regards the method proposed by the CDC. Furthermore, laboratory work and the possibility of false negative results due to the absence of colonies with β hemolysis is decreased by CR.

P625 Evaluation of different culture media, swabs and sampling techniques for rapid detection of vaginal and rectal group B streptococci in pregnant women

N. El Aila, I. Tency, G. Claeys, B. Saerens, M. Temmerman, R. Verhelst, M. Vaneechoutte (Ghent, BE)*

Objectives: The aim of this study was to compare different selective media for group B *Streptococcus* (GBS) detection in pregant women, to compare different sampling techniques, i.e. combined vaginorectal swabs with separate vaginal and rectal swabs and to compare two different types of swabs.
Methods: Seventy women were included, and four swabs were collected from each. Three Eswabs were used, one for vaginal, one for rectal and one for combined vaginorectal sampling. In addition, a classical swab was used for vaginal sampling. All four swabs were inoculated indirectly onto chromID Strepto B agar, Granada agar and Columbia CNA after overnight incubation in Lim broth. The vaginorectal swab was also inoculated directly on chromID Strepto B agar, Granada agar and Columbia CNA. Red colonies on chromID Strepto B agar, orange pigmented colonies on Granada agar or hemolytic colonies on Columbia CNA, were confirmed as GBS using the CAMP test and GBS specific PCR.
Results: Fourteen women (20%) were colonized with GBS. Of these, 13 were positive after direct culture and one after enrichment. Six harbored GBS in both rectum and vagina, six only in the rectum and for two only the vaginorectal swab was positive. For chromID Strepto B agar three women were false positive with red colonies identified as other streptococcal and enterococcal species.
One false-negative result on Granada agar corresponded to a non-haemolytic and non-pigmented GBS strain which was correctly identified on chromID Strepto B agar. No differences could be observed between the Eswab and the classical swab for sampling the vagina.
Conclusion: chromID Strepto B agar correctly identified one more strain than Granada agar, but yielded false positive results for three women. GBS colonies can be easily visualized and are clearly apparent in 24 h after direct inoculation. Unexpectedly, vaginorectal swabbing was most sensitive, since it detected GBS in two additional women, compared to separate swabbing of rectum and vagina. No difference was found

between Eswab and classical swab for vaginal sampling. In conclusion, Granada agar may be prone to false negative results whereas chromID Strepto B agar may be prone to false positive results but both of them are superior to Columbia CNA and excellent for rapid detection of group B streptococci in vaginal, rectal and vaginorectal samples.

P626 *Kingella kingae* culture: comparative study among different BacT/Alert haemoculture bottles

A. Gené, A.C. Aguilar (Barcelona, ES)*

Objectives: *Kingella kingae* isolation has increased since osteoarticular samples from children are cultured in hemoculture bottles.
The aim of this study was to compare the ability to support *K. kingae* growth of three different bottles of BacT/Alert hemoculture system (Biomerieux®), taking into account the bacterial concentration of the sample and the addition of blood supply in the culture medium.
Methods: The hemoculture bottles used were: SA (Standard aerobic), FA (FAN aerobic, containing activate charcoal) and PF (Pediatric FAN, containing activate charcoal).
Eighteen strains of *K. kingae* isolated in our hospital from osteoarticular infection in 7–23 month old patients were studied.
Each strain was progressively diluted to obtain six different concentrations (A – F) of approximately 10^8, 10^4, 10^3, 10^2, 10 and 1 colony forming units / ml. Quantitative cultures in blood agar plates were done to control the final concentration of each dilution.
In two SA, FA and PF bottles, 0.4 ml of each dilution was inoculated. In one bottle of each type 1 ml of sterile human blood was added. The bottles were processed in the BacT/Alert automatic incubator. Negative cultures were discarded after seven days. All strains were double tested (Series a and b).
Results: Including both series, in the bottles SA, FA and PF with blood supply growth was detected in 84%, 25% and 38% and without blood supply in 34%, 6% and 6% respectively.
A direct correlation between bacterial concentration of the inoculums and bacterial growth, and inverse correlation between bacterial concentration and incubation time to detect growth were observed.
Conclusion: According to our results, BacT/Alert SA bottles, in combination with blood supply, is a good method to isolate *K. kingae* from inoculums with low bacterial concentration. The ability to support *K. kingae* growth is higher in SA than in SF and PF bottles. Addition of blood supply in the culture medium increases the growth capacity, mainly in those with low bacterial concentration, and decreases the incubation time to isolate this microorganism.

Type of bottle	Series	Dilutions. No. of growing strains (Range of hours to detect growth)					
		A	B	C	D	E	F
SA + blood	a	18 (9–13)	18(15–26)	18 (18–29)	18 (20–34)	14 (22–43)	4 (22–29)
	b	18 (7–14)	18 (14–28)	18 (16–38)	18 (18–50)	16 (20–58)	3 (27–41)
SA	a	16 (11–96)	7 (27–115)	4 (42–113)	3 (61–84)	2 (80–94)	0
	b	17 (10–74)	7 (32–101)	5 (35–125)	6 (40–118)	4 (44–98)	2 (58–74)
FA + blood	a	14 (9–113)	5 (24–82)	5 (29–106)	4 (31–130)	3 (35–89)	0
	b	11 (9–103)	6 (25–115)	4 (28–139)	1 (34–)	1 (42–)	0
FA	a	5 (17–77)	2 (48–50)	1 (67–)	1 (60–)	0	0
	b	3 (39–94)	0	1 (96–)	0	0	0
PF + blood	a	17 (8–65)	9 (31–127)	9 (31–118)	6 (33–146)	6 (39–91)	2 (37–96)
	b	14 (8–132)	6 (21–60)	7 (24–168)	3 (24–96)	3 (29–110)	0
PF	a	8 (9–146)	0	0	0	0	0
	b	5 (36–108)	0	0	0	0	0

P627 Clinical relevance of rapid identification of Gram-negative pathogens causing bloodstream infections using a new peptide nucleic acid fluorescent hybridization assay

P. Della-Latta, S. Whittier, F. Wu (New York, US)*

Objectives: Gram-negative bacilli (GNB) causing bloodstream infections (BSI) can be life threatening in hospitalized patients. Rapid identification (ID) of common GNB pathogens can impact appropriate empiric therapy and infection control precautions, and is therefore critical to improve patient care. This study evaluated the performance of the first rapid assay to identify 3 major GNB, *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA), from newly positive BC. Empiric therapy for PA differs from that for EC or KP and rapid detection of KPC carbapenemase by real-time PCR further impacts prudent empiric therapy and infection control decisions.
Methods: From Oct. to Nov. 2009, 76 BC bottles (28 patients) newly smear-positive for GNB were tested with a new rapid tri-color fluorescence in situ hybridization (FISH) assay, the GNR Traffic Light PNA FISH (GNRTL) (AdvanDx). The peptide nucleic acid fluorescent (PNA) probes that target rRNA sequences of EC, KP and PA appear as green, yellow or red cells, respectively, when examined by fluorescent microscopy. Culture ID was performed using the Vitek 2 system (bioMérieux). KPC production was tested directly from BC by real-time PCR. Patient charts were reviewed retrospectively for therapeutic changes based on pathogen ID.
Results: Of 76 GNB from BC, 29 were EC, 12 KC, 2 PA and 5 had both EC and KP. Of 28 patients with BSI due to GNB, 73% were infected with the targeted pathogens. The sensitivity, specificity, positive and negative predictive values for GNRTL ID compared to culture were 100%. No false-positives or false-negatives occurred and co-infection (EC and KP) was rapidly detected in 3 patients. One KP was KPC-producing, which resulted in a therapeutic change from piperacillin–tazobactam to polymyxin B. PA detection resulted in the addition of tobramycin to regimens.
Conclusions: GNRTL is a highly sensitive and specific assay for identifying specific GNB from BC in real-time. The ability to distinguish PA from enteric GNB is clinically useful in targeting appropriate pathogen specific therapy. GNB were identified within 1.5 hrs compared to 1–3 days with culture.

P628 Identification of *Burkholderia pseudomallei* from 2 types of media using Vitek 2 software version 4.03

T. Urbanski, M. Costello, P. O'Loughlin (Brisbane, AU)*

Objectives: *Burkholderia pseudomallei*, the causative agent of Melioidosis is listed as a Category B agent that could potentially be used in a Bioterrorist attack. Melioidosis is potentially fatal. It is important that this disease is quickly diagnosed and treated. The resistance to a wide range of antibiotics makes early detection important for therapeutic management. Failure to Identify has been reported due to lack of knowledge and inadequate test-systems for this organism. The Vitek 2 is rapidly becoming integrated into microbiology laboratories with a requirement for reliable identification. Vitek 2 GN cards and an upgraded software database to version 4.03 and above are now in use. A follow up study of work done by Lowe, Haswell and Lewis, 2006 was required to assess Vitek 2 performance to identify the same collection of isolates from clinical specimens within Australia and a PCR method was used to validate this work.
Methods: 100 *B. pseudomallei* isolates, were recovered from −70ºC storage. Sub-cultures on columbia horse blood and sheep blood agar were analysed on the Vitek 2 using GN cards and standard procedures. Identification results were assessed for the numbers of correct identifications from the 2 agar types. PCR was used for identification as described by Novak et al., 2006.
Results: Isolates growing on horse blood and sheep blood agar gave 84 and 82 correct identifications; 9 and 10 low discrimination; 7 and 7 incorrect identifications; and 0 and 1 inconclusive identification respectively and no statistical significant difference of identification using the 2 media types ($p > 0.05$). The 7 misidentified isolates gave an identification of *Burkholderia cepacia*. All 10 low discrimination isolates were between *B. pseudomallei* and *B. cepacia* with the exception of one isolate that could not be discriminated between *B. pseudomallei, B. cepacia* and *Pseudomonas aeruginosa*. All isolates were confirmed as *B. pseudomallei* by PCR testing.
Conclusions: No statistical significant difference could be attributed to the 2 media types. An improvement in Vitek 2 database has been demonstrated. A lower rate of incorrect identifications was seen compared to previously published data. However, users should still be aware of potential misidentifications with *B. cepacia.* Confirmatory testing by PCR offers a fast, reliable method for discrimination.

P629 Analysis of recombination events in DNA mismatch repair genes among *Escherichia coli* strains belonging to different phylogroups with different mutation frequencies

J.M. Gonzalez, M.C. Turrientes, M.R. Baquero, R. Cantón, J.C. Galán, F. Baquero (Madrid, ES)*

Objective: The genetic load imposed by the high frequency of mutations might lead to long-term deleterious effects for the mutator population. In this work, we analyze another possibility, "recombinational rescue" rate for wild-type mismatch repair (MMR) genes.
Methods: MMR genes including mutS, mutL, and mutH, as well as those involved in oxidative damage (GO) mutT and mutY, and two housekeeping genes (arcA and fumC) were sequenced in 66 human Escherichia-coli isolates with different mutation frequencies (17 hipo-mutators; 23 normo-mutators; 21 weak-mutators; 5 strong-mutators) and different phylogenetic groups B2 (17), D (16); A (19); and B1 (14). Sixty-nine sequences (including 3 reference sequences) were aligned using ClustalW program, and the best-fit model of nucleotide substitution was selected using jModelTest program. Alignments were analyzed using PhyML, software that estimates maximum likelihood phylogenies. Recombination among aligned sequences was detected using RDPv.3.27 program.
Results: No evidences of recombinational events were found analyzing the housekeeping genes, nor in mutH (MMR) and mutT (GO repair system). Although recombination events were detected in mutY gene (26%) the highest frequency of these events ($p < 0.001$) was detectable in the MMR genes: mutS (55.07% of sequences analyzed) and mutL (44.93%). Recombination events in mutS gene were higher in weak mutators (71.43%), but interestingly in mutL gene weak mutators showed the lowest rate of recombination ($p < 0.05$). To avoid phylogenetic noise the sequences with recombination events were eliminated. In this new scenario, D group is still extremely heterogeneous suggesting that new phylogenetic groups must potentially be accepted. Phylogenetic trees of non-recombination events showed higher number of incongruences (when a gene was not clustering with its phylogenetic group) among mutator strains ($p < 0.05$).
Conclusions: High rates of intragenic recombination occur for the MMR gene mutS, and mutL (high recombination in mutS with low recombination in mutL seems to be a particular feature of weak-mutators). Mutator strains showed significantly higher genetic incongruence. These results suggest that recombinational variation of MMR genes might provide alterations in the mutation frequencies of human *E. coli* isolates, including "recombinational rescue" of hypermutable strains. The classification into four major groups appears to be an oversimplification of a more complex reality.

P630 The use of commercial system in the identification of Gram-negative non-fermenting bacilli isolated from cystic fibrosis patients

C.R. Saraiva, L.V. da Silva Filho, D. de Oliveira Garcia (São Paulo, BR)*

Cystic fibrosis (CF) is characterised by chronic lung infections. Respiratory tract of CF patients are often colonised or infected by specific microorganisms. Gram-negative non-fermenting (NF) bacilli, such as, *Pseudomonas aeruginosa*, *B. cepacia* complex and *Stenotrophomonas maltophilia* are the most prevalent microorganisms and other NF, including some atypical or of difficult identification, are occasionally isolated. NF differ in the potential pathogenic and transmissibility, so, the correct identification of the species is critical for the treatment.
Objectives: This study evaluated the API 20 NE system and classical phenotypic and genotypic (PCR) method in the identification of NF isolated from CF outpatients being seen at Pediatric Pulmonology Unit (Uiversity of São Paulo Medical School).
Methods: Sixty-seven NF with inconclusive results by classical phenotypic methods using an extensive biochemical serie were analysed by API 20NE system. *S. maltophilia* and *Achromobacter* spp were confirmed by PCR using specific primers to 23S rRNA and 16S rRNA genes, respectively.
Results: The identification by API 20NE system showed 34.3% *Achromobacter* spp, 10.4% *Ochrobactrum anthropi*, 10.4% *S. maltophilia,* 9% *Ralstonia pickettii*, 6% *Acinetobacter* spp, 6% *Burkholderia cepacia*, 4.5% *Comamonas testosteroni*, 4.5% *P. aeruginosa*, 3% *Alcaligenes faecalis*, 3% *Delftia acidovorans*, 3% *P. stutzeri,* 1.5% *Moraxella* spp, 1.5% *Rhizobium radiobacter*, 1.5% *Wautersia paucula* 0.6% *Bordetella bronchiseptica*, 0.6% *Brevundimonas vesicularis*. PCR confirmed 90% *Achromobacter* spp and 42% *S. maltophilia.* All the other identifications were compared with the morphological characteristics and complementary biochemical tests and all of them showed inconclusive results.
Conclusion: API 20NE system was unable to identify correctly microorganisms atypical or rarely isolated from respiratory tract of CF patients according to the morphological characteristics and complementary biochemical tests. The identification needs to be confirmed by 16S rRNA sequencing. An association of phenotypic and genotypic methods is more efficient to identify correctly rare or atypical NF.
Financial support: FAPESP no 2005/01625−8 and PPG/CCD/SES.

P631 Using an optimized protocol of the Corbett CAS1200 automated liquid handling system as a fast PCR setup platform, reduces hands-on and process time significantly

E. van Zanten, S. Hofs, A.M. Kooistra-Smid (Groningen, NL)*

Objectives: In the past few years our laboratory has witnessed an increase in the number of samples analyzed, and the number of pathogens screened for, using molecular methods. Manual performance of the PCR setup has put a strain on the laboratory technicians performing these PCR assays, for both workload and quality maintenance. We therefore implemented an optimized protocol of the CAS1200 liquid handling system to automate the entire PCR setup process reducing workload, hands-on time and increasing quality control.
Methods: The Corbett Robotics4 software has two important features that can speed up the PCR setup significantly: the option to re-use tips and the possibility to implement a "sample bank duplication step" (SbDS). These features reduce the number of robotic movements, and allow the platform to multi-dispense small volumes from a single large volume uptake. The re-use of a single tip and SbDS were investigated for the possibility of carry over of primer/probe from one well to the next, regarding false positive results. We validated the CAS1200 platform by prospectively analyzing 180 clinical samples in our internally controlled triple-multiplex real-time PCR assays, screening for 8 enteric pathogens, and comparing the results with manual PCR setup. The automated PCR setup dispenses three different PCR master mixes in a PCR tray, then adds DNA isolate directly from DNA isolation vessels, using a single tip per DNA isolate, to the PCR mix using SbDS, and finally dispenses the remainder of the DNA isolate in a storage vessel.
Results: No carry-over contamination was observed using the automated PCR setup. Re-using tips for both apportioning the PCR mix and DNA isolates yielded reproducible volumes. Of the 180 samples, 43 samples were positive in both methods. Manual setup yielded one (Ct 38.7) and automated setup two (Ct 36.6 and 38.7) additional positive samples respectively. The mean difference in Ct values was 0.1 (range −3.4 to 2.5) in favour of manual PCR setup. Automated PCR preparation setup could be completed within approximately 17 minutes, where as a trained technician performs the manual PCR setup in approximately 45 minutes.
Conclusion: The use of the optimized protocol of the CAS1200 liquid handling system results in a fast PCR setup that significantly reduces hands on time and workload. Moreover, the risk on human errors decreases when dealing with large sample quantities and complex PCR setups.

P632 Evaluation of a chromogenic medium for extended-spectrum β-lactamase-producing Enterobacteriaceae

P. Lagacé-Wiens, F. Tailor, P. Baudry, D. Hoban, G. Zhanel for the Canadian Antimicrobial Resistance Alliance*

Objectives: ESBL-producers are increasingly considered an infection control problem worldwide. Simple and specific means of screening for

ESBL-producing *Escherichia coli* and *Klebsiella* sp. are complicated by the fact that cephalosporin resistance in Enterobacteriaceae may be mediated by other mechanisms. The purpose of this study was to evaluate the performance of a differential chromogenic screening medium selective for ESBL-producers with a large library of genetically characterised cephalosporin-resistant *E. coli* and *K. pneumoniae*.

Methods: Isolates were from 12 medical centers across Canada. 213 *E. coli* and 17 *K. pneumoniae* with ESBL or AmpC mediated cephalosporin resistance were studied. *E. coli* and *K. pneumoniae* were screened using ceftazidime and/or ceftriaxone MIC $\geqslant$1 ug/ml. ESBL genes (CTX-M, SHV, TEM, OXA), AmpC promoter/attenuator alterations and presence of acquired AmpC-type genes (ACT-1/MIR-1-related, DHA-related, FOX-related and CMY-2-related) were detected by PCR and, where needed, sequencing. CHROMagar-ESBL (CHROMagar, Paris, France) was inoculated with 150 cfu of each test organism. Growth characteristics, including colour and colony counts were obtained.

Results: 114 ESBL-*E. coli* (1 each of CTX-M-1, 9, -24 and -65, 2 CTX-M-2, 24 CTX-M-14, 77 CTX-M-15, 4 CTX-M-27, and 3 SHV-2a), 92 AmpC-*E. coli* (46 CMY-2 and 45 promoter/attenuator mutants), 8 co-expressing ESBL and AmpC-*E. coli* (4 CTX-M-15, 2 CTX-M-14, 1 each of CTX-M-3 and -12, all with promoter/attenuator mutations), 17 ESBL-*K. pneumoniae* (co-expression of CTX-M and SHV-type ESBLs) and 50 wild-type *E. coli* were studied. Growth on medium is described in Table 1. 2.3% of *E. coli* had an atypical chomogenic reaction with no colour produced. All *K. pneumoniae* had a typical chromogenic reaction. All uninhibited AmpC hyperproducing organisms had >100 colonies/plate and for 7/10, the mechanism of cephalosporin resistance was the CMY-2 gene. One ESBL-*E. coli* was inhibited by the medium. This isolate expressed the TEM-12 gene and had a ceftriaxone MIC of 0.12 ug/ml and a ceftazidime MIC of 8 ug/ml.

Conclusions: For *E. coli* with ceftriaxone and/or ceftazidime MIC $\geqslant$ 1 ug/mL, sensitivity for ESBL-producers is 99.2%, specificity is 89.0%. Sensitivity for *K. pneumoniae* was 100%, but numbers were small. This medium may facilitate the screening of ESBL-carriers, particularly in areas where prevalence of ESBL producers is relatively low compared to AmpC or other class C cephalosporinase-producing organisms.

Organism (n)	Any growth on medium, n (%)	Sensitivity (for ESBL)	Specificity (for ESBL)
ESBL-*E. coli* (114)	114 (100)	99.2%	89%
AmpC *E. coli* (91)	10 (11.0)	99.2%	89%
ESBL and AmpC *E. coli* (8)	7 (87.5)	99.2%	89%
ESBL[1] *K. pneumoniae* (17)	17[1] (100)	100%	N/A
Wild-type *E. coli* (50)	0 (0)		

[1]3 *K. pneumoniae* were phenotypically ESBL-producers but had only SHV-1 β-lactamase.

P633 Extended-spectrum β-lactamase-producing Enterobacteriaceae: diagnostic performance of the chromogenic medium Brilliance®-ESBL

Y. Gil, A. Burillo, J. Gómez-Garcés (Madrid, ES)*

Objectives: The rapid spread of extended-spectrum β-lactamases (ESBL) among the Enterobacteriaceae (EB) is currently a cause of concern for the management of infections caused by these microorganisms.

In this study, the use of Brilliance®-ESBL (Oxoid) to detect clinical isolates of ESBL-producing EB was compared to a reference test (E-test strips, bioMérieux). We also tested the method on urine samples spiked with different concentrations of ESBL-producing EB.

Methods: Sixty clinical isolates of ESBL-producing EB, as determined by an automated system (Vitek2®, bioMérieux), and 20 clinical isolates of non ESBL-producing EB were plated on Brilliance®-ESBL plates at final concentrations of 10^5 cfu/ml and ESBL production checked in parallel using ceftazidime or cefotaxime E-test strips with or without clavulanic acid. *Klebsiella pneumoniae* ATCC 700603 was used as a positive control and *Escherichia coli* ATCC 25922 was used as a negative control. The isolates were also used to inoculate culture-negative urine samples at three different final concentrations, 10^5, 10^3 and 10 cfu/ml, which were simultaneously plated on Brilliance®-ESBL and blood agar.

Results: After 24 h of incubation, the growth of 100% of the ESBL+ strains on Brillance® medium was detected. All strains of *E. coli* produced night blue colonies, while non-*E. coli* EB were light green (*K. pneumoniae* or *Enterobacter cloacae*) or tan (*Proteus vulgaris*).

Of the non ESBL-producers (20), 7 strains of *E. coli* presumably producing TEM-1, TEM-2 or SHV-1 and 4 strains of *E. coli* expressing AmpC did not grow on Brilliance®, while the remaining 8 strains of *E. cloacae* and 1 of *E. coli*, derepressed which overexpressed AmpC, did grow in the chromogenic medium. At each concentration tested in the urine samples, similar counts were obtained to those rendered by standard media such as blood agar.

Conclusions:

1. The performance of Brilliance®-ESBL was excellent for isolating ESBL-producing EB strains.
2. Derepressed *E. cloacae* strains hyperproducing cephalosporins are able to grow in this medium.
3. Brilliance®-ESBL correctly identified both groups of bacterial species examined (*E. coli* vs. non-*E. coli*), although it was unable to distinguish between the latter (*K. pneumoniae* or *E. cloacae)*.
4. Brilliance®-ESBL showed the same detection limits as non-specific media, yielding similar counts to those obtained on blood agar.

P634 The potential of phenotypic methods for detecting ESBL- and AmpC-type broad-spectrum β-lactamases

M. Htoutou Sedlakova, V. Hanulik, M. Chroma, K. Hricova, M. Kolar, R. Schaumann, A.C. Rodloff (Olomouc, CZ; Leipzig, DE)*

Objectives: At present, enterobacteria producing broad-spectrum β-lactamases belong to the feared bacterial pathogens. Of particular clinical importance are ESBL and AmpC enzymes increasing the risk of failure of antibiotic therapy and related morbidity and mortality. The reliable laboratory detection of these enyzmes is still challenging, especially in the case of AmpC enzymes. The aim of the work was to compare the sensitivity of selected phenotypic methods for detecting ESBL and AmpC production.

Methods: A total of 106 strains of the Enterobacteriaceae family were tested, in which molecular biology methods confirmed the presence of genes encoding for ESBL (85 strains) or for AmpC (21 strains). The strains were identified using the Phoenix automated system (Becton Dickinson). In ESBL-positive strains, the sensitivity of the ESBL E-test (AB Biodisk) and the modified double-disk synergy test (DDST) was evaluated. AmpC strains were tested by the modified AmpC disk method using 3-aminophenylboronic acid. For simultaneous detection of ESBL and AmpC, the microdilution method and determination of minimum inhibitory concentrations (MICs) with a modified set of antimicrobial agents, including combinations with sulbactam, tazobactam and 3-aminophenylboronic acid, were used. Furthermore, the accuracy of determination of ESBL and AmpC production by the Phoenix automated system was tested.

Results: The sensitivity of detection of ESBL by the Phoenix automated system was high (99%); however, it only reached 38% in the group of AmpC-positive strains. High sensitivity was also achieved by tests specific for particular types of broad-spectrum β-lactamases: the sensitivity of the ESBL E-test was 95%; the modified DDST yielded 100% sensitivity for ESBL producers and the AmpC test correctly detected 95% of AmpC-positive strains. The sensitivity of the modified microdilution method was 94% and 95% for ESBL and AmpC β-lactamases, respectively.

Conclusion: It is clear that the detection of broad-spectrum β-lactamases must be based on specific phenotypic methods such as, in particular, the modified DDST, ESBL E-test and AmpC disk method. The microdilution method with a modified set of antibiotic agents and the comparison of relevant MIC values seem also suitable as high sensitivity in the detection

of the ESBL and AmpC enzymes can be achieved with easy to perform methodology.
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P635 A selective screening broth for rapid detection of extended-spectrum β-lactamases

G. Wilson, K. Irvine, H. Hanaki, R. Kubo (Stirling, UK; Tokyo, JP)*

Objectives: The objective was to develop a rapid method of screening hospital patients for ESBL carriage. Development of a selective ESBL broth, which changes colour (red to yellow) in the presence of an ESBL, enables the detection and presumptive identification of an ESBL-producing organism.
Methods: A total of 264 controls were used to evaluate the screening broth. This included; 160 phenotypically confirmed ESBL-producing organisms and 104 negative controls. In addition, 5 NCTC and ATCC organisms were also tested against the broth.
The broth was tested against 510 unknown clinical samples (groin swabs). After overnight incubation positive broths were subcultured in order to test for ESBL production. The broth was centrifuged and the deposit tested with the chromogenic cephalosporin HMRZ-86 (Kanto Chemical Company, Japan). A coloured reaction (yellow to red) is produced in the presence of an ESBL enzyme within 15 min. A positive result was then followed by neutralisation with clavulanic acid to presumptively confirm ESBL. The Jarlier test was used to detect ESBL production and all isolates were identified by the VITEK2 (bioMérieux, France). Groin swabs were also cultured onto a growth control (MacConkey agar, Oxoid, England) for isolation of any Enterobacteriaceae; all isolates were identified by the VITEK2, and checked for ESBL production. A subset of 139 clinical samples were incubated and examined after a 4 h period.
Results: A total of 48 ESBL-producing isolates were detected from the clinical samples. The broth alone detected all 48 ESBL-producing isolates, resulting in 100% sensitivity, however the specificity of the broth was low (84.2%) due to 73 false positives. This resulted in a low positive predictive value (PPV) of 39.6%. The combined use of the broth and HMRZ-86 neutralisation test improved the specificity (98.9%), and PPV (89%) of the test. The resulting sensitivity of the test was reduced to 85.4%. Clinical samples tested at a 4 h incubation period showed that 40% of positive broths contained an ESBL-producing organism, confirmed by the HMRZ-86 neutralisation test.
Conclusion: The combined use of the ESBL broth with the HMRZ-86 neutralisation test provides a specific and relatively sensitive method for the presumptive detection of ESBL-producing organisms. There is also the potential for the ESBL broth to be used as a rapid 4 h test.

P636 Evaluation of four screening protocols for detection of extended-spectrum β-lactamase-producing Enterobacteriaceae

B.M. Diederen, C. Cheung, S. Euser, J.W. Cohen Stuart (Haarlem, Utrecht, NL)*

Objective: We evaluated the use of four different screening protocols for the detection of extended-spectrum β-lactamase producing (ESBL) Enterobacteriaceae.
Methods: From January 2009 to May 2009, surveillance culture samples from ICU patients at two teaching hospitals were included. In addition, random fecal samples obtained from GP and nursing home (NH) residents were tested. Specimens were obtained with an Amies swab and subsequently, each specimen was homogenized in a liquid Amies medium (Eswab, Copan). Ten microliter suspension was inoculated in a single session onto four culture media: directly on a chromogenic medium (Method A. ESBL-ID; bioMérieux), and into the three different enrichment broth regimens: trypticase soy broth (TSB) supplemented with 1 mg/l ceftazidime (Method B), TSB supplemented with 1 mg/l cefotaxim (Method C), and non-selective TSB (Method D). After incubation for 24 h at 36ºC, 10 µl of broth was streaked on a ESBL ID plate in the same way. All plates were incubated for 24 h at 36ºC. All oxidase negative colonies presenting different morphological aspects and growing on ESBL-ID after 18 to 24 h were identified with Vitek2. Confirmation of ESBL-producing isolates was performed by combined double disks according to national guidelines.
Results: A total of 514 samples obtained from 384 patients was tested (ICU patients: 202 samples, 72 patients; GP: 208 samples, 208 patients; NH: 94 samples, 94 patients). A total of 23 samples obtained from 21 patients tested positive for ESBL producing Enterobacteriaceae (overall prevalence 5.5%; 21 *E. coli*, 1 *K. pneumoniae*, 1 *E. cloacae)*. Sensitivity of method A, B, C, and D on a sample level were estimated as 70% (16/23; 95% CI, 49 to 85%), 82% (19/23; 95% CI, 62 to 94%), 82%(19/23; 95% CI, 62 to 94%), and 74% (17/23; 95% CI, 62 to 94%), respectively. On a patient level, sensitivity of method A, B, C, and D were estimated as 71% (15/21; 95% CI, 50 to 86%), 86% (18/21; 95% CI, 65 to 96%), 81%(17/21; 95% CI, 59 to 93%), and 76% (16/21; 95% CI, 62 to 94%), respectively. There were no statistically significant differences between specificities and sensitivities using the McNemar test for paired samples or Fisher's exact test.
Conclusions: From this clinical comparison, we conclude that a direct inoculation on a chromogenic medium is a simple, rapid, and acceptable accurate method for performing active surveillance for ESBL in a variety of health care populations.

P637 Use of an HMRZ-86 supplemented broth for the direct detection of extended-spectrum β-lactamase producing Enterobacteriaceae in clinical samples

N. Gordon, A. Sivaramakrishnan, R. Kubo, D. Wareham (London, UK; Tokyo, JP)*

Objectives: HMRZ-86 is a novel cephalosporin susceptible to hydrolysis by extended spectrum and metallo-β-lactamases, resulting in an easily observed colour change from yellow to red. It has previously been used for the detection of ESBL production from bacterial colonies taken from agar plates (Cica-β-Test). In this study we evaluated the use of an HMRZ-86 based broth for the direct detection of ESBL producers from clinical specimens.
Methods: the ability of nutrient broth supplemented with cefpodoxime, vancomycin and HMRZ-86 to detect ESBL production by a number of type strains was first evaluated using inocula from 10e7–10e3 CFU/ml. The broth was then assessed for the detection of ESBL producers in perineal swabs (n = 94) obtained for routine MRSA screening. After inoculation and incubation for 18 h at 37ºC, broths were inspected for colour changes, subcultured to CHROMagar Orientation and compared with growth obtained by inoculating swabs directly onto CHROMagar Orientation supplemented with cefpodoxime. Isolates were identified by oxidase testing and API20E, with ESBL production confirmed by double disc tests containing cefpodoxime or cefipime ± clavulanate.
Results: Using type strains NCTC 13353 (*Escherichia coli* blaCTX-M15), ATCC 700603 (*Klebsiella* pneumonia blaSHV-18) and ATCC 25922 (*E. coli*, ESBL negative), a red colour change was detectable down to 10e4 CFU/ml. 13 broths were positive following direct inoculation with perineal swabs. Of these, 9 were identified as *Pseudomonas* spp. The remaining 4 were *K. pneumoniae*, *E. coli* and *Enterobacter cloacae*, 3 of which were confirmed as ESBL producers. One of the *E. cloacae* isolates was ESBL negative but phenotypically appeared to be a hyperproducer of AmpC. The sensitivity of the HMRZ broth for detection of ESBLs was 100% with a specificity of 89%. To control for the possibility of AmpC production resulting in false positives, all thirteen isolates were re-inoculated into broth containing HMRZ-86 supplemented with cloxacillin. The confirmed ESBL producers gave a positive result at 18 h, while the remaining isolates caused no colour change.
Conclusions: HMRZ-86 was highly sensitive for the detection of ESBLs from perineal swabs, but lacked specificity due to the presence of AmpC producers. This was improved by the addition of the AmpC inhibitor cloxacillin. HMRZ-86 may be a useful substrate for the rapid detection of high prevalence class A ESBLs directly in clinical samples.

P638 Evaluation of Brilliance ESBL, a novel chromogenic agar for the detection of extended-spectrum β-lactamases producing Enterobacteriaceae

T.D. Huang, P. Bogaerts, C. Berhin, A. Guisset, Y. Glupczynski (Brussels, Yvoir, BE)*

Objective: To evaluate the performance of Brilliance ESBL® (OX; Oxoid) selective chromogenic agar for the detection and the presumptive identification of extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae (EB).
Methods: In a preliminary study, we challenged a collection of 200 Gram-negative bacterial including 156 EB strains with well-defined resistance mechanisms against OX and ChromID ESBL® (BM; bioMérieux) chromogenic agar to evaluate the growth selectivity and the chromogenic features of the media. In a second part, 528 clinical samples (including 344 fecal specimens) obtained from 424 ambulatory and hospitalized patients were plated onto OX, BM and MacConkey agar to which a disk of ceftazidime was added (MCC) for the screening of ESBL-producing EB. All colonies growing on any of the three media after full 24-hour incubation were identified and tested for susceptibility by Phoenix® System (Becton-Dickinson). ESBL confirmation was performed by combination disks method. Characterization of resistance mechanisms was determined by PCR of TEM, SHV, CTX-M, OXA, and AmpC genes with amplicon sequencing.
Results: Of the 156 EB isolates from the collection isolates, all 98 ESBL producers were detected but 50 strains harbouring various non-ESBL resistance mechanisms were also recovered on both OX and BM; 8 fully susceptible isolates did not grow on any of the two selective media. Of the 528 clinical samples screened, 144 (27%) yielded growth on at least one of the three media. A total of 182 isolates including 109 (60%) EB were recovered and 70 of these (from 59 specimens) were confirmed as ESBL-producing isolates. The sensitivities were 74.6%, 94.9% and 94.9% for MCC, BM and OX respectively. The specificities calculated for the ESBL-negative samples reached 94.9%, 95.5% and 95.7% for MCC, BM and OX respectively when only chromogenic enterobacterial colonies were considered on the two chromogenic media.
Conclusions: OX and BM yielded comparable performances and had a higher sensitivity than MCC for the detection of ESBL-producing EB from clinical specimens. The high negative predictive value (99.3%) found for OX suggested that this medium could represent an excellent screening tool for rapid exclusion of carriage of ESBL producers. The selection of enterobacterial isolates only for ESBL confirmation based on chromogenic features could limit the unnecessary workout.

P639 Evaluation of the UF1000i flow cytometer as a means of reducing urine cultures and predicting the micro-organisms involved in urinary tract infections

J.M. Acedo, C. Artaza, M. Morito, J. Fernández, J. Valverde, A. Delgado-Iribarren (Madrid, ES)*

Objectives: The gold standard method for diagnosis of UTI is urine culture but it is time-consuming and fewer than 25–30 percent of urine samples sent to the laboratory are proven positive. Rapid screening methods such as the UF-1000i flow cytometer have been introduced to reduce the number of urine samples requiring culture. This method may also give an approximation of the microorganisms involved in UTI because the scattergram B1 differs with bacilli and cocci. We evaluated these two interesting topics of flow cytometry for diagnosis of UTI.
Methods: Testing with the UF1000i system was done following the instructions provided by the supplier (bioMérieux, France). The reference method used was the semi-quantitative urine culture on CLED agar (bioMérieux, France). Bacterial identification was performed automatically with MicroScan WalkAway (Siemens). A total of 500 urine samples from general practice patients representing all age groups were collected and analyzed. Culture results were reported as no growth; contaminated (if there were 3 or more colony types without a dominant species); positive (culture with $\geqslant 10^5$ or 10^4 to $<10^5$ colony-forming units/mL (cfu/mL) of a urinary pathogen in pure culture, two or more potentially pathogenic bacterial species were isolated when the individual counts were $\geqslant 10^4$ cfu, or when the count for one organism was $\geqslant 10^4$ cfu/mL and was clearly predominant).
Bacterial orientation: 2 independent observers classified the scattergram B1 in samples with more than 25.000 cfu/ml into four groups: Bacilli, Cocci, Both, Not classifiable.
If there were discrepancies between the observers, a third observer revised the scattergram and determined how to classify the sample. If there were discrepancies between the three observers the sample was excluded (2 samples). Finally 125 out of 127 urine samples with more than 25.000 cfu/ml were evaluated.
Results: The NPV and sensitivity calculated during this study are in accordance with data obtained during previous studies, they are more than 92% and 95% respectively.
Conclusions: The good NPV obtained allowed reporting of negative results without culture being performed, reducing the urine cultures by 35% and reducing the turnaround time for these samples from 24 h to same-day reporting.
Bacterial orientation of the microorganisms involved in UTI may be achieved with UF1000i, especially with Staphylococci and Gram-negative rods.

Bacterial orientation	n	Correct		Not classifiable		Wrong	
		n	%	n	%	n	%
Cocci and bacilli	39	23	59	7	17.9	9	23.00
Bacilli	69	52	75.3	3	43.5	14	20.3
Cocci	17	8	47.00	0	0	9	53
Streptococci	11	2	18.2	0	0	9	81.8
Staphylococci	6	6	100	0	0	0	0

P640 Antibiotic screening of urine culture – a useful quality audit

W. Godwin, S. Badarudeen, A. Godwin (Doha, QA)*

Interpretation of urine cultures depends on various factors. Antibiotic history is an important factor that affects the performance of urine cultures in the laboratory. We decided to test the urine specimens for antibiotics and compare it with the request forms sent to our laboratory. The technique, incidence, implications, involved in processing of specimens with incomplete antibiotic history received in the laboratory are discussed. This internal audit practice stresses the need for quality requests which play a major role in the clinical interpretation of culture specimens.
Material and Method: Detection of antibacterial substance in the urine. We modified the Urine Antibacterial Substance Assay (UABA), a technique described by Sombrero L et al by using two Muller Hinton agars each inoculated with a lawn culture of standard strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.
Results: A total of 14680 consecutive urine specimens for culture and sensitivity received in the laboratory during the study period from 1st July 2008–30th June 2009 were tested with the UABA assay. 2494 (16.99%) samples yielded positive cultures. 388 (2.64%) samples were positive for UABA of which 165 (42.53%) were received from the out patient departments (OPD) and 223 (57.5%) from the wards. Of the positive samples of UABA 222 (57.22%) had antibiotic history written on the requisition form and 208 (93.7%) of the samples reported no growth. Compliance to writing antibiotic history in the wards 183 (82.1%) was higher than OPD's 39 (23.64%). 115 (29.6%) of the positive UABA had pus cells >5/HPF.
Conclusion: A periodic internal audit of antibiotic assay in urine cultures provides baseline data permitting blind assessment of areas of culture based clinical diagnostic work that are not readily amenable to other quality assurance methods. It raises awareness to the importance of quality and patient safety issues involved with history taking using an evidence-based protocol and aids in the review of

validating physicians' compliance with an established protocol. Reaudit interventional strategies can be planned, as part of a continuous quality improvement program to affect change in physicians' practices when there is a drop from established benchmark. This can lead to overall quality improvement in the process of care and save valuable financial resources.

P641 Evaluation of the Sysmex UF1000i urineflowcytometer in the diagnostic work-up of urinary tract infection

W. van der Zwet, F. Canbolat, J. Hessels, M. Deckers (Deventer, NL)*

Objectives: Automation and standardisation of sediment analysis of urine samples by flow-cytometry might serve as an alternative to traditional labour-intensive laboratory methods such as microscopic examination and bacterial culture. We evaluated the Sysmex-UF1000i urine flowcytometer and compared results to standard laboratory methods. Special interest was focused on the negative predictive value and percentage of cultures that could be left undone.
Methods: The Sysmex UF1000i is a urine flow-cytometer that uses two separate channels for counting blood cells and bacteria. Two fluorescent dyes, one binding to cell membranes and one binding to nucleic acids, are added to uncentrifuged urine. The urine is subsequently flushed as a laminar flow through the two analytical channels in which the emitted pulses, light scattering and fluorescence are measured by a photodiode and enhanced by a photomultiplier. Urine samples were investigated with the Sysmex-UF1000i, manual microscopy of urine sediment, Gram-stain and bacterial culture (golden standard).
Results: Three hundred fifty eight urine samples were analyzed. Reproducibility for detection of white (WBC) and red blood cells and bacteria with the UF1000i was good, whereas detection of yeasts proved unreliable. Depending on the definition of urinary tract infection (UTI) used, the negative predictive value and the percentage of false-negative results were 100% and 0% (UTI defined as $>10^5$ Colony Forming Units (CFU)/ml) and 99% and 1.3%, (UTI defined as $>10^4$ CFU/ml) respectively. Pre-screening with the Sysmex UF 1000i results in a reduction of bacterial cultures of 42%.
Carry-over of bacteria between consecutive samples due to a fixed sample needle was observed, but did not result in false-positive interpretation of Sysmex UF 1000i results. Evidently, because of the occurrence of carry-over, samples that have been analyzed in the Sysmex UF 1000i cannot be used for subsequent urine culture.
Conclusion: In conclusion, the Sysmex-UF1000i offers the possibility of screening high numbers of urine samples in a fast and standardized way resulting in a reduction in workload and speeding up the diagnostic process. As in other non-culture methods, the UF 1000i should not be applied in patients in whom yeast infection is suspected or patients that are leukopenic.

P642 Preliminary evaluation of Sysmex UF1000i flow cytometer in discriminating Gram-negative and Gram-positive micro-organisms in urine

R. De Rosa, M. Avolio, P. Stano, S. Grosso, G. Bruschetta, A. Camporese (Pordenone, IT)*

Objectives: Urine culture test is the gold standard to identify the aetiological agent of UTI, however it does not supplie same-day results. Sysmex UF 1000i urine cytometer provides results in a few minutes; we aimed to explore preliminary B_FSC and B_FLH parameters in discriminating Gram negatives and Gram positives respect of bacterial identification by urine culture.
Methods: Sysmex UF1000i (Sysmex Co. Japan) a new fluorescence flow cytometer intended for urinalysis purpose has a specific analitical channel for bacteria counting. From this channel two parameters, the B_FSC (Bacteria Forward Scatter) and the B_FLH (Bacteria Fluorescent Light Intensity), expressed in arbitrary units (analytical channel, ch), provide informations on the size and the nucleic acid content of bacteria particles. 1349 samples from inpatients and outpatients were examined by Sysmex UF1000i and cultured on CLED and CNA agar plates (Kima, Padua, Italy); in the positive samples bacterial identification was performed by Vitek 2 system (bioMérieux, Florence, Italy). Data between Gram-negative and Gram-positive bacteria were evaluated, comparing the distribution of B_FSC and B_FLH values in the two groups.
Results: 1003 samples were negative (74.4%). Out of 346 bacterial culture positive (25.6%), 229 samples presented a growth of Gram negatives, 45 samples growth of Gram positive bacteria and 72 samples showed mixed growth (a Gram positive and a Gram negative or contamination). The median B_FSC in Gram-negatives was 22.1 ch., in Gram-positive bacteria 44.6 ch. ($p < 0.0001$). The 95% distribution of B_FSC was 11.5–70.4 ch. in Gram-negatives, 17.6–127.5 in Gram-positives. Out of 274 samples, 168 (61.3%) had a B_FSC value <30 ch.: 163 (97.0%) were from samples with Gram-negatives, 5 (3.0%) from samples with Gram-positives. The median B_FLH in Gram-negative bacteria group was 85.5 ch., in Gram-positives 94.6 ch. ($p = 0.027$); the 95% distribution of B_FLH in the two groups were: 72.3–177.8 ch. in Gram-negatives, 75.2–178.8 in Gram-positives. B_FSC value showed at a cut-off value of 30 ch. a high discriminating power between the two groups.
Conclusion: In this preliminary evaluation the B_FSC and B_FLH seem to be interesting parameters in morphological assessment of bacteria allowing to obtain in few minutes information as well as on bacterial counting also on aetiological agent of urinary tract infection, needing however to be further investigated in more extensive studies.

P643 Use of a polymerase chain reaction assay for rapid detection and identification of *V. vulnificus*

I. Cañigral, Y. Moreno, A. Adrados, A. Gonzalez, M.A. Ferrús (Valencia, ES)*

Objectives: *Vibrio vulnificus* is a Gram-negative bacterium found in warm seawater.
The bacterium is a worldwide highly invasive human pathogen with highest mortality rates (up to 50%). Human infection are acquired though consumption of contaminated seafood or though skin wounds. The main symptoms are gastroenteritis, wound infection and septicemia, frequently leading to limb amputation and fatality. It is considered as an important emerging environmental and food safety issue around the world.
The objectives are optimize and to test Polymerase Chain reaction (PCR) method for rapid detection and identification *Vibrio vulnificus* in clinical and environmental samples.
Methods: Primers vvh785 and vvh 990, that amplify a fragment with 205bp of a haemolysin A gen (vvhA) were used. PCR conditions were optimized and annealing temperature was established at 56ºC. The reaction was applied to 20 *Vibrio vulnificus* clinical and environmental strains. It was applied directly to inoculated saline, water and seafood samples too in order to test its potential for rapid detection. 10 sea water samples obtained from Valencia coasts and 40 seafood samples, including oysters, clams, mussels and goose barnacle, were analyzed.
Results: PCR protocol yielded a fragment of 205 bp. Reaction identified all the *Vibrio vulnificus* strains tested. Detection limit in inoculated samples was 10^2 c.f.u in saline water and 10^4 c.f.u in complex matrix such as seafood. Among the 50 samples ten samples were positive by PCR and 2 strains of *Vibrio vulnificus* were isolated.
Conclusion: This PCR method allows for rapid and specific detection and identification of *V. vulnificus* and could be useful for diagnostic and epidemiological purposes, determining the potential health risk due to contaminated seafood and water.

P644 Long-term tigecycline therapy (6 weeks) for complications of *E. coli* bacteremia

S. Hagenauer-Drektraan, C. Wenisch, G. Seiberl (Vienna, AT)*

Introduction: *E. coli* is one of the most common organisms associated with bacteremia, but rarely causes complications under sufficient therapy.

We report a case of bacteremia with fully susceptible *E. coli* complicated by discitis despite treatment with β-lactam antibiotics.

Case report: A 70 year old male patient was admitted due to sudden onset of generalized weakness. On admission he had hypotension, tachycardia, and scleral icterus. A few hours after admission the patient became febrile. Laboratory test results revealed biliary obstruction and elevated erythrocyte sedimentation and CRP as well as leucocyturia. Abdominal ultrasound showed cholecystolithiasis and three layered gallbladder wall suggestive for cholecystitis but without signs of intrahepatic dilatation of the biliary tract.

Piperacillin/tazobactam 3.5g tid was started. On day 3 the patient developed septic shock. Blood cultures grew Gram negative rods and the antibiotic regimen was changed to ertapenem, presuming an ESBL producing organism. Blood cultures subsequently grew fully susceptible *E. coli*, but aspiration culture of a subcutaneous pustule, which developed during the 1st week of therapy revealed ESBL producing *E. coli* and *Staphylococcus aureus* (MSSA). The urine was sterile. The patient's condition improved and antibiotic treatment was stopped after 2 weeks. The following day the patient became febrile and complained about severe pain at palpation of the lower lumbar spine. An MRI scan showed contrast enhancement of L4 and L5 and the L4-L5 intervertebral disc (Figure 1.). The disc was punctured, but aspiration cultures were sterile. Due to the ESBL producing *E. coli* and *Staphylococcus aureus* in a pustule, antibiotic treatment was started with tigecyclin. The patient was placed in a thoracic lumbar sacral orthosis. Intravenous therapy with tigecyclin was continued for 6 weeks, followed by oral therapy with ciprofloxacin and clindamycin for additional 6 weeks. Orthopaedic training resulted in 100% rehabilitation.

Conclusions: *E. coli* is the second most common causative agent of septic discitis. Therefore any prolonged episode of fever and elevated CRP and erythrocyte sedimentation following *E. coli* bacteremia should raise the suspicion of septic discitis and must be followed by directed physical examination, diagnostic imaging and aspiration.

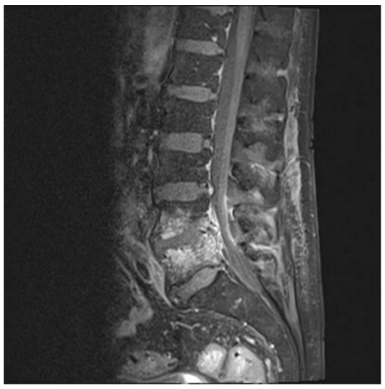


Figure 1. Contrast enhancement of L4 and L5, intervertebral disc, and paravertebral soft tissue and also paraspinal muscles lateral to the p. spinosi.

P645 Tigecycline activity tested against infrequently recovered clinical species of non-enteric Gram-negative bacilli

R. Mendes, H. Sader, M. Stilwell, R. Jones (North Liberty, US)*

Objectives: To assess tigecycline (TIG) activity and potency against non-enteric Gram-negative bacilli (NEGNB) clinical isolates. NEGNB usually display multidrug-resistance (MDR) phenotype due to upregulated efflux and chromosomal β-lactamases belonging to Classes A, B, C or D, limiting the therapeutic options for treating infections caused by these organisms. TIG has demonstrated a broad spectrum of activity against Gram-positive and -negative bacterial pathogens, including many MDR isolates.

Methods: A total of 2,996 clinically-significant isolates of NEGNB (23 species) were collected from 142 hospitals in 32 countries as part of the SENTRY Antimicrobial Surveillance Program over a ten year sampling period (2000–2009; North America [32.1%], Europe [26.8%], Asia-Pacific region [23.4%] and Latin America [17.7%]). Isolates were submitted to a coordinator laboratory where species identifications were confirmed using standard algorithms and Vitek 2, and tested for susceptibility (S) against TIG and comparators by reference CLSI methods (M07-A8, 2009). CLSI and EUCAST interpretative criteria were applied when available.

Results: Isolates were recovered mostly from bacteremia (58.1%), pneumonia (29.7%) or skin and skin structure infections (10.4%). TIG was most active against Pasteurella multocida (MIC90, 0.12 mg/L) and various *Acinetobacter* spp. (MIC90, 0.5 mg/L). *Aeromonas* spp., *Rhizobium radiobacter*, *Ralstonia pickettii* and *Sphingomonas paucimobilis* were also very susceptible to TIG (MIC90, 0.5 mg/L). In contrast, *Burkholderia cepacia*, *P. fluorescens/putida*, Chryseobacterium spp. and Elizabethkingia spp. (MIC90, 4–>4 mg/L) generally exhibited elevated TIG MIC values. TIG (MIC50/90, 0.5/2 mg/L; 95.5% inhibited at ⩽2 mg/L) and trimethoprim/sulfamethoxazole (TMP/SMX) (MIC50/90, ⩽0.5/1 mg/L; 96.1% S) were the most active compounds tested against *Stenotrophomonas maltophilia*; while ceftazidime (MIC50/90, 16/>16 mg/L; 45.1% S), levofloxacin (MIC50/90, 1/4 mg/L; 83.3% S) and ticarcillin/clavulanate (MIC50/90, 32/>128 mg/L; 38.7% S) showed more limited activity against this organism.

Conclusions: TIG showed potent *in vitro* activity against many NEGNB for which there are very limited therapeutic options and S data to guide therapy. Against *S. maltophilia,* TIG activity was comparable to that of TMP/SMX. The results of this study indicated that TIG may have an important role in the treatment of infections caused by these species.

Organism (no. tested)	MIC (mg/L)	
	50%	90%
Achromobacter xylosoxidans (130)	0.5	2
Acinetobacter haemolyticus (13)	0.12	0.5
A. junii (47)	0.12	0.5
A. lwoffii (216)	0.25	0.5
Aeromonas spp.[a] (211)	0.25	0.5
Alcaligenes faecalis (23)	0.5	2
Burkholderia cepacia (198)	1	4
Chryseobacterium indologenes (26)	4	>4
Elizabethkingia meningosepticum (39)	2	>4
Ochrobactrum anthropi (14)	0.5	1
Pasteurella multocida (53)	0.06	0.12
Pseudomonas spp. (83)[b]	0.25	2
P. fluorescens/putida (191)	2	>4
Ralstonia pickettii (17)	0.25	0.5
Rhizobium radiobacter (18)	0.12	0.5
Sphingomonas paucimobilis (32)	0.12	0.5
Stenotrophomonas maltophilia (1685)	0.5	2

[a]Includes *A. caviae* (34), *A. hydrophila* (118), *A. salmonicida* (1), *A. sobria* (15), *A. veronii* (6) and *Aeromonas* spp. (37).
[b]Includes *P. mendocina* (14), *P. oryzihabitans* (27) and *P. stutzeri* (42).

P646 *In vitro* activity of daptomycin combined with other antimicrobial agents against Gram-negative bacteria

B. Körber-Irrgang, M. Kresken (Rheinbach, DE)*

Objectives: Daptomycin (DAP), a cyclic lipopeptide, exerts rapid bactericidal activity against clinically important Gram-positive bacteria. Combination therapy might be beneficial to cover also Gram-negative pathogens in mixed infections. We evaluated the *in vitro* activities of DAP in combination with 11 other relevant drugs against clinical isolates of *Escherichia coli* (ECO), *Klebsiella pneumoniae* (KPN), *Klebsiella oxytoca* (KOX), *Enterobacter cloacae* (ECL), *Pseudomonas aeruginosa* (PAE) and *Acinetobacter baumannii* (ABA).

Methods: A total of 50 organisms including strains with various resistance phenotypes were studied. Synergy testing was performed

by using the checkerboard broth microdilution method. DAP in combination with amikacin (AMK), tobramycin (TOB), ceftazidime (CAZ), ceftriaxone (CRO), piperacillin–tazobactam (P/T), meropenem (MEM), imipenem (IMP), ciprofloxacin (CIP), moxifloxacin (MOX) or fosfomycin (FOS) were tested against strains of ECO (n = 10), ECL (n = 10), KPN (n = 6) and KOX (n = 4) (in total 300 drug combination tests), while DAP in combination with AMK, TOB, CAZ, P/T, MEM, IMP, CIP, MOX or colistin (COL) was tested against 10 strains each of PAE and ABA (in total 180 drug combination tests). The fractional inhibitory concentration indices (FICIs) were calculated to interpret the results. Synergism was defined as FICI $\leqslant 0.5$, indifference as FICI > 0.5 to $\leqslant 4$, and antagonism as FICI > 4.
Results: Antagonism was not detected with any combination. Of the 300 drug tests performed with the enterobacterial strains, 284 revealed an indifferent effect, while synergism of DAP plus CIP, CRO, MEM or MOX was observed for 10, 3, 2 and 1 strain, respectively. For non-fermenting bacteria indifference was observed for nearly all drug combinations, while synergism was seen for 2 ABA with COL and 1 ABA with TOB.
Conclusion: DAP did not antagonise the activity of antibacterial agents directed to Gram-negative bacteria and may thus be combined with these drugs for the treatment of bacterial mixed infections.

Molecular virology – diagnostics

P647 Real-time, random access detection of influenza A/B and RSV A/B in respiratory specimens using nanoparticle probes

P.J. Jannetto, K.A. Vaughan, N.A. Ledeboer (Milwaukee, US)*

Introduction: Multiplexed molecular detection of influenza A, influenza B, and respiratory syncytial virus (RSV) is becoming increasingly available with a number of commercial assays on the market. Numerous studies have demonstrated increased sensitivity of these techniques compared to culture and antigen-based detection methods. This study evaluated the sensitivity and specificity of Nanosphere's Verigene System for the detection of respiratory viruses.
Methods: Nasopharyngeal specimens were collected prospectively at multiple sites during the 2008–9 and 2009–10 respiratory seasons. The presence of influenza A/B and RSV was determined using culture/DFA and the residual samples frozen. The culture/DFA results for the samples were blinded to the investigators until the conclusion of the study. Discrepant results were resolved using bi-directional sequencing. Further studies were conducted comparing the analytical sensitivity and percent agreement between the Verigene (VG) and automated Verigene SP (SP) system. Finally, studies were conducted evaluating performance of the SP for detection of 2009 novel H1N1.
Results: Of the 720 specimens enrolled in the clinical trial, 123 were culture/DFA positive for Influenza A, 31 were positive for influenza B, 49 were positive for RSV, and 517 specimens were culture negative. In comparison to culture, the VG assay was 96.6% sensitive, 93.6% specific. There were 133 discrepant specimens between VG and culture. Bidirectional sequencing revealed that the VG assay yielded 108 additional positives otherwise negative by culture/DFA. After discrepant resolution the VG sensitivity was 98.7% and specificity was 99.3% with a positive and negative predictive value of 94.2% and 99.7% respectively. A sample set representing influenza A/B, and RSV was prepared to compare the semi-automated VG with the automated SP. A total of 62 unique samples were obtained and tested at three sites. The sites demonstrated overall positive agreement of 97.9% and negative agreement of 100%. The SP assay has also successfully detected 2009 novel H1N1 from clinical specimens with high sensitivity and specificity, compared to the CDC PCR.
Conclusion: Data from the study demonstrates that the VG assay and its automated SP successor are highly sensitive and specific alternatives to culture. The benefit of this system over others is complete automation of the test, including extraction, amplification, and detection.

P648 Subtyping (H1/H3) of seasonal influenza A viruses by multiplex real-time PCR assay

J. Acosta, S. Vazquez, J.R. Otero, L. Folgueira (Madrid, ES)*

Objectives: The emergence of seasonal influenza A H1N1 viruses resistant to oseltamivir points out the need for rapid and sensitive subtyping methods, in order to prescribe appropriated antiviral treatment. The aim of this study was to identify the subtypes of seasonal influenza A circulating in our patients over a period of three respiratory viruses seasons and to assess the utility of a multiplex real-time PCR as a routine method for subtyping Influenza A viruses.
Methods: From January 2007 to May 2009, 162 influenza A positive respiratory samples by means of antigen detection or rapid culture in shell vial, were collected and stored in aliquots at −70ºC until RNA extraction. RNA was isolated from 200 ml of respiratory samples (nasal aspirates and nasopharyngeal swabs) by nucliSENS easyMAG® instrument (Biomerieux). After retrotranscription, real-time PCR was performed in a LightCycler® instrument version 2.0 (Roche Molecular Biochemicals). A region of the matrix (M1) gene of influenza A virus, two specific sequences of subtype H1 and H3 hemagglutinin (HA), and an internal control were amplified in independent capillary reactions by uniform cycling parameters: 10 min at 95ºC and 50 cycles of 15 s at 95ºC and 60 s at 60ºC. The PCR product was detected by specific TaqMan probes. H1/H3 subtyping assay was performed in a multiplex format, using probes labeled with two different fluorescent reporter dyes.
Results: The number of specimens studied during the respiratory virus seasons of three calendar years, 2007, 2008 and 2009 were 98, 34 and 30 respectively. All the samples showed M1 gene amplification. The results demonstrated subtype H1 in 41 samples (25.3%) and subtype H3 in 117 samples (72.2%); in 4 samples subtype could not be determined (2.5%). H1 subtype represents almost one third of influenza A positive samples during years 2007 and 2008, whereas it was detected in less than 5% of samples collected during 2009.
Conclusions: The assay based on a multiplex real-time PCR used in this study provides a rapid, feasible and cost-effective approach for subtyping (H1/H3) of seasonal influenza A viruses. This laboratory diagnostic tool provides information relevant to make optimal drug treatment recommendations.

P649 Detection and discrimination of influenza A and B, and respiratory syncytial viruses using the 3M integrated cycler

M. Aye, J. Chen, Y. Xie, S. Dempsey, H. Mai, E. Eleazar, M. Exner (Cypress, US)*

Objective: Recently, it has become evident that many rapid tests designed to detect respiratory viruses lack sensitivity. As a result, a clinical need has arisen for more sensitive tests that will detect and discriminate influenza viruses. Molecular techniques have been shown to be more sensitive than culture or rapid lateral flow methodologies. Therefore, a multiplex RT-PCR assay was designed to detect influenza A viruses (of all hemagglutinin and neuraminidase types), influenza B viruses, and Respiratory Syncytial virus (RSV).
Methods: Primers were designed to target conserved regions of influenza A, B, and RSV. Specificity of the assay was tested by running a panel of known influenza strains and against a set of clinical specimens that were verified to be positive for the target viruses. In addition, a panel of other respiratory specimens was tested to determine whether there was any cross reactivity with these organisms. Assay sensitivity was determined analytically by limit of detection studies, and sensitivity was compared to culture and DFA methods.
Results: The assay was able to detect multiple influenza types and strains including seasonal H1 and H3 strains, H1N1 (2009), and H5N1. All influenza B strains tested were detected, as were all strains of RSV-A and RSV-B that were tested. The assay sensitivity was excellent, and it was shown to be more sensitive than culture and DFA for all viruses.
Conclusions: The use of this assay on the 3M integrated cycler provides a sensitive, rapid and high throughput method for detecting

and discriminating influenza A, B, and RSV viruses. The portability and ease of use of the instrument make it useful as a first line tool for diagnosis of respiratory infections.

P650 Multiplex molecular diagnostics of respiratory infections during the 2009 H1N1 pandemic: what else is out there?

A. Dumoulin, H.H. Hirsch (Basel, CH)*

Objectives: The aim of this study was to investigate the causes of respiratory infections in samples from patients with suspected H1N1v(2009) infection, using RespifinderPLUS (RF), a commercial multiplex assay detecting Influenza-A/B, Influenza-H5N1, RSV-A/B, Parainfluenza-1–4, Coronavirus-OC43/229E/NL63, Rhinovirus, Adenovirus, Metapneumovirus, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Bordetella pertussis*.
Methods: Samples collected during the initial wave (spring and summer) of the 2009 H1N1v pandemic (n = 141) as well as during the second wave (autumn and winter 2009) were analysed in parallel with a home-brew, validated H1N1v real-time PCR. Turnaround time of the RF analysis was 24 h. Hands-on time was 3 h.
Results: In the 141 samples collected during the initial wave of the 2009 H1N1v pandemic, the proportion of positive samples was 63%: 40 Rhinovirus (28%), 25 Influenza-A (18%, all H1N1v), 5 Adenovirus, 5 Metapneumovirus, 3 Influenza-B, 2 Parainfluenza, 2 Coronavirus, 1 RSV-B, 1 *Mycoplasma* pneumonia, 52 negative. The analysis results of the samples collected during the second wave will be presented. Preliminary results indicate a much larger proportion of H1N1v positive samples (PCR positivity rate 34%), as well as an increase in Parainfluenza positive samples (10%).
Conclusion: RF analysis of respiratory samples collected during the initial phase of the 2009 H1N1v pandemic revealed a high incidence of Rhinovirus during spring and summer, whereas H1N1v was present in only 8% of the samples. A strongly increased proportion of positive H1N1v samples is expected in the samples of the second phase.

P651 Detection of six groups of human adenoviruses by quantitative real-time polymerase chain reaction with one set of primers

C.Y. Lee, Y.J. Chan, C.H. Chang, J.C. Lin, C.L. Lee, T.Y. Chou (Taipei City, TW)*

Objective: Human adenoviruses (HAdVs) are associated with a broad spectrum of clinical illnesses and are endemic in the pediatric population in particularly. At least 51 serotypes of adenoviruses has been identified and classified into six groups (A to F) at present. Because of few cluster homologies within AdVs species and between species, it is challenging to develop real-time quantitative AdVs polymerase chain reaction (PCR) assays effectively covering all AdVs types in one reaction. In this study, a real-time PCR assay to quantitatively detect all six groups of human adenoviruses is presented.
Methods: One set of designed primers was constructed to target the relative conserved region of AdV hexon region with 130bp in size after alignment. Real time PCR was performed with an ABI Prism 7000 sequence detection system. Six representative AdV strains of each group (AdV-C1, AdV-E4, AdV-B7, AdV-D9, AdV-A12, AdV-F40) and stored culture AdV isolates in Taipei Veterans General Hospital during 2004–2008 were tested. For specificity testing, herpes simplex virus type 1 and 2 (HSV-1, 2), cytomegalovirus (CMV), BK and JC virus as negative controls were tested for cross-reaction. For comparison, two published real time quantitative PCR assays were conducted as well.
Results: A total of 119 clinical AdV isolates during the 5-year-period, comprising 100 respiratory specimens, 11 stool or rectal specimens, 3 urine specimens, 1 blood specimen, 1 vaginal specimen and 1 cerebrospinal fluid specimen, were analyzed. The serotypes distribution of these clinical isolates, which were identified by Taiwan Center of Diseases Control, belonged to serotypes -C1, -C2, -B3, -E4, -C5, -C6 and -B7. The detection rate of the designed primers of this study for the DNA of six representative strains and the 119 culture isolates is 100%, as effectively as the published methods. There was no cross reactivity against HSV-1 and 2, CMV, BK and JC virus.
Conclusions: A potential effective real time quantitative PCR assay is important for rapid clinical diagnosis of adenovirus infection, especially amongst the immunocompromised hosts. The new developed real time PCR assay allows rapid and sensitive quantification of all defined groups of human adenoviruses to date. In addition, this assay is non-inferior to the published methods.

P652 Comparison of two mega-multiplex-PCR respiratory pathogen panels: the FilmArray™ 20-pathogen panel versus Luminex xTAG® 12-pathogen panel

R. Selvarangan, G. Metzler (Kansas City, US)*

Background: FilmArray RP is a user friendly multiplex-PCR system for comprehensive detection of 20 different bacterial and viral respiratory pathogens and subtypes in clinical specimens within one hour from receipt. Pathogens detected are influenza A (Flu A, H1 or H3), influenza B (Flu B), Respiratory syncytial virus (RSV), Adenovirus (AdV), Parainfluenza viruses (PIV 1, PIV1, PIV3, PIV4), human metapneumovirus (hMPV) Rhinovirus (RhV), Coronaviruses (NL63, HKU1, OC43, 299E), human BoCa virus (BV), *M. pneumoniae* (Mpne), *C. pneumoniae* and *B. pertussis.*
Objective: To evaluate performance of FilmArray RP using respiratory specimens from children that were previously tested by Luminex xTAG multiplex PCR (xTAG RVP) capable of detecting 12 different respiratory viruses and their subtypes.
Method: Frozen aliquots of xTAG RVP-tested specimens (n = 67) were used for the comparison study. About 250 ul of respiratory specimen was processed and inoculated into a hydrated FilmArray RP pouch and loaded on the instrument. Five realtime PCR-tested pertussis specimens were also used for evaluation.
Results: FilmArray RP results agreed with 63/67 (94%) specimens tested by xTAG RVP. In the 67 specimens tested, xTAG RVP detected a total of 81 viruses while FilmArray RP detected 98 pathogens (see Table 1); 12 of these pathogens are either not reportable (NL63 = 4, HKU1 = 1, 229E = 1) or absent on xTAG RVP panel (BV =4, PIV4=1, Mpne=1). Co-infection with more than one pathogen was detected in 18% and 42% of the specimens tested by xTAG and FilmArray RP respectively. FilmArray RP detected *B. pertussis* accurately in 3 positive specimens and detected HKU1 and OC43 in 1 of 2 pertussis-negative specimens.
Conclusion: FilmArray RP had a higher detection rate for pathogens when compared to xTAG RVP; this may be attributed to the use of highly sensitive nested PCR and addition of new pathogens. Limitation of the current FilmArray RP is low-throughput of one sample per hour on a single instrument; however hands-on-time of less than five minutes and rapid turn-around-time (TAT) of about 1 hour makes this assay user friendly and rapid compared to the >1hr set-up time and 8 hr TAT for xTAG RVP. A cluster of 4 FilmArray instruments may allow processing of 28 to 84 specimens in one to three laboratory shifts making it a suitable option for medium to large sized laboratories. The FilmArray RP assay is easy to setup and provides rapid and highly sensitive detection of respiratory pathogens.

Table 1: xTAG-RVP and FA-RVP results from 67 respiratory specimens

Results	FluA	FluB	RSV	AdV	hMPV	PIV	RhV/EV	CoV
xTAG-RVP	5	9	10	6	10	18	21	NR*
FA-RVP	5	9	13	7	10	20	23	6

* Not reportable in FDA approved xTAG version.

P653 Validation of a pre-market version of the Idaho Technology Inc. FilmArray™ Respiratory Panel for the detection of respiratory pathogens in well-characterized human respiratory specimens

R.S. Buller, J. Jimenez, M. Poritz, K. Nilsson, K. Ririe, G.A. Storch (Saint Louis, Salt Lake City, US)*

Objectives: Respiratory infections are a major cause of illness worldwide. Unbiased molecular detection of respiratory pathogens is complicated by the fact that multiple agents sharing few conserved sequences cause respiratory infections. Massive multiplexed PCR assays for respiratory pathogens exist but are labor intensive and there is a prolonged time to produce a result. Idaho Technology Inc.(ITI) has developed a real-time PCR instrument called the FilmArray and an associated reagent pouch which together are capable of simultaneously detecting multiple organisms in a sample. The FilmArray pouch contains freeze-dried reagents to perform nucleic acid extraction amplification, reverse transcription, and nested, multiplex real-time PCR. The Respiratory Panel (RP) pouch identifies 20 common and emerging viral and bacterial pathogens and results are available in less than an hour. Our objective was to help validate the RP by testing a set of well-characterized specimens.
Methods: 86 specimens positive for adenoviruses, influenza A, influenza B, parainfluenza virus, rhinovirus, respiratory syncytial virus, coronavirus, human metapneumovirus, *Bordetella pertussis*, or *Mycoplasma* pneumonia, as determined by PCR and conventional methods or PCR alone, were tested by the RP assay and the results compared to the earlier testing.
Results: 68 specimens positive for a respiratory virus were tested. 9 of 68 (13%) produced invalid FilmArray results due to control failures. Of the valid runs, 58/59 (98%) agreed with the previous results. Additional viruses were detected in 16 (28%) of the positive specimens, including 11 with 1 extra virus and 5 with 2 additional viruses. 18 specimens positive for either *B. pertussis* or *M. pneumoniae* were also evaluated by the FilmArray. 3 of these 18 (17%) specimens produced invalid results due to control failures with 10/15 (67%) valid runs yielding results that agreed with previous testing. Detection of individual agents and data from the ongoing resolution of discrepant and FilmArray failed results will be presented.
Conclusion: The ITI FilmArray RP is simple to use with minimal hands-on time and has the ability to quickly detect a wide variety of viral and bacterial agents of respiratory disease. The majority of instances where the FilmArray RP failed to detect the expected agent were due to one or more control failures, an issue that ITI is working to resolve prior to formal clinical studies.

P654 Rapid and sensitive detection of viral infection and co-infections in upper respiratory tract of patients with flu-like illness symptoms using PCR DNA microarray systems

F. Renois, N. Leveque, L. Andreoletti (Reims, FR)*

Background: In France, a specific molecular diagnosis for the detection for the new pandemic influenza A/H1N1v strain is recommended for 4 categories of patients with symptoms of flu-like illness including infants aged less than 12 months, symptomatic pregnant women, symptomatic infants or adults suffering from chronic cardio-respiratory pathologies or diabetes or for medical workers in daily contact with immunocompromised patients.
Objective: In the present study, we determined the presence of influenza A/H1N1v and 17 other respiratory viruses in nasopharyngeal samples of the four above described categories of patients visiting the Reims (Champagne-Ardenne) university hospital for symptoms of influenza flu-like illness.
Patients and Methods: From the 1st to 15th October 2009, we prospectively tested 95 nasal swabs or nasopharyngeal aspirates taken from adults (n = 57; 40.2y, SD=20.3y) or infants (n = 38; 5.3y, SD=4.3y) suffering from acute flu-like illness symptoms using two commercially available PCR-DNA microarray detection systems (Pneumovir and Fluavir detection assays, Genomica, Spain).
Results: Of the 95 respiratory samples tested by PCR-DNA microarray systems, 30 (31%) were positive for the detection of influenza A/H1N1v infection. Among these cases of influenza A/H1N1v infections, 25 (83%) were mono-infections whereas 5 cases (17%) were multiple infections associating influenza A/H1N1v with Coronavirus, Bocavirus (BoV), RSV or Human rhinoviruses (HRVs). Of the 95 respiratory samples tested, 35 (37%) were positive for other respiratory viruses than influenza A/H1N1v. Among these patients, we observed 30 mono-infection cases [HRVs (60%), PIV (20%), A/H3N2 (6%), Coronavirus (4%), BoV (4%)] and 5 multiple infection cases where HRVs were the most frequently virus detected. No specific viral mono-infection or mixed viral infections appeared to be significantly associated with the severity of the disease or secondary hospitalization in intensive care unit ($P > 0.5$).
Conclusion: The use of PCR-DNA microarray systems in clinical virology practice allows a rapid and accurate detection of conventional and newly discovered viral respiratory pathogens in infants and adults suffering from flu symptoms potentially related to an influenza A/H1N1v infection. Moreover these molecular assays are of major interest to develop new reliable weekly epidemiological survey systems for respiratory viral infections in hospitalized and non hospitalized infants and adults.

P655 Timely diagnosis of respiratory tract infections: evaluation of the performance of the Respifinder assay compared to the RVP assay

M. Raymaekers, B. De Rijke, I. Pauli, A-M. Van den Abeele, R. Cartuyvels (Hasselt, Ghent, BE)*

Objective: Lower respiratory tract infections are a common cause of hospitalization in infants and young children and are typically caused by viral or bacterial pathogens. Diagnostic methods such as culture and serology are time consuming and have several other drawbacks such as limited sensitivity, long turn-a-round time and limited number of detected pathogens. Nucleic acid amplification methods can increase sensitivity and enable the initiation of appropriate interventions without delay.
Broad-spectrum detection and identification circumvent the use of individual diagnostic DNA or RNA based assays. At present, several commercial assays are available for broad-spectrum detection. We compared the performance of the Respiratory Virus Panel assay (RVP) (Luminex Molecular Diagnostics) with that of the Respifinder assay (Pathofinder) for the detection of respiratory pathogens.
Methods: A total of 106 EQC samples of 9 QCMD Quality Control panels were analysed, of which 95 samples were expected to be positive. Extraction was performed with easyMag (Biomérieux) using the generic 2.0.1 protocol. The panels were analysed with the Respifinder assay and the RVP assay according to the manufacturer's instructions.
Results: RVP was positive in only 31 samples. For 8 samples an inconclusive result was obtained. All samples containing adenovirus, Coronavirus NL63, Coronavirus OC43 and Coronavirus 229E were false negative with RVP. No false positive results were found. Hence, sensitivity was 32.6% and specificity 100%.
A positive result was found with the Respifinder assay in 75 samples. For 3 weak positive samples an inconclusive result was obtained. For 2 adenovirus type 31 samples the analysis was also false negative. No false positive results were found. Hence, sensitivity was 79% and specificity 100%.
Conclusion: Both the RVP and Respifinder assay have an excellent specificity. Sensitivity was 32.6% and 79% for the RVP assay and Respifinder assay respectively. In conclusion, the results of our study seem to indicate a better sensitivity for the Respifinder.

P656 PCR and microarray-based Prove-it™ Herpes assay for rapid herpesvirus diagnostics from clinical samples

A-K. Järvinen, M. Mäki, L. Mannonen, R. Vainionpää, J. Kauppinen, R. Lienhard, M. Tritten, G. Cannon, K. Moilanen, M. Häkkinen, M. Lappalainen (Helsinki, Turku, Kuopio, FI; La Chaux-de-Fonds, CH; Dublin, IE)*

Objective: We conducted an evaluation study for the Prove-it™ Herpes assay (Mobidiag) to compare results to those of current PCR-based herpes diagnostics. Prove-it™ Herpes identifies seven herpesviruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7) in a single assay in three hours, whereas most current PCR-based commercial or homebrew methods search for only one or few viruses at a time.
Methods: Altogether, 497 cerebrospinal fluid samples were analyzed by both methods in five laboratories in Finland, Ireland, and Switzerland. Furthermore, one of the laboratories analyzed Quality Control for Molecular Diagnostics (QCMD) 2009 proficiency sample panels for VZV, HSV, EBV, CMV, and HHV-6. In Prove-it Herpes standard workflow, extracted DNA was amplified by PCR followed by hybridization and detection by Prove-it™ Advisor software according to manufacturer's instructions. Reference PCR assays were performed as determined by individual laboratories. Discordant results were studied by additional reference PCRs and DNA sequencing.
Results: The results for QCMD samples were excellent with over 90% of the samples identified correctly. In cerebrospinal fluid material, Prove-it™ Herpes revealed multiple additional viral targets, either alone or in combinations, when compared to reference PCR workflows. In 12 confirmed multi-infections combinations such as HSV-2 with HHV-7 were detected. HHV-7 was observed in nine cases together with other herpesviruses and in eight cases alone. Prove-it™ Herpes revealed also individual targets not suspected initially by patient's profile such as HHV-6, EBV and CMV. The final sensitivity and specificity for Prove-it™ Herpes assay were 92% and 98%. These results, especially HHV-7 cases, yield also interesting data for further investigations.
Conclusions: Prove-it™ Herpes was considered as a rapid and robust diagnostic platform that was easily implemented into laboratory workflow. As a qualitative screening assay, Prove-it™ Herpes was deemed especially suitable for diagnostics of cerebrospinal fluid samples. However, the results from other sample types have also been promising. The broad target coverage and small sample volume required by the assay could benefit diagnostics, and especially, the treatment of life-threatening infections of the central nervous system.

P657 Evaluation of a commercial real-time hepatitis B assay in combination with NucliSens easyMAG nucleic acid extraction

K. Bergs, D. Ursi, H. Goossens, M. Ieven (Edegem, BE)*

Introduction: The Abbott RealTime Hepatitis B virus (HBV) assay, is a quantitative assay, to be used with the Abbott m2000sp extraction module. The NucliSens easyMAG (bioMérieux) is an open system for automated isolation of nucleic acids, based upon the BOOM technology.
Objectives: To evaluate the performance of the easyMAG nucleic acid extraction system for routine use with the Abbott RealTime HBV assay.
Materials and Methods: Serum samples were extracted using the easyMAG Specific B protocol with a proteïnase K pre-treatment, with an input volume of 200 µl serum and 3 µl internal control (IC) and an elution volume of 110 µl.
Precision was evaluated by intrarun and interrun testing according to Rabenau et al. (JCV 2007;40:93–98) using the HBV low positive control (HBVLP) and HBV high positive control (HBVHP). Linearity was determined by a 10-fold dilution series of a strong positive sample.
To test accuracy, 31 samples (6 negatives, 25 positives) were sent to another laboratory that used the standard Abbott m2000 extraction and amplification protocol and results were compared.
Results: For all samples the Ct values of the IC were within the specifications of the assay. Mean intrarun variationcoefficient for HBVLP and HBVHP was 1.95% and 0.24% respectively. Mean interrun variationcoefficient was 5.03% and 1.27% respectively. These are within the limits of the manufacturer.
The linearity resulted in a correlation coefficient of 0.999. Concerning the accuracy experiments, the 6 negative samples were also negative after easyMAG extraction. Twenty-four out of 25 positive samples (96%) did not differ more than 0.5 log IU/ml.
Conclusion: The Abbott RealTime HBV assay can be used in combination with easyMAG extraction.

P658 Evaluation of a new commercial PCR-DNA microarray for rapid and simultaneous detection of 9 viruses responsible for central nervous system infections

N. Leveque, A. Van Haecke, F. Renois, L. Andreoletti (Reims, FR)*

Objectives: Viruses are the main etiological cause of central nervous system infections. Rapid testing is recommended in order to improve therapeutic management in case of viral infection. In the present work, we evaluated a new PCR-DNA microarray system allowing the rapid and simultaneous detection of 9 neurotropic viruses: HSV-1 and -2, VZV, EBV, HCMV, HHV-6, -7, -8 and enteroviruses.
Methods: Clart® Entherpex kit (Genomica, Madrid, Spain) is based on viral genome-specific fragments amplification by multiplex PCR and its subsequent detection via hybridization with microorganism-specific binding probe arrays. Evaluation of the kit was first carried out using the 2009 samples of the Quality Control for Molecular Diagnostics (Glasgow, Scotland). Four quality controls for HSV-1 and -2, VZV, EBV, HCMV, HHV-6 and enteroviruses ranging from 102 to 103 copies/ml were independently tested 4 times each in order to assess the sensitivity and the reproducibility of the assay. Moreover, 20 cerebrospinal fluids (CSF) tested positive for enteroviruses and 27 tested positive for an herpesvirus (7 HSV1, 2 HSV2, 8 VZV, 4 EBV, and 6 HHV6) were retrospectively analyzed. The viral load in the CSF was also determined.
Results: Clart® Entherpex kit detected in all cases (4/4) the quality controls >500 copies/ml for HHV6 and >1000 copies/ml for the other Herpesviruses tested. The sensitivity was estimated from 166 copies/ml for HHV6 to 220 copies for the HSV1. For enteroviruses, quality controls corresponding to 280 and 390 copies/ml were never detected during the 4 analyzes performed while a viral load of 1480 copies/ml was detected in 3 times of 4. These results placed Clart® Entherpex kit on the same level of sensitivity that the other commercially available kits evaluated by the QCMD. From the clinical samples, Clart® Entherpex kit detected 26 of the 27 herpesvirus infections of the central nervous system and all the 20 enterovirus positive CSFs selected. Only one infection due to EBV characterized by an undetectable viral load (<500 copies/ml) was not diagnosed.
Conclusion: This new PCR-DNA microarray system is a sensitive and specific test allowing rapid (in one day's work) and simultaneous detection from the CSF of the main RNA (enteroviruses) and DNA (Herpesviridae) neurotropic viruses.

P659 Comparison of two fully automated QIAGEN extractors for a range of viral targets, including swine lineage influenza A H1N1v

A.V. Lee, R.J. Manuel, D.A. Clark (London, UK)*

Objectives: Automated DNA/RNA extractors are becoming increasingly common and 2 such platforms from Qiagen are the QIAsymphony SP and BioRobot MDx. Here we compare these automated platforms for use in routine laboratories.
Methods: A range of sample types were used and the nucleic acid extracts tested on "in-house" PCR assays. Four real time PCR tests were used: quantitative assay for cytomegalovirus (CMV) on 80 positive and negative stored samples; 108 stored positive respiratory samples for an influenza multiplex, including swine lineage sH1 (3 targets) and swine lineage specific sN1 targets; 48 stored positive samples were tested using a herpes simplex virus (HSV) type 1, HSV type 2 and varicella zoster virus (VZV) multiplex.

Results: Overall the two platforms gave similar results for each assay. For CMV viral loads, 66/80 positive and negative stored samples had a mean difference (QIAsymphony – MDx) of 0.083 log10 c/ml (s.d. 0.365) with a correlation of 0.944. For the influenza multiplex and swine specific H1 and N1 assays, 130/157 positive PCR targets showed a mean difference of –0.595 cycles (s.d. 2.563) with a correlation of 0.873. For HSV1/HSV2/VZV, 48/48 samples showed a mean difference of 1.741 cycles (s.d. 3.425) with a correlation of 0.777.
Conclusions: In total, 244/285 (85.6%) samples tested gave concordant results between the QIAsymphony® SP and BioRobot MDx extractors, with 6 previously negative PCR targets being detected as positive by the BioRobot MDx.
Both extractors have a load and walk-away system, full sample and reagent barcode tracking and intuitive software that is easily operated. The QIAsymphony® SP has a continuous loading system that enables 24–96 samples to be loaded compared to the batch-based system of the BioRobot MDx which processes 32–96 samples in each run. Both instruments are present in our laboratory and in routine use, with the advantages of each system being utilised for different requirements. Our comparison shows that both extractors can be used interchangeably which will prove beneficial in outbreak situations.

P660 New SmartFinder assay for the real-time detection of 19 respiratory pathogens

M. Reijans, M. Noben, J. Ossel, G. Simons (Maastricht, NL)*

Objective: Molecular diagnostics play an increasingly important role in the detection of infectious agents. Up to now, multiplex realtime PCR assays are able to detect up to 5 targets in one reaction.
Here we present a new SmartFinder assay which enables the detection of 19 respiratory infectious agents in one realtime PCR reaction.
Method: The SmartFinder assay makes use of MultiFinder probes. In a MultiFinder assay the different probes are identified based upon the specific length of each probe by size-fractionation. The SmartFinder assay uses realtime PCR as detection platform. Fluorescent labelled hybridization probes detect the different MultiFinder probes using a realtime PCR machine. The combination of different fluorescent labels and specific melting temperatures allow the development of highly complex realtime PCR assays. The assay detects 19 pathogens in one reaction thereby differentiating between Influenza A, Influenza B, RSV A, RSV B, Adeno, Rhino, Parainfluenza 1–4, Coronavirus 229E + NL63 + OC43, Human metapneumovirus, BOCA virus, *M. pneumoniae, C. pneumoniae, L. pneumophila,* and *B. pertussis.*
Results: The proof of principle was demonstrated using 70 QCMD samples and selected clinical samples. The results were compared with a standard RespiFinder assay based upon detection using capillary electrophoresis. All 19 different targets were detected. The two assays showed a high degree of correlation. Mixed etiologies were mimicked by combining different targets. Most of the mixed infections could be readily detected.
In addition, a large cohort of more than 80 clinical samples was tested with the SmartFinder assay and RespiFinder. Samples positive for any of the two assays where retested with specific realtime PCR's. The two assays showed a high concordance. Most of the positive samples could be confirmed by realtime PCR.
All analyses were done on a LightCycler 480 machine.
Conclusions: The new SmartFinder assay shows a high concordance with realtime PCR and RespiFinder. Post PCR handling and analysis time in the SmartFinder assay were reduced compared to the RespiFinder generating a faster outcome. In addition, the closed, realtime detection reduces contamination risk. This study proves that the SmartFinder technology shows great potential in fast and easy multiparameter screening of clinical samples for infectious agents.

STI – molecular diagnostics

P661 Comparison between an opa-based real-time PCR and the Cobas Amplicor test for the detection of *N. gonorrhoeae*

M.V. Elzi, M. Gorgievski-Hrisoho (Berne, CH)*

Objectives: Aim of this study was to compare the performance of a real-time PCR targeting a 90-bp region on the *N. gonorrhoeae* opa gene (Tabrizi et al. 2005) with the COBAS AMPLICOR test for detection of *N. gonorrhoeae.*
Methods: 421 clinical samples were analysed using an opa-based PCR (Tib Molbiol, Berlin, Germany) on the COBAS TaqMan Analyzer (Roche Diagnostics, Rotkreuz, Switzerland), and the COBAS AMPLICOR CT/NG test (Roche Diagnostics). DNA extraction was performed using the AMPLICOR CT/NG sample preparation kit (Roche Diagnostics) for swabs, and the NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France) for urine, ascites and synovial fluid. Analysis of COBAS TaqMan data was performed using the Utility Channel Data Analyzer Software (Roche Diagnostics). Positive samples were submitted to a confirmatory laboratory (Institute of Medical Microbiology, Zürich, Switzerland) for further testing (PCR targets: 16S rRNA gene, cppB gene and porA gene).
Results: We analysed 421 clinical samples (161 urines, 120 pharynx, 78 rectal swabs, 57 urogenital swabs, 4 intraabdominal samples, 1 synovial fluid) and 10 samples of QCMD 2009. Overall, *N. gonorrhoeae* was detected in 5/421 (1.2%) clinical samples using the opa-based method, and in 82/421 (19.5%) clinical samples using the COBAS AMPLICOR test. According to the confirmatory laboratory, all positive samples detected with the opa-based method, compared to only 5 of 82 (6.1%) positive samples from the COBAS AMPLICOR test were correct. Agreement between the COBAS AMPLICOR NG test and the opa-based test was noted in 81.7% of clinical samples (339 tests negative and 5 positive using both methods). False positive results were detected in 77 of 82 (93.9%) clinical samples using the COBAS AMPLICOR NG test and in 0 of 5 clinical samples using the opa-based method. We detected no additional positive clinical samples with the opa-based method. However, in contrast to the COBAS AMPLICOR test, the opa-based method allowed us to correctly identify 2 QCMD samples as positive.
Conclusions: The opa-based PCR allowed a significant reduction in the number of false positive results. In comparison to the COBAS AMPLICOR NG test, that is known to cross-react with some non-pathogenic Neisseria species, the opa-based PCR showed an increased specificity without impairment of sensitivity.

COBAS AMPLICOR NG test	*opa*-based PCR		
	Negative	Positive	Total
Negative	339	0	339
Positive	77	5	82
Total	416	5	421

P662 Screening for *Neisseria gonorrhoeae* in urine samples using real-time PCR

C. Berglund, L. Carlsson, M. Bäckman (Täby, Stockholm, SE)*

Objectives: In Sweden, an increase of *Neisseria gonorrhoeae* (NG) cases has been observed during recent years, especially among young women. However, the sexually transmitted disease *Chlamydia*, caused by *Chlamydia trachomatis* (CT), still remains more prevalent. NG is routinely diagnosed by culturing methods whereas CT generally is detected in urine by nucleic acid tests. A screening method for both CT and NG has been requested since sampling for NG is invasive and painful.
Methods: The Abbott RealTime m2000 CT/NG assay was evaluated on ten serological variants of NG as well as on four culture collection

strains that were diluted in urine (approximately 1×10^8, 2.5×10^5, 800 or 2 organisms per 400 µl input). In addition, the QCMD 2008 NG EQA Programme and dilutions of the AmpliTrol CT/GC DNA control (Bio-Rad Laboratories) were tested. All runs were repeated at least twice and in both presence and absence of CT.
Results: All included serological variants could be detected at expected concentrations (1×10^8: mean CT = 19.4 (+++), 2.5×10^5: mean CT = 27.7 (++), 800: mean CT = 35.8 (+), 2: CT > 40 (–). Analysis of the 2008 QCMD panel resulted in a performance of 96.7%. One of the low positive samples had a CT of 40.44 and was regarded as negative in one of three runs.
Conclusion: Abbott RealTime m2000 CT/NG provided rapid, effective and safe testing for NG in our study. The major advantages included: (i) testing on urine which simplifies sampling and subsequently may lead to increasing sample volumes, (ii) Abbott multi-collect Specimen Collection Kit allow long sample storage (14 days) prior to analysis, which is in contrast to culturing methods where NG is extremely sensitive for storage, (iii) a large amount (96) samples could be analysed in a short time allowing negative results to be released within one day. Disadvantages include: lack of bacterial isolates in order to perform epidemiological typing and susceptibility testing, (ii) the method is validated only for genital and urine samples.

P663 Comparison of the Roche prototype Cobas® 4800 CT/NG assay to the Cobas Amplicor CT/NG assay

G. Tyrrell, K. Kalan, G. Yung, J. Fuller (Edmonton, CA)*

Objective: The Medical Microbiology Laboratory at the University of Alberta Hospital routinely assays urine and dry swab specimens using the MagNA Pure instrument for sample preparation and COBAS AMPLICOR CT/NG test for the detection of *Chlamydia trachomatis* (CT) and Neisseria gonorrhoea (NG). The cobas® 4800 is a highly automated system that performs sample preparation; real-time PCR amplification; and simultaneous detection of CT and NG as well as an internal control in a single tube. This study compares the current COBAS AMPLICOR CT and NG test with the new cobas® 4800 system for detection of CT and NG from cervical swabs and urine specimens to evaluate the performance of these two tests in terms of sensitivity, specificity, PPV, and NPV.
Methods: 541 CT and 279 NG banked specimens of urine or dry swabs, were retested with the cobas® 4800 CT/NG test. An aliquot of the frozen specimen (400µl) previously tested by the COBAS AMPLICOR CT/NG test was diluted with 450µl saline solution and mixed with 850µl of the cobas® PCR Media, before being loaded onto the cobas 4800 system. The volume adjustment was to ensure equal amounts of specimen were present in the PCR reactions using two the different assays. Correlation of the CT and NG test results between the two assay systems is presented.
Results: Of the 541 CT specimens assayed, 7 specimens were excluded from the data analysis due to samples precipitating out of the solution or unknown COBAS AMPLICOR results. 536 specimens were assayed for CT. The CT results showed 98.3% concordance (527/536 samples, kappa=0.861) between the two tests. Banked CT-positive samples without COBAS AMPLICOR NG ordered were excluded from the NG analysis. Of the 279 remaining specimens which also had NG results, 9 mixed CT and NG infections were observed with the COBAS AMPLICOR CT/NG test, whereas 17 mixed CT/NG infections were observed with the cobas® 4800 CT/NG test. A concordant rate of 96.8% was observed (270/279, kappa=0.603) for NG results from banked CT positive samples.
Conclusion: The cobas® 4800 CT/NG test and the COBAS AMPLICOR CT/NG assays correlated well in this analysis. The cobas® 4800 system is highly automated and easy to use for high volume testing of CT/NG.

P664 Performance of the Aptima CT and GC assays on the Panther instrument

M.K. Vi, J. Pham, C. Nguyen, B. Vinluan, C. Clark, A. Worlock (San Diego, US)*

Objectives: To evaluate the analytical performance of the Panther Instrument, a new, fully automated molecular diagnostic analyzer under development at Gen-Probe.
Methods: Reproducibility was assessed for the Gen-Probe APTIMA COMBO 2 (AC2), APTIMA CT (ACT), and APTIMA GC (AGC) Assays on the Panther Instrument. Positive reproducibility panels were made using pooled naturally infected positive urine specimens diluted 1:1000 with pooled negative urine specimens. Panels consisted of a *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC) and dual positive panel members, as well as a negative panel member. Each panel member was tested in replicates of 3 on two prototype Panther Instruments over 10 days, giving a total of 60 replicates for each assay. Platform comparison panels were made using individual positive specimens diluted into individual negative specimens. The positive panel member dilutions ranged from 1:100 to 1:1,000,000 for the AC2 and AGC Assays and 1:100 to 1:10,000,000 for the ACT Assay. The total number of positive panel members was 42 for the AC2 Assay (CT), 62 for the AC2 Assay (GC), 51 for the ACT Assay, and 60 for the AGC Assay. The positive panels were run on both a prototype Panther Instrument and Gen-Probe's TIGRIS System in replicates of one, along with between 84 and 92 individual known negative specimens.
Results: Total precision for reproducibility panels as measured by %CV was 4% for CT, 2% for GC and 6% for dual positive samples using the AC2 Assay, 4% for GC positive samples using the AGC Assay, and 11% for CT positive samples using the ACT Assay. The % agreement between the Panther Instrument and TIGRIS System results for the platform comparison panels was 95% (41 of 43) for CT positive panels, 100% (62/62) for GC positive panels and 100% (92/92) for negative panel members using the AC2 Assay. The % agreement was 100% (60/60) for GC positive panels and 100% (84/84) for negative panels using the AGC Assay. The % agreement was 100% (51/51) for CT positive panels and 100% (92/92) for negative panels using the ACT Assay.
Conclusions: The fully automated Panther molecular diagnostic analyzer under development at Gen-Probe provides precise results comparable to the TIGRIS System when running the AC2, ACT and AGC Assays. In addition to superior assay performance, the Panther Instrument offers the optimum throughput, workflow and turn around time that is ideal for the low-to-mid volume molecular testing laboratory.

P665 A multiplexed isothermal amplification assay for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

D. O'Neil, V. Doseeva, T. Forbes, J. Wolff, K. Upton, K. McPartland, D. Loeffert, I. Nazarenko (Gaithersburg, US)*

Objectives: Our objective was to develop a sensitive, highly specific, multiplexed assay for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). We have combined Qiagen's proprietary Hybrid Capture® (HC) technology for sample processing with isothermal helicase dependent amplification (tHDA) and endpoint fluorescence detection to develop a multiplex assay for the detection of CT and NG in clinical samples. This product is currently at a research prototype stage and is not yet commercially available.
Methods: Hybrid Capture sample preparation involved hybridization of sequence-specific oligoribonucleotide probes to target DNA. RNA:DNA hybrids were captured by HC antibodies conjugated to magnetic beads, which can be placed directly into the amplification reaction without prior elution.
Captured target is amplified by tHDA, an isothermal technology which utilizes a thermostable helicase to unwind double-stranded DNA, followed by priming of the single-stranded target and extension by DNA polymerase. This amplification technology eliminates the need for

thermocycling. tHDA products are detected in a closed-tube format by endpoint fluorescence detection with dual-labeled probes.
Our model assay has two CT amplification targets, including the cryptic plasmid and the outer membrane protein (omp) gene. Dual targets ensures against deletion or mutation of the target sequence causing false negative results. NG amplification target is the outer membrane opacity protein (opa), a multi-copy gene.
Results: Using this research prototype test system, we detected as little as two CT elementary bodies, and less than ten NG cells per mL of sample. Targets were detectable in multiplex, and each target was detectable in the presence of an excess (10^5) of the other. All CT serovars A-K, and L1-L3 were amplified and detected at equivalent sensitivity. The method is suitable for the processing of samples in many different media, among them: urine, PreservCyt®, STM and SurePath. The clinical utility of this assay was demonstrated on a limited number of clinical samples.
Conclusions: The combination of sequence-specific sample preparation and isothermal target amplification allows for a multiplex CT/NG assay which delivers high analytical sensitivity and specificity. The combination of short turn-around time (under three hours), isothermal reaction conditions, and closed-tube format make the assay well suited to adaptation for future high-throughput automation.

P666 Evaluation of automated DNA extraction and real-time PCR detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the Abbott m2000 assay

C. Visser, J. de Haan, A. de Cuijper, B. Duim (Amsterdam, NL)*

Objectives: For routine diagnostics of *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Ng) commercial assays have been developed. One is the Abbott m2000 assay that allows DNA extraction from 90 clinical specimens and real-time PCR detection of Ct and Ng. The assay targets the cryptic plasmid of *C. trachomatis* and allows detection of the recently developed deletion variant, and for *N. gonorrhoeae* detects the opaA gen.
Methods: A total of 618 specimens consisting of male and female urines and endocervical swabs collected in M4RT medium, were tested for the presence of Ct and Ng nucleic acids using Abbott RealTime CT/NG reagents on an m2000 platform and the in our lab routinely performed Roche Cobas Amplicor test. Samples positive for Ng were confirmed with a real-time assay targeting the opaA gene. The evaluation assessed the performance characteristics of the Abbott m2000 assay and the ability of the assay to improve laboratory workflow and throughput.
Results: 44 specimens were Ct positive with the Abbott real-time test. Of these samples 3 were negative with the Cobas Amplicor test. For detection of CT the Abbott real-time showed a sensitivity of 100% (44/44) and a specificity of 99% (551/554). 12 specimens were Ng positive with the Abbott real-time test. Without confirmation of the positive Ng specimens detected with the Cobas Amplicor with the opaA real-time assay, the sensitivity and specificity of the Abbott real-time test were 27% (12/44) and 100% (438/438), respectively. After confirmation of the positive Cobas Amplicor samples, the sensitivity of the Abbott real-time improved to 100%.
Conclusions: The Abbott m2000sp demonstrated greater sensitivity than the Cobas Amplicor, and no confirmation of Ng positive specimens is required. Abbott m2000 combines high throughput with minimal labor and supports a procedure that is easily learned. This makes it a suitable system for routine detection of Ct/Ng in endocervical swabs and urine.

P667 Comparison of the new Versant® CT/GC DNA 1.0 Assay (kPCR) and Roche Cobas® TaqMan® (CTM) assay for the detection of *Chlamydia trachomatis* in urine samples

A. Lindauer, K. Beyser, G. Warnat (Weiden, Eschborn, DE)*

Objectives: Performance of the VERSANT® CT/GC DNA 1.0 Assay (kPCR), which can simultaneously detect *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in swab and urine specimens, was evaluated and compared with the Roche COBAS® TaqMan® CT Test (CTM). Secondarily, the stability of urine samples in the presence or absence of the Siemens VERSANT Urine Transport Kit (UTK) was also evaluated.
Methods: 372 samples from preselected CTM positive pool urine samples (pools of 5) were transferred into Siemens VERSANT® Urine Transport Kits (UTK) and tested on the VERSANT® kPCR Molecular System with the VERSANT assay. The Roche COBAS TaqMan assay was used as a comparative method. Specimens with results that were discrepant by the two assays were subjected to repeat testing in replicates of two in both methods. Ten (10) low positive urine samples with late CTM cycle threshold values (Ct 35–40) were also subjected to elevated temperature (37ºC) for up to 96 hours stored in the VERSANT UTK or neat urine and then tested in the VERSANT assay to determine effects on Ct values.
Results: A total of 367 samples were evaluable of which 86 were CT-positive and 281 were CT-negative. After resolution testing, the VERSANT assay detected 85 of 86 of the positive samples and 279 of the 281 negative samples resulting in a sensitivity and specificity of 98.83% and 99.28%, respectively. In comparison, the CTM assay detected 83 of 86 of the positive samples and 276 of 281 of the negative samples resulting in a sensitivity and specificity of 96.51% and 98.22%, respectively. Furthermore, the VERSANT assay detected positive samples 7 to 10 Ct's earlier than the CTM assay in urine samples undergoing stability testing. CT positive urine samples stored in the presence of VERSANT UTK and subjected to temperatures of 37ºC for up to 96 hours were shown to be stable. Also for the native urine samples nearly the same stability could be shown.
Conclusion: These results indicate that the automated VERSANT assay detects more true positive and true negative samples than the CTM assay even when using CTM preselected urine samples from a screening population. The VERSANT UTK, which requires no off-line processing steps, offers the advantage of stable transport and storage for urine samples up to 37ºC. The sensitivity of the VERSANT CT/GC DNA 1.0 Assay combined with automated throughput establishes this assay as a quality diagnostic tool including the detection of urine pool samples.

P668 The role of the microbiology laboratory in the diagnosis of lymphogranuloma venereum illustrated in 4 cases at a university hospital, Basel, Switzerland

D. Goldenberger, M. de Roche, U. Flückiger, A. Blaich, R. Frei (Basel, CH)*

Objectives: In context with the ongoing epidemic of lymphogranuloma venereum (LGV) and its unspecific clinical presentation, there is a need for a fast and reliable diagnosis of LGV. We applied a simple laboratory-based algorithm for detection of *Chlamydia trachomatis* L1, L2, and L3 and performed a retrospective search for LGV cases at our clinics.
Methods: We established a duplex real-time PCR for simultaneous detection of *C. trachomatis* L1, L2, L3 targeting the pmpH gene and L2/L2b targeting the omp1 gene, respectively. For routine LGV diagnosis, we first performed a commercially available *C. trachomatis* PCR (cryptic plasmid, Cobas Amplicor) in anorectal swabs or biopsies. If the PCR was positive, we then routinely tested for the LGV agents. To identify LGV cases retrospectively, we ran a search for *C. trachomatis*-positive, non-urogenital and non-occular specimens in our laboratory information system.
Results: We identified two LGV cases in the year 2008. Patient 1 presented with ulcer and inguinal lymphadenopathy. Patient 2 suffered from severe ulcerative proctitis with delayed clinical diagnosis of LGV. A total of 7 paraffin-embedded tissue specimens of these patients were analyzed. All samples showed *C. trachomatis* L2/L2b-DNA. The two diagnoses were completed with the omp1 genotyping result of *C. trachomatis* L2b and positive serology findings. Applying our laboratory algorithm, we additionally detected 2 cases with *C. trachomatis* L2/L2b from 3 *C. trachomatis*-positive anorectal samples. The 4 patients were between 41 to 50 years old and men who have sex with men, one of whom was HIV-positive.

Conclusion: Our laboratory-based approach is a promising tool to detect LGV in a rapid and reliable way. This is crucial for the clinician to initiate an appropriate antibiotic therapy. We also show that our tests can be applied successfully to formalin-fixed biopsies.

Detection of *C. difficile*

P669 Clinical and laboratory evaluation of a real-time PCR for *Clostridium difficile*

E. de Jong, A. de Jong, C. Bartels, C. van de Rijt, W.J. Melchers, P.D. Sturm (Nijmegen, NL)*

Objective: *C. difficile* associated diarrhea (CDAD) is the major cause of nosocomial diarrhea. A rapid and sensitive diagnostic test is necessary to make the diagnosis. Many laboratories use enzyme immunoassays (EIAs) for rapid diagnosis of CDAD since cell cytotoxicity test(CTT) and toxigenic culture (TC) take 24 hours and more. The performance EIAs vary widely with sensitivity and specificity ranging from 50 to 99% and 70 to 100%, respectively. Few studies have described PCR with sensitivity ranging from 86 to 97% and specificity from 96 to 98%. A real-time PCR detecting *Clostridium difficile* toxin A and B was evaluated prospectively in hospitalized patients with diarrhea and healthy volunteers.
Methods: Stool samples from 150 consecutive hospitalized adult patients were included. PCR was compared to CTT of stool samples, TC, immunochromatography (ICT) (Meridian), and ELISA (Vidas). Clinical data were collected prospectively using a standardized questionnaire. PCR was performed on the stool samples from 75 healthy volunteers.
Results: The median age of the patients was 61 yrs and 49% were male. TC was positive in 17 (11%) patients. Using TC as the gold standard, sensitivity/specificity of PCR, CTT, ICT, and ELISA was 100/99.2, 70/100, 47/99.2, and 58.8/89.4%, respectively.
Patients were categorized as positive CCT/TC/PCR (12); positive TC/PCR only (5); negative TC (133). All patients with positive TC/PCR had clinical diarrhea. No differences were present with respect to current or prior antibiotic use, type of antibiotic, presence of diarrhea defined as >2 loose stools per day for a duration of >48 or >24 hrs, aspect of stool, fever, abdominal pain, tube feeding, proton pump inhibitors, chemotherapy, immunocompetency, admission ward, inflammatory bowel disease, leucocytes, and CRP. The median age of the volunteers was 21 yrs and 29% were male. None of the healthy volunteers were positive on PCR evaluation.
Conclusion: PCR is rapid and sensitive for the detection of *C. difficile*. The sensitivity of CTT was only 70% and cannot be used as gold standard. EIAs had low sensitivity and specificity and were not complementary to other tests. Clinical prediction of CDAD was not possible. Clinical significance of a positive result for TC/PCR only could not be established, however all healthy volunteers had negative PCR results.

P670 Evaluation of real-time PCR tcdC gene detection assay for the diagnosis of *Clostridium difficile* infection

T. Wong, M. Romney, G. Ritchie, S. Pengilly, S. Champagne (Vancouver, CA)*

Objectives: The tcdC gene within the *Clostridium difficile* pathogenicity cassette is a negative regulator for toxins A and B expression. Deletions in the tcdC gene are associated with more virulent strains of *C. difficile* including ribotype 027. In this prospective study, a *C. difficile* GDH antigen enzyme-linked immunoassay (Techlab, Blacksburg, VA), a *C. difficile* Toxin A/B enzyme-linked immunoassay (Techlab, Blacksburg, VA), cell cytotoxicity assay, *C. difficile* culture and an in-house real-time PCR amplifying the tcdC gene were evaluated.
Methods: Faecal samples from hospitalised patients with a query diagnosis of *C. difficile* infection (CDI) were collected for two consecutive months at St. Paul's Hospital, Vancouver, Canada. In total, 430 faecal samples were obtained from 343 patients. Samples were first tested according to the hospital's three step "standard operating protocol" (SOP), which consists of (1) GDH antigen EIA, (2) Toxin A/B EIA and (3) cell cytotoxicity assay. All faecal samples were cultured on *C. difficile* agar and then frozen for further studies. Samples were then tested with an in-house tcdC gene real-time PCR for performance evaluation. Discordant results were resolved by performing an ethanol shock treatment; recovered isolates underwent toxigenic culture assay, which is considered the gold standard for statistical analysis.
Results: There were 329 concordantly-positive, 57 concordantly-negative, and 44 discordant results for a total concordance/discordance rate of 89.8% / 10.2%. After resolution of discordant results, the sensitivity for culture, SOP, and PCR were 87.4%, 85.5%, and 98.8%, respectively. The specificity for culture, SOP, and PCR were 96.8%, 99.1%, and 97.4%. The positive and negative predictive values for culture, SOP, and PCR were 87.4%/96.8%, 95.6%/94.0%, and 90.5%/99.7%. For the 44 discordant results, agreement with the gold standard for culture, SOP and PCR was 50.0%, 43.2%, and 77.3%.
Conclusion: We concluded that this in house real-time PCR for the detection of *C. difficile* tcdC gene is both sensitive and specific in our hospital setting for patients with a query diagnosis of CDI. For resolving discordant results, *C. difficile* tcdC gene real-time PCR had the highest concordance rates when compared to toxigenic culture.

Performance evaluation of *C. difficile* culture, SOP* and tcdC gene PCR

	Sensitivity %	Specificity %	PPV %	NPV %
Culture	87.4	96.8	87.4	96.8
SOP*	85.5	99.1	95.6	94.0
tcdC gene PCR	98.9	97.4	90.5	99.7

*SOP = St. Paul's Hospital three step standard operating protocol involving (1) GDH antigen EIA (2) toxin A/B EIA (3) cytotoxicity assay.

P671 Comparative evaluation of *Clostridium difficile* detection by three distinct real-time PCR techniques

C.W. Knetsch, D. Bakker, R.F. de Boer, S. Hofs, A.M. Kooistra-Smid, K. Eastwood, M. Wilcox, E.J. Kuijper (Leiden, Groningen, NL; Leeds, UK)*

Objectives: Incidence of *Clostridium difficile* infections (CDI) has increased in the past decade. Assays for the rapid diagnosis of CDI are preferable. Previously, we have developed a real-time PCR, which targets the *C. difficile* toxin B gene. The aim of this study was to compare this toxin B real-time PCR with two other real-time PCR techniques that detect the presence of *C. difficile* toxin genes and determine their utility as rapid diagnostic test to diagnose CDI.
Methods: In total, 535 routine diagnostic diarrheal samples were tested prospectively using cytotoxin assay (CYT) and cytotoxigenic culture (CYTGC) and retrospectively using 2 in house developed real-time PCRs (LUMC, LvI) and a commercially available PCR (GeneOhm, Becton and Dickenson). Specimens were stored at 4ºC for 1 week and then frozen at −20ºC. For the in-house developed real-time PCRs, faecal samples were pre-treated with STAR buffer and chloroform to optimize DNA extraction. DNA was extracted from faeces using automated DNA extraction procedures. For each real-time PCR technique sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined by using two gold standards; cytotoxigenic culture and cytotoxicity assay.
Results: Compared with CYTGC, sensitivity, specificity, PPV and NPV values were: for real-time PCR LUMC 96.0%, 88.0%, 66.0% and 98.9%, for real-time PCR LvI 100.0%, 89.4%, 69.7% and 100.0% and for BD GeneOhm 88.3%, 96.9%, 86.5% and 97.4%. Compared with CYT, sensitivity, specificity, PPV and NPV values were: for real-time PCR LUMC 96.7%, 85.9%, 59.2% and 99.2%, for real-time PCR LvI 98.9%, 87.3%, 62.7% and 99.7% and for BD GeneOhm 90.7%, 95.8%, 81.3% and 98.1%.

Conclusion: The three real-time PCRs have similar performances but BD GeneOHM real-time PCR had the best PPV. Due to the high NPVs all three real-time PCRs can be applied as a first screening test for CDI. However, the low PPVs of all PCRs require a second confirmation test.

P672 Comparison of real-time polymerase chain reaction (Cepheid GeneXpert) with enzyme-immunoassay (Meridian Premier A/B) for the laboratory diagnosis of toxigenic *Clostridium difficile*

S. Goldenberg, T. Dieringer, G. French (London, UK; Rochester, US)*

There is increasing concern over the performance of Enzyme Immunoassay (EIA) tests for the laboratory diagnosis of *Clostridium difficile* Infection (CDI). The optimal method for diagnosis is yet to be defined and may vary from centre to centre, however there is a move towards the use of newer Polymerase Chain Reaction (PCR) based tests.

We compared the Cepheid Genxpert PCR test (which detects Toxin B gene and is able to give a presumptive identification of ribotype 027) with the Meridian Premier EIA for Toxin B in 224 consecutive diarrhoeal stool samples. Results were validated against a gold standard of toxigenic culture (TGC).

57 specimens were TGC positive. This 25.4% positivity rate (created by stratification) was much higher than our institution's background rate and we therefore used a modified method for calculating performance characteristics in order not to distort figures for sensitivity and specificity. Overall agreement between EIA and TGC was 92% (206/224) and between PCR and TGC was 97.3% (218/224). The EIA had a sensitivity of 65.7%, specificity of 98.0%, positive predictive value (PPV) of 84.2% and negative predictive value (NPV) of 94.6% compared with TGC. The PCR had a sensitivity of 100%, specificity of 96.7%, PPV of 90.5% and NPV of 100% compared with TGC. (See table.)

6 specimens gave a PCR positive result but were EIA negative and failed to yield *C. difficile*. These may have been false-positives but it is possible that toxigenic *C. difficile* was present in the sample but in insufficient quantities or condition to be detected by other methods. (The number of viable bacteria within the sample may have reduced during transportation or storage).

The PCR assay identified 10 samples (17.5% of the samples that yielded toxigenic *C. difficile*) as ribotype 027. 9 of these were indistinguishable from ribotype 027 by PCR-ribotyping, however one was a different ribotype.

In our hands the Cepheid GeneXpert had a significantly improved sensitivity and specificity compared with the Meridan Premier EIA for detection of toxigenic *C. difficile* in diarrhoeal stool samples.

It is clear that suboptimal diagnostic *C. difficile* assays undermine the effectiveness of control measures and the usefulness of surveillance systems.

Further research is warranted to determine the most accurate method of diagnosis possibly including a PCR test as part of multistep procedure.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Meridian Premier A/B EIA	65.7	98	84.2	94.6
Cepheid GeneXpert RT-PCR	100	96.7	90.5	100

P673 Performance comparison of Xpert *C. difficile*, ImmunCard enzyme immunoassay, and culture for the detection of *C. difficile* in stool

P. Legrand, Z. Ould Hocine, N. Launay, F. Maillebuau, J.M. Le Glaunec, L. Deforges, N. Finnström, M. Marmiesse (Creteil, Maurens-Scopont, FR)*

Objective: Various tests can detect the presence of *C. difficile* and/or its toxins in feces to establish the diagnosis in diarrhea following the use of antibiotics. Many laboratories routinely use a rapid enzyme immunoassay (EIA). Recently the Xpert *C. difficile* test was developed. It runs on the automated real-time PCR instrument GeneXpert (Cepheid). The assay is very simple and easy to use and has proven to have an excellent sensitivity (at least as good as the cytotoxicity assay). It allows the detection of the toxin B gene, binary toxin gene and the nt117-deletion in the tcdC gene.

We wanted to evaluate the added value provided by the diagnostic test Xpert *C. difficile* compared to the EIA ImmunoCard Toxins A & B (IC) (Meridian Bioscience) in routine laboratory work. A CCFA culture (bioMérieux) was also performed and the toxigenic nature of the strain was determined by enzyme immunoassay.

Methods: 172 post-antibiotic diarrhoeal stool samples were tested consecutively over a period of four months by Xpert, IC and culture according to the manufacturers' instructions.

Results: For 34 stool samples (19.8%), at least one of the techniques detected a toxigenic *C. difficile*. No other enteropathogenic organisms were detected. The detection of *C. difficile* was made in 19.2% of the stool samples (33/172) by Xpert, 15.7% (27/172) by IC and 16.9% (29/172) by culture.

Seven stool samples were positive by Xpert and negative by IC (diagnostic gain of 20.6%). Three of them had a positive culture for toxigenic *C. difficile* Strain. Only one positive stool sample by culture and IC was initially interpreted by Xpert as negative, but was positive after a second test performed after the stool had been homogenized. Toxigenic *C. difficile* strain could not be isolated from any stool samples determined negative by Xpert.

For one sample Xpert detected the binary toxin and the tcdC deletion, characteristic of the PCR-ribotype O27. This was confirmed by PCR-ribotyping of the strain.

Conclusion: The Xpert *C. difficile* test is much more sensitive than the IC test. It is easy to use, gives a result in less than an hour and can detect the ribotype O27. It may therefore contribute to a better diagnosis of *C. difficile* infections and hence limit the spread.

P674 Molecular analysis of toxigenic *Clostridium difficile* isolates from patients with nosocomial diarrhoea

M. Salari (Tehran, IR)*

Objective:*Clostridium difficile* is an identified cause of antibiotic-associated diarrhea, antibiotic-associated colitis, pseudomembranous colitis and nosocomial diarrhea. the objectiveof this survey was to determine molecular analysis of toxigenic *C. difficile* solates from patients with nosocomial diarrhea in Tehran tertiary medical centers.

Methods: In this study, 1822 specimans of patients with nosocomial diarrhea that were hospitalized in Emam khomeini hospital, Shariati hospital and Children medical center and also100 hospital environmantal specimens were collected. The specimens were cultured on a selective cycloserine cefoxitin fructose agar (CCFA) and incubated in anaerobic conditions, at 37ºC for 2 days. *C. difficile* Isolates were characterized by conventional biochemical tests. Bacterial cytotoxicity was assayed on tissue culture and also all strains were typed by PCR-ribotyping method.

Results: Of the total specimens of patients and hospital enviroment, 124 and 17 toxigenic *C. difficile* were isolated, respectively. Results of statistical analysis show no significant differences between the rate of isolated toxigenic *C. difficile* from patients, sex and age group of patients ($P > 0.05$). A total of 28 different ribotypes was detected among the clinical isolates by PCR ribotyping method. The predominant ribotypes of isolated toxigenic *C. difficile* were 13, 14, and 15. Ribotypes 13–17 were five distinct clones that were circulating in all three hospitals.

Conclusion: Findings of this study show that the toxigenic *C. difficile* isolates in Iran have different PCR-ribotyping patterns. Therefore, further studies to evaluate PCR-typing are suggested.

P675 A comparison of the dominant strains of *Clostridium difficile* in Scotland: growth rate, toxin production and sporulation

P. Vohra, I.R. Poxton (Edinburgh, UK)*

Objectives: To determine the differences in potential virulence of *Clostridium difficile* ribotypes most prevalent in Scotland (106, 001 and 027) using phenotypic traits and genomic analysis.
Methods: The growth of *C. difficile* ribotypes 106, 001 and 027 was observed along with 2 reference strains, 630 and VPI10463, over a 24 h period by measurement of OD600 every 4 h. Corresponding toxin production was studied by ELISA using the *C. difficile* TOX A/B II™ kit (Techlab) and associated spore production was determined by viable counts for the same time points. The expression of the genes coding for the *C. difficile* toxins, tcdA and tcdB, and the initiator of sporulation, spo0A, was analysed by Real-Time RT-PCR.
Results: The growth of all ribotypes was similar, but 106 and 001 appeared to have accelerated growth at 4 h compared to 027. Toxin production was markedly different between the strains. By the stationary phase, 027 produced at least 10-fold more total toxin (A and B) than the others. 106 produced the most spores during the stationary phase, followed by 027 and 001. Transcriptional analysis revealed that tcdA expression was the highest in 027, almost double that observed in 106 and 001, but that of tcdB was markedly higher in 106. spo0A transcription was the highest in 001, followed by 027 and 106.
Conclusions: Ribotypes 106, 001 and 027 are currently the most prevalent in Scotland. The growth of these strains demonstrates that greater cell numbers do not contribute to greater virulence. However, higher spore production, as seen in 106, 001 and 027, is significant in terms of spread in the environment. Large amounts of toxin production, however, can lead to greater virulence, as seen in 027 and even in 106 and 001 that do not produce as much toxin as 027, but produce significantly more toxin than reference strain 630. It is of interest to note that in 027, tcdA transcription was significantly higher than that of tcdB. This was also observed in 001 and 106, though not as prominently. This suggests that even though toxin B is essential for virulence, production of larger amounts of toxin A may increase virulence. spo0A transcription was also higher in the epidemic strains and this is also a good indicator of differences between the strains. The epidemic ribotypes 106, 001 and 027, therefore, appear to have increased potential to spread in the environment and cause more severe disease.

P676 Quantitative real-time PCR measurement of the impact of fidaxomicin or vancomycin treatment of *Clostridium difficile* infection on the intestinal microbiome, compared with normal controls

T. Louie, K. Cannon, M.St. Denis, B. Byrne, L. Ward (Calgary, CA)*

Objective: The extent of impairment of the normal intestinal microbiome in patients with *Clostridium difficile* infection (CDI) is not well described. Selective bacterial killing of *C. difficile* (Cd) with sparing of the normal flora is hypothesized to produce better treatment outcomes. Persistence and regeneration of the normal flora is thought to prevent recurrence of disease. qPCR was performed to measure shifts in major component organisms in fecal samples obtained during the conduct of a phase 3 clinical trial which showed that fidaxomicin was equally as effective as vancomycin for disease resolution, but recurrence of CDI was reduced by ~50%.
Methods: Treatment naïve patients (n = 20) with first episode or first recurrence of CDI were randomized to receive fidaxomicin (Fdx) 200 mg BID or vancomycin (Van) 125 mg QID p.o. for 10 days. Fecal samples (>5 g) were retrieved at study entry and days 10, 14, 21 and 28. Patients did not receive other antimicrobials. Bacterial DNA was extracted from 2–300 mg samples with a QIAamp DNA Mini Kit. *Bacteroides* /*Prevotella* (Bact), *Clostridium coccoides* (Cc), *C. leptum* (Clep), *E. coli*, *Lactobacillus* and *Enterococcus* group specific primers were used to examine 10–20 ng of gDNA using the BioRad iQ5 / SYBR green master mix RT-PCR method with melting point assessment. The accuracy of qPCR quantitation was determined by testing normal stool controls (n = 10), spiked known quantitative samples and quantitative cultures for Cd, *B. fragilis* group, and coliforms.
Results: In normal stool controls, CFU/gm (SD) of Bact, Cc and Clep were 10.2(0.6), 9.0(0.5), and 7.5 (1.2), respectively, 2–3 log10 higher than counts at diagnosis of CDI. Fdx and Van were equally effective in reducing *C. difficile* counts from 7 to $<2\log_{10}$ CFU/g during treatment. In CDI patients, Fdx allowed the major components to persist, whereas Van had an enduring suppression of Bact, and temporary suppression of both Cc and Clep. Coliform and enterococcal counts were increased $1–2\log_{10}$/g compared to controls; vancomycin temporarily suppressed enterococcal counts.
Conclusion: Compared to normal controls, CDI patients had a 100 to 1000 fold reduction in microbiome counts at CDI diagnosis. Vancomycin suppressed *Bacteroides*/prevotella group organisms throughout the 4 week observation, and also affected *C. coccoides* and *C. leptum* group bacteria. The relative preservation of components of the normal microbiome during and after Fdx treatment may account for reduced recurrences.

Log_{10} CFU/gm ± SD by qPCR, N = 10/group

	Day 0	Day 10	Day 14	Day 21	Day 28
Bact/prev					
Van	8.0±2.5	5.3±0.9**	5.8±1.8**	7.0± 2.3*	6.8± 2.8[a]
Fdx	8.3±1.9	9.3±1.3	9.4±1.2	9.3±1.4	9.2±1.3
C. coccoides					
Van	7.8±1.7	4.7±0.7**	6.7±2.2*	8.5±0.8	8.6±1.1
Fdx	8.2±0.7	8.4±0.5	8.6±0.5	8.7±0.6	8.7±0.4
C. leptum					
Van	5.1±2.2	2.1±1.2*	3.7±1.6	5.9±1.0	6.2±1.1
Fdx	5.2±2.8	4.9±2.4	4.7±2.3	5.7±2.2	5.9±1.3

**$p \leqslant 0.0001$, *$p \leqslant 0.03$, [a]$p = 0.04$ compared to Fdx.

P677 Development of unique one-step lateral flow test for the detection of *Clostridium difficile* toxin A and B in faeces

S. Rameil, D. Burghardt, J. Winkle, P. Schubert (Darmstadt, DE)*

Objectives: *Clostridium difficile* (C. diff.) is the predominant cause of antibiotic-associated colitis and the leading cause of diarrhoea at hospitalised patients. The toxicity of C. diff. is caused by its toxin A and B. As early and rapid diagnosis is associated with better prognosis, rapid, sensitive and easy to handle test systems are highly desirable. Considering these demands we choose the Lateral flow (LF) test format as it is highly convenient for the operator. Furthermore it reduces the liquid handling steps, sample preparation steps and hands-on time to a minimum. Results of a comparative study performed with 181 clinical stool samples tested by four commercially available ELISAs and PCR as gold standard demonstrate an excellent performance and high quality of the C. diff.-LF prototype. Sencondly we analysed the analytical sensitivity of the C. diff.-LF prototype for both Toxins A and B of C. diff..
Methods: LF-Assay: Test procedure of the C. diff.-LF prototype was as follows:
1. mix stool with mobile solvent (ready to use) thoroughly (1:20)
2. 5 min sedimentation of prepared stool suspension at room temperature
3. transfer 150 µL supernatant (of 2.) on the application area of the LF strip and read result after 15 min

ELISAs: ProSpecT® *C. difficile* Toxin A/B Microplate Assay (Oxoid), Premier™ Toxins A and B (Meridian), Serazym® *Clostridium difficile* Toxin A+B (Seramun) and C. DIFFICILE TOX A/B II™ (Inverness).
PCR: Fast *Clostridium difficile* A/B Realtime (IFP).
All commercially available assays were used according manufacturers instruction.
Samples: 181 (116 PCR negative and 65 PCR positive) stool specimens of hospitalised diarrhoea patients were tested. Samples were stored at –20ºC until analysed.

The pure C. diff. Toxins A and B were obtained from tgcBIOMICS GmbH (Mainz, Germany).
Results: See the tables.
Conclusion: Sensitivity and specificity of the LF-test (60%; 97%) is comparable to the results of the Oxoid ELISA (62%; 100%) which was the best of the used immunoassays. This is especially remarkable as the LF-test takes only 20 min until result. It does not require any washing step and needs only a minimum of liquid handling steps. Furthermore the LF test demonstrated a great analytical sensitivity for Toxin A (0.2 ng/mL) and Toxin B (0.1 ng/mL) in stool matrix without particular sample preparation.

Table 1: Sensitivity and specificity of four ELISAs and LF-test (PCR as standard)

Test	Sensitivity (%)	Specificity (%)
ELISA, Oxoid	62	95
LF-test, R-Biopharm	60	96
ELISA, Seramun	46	92
ELISA, Inverness	46	100
ELISA, Meridian	42	97

Table 2: Analytical sensitivity of the LF-test for *C. diff.* Toxin A and B in stool matrix

$ng \cdot mL^{-1}$	0	0.05	0.1	0.2	0.4	0.8
Toxin A	–	–	–	+	+	+
Toxin B	–	–	+	+	+	+

P678 Evaluation of a chromogenic culture medium for detection of *Clostridium difficile*

J.D. Perry, K. Asir, D. Halimi, S. Orenga, R. Carlton, J. Evans, F.K. Gould (Newcastle upon Tyne, UK; La Balme-les-Grottes, FR)*

Objectives: To evaluate a novel prototype chromogenic medium for detection of *Clostridium difficile* from stool samples from patients with hospital acquired diarrhoea.
Methods: A prototype chromogenic medium for *C. difficile* (chromID™ *C. difficile* prototype = IDCd) was evaluated with 268 diarrhoeal samples positive for *C. difficile* toxin by VIDAS immunoassay and 100 diarrhoeal samples negative for *C. difficile* toxin by VIDAS immunoassay. 10 microlitres of stool suspension was inoculated onto IDCd and a commercially available selective medium (CLO medium; bioMérieux). All media were incubated anaerobically at 37ºC for a full 24 h period and then removed into air for interpretation and selection of colonies for testing. After a maximum of 30 minutes in air, culture plates were re-incubated anaerobically for a further 24 h and re-examined. Alcohol treatment was avoided to assess the selectivity of the media. All suspect strains of *C. difficile* were identified using standard biochemical tests and were further confirmed by ribotyping.
Results: *C. difficile* was recovered from 230 'toxin-positive' stool specimens (86%) using a combination of both media and all isolates were recovered on IDCd after 24 h incubation. 97% of isolates generated black colonies after 24 h incubation and only 1% remained colourless after 48 h incubation. Occasional strains of *Clostridium* clostridioforme and *Bacteroides* spp. also formed black colonies. On CLO medium, only 59% of isolates could be recovered after 24 h incubation and after 48 h incubation, 24% of isolates remained uncultured. From 100 'toxin-negative' samples, six isolates of *C. difficile* were recovered on IDCd compared with only two on CLO medium.
Conclusions: There is increasing interest in the routine culture of *C. difficile* either as part of a diagnostic algorithm or to obtain isolates for epidemiological typing. Chromogenic media have proven efficacy for the enhanced recovery of clinical pathogens such as methicillin-resistant *S. aureus*. The chromID™ *C. difficile* prototype medium is the first chromogenic medium described for isolation of *C. difficile* and shows good potential for detection of this increasingly important pathogen within only 24 hours.

P679 ChromID™ *C. difficile*, the first chromogenic medium for detection and direct identification of *Clostridium difficile*. Comparison with three commercially available selective media

D. Halimi, M.P. Bourguignon, V. Sauvonnet, J.D. Perry (La Balme Les Grottes, Newcastle, FR)*

Objective: To evaluate the performances of a novel chromogenic medium for detection and direct identification of *C. difficile* in comparison with 3 commercially available selective media. On the new medium, the strains of *C. difficile* produce grey to black colonies.
Methods: chromID™ *C. difficile* prototype medium was compared to CDSA (BD), CDA (Oxoid) and CLO (bioMérieux). A total of 96 strains (47 *C. difficile* and 49 non-*C. difficile* strains) were inoculated directly onto the media which were incubated in anaerobic conditions. After 24H the plates were examined and were re-incubated anaerobically for a further 24H and re-examined.
Results: After 24 h of incubation, sensitivity of detection of *C. difficile* (growth and colour or growth and characteristic morphology) was higher with the prototype medium (94% of strains generated black colonies) than with CLO medium (85%), CDSA (13%) and CDA (45%). After 48 h, the sensitivity increased to 98% on the prototype medium and respectively to 96%, 38% and 6% (loss of the characteristic aspect) for the other media. After respectively 24 and 48 h of incubation, specificity was 92 and 92% with the prototype medium, 96 and 96% with CDSA, 86 and 78% with CLO, and 61 and 41% with CDA.
Conclusion: chromID™ *C. difficile* prototype medium is a chromogenic medium that is accurate and easy to use for the detection of *C. difficile*. The high specificity and sensitivity of this prototype medium, after only 24 h of incubation, make it the medium of choice for the direct identification of *C. difficile*.

P680 A two-step algorithm for the diagnosis of *Clostridium difficile* infection: screening with a rapid immunoassay for the detection of glutamatedehydrogenase and toxins A and B followed by a real-time PCR for *C. difficile*

J. Van Broeck, C. Hubert, M. Vast, M. Delmée (Brussels, BE)*

Introduction: The Techlab ® C. diff Quik Chek Complete™ (Techlab Blacksburg, VA, USA) is a rapid immunoassay for the detection of Glutamatedehydrogenase and toxins A and B in fecal specimens, using specific antibodies. The BD Gene-Ohm™ Cdiff (BD Diagnostics, San Diego, CA, USA) is a real-time PCR assay targeting the toxin B gene (TcdB) of *Clostridium difficile* with a fluorogenic target-specific hybridization probe for the detection of the amplified DNA. With toxigenic culture (TC) as gold standard, we evaluated the performances of the combination of these tests for the diagnosis of *C. difficile* infections (CDI) on stool specimens.
Materials and Methods: Stools were from inpatients of the St Luc-UCL University hospital (890 beds), older than 2 years and suffering from diarrhoea. Cell cytotoxicity (CTA) was performed on MRC5 cells. Cultures were performed on CCFA. In case of positive culture and negative CTA, colonies were tested for '*in vitro*' toxin production (TC). Some cultures were repeated on CCFA with added taurocholate (TCCFA). Real-time PCR and the rapid immunoassay were performed according to the manufacturer's instructions.
Results: A total of 607 stool specimens collected in 2008 were tested. Eighty four samples were shown to contain toxigenic *C. difficile* by CTA and/or toxigenic culture (prevalence: 13.9%). The sensitivity, specificity, PPV and NPV of BD Gene-Ohm Cdiff™ were respectively: 91.7%, 96.5%, 81.1% and 98.5%. Those of CTA were 69.0%, 100%, 100% and 95.3%. Unresolved result was recorded in 2 instances. PCR tests were repeated, the two results remained unresolved and were excluded from our calculation. Screening with C. diff Quik Chek Complete™ followed by the BD Gene-Ohm Cdiff™ on GDH positive, toxins A

and B negative samples gave sensitivity, specificity, PPV and NPV of respectively: 91.5%, 99.0%, 93.8% and 98.7%.
Conclusion: The combination of theTechlab ® C. diff Quik Chek Complete™ and the BD Gene-Ohm Cdiff™ demonstrated a very good sensitivity much superior to that of CTA and a good specificity. It is a rapid method allowing result in less than two hours.

P681 Validation of Copan faecal swab for the detection of *Clostridium difficile* from faecal specimens with multiple diagnostic assays

S. Castriciano, J. Kafka, J. Padman, C. Lee (Hamilton, CA)*

Objective: *Clostridium difficile* (CD) infection is the leading cause of nosocomial antibiotic-associated diarrhoea in adult, and paediatric patients. There are a number of commercially available tests to detect the CD toxin A and/or B and the common antigen.
A collection device with a liquid base transport medium compatible with different laboratory methods for the collection and storage of faecal specimen would be beneficial. This study was conducted to determine the compatibility of the Fecal Swab kit (FS) (Copan Italia) with tests, which detect CD toxins A/B and the common antigen, CD glutamate dehydrogenase.
Methods: 35 frozen faecal specimens, which tested positive previously for toxin A/B by CD TOX A/B® II EIA, were used to validate the Fecal Swab (FS) kit, which consists of a flocked swab for the collection of the faecal sample and a 2 ml tube of semi-solid Cary Blair for transportation. The swab was tested to determine the amount of faecal material collected, and to ensure that the concentration of faecal material required in each testing method was not affected by the dilution factor introduced by the medium. The flocked swab of the FS kit was used to prepare the assimilated clinical specimens. The kits used in this validation were: C. DIFF QUIK CHECK®, C. DIFF TOX A/B II®, TOX A/B QUIK CHECK™, C. DIFF QUIK CHEK COMPLETE™, CD Toxin/Antitoxin test (TechLab), ImmunoCard CD Toxin A&B (Meridian), ProGastro™ Cd (Prodesse, Inc.), and an in-house CD real-time PCR. Testing of commercial kits was done according to the manufacturers' specifications. A negative FS kit was tested with each kit to check for interference. For the in-house real-time PCR, nucleic acid was extracted from 140 ul of FS sample to a 55µl elution buffer with the easyMAG (bioMérieux); 2 ul of purified nucleic acid was tested by real time PCR on the RotorGene 6000 (Corbett).
Results: The flocked swab collects ~150 mg of faecal material and no interference was found when uninoculated FS kits were tested with all methods. Out of 35 samples 33 were positive and 2 were negative with all methods including the two PCR methods. 2 samples which tested positive originally with the CD Tox A/B® II when retested were negative for the toxin.
Conclusions: Copan Fecal Swab kit, a Liquid Based Microbiology pre-analytic device, can be used to collect faecal specimens for the detection of CD toxin A/B, CD glutamate dehydrogenase, toxin A, B, cytotoxin in cell culture and nucleic acid by PCR.

P682 Comparison of a *Clostridium difficile* antigen detection test with toxigenic culture by PCR for the laboratory diagnosis of community-acquired *C. difficile* infections

K. Tan, C. Pienaar, M. Kelly (Vancouver, Burnaby, CA)*

Objective: Fifty percent of *Clostridium difficile* (CD) infections (CDI) are community acquired (CA CDI), and accurate laboratory diagnosis of CA CDI is essential. Laboratory diagnosis is based on detection of CD toxins in stool specimens and/or by toxigenic culture. CD toxins may be detected by cell culture cytotoxin assay or by enzyme immunoassay (EIA); however, both tests are less sensitive than toxigenic culture. Due to the high proportion of CD-negative specimens, screening for the CD specific antigen, glutamate dehydrogenase (GDH) before proceeding with toxin testing may provide a rapid and cost-effective approach to the laboratory diagnosis of CDI. In this study we evaluated a commercially available antigen-EIA test (C. DIFF CHEK, TechLab) compared to toxigenic culture by PCR as the gold standard.
Methods: The evaluation was performed over a 21-day period at a large community laboratory. To insure an adequate number of positive specimens in the evaluation, all stool specimens submitted for CD testing were first assessed by an automated toxin-EIA (VIDAS *Clostridium difficile* A & B, Biomerieux). 50 consecutive toxin EIA positive specimens and 150 consecutive toxin EIA negative specimens were tested by toxigenic culture and the antigen EIA test. The antigen-EIA test was performed according to manufacturer's recommendations. Toxigenic culture was performed by inoculating the specimens on selective CD agar, followed by a validated in-house PCR assay for confirmatory identification of CD and the presence of toxin A and B genes.
Results: Of the 200 specimens included in the study, 55 were positive and 145 were negative by toxigenic culture. Compared to toxigenic culture, the antigen EIA test demonstrated 89% sensitivity, 94% specificity, 79% positive predictive value (PPV) and 97% negative predictive value (NPV). Results of the antigen EIA test could be reported the same day that the test was performed, and the test was not technically demanding.
Conclusions: With sensitivity of 89% and NPV of 97% compared to the toxigenic culture gold standard, the CD antigen detection test may provide an acceptable screening test for the laboratory diagnosis of CDI. However, the low specificity and PPV of the CD antigen detection test indicate that confirmatory toxin testing of antigen positive specimens is required.

P683 Evaluation of an ELFA assay for direct detection of toxins from *Clostridium difficile* colonies

V. Lalande, B. Burghoffer, C. Eckert, F. Barbut, J.C. Petit (Paris, FR)*

Toxigenic culture is considered to be a very sensitive method for the diagnosis of *Clostridium difficile* infections. However, this technique is labor-intensive when using the cytotoxicity assay with a filtrate of a five-day broth culture.
Objective: To evaluate an ELFA assay, originally designed for toxins A and B detection in stools (VIDAS® CD A&B; bioMérieux, France), for toxin detection directly from colonies.
Methods: In the first part of the study, we used a collection of 18 toxigenic strains previously characterized by toxinotyping (14 A+B+ strains, 2 A–B– strains, and 2 A–B+ strains). These strains were subcultured on three selective media: taurocholate cycloserine cefoxitin agar routinely used by the site (TCCA), CLO® (bioMérieux) and chromID (bioMérieux), a new chromogenic medium under development. Colonies were tested on the VIDAS system according to the manufacturer's recommendations.
In the second part of the study, we collected 25 positive prospective fresh stools from hospitalized patients. Stools were selected on the positivity of the cytotoxicity assay or toxigenic culture.
The stools were then subcultured on the three media (TCCA, CLO and chrom ID) and toxins were detected on the VIDAS directly from an inoculum obtained with 5 colonies (3 McFarland) taken from each medium.
Results: Among the 16 toxigenic A+B+ and A–B+ strains no negative results were obtained. For all media, equivocal results were found independently of the toxinotype. Based on the cut off used for the stools, the sensitivity on the VIDAS is 86.6% with CLO and chromID and 80% with TCCA culture.
In the second part of the study using stool specimens, VIDAS testing performed on colonies showed concordant results in 48% of cases for the three media. The positivity rate for toxin detection was respectively 60%, 65% and 82.6% for TCCA, CLO and chromID. Equivocal results were founded respectively for 30%, 15% and 4% for TCCA, CLO and chromID. For 3 stool specimens, CLO medium did not enable growth of *C. difficile*.
Conclusion: The TCCA and chromID media showed very good performance for the growth of *C. difficile*. The VIDAS CD A&B assay can be used to detect toxins directly from colonies isolated on the 3

media tested. Further analysis are needed to establish the level of cut off for toxin detection on colonies.

P684 *Clostridium difficile* associated diarrhea uncertainties in testing algorithms and the role of Toxin gene detection

G. Smith, H. Reddy, P. Dexter, N. Clarke, C. Tremlett, A. Johnson, V. Sivaprakasam (Hereford, Norwich, UK)*

Objectives: The Department of health of UK launched mandatory surveillance for clostridium difficile associated diarrhea (CDAD) in January 2004, in response to notable increase in morbidity and mortality linked to CDAD becoming more apparent since 1999. This resulted in an increasing array of assays for the laboratory diagnosis of CDAD. Against this background we set to define an optimum testing algorithm for the laboratory diagnosis of CDAD with the use of clostridium difficile gene detection system (PCR assay) by GeneXpert (Cepheid), with the single objective to achieve zero tolerance to hospital acquired CDAD.
Methods: 261 Stool samples from patients with loose stools attending Hereford hospitals NHS trust in UK, between January and August 2009 were tested for the presence of *Clostridium difficile* toxin by VIDAS assay (Biomerieux) and cell culture cytotoxin assay (CTA) and the clostridium difficile toxin gene by PCR assay.
Results: With reference to CTA as the gold standard the sensitivity and specificity of the VIDAS assay was 83.92% and 75.72 and that of PCR assay was 100% and 77.66% respectively. The negative predictive value and positive predictive value of VIDAS was 94.54% and 48.45% and that of PCR was 100% and 54% respectively. In 37 samples VIDAS and PCR were coherently positive where CTA was negative.
Conclusion: The superiority of the GeneXpert gene detection assay over VIDAS in terms of efficacy though significant is not exceptional. In terms of efficiency (user comfort, turn around time and no equivocal results) we feel the PCR assay is far more superior. The ability to identify the ribotype 027 strain at the same time is beneficial to prevent harmful cohorting. The CTA performance disappointed, given the coherence of VIDAS and PCR for 37 "false-positive". Since our patient population had a high pre-test probability of CDAD, we feel that the PCR assay has outperformed the CTA and we wonder whether it is time to crown the PCR assay as the gold standard. Future similar studies could reaffirm our suggestion using toxinogenic culture as a comparator instead of CTA alone.

Plasmids, integrons, transposons associated with resistance genes

P685 Biological fitness effects of integrons in the absence of antibiotic-selective pressure

S.S. Domingues, P. Vieira, I. Starikova, G.J. da Silva, K.M. Nielsen (Coimbra, Aveiro, PT; Tromsø, NO)*

Objective: The biological cost of antibiotic resistance is the key parameter determining the emergence, spread and stability of resistant bacteria. The aim of this study was to assess the *in vitro* fitness cost conferred by class 1 integrons in *Acinetobacter*.
Methods: *Acinetobacter baylyi* BD413 was used as recipient bacterium in natural transformation assays with integron-carrying donor DNA. Acquisition of classical class 1 integrons was confirmed by PCR and the antimicrobial susceptibility of transformants was evaluated by E-test. The fitness of integron-carrying transformants was estimated by pairwise competition experiments with the recipient bacterium in a minimal medium during 24 h. Four competition replicates were done for each transformant. The relative fitness (W) was calculated as the ratio of the Malthusian parameter of each competitor. The resistance genes confer a disadvantage if $W < 1$ and an advantage when $W < 1$.
Results: Four distinct integrons that carried the following resistance genes were evaluated: blaOXA-30+aadA1 (transformants 2.26.1, 11.1.1, I2.1.1); dfrA12+aadA2 (transformant I3.1.1); aacA4+blaPSE+aadA2 (transformant I8.1.1); aadB (transformants 4.10.1, 10.1.1). Accordingly to the CLSI guidelines, 11.1.1 and I2.1.1 were susceptible (S), 2.26.1 was intermediate and I8.1.1 was resistant (R) to ampicillin; I8.1.1 was S and 4.10.1 and 10.1.1 were R to gentamicin; 4.10.1 and 10.1.1 were R to kanamycin; 2.26.1, 11.1.1, I2.1.1 and I3.1.1 were S and I8.1.1 was R to spectinomycin; all transformants were R to sulfamethoxazole. The mean W of integron-carrying strains ranged from 0.99 to 1.17. However, only one of the mean fitness changes was statistically significant. More replicates are necessary to establish any (if) minor but significant changes.
Conclusion: Intervention policies aimed at combating antibiotic resistance often rely on the assumption that resistance genes are costly and will be lost from pathogenic bacterial populations when antibiotic pressure is removed. Although our results are only statistically significant for 1 transformant, they clearly point out that the acquired integrons and resulting changes in resistance profiles did not lead to major relative fitness reductions in *Acinetobacter*. These results might help to explain integron's wide dissemination and persistence. To our knowledge, this is the first time that a fitness study of integrons is conducted in transformants acquired by natural transformation.

P686 Plasmid addiction systems targeting DNA gyrase: drivers of fluoroquinolone resistance?

M. Doumith, M.J. Ellington, P. Hawkey, N. Woodford (London, Birmingham, UK)*

Objectives: Plasmid addiction toxin-antitoxin (TA) systems enforce plasmid retention at cell division. Like fluoroquinolones, some toxins, including CcdB, target DNA gyrase. Toxin resistance can be mediated via mutations in the gyrase, which led us to hypothesize that: (i) some of these changes might contribute to, or predispose towards fluoroquinolone resistance, and (ii) Qnr determinants might protect DNA gyrase against these toxins. We tested these hypotheses.
Methods: The CcdAB TA system was used as model. The pBAD and pBBR1MCS-2 vectors were used for dose-dependent L-arabinose-induced CcdB expression and for Qnr protein expression in *E. coli* Top10 strain, respectively. MICs were determined by agar dilution or by Etest. DNA manipulations and sequencing were by standard methods.
Results: Induction of CcdB expression led to colonies being smaller and slow-growing than normal. Sub-inhibitory expression of CcdB selected mutants of variable colonial sizes able to withstand expression of the toxin at different levels. The mechanism of CcdB resistance in these mutants did not involve mutations in GyrA, GyrB, ParC or ParE, and fluoroquinolone MICs were unaffected. Co-expression in trans of CcdB and a Qnr determinant (A, B or S-type) did not allow the *E. coli* strain to withstand higher expression levels of CcdB, indicating no protection against its toxic effects. The glutamine residue at position two was shown to play a critical role in toxicity of CcdB.
Conclusions: Our results show that resistance to the CcdB TA systems can be attenuated by mechanisms other than changes in the gyrase. Furthermore, resistance had no effects on fluoroquinolone MICs, and was not enhanced in the presence of Qnr proteins.

P687 Tn21-like transposons found in non-typhoidal *Salmonella enterica* isolates of clinical origin carrying class 1 integrons

I. Rodríguez, P. García, I. Montero, A. Herrero, N. Martínez, J. Beutlich, M.C. Martín, R. Rodicio, B. Guerra, M.C. Mendoza, M.R. Rodicio (Berlin, DE; Oviedo, Villaviciosa, ES)*

Objectives: In clinical isolates of *Salmonella enterica* belonging to different serotypes, the structures of class 1 integrons inserted within Tn21-like transposons and their involvement in multidrug resistance were established.
Methods: The variable regions of class 1 integrons were amplified by PCR using specific primers, and subsequently sequenced to identify the gene cassettes inserted therein. PCR mapping, cloning and sequencing were used to ascertain the organization of the Tn21-like transposons carrying class 1 integrons.

Results: Different configurations of class 1 integrons inserted within Tn21-like transposons were detected: (a) multidrug resistant S. Brandenburg and S. Ohio isolates positive for tet(B) contained a defective class 1 integron with the 1600 bp/dfrA1-aadA1 variable region, lacking IS1326, IS1353, tniBΔ1 and tniA (all carried by In2, the integron of Tn21). This integron was included within a Tn2670-like transposon (created by incorporation of Tn21 into Tn9), interrupted by a defective Tn10; (b) multidrug resistant S. Brandenburg isolates, positive for a Tn1721-like transposon carrying tet(A), harboured a class 1 integron with the variable region 1600 bp/dfrA1-aadA1, which lacked IS1326 and IS1353, and was located within Tn21-like transposons not always inserted into Tn9; (c) multidrug resistant S. Virchow isolates contained a class 1 integron with the 1000 bp/aadA1 variable region, lacking IS1353, and located within a Tn2670-like transposon; (d) in multiresistant S. Panama and S. Virchow isolates, the class 1 integron 2300 bp/estX-smr-aadA1, without IS1353, was also part of a Tn2670-like transposon; (e) in multidrug resistant isolates of S. Typhimurium, a 2000 bp/blaOXA-1-aadA1 integron, carrying IS1326, IS1353 and the tni module, was inserted within a Tn2670-like transposon adjacent to a defective Tn10; (f) in multidrug resistant S. Typhimurium monophasic isolates (antigenic formula [4,5,12:i:–]) a class 1 integron 1000 bp/aadA1 (lacking IS1326, IS1353 and the tni genes) was associated with a defective Tn21-like transposon without the transposase gene tnpA.
Conclusion: All class 1 integrons reported in this work were part of Tn21-like structures carried by large plasmids. These transposable elements are often associated with other transposons, creating new and more complex structures responsible for the multidrug resistance phenotype of the analyzed isolates.

P688 Detection and characterization of class 1 integrons of Gram-negative bacteria isolates from domestic, wild and food-producing animals

S.S. Domingues, C. Dias, M.J. Saavedra, G.J. da Silva (Coimbra, Vila Real, PT)*

Objective: Several antimicrobial agents used in animal and human medicine belong to the same antibiotic families and occurrence of cross-linked resistances has been identified. The aim of this study was to detect and characterise class 1 integron in Gram-negative bacteria collected from animal samples of different origin.
Methods: 76 samples were isolated from caecum, faeces, intestine, oviduct, skin or vaginal fluid of cats, dogs, jennies, deer, eagles, emus, fox, owls, snake, squirrels, chickens, gilthead breams, rabbits and trouts. The bacterial species were grown in selective culture media and identified by API20E, API20NE or by sequence of the 16S rDNA or gyrB genes. Antimicrobial susceptibility was determined by the disc diffusion method. Strains intermediate or resistant to co-trimoxazole (SXT), one of the most widely association of antibiotics used in animal husbandry, were screened by PCR for intI gene presence. Positive isolates were subject to PCR for detection of classic class 1 integrons. Gene cassettes were identified by genomic sequencing.
Results: 57% of the isolates were intermediate or resistant to SXT. Of these, 67% (29) were positive for intI and 37% (16) for class 1 integrons. Integrons were detected in strains of 2 chickens, 7 rabbits, 1 dog, 1 fox and 5 owls. 8 gene cassettes were identified in 4 different integron arrangements: dfrA12+orfF+aadA2, in 1 *Citrobacter braakii* (1 rabbit), 2 *Citrobacter* freundi (1 owl and 1 rabbit), 3 *Enterobacter cloacae* (3 rabbits) and 3 *Escherichia coli* (2 chickens and 1 rabbit); dfrA1+aadA1, in 1 *Aeromonas hydrophila*/caviae (1 owl), 1 *Aeromonas* salmonicida (1 owl), 1 Escherichia fergusonii (1 owl) and 2 *Pseudomonas fluorescens* (1 fox and 1 owl); aadA6+orfD, in 1 *Pseudomonas aeruginosa* (1 dog); and dfrA7, in 1 *Escherichia coli* (1 rabbit).
Conclusion: The results show that the same integron is present in different bacterial species suggesting the horizontal genetic transfer of the whole structure. The same gene cassette arrays have also been reported in human isolates. Moreover, these were isolated from animals that inhabit in diverse environments, highlighting that class 1 integrons are well disseminated among wild and domestic animal isolates, acting as integron-borne resistance gene reservoirs. These may have serious implications in human health, not only because integrons can end up in food products but also because manure is often used as a fertilizer with possible contamination of soil and water.

P689 Occurrence and dissemination of class 1 integrons in *Klebsiella pneumoniae* isolates in a hospital in Aveiro, Portugal

S. Ferreira, C. Santos, E. Ramalheira, S. Mendo (Aveiro, PT)*

Objective: *Klebsiella pneumoniae* is a common nosocomial agent responsible for infections difficult to treat. In the hospital environment, resistant strains are not confined to the patient but they can also colonize inanimate surfaces, thus spreading resistance. The aim of the present work was to evaluate the presence of class 1 integrons in *K. pneumoniae* strains collected from different sources, in the Hospital Infante D. Pedro EPE, Portugal.
Methods: The isolates were recovered from inpatients and inanimate surfaces within the hospital wards. Inpatients samples were collected from different biological products (urine, sputum and blood) analyzed in the laboratory. Inanimate surfaces samples were collected with sterile cotton swabs rubbed in the facility surfaces (toilet, lavatory and door knob) and placed in rich medium, at 37ºC for 24 h. Dilutions were plated in appropriate media and incubated as previously. Individual colonies were identified using the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (bioMérieux, Marcy L'Étoile, France). Susceptibility testing was carried out according to guidelines of CLSI standards. The presence of the integrons was detected by PCR, using specific primers. Resulting nucleotide sequence of the PCR amplicons was analyzed with the Blast and Clustal W programs.
Results: Class 1 integrons were detected in 59% of the *K. pneumoniae* isolates collected from both sources. A total of 18 gene cassettes occurring in 3 different arrays were identified; the variants aadA and dfrA genes were the most frequently found. A particular array (dfrA17:aadA2) was found in isolates from both sources.
Conclusions: These results suggest that new measures should be implemented to control the dissemination of antibiotic resistance genes in the hospital environment.

P690 Distribution of the gene cassette promoter variants of class 1 integrons in *Escherichia coli* strains isolated from clinical and community samples in Spain and France

L. Vinué, T. Jové, C. Torres, M.C. Ploy (Logroño, ES; Limoges, FR)*

Background: Class 1 integrons are widespread genetic elements that capture gene cassettes (GC) that mainly encode antibiotic resistance genes, by an integrase-mediated site-specific recombination. They play a major role in the dissemination of antibiotic resistance in Gram-negative bacteria. Integrated genes are usually promoterless and are expressed from a common promoter Pc. Our previousin silico analysis showed that 13 variants of Pc exist, providing a wide range of gene cassettes expression levels. However, the Pc variants distribution in clinical strains is unknown. Furthermore, there is a negative correlation between Pc strength and integrase excision activity: the weaker the Pc variant is, the more active the IntI1 is.
Methods: We studied 86 *E. coli* strains containing class 1 integrons, isolated from clinical samples (n = 59) or from stools in the general population (n = 27) either in Spain or France. The phylogenetic groups, GC arrays and Pc variants were characterized by PCR and sequencing.
Results: From the 88 integrons detected (two strains carried two different class 1 integrons), we identified only 4 Pc variants with a wide distribution range. The weaker Pc variants, PcW and PcH1 were predominant (n = 50 and 30, respectively), the stronger variants, PcWTGN-10 and PcS, remaining rare (n = 6 and 2, respectively). There was no clear correlation between the Pc variant and the isolates' origin or phylogenetic group, nor between the Pc variant and the GC arrays.
Conclusion: Interestingly, the weak PcW was the most frequent variant in both the *E. coli* clinical and community isolates. This suggests that

the presence of an efficient integrase for GC exchange may be more beneficial for the adaptation to often varying conditions rather than an efficient Pc promoter leading to a high level of resistance.

P691 Characterization of a novel class 3 multi-resistant integron isolated from a hospital effluent

O. Barraud, M. Casellas, B. Mayjonade, D. Chainier, C. Dagot, M.C. Ploy (Limoges, FR)*

Objectives: Integrons are bacterial genetic elements able to capture and express antibiotic resistance genes contained within mobile gene cassettes. Three main classes of multiresistant integrons (MRI) have been described: class 1 and class 2 are the most prevalent but little is known concerning class 3. We described a new class 3 integron with a novel gene cassette array from an *Enterobacter cloacae* retrieved from an hospital effluent.
Methods: An effluent sample was collected in April 2009 from the Limoges University Hospital Center, France, and inoculated onto Drigalski agar plates. Total DNA of the isolated strains was extracted by boiling. Class 1, 2 and 3 MRI were detected from each strain using real-time-PCR. Susceptibility testing was determined using Mueller-Hinton agar according to the recommendations of the French Society for Microbiology. Class 3 MRI cassette content was determined by PCR and sequencing.
Results: Forty one different bacterial strains were isolated from the effluent sample: 27 harbored class 1 MRI, no strain harbored class 2 MRI and one strain of *Enterobacter cloacae* harbored a class 3 MRI. This strain expressed a high-level penicillinase and was resistant to tobramycin, netilmicin and amikacin. Cassette content revealed a new array with two cassettes: blaOXA10 and aac(6′)-Ib.
Conclusion: This study describes the first class 3 MRI detected in France. Its cassette array has never been previously described, with a functional blaOXA10 gene encoding an oxacillinase, followed by an aac(6′)-Ib gene conferring resistance to aminoglycosides. This study suggests that effluents could be a reservoir of class 3 MRI.

P692 Association of the extended-spectrum β-lactamase blaTLA-1 gene to a novel ISCR element, ISCR20

B. Bercot, L. Poirel, J. Silva, P. Nordmann (Le Kremlin-Bicetre, FR; Morelos, MX)*

Objective: TLA-1 is an Ambler class A extended-spectrum β-lactamase reported from an *Escherichia coli* R170 clinical isolate described from Mexico City in 2000. The aim of the study was to characterize the genetic elements associated with of the blaTLA-1 gene from *E. coli* R170 and from a collection of TLA-1-producing Enterobacteriaceae recovered from various hospitals from Mexico City.
Methods: The 150-kb plasmid RZA92 (pRZA92) of *E. coli* R170 was extracted by using the Qiafilter Maxi plasmid purification kit and sequenced. The blaTLA-1 gene initiation site of transcription was mapped using a 5' rapid amplification of cDNA ends PCR technique. TLA-1-producing enterobacterial isolates were typed by the PFGE technique. Plasmids were transferred by conjugation to azide-resistant *E. coli* J53 strain. Plasmids were extracted from the tranconjugants by the Kieser technique, transferred and hybridized with a TLA-1 probe. Their incompatibility group (Inc) were determined by PCR.
Results: Analysis of the nucleotide sequence (5,925 bp) of pRZA92 surrounding the blaTLA-1 gene did not contain any insertion sequence forming a transposon or any class 1 integron structure but evidenced a novel ISCR element, ISCR20, coding a 406 amino-acids transposase. A third of the transposase has respectively 33%, 30% and 27% of identity with that of the ISCR1, ISCR2 and ISCR3. This element is inserted 232 bp upstream of the initiation start codon of the blaTLA-1 gene and brings it its own promoter. An open reading frame (orf) encoding a methyl-accepting chemotaxis protein element, but no ISCR20 element, was evidenced upstream of the blaTLA-1 gene. An orf encoding a reverse transcriptase/maturase (group II intron) preceded the ISCR20 element. After PFGE analysis, eight non-clonal clinical isolates TLA-1-producing Enterobacteriaceae were identified. For seven isolates, the blaTLA-1 gene was located on conjugative plasmids of various sizes (ca. 110 kb to 150 kb) belonging to various group of incompatibility (IncA/C, IncF, IncL/M or IncN). The blaTLA-1 gene was transferred from the seven isolates to *E. coli* J53 by conjugation. The same ISCR20 element was identified upstream of the blaTLA-1 genes in seven isolates.
Conclusion: The transposase encoded by the ISCR element may transpose an adjacent DNA via a rolling-circle (RC) transposition mechanism. Our study highlights a novel element, ISCR20, which is involved in the expression of the blaTLA-1 gene and may allow its transfer.

P693 A novel type of IncQ plasmid harbouring a class 3 integron from *Escherichia coli*

L. Poirel, A. Carattoli, T. Bruderer, R. Frei, S. Bernabeu, P. Nordmann (Le Kremlin Bicetre, FR; Rome, IT; Basel, CH)*

Objectives: The genes encoding the extended-spectrum β-lactamases (ESBLs) of the GES type are usually part of class 1 integron structures. Those integrons are themselves usually identified onto Tn21/Tn402 transposon structures. Our study was initiated by the identification of a blaGES-1 gene inside a peculiar class 3 integron structure.
Methods: *Escherichia coli* isolate TB7 had been isolated in 1998 in Switzerland from an hospitalized patient. Genomic DNA *E. coli* TB7 was extracted and used for PCR screening of known ESBL genes. Plasmids were extracted using the Kieser technique. Whole sequencing of the natural plasmid harbouring the blaGES-1 gene was achieved by a primer-walking approach.
Results: *E. coli* TB7 harboured a blaGES-1 gene that was not bracketed by class 1 integron features. Whole sequence analysis of plasmid pTB7 identified a gene encoding the class 3 integrase Int3 upstream of the blaGES-1 gene cassette, sharing 60% amino acid identity with the class 1 integrase Int1. Downstream of blaGES-1, the blaOXA-10/aac(6′)-Ib fused gene cassette was identified. The extremities of the class 3 integron were not clearly defined, but suggested that a deletion of those extremities had occurred during evolution. The backbone of plasmid pQ7 contained five open reading frames; RepC, RepA, a fused relaxase-primase protein, MobC, and an hypothetical protein, showing a replication machinery similar to that of other IncQ-type plasmids, including the oriV and the iteron-based incompatibility determinant. However, plasmid pTB7 exhibited replicase genes and iteron sequences that significantly differed from those of all the previously reported IncQ plasmid. The comparative analysis of the replication proteins of pQ7 showed it was homologous to two other plasmids; pGNB2 recovered from a wastewater treatment plant from an unknown host strain and harbouring the quinolone resistance gene qnrS2, and pWES-1 identified from *Salmonella enterica* Westhampton in France and carrying the ESBL gene blaCTX-M-53.
Conclusion: We propose here that pQ7 and its two related plasmids may be classified into a novel sub-family of IncQ plasmids, defined as IncQ-2 type, that correspond to non-conjugative and broad-host range resistance plasmids.

P694 Spread and evolution of ST131 and IncFII-blaCTX-M-15 and emergence of IncA/C-blaCTX-M-15 and IncN-blaCTX-M-28 plasmids in widespread *Escherichia coli* clones

Â. Novais, D. Viana, F. Baquero, R. Cantón, T.M. Coque (Madrid, ES)*

Objectives: Widespread IncFII plasmids and the ST131 *Escherichia coli* (EC) clone have determined the global expansion of CTX-M-15. Nevertheless, IncA/C and IncHI2 plasmids and other enterobacterial hosts are being increasingly involved in the secondary spread of this ESBL. Comprehensive molecular characterization of recent isolates and plasmids harbouring blaCTX-M-15 and related blaCTX-M-28 was performed.
Methods: Thirty CTX-M-15 and three CTX-M-28 (differing from CTX-M-15 in the aminoacid change D288N) EC producers recovered in our

institution in 2008 were studied. Population structure was established by PFGE, MLST and phylogroup identification. blaESBL genes and its genetic environments were identified by PCR and sequencing. Transfer of bla was carried out by broth/filter matings. Plasmid characterization included S1-PFGE, comparison of RFLP patterns and Inc group identification (PCR-sequencing-hybridization). Genes associated with blaCTX-M-15-IncFII plasmids (blaOXA-1, blaTEM-1, aac(6′)-Ib-cr) and quinolone resistance (qnrA, qnrB, qnrS) were also investigated.
Results: Isolates were mostly recovered from urine (n = 26/33, 79%) in outpatients (n = 19/33, 58%) or inpatients located at medical wards (n = 9/33, 27%). Isolates were identified as B2-ST131 (n = 21, 64%; 3 PFGE-types), widespread clones of phylogroups A (4 ST410, 3 ST1284, 1 ST167) and D (1 ST405) and non-widespread clones of group B1 (n = 2). Plasmid content varies within ST131, ST410 and ST1284 clones (IncFII and/or IncA/C or IncN plasmids). blaCTX-M-15 gene was transferred by conjugation in 91% of the cases, being located in IncFII (n = 23, 70–110 Kb, reppC15–1a, 3 related RFLP) or IncA/C plasmids (n = 2, 230 Kb, repp2039) among ST131, A-EC or B1-EC clones. Identical genetic environments (ISEcp1-blaCTX-M-15-orf477) and the presence of blaOXA-1, blaTEM-1 and aac(6′)-Ib-cr were detected in IncFII and IncA/C plasmids. The blaCTX-M-28 (deltaISEcp1-IS5-blaCTX-M-28) was located in a 50kb-IncN plasmid (n = 3, repR64) recovered from B2-ST131, A-ST410 and D-ST405. qnrS1 was identified in 45% of CTX-M-15 producers, mainly ST131 isolates, and located in blaCTX-M-15-IncFII or co-existing IncA/C (repp2039) and IncN (reppKP96) types.
Conclusion: Current spread of blaCTX-M-15 and blaCTX-M-28 in our setting is associated with a variety of genetic platforms within prevalent EC clones. This study highlights the evolution of ST131 by acquisition of different plasmids and antibiotic resistance mechanisms.

P695 Contemporary spread of CTX-M-14-*Escherichia coli* in Spain is mainly associated with persistent IncK and IncI1 and emerging IncN plasmids

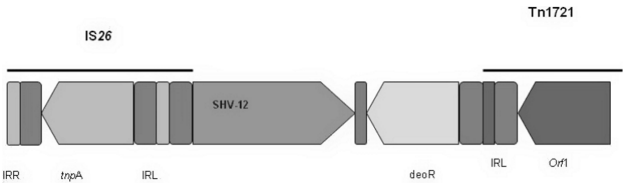
Â. Novais, D. Viana, F. Baquero, R. Cantón, T.M. Coque (Madrid, ES)*

Objectives: CTX-M-14 represents one of the most frequent ESBLs in different countries, including Spain, where it is increasingly detected. Spread of an epidemic IncK-blaCTX-M-14a plasmid among *E. coli* (EC) strains was documented until 2005. The aim of this study was to evaluate the current epidemiological scenario to understand persistence and the steady increase of CTX-M-14-EC producers.
Methods: Thirty-six CTX-M-14 *E. coli* producers collected in 2008 were studied. Relationship among isolates was established by XbaI-PFGE and identification of phylogenetic groups. blaESBL genes were identified by PCR and sequencing. Transfer of bla was carried out by broth/filter matings. Plasmid analysis included determination of size and content (S1-PFGE), incompatibility group [characterization of replicases (rep) and relaxases (rel) by PCR, sequencing and hybridization] and comparison of RFLP patterns.
Results: Most isolates were recovered from urines (n = 32/26, 89%) of outpatients (56%) or inpatients located at medical wards (33.3%). A high clonal diversity was detected with 36 PFGE-types corresponding to B1 (n = 17, 47%), A (n = 10, 28%; 5 A1, 5 A0), D (n = 8, 22%) and B2 (n = 1, 3%) phylogroups. Conjugative transfer was achieved in 61% of the cases. Two blaCTX-M-14 variants (34 blaCTX-M-14a and 2 blaCTX-M-14b) were identified in 1 IncK (n = 13, blaCTX-M-14a), 2 IncI1 (n = 2, blaCTX-M-14a/b), 1 IncB/O (n = 1, blaCTX-M-14b, reppEI545) and 1 IncN (n = 3, 35kb, blaCTX-M-14a, repR64) plasmids. IncK plasmids comprise the epidemic pRYC105 first detected in our institution in 2001 (75–85kb, blaCTX-M-14a, repR64+repR387+relpSERB1) which was found in 36% of the isolates corresponding to A, B1 and D EC-clones; and a closely related variant (80–100kb, blaCTX-M-14a, repR387 and relpSERB1) identified in A and B1 isolates (n = 8), which consistently contained 2 additional plasmids positive for FII+FIB (120–230kb) or N replicons (30–60kb). Two IncI1 plasmids of 85kb (blaCTX-M-14a, repR64+relpSC138) and 110kb (blaCTX-M-14b, repR64+rel R64) were detected.
Conclusion: We described contemporary spread and persistence of the epidemic pRYC105-IncK and IncI1 plasmids and the emergence of other plasmid types (IncN, IncB/O) harbouring blaCTX-M-14 variants. This study illustrates the impact of multiple acquisition events, recombination and plasmidic selection for the successful spread of a particular antibiotic resistance gene.

P696 Genetic environment of blaSHV-12 ESBL in three *E. coli* clinical isolates: an IS26 structure truncated by a Tn1721

M. Fernández Vázquez, H. Iglesias de Sena, J.A. García Rodríguez, J.L. Muñoz Bellido (Salamanca, ES)*

Objectives: SHV-12 is one of the most prevalent enzymes within the SHV family. It has been reported all over the world in *K. pneumoniae* and in *E. coli*, and has been shown to be the most frequent ESBL in enterobacteria other than *Escherichia coli* and *Klebsiella* spp. blaSHV-12 gene usually appears bracketed by two IS26, and has been associated with genetic elements conferring resistance to fluoroquinolones (qnr). Here we describe a newer genetic environment (GE) of blaSHV-12 in three clinical isolates of *E. coli*, previously undescribed for this ESBL.
Methods: MICs were determined according CLSI guidelines. Conjugation was performed by the filter mating method. ESBLs genes were characterized by PCR and sequencing. The GE of blaSHV-12 was determined by using a gene-walking strategy. Genetic diversity was determined by RAPD.
Results: RAPD showed the three isolates were not genetically related. All three yield a transconjugant strain (TS) harbouring the same structure (Figure). Isolate 3 also harboured a blaCTX-M-14. MICs of ceftazidime, cefotaxime and cefepime were respectively: for isolate 1: >16 mg/l, >8 mg/l and ⩽1 mg/l; TS-1: >16 mg/l, 4 mg/l and ⩽1 mg/l; isolate 2: >16 mg/l, >8 mg/l and 2 mg/l; TS-2: 16 mg/l, ⩽1 mg/l and ⩽1 mg/l; isolate 3: >16 mg/l, >8 mg/l and 16 mg/l; and TS-3: 8 mg/l, ⩽1 mg/l and ⩽1 mg/l. The analysis of plasmid incompatibility groups present in the six strains revealed that the group I1/Ig was the common group for all of them. blaSHV-12 was associated to an IS26 upstream, but 137 pb downstream deoR gene, the structure was truncated by an Tn1721. This organization has been described previously only in two *P. aeruginosa* isolates harbouring a blaSHV-2a (Access. No. AF74954 and AM988779). There is no information in these cases about plasmidic behaviour, or incompatibility groups.
Conclusions: New genetic environments associated to ESBLs are being progresively described. The diversity of these genetic environments and their association to insertion sequences, transposons and other mobile genetic elements are probably associated to the wide diffusion of these enzymes.



P697 Characterization of plasmids carrying CMY-2-like genes in *Escherichia coli* from the United Kingdom

J. Curry, N. Woodford, P.D. Stapleton (London, UK)*

Objectives: CMY-2-like β-lactamases, in common with other class C cephalosporinases, confer resistance to third-generation cephalosporins, cefoxitin and are refractory to inhibition by clavulanate. They are encoded by blacmy-2 genes that originate from ampC found on the chromosome of *Citrobacter freundii*. Mobilisation of the ampC gene on to plasmids has led to its dissemination into a number of hosts including *Salmonella* spp., *Klebsiella* spp. and *Escherichia coli*. This study aimed to characterise the plasmids encoding CMY-2-like enzymes in the UK by investigating a defined set of *E. coli* isolates from diverse geographical locations.

Methods: Single *E. coli* isolates from each of nineteen different hospitals in the UK were investigated; all were known to carry blaCMY2-like genes. Plasmids harbouring these genes were identified by a combination of plasmid profiling, DNA:DNA hybridisation with a blaCMY-2 probe, and plasmid transfer studies. PCR-based replicon typing and plasmid multi-locus sequence typing (pMLST) was used to classify the plasmids.
Results: Most (17) of the 19 isolates carried blaCMY-2, and 2 carried its close relative, blaCMY-23. Insertion sequence element ISEcp1 was identified 116 bp upstream from the initiation codon of the blaCMY-2-like genes in 16 of 19 isolates indicating a common insertion point. ISEcp1 from one of the isolates carrying the blaCMY-23 gene was interrupted by IS5. The isolates each carried multiple plasmids with sizes ranging from 1.1 to 168 Kb. Plasmids of incompatibility groups I1 (16/19 isolates) and F (16/19 isolates) were the most common followed by the A/C2 subgroup (4/19 isolates). The blaCMY-2 genes were transferable by conjugation to an *E. coli* recipient from 16 donor isolates. All transconjugants carried IncI1 plasmids; 6 also acquired IncF or IncA/C2 types present in their parental donor; no transfer was detected from three isolates, including those carrying blaCMY-23. Classification of 10 IncI1 plasmids by pMLST revealed two isolates had identical profiles (ST2), but the other eight plasmids all had unique sequence types; all ten carried blaCMY-2.
Conclusions: A diverse set of IncI1 plasmids is responsible for the spread of blaCMY-2-like genes in *E. coli* from the UK. This study illustrates the power of pMLST for differentiating IncI1 plasmids responsible for the spread of antimicrobial resistance genes.

P698 Comparison of fully sequenced plasmids harbouring different CTX-M variants in German clinical isolates of *Escherichia coli*

A. Cullik, W. Witte (Wernigerode, DE)*

Objectives: In recent years the number of outbreak and community associated extended-spectrum b-lactamases (ESBL) producing Enterobacteriaceae is constantly increasing. In Europe the CTX-M type ESBL of subgroups 1 and 9 are primarily found. They are often located on large low-copy plasmids belonging to incompatibility groups F, I1, N, HI, A/C and L/M. In order to learn about the mechanisms of acquisition and dissemination of blaCTX-M in enterics, we sequenced two IncN plasmids, pKC394 and pKC396, harbouring each one blaCTX-M gene of the CTX-M subgroups 1 and 9, respectively.
Methods and Results: By PCR sequencing blaCTX-M-1 and blaCTX-M-65, respectively, were identified in two 3rd generation cephalosporin resistant *E. coli* and found to be located on IncN plasmids. The plasmids obtained by conjugation were sequenced on a 454 Sequencer. They were approximately 45 000 (pKC396, blaCTX-M-65) and 50 000bp (pKC394, blaCTX-M-1) in size showing identical backbone regions, similar to previously sequenced antibiotic IncN plasmids R46, pMUR050 and pC15−1a. The integration of blaCTX-M was probably mediated by different insertion sequences (IS903 for blaCTX-M 65; IS26 for blaCTX-M-1) and occurred at different sites of the plasmid backbone. These sites are the same in R46 and pKP96, but they harbour different antibiotic resistance cassettes. Unexpectedly, the pKC394 sequences showed the blaCTX-M-1 as well as blaCTX-M-65 gene which was confirmed by PCR and southern hybridization experiments. The blaCTX-M-65 integration site in pKC394 and pKC396 was identical.
Conclusions: The CTX-M/IS mobilization units seem to have different targets sites for integration in the plasmid backbone. This is probably due to their association of different IS elements and their corresponding DNA recognition sequences. The integration of two different blaCTX-M genes into the plasmid pKC394 is a new phenomenon that could entail a increased resistance rates to cephalosporins, especially with inhibitor, or fitness benefit like described for another β-lactamase, blaTEM.

P699 Occurrence and dissemination of genetic resistance determinants among Gram-negative isolates collected from inanimate surfaces in a hospital in Aveiro, Portugal

C. Santos, T. Caetano, E. Ramalheira, S. Ferreira, S. Mendo (Aveiro, PT)*

Objective: In hospital environment, the arising of antibiotic resistant strains is not only confined to patients but also to the surrounding environment. This is a serious problem worldwide given the selective pressure exerted by overuse of antibiotics. The present study aimed to assess the occurrence of antibiotic resistance determinants in bacterial strains collected from inanimate surfaces.
Methods: Samples were collected with sterile cotton swabs rubbed in the toilet, lavatory and door knob and placed in rich medium, 37ºC o/n. Dilutions were plated in McK agar and incubated as previously. Phenotypically different colonies were selected and their clonal relationship was determined by rep-PCR. Individual clones were identified using VITEK2 system and VITEK2 AES (bioMérieux, Marcy L'Étoile, France). Susceptibilities were determined according to guidelines of CLSI standards. Class 1, class 2 integrons and β-lactamases were screened by PCR, using specific primers. Nucleotide and deduced aminoacid sequences were analyzed with Blast and ClustalW programs and compared to others in the database.
Results: Eighty-five genetically different strains belonging to genera Escherichia, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Acinetobacter*, *Serratia*, *Proteus*, Alcaligenes, *Stenotrophomonas* and *Morganella*, were identified. The intI1 gene was amplified in 29% of the isolates and intI2 gene was not detected. A total of 15 different arrays identified: 35% were novel combinations of gene cassettes, never described before; 65% were already described in different countries and species, but in biological samples of patients or animals. Screening for β-lactamases and metallo-β-lactamases (MBLs) enzymes revealed the presence of CTX-M (7.05%), OXA (9.41%), SHV (18.82%), TEM (87.06%), VIM (5.88%) and IMP (1.17%) variants. The most prevalent β-lactamases were TEM-1 and SHV-12 variants, followed by MBL of VIM-2 type that appeared always associated with class 1 integron.
Conclusions: Class 1 integrons are widely spread in the hospital environment, particularly among opportunistic strains, as well as the β-lactamases. These results are worrisome, since the studied isolates were collected from inanimate surfaces, and possess different resistance determinants that were described worldwide. The results show that antibiotic resistance genes can be easily disseminated within the hospital environment, and may be a possible source of infection in compromised patients.

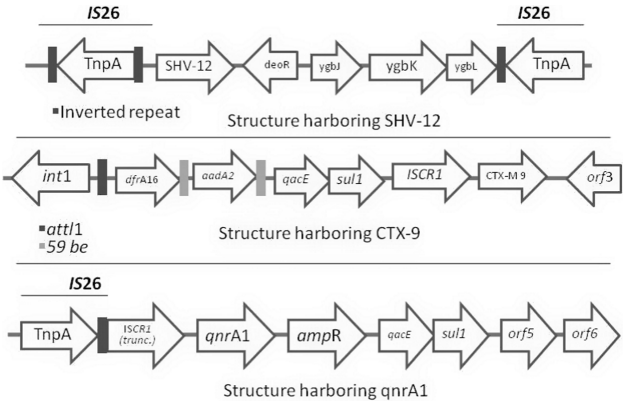
Extended-spectrum β-lactamases

P700 New genetic environment of quinolone resistance gene qnrA, in a qnrA1 and double ESBL-producing *K. oxytoca* clinical isolate

M. Fernández Vázquez, N. Gutiérrez Zufiaurre, J.A. García Rodríguez, J.L. Muñoz Bellido (Salamanca, ES)*

Objective: We describe the genetic environment (GE) of qnrA1 and ESBLs in a *K. oxytoca* harbouring qnrA and two ESBLs.
Methods: MICs were determined according CLSI guidelines. Conjugation was performed by the filter mating method. qnrA1 and ESBLs genes were characterized by PCR and sequencing. The GE of qnrA1 and ESBLs genes were determined by using a gene-walking strategy. gyrA, gyrB, parC and parE genes were sequenced.
Results: MICs of the *K. oxytoca* isolate were: amoxicillin, >16 mg/l; cefoxitin >8 mg/l; ceftazidime, >16 mg/l; cefotaxime, >8 mg/l; nalidixic acid, 16 mg/l; ciprofloxacin, 0.12 mg/l. gyrA, gyrB, parC and parE genes were wild-type. The isolate harboured qnrA1, CTX-M-9 and SHV-12. Transconjugant MICs were: ceftazidime, >16 mg/l; cefotaxime, >8 mg/l; nalidixic acid, 8 mg/l; ciprofloxacin, 0.1 mg/l. There was an unique plasmid sized >29,950 pb (incompatibility group HI2). SHV-12 was

associated to a IS26, with a GE described in pMUB9 (Access. No. EF370423). CTX-M 9 was included in a In60 class-1 integron (Access. No. AF174129.3). qnrA1 was included in a structure similar to the In36 (Access. No. AY259085.1) downstream from the ISCR1, excepting because IS6100 was not found downstream orf6. Nevertheless, the ISCR1 3′ end is truncated in its first 144 bases by a IS26, probably displacing the elements upstream of ISCR1 in the In36 backbone.
Conclusions: *K. oxytoca* harbouring both ESBLs and qnr simultaneously have been infrequently reported. The GE reported for qnrA1 has not been reported previously.



P701 Identification of extended-spectrum β-lactamase producing Enterobacteriaceae using the Hyplex® ESBL-ID multiplex PCR-ELISA system

A. Senok, M. Al-Zarouni, N. Al-Zarooni, D. Panigrahi (Sharjah, AE)*

Objectives: Rapid detection and genotyping of extended-spectrum β-lactamase (ESBL) types is required particularly in high prevalence settings. This study was carried out assess the use of Hyplex® ESBL ID multiplex PCR-ELISA system (h-ES-ID) in our setting and compare it with conventional methods. In addition, the antibiotic resistance profile of identified ESBL isolates to carbapenems and tigecycline was determined.
Methods: ESBL Enterobacteriaceae identified from seven secondary and tertiary hospitals in the United Arab Emirates from June 2008-March 2009 were included in the study. Screening for ESBL phenotype was by double disk diffusion method according to CLSI guidelines and confirmed using the VITEK-2 (bioMérieux) and MicroScan Walk-Away automated methods as well as ESBL-Etest. All isolates were tested using the h-ES-ID which is designed to amplify TEM, blaSHV, blaCTX-M and blaOXA bacterial DNA by multiplex PCR and subsequent hybridisation of the PCR products to specific oligonucleotide probes. These consist of a consensus probe for TEM, SHV, CTX-M and OXA and a CTX-M specific probe for identification of ESBL isolates with CTX-M gene. The pattern of antibiotic susceptibility of CTX-M isolates to a panel of antibiotics including ciprofloxoacin, imipenem, meropenem and tigecycline was determined.
Results: A total of 292 ESBL-producing Enterobacteriaceae were identified during the study period using the double disk diffusion and automated methods. Majority of these were *E. coli* (n = 228) and *K. pneumoniae* (n = 63). Of these 288 (98.6.%) exhibited the ESBL phenotype using the Etest. The h-ES-ID assay identified 287 (98.2%) ESBL isolates of which 95% (n/N = 272/287) were CTX-M types. Four isolates identified as ESBL positive by the double disk and automated methods were negative by both E-test and PCR. All isolates were sensitive to imipenem and meropenem. Ciprofloxacin resistance was 80% among CTX-M positive isolates compared to 37.5% among CTX-M negative isolates. Four CTX-M positive isolates showed resistance to tigecyline. These included two *K. pneumoniae* and one *Enterobacter cloacae* with MIC90: >4μg/ml, and one *K. pneumoniae* with MIC90: 3μg/ml.
Conclusion: The h-ES-ID is a reliable test for the rapid detection of ESBL isolates with the additional advantage of detecting those harboring the CTX-M gene. The emergence of resistance to tigecycline among the CTX-M isolates is of clinical significance and should be monitored.

P702 Identification of CTX-M-15-producing O25b-ST131 *Escherichia coli* isolates on the basis of distinct antibiotic susceptibility and metabolic profiles

T. Gibreel, F. Bolton, J. Cheesbrough, A. Fox, A. Dodgson, M. Upton (Manchester, Preston, UK)*

Objectives: The recent global dissemination of O25:H4-ST131 CTX-M-15 producing uropathogenic *E. coli* (UPEC) isolates with a high virulence potential represents a major health problem. The aim of this study was to assess the possible association of key UPEC strains with phenotypic profiles and to recognize putative predictor markers for identification of the ST131 clone.
Methods: A total of 310 isolates representing the UPEC population in the northwest region of England were collected in the period 2006–2009. Genotyping using multilocus sequence typing (MLST) and phylogenetic group PCR was carried out in addition to determination of biochemical activity and antibiotic susceptibility profiles using Vitek® system. ST131 isolates were screened using an O25b-ST131 CTX-M-15 allele specific multiplex PCR.
Results: A nine-digit numerical code was generated using the results of 27 biochemical tests. A total of 144 different profiles were detected, of which 22 were solely associated with ST131 isolates. Aggregative bioscores were significantly higher among ST131 isolates (median 19 range 14–23) than among other commonly detected clonal groups.
Isolates were grouped based on the biochemical profiles and clusters were examined for associations with ST and antibiogram profiles. This revealed a large ST131 cluster comprising of 69 (90%) isolates that were characterized as having a multiple resistance antibiogram.
Stepwise multivariate logistic regression analysis revealed resistance to fluoroquinolones as the strongest predictor of ST131, in addition to three biochemical tests (production of α-galactosidase, 5-keto-D-glucosnate and fermentation of saccharose) with an overall accuracy of 94.2%. Among the ST131 clonal group isolates, production of tyrosine arylamidase appeared to be the only significant predictor of CTX-M-15 producing O25b-ST131 strains with an over all accuracy of 94%.
Conclusion: ST131 clonal group in general, and O25b-ST131 CTX-M-15 producing stains in particular, are characterized by unique biochemical activities that can, to a certain extent, be used to predict multiple drug resistant ST 131 clonal group strains.

P703 Characterization and location of the blaCTX-M-15 gene in enteroaggregative *Escherichia coli* causing traveller's diarrhoea

E. Guiral, S. Soto, E. Méndez-Arancibia, P. Salvador, J. Vila (Barcelona, ES)*

Objectives: Enteroaggregative *Escherichia coli* (EAEC) are one of the most important bacteria causing traveller's diarrhoea. Although multiresistant EAEC clinical isolates have been shown, no EAEC isolates resistant to third generation cephalosporins have been reported up to now. In a previous study, 5 out of 51 EAEC isolates causing traveller's diarrhoea from patients who had travelled to India were found to be resistant to these cephalosporins. The aim of this study was to investigate the mechanisms of resistance to the third-generation cephalosporins in these 5 EAEC clinical isolates.
Methods: Five *E. coli* strains were collected from stool samples from patients with traveller's diarrhoea. The presence of SHV, TEM, CTXM ESBL and an Insertion Sequence (IS) related to those genes was determined by PCR and sequenced using specific primers. Genetic location of the gene encoding an ESBL was determined by reverse PCR and conjugation. Conjugation was performed using both the K7 759 lac- kanamycin-resistant and J53 rifampicin-resistant *E. coli* strains. The epidemiological relationship among these five EAEC strains was studied

by PFGE, REP-PCR and MLST. The phylotype of the five strains was determined by multiplex PCR.
Results: A blaCTX-M-15 gene was detected in all EAEC strains. By reverse PCR, it was found that the gene could be related to the ISecp1 upstream, which was comfirmed by a combined PCR. Transconjugants were obtained for three out of five strains. The PFGE and the REP-PCR showed that these strains were non-epidemiologically related. Three strains belonged to phylotype D and the other two to phylotype B2. The MSLT study showed that the three strains belonging to phylotype D were included in the Clonal Complex ST38.
Conclusion: This is the first time that the blaCTX-M-15 gene has been detected in EAEC clinical isolates causing traveller's diarrhoea. This gene was found in five epidemiologically unrelated EAEC strains although three of them were from the same Clonal Complex and had the same phylotype. The five strains had the ISecp1 element upstream of the gene blaCTX-M-15. This gene was located in a transferable plasmid in three of the strains.

P704 Prevalence of CTX-M extended-spectrum β-lactamase *Escherichia coli* in chickens and turkeys in Great Britain between 2006 and 2009

L.P. Randall, C.S. Clouting, H. Wearing, M.J. Toszeghy, R. Horton, N. Coldham, F.A. Clifton-Hadley, C.J. Teale, R.H. Davies (New Haw, UK)*

Objectives: In recent years *E. coli* strains carrying CTX-M genes have been isolated from chickens in Belgium, China, France, Japan, Portugal and Spain. In view of the detection of such strains in poultry in a number of different countries, two separate surveys for CTX-M *E. coli* in chickens and turkeys were performed in Great Britain in 2006–7 (turkeys) and 2008–9 (chickens) to determine the prevalence of CTX-M *E. coli* in poultry.
Methods: For the chicken survey, caecal contents from 388 chickens from 22 abattoirs were sampled. For the turkey survey, 442 turkey flocks comprising 125 breeder and 317 meat flocks from 337 farms were sampled. Potential CTX-M positive isolates were recovered on CHROM agar ECC (CHROMagar France) with addition of 1 or 2 mg/L cephalosporin antibiotic or, for chicken samples only, also on CHROMagar CTX (CHROMagar, France). Presumptive ESBLs were tested for the presence of blaCTX-M gene by PCR and CTX-M positive isolates were sequenced to determine the CTX-M type, PFGE typed, tested for the ability to conjugation and tested for the presence of the O 25 serotype.

Table 1. Farms (Turkeys only) or abattoirs (chickens only) positive for CTX-M *E. coli* showing CTX-M types

Samples collected from Turkey Farms			Samples collected from broiler chickens at slaughter	
Type of farm	Farm code	CTX-M types	Abattoir code	CTX-M-types
Meat	2	14	AC	1
Meat	3	14	AE	1 & 15
Meat	4	14	AG	1
Meat	5	14	AH	1 & 15
Breeder	6	14	AI	1 & 3
Meat	7	14	AL	15
Meat	8	14	AM	3
Breeder	9	14	AS	1
Meat	10	14	AW	15
Meat	14	1	BB	1
Meat	11	14	BD	1
Meat	13	55	BJ	1
Meat	1	1 and 15		
Meat	16	14		
Meat	15	1		
Meat	18	1		
Meat	17	1		
Meat	12	1 and 15		

Results: Only 3.6% of individual chickens, 6.9% of turkey breeding flocks and 5.2% of turkey meat flocks were found to have CTX-M positive *E. coli*. CTX-M types 1, 3 and 15 were isolated from chickens, with CTX-M type 1 being predominant. CTX-M types 1, 14, 15 and 55 were isolated from turkeys with CTX-M types 1 and 14 being predominant (Table 1). In turkeys in particular, there was an association of some CTX-M types with some of the shared ownerships. CTX-M positive isolates from both chickens and turkeys were shown to be clonally diverse by PFGE and most isolates readily transferred their CTX-M gene to *Salmonella* by conjugation. None of the CTX-M positive isolates was serogroup O 25. In the chicken survey, CHROMagar CTX was the most effective agar for isolating CTX-M strains compared to other agars used.
Conclusion: There was a suggestion of different CTX-M enzymes circulating within chicken populations compared to those in turkeys and the plasmids carrying these enzymes appeared to have spread into clonally diverse *E. coli* strains. The prevalence of CTX-M *E. coli* in individual chickens and in turkey flocks was low, and on the basis of strains being negative for serogroup O 25, none of the CTX-M 15 isolates were O25-ST131 CTX-M 15, a human epidemic strain. Further work is planned to investigate MLST and replicon types of CTX-M isolates.

P705 Molecular analysis of CTX-M-15 producing *Escherichia coli* Belgian epidemic clone

I. Montesinos, P. Bogaerts, R. De Mendonça, A. Deplano, H. Rodriguez-Villalobos, M.J. Struelens, Y. Glupczynski (Brussels, BE; Stockholm, SE)*

Objectives: Pandemic clones of ST 131 CTX-M-15 producing *E. coli* have been identified worldwide and represents a major public health problem. Previous studies showed the dissemination of a major clone of the virulent B2 group CTX-M-15 producing *E. coli* (CTX-M-15-EC) in a large number of Belgian hospitals. We analysed this clone to elucidate its phylogenetic origin and CTX-M-15 plasmid structure.
Methods: Ten CTX-M-15-EC isolates of Belgian epidemic clone (PFGE type 6) were analyzed. Strains were genotyped by MLST. Plasmids were extracted and transformed into *E. coli* TOPO10 electrocompetent cells. MICs were determined by E-test. The presence of extended spectrum-β-lactamases was confirmed by double combination disk test (DDT) and by PCR. The genetic environments of blaCTX-M-15 were analysed by PCR mapping and sequencing.
Results: MLST analysis showed that CTX-M-15-EC isolates from Belgian clone 6 belong to the ST131 lineage. Transformants were obtained from all isolates. We found two groups of plasmids harbouring blaCTX-M-15 as determined by PCR mapping. In the first group (n = 8), sequencing of a 5kb fragment showed 100% sequence identity to the plasmid pEK516 (64 Kb), containing the transposase of the insertion sequence ISEcp1, tnpA, tnpR and blaTEM upstream of blaCTX-M-15. The second group (n = 2) showed 100% sequence identity over 7kb to the plasmid pEK499 (117 Kb) which encompasses an upstream region containing the transposase of the insertion sequence IS26 instead of ISEcp1, tnpM, int, dfrhVII, addA and sulI. Both plasmids share a truncated ORF 477 downstream of blaCTX-M-15. The transformants harbouring pEK499 showed negative DDT test and lower MIC values to ceftazidime, cefepime and cefotaxime (<2 mg/l), contrasting to the clinical isolates.
Conclusions: Molecular analysis of Belgian epidemic B2 CTX-M-15 producing *E. coli* strains showed its link to the international *E. coli* ST131 lineage. Different plasmids were observed in the same genetic lineage ST 131. This may contribute to the epidemiological success of this clone.

P706 Multicentre study extended-spectrum β-lactamase producing *Escherichia coli* in Spain: wide dissemination of CTX-M-15-producing O25b-B2 strains

J. Oteo, V. Bautista, N. Lara, M. Arroyo, S. Fernández, M. Pérez-Vázquez, O. Cuevas, S. García-Cobos, B. Aracil, J. Campos and the ESBL-REVERA-EARSS Study Group (REIPI)*

Objectives: ESBL-producing *E. coli* isolates have increased in last years in Spain. Recently, the local spread of the CTX-M-15-producing O25b-phylogroup B2-ST131 strain has been also described in Spain. The aim

of this study is to characterize the ESBL production in Spanish *E. coli* isolates, mainly focussed on the distribution of O25b-phylogroup B2 strains.
Methods: A national prospective multicenter study was designed to collect ESBL-producing *E. coli* from clinical samples between February 2008-May 2008. The 28 participant hospitals (representatives of the main Spanish geographic areas) were asked to collect up to 10 consecutive, non-duplicated isolates. Antibiotic susceptibility testing and ESBL detection was performed following CLSI guidelines. ESBLs were characterized by PCR and sequenciation. Phylogroups were determined by a multiplex PCR assay. The O25b type detection was carried out by an allele-specific PCR.
Results: A collection of 229 ESBL-producing isolates was studied. 181 (79%) were from urine, 135 (58.9%) were from community-acquired infections, and 153 (66.8%) were from patients >65 years old. Most prevalent ESBL types were: CTX-M-15 (41.5%), SHV-12 (31%) and CTX-M-14 (23.1%). Two different ESBLs were detected in 11 isolates: CTX-M-15 + SHV-12 (5), CTX-M-14 + SHV-12 (5), CTX-M-15 + CTX-M-14 (1). Seventy-six isolates (33.2%) belonged to phylogroup A, 47 (20.5%) to group B1, 75 (32.7%) to group B2, and 31 (13.5%) to group D. The most frequent phylogroup in CTX-M-15 producers was B2 (57.9%), in CTX-M-14 producers was B1 (34%) and in SHV-12 producers was A (49.3%). Resistance to ciprofloxacin, cotrimoxazole and gentamicin was 81%, 60% and 12.6%, respectively, in CTX-M-15 producers; 80.3%, 63.4% and 9.9% in SHV-12 producers; and 71.7%, 77.4% and 17% in CTX-M-14 producers. A total of 53 CTX-M-15-producing O25b-phylogroup B2 isolates were detected, but also 12 SHV-12, 2 CTX-M-14, 1 CTX-M-9 and 1 SHV-2 producers were O25b-B2. O25b-B2 *E. coli* producing CTX-M-15 were detected in 19 hospitals of 9 different geographic areas.
Conclusions: ESBLs more frequent in clinical *E. coli* isolates in Spain were CTX-M-15, CTX-M-14 and SHV-12. CTX-M-15 isolates belonged mainly to virulent phylogroup B2 while CTX-M-14 and SHV-12 belonged mainly to phylogroups A and B1. The 55.8% of CTX-M-15 isolates were O25b-B2 isolates. O25b-B2 *E. coli* isolates also carried other ESBLs. O25b-B2 *E. coli* producing CTX-M-15 is spreading in different Spanish geographic areas.

P707 Identification and characterization of epidemic CTX-M-producing Enterobacteriaceae in a United Kingdom region of 10-million population

L. Xu, S. Shabir, T. Bodah, C. McMurray, K. Hardy, P. Hawkey, K. Nye (Birmingham, UK)*

Objective: An epidemic strain (strain A) of *E. coli* was identified by a previous national survey of CTX-M producing ESBLs, conducted by the UK Health Protection Agency. The aim of this study was to establish the incidence of strain A and the prevalence of other epidemic strains harbouring bla-CTX-M using a regional survey in the West Midlands.
Methods: A 2 month survey of CTX-M-producing ESBLs in Enterobacteriaceae was carried out. A total of 370 consecutive non-duplicate isolates were collected from 13 loboratories which serve a population of 10 million. The 345 confirmed ESBL isolates were screened for the presence of blaCTX-M by mutiplex PCR. Genotypes were identified using Denaturing High-Performance Liquid Chromatography (DHPLC). Insertion sequence typing of IS26 was performed. Clonal relationships were studied by PFGE. A duplex PCR method was used to identify the O25b-ST131 *E. coli* clone isolates.
Results: Of the 294 (85.2%) isolates carrying blaCTX-M 175 (59.5%) were from hospital and 119 (40.5%) were from community patients. These comprised 232 *E. coli*, 58 *Klebsiella* spp., 2 *E. aerogenes,* 1 *E. cloacae* and 1 *P. vulgaris.* The majority of isolates were from urinary tract infections (241, 82.0%). Multiplex PCR showed that 284 isolates (96.6%) expressed CTX-M group 1 enzymes. The remaining 10 isolates harboured group 9, 2 and group 25/26 genes. All group 1 isolates had blaCTX-M-15 profiles. IS26 was identified in 62 isolates (21%). PFGE analysis found 23 clusters (RE 1–23) containing 213/225 (95%) blaCTX-M positive *E. coli* which were distributed between hospital and community isolates, 55% and 45% respectively. Of the clustered strains, 102 (60%) constituted the largest clone RE1 and shared the epidemic strain A PFGE profile; all harboured blaCTX-M-15 except one which carried blaCTX-M-9. A total of 66% *E. coli* (154/232) were O25b-ST131 positive. The majority of the strains, clustered in the largest PFGE clone RE1 (99 of 102) belonged to O25b-ST131.
Conclusion: Our survey shows for the first time that the largest cluster in the West Midland is the CTX-M-15 producing RE1 clone (strain A). This clone has not only persisted for more than two years after it emerged in one of local hospitals, but has also spread throughout the region. Almost all RE1 strains belong to the O25b-ST131 lineage (99/102) providing further evidence that this lineage plays a pivotal role in the clonal dispersal of CTX-M-15-producing ESBLs worldwide.

P708 Sequence analysis of extended-spectrum β-lactamase genes harboured by invasive isolates of *Escherichia coli* in Kuwait

G. Al Hashem, N. Al-Sweih, W. Jamal, M. Shahin, V.O. Rotimi (Safat, KW)*

Objective: This study was undertaken to analysis the genes encoding ESBL phenotypes in invasive isolates of *Escherichia coli* obtained from patients managed in Kuwait hospitals.
Methods: One hundred and thirteen ESBL-positive *E. coli* were studied. ESBL production was confirmed by PCR. DNA extracts of ESBL-positive isolates were screened for the presence of blaCTX-M, blaTEM and blaSHV genes using the following primers: MA-1 – 5′-SCSATGTGCAGYACCAGTAA-3′ and MA-2 5′-CCGCRATATGRTTGGTGGTG-3′ (blaCTX-M), Cf-TCGGGGAAATGTGCGCG and Dr-TGCTTAATCAGTGAGGCACC (blaTEM) and OS-5f TTATCTCCCTGTTAGCCACC and OS-6r GATTTGCTGATTTCGCTCGG (blaSHV). Strains with PCR amplicons positive for these bla genes were sequenced using Applied Biosystems sequencer and the nucleotides analyzed with software available at website http://www.ncbi.nlm.nih.gov/blast. Selected CTX-M-, TEM- and SHV-positive strains were evaluated for transfer of genetic determinants by conjugation experiments and associated plasmid DNA extracted and analyzed by agarose gel electrophoresis.
Results: Out of the 113 ESBL-positive isolates, 88 (77.9%), 46 (40.7%), 8 (7.1%) and 59 (52.2%) harbored blaCTX-M, blaTEM, blaSHV, and blaISEp1, respectively. Forty-four (50%) of the CTX-M-positives harbored blaTEM and 5 harbored blaSHV., but only 4 carried the 3 genes simultaneously. Sequence analysis showed that the most common CTX-M-type was CTX-M-15 representing 84.1%, followed by CTXM-14 (6.8%), CTX-M-14b (5.7%) and TOHO-1 (3.4%). Of these, 66 (89.2%) were found in isolates from the 3 predominant nationalities; Kuwaitis (36.5%), Egyptians (32.4%) and Indians (20.3%). The CTX-M phenotypes were easily transferrable to *E. coli* J53 (rif^r) recipeint. Three of the SHV-producing harboured blaSHV-1 and the remaining blaSHV-11 while 27 of the TEM-producing strains harboured blaTEM-1, 2 blaTEM-170, 1 blaTEM-174 and remaining contained no sequence.
Conclusions: A large proportion of the invasive ESBL-positive *E. coli* isolates in Kuwait produced the CTX-M-type β-lactamases and about half of them also produced the TEM-type enzymes simultaneously. SHV ESBL is relatively rare. However, all these ESBL phenotypes are transferable by conjugal plasmids.

P709 Characterization of extended-spectrum β-lactamase types produced by clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Turkey: results of a multicentre surveillance study (Hitit 2)

Z. Gulay, D. Gur, M. Bicmen on behalf of The Hitit 2 Study Group*

Objective: (i) to investigate specific ESBL types produced by clinical *E. coli* (Ec) and *K. pneumoniae* (Kp) isolates in order to understand their prevalence and distribution in Turkey (ii) to detect new ESBLs that has a potential for spread (iii) to understand the epidemiology of

multiresistant ST131:O25b clone which contributes to the increasing prevalence of CTX-M-15.
Methods: A total of 319 clinical isolates which were confirmed as ESBL producers by E Test, were collected from 12 centers located in 10 different cities. bla genes were amplified by PCR, cloned if necessary and sent for sequence analysis. *E. coli* ST131:O25b clone was investigated among CTX-M-15 producers by a PCR method developed by Clermont et al. Plasmid analysis and in some cases ERIC-PCR were also performed.
Results: CTX-M β-lactamases were detected in 97.4% of Ec and 95.1% of Kp. Among CTX-Ms, CTX-M- 15 predominated in both species (77.7% in Ec and 68.2% in Kp), followed by CTX-M-3 and CTX-M-1(overall prevalances 22.5% and 3.1%). CTX-M-14 was detected in an Ec isolate for the first time in Turkey. SHV-type ESBLs were found in 14.8% in Kp and 2.6% in Ec. The ESBLs with higher prevalances were SHV-12 and SHV-5 in Kp. The only TEM type ESBL was TEM-15 found in a Kp isolate. 5.3% of Kp SHV producers also produced a CTX-M (15 or 3). Several SHV-type enzymes belonging to group 2be (SHV-31, SHV-38) and group 2b (SHV-11, SHV-33, SHV-62, SHV-65) were detected for the first time in Turkey. Carbapenem resistance which is due to OXA-48 production was found in 3.1% of Kp isolates and a small outbreak caused by OXA-48 producing Kp was detected in one of the centers. Among 119 Ec isolates which produce CTX-M-15, 51 (42.8%) belonged to the ST131:025b clone. 86.3% of these isolates possessed an identical ERIC-PCR pattern although collected from 10 different centers. ESBLs were carried on plasmids with sizes 91–16 kb sometimes along with quinolone resistance.
Conclusions: (i) CTX-M-15 clearly predominates among ESBLs detected in Enterobacteriaceae isolated in Turkey. (ii) Several β-lactamases including CTX-M-14 that are new for Turkey were identified.(iii) Carbapenem resistance due to OXA-48 may become an emerging problem.(iv) The international pandemic clone Ec ST131:O25b which produces CTX-M-15 is widely disseminated in Turkey.

P710 Prevalence of extended-spectrum β-lactamases in *Escherichia coli* strains isolated from Peruvian infants

S.G. Mosquito, M.J. Pons, R. Maves, Y. Saenz, E. Mercado, M. Vargas, L.J. Del Valle, C. Torres, T. Ochoa, J. Ruiz (Lima, PE; Barcelona, ES; Logroño, ES)*

Objectives: To determine the frequency of extended spectrum β-lactamases (ESBLs) in diarrhoeagenic *Escherichia coli* strains isolated from children under one year of age with and without diarrhoea in Peru.
Methods: A total of 293 ampicillin-resistant *E. coli* isolates were obtained between October 2006 and July 2008 as part of a prospective cohort study of diarrhoea in 1,034 children under one year of age. Specimens were tested for the presence of ESBLs by double disk synergy testing with cefotaxime (CTX), ceftazidime (CAZ) and, amoxicillin–clavulanic acid (AMC). From these, 230 isolates were classified as diarrhoeagenic *E. coli* (DEC) and 63 as non-DEC by multiplex real-time PCR testing for diarrhoea virulence factors. The β-lactamase genes blaTEM, blaSHV, blaOXA-1-like, and blaCTX-M were identified by PCR. Posterior sequencing was performed for ESBL-positive isolates. Additionally, 95 ampicillin-resistant but ESBL-negative isolates were analysed by PCR in order to determine the prevalence of blaTEM, blaSHV, blaOXA-1-like and blaCARB.
Results: Only two ESBL-positive isolates (0.7%) were identified, both enteropathogenic *E. coli* (EPEC). The first isolate was CAZ-resistant, CTX-intermediate, and positive for the blaTEM and blaSHV genes. The second isolate was CTX-intermediate and CAZ-susceptible; this isolate only harboured blaSHV-12. Neither blaOXA-1-like nor blaCTX-M were detected in these isolates.
The prevalence of blaTEM, blaSHV, and blaOXA-1-like among non ESBL isolates was 48%, 42%, and 5%, respectively. No blaCARB genes were detected. The presence of blaTEM (34%[12/35] vs. 57%[34/60])differed between control and diarrhoea groups respectively. No differences were observed in the presence of β-lactamase genes among DEC or non-DEC isolates from control participants.
Conclusion: There is a low prevalence of ESBL-positive *E. coli* isolates in Peruvian children but a high frequency of blaTEM and blaSHV genes among non-ESBL isolates. The blaTEM gene is most prevalent in specimens from diarrhoea samples. Continued surveillance of the prevalence of ESBL-positive isolates is necessary to monitor the evolution of drug resistance in DEC.

P711 First description of *Escherichia coli* producing CTX-M-15 extended-spectrum β-lactamase in outpatients from south-eastern Nigeria

I. Iroha, S. Neumann, L. Marlinghaus, M. Korte, F. Szabados, S. Gatermann, M. Kaase (Bochum, DE)*

Objectives: The prevalence of extended-spectrum β-lactamases (ESBL) in *E. coli* is increasing worldwide, mainly due to the spread of ESBLs of type CTX-M-15. Plasmids with CTX-M-15 often also carry aac(6′)-Ib-cr and OXA-1 β-lactamase genes.
Methods: A total of 44 single patient ESBL *E. coli* strains collected from two hospitals located in Enugu (n = 28) and Abakaliki (n = 16) in south-eastern Nigeria were used in this study. Confirmation of the ESBL phenotype was done by a double-disk synergy test. Initial species identification was confirmed by MALDI-TOF analysis. Identification of ESBL genes was performed by specific PCR tests and subsequent sequencing. In addition, PCR for OXA-1 β-lactamase was performed. Screening for aac(6′)-Ib-cr was done by a specific PCR followed by restriction with BseGI.
Results: Of the 44 ESBL *E. coli* strains 34 (77.2%) were isolated from urine, 6 (13.6%) from vaginal swabs and 4 (9.0%) from wound swabs. 30 (68.1%) strains were isolated from female patients and 16 (36.4%) strains were from outpatients. The age range of patients was 7 to 72 years, but the majority of patients (n = 42; 95.5%) were under the age of 30. Using specific PCRs we could demonstrate CTX-M-1 cluster enzymes in 43 (97.7%) of strains, three of which were selected for sequencing and found to be CTX-M-15. A positive PCR for OXA-1 was found in 40 (90.9%) strains and aac(6′)-Ib-cr could be demonstrated in 40 (90%) strains.
Conclusion: For the first time we could demonstrate CTX-M-15, found almost always in association with OXA-1 and aac(6′)-Ib-cr, as the dominant ESBL type in *E. coli* strains from south eastern Nigeria. Remarkably a high proportion of the tested strains were from outpatients. For the first time aac(6′)-Ib-cr was detected in *E. coli* strains from Africa.

P712 Characterization of β-lactamase and plasmid content of nosocomial and community *Escherichia coli* from Rio de Janeiro, Brazil

M. Queiroz, P. Antunes, J. Mourão, E. Machado, L. Peixe (Rio de Janeiro, BR; Porto, PT)*

Objectives: Extended spectrum b-lactamases (ESBL) in Enterobacteriaceae are recognized as a major public health problem worldwide. Particularly in Latin America, CTX-M ESBLs are endemic and largely distributed. Our aim was to characterize the b-lactamase and plasmid content of *Escherichia coli* isolates recovered from different community sources and a hospital setting (1998–2001) in Rio de Janeiro, Brazil.
Methods: We analysed a total of 44 *E. coli* isolates obtained from community, including raw vegetables (n = 14) and food handlers hands (n = 6), and hospital, including fecal isolates of hospitalized patients (n = 4), clinical specimens (n = 9) and hospital diets (n = 11). Clonality was established by PFGE (XbaI). Susceptibility to antimicrobial agents and b-lactamase characterization was performed. Resistance genes and class 1 integrons were detected by PCR and sequencing. Plasmid analysis included conjugation assays, determination of size (S1-PFGE) and content (incompatibility groups by rep-PCR typing, hybridization and sequencing).
Results: A positive synergism test was observed for 9 isolates and 7 ESBL-producing human isolates were detected. All produced b-lactamases with a pI of 5.4 and 7.3 to 8.2 and gave rise to positive

amplicons for blaCTX-M groups and blaTEM being negative for blaSHV. By sequencing, CTX-M-2 was detected in isolates from rectal swab of hospitalized patients (n = 4), CTX-M-59 (n = 1) and CTX-M-9 (n = 2) in clinical specimens and TEM-1 was detected in all all (n = 7). We identified different clones among all ESBL-producing *E. coli*, which were multidrug-resistant (MDR) and contained different plasmid types (IncI1, IncA/C, IncFrepB, IncK, IncP and/or IncFIB). Among community isolates, only 3 isolates from vegetables were MDR (ampicillin, tetracycline, chloramphenicol, sulfametoxazol, streptomycin), though non ESBL-producers, and one isolate carried a 1000 bp (aadA1) integron. Interestingly, these isolates also harboured several plasmid types (IncI1, IncFrepB, IncK, IncFIIs, IncY, Inc FIA and/or IncFIB), providing genetic platforms for acquisition of diferent antibiotic resistance genes.
Conclusion: Our results corroborate the previously reported prevalence of CTX-M group 2 in ESBL-producing *E. coli* isolates from the hospital environment (colonization and infection) in Brazil. Furthermore, we found a diversity of plasmids that might act as substracts for the acquisition and transfer of antibiotic resistance genes and elements.

P713 High rates of intestinal colonization with extended-spectrum β-lactamase-producing Enterobacteriaceae in patients at a tertiary-care hospital in Israel

M. Gazin, J. Vervoort, M. Kazma, C. Lammens, R. Lameir, Y. Carmeli, H. Goossens, S. Malhotra-Kumar (Antwerp, BE; Tel-Aviv, IL)*

Objectives: Nosocomial infections caused by extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-En) are increasing worldwide. The influx of these bacteria into hospitals has major implications for infection-control. We studied the prevalence and molecular epidemiology of ESBL-En isolated from stool samples of hospitalized patients at the Tel-Aviv Sourasky Medical Center, Israel.
Methods: Stool samples (n = 121) were screened for presence of ESBL-En on the BLSE bi-plate (MacConkey/Drigalski agar supplemented with ceftazidime and cefotaxime, respectively). Isolates growing on BLSE agar were purified and phenotypic presence of ESBLs confirmed by the double disc synergy test (DDST; cefotaxime, ceftazidime, cefepime, and amoxicillin/clavulanate). Isolates were identified by conventional and semi-automated (Api20NE/E, bioMérieux) biochemical testing. β-lactamase genes (blaTEM, blaSHV and blaCTX-M) were detected by consensus PCRs. blaCTX-M amplicons were typed by bidirectional sequencing.
Results: Forty-three (35.5%) stool samples showed presence of ESBL-En. CTX-M, TEM, and SHV genes were found in 38 (88.4%), 30 (69.8%), and 13 (30.2%) stool samples, respectively. Upto 4 different ESBL-En species could be recovered from a single stool sample. One, 2, 3, and 4 different ESBL-En species were recovered from 27, 12, 2, and 2 stool samples, respectively. Of the 63 ESBL-En isolates studied, majority were *E. coli* (n = 20, 31.7%) *Klebsiella pneumoniae* (n = 17, 27.0%), and *Enterobacter cloacae* (n = 7, 11.1%). CTX-M genes were present either alone or in combination with another bla gene in 51 (81.0%) of the 63 ESBL-En analyzed. Of the 51 CTX-M genes, 35 (68.6%) were identified as CTX-M group 1 (CTX-M 15/28/3), 5 (9.8%) as CTX-M group 2 (CTX-M 2/20), 3 (5.9%) as CTX-M group 9 (CTX-M14/21/17/27), and 1 (2.0%) as CTX-M group 25 (CTX-M 25/26), while 7 (13.7%) genes could not be assigned to any CTX-M group.
Conclusions: Our results show that CTX-M group 1 enzymes are the predominant ESBL type present in commensal ESBL-En in Israel. The high rates of intestinal colonization with ESBL-En in Israeli patients underscore the importance of screening for ESBL-En carriage on hospital admission.

P714 Identification and dissemination of β-lactamases in Gram-negative enteric bacteria in Nigerian hospitals

D.O. Ogbolu, M.A. Webber (Ogbomosho, NG)*

Objective: β-lactamases are the predominant cause of resistance to β-lactam antibiotics in Gram negative bacteria and are a major cause of nosocomial infections associated with high mortality all over the world particularly in developing countries. This study however investigated the dissemination of different β-lactamases in Gram negative enteric bacteria isolated from clinical specimens of patients attending tertiary hospitals in Nigeria.
Methods: One hundred and thirty-four Gram-negative enteric bacilli (of 13 species) were analysed for antimicrobial susceptibility, phenotypic and genotypic detection of various β-lactamases and analysis of plasmids carrying Extended Spectrum β-lactamase (ESBL) enzymes.
Results: Of the 134 isolates, 111 (82.8%) were found to be β-lactamase positive while 28 (20.9%) were found to carry an ESBL. PCR revealed that TEM was present in 109 of 134 isolates (81.3%), SHV in 33 (24.6%), OXA in 15 (11.2%) and CTX-M in 25 isolates (18.7%), while all the genes were present in 6 (4.5%) of the isolates. AmpC enzymes were present in 29 of 44 ESBL producing isolates (65.9%). Multiplex PCR showed that 6 isolates were positive for ampC and these genes were only found in isolates with CTX-M genes. Sequencing identified these genes as ACT-1, DHA-1 and CMY-2. Of 13 representative plasmids investigated 9 (69.2%) were self transferable when selected by a β-lactam and the plasmids once transferred coded for β-lactam resistance. Restriction enzymes revealed transformants all carried an identical plasmid of approximately108 kb (Inc F).
Conclusion: There is diversity of β-lactamases genes in Gram-negative enteric bacilli; TEM, SHV, OXA, CTX-M-1(15), CTX-M-1(3) and AmpC types; ACT-1, DHA-1 and CMY-2. These genes are disseminated by a dominant conjugative plasmid.

P715 Diversity of β-lactamase families in Enterobacteriaceae isolates of Portuguese healthcare facilities

D. Jones-Dias, V. Manageiro, D. Louro, E. Ferreira, M. Caniça for the ARSIP (Antimicrobial Resistance Surveillance Program in Portugal)*

Objectives: β-lactamases are one of the most important mechanisms of resistance in Gram negative bacteria. We aimed to investigate β-lactamase producing-Enterobacteriaceae recovered from health care facilities in Portugal, to evaluate how the use of β-lactams is compromised in Portugal, due to this resistance mechanism.
Methods: A total of 125 strains of Enterobacteriaceae were characterized as ESBL producers over a 4 year period (2004 to 2008) in 12 health care facilities and tested for susceptibility to 32 antibiotics. Detection of TEM, SHV, OXA, CTX-M, GES and AmpC encoding genes was carried out by PCR. All positive isolates for CTX-M, GES and AmpC enzymes were submitted to gene sequencing. TEM and SHV encoding genes were identified when CTX-M was negative. Isoelectric focusing was performed to determine the expression and pI of chromossomal AmpC β-lactamases.
Results: A total of 118 isolates were non-susceptible to cefotaxime, ceftriaxone, ceftazidime, cefpodoxime and cefixime; 120 isolates were non-susceptible to aztreonam and 27 to cefoxitine. Regarding carbapenems, only one isolate revealed to be non-susceptible to imipenem. Only 19 isolates presented susceptibility to amoxicillin/clavulanic acid and 41 to piperacillin/tazobactam combinations. A total of 103 isolates were multidrug resistant. Although all the isolates presented ESBL phenotype, this was confirmed only in 109 of them. Indeed, molecular methods identified 99 strains possessing bla-CTX-M (blaCTX-M-1, blaCTX-M-9, blaCTX-M-14, blaCTX-M-15 and blaCTX-M-32 genes), 5 strains possessing bla-SHV-12, 5 with bla-TEM (blaTEM-10, blaTEM-24 and blaTEM-52) and one with blaGES-1 gene, all encoding ESBL enzymes; additionally, we also found 9 strains with hyperproduced penicillinases like SHV-28, SHV-1 and TEM-1, probably responsible for false positive ESBL phenotypes. The IEF method revealed that 11 strains possessed a chromossomal AmpC enzyme and we also detected 2 plasmid-mediated AmpC enzymes (DHA-1 and CMY-2). Overall, CTX-M-15 β-lactamases were predominant and detected in multiple hospital wards, distinct hospitals and various regions of the country.
Conclusion: In conclusion, the frequent co-production of two or more β-lactamases, the diversity of the enzymes identified and the high level of resistance to extended spectrum cephalosporins detected suggests real threat and the need to take action.

P716 Five-year trends in the prevalence and types of ESBLs and antimicrobial susceptibility of ESBL-producing nosocomial strains of Enterobacteriaceae in Russia

M. Sukhorukova, V. Kozyreva, N. Ivanchik, M. Edelstein, R. Kozlov on behalf of the ROSNET Study Group*

Objectives: To determine the trends in prevalence of ESBLs of different types and antimicrobial susceptibility of ESBL-producing Enterobacteriaceae in Russian hospitals over the period of 2002–2007.
Materials and Methods: A total of 1,373 and 1,035 non-duplicate nosocomial isolates of Enterobacteriaceae were collected, respectively, from 31 and 36 hospitals of various geographic regions of Russia in 2002–2004 and 2006–2007. Antibiotic susceptibilities were determined by agar dilution method and interpreted according to EUCAST clinical breakpoints. All isolates were screened for ESBL-production using MIC and disk synergy tests with cefotaxime, ceftazidime, cefepime and clavulanate. Isolates with ESBL phenotype were tested for class A ESBL genes using three real-time PCR assays: one for detection of CTX-M-1, -2, -8 and -9/25 cluster enzymes, and the other two targeting ESBL mutations in the genes for TEM (E104K, R164C/H/S, A237T, G238S, E240K) and SHV (D179A/N/G, G238A/S, E240K) β-lactamases. ESBL genes from 100 selected isolates were characterised by sequencing.
Results: Between 2002–2004 and 2006–2007, the prevalence of ESBLs in nosocomial Enterobacteriaceae increased from 52.3% to 69.3%; from 81.2% to 90.0% in *K. pneumoniae*; and from 49.2% to 67.4% in *E. coli*. The species distribution of ESBL producers remained fairly constant with *K. pneumoniae* and *E. coli* being predominant species: 47.5%-46.3% and 26.6%-28.6%, respectively. The changes in frequency of ESBL groups were: 41.7% to 60.2% for CTX-M-1-cluster (mainly CTX-M-15 and -3); 18.4% to 14.1% for SHVs (mainly SHV-12 and -2); 2.6% to3.6% for CTX-M-9 cluster (CTX-M-14); 0.4% to 0.2% for CTX-M-2 cluster (CTX-M-5); and 0.1% to 0.1% for TEMs (TEM-29 and -143). The resistance rates of ESBL producers in two study periods were, correspondingly: 87.6% and 85.2% to amoxicillin–clavulanate, 40.4% and 32.8% to piperacillin-tazabactam, 4.3% and 6.7% to ertapenem, 0.0% and 0.0% to imipenem and meropenem, 39.1% and 44.4% to amikacin, 89.0% and 84.7% to gentamicin, 54.8% and 73.6% to ciprofloxacin, 73.1% and 66.0% to co-trimoxazole.
Conclusions: To our knowledge, this study documents one of the highest rates of ESBL production reported in national surveillance studies that increased significantly between 2002–2004 and 2006–2007. The dominance of CTX-M-1 cluster ESBLs, the increase in resistance to ciprofloxacin and consistency of carbapenem activity against ESBL producers are the main trends identified in this study.

P717 Clonal spread of CTX-M producing *Klebsiella pneumoniae* in a Croatian hospital

M. Vranic-Ladavac, N. Beader, Z. Bosnjak, N. Barisic, S. Kalenic, B. Bedenic (Pula, Zagreb, HR)*

Objectives: Study was conducted to analyze the presence of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* associated with nosocomial outbreak at Croatian Hospital Pula. During 2007, 162 *K. pneumoniae* isolates with reduced susceptibility to third generation cephalosporins were collected from hospital. Sixty of these strains were chosen for further analysis.
Methods: Double-disk synergy test was used to detect ESBLs. Minimum inhibitory concentrations (MICs) were determined by broth microdilution method according to CLSI. The transferability of ceftazidime resistance was tested by conjugation (broth mating method). PCR was used to detect alleles encoding ESBL enzymes. The genotypes of the strains were compared by analysis of banding patterns generated by pulsed-field gel electrophoresis (PFGE) of Xba I-digested genomic DNA.
Results: ESBLs were found by DDST in all isolates. All strains were resistant to cefuroxime, ceftazidime, cefotaxime, ceftriaxone, aztreonam, piperacillin/tazobactam and ciprofloxacin. There was variable susceptibility/resistance to cefepime and gentamicin. No resistance to ceftazidime/clavulanate and carbapenems was observed. Only six strains transferred resistant to *E. coli* recipient strain with low frequency. Resistance to tetracycline was cotransfered to recipient strain alongside with cefotaxime resistance from six strains and to sulphametoxazole from four strains. Resistance to cefoxitin and ciprofloxacin was not transferable.
All isolates yielded an amplicon of 545 bp with consensus MA primers. Multiplex PCR was positive for group 1 of CTX-M β-lactamases. Sequencing of selected amplicons revealed the presence of blaCTX-15/28 with coding regions containing identical nucleotide sequences. Our isolates contained ISEcpI insertion sequence located upstream of the blaCTX-M-15 gene, which has been recently demonstrated to mobilize 3'adjacent genes to transfer between DNA replicons.
Conclusion: The results of this work provided insights into the molecular epidemiology of the spread of ESBLs in *Klebsiella pneumoniae* involved in an outbreak at Croatian Hospital. The hospital antibiotic policy results in ceftriaxone being most heavily prescribed third generation cephalosporin which might be expected to select for cefotaximases such as CTX-M-15.
The spread of CTX-M producing Enterobacteriaceae in Croatia may indicate difficulty in controlling this emerging resistance determinance whatever the antibiotic policy is.

P718 Increase of extended-spectrum β-lactamase-producing *Escherichia coli* in the Tyrol – a matter of clonality?

L. Bruder, K. Grif, D. Orth, C. Lass-Flörl, R. Würzner (Innsbruck, AT)*

Introduction: β-lactamases represent a major defence mechanism of Gram-negative bacteria against β-lactamase antibiotics. With the development of β-lactamase resistant antibiotics bacteria developed new mechanisms to overcome these new agents and responded with the production of extended-spectrum β-lactamases (ESBLs). ESBL producing *E. coli* are increasing dramatically nowadays and have become a public health concern due to limited antibiotic options.
The aim of our study was to characterize ESBL producing *E. coli* in the Tyrol by investigating clonality via two different molecular typing methods and by determining the β-lactamase profile.
Methods: One hundred twenty two ESBL producing *E. coli*, mostly from urine samples (n = 97), from patients of the University hospital Innsbruck (n = 60), district hospitals (n = 20) and practitioners (n = 41) in the Tyrol (Austria) were collected from January to April 2009. Molecular comparison of the ESBL producing *E. coli* strains was done by repetitive PCR method (repPCR) (DiversiLab™) and Pulsed-Field Gel-Electrophoresis (PFGE). Serotyping of representative strains was performed according to the method of Ørskov & Ørskov. Characterisation of β-lactamases was done by microarray analysis (Identibac®).
Results: Among 121 ESBL producing strains repPCR revealed one main clone comprising 99 isolates, twenty-two isolates showed different band patterns. PFGE corroborated these finding, but was even more discriminatory and could distinguish within the main clone, consisting of 90 of the 99 repPCR main clone isolates, between two subclones with subclone I consisting of 37 isolates and subclone II of 53 isolates. Serotyping of representative strains of both subclones showed, that they all belonged to the serotype O25:H4. Data of microarray revealed different combinations of β-lactamases in ESBL producing strains of the main clone with TEM and CTX-M-type β-lactamases being most prevalent.
Conclusion: ESBL producing *E. coli* in patients from hospitals and outpatients in the Tyrol show clonal distribution. Thus transmission of the same ESBL producing strain among patients might be the reason for the dramatic increase in this region in recent time.

P719 Variability and stability of bacteriologic and molecular traits within *Escherichia coli* isolates of clone ST131

O. Boulet, L. Landraud, E. Frapy, V. Leflon-Guibout, E. Marcon, X. Nassif, M. Nicolas-Chanoine (Clichy, Nice, Paris, FR)*

Objective: *E. coli* phylogenetic group B2 clone ST131 producing CTX-M-15 has been reported worldwide. However, isolates non extended-spectrum β-lactamase (ESBL) producers have also been identified recently. The purpose of this study was to compare different bacteriologic and molecular traits in CTX-M-15- and non ESBL-producing isolates of clone ST131.
Methods: Fifteen isolates [3 CTX-M-15 producers from the international collection (IC) previously published, 7 from stools (S) of healthy subjects and 5 from bacteremia (B)] were studied. Antibiotic susceptibility [amoxicillin (AMX), nalidixic acid (NAL), cotrimoxazole (SXT) and fosfomycin (FOS)] was determined by the agar diffusion method. β-lactamase content and mutations in gyrA and parC were determined by PCR and sequencing. Biofilm production was quantified by crystal violet assay. Virulence factor (VF) genes were identified by PCR and the presence of 2289 sequences issued from the flexible gene pool of 2 UPEC reference strains, strain RS218 and strain K2 were analyzed by macroarray.
Results: Of the 15 isolates, 1 (S) was susceptible to AMX, 5 (3 S, 2 B) to NAL, 10 (2 IC, 6 S, 2 B) to SXT and all but one (IC) to FOS. Five isolates (3 IC, 2 B) had CTX-M-15 with the chromosomal cephalosporinase hyper-production in 1 case. Eight (5 S, 3 B) isolates had TEM-1 and 1 (S) OXA-1. NAL resistance was related to GyrA substitutions S82L-D87N alone in 3 S isolates and associated with ParC substitutions S80I-E84V in 7 isolates (3 IC, 1 S, 3 B). Rate of biofilm production varied from 0.02 to 2.2. All isolates but one (B) had no adhesin coded by pap genes and no hemolysin gene but all had fimH and 2 siderophore related genes. Macroarray confirmed the small number of VF in all isolates (about 33% of the 204 VF sequences tested) whereas it showed a higher proportion of the other sequences tested (more than 50% of the structure proteins, IS/transposases, and metabolic enzymes tested).
Conclusions: Isolates of clone ST131 show variable bacteriologic traits such as antibiotic susceptibility, β-lactamase content, biofilm production. Inversely, their genetic background evaluated on more than 2200 gene sequences shows a certain stability. A noticeable common trait of these isolates is the small number of VF genes in comparison with strains of group B2 and the absence of pap and cnf1 genes in the great majority of them.

P720 Phenotypic and genotypic analysis of a novel ESBL phenotype (cefepimase)

F. Boyle, D. Morris, S. Kariuki, G. Revathi, G. Healey, M. Cormican (Galway, IE; Kenya, KE)*

Objectives: We have reported a novel ESBL phenotype (cefepimase) characterised by greater resistance to cefepime (FEP) than to cefotaxime with significant inhibition by clavulanic acid (CA). This phenotype was observed in 19 isolates of S. Typhimurium − 17 from patients in Kenya, 1 from an Irish patient and 1 from a patient from Malawi. The isolates have β-lactamases with isoelectric points (pI's) of 5.2 and 7.2. BlaTEM-1, blaOXA-1 and ISCR1 were identified on an IncHI2/ IncW multi replicon 39kb plasmid (pFEP39) present in all isolates. All isolates harboured a c. 2kb Class 1 integron (C1I). Transfer of the phenotype by electroporation of plasmid pFEP39 to S. Dublin was achieved.
Methods: Shot gun cloning of BamH1/ Xba1 digested 39kb plasmid (pFEP39) into the corresponding sites of pBK-CMV was carried out and transformed into Top10 cells selected on FEP containing agar. Sequencing of a c. 5kb insert from recombinant plasmid (pFEP39−1dr) was performed using T3 and T7 primers which flanked the multiple cloning site of the pBK-CMV expression vector, which was carried out on an ABI 3730 capillary sequencer by Sequiserve, Vaterstetten, Germany.
Results: Shotgun cloning resulted in a c. 5kb insert (pFEP39−1dr) conferring resistance to cefepime (MIC 16μg/ml) with significant inhibition by CA (MIC 0.38 μg/ml) compared to a FEP MIC of <0.25μg/ml for native Top10 cells. Sequencing results identified a Pant/Pc (TGGACA17TAAGCT, −35/−10 sequence), promoter combination directly upstream of blaOXA-1 in the c. 5kb insert, downstream of which there was an activated P2 promoter combination (TTGTTA17TACAGT −35/−10 sequence) due to the presence of a 'GGG' triplet immediately upstream of the −10 signal within the 17 nucleotide spacer region. No other β-lactamases identified in the insert.
Conclusions: OXA-1 is reported as having capacity to hydrolyse cefepime but is not inhibitable by clavulanic acid. However the "cefepimase" phenotype inhibited by clavulanic acid is transferred with a plasmid construct in which blaOXA-1 (with the hybrid Pant/c/P2 promoter immediately upstream) is the only β-lactamase gene present. This promoter combination has been shown to dramatically increase expression of the proximal gene in the cassette.

P721 Mutational changes in different CTX-M β-lactamases able to confer resistance to amoxicillin–clavulanate combination

A. Ripoll, M. Rodríguez-Domínguez, A. Novais, M.C. Turrientes, F. Baquero, R. Cantón, J.C. Galán (Madrid, ES)*

Objectives: The aim of this work is to test the possibility of emergence of mutational resistance to amoxicillin–clavulanate (AMC) combination in CTX-M β-lactamases in an *in vitro* evolution assay using an Eschericha coli hypermutable strain carrying different blaCTX-M genes and challenged by increasing AMC concentrations.
Methods: Six different blaCTX-M genes (blaCTX-M-1; -2; -3; -9; -10; and -14) representing the three more frequent groups of CTX-M (CTX-M-1; CTX-M-2; and CTX-M-9) were cloned in the same plasmid vector (pBG18) and expressed in a non-mutator strain (MI1443, ampC defective) and its isogenic strong-mutator derivative GB20 (MI1443 mutS::Tn10). GB20 carrying the different blaCTX-M genes, was submitted to increasing concentrations of amoxicillin (1–128 mg/L) and a fixed concentration of clavulanate (2 mg/L) in 12 replicate cultures. Then, cultures growing at 128/2 were exposed to growing concentrations of clavulanate (128/2 to 128/64). Plasmid pools from 53 independent cultures at different AMC concentrations (128/64, 128/8 and 64/2 mg/l) were extracted, transformed into MI1443 using as selector agent 2x the original MIC to AMC. Finally 158 clones were selected, retransformed and sequenced. MIC for AMC, ampicillin, cefuroxime, cefotaxime, ceftazidime, cefepime and imipenem were determined for each wild-type β-lactamase and their derivative mutants according CLSI guideline.
Results: Three types of AMC-resistant mutants were obtained: i) at 128/64 mg/L a single S130G change was only found in CTX-M-1; ii) at 128/8 mg/L S130G and K234R changes were detected as single changes in CTX-M-1 and CTX-M-9 groups; iii) at 64/2 mg/L three independent mutants were detected, containing S130G and S237G in CTX-M-1 group or S130G and K234R in CTX-M-9 group. For each CTX-M group, two independent mutants were obtained, except in CTX-M-2, that not yielded any AMC-resistant mutant in this assay. S130G mutation increased 8–32 times the MIC to AMC and was detected in 88% of all AMC-resistant mutants. This mutation drastically decreased cephalosporin resistance. K234R and S237G mutations conferred increased to 4x MIC to AMC, although S237G change not affect to activity against cephalosporins.
Conclusion: Reduced susceptibility to AMC is shown to be caused by S130G, S237G, and K234R mutations in CTX-M β-lactamases. CTX-M-1 group might be prone to produce AMC-resistant variants than other groups.

P722 Characterization of the new inhibitor-resistant SHV-107 β-lactamase in a clinical isolate of *Klebsiella pneumoniae*

V. Manageiro, L. Albuquerque, M. Caniça (Lisbon, Coimbra, PT)*

Objectives: β-lactamase inhibition is used as clinically strategy to fight the problem of β-lactam resistance. However, more than 30 inhibitor resistant (IR) β-lactamases from TEM and SHV families have been detected so far. In this study we performed a phenotypic, molecular,

and biochemical characterization of the IR SHV-107 carrying the amino acid substitution Thr235Ala.
Methods: *K. pneumoniae* INSRA6884 was isolated from sputum of a 92 years old female in an internal medicine service of a Portuguese hospital, in 2006. Antimicrobial susceptibility was determined by an agar dilution method. β-lactamase encoding genes were screened by PCR and identified, using specific primers. Transformant *E. coli* BL21 (DE3) harbouring the recombinant SHV-encoding plasmid was obtained by electroporation. SHV-107 was extracted and purified by ion exchange and gel filtration. The kinetic constants were obtained by a computerized microacidimetric method. Isoelectric point was estimated by isoelectrofocusing.
Results: The clinical *K. pneumoniae* strain exhibited non-susceptibility to all penicillins tested (MICs of 64 to >2048 ug/ml). The MICs of penicillins were weakly reduced by clavulanic acid (from 2048 to 512 ug/ml), while tazobactam totally restored the piperacillin susceptibility. Molecular characterisation identified a blaGES-7 gene, and the new blaSHV-107, which sequence presented, according to Ambler numbering, the amino acid substitutions L35Q and T235A, in relation with SHV-1. The *E. coli* BL21 (pBK-SHV-107) transformant, producing a β-lactamase with a pI of 7.6, exhibited a β-lactam resistance phenotype similar to that of the clinical strain with respect to penicillins and amoxicillin/clavulanic acid combination. The kinetic parameters of the purified SHV-107 enzyme revealed strong affinity to penicillins. However, catalytic efficiency for these antibiotics was lower for SHV-107 than for SHV-1. No hydrolysis was detected against extended-spectrum cephalosporins. The IC50 for SHV-107 was 1.53 uM for clavulanic acid, 9-fold higher than for SHV-1 (0.17 uM).
Conclusion: In this study we characterized a clinical *K. pneumoniae* strain co-expressing two β-lactamases: ESBL GES-7 and the new IR SHV-107. As evaluated by the kinetic data of SHV-107 β-lactamase, T235A mutation in SHV enzymes not only is responsible for the loss of the cephalosporinase activity, but is also important to the interaction with the β-lactamase inhibitors.

P723 PER-6, an expanded-spectrum β-lactamase from *Aeromonas allosaccharophila*

L. Poirel, D. Girlich, P. Nordmann (Le Kremlin Bicetre, FR)*

Objectives: Screening for potential expanded-spectrum β-lactamase (ESBL)-producing bacteria in aquatic environments.
Methods: Water samples (n = 3) were recovered in January 2009 from the Seine river, Paris, France, filtrated, resuspended in sterile water and then plated onto MacConkey agar plates containing ceftazidime (2 µg/ml). Isolates were identified by the API 32GN system and by sequencing the 16S rDNA genes. Cloning was performed by partial digestion of total DNA, followed by ligation into plasmid pBKCMV and expression into *E. coli* DH10B. Biochemical characterization was performed by UV spectrophotometry.
Results: *Aeromonas allosaccharophila* isolate AL-1, resistant to broad-spectrum cephalosporins but susceptible to cefoxitin and moxalactam, was recovered. A typical synergy image between discs containing clavulanic acid and ceftazidime suggested the production of an ESBL. Cloning and sequencing identified blaPER-6 encoding a novel ESBL, that was chromosomally-located. No integron nor transposon was identified in the close vicinity of the blaPER-6 gene. PER-6 possessed 86% and 92% amino acid identity with PER-1 and PER-2, respectively, known to be widespread among clinically-relevant Gram negative isolates (*P. aeruginosa*, *A. baumannii*...). PER-6 possessed hydrolytic properties of an ESBL, together with the ability to hydrolyse carbapenems (imipenem, meropenem, and ertapenem) slightly, whereas PER-1 and PER-2 did not. The identification downstream of both blaPER-6 and blaPER-1 genes of similar glutathion S-transferase encoding genes suggests a common ancestor origin for these two ESBL genes.
Conclusion: This study emphasises the spread of PER-type ESBLs in *Aeromonas* species. In addition, it indicates that a PER-type β-lactamase, through some specific amino acid substitutions, may possess a weak ability to hydrolyse carbapenems. Finally, the recent identification of PER-1 in *Aeromonas media* from a Swiss alpine lake together with that of PER-6 in *A. allosaccharophila* from the Seine river here underlines the spread of these PER-type determinants in the environment on the European continent, with a reservoir being likely a waterborne bacterial species.

P724 Detection of ESBL presence in carbapenem-resistant *K. pneumoniae* strains in the clinical laboratory

H. Moraitou, I. Galani, M. Makarona, A. Mavrommati, P. Tsarpali, P. Grillas, N. Soufla, A. Georgousi, V. Spyropoulos, S. Kanavaki (Athens, GR)*

Objectives: The aim of this study was to evaluate phenotypical methods of detection of ESBL presence in carbapenemase-producing *K. pneumoniae* isolates.
Materials and Methods: A total of 27 multidrug resistant *K. pneumoniae* strains were collected from January to September 2009, from various units of our Hospital. All isolates had a resistance phenotype compatible with carbapenemase production. Susceptibility testing was performed by Kirby Bauer, E-test and Vitek2 automated system, according to CLSI guidelines. All strains were further tested phenotypically for the production of metallo-β lactamase by imipenem/EDTA test and KPC by boronic acid/imipenem disc test. ESBL production was tested both by DDST (ceftazidime-CAZ, cefotaxime-CTX, aztreonam-ATM, ceftriaxone-CRO at 20 mm distance from a disk containing amoxicillin/clavulanate) and CLSI ESBL confirmatory test using both clavulanic acid (CA) and boronic acid (BA). The latter was performed by placing disks containing CAZ with or without CA, with the addition of a final amount of 400 micrograms BA on each disk. The test was considered positive when an increase in inhibitory zone around CAZ/CA disk was 5 mm or greater than CAZ alone. All strains were subjected to PCR for the detection of blaVIM, blaKPC and SHV-type genes.
Results: Molecular methods proved that 3/27 strains (11.1%) harbored blaVIM, 24/27 blaKPC (88.9%). SHV-type ESBLs were detected in all isolates.
DDST was positive in all 3 VIM(+) strains (specificity and sensitivity 100%), and in 6/24 (25.0%) of KPC(+) strains. The CLSI ESBL confirmatory test with CA and BA was negative in VIM(+) strains and positive in 23/24 KPC(+) strains (95.8%). As all *K. pneumoniae* harbored ESBLs, no false positive results were detected by phenotypic methods.
Conclusions: Phenotypical methods of detection of resistance mechanisms are of great importance in a routine clinical microbiology laboratory, where no molecular confirmation methods are any time available. Our results show that in the case of multidrug resistant *K. pneumoniae* isolates, both DDST and the CLSI ESBL confirmatory test with CA and BA can safely detect ESBL production. These tests are both included in our routine methodology, together with imipenem/EDTA test and imipenem/BA test, in order to rapidly identify resistance mechanisms and evaluate epidemiology of resistance in our Hospital.

P725 Molecular detection of SHV and AmpC (CITM, FOX)-type β-lactamase in clinical isolates of *Escherichia coli*

A. Sabbaghi, J. Fallah Mehrabadi, H. Molla agha Mirzaei, F. Fard Saneei, M.M. Soltan Dallal (Zanjan, Tehran, IR)*

Objective: Co-production of extended spectrum b-lactamase (ESBLs) and AmpC enzymes has been detected as one of the most important mechanism of resistance to b-lactam antibiotics among some pathogens especially *E. coli*. In recent years, the prevalence of b-lactamase producer organisms is increased and it led to a problem for diagnosis via Disk diffusion method. On the other hand, SHV, CITM and FOX genes have several subfamilies, and designing universal primers could be valuable to detect all of them. Therefore, the aim of this study was to detection of SHV and AmpC (CITM, FOX)-type b-lactamase genes by using specific primers through PCR.

Materials and Methods: More than 500 clinical samples were collected from hospitals of Tehran and 265 *E. coli* isolates were detected by standard biochemical tests such as IMVIC. Subsequently, these isolates were screened for β-lactamase production by Disk diffusion method and confirmatory test (Combined Disk). Resistant isolates was evaluated for molecular assessing with PCR.
Results: In Disk Agar Diffusion test, 128 (64%) *E. coli* isolates which resistant to ceftazidime and cefotaxim were selected and followed by Combined Disk (ceftazidime, cefotaxim and clavulanic acid) assay. In Combined Disk, among 128 screened isolates, 115(89.8%) and 13 (10.2%) isolates were as ESBLs and AmpC producers, respectively. PCR was performed on all 128 resistant isolates and results were showed among 115 and 13 isolates, 7 (6.1%) and 13 (100%) to have bla SHV and bla CITM, respectively. Fox Gene was not detected in any samples.
Conclusion: According to the recommendation of the Clinical and Laboratory Standards Institute (CLSI), isolates which showed negative confirmatory test are potentially producer of AmpC. This survey improved CLSI recommendation because among 13 AmpC producer isolates, the result of CITM PCR was 100% positive. SHV gene was detected in just 6.1% of isolates and others β-lactamase genes may be have a role in b-lactam antibiotics along with other isolates.

AmpC β-lactamases and carbapenemases in Enterobacteriaceae

P726 Inhibitor-based methods using boronic acid compounds for detection of AmpC β-lactamases and extended-spectrum β-lactamases-producing *Escherichia coli* and *Klebsiella* spp.

N. Yilmaz, N. Agus, E. Bozcal, O. Oner, A. Uzel (Izmir, TR)*

Objectives: The increasing prevalence of extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC) enzyme is of clinical concern. Methods for the identification of strains that produce ESBL have been developed and applied for routine use, but no practical methods for identification of pAmpC β-lactamases producers have been established to date. It has been stated that the AmpC β-lactamases when present along with ESBL can mask the phenotype of the latter. We evaluated highly sensitive methods using boronic acid (BA), an AmpC enzyme inhibitor, to detect ESBL and p-AmpC production
Material and Methods: A total of 273 clinical isolates of *Klebsiella* spp. (n: 82) and *Escherichia coli* (n:191) were analyzed. Antibiotic susceptibility testing was performed by the Kirby-Bauer method according to CLSI protocols. An organism demonstrating a zone diameter around the cefoxitin (FOX), and/or ceftazidime (CAZ), and/or cefotaxime (CTX) disk containing with BA $\geqslant$5 mm than containing alone these disks were considered an AmpC producer (BA test). The CLSI confirmatory method using CAZ and CTX disks with and without clavulanic acid (CLA) was also carried out. Similar to the CLSI confirmatory test, an organism exhibiting $\geqslant$5 mm zone size increase around the CAZ-CLA or CTX-CLA disk combination with BA compared to the CAZ or CTX disk alone was considered positive for ESBL production (CLSI confirmatory test with BA).
Results: A total of 102 isolates harbored only ESBLs, 112 harbored both ESBLs and pAmpC, and 17 harbored only pAmpC enzyme. Sixty-six of the 127 FOX resistant clinical isolates yielded positive AmpC by BA test. The majority of AmpC isolates co-produced (84%) ESBL enzyme. The CLSI confirmatory test detected 215 (78%) ESBL-positive isolates. In the test with both CLA and BA detected 228 ESBL-positive (83%) isolates. In this test population 11 (65%), 10 (59%), and 12 (70%) AmpC producing clinical isolates were susceptible by routine testing to CAZ, CTX and cefepime, respectively.
Conclusion: The exact detection of ESBLs and pAmpC in isolates is important for both treatment and epidemiology. The BA disk test accurately detects the isolates that harbor both AmpCs and ESBLs. In particular, the method allowed detection of an ESBL even when potentially masked by a pAmpC.

P727 Prevalence and molecular characterization of plasmid-mediated AmpC β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella* spp. in Rhone-Alpes area, France

Z. Driche, H. Salord, A. Carricajo, M. Perouse de Montclos, O. Raulin, C. Roure-Sobas, F. Laurent, S. Tigaud (Lyon, Saint Etienne, FR)*

Objectives: The aim of this study was i) to investigate the prevalence of plasmid-mediated AmpC β-lactamase in *E. coli* and *Klebsiella* spp from five different hospitals in Rhone-Alpes area, France, ii) to determine which types of plasmid-mediated AmpC-β-lactamases were harbored.
Methods: Between January 1st and May 31, 2009, a total of 3460 consecutive clinical isolates of *E. coli* and *Klebsiella* spp were screened for resistance to cefoxitin (I or R) by phenotypic methods. A method using a double diffusion test on cloxacillin plates was used to screen isolates possibly harbouring the plasmid-mediated AmpC β-lactamase. The plasmid-mediated resistance determinants in these isolates were screened by multiplex PCR (CMY, DHA, MOX, EBC, FOX, ACC). When positive, sequencing was performed to accurately identify the genes.
Results: A total of 205 *E. coli* and 54 *Klebsiella* spp cefoxitin-R or I isolates were initially included. Seventy-eight isolates were selected by the cloxacillin methods and in 26 of those isolates (*E. coli*, n = 22; *Klebsiella* spp, n = 4) a plasmid-mediated AmpC β-lactamase was detected by PCR (9.6%). In *E. coli* (n = 22), we detected 20 CMY-2 or CMY-2 like AmpC β-lactamase, one ACT-6 and one DHA-1. In *Klebsiella* spp (n = 4), we detected were detected two DHA-1, one ACT-6 and one ACT-1.
Conclusion: Our study demonstrated the occurrence (0.75% prevalence) of plasmid-mediated AmpC β-lactamases in *E. coli* and *Klebsiella* spp in Rhône Alpes area. The CMY-2 AmpC β-lactamase types in *E. coli* are the most prevalent and this may be the first evidence of a dissemination of this threatening mechanism. On the contrary, *Klebsiella* genus seemed to be more rare but more diverse. Hence, continuous surveillance of the prevalence and evolution of emerging AmpC β-lactamase is of major concern.

P728 Multilocus sequence typing of DHA-1-producing *Klebsiella pneumoniae* isolates in a Spanish hospital

K. Diestra, E. Miró, C. Martí, D. Navarro, J. Cuquet, P. Coll, F. Navarro (Barcelona, ES)*

Objective: *Klebsiella pneumoniae* is an important opportunistic pathogen with the ability to spread rapidly and cause nosocomial outbreaks. The objective of this study was to investigate the molecular epidemiology of *K. pneumoniae* isolates with plasmid mediated AmpC type cephalosporinase that were isolated in a Spanish hospital.
Methods: A total of 26 *K. pneumoniae* isolates with induced resistance to extended spectrum cephalosporinase were cultured from patients hospitalized in the Granollers Hospital (Barcelona, Spain), between June 2007 and January 2008. bla genes were characterized by multiplex PCR and sequencing. Isolates were typed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) according to the Institut Pasteur scheme for *K. pneumoniae*. Only one isolate of each PFGE pattern was selected for MLST.
Results: PCR and sequencing studies revealed that the bla gene encoding the DHA-1 AmpC-like β-lactamase was present in all 26 isolates. Three of them had also the blaCTX-M-15 ESBL gene. The 26 isolates were classified into 11 PFGE patterns and five clusters: one cluster with seven isolates (A), one with four isolates (B) and three clusters with three strains (C, E and J).
By MLST the clusters A, B, and C were classified to sequence type ST17, cluster J (carrying both blaCTX-M-15 and blaDHA-1 genes) to ST326 and cluster E to a novel ST. Others PFGE patterns corresponded to ST13, ST37 and four others new STs (numbers pending from Institut Pasteur MLST Database).
ST326 carrying a CTX-M-15 ESBL alone was previously found in two different hospitals from Barcelona.

Conclusion: This study reports the first detection and characterization of ST for *K. pneumoniae* carrying blaDHA-1 gene in Spain. *K. pneumoniae* ST326 seems to have acquired blaDHA-1, blaCTX-M-15 or both genes in a limited period of time and geographical area.

P729 Plasmids carrying AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes: findings from a Spanish hospital, 1999–2007

C. Mata, E. Miró, B. Mirelis, P. Coll, F. Navarro (Barcelona, ES)*

Objective: This study aimed to determine the plasmid families involved in the dissemination of ampC genes.
Methods: In a study conducted at a Spanish hospital from 1999 to 2007 we found a total of 117 Enterobacteriaceae with acquired ampC genes among 27,119 isolates lacking inducible chromosomal ampC genes.
Plasmid analysis was carried out in the transconjugants, when possible, or in the donors' strains by PCR-based replicon typing, S1-PFGE analysis and Southern hybridization. Plasmid sizes were calculated by using Fingerprinting II Informatix™ software.
Results: Among the 117 isolates studied, 97 transconjugants were obtained (83%). Southern hybridization analysis was only possible in 94 strains, and in 8 strains a chromosomal location was suggested. The remaining 15 were unresolved, 3 of them being lysed. The 94 solved strains showed that ampC genes were located on large plasmids of different sizes. The predominant plasmids present in the 78 strains harbouring CMY-2 or CMY-2 plus an ESBL belonged to: A/C (33.3%: plasmid sizes ranged from 150 to 170kb in 66.7% of cases), I1 (20.5%: sizes from 82 to 100kb in 81.2%) and K/B (10.3%: from 81 to 86kb in 81.2%). bla(CMY-2) gene was also found in multireplicon plasmids (12.8%) and in one case in an IncF group (1.3%). However, all but one of the plasmids associated with DHA-1 or DHA-1 plus ESBL belonged to the L/M group. In most cases, L/M was the only replicon present (63.3%: with plasmids ranging in size from 70 to 80kb in 55.5% of cases) or forming multireplicon plasmids (20%). Genes codifying ACC-1, CMY-4 and CMY-27 were harboured by I1, IncF group or non-typeable plasmids while a chromosomal location was suggested in CMY-25 and CMY-40 (Table 1).

Table 1. Plasmid families associated with *ampC* genes in Enterobacteriaceae lacking inducible chromosomal *ampC* genes:

Enzymes (No.)	Replicons[a]	Species (No.)
CMY-2 / CMY-2+ESBL (78)	A/C	*E. coli* (15), *K. pneumoniae* (2), *P. mirabilis* (8), *S. enterica* (1)
	I1	*E. coli* (13), *P. mirabilis* (2), *S. enterica* (1)
	K/B	*E. coli* (7), *P. mirabilis* (1)
	I1-K/B	*E. coli* (3)
	I1-F	*E. coli* (3)
	A/C-F	*E. coli* (3)
	A/C-I1	*E. coli* (1)
	F	*E. coli* (1)
	CL	*P. mirabilis* (4)
	NS	*E. coli* (4), *K. pneumoniae* (1), *P. mirabilis* (6)
	L	*E. coli* (2)
DHA-1 / DHA-1+ESBL (30)	L/M	*K. pneumoniae* (8), *E. coli* (7), *K. oxytoca* (3), *P. mirabilis* (1)
	L/M-F	*E. coli* (2), *K. pneumoniae* (1), *K. oxytoca* (1)
	N	*E. coli* (1)
	L/M-N	*P. mirabilis* (1)
	L/M-NT	*K. pneumoniae* (1)
	NS	*E. coli* (2)
	CL	*E. coli* (1)
	L	*E. coli* (1)
ACC-1 (3)	I1	*E. coli* (1)
	NT	*E. coli* (1), *K. pneumoniae* (1)
CMY-4 (2)	F	*E. coli* (1)
	NT	*E. coli* (1)
CMY-27 (2)	F	*E. coli* (1)
	NT	*E. coli* (1)
CMY-25 and CMY-40 (2)	CL	*K. pneumoniae* (1), *E. coli* (1)

[a]F: includes one or more rep of the IncF group; CL: possible chromosomal location; NS: not solved; L: lysed; NT: not typeable by the PCR-based replicon typing described by Carattoli et al.

Conclusions: Genes were mobilized by conjugation in 83% of strains with acquired ampC genes. The results showed a relationship between each ampC gene and the plasmid involved. Plasmids belonging to the A/C, I1 and K/B incompatibility group were the most prevalent plasmids carrying the bla(CMY-2) gene, while plasmids of the L/M incompatibility group were associated to the bla(DHA-1) gene. In addition, ampC genes were located on large plasmids of different sizes, the incompatibility group A/C being the most heterogeneous group. The present study corroborates that vectors involved in the mobilization of ampC genes have a great ability to spread between species.

P730 Plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes: prevalences at a university hospital, Basel, Switzerland 2006–2009

H. Adler, D. Hohler, E. Schultheiss, R. Frei (Basel, CH)*

Objectives: Enterobacteriaceae producing plasmid-mediated AmpC β-lactamases have emerged worldwide. However, European data on prevalence and development over years are still rare. The aim of the study was to determine the prevalence of plasmid-mediated AmpC β-lactamases in clinical isolates of Enterobacteriaceae naturally lacking an inducible chromosomal ampC gene (i.e. *Klebsiella* spp., *Proteus mirabilis*, *Salmonella enterica*, *Shigella* spp. and *E. coli*).
Methods: Between January 2006 and August 31, 2009 a total of 10,777 consecutive, non repetitive clinical isolates of Enterobacteriaceae were screened for ESBLs. Using CLSI procedures, at least 3 of the following substances were tested: ceftazidime, cefpodoxime, aztreonam, ceftriaxone or cefotaxime. In a second step, organisms naturally lacking an inducible chromosomal ampC gene that were positive in the initial screen assay were tested for resistance to cefoxitin. Cefoxitin resistant isolates were considered suspicious for possessing a plasmid-mediated AmpC β-lactamase. They were subsequently tested by ampC multiplex PCR (Perez-Perez and Hanson, 2002, J Clin Microbiol 40:2153–62) and the amplicons were sequenced.
Results: In the years 2006, 2007, 2008 and in the first 8 months of 2009, prevalences of plasmid-mediated AmpC β-lactamases were 0.17%, 0.18%, 0.24% and 0.23%, respectively. Results are shown in table 1. Molecular types were as follows: 4 CMY-2 in 2006, 4 CMY-2 and 1 DHA-1 in 2007, 4 CMY-2 and 3 DHA-1 in 2008, 3 CMY-2, 2 DHA-1 and one ACC-1 in 2009.
Conclusion: The prevalence of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking an inducible chromosomal ampC gene has remained very low. However, between 2006 and 2009 the diversity of molecular types increased.

Table 1: Prevalence of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal *ampC* genes

Organism	Organisms with plasmid-mediated AmpC/Organisms without plasmid-mediated AmpC (%)				
	2006	2007	2008	2009*	2006–2009*
Escherichia coli	4/1776 (0.23)	4/2124 (0.19)	5/2202 (0.23)	3/1977 (0.15)	16/8079 (0.20)
Proteus mirabilis	0/115 (0)	0/132 (0)	0/142 (0)	1/148 (0.68)	1/537 (0.19)
Klebsiella spp.	0/484 (0)	1/561 (0.18)	2/562 (0.36)	2/514 (0.39)	5/2121 (0.24)
Salmonella	0/7 (0)	0/6 (0)	0/10 (0)	0/3 (0)	0/26 (0)
Shigella spp.	0/1 (0)	0/2 (0)	0/7 (0)	0/4 (0)	0/14 (0)
All	4/2383 (0.17)	5/2825 (0.18)	7/2923 (0.24)	6/2646 (0.23)	22/10,777 (0.2)

*January 1 to August 31, 2009.

P731 The sequence type ST131 covers half of the ESBL-producing *Escherichia coli* strains in Southwest Finland

J. Jalava, K. Vuorenoja, H. Marttila, A. Hakanen, O. Meurman, K. Rantakokko-Jalava (Turku, FI)*

Objectives: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strains are increasing rapidly in Southwest Finland. The purpose of this work was to study the role of internationally recognized ESBL-producing clones, like *E. coli* ST131, in the emergence of ESBL-positive strains.
Methods: Altogether 58 ESBL-producing *E. coli* strains isolated between the years 2004 and 2008 were typed with repetitive sequence-based PCR assay (rep-PCR) using DiversiLab system. A portion of the strains analysed with the rep-PCR were further analysed with MLST in order to identify the sequence type of rep-PCR clusters. The most important ESBL genes (CTX-M, TEM and SHV) were detected by PCR.
Results: In this study a cluster was defined as strains that showed more than 96% similarity in rep-PCR. Altogether 24 rep-PCR clusters were identified. The biggest rep-PCR cluster included 28 (48%) strains and the relative proportion of this cluster increased during the study period. Eight

strains belonging to this rep-PCR cluster were typed with MLST and they all were of sequence type 131. Twenty-four (86%), three (11%) and one (3%) of these strains had CTX-M-1-type, CTX-M-9-type and SHV-type ESBL gene, respectively. Each of the other rep-PCR clusters covered only 2 to 5% of all isolates. Thus far MLST has been performed for eight strains belonging to seven different minor REP-PCR clusters and seven different sequence types have been identified. Among these CTX-M-1, CTX-M-9, CTX-M-2, and SHV-type ESBL genes were detected in 13 (43%), 10 (33%), 1 (3%) and 6 (20%) strains, respectively.
Conclusions: When studied with rep-PCR and MLST, ESBL-producing *E. coli* strains in Southwest Finland showed polyclonal origin. However, also a single rep-PCR cluster covering almost half of the strains was identified. This large rep-PCR cluster equals to *E. coli* sequence type ST131, which is known to be widely spread. A great majority of these strains had CTX-M-1-type ESBL-genes, and although CTX-M-9-type and SHV-type genes were also detected, the distribution of ESBL genes differed in this main clone compared to minor clones. Our data indicates that the spread of ESBL-carrying clone ST131 plays a major role in the emergence of ESBL-positive strains in Southwest Finland. Further studies on the prevalence of ST131 strains among ESBL-negative isolates of clinical origin are also needed to confirm this finding.

P732 Prevalence study of β-lactamase genes TEM-1, SHV-1 and CTX-M in strains of extended-spectrum β-lactamases producing Enterobacteriaceae by multiplex PCR

M. Moosavian, B. Deiham (Ahvaz, IR)*

Objective: Study of antimicrobial resistance patterns of Enterobacteriaceae isolated from clinical samples to seven of different antibiotics during a 14 month period and also survey of Extended Spectrum β-Lactamases (ESBLs) phenotype production and TEM, SHV and CTX-M genes by Multiplex PCR method.
Methods: In this study 420 Enterobacteriaceae strains isolated from 8000 clinical specimens (except stool) were examined. The isolates were identified by biochemical tests and then primary antimicrobial susceptibility test was performed by Kirby-Bauer disk diffusion method. Seven antibiotic disks used in this test, such as Cefotaxime, Ceftriaxone, Ceftazidim, Cefepim, Cefepirom, Imipenem and Meropenem. ESBLs producing strains detected by phenotypic confirmatory test using single or combined Ceftazidim/ Cefotaxime – Clavulanic acid disks. Then DNA extraction was done by DNP kit procedure and finally presence of blaTEM, blaSHV and bla CTX_M genes were evaluated by Multiplex PCR Method.
Results: We identified 420 bacterial strains of Enterobacteriaceae which were isolated from clinical specimens, such as blood, wound and eye samples. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (Kl. Pneumoniae) were the most common isolates with frequency of 64.5 and 20 percent, respectively. Drug resistance patterns of these strains showed that resistance to Cefotaxime, Ceftriaxone, Ceftazidim, Cefepim, Cefepirom were 44, 44, 42, 39.5 and 39 percent, respectively. Confirmatory test showed 128 strains (30.5%) produced ESBLs, including *Klebsiella* 45.4 and *E. coli* 28.8 percent. Multiplex PCR of the genes among positive ESBLs bacteria (73 strains) showed TEM gene in 65.5% and SHV in 15% strains. Also, 14 isolates (19%) had both TEM & SHV β-lactamase genes, but CTX-M was not detected in isolated bacteria.
Conclusion: Results from our study showed that the prevalence of ESBLs, β-lactamase genes and antibiotic resistance patterns were noticeable among of Enterobacteriaceae isolates, especially *E. coli* and Kl. pneumoniae. So, we suggest that combined therapeutic regimens such as β-lactamase antibiotics and β-lactamase inhibitors or carbapenems be limited only to patients with serious infections and be designed based on the antibacterial susceptibility test.

P733 Identification of a blaCTX-M-15 duplication in a nosocomial *Escherichia coli* isolate from Germany

A. Cullik, W. Witte (Wernigerode, DE)*

Objectives: The CTX-M type enzymes are the most prevalent group of extended spectrum b-lactamases (ESBL) in Enterobacteriaceae. Especially blaCTX-M-15 and blaCTX-M-1 are spread worldwide which is due to horizontal transfer of the blaCTX-M genes by different plasmids. During distribution analysis of nosocomial ESBL-producing *E. coli* an isolate carrying two blaCTX-M-15 copies was found.
Methods: The plasmids of *E. coli* transconjugants obtained after conjugation on cefotaxime/sodium azide selection agar were checked by PCR sequencing of blaCTX-M genes and replicon typing. To investigate the closer blaCTX-M environment gene walking and Sanger sequencing of cloned PCR products were performed.
Results: The *E. coli* isolate 390/06 harboured blaCTX-M-15 on a plasmid of IncI1 group. Upstream of a blaCTX-M-15 gene an ISEcp1 remnant, disrupted by an intact IS26 was identified. A second blaCTX-M-15 copy was directly attached upstream of the IS26 element.
Conclusions: A tandem configuration of blaCTX-M-15 has not been reported so far. These two blaCTX-M-15 copies seem to lead to increased resistance rates and can easily be distributed by conjugation. Both are important criteria for the successful spread and stable inheritance of the blaCTX-M-15 gene in a population under selective antibiotic pressure.

P734 Identification of CMY-4 AmpC β-lactamase-producing *Proteus mirabilis* clinical strains in Bulgaria

S. Sabtcheva, T. Saga, T. Kantardjiev, M. Kaku (Sofia, BG; Sendai, JP)*

Objective: To characterize bla genes responsible for acquired AmpC-type β-lactamase resistance of *Proteus mirabilis* clinical isolates recovered in Bulgaria.
Methods: Four epidemiologically unrelated *Proteus mirabilis* strains, resistant to cephalosporins, cephamycins and β-lactam/β-lactamase inhibitor combinations, were isolated from urine specimens at a Bulgarian cancer hospital between 2000 and 2005. The disk potentiation test using a Kirby-Bauer cefotaxime disk in combination with 3-aminophenylboronic acid was performed to confirm the production of AmpC-type enzymes. Multiplex polymerase chain reaction (PCR) using six sets of ampC-specific primers (MOXMF/R, CITMF/R, DHAMF/R, ACCMF/R, EBCMF/R, FOXMF/R) followed by sequence analysis of PCR amplicons were used to identify ampC genes. Genotyping, by pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with SfiI was performed to determine genetic relatedness of isolates. The transferability of cefoxitin resistance was examined by conjugation. The genetic basis of AmpC determinants was study by using the endonuclease I-CeuI technique.
Results: CMY-4 AmpC-type β-lactamase was identified in all isolates. Genotyping showed that one of the isolates was genetically different, while the remaining three isolates were clonally related. The blaCMY-4 gene was not transferable to *Escherichia coli* by conjugation. In all isolates it was chromosomally located and associated with an ISEcp1-like element.
Conclusion: This is the first report of CMY-4-producing Enterobacteriaceae in Bulgaria and the first characterization of an acquired AmpC-type β-lactamase in Bulgarian isolates.

P735 Diversity of plasmid-mediated AmpC β-lactamases among clinical isolates of Enterobacteriaceae lacking inducible chromosomal ampC gene from Portuguese hospitals

V. Manageiro, D. Jones-Dias, G.A. Domingues, E. Ferreira, D. Louro, M. Caniça for the Antimicrobial Resistance Surveillance Program in Portugal (ARSIP)*

Objectives: In Portugal, little is known on plasmid-mediated AmpC β-lactamases. Hence, we purpose with this study to investigate plasmid-

mediated AmpC β-lactamases (PMAB) in clinical Enterobacteriaceae isolates lacking inducible chromosomal ampC genes in Portugal.
Methods: A total of 2576 clinical isolates of various species of Enterobacteriaceae lacking inducible chromosomal ampC genes were collected between January–June 1999 and March 2004–August 2009 from patients in Portuguese hospitals. Screening of antimicrobial susceptibility of clinical isolates was performed by disc diffusion method. Clinical isolates with resistance or with decreased susceptibility to cefoxitin (inhibition zone diameter <18 mm), showing synergism between boronic acid and clavulanic acid and/or between cloxacillin and ceftazidime (and/or cefoxitin) were considered presumptively PMAB. The presence of acquired ampC genes was confirmed by multiplex PCR. Positive results were sequenciated. Transconjugants were achieved by conjugation experiments.
Results: Among the 2576 isolates tested, 6.4% (109 *E. coli*, 43 *K. pneumoniae*, 11 *K. oxytoca,* 2 *P. mirabilis* and 1 *Pantoea agglomerans*) were assumed to be PMAB and were selected for further analysis. Seventy-six of the 166 positive isolates (45.8%) were confirmed to be PMAB-producers by multiplex PCR (52 blaDHA-, 24 blaCMY-2-, 5 blaMIR-, 1 blaACT- and 1 blaFOX-type genes). From that, 5 *E. coli* strains and 1 *P. mirabilis* co-produced more than one PMAB. The sequence of one CMY-type β-lactamase encoding gene differed from blaCMY-2 by nine mutations, leading to the A49T, R105S, R125T, H153R, T143A, Q213K, P208A, A236V and H262R amino acid substitutions. This new PMAB was designated CMY-46.
Conclusion: AmpC β-lactamases are a major group of enzymes of clinical importance (along with Class A β-lactamases). This study documents for the first time the occurrence and dissemination of these enzymes in Portuguese hospitals. As noticed in other countries, PMAB was described mostly in *K. pneumoniae* and *E. coli* isolates, with DHA-1 β-lactamase predominant, followed by CMY-2. Our work suggests that reduced susceptibility to cefoxitin in the Enterobacteriaceae may be an indicator of AmpC activity, but it should be confirmed by other tests, namely multiplex PCR. Infection with AmpC producing bacteria is of great concern, and detection of such enzymes is consequently of epidemiological importance.

P736 Emergence of KPC-producing *Klebsiella pneumoniae* in Germany

Y. Pfeifer, C. Wendt, R. Schiller, M. Höck, R. Podschun, T.A. Wichelhaus, K.P. Hunfeld, W. Witte (Wernigerode, Heidelberg, Berlin, Kiel, Frankfurt/M., DE)*

Objectives: The dissemination of multidrug-resistant *Klebsiella pneumoniae* producing KPC carbapenemase is an increasing problem worldwide. Since the first emergence of KPC in 2001 in New York (USA) these enzymes were described in *K. pneumoniae* from many countries like Israel, China, France, Italy, Greece and the UK. In 2008 the first isolates occurred in Germany. Here we report on molecular-epidemiological analysis of KPC-producing *K. pneumoniae* isolated in different German hospitals 2008–2009.
Methods: Twelve multidrug-resistant isolates of *K. pneumoniae* from seven different hospitals were analysed. Among them were two isolates from an outbreak in spring 2008 in one hospital.
PCR amplification and sequencing of various β-lactamase, carbapenemase and porin genes was performed. The clonal relatedness of all isolates was analysed by molecular typing using PFGE (Xba1 restriction).
Results: All above mentioned isolates were resistant to fluoroquinolones, sulfonamides and β-lactams including carbapenems. They remained susceptible only to gentamicin and colistin and showed intermedia MIC values for tigecyclin. PCR and sequencing revealed the presence of KPC-2 (n = 7) and KPC-3 (n = 5). All isolates contained SHV-11 and the KPC-3 producer additionally the TEM-1 β-lactamase. Sequence analysis of the porin genes showed in all isolates a typical g-insertion in the ompK35 gene leading to a premature stop of translation. The similarity of PFGE-macrorestriction patterns of the isolates were more than 80%. Three KPC-3 isolates from two hospitals located in the region were identical and they were closely related to two KPC-3 isolates from another region, respectively. The KPC-2 producing *K. pneumoniae* were closely related as well as probably related to the KPC-3 producers.
All outbreak isolates contained KPC-2. The index patient was found to be hospitalized in Greece before. Because of severe courses of infection and several cases of death strict precautions like isolation were implemented and a screening system was established in this hospital.
Conclusion: Infections with multidrug-resistant *K. pneumoniae* are still rare in Germany. Probably one or more strains were introduced from other countries and now occur sporadically in the whole of Germany. Because of limited therapeutic options a surveillance of multidrug-resistant Enterobacteriaceae is an urgent need to prevent further dissemination.

P737 Emergence and prevalence of KPC-producing *Klebsiella pneumoniae* isolates in a tertiary Greek hospital

S. Tsiplakou, A. Stylianakis, V. Papaioannou, F. Zouboulglou, D. Paraskevopoulou, P. Giakkoupi, A. Vatopoulos, A. Koutsoukou (Athens, GR)*

Objectives: KPC-producing *Klebsiella pneumoniae* isolates are increasingly encountered and result in carbapenem resistance, thereby posing a major problem for treating multidrug resistant isolates. The aim of the present study was to determine the emergence and spread of KPC producers in our hospital.
Methods: From 2006 to 2008 a total of 238 *Klebsiella pneumoniae* isolates exhibiting MIC values >1 mg/L to imipenem and/or meropenem were screened by the imipenem-EDTA MBL Etest and cloverleaf (modified Hodge) test. PCR analysis and sequencing was performed for those isolates that were phenotypically positive for carbapenemase production, yielded a positive cloverleaf test and were negative for metallo-β-lactamase (MBL) production. *Klebsiella pneumoniae* clinical isolates were collected from blood (55), urine (41), pus (58), catheters (49) and bronchial secretions (35) cultures of patients hospitalised in different wards of our hospital, mainly in ICUs, and were tested for susceptibility via the Vitek 2 system (Biomerieux, France) and Etest strips (AB Biodisc, Sweden).
Results: All isolates were multidrug resistant to β-lactams, especially to extended spectrum cephalosporins, quinolones and aminoglycosides, but the majority was susceptible to gentamicin, tetracycline, tigecycline and colistin. The MIC values of imipenem and meropenem ranged from susceptible to non-susceptible. The imipenem-EDTA MBL Etest was positive for MBL production for 181 isolates. PCR and sequencing revealed the presence of KPC-2 gene in 57 isolates. The distribution of isolates during the three-year period was the following: in 2006 all 80 isolates were MBL producers. In 2007 89 out of 90 isolates produced MBL and one was KPC positive. In 2008 12 out of 68 isolates produced MBL and 56 of 68 were KPC-producers.
Conclusions: Considering the fact that in the first two years of the study all carbapenem non susceptible isolates were MBL producers, the emergence and significant increase of KPC-producing *Klebsiella pneumoniae* isolates not previously identified in our hospital poses a significant problem for the treatment of serious infections.

P738 First clinical isolate of blaIMI-2-producing *Enterobacter asburiae* in France

G. Cuzon, D. Tandé, D. Le Nen, R. Le Berre, G. Le Lay, P. Nordmann, T. Naas (Le Kremlin Bicetre, Brest, FR)*

Objectives: Carbapenem resistance in Enterobacteriaceae is mainly related to acquired carbapenem-hydrolyzing β-lactamases (1). These β-lactamases can be either metallo β-lactamases (IMP, VIM), expanded-spectrum oxacillinases (OXA-48), or Ambler class A enzymes (NMCA, IMI, SME, GES, and KPC). Genes encoding IMI-2 β-lactamases were found on plasmids in *Enterobacter asburiae* from United States rivers and in one clinical isolate of *E. cloacae* from China. Here we describe a single clinical isolate of IMI-2-producing *E. asburiae.*

Methods: Bacterial isolates were characterized by standard biochemical methods, susceptibility testing (disk diffusion), by Pulsed Field Gel Electrophoresis, and plasmid analysis. Antibiotic resistance genes were sought by PCR and sequencing.
Results: A 23 year old man, was hospitalized for an open dislocation of the left index finger, subsequent to a tumble into a river. The wound was disinfected, the finger reinserted, and was discharged the next day with only local desinfections. Twenty days later he was rehospitalized for cutaneous necrosis of the scare, and an osteotitis requiring amputation of the left index finger. Bacterial cultures of pus, soft skin tissu and bone revealed the presence of *Aeromonas hydrophila* and *Enterobacter asburiae*. The patient was treated successfully with ceftazidime (one week) and ciprofloxacin for three months. *E. asburiae* isolate Bre-1 displayed reduced imipenem susceptibility, a synergy image between carbapenems and with clavulanate, and crude extracts displayed significant Imipenem hydrolysis. A clavulanic acid-inhibited β-lactamase IMI-2 was identified. The blaIMI-2 gene was located on a self-transferable 66-kb plasmid, of similar size as the one initially characterized by Aubron et al. (EID, 11, 2005). PCR analysis identified an upstream LysR-type regulator gene that explained inducibility of IMI-2 expression. Futhermore, the genetic environment of blaIMI-2 identified two IS2-like elements.
Conclusion: This is the first description of an inducible and plasmid-encoded IMI-2-producing *E. asburiae* clinical isolate. This study raises the question of the importance of this reservoir in Enterobacteriaceae as well as the origin of this plasmid-located carbapenemase gene that may be transferred among other enterobacterial pathogens.

P739 Prevalence of extended-spectrum β-lactamases and of carbapenemases in Gram-negative clinical isolates from Belgian ICUs

Y. Glupczynski, P. Bogaerts, T.D. Huang, C. Lammens, C. Berhin, J. Van Eldere, H. Goossens (Yvoir, Antwerp, Leuven, BE)*

Objectives: The objectives were to determine the prevalence and to characterize the genotypes of ESBLs and of carbapenemases in a collection of Gram-negative isolates recovered from patients hospitalized in ICU in 14 Belgian hospitals in 2008–2009.
Methods: Non-duplicate, consecutive Gram-negative clinical isolates were collected from patients hospitalized in ICUs for ⩾48 h and with a suspicion of deep-seated infections. MICs to 8 antimicrobials including extended spectrum cephalosporins and carbapenems were determined by microbroth dilution method according to CLSI guidelines. ESBLs were screened by double combination disk test on all Enterobacteriaceae (EB) isolates with ceftazidime (CAZ) MICs >1 mg/L and were characterized genotypically by IEF and by Ligase Detection Reaction and low-density DNA micro-array (Check-Points®, The Netherlands). CAZ-R non-ESBL *E. coli* and *K. pneumoniae* isolates were further analyzed by multiplex PCR for plasmidic AmpC coding genes. *Pseudomonas* and *Acinetobacter* spp. isolates with an imipenem (IMI) or meropenem (MERO) MIC ⩾8 mg/L and EB with IMI or MERO MIC ⩾1 mg/L were tested by PCR-sequencing for the presence of class A, B, and D carbapenemases.
Results: ESBLs were detected by double combination discs in 55/571 (9.6%) EB (range of ESBL by centre: 2.1–23.8%), being mostly found in *E. coli* (n = 24), *E. aerogenes* (n = 14) and in *E. cloacae* (n = 10). CTX-M-groups (mainly CTX-M-15 and CTX-M-9) accounted for nearly 50% of ESBLs followed by SHV (SHV-4, SHV-5/-12) and TEM types (TEM-24/TEM-52) in 30% and 20% of the isolates, respectively. Four plasmidic AmpC were detected in 3 *E. coli* (CMY-2) and 1 *K. pneumoniae* (DHA-1) strains. No carbapenemase was found in any of the 7 carbapenem-non susceptible EB isolates, while MBLs (VIM-2 like) were detected by PCR-sequencing in 5/174 (2.9%) *P. aeruginosa* and OXA-23 or OXA-40 carbapenemases were found in 3/21 (14.3%) *A. baumannii*.
Conclusion: The prevalence of ESBLs among EB averaged 10% in Belgian ICUs but showed wide variations between centres. *E. coli* accounted for more than 40% of all ESBL-producing EB and CTX-M types were the most prevalent. Acquired transferable carbapenemases were detected albeit at a low frequency in *Pseudomonas* spp (4 centres) and in *Acinetobacter* spp. (1 centre). Further epidemiological surveillance is needed to monitor trends in the resistance mechanisms of Gram-negative bacteria in Belgian ICUs.

P740 Further identification of *Klebsiella pneumoniae* producing carbapenem-hydrolyzing β-lactamase OXA-48 from Tunisia

G. Cuzon, T. Naas, A. Lesenne, M. Benhamou, P. Nordmann (Le Kremlin Bicetre, Antony, FR)*

Objectives: Emergence and dissemination of Enterobacteriaceae isolates harboring carbapenemases in various geographic regions represents a significant threat to the management of nosocomial infections. The Ambler class D OXA-48 β-lactamase has initially been identified from a carbapenem-resistant *K. pneumoniae* isolate from Istanbul, Turkey and subsequently from Lebanon, Belgium, UK, and Israel. Here, we report an OXA-48 producing *K. pneumoniae* isolate from Tunisia.
Methods: Bacterial isolates were characterized by standard biochemical methods, disc diffusion susceptibility testing, by Pulsed Field Gel Electrophoresis, and plasmid analysis, Carbapenemase expression was studied spectrophotometrically. Antibiotic resistance genes were sought by PCR and sequencing.
Results: On August 31, an 86-year-old man with severe chronic obstructive pulmonary disease, was hospitalized in Tunisia for fever, polypnea, and bronchiectasis. His state of health worsened and he was transferred to the Intensive Care Unit of the hospital Privé d'Antony (Paris Suburbs), France, for sepsis syndrome. Five days after imipenem treatment started, sputum and blood culture revealed an ESBL-producing *K. pneumoniae* HPA-1 with reduced susceptibility to imipenem. *K. pneumoniae* HPA-1 was resistant to expanded-spectrum cephalosporins, fluoroquinolones, rifampin, all aminoglycosides (except amikacin), tetracycline and to ertapenem, but was of intermediate susceptibility to meropenem and imipenem. The patient was successfully treated with amikacin. Crude β-lactamase extract of *K. pneumoniae* HPA-1 showed significant imipenem hydrolysis activity. PCR experiments followed by sequencing identified β-lactamase genes blaOXA-48, bla TEM-1, blaSHV-1 and blaCTX-M--15. Plasmid analysis revealed a ca. 70-kb self-conjugative plasmid harboring blaOXA-48. Two IS1999 elements were found surrounding the blaOXA-48 gene in a similar manner to that found in the prototype *K. pneumoniae* 11978 strain from Turkey.
Conclusion: This is the first evidence of OXA-48-mediated carbapenem-resistance in Enterobacteriaceae in Tunisia, and further evidence of spread of this novel and powerful resistance determinant in Mediterranean countries.

P741 Molecular characterization of the first carbapenem-resistant *Klebsiella pneumoniae* isolate Va22038 expressing blaOXA48 β-lactamase in Germany

J. Weinberg, A. Heisig, P. Heisig (Hamburg, DE)*

Objectives: The increasing frequency of Enterobacteriaceae resistant to carbapenems is a major cause of concern and demands detailed understanding of the molecular basis and epidemiology of carbapenem resistance. Thus, this work, aims at identifying the molecular mechanism(s) of carbapenem resistance in a clinical isolate of *Klebsiella pneumoniae* from Germany in 2004.
Methods: Clinical isolate *K. pneumoniae* Va22038 has been described [1]. Susceptibilities to antimicrobial drugs were determined as minimal inhibitory concentrations (MICs) according to guidelines of the CLSI. Plasmid-DNA was isolated by both, the Crosa method and alkaline lysis method and analysed after agarose gel electrophoresis. The outer membrane proteins (OMPs) of the clinical isolate were isolated by ultrasonic and sodium lauroyl sarcosine (sarcosyl) treatment, separated on an SDS-PAGE gel, stained and visualized by a coomassie brilliant

blue. Screening for genes encoding β-lactamases has been accomplished by gene family-specific PCRs.
Sequencing of respective amplicons has been done by means of dye-terminator sequencing.
Results: The MIC values of different β-lactam-antibiotics were elevated, including imipenem, ertapenem, and meropenem (16, 32, and 8, respectively). The plasmid analysis revealed a single 65kb plasmid. The OMP profile lacked characteristic bands of OmpK35 and OmpK36. A PCR using primers specific for blaOXA resulted in a characteristic 744nt fragment. DNA sequence analysis of an internal blaOXA-gene fragment yielded 100 per cent homology to that of blaOXA-48. Further sequence analysis of the adjacent regions of blaOXA-48 revealed two flanking putative transposase-genes.
Conclusion: This is the first report of a clinical isolate expressing the OXA-48 β-lactamase in Germany. The MIC profile of carbapenems indicates an interplay of this carbapenemase with a reduced expression of the major OMPs OmpK35 and OmpK36.
The blaOXA-48 gene location between two transposase genes implies that it is part of a composite transposon. This view is suppoorted by results of PCR fragment analysis and sequencing of the corresponding genetic context which resembles that of *K. pneumoniae* 11978, the first isolate reported to harbour an blaOXA-48 gene as part of the composite transposon Tn1999.

P742 First report of the co-existence of blaOXA-48 or blaOXA-48-like gene with blaNDM-1 in Enterobacteriaceae from India

K. Karthikeyan, M. Toleman, C.G. Giske, M. Thirunarayan, K. Kumaraswamy, N. Narayam, P. Krishnan, T. Walsh (Chennai, IN; Cardiff, UK; Stockholm, SE; Haryana, IN)*

Objectives: Carbapenemases are rapidly emerging in Enterobacteriaceae in India causing serious treatment problems. This is probably due to the combination of poor antibiotic prescription control, poor sanitation and a large population. This project was initiated to determine the mechanism responsible for the carbapenem resistance in four enteric clinical isolates obtained from the ICU of a tertiary care hospital in Chennai in 2009.
Methods: Speciation and antibiotic testing was determined using a Phoenix automated machine. Double disc synergy tests and Modified Hodge tests were used for detection of metallo-β-lactamases (MBL) and non-MBL carbapenemases, respectively. PCR screening involved using custom primers for all known carbapenemases. Transconjugations were performed using *Escherichia coli* J53 as the recipient. Pulsed field gel electrophoresis with partial S1 nuclease digestion and probing of agarose gels with radio-labeled 32P blaNDM-1 and blaOXA48 gene DNA probes was used to detect the genomic location of the respective carbapenemase genes. blaOXA-48 and blaNDM-1 genes were sequenced and their local genetic environment investigated with specific custom designed primers. Plasmid replicon typing was employed to determine the Inc types.
Results: The four isolates; 3 *Klebsiella pneumoniae* and an *E. coli* strain, displayed a multi-drug resistant profile including resistance to all the β-lactam antibiotics, aminoglycosides and quinolones and was only sensitive to tigecycline and colistin. All isolates gave positive DDST and MHT results and gave PCR products of expected sizes for both blaOXA-48 and blaNDM-1. In-gel hybridization of PFGE gels following partial S1 digestion determined that blaOXA-48 was found on 250kb plasmid, whereas blaNDM-1 was found on plasmids of varying sizes (50kb-145kb) in the different strains. Transconjugants harboured plasmids of identical size to the blaOXA-48 and blaNDM-1 plasmids and the blaNDM-1 plasmids were determined to be of rep A/C type. blaOXA-48 (100% ID) was found in the *E. coli* strain whereas a novel variant of the blaOXA-48 gene (94% ID and 4 amino acid substitutions) was found in the *Klebsiella* strains.
Conclusion: *K. pneumoniae* and *E. coli* isolates have been found in an ICU in Chennai that carry two carbapenemase genes giving very broad spectrum antibiotic resistance profiles. The emergence of these powerful resistance mechanisms in India is a cause for great concern as treatment options are virtually exhausted.

P743 First isolation of VIM-5 producing *Enterobacter cloacae* in Chennai, India

K. Karthikeyan, T. Walsh, M. Thirunarayan, P. Krishnan, M. Toleman (Chennai, IN; Cardiff, UK)*

Objectives: The emergence of enteric organisms harbouring carbapenemases is a significant threat in the control of hospital infections as carbapenems are the reserved for serious infections when other options are depleted. Two carbapenem resistant *Enterobacter cloacae* clinical isolates were obtained from the ICU of a tertiary care hospital in Chennai in 2009. This project was initiated to determine the mechanism responsible for the carbapenem resistance.
Methods: Speciation and susceptibility testing was determined by automated systems (Phoenix). The presence of a metallo-β-lactamase (MBL) was determined using the Hodge double disc synergy test (DDST). Genomic DNA was screened for known MBL genes by PCR using custom designed primers based on sequences of all known MBL genes. The local genetic locus of the blaVIM-5 gene was determined by PCR mapping and sequencing with primers designed to genes commonly found in the class1 integron and the transposon, Tn5090. The genetic location was further determined by pulsed field gel electrophoresis combined with S1 nuclease digestion and probing with radio-labelled DNA probes and a random flanking primer PCR approach. Transconjugation and transformation experiments utilized Escherichia. coli J53 and Top10 cells as recipients.
Results: Both isolates were classified as *E. cloacae* displayed a multi-drug resistant phenotype; were resistant to all β-lactams, aminoglycosides and quinolones and were sensitive to only tigecycline and colistin. The carbapenemase gene blaVIM-5 was found in an unusual Class 1 integron that lacked the usual 3′ conserved sequence and was found on a complete Tn5090-like transposon. Two copies of this transposon were found on a large 325kb plasmid. The transposons were inserted at two points within this plasmid both near resolvase genes. The integron variable region contained an additional aac6′II gene cassette in the second position. Transconjugants and transformants had similar resistance patterns and in both cases the blaVIM-5 gene was located on a plasmid of identical size. The frequency of transconjugation was low i.e. approximately 1X10–8.
Conclusion: This is the first report of VIM producing Enterobacteriaceae in India. MBL producing pathogens are emerging rapidly in India and their spread is likely to be facilitated by the large population, poor sanitary conditions and little or no antibiotic prescription control.

P744 High-resolution crystal structures of SFC-1 and acyl-intermediate in complex with meropenem: structural basis for carbapenemase activity in class A β-lactamases

F. Fonseca, A. Correia, J. Spencer (Aveiro, PT; Bristol, UK)*

Objectives: Carbapenems are resistant to hydrolysis by most β-lactamases and are used as last resort antibiotics in treatment of multidrug resistant bacteria. SFC-1 is a class A serine carbapenemase from *Serratia fonticola*, an environmental bacterium and occasional pathogen. Unlike the majority of such enzymes, SFC-1 also efficiently hydrolyzes third generation cephalosporins and is only weakly inhibited by clinically useful inhibitors. Here we present crystal structures of wild-type SFC-1 and of complexes of site-directed mutants with the carbapenem meropenem in unhydrolysed and acylenzyme intermediate forms.
Methods: S70A and E166A active site mutants were generated using the QuikChange Site-Directed Mutagenesis kit. SFC-1 and mutant proteins were purified from induced cultures of *E. coli* BL21 (DE3). Crystals of SFC-1 were obtained by hanging drop vapour diffusion from 18–22% w/v polyethylene glycol 3350, 0.2M sodium acetate. Crystals of SFC-1 mutants were obtained by seeding using crystals of the wild-type SFC-1. Acylenzyme-intermediates and unhydrolysed complexes were prepared by soaking crystals in the mother liquor solution containing meropenem and ethylene glycol as cryoprotectant. X-ray data were collected at SRS and DLS beamlines. Molecular replacement was performed with Phaser

using KPC-2 or wild-type structures as search models. Model building and refinement were carried out using CCP4.
Results: Wild-type SFC-1 crystallised in space group P21 with 2 molecules in the asymmetric unit. SFC-1 mutants crystallized in space group P212121 with 1 (E166A; acylenzyme) or 2 (S70A; substrate complex) molecules in the asymmetric unit. SFC-1 has the disulfide bond and other conserved features of the class A carbapenemase active-site. Meropenem is oriented in the active site by Thr-236 and Thr-238, placing it close to Ser-130 for proton transfer. In the carbapenem-inhibited class A enzymes (e.g. SHV-1) interaction with Arg-244 imposes a different orientation on bound meropenem, resulting in flexible binding at a distance from Ser-130, impairing proton transfer.
Conclusion: The overall structure of SFC-1 closely resembles those of other known class A β-lactamases. However, these first structures of a class A carbapenemase with bound carbapenem reveal that these enzymes change the orientation of bound meropenem to promote catalysis, without gross distortion of overall structure.

P745 Detection of AmpC producers: comparison and evaluation of phenotypic methods in *Klebsiella pneumoniae* clinical isolates

A. Paradela, S. Ferreira, E. Ramalheira, S. Mendo (Aveiro, PT)*

Objectives: This study aims to evaluate the performance of 3 phenotypic methods for AmpC detection in *K. pneumoniae*: cefoxitin Hodge-test, Induction test and Etest strips.
Methods: Strains resistant to extended-spectrum cephalosporins and/or broad spectrum penicillins and susceptible to carbapenems, were selected. *E. coli* ATCC 25922 and a *S. marcescens* were used as AmpC non-producer and producer, respectively. Strains were identified and antibiotic susceptibilities determined by Vitek2 system and Vitek2 AES (bioMérieux, Marcy L'Étoile, France). ESBL producers were confirmed by Etest™ (AB Biodisk) ESBL with CT/CTL, TZ/TZL and PM/PML strips, according to manufacturer's instructions. Additionally, all isolates were screened with Etest™ (AB Biodisk) AmpC strips CN/CNI. For the Hodge test, a Mueller–Hinton (MH) agar plate was swab inoculated with *E. coli* ATCC 25922 adjusted to 0.5 McFarland. A cefoxitin disk was placed at the center of the plate and the test strains were heavily streaked outwards from the disk. After incubation, the radius of the inhibition zone of the indicator strain, and the decreased radius of the inhibition zone along the growth of test strain were measured. In the induction test, aztreonam, cefotaxime and amoxicillin–clavulanic acid disks were placed 10 mm apart from margin to margin in a MH plate inoculated with the test strain. After incubation the plate was observed and the presence of a truncated inhibition zone was interpreted as a positive.
Results: 7 isolates were determined as ESBL positive and 5 negative by Vitek2 compact AES. The phenotype consistent with both ESBL and AmpC was detected in 2 isolates. 7 and 5 isolates were positive for AmpC production by strips CN/CNI strips and Induction test, respectively. Hodge test results showed 6 isolates were positive, whereas 1 isolate showed minimal distortion. All AmpC producers were found to be resistance to cefoxitin. Discrepancies between the different methods were observed in 3 isolates. These results were confirmed by PCR and nucleotide sequence determination.
Conclusion: Although cefoxitin sensitive AmpC producers have been reported, these results indicate that screening methods using cefoxitin are still useful standardized tests. Etest CN/CNI strips was an easy, reliable and rapid method of AmpC producers detection. These results highlight the need of implementation of routine screening for AmpC producers by phenotypic methods.

P746 New DNA microarray test for rapid detection of KPC, OXA-48, VIM, IMP carbapenemase and TEM, SHV, CTX-M ESBL-producers

T. Naas, G. Cuzon, S. Gardin, P. Nordmann (Le Kremlin Bicetre, FR)*

Background: Extended-spectrum β-lactamases (ESBLs) have rapidly emerged worldwide. More recently also various carbapenemases have spread in many regions. Rapid identification of bacteria expressing these enzymes therefore becomes more and more important. We evaluated a new qualitative *in vitro* molecular diagnostic test "Check-CARBA ESBL" for specific identification of TEM and SHV-type ESBLs, and detection of CTX-M, KPC, OXA-48, VIM, IMP producers.
Methods: We evaluated the new micro-array based "Check-CARBA ESBL" test (Check-Points, Wageningen, Netherlands) that employs highly specific DNA markers to identify the β-lactamase genes of TEM, SHV, CTX-M, KPC, OXA-48, VIM and IMP, and discriminates ESBL and non-ESBL TEM and SHV-genes. We used 67 well-characterized Gram-negative rods (Enterobacteriaceae, *Pseudomonas* sp., *Acinetobacter* sp.) expressing various β-lactamases: 4 SHV, 5 TEM, 6 CTX-M, 10 OXA-48, 20 KPC, 10 VIM-, 10 IMP. 32 wild-type isolates or isolates harbouring other β-lactamase genes were used as controls. Total DNAs were extracted using Qiagen DNA mini kit.
Results: The Check-CARBA ESBL assay allowed fast and unambiguous detection of all TEM, SHV and CTX-M ESBL genes present. In addition, the clinically most relevant carbapenemases were also reliably detected. The assay enabled easy distinction between non ESBL TEM and SHV variants and their ESBL derivatives. None of the other tested β-lactamase genes were detected (OXA-18, VEB, PER, GES) underlining a good specificity. The assay was performed with several enterobacterial isolates, but also on non-fermenting Gram negative rods, such as *P. aeruginosa* and *A. baumannii*. A 1/10 dilution of the total DNA was required for optimal results for non-fermenting isolates.
Conclusion: The Check-CARBA ESBL test represents a powerful high-throughput tool for rapid identification of carbapenemases and ESBLs in all the clinical isolates tested. Results were obtained within the same working day, allowing rapid implementation of isolation measures and appropriate antibiotic treatment.

MDR mechanisms in Enterobacteriaceae

P747 NaCl and clonazepam induce low-levels of multidrug resistance in *Escherichia coli* strains through sdiA and soxS overexpression

M.M. Tavío, V. Aquili, M. Perilli, G. Amicosante, G. Celenza, J.B. Poveda (Las Palmas G.C., ES; L'Aquila, IT)*

Objectives: Low-levels of multidrug resistance (LL MDR) can serve as intermediate steps for the development of increased clinically relevant resistances. The aim of this study was to analyze the induction of LL MDR by NaCl and clonazepam, which is one of the commonest benzodiazepines in clinical treatments, in *E. coli* strains and regulator genes involved. Results were compared with those previously described utilizing diazepam, another benzodiazepine, in order to explain LL MDR induced by non-antimicrobial drugs.
Methods: The effect of concentration ranges of NaCl (10.7–342 mM) and clonazepam (0.0002–0.0625 mM) on antimicrobial agent MICs, outer membrane protein expression analyzed by SDS-PAGE, 10% cyclohexane tolerance by liquid-medium assay, and transcript levels of acrB, marA, soxS, and sdiA genes by reverse transcription of total RNA and PCR of cDNA, were evaluated in the wild type AG100 strain and three susceptible *E. coli* clinical isolates. AG100 (induced with 5 mM salicylate or 0.2 mM paraquat) was used as control.
Results: LL MDR induced by NaCl or clonazepam in the four *E. coli* studied strains were characterized by OmpF loss, maintenance of bacterial growth in the presence of 10% cyclohexane, and increased transcript levels of acrB, sdiA, and soxS, but not marA. Such changes occurred in a reversible and concentration-dependent manner. Thus, the highest concentrations of NaCl or clonazepam that the studied strains tolerated, induced the highest cyclohexane tolerance levels and transcript levels of the analyzed genes, increasing 10.4–16 fold sdiA, 4.2–9 fold soxS, and 3.8–4.5 fold acrB compared with their respective transcript levels in non-induced AG100.
Conclusion: NaCl and clonazepam induced LL MDR, OmpF loss, increased cyclohexane tolerance, and overexpression of the RND transporter acrB, the quorum-sensing regulator sdiA, and soxS gene

in the four studied *E. coli* strains, in a reversible and concentration-dependent manner, fitting with those described for diazepam.

P748 Real-time PCR analyses of ramA expression in tigecycline non-susceptible isolates

M. Hackel, C. Lascols, S. Bouchillon, B. Johnson, D. Hoban, J. Johnson, R. Badal, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Objectives: Tigecycline is a synthetic analogue of the tetracyclines which exhibits significant antibacterial activity against Gram-negative and Gram-positive bacteria and is active against strains carrying either one or both of the two major forms of tetracycline resistance: tetracycline-specific efflux pumps and ribosomal protection. While *Klebsiella pneumoniae* (KPN) is generally susceptible to tigecycline, decreased susceptibility has been associated with the multidrug efflux pump AcrAB. RamA belongs to a family of transcriptional activators which are known to have a role in the up-regulation of this pump. Overexpression of ramA or its close homologs has been correlated with increased expression of the AcrAB efflux pump in several Gram-negative species. This study evaluates the expression of ramA in 42 tigecycline non-susceptible KPN isolates from the Tigecycline Evaluation Surveillance Trial (TEST).
Methods: MICs were performed by broth microdilution following CLSI guidelines. 42 clinical isolates with tigecycline MICs $\geqslant$8 mcg/ml were analyzed for the expression of ramA by RT-PCR using previously published primers and probes. Expression of the target gene was normalized to that of housekeeping gene rrsE, a 16S rRNA gene.
Results: 4 of the 42 KPN tested had MICs of $\geqslant$16, while 38 had MICs of 8. The average normalized level of expression of the ramA gene was 11.87, using a tigecycline susceptible strain as the reference condition with an expression of 1. Overexpression was found in all these non-susceptible isolates, with no differences between those with MICs of 8 or 16.
Conclusions: These results confirm the previously established role of ramA in reduced tigecycline susceptibility in *Klebsiella pneumoniae*. As the expression of acrAB has been shown to be subject to multiple levels of regulation, the exact function of ramA will require further experiments.

P749 Energetics, pH and intrinsic efflux pump systems of Gram-negative pathogens

G. Spengler, M. McCusker, M. Martins, A. Martins, L. Rodrigues, J. Ramos, M. Viveiros, I. Couto, J. Pàges, S. Fanning, L. Amaral (Lisbon, PT; Dublin, IE; Marseille, FR)*

Objectives: Multidrug resistant Gram-negative clinical isolates owe their resistance primarily to over-expressed efflux pumps (EPs). Although in recent years EPs of these bacteria have received increasing attention, surprisingly, few studies are conducted under physiological conditions. Furthermore, physiological parameters that normally effect enzyme function including pH, metabolic energy, etc., are rarely explored for possible effects on EP activity. The question of whether these physiological parameters impact the activity of EPs of important Gram-negative pathogens is partially answered by the study to be described.
Methods: The ability of wild-type reference strains of *Salmonella* Enteritidis, *Escherichia coli* and *Enterobacter aerogenes* to extrude the common substrate ethidium bromide (EB) in saline at varying pH or in deionised water was assessed using the semi-automated fluorometric method. The capacity of each isolate to extrude EB in liquid medium and on solid medium at varying pHs was similarly assessed using a novel microplate-based EB method and the EB agar cartwheel method, respectively.
Results: The capacity of each strain to extrude EB is consistently affected by pH. At pH 5, extrusion of EB measured by any method is shown to be optimized, is independent of metabolic energy and is increasingly affected by concentrations of carbonyl cyanide m-chlorophenylhydrazone (CCCP) which uncouples the proton motive force (PMF). In contrast, at pH 8, extrusion is dependent upon metabolic energy, and is affected by inhibitors of ABC transporters orthovanadate and verapamil. In deionised water, efflux is exclusively dependent upon metabolic energy (glucose or ethanol).
Conclusions: Gram-negative bacteria contain two general EP systems – one that functions at low pH (represented by the RND superfamily of transporters) and one that functions at high pH (a putative ABC transporter). These data are consistent with those obtained by others using purified AcrB which demonstrate a high dissociation of a substrate at low pH and low dissociation at high pH, and for the stable association of the EP itself that is greatest at low pH and unstable at high pH. The relationship of efflux to pH and the relationship of the latter to the PMF, the activity of F1F0-ATPase, and the protons required to energize the efflux pump systems will be presented.

P750 The role of mar mutations for the fitness cost and its compensation in fluoroquinolone-resistant *Escherichia coli*

B. Arntjen, A. Heisig, P. Heisig (Hamburg, DE)*

Objective: Selection of highly fluoroquinolone resistant mutants with ciprofloxacin from a quinolone susceptible isolate of *E. coli* (WT) has yielded mutants MIII and MIVa carrying target mutations in genes gyrA and parC in addition to a marR knock out deletion. This combination of mutations is assumed to be responsible for the multiple-antibiotic resistance (mar) phenotype and a distinct loss of fitness of mutants MIII and MIVa. Growing these mutants over 300 generations in the absence of selective pressure resulted in mutants MIII-300-S16 and MIVa-300-S32 characterized by a restored fitness and a partial loss of the mar phenotype. DNA sequence analysis revealed a different marA point mutation in each mutant. The aim of this study was to investigate the impact of these marA mutations on fluoroquinolone resistance and the restoration of the fitness.
Methods: Individual marA point mutations were introduced into the chromosome of parent strains WT and its marR deletion derivative WTIII by site-directed mutagenesis. MICs were determined in the absence and presence of sodium salicylate. The measurement of fitness *in vitro* was performed as a pairwise growth competition experiment. Expression rates of genes marA and acrA were determined by qRT-PCR.
Results: MICs of novobiocin, ciprofloxacin, tetracycline, nalidixic acid and cloramphenicol were decreased by fourfold for marA mutants of WTIII, but only twofold for marA mutants of WT in the presence of sodium salicylate as a known activator of marA expression. In a pairwise competition experiment with WT, WTIII showed a significantly reduced fitness (delta logCFU= 3). Introducing the marA mutations into WTIII completely compensated this loss of fitness (delta logCFU= 0). Gene expression analysis revealed a more than twenty fold overexpression of marA in WTIII and its marA mutants compared to WT. However, the gene acrA encoding the multiple drug resistance efflux pump AcrAB was overexpressed only in WTIII (fourfold). In marA mutants of WTIII acrA expression was comparable to the level determined for WT. These data support the idea of the marA mutations confering a loss of function.
Conclusions: The mar operon plays an essential role not only for the development of high-level fluoroquinolone resistance in *E. coli*, but also for the fitness of such isolates. These results stress the importance of a balanced regulation of the mar operon.

P751 Characterization of a proposed RamR binding site involved in regulation of multiple drug resistance in *Salmonella* Typhimurium and *Salmonella* Hadar using a reporter gene assay

F. Pott, A. Heisig, P. Heisig (Hamburg, DE)*

Objectives: *In vitro*-selected second-step mutants of *Salmonella* Typhimurium (CII) and S. Hadar (DIIa) express a multiple drug resistance (MDR) phenotype associated with overexpression of AcrAB-TolC efflux pump and its activator RamA. This study aimed at analyzing the regulation of ramA by characterizing a postulated stem-loop-structure of the putative binding site of the repressor RamR upstream of ramA.

Methods: The DNA sequence of ramR and the ramR-ramA intergenic region (IGR) was determined by cycle-sequencing. To study the impact of mutations on the ramA overexpression a set of plasmids was constructed with promoter pramA and the adjacent RamR-binding site fused to the b-lactamase blaTEM-1a reporter gene. Mutations in the putative binding site were introduced into the plasmids by replacing the wildtype IGR by those of mutants CII and DIIa to yield plasmids pHPFP10–11 and -12, respectively. Variants were generated by site-directed mutagenesis (pHPFP10–13 to -16). Gene expression was assayed by determining minimal inhibitory concentrations (MICs) of ampicillin (AMP) for salmonellae carrying the different reporter gene plasmids. A ramR-knockout mutant was generated by inserting a kanamycin resistance-cassette into ramR.
Results: Mutants CII and DIIa show deletions upstream ramA of 22 basepairs (bp) and 1bp, respectively. This deletes 15bp of the 20bp-sized RamR binding site in CII and 1bp in DIIa. Cells carrying the corresponding plasmids pHPFP10–11 and -12 showed an 8-fold increase in MIC of AMP whereas knockout of the ramR gene only leads to a 4-fold increase. Reconstituting the number of bases in the loop (pHPFP10–13) does not alter the MIC compared to pHPFP10–12. A different 1bp-deletion in the loop (pHPFP10–14) and deletions altering the stem (pHFP10–15, -16) show increases in MIC of 7- and 6-fold, respectively.
Conclusions: The results of the reporter gene assay (pHPFP10–11 and -12) indicate that both deletions in CII and DIIa increase the ramA-expression (8-fold MIC). Any other alteration (pHPFP10–13 to -16) including the restoration of the loop size by a different bp, deletion of another nucleotide or variations of the stem yielded comparable MIC increases (6- to 8-fold), while inactivation of the ramR gene had a reduced effect (MIC increase 4-fold). These results indicate that the binding of repressor RamR to its binding site is strictly sequence-dependent. Furthermore, the existence of ramA-regulators besides RamR is likely.

Strain	Plasmid	Ampicillin MIC (μg/ml)	Relative increase in MIC compared to plasmid pHPFP10-10
S. Typhimurium CI	pHPFP10-10	512	1
S. Typhimurium CI ramR::kanR	pHPFP10-10	2048	4
S. Typhimurium CI	pHPFP10-11	4096	8
S. Typhimurium CI	pHPFP10-12	4096	8
S. Typhimurium CI	pHPFP10-13	4096	8
S. Typhimurium CI	pHPFP10-14	3584	7
S. Typhimurium CI	pHPFP10-15	3072	6
S. Typhimurium CI	pHPFP10-16	3072	6

P752 Metabolomic profiling of the multidrug resistance transcription regulator, RamA, in *Klebsiella pneumoniae*

T. Schneiders, M. Abu Oun (Belfast, Surrey, UK)*

Objectives: RamA is an AraC/XylS family transcriptional activator where it's over expression is associated with a multidrug resistance phenotype via the acrAB efflux pump. In *Salmonella* it has been shown that a deletion of the ramA renders the bacterium susceptible to the effects of the putative efflux pump inhibitor chlorpromazine. Whilst it has been repeatedly shown that RamA overexpression and its effect on AcrAB results in the efflux of a variety of compounds there has been no data on the effect of RamA overexpression on the metabolomic profile of these pathogenic bacteria. Therefore in order to understand the role of this key regulator in the development of antibiotic resistance, metabolomic analyses of RamA overexpression was undertaken in *K. pneumoniae*.
Methods: Genetically modified mutants in the ramA locus (delta ramA and delta ramR which is a ramA overexpresser) and the parental *Klebsiella pneumoniae* strain Ecl8 were used in the Biolog analyses. The three bacterial strains were analysed using the OmniLog Phenotype Microarray System a high-throughput assay that allows the analyses of carbon, nitrogen, phosphorus, sulphur and dipeptide substrate utilisation of bacterial cells. All fluids, reagents and PM panels were supplied by Biolog and used according to the manufacturer's instructions. Data was analysed using the Genespring software.
Results: Generally the delta ramA mutant produced a similar biochemical profile to the parental wild type Ecl8. The biochemical tests where the lack of ramA impacted significantly on were in the presence of osmolytes and growth in high pH. Approximately 43 significant differences in substrate metabolism were found when the ramA overexpresser was compared to either the delta ramA or parental wildtype. The ramA overexpresser was particularly impaired when grown in the presence of several nitrogen sources. Interestingly the ramA overexpresser exhibited less pH sensitivity than the delta ramA mutant.
Conclusion: In this study the metabolomic impact of ramA overexpression, was determined in the nosocomial pathogen *K. pneumoniae*. Our transcriptomic analyses shows that various membrane components are up and down regulated in response to ramA overexpression which supports the effects noted in the efflux/influx of the different metabolic substrates identified in the Biolog screen. These data clearly underscore the metabolic cost of ramA overexpression and its effect on multidrug resistance.

P753 Novel AraC regulator in mediating the multidrug-resistant phenotype via the OqxAB efflux pump in clinical and environmental isolates of *Klebsiella pneumoniae*

M. Veleba, T. Schneiders (Belfast, UK)*

Objectives: AraC/XylS family consists of transcriptional regulators that are involved in a myriad of cellular functions. A subset of these transcriptional regulators typified by the MarA/SoxS and RamA proteins confer a multidrug resistance phenotype via the upregulation of the acrAB efflux pump. In this study we have identified another AraC type regulator within the *Klebsiella pneumoniae* genome that may be involved in conferring a multidrug resistant phenotype via the OqxAB pump.
Methods: RNA was extracted from a combination of isogenic mutants and clinical isolates using the Qiagen RNaeasy Kit. RNA integrity was assessed using nanodrop and Agilent Nanochip systems. The RNA was transcribed into double stranded cDNA prior to labelling with Cy3. The cDNA was hybridised to the Nimblegen expression array platform designed from the *K. pneumoniae* MGH 78578 genome.
Results: Our microarray results demonstrated that the novel AraC regulator was overexpressed in isogenic strains that differed only in ramA expression levels. However ramA overexpression was not linked to the upregulation of this novel AraC regulator. Bioinformatic analyses of the genomic organisation of the area surrounding the novel regulator indicated that it controls an efflux pump annotated as oqxAB. RT-PCR analyses of clinical and environmental isolates demonstrated that upregulation of the novel AraC regulator is linked to the upregulation of the oqxAB efflux pumps. Bioinformatic and RT-PCR analyses support this association as mutations within the AraC regulator also result in the upregulation of the oqxAB pump.
Conclusion: In this study we have identified a previously uncharacterised transcriptional regulator of the efflux pump oqxAB in *Klebsiella pneumoniae*. A combination of factors underscore the importance of understanding the regulation of the oqxAB gene by this novel AraC regulator; firstly oqxAB has been previously identified as a significant efflux mechanism in conferring resistance to the growth promoter olaquindox and ciprofloxacin and secondly is chromosomally encoded in nosocomial pathogens such as *Klebsiella* spp. and *Burkholderia* spp. but has acquired genetic mobility as a plasmid in *E. coli*. Therefore the role of oqxAB and its novel AraC regulator represent an emerging mechanism of resistance to clinically relevant antibiotics in *K. pneumoniae*.

Quinolone resistance in Enterobacteriaceae

P754 Aac(6′)-Ib-cr genotyping by simultaneous high-resolution melting analysis of an unlabelled probe and full length amplicon

P. Andersson, J.M. Bell, J. Turnidge (North Adelaide, AU)*

Objectives: There are several variants of the gene encoding aminoglycoside acetyltransferase aac(6′)-Ib, and of greatest clinical concern is

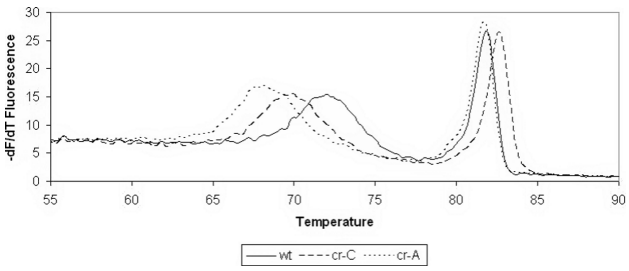
the aac(6′)-Ib-cr variant which extends the enzyme targets to include fluoroquinolones in addition to aminoglycosides. The aac(6′)-Ib-cr variant is characterised by amino acid changes at codon 102 (Trp → Arg) and codon 179 (Asp → Tyr). There are two described aac(6′)-Ib-cr variants which differ from the wild type aac(6′)-Ib (wt) at nucleotide 304 by a T → C (cr-C) or T → A (cr-A) change. Both nucleotide changes result in a Trp → Arg amino acid substitution. We sought to design a rapid and simple assay able to distinguish between the three known alleles found in the aac(6′)-Ib gene at codon 102.

Methods: A 58bp fragment was amplified using asymmetrical primer concentrations in the presence of a 25bp unlabelled probe with a perfect match to the wt allele. The combined information from simultaneously acquired high resolution melting data of the full length amplicon and the unlabelled probe, allows unambiguous genotyping, even of discrete T → A nucleotide changes.

Results: Two melting events were observed: one around 65–75ºC, describing the probe disassociation and one around 79–84ºC, describing whole amplicon melting (see figure). In brief, the probe and amplicon melting points were: 72.0ºC and 81.9ºC for wt; 69.7ºC and 82.7ºC for cr-C; 68.8ºC and 81.7ºC for cr-A. Heterozygote isolates containing both wt and cr alleles showed a distinct shoulder on the amplicon melting peak, due to the melting of heteroduplex fragments. The probe melting event for heterozygotes showed two distinctive peaks representative of the two individual allele probe peaks.

The assay was validated using 211 isolates with known aac(6′)-Ib status and genotype, and further tested on 732 isolates with unknown aac(6′)-Ib status and genotype. The assay performed reliably in all tests.

Conclusion: We have developed a one-step closed-tube assay for the detection and genotyping of aac(6′)-Ib-cr, capable of differentiating between the two genetic variants responsible for the aac(6′)-Ib-cr phenotype, which from an epidemiological perspective is of importance. The assay is rapid at <2 hours, costs less than US $1 per isolate and addresses the need of an efficient detection system for the monitoring of the growing prevalence of clinically significant aac-Ib-cr variants.



P755 Diffusion of plasmid-mediated quinolone resistance determinants in CTX-M-type extended-spectrum β-lactamases in hospitals and the community in the Saint-Etienne region, France

N. Filippa, A. Carricajo, G. Aubert (Saint Etienne, FR)*

Objectives: Fluoroquinolone resistance has been widely reported due to unbridled clinical use of these compounds and is usually chromosomally mediated. However, plasmid-mediated resistance has emerged and is now described worldwide. The aim of this study was to evaluate the prevalence of determinants of plasmid-mediated quinolone resistance (PMQR) (qnrA, qnrB, qnrS, aac(6′)-Ib-cr, and qepA) among clinical isolates of CTX-M type strains in the Saint-Etienne region (population: 600 000) comprising 11 analytical laboratories between October 2007 and March 2008.

Methods: A total of 103 non-duplicated isolates of CTX-M-positive Enterobacteriaceae were screened using specific primers for qepA and aac(6′)-Ib-cr and multiplex PCR for qnrA, qnrB and qnrS genes; The aac(6′)-Ib-cr variant was further identified by digestion with BstCI. PMQR-positive isolates were analyzed for antimicrobial susceptibility and presence of mutations in the quinolone-resistance determining region (QRDR) of gyrA gene.

Results: During the study period, 103 isolates were analyzed comprising 87 *E. coli* and 16 *K. pneumoniae* mostly CTX-M15 (36.9%), CTX-M1 (28.2%), CTX-M14 (20.4%) but also CTX-M2 (5.8%), CTX-M57 (2.9%), CTX-M32 (2.9%), CTX-M27 (2.6%) and CTX-M8 (0.9%). Twenty-seven isolates (26.2%) were positive for aac(6′)-Ib gene and 25/27 (92.6%) were aac(6′)-Ib-cr mutant. Of these 25 aac(6′)-Ib-cr, 23 were detected in CTX-M15 (19 *E. coli* and 4 *K. pneumoniae*), 1 in CTX-M14 (*E. coli*) and 1 in CTX-M54 (*E. coli*). 29.6% of hospitals isolates were positive for aac(6′)-Ib-cr compared to 21.6% in the community and 8.3% in tertiary care units. Of 103 isolates, only 1 qnrS (0.9%) was identified in a *K. pneumoniae* CTX-M15 isolate (in association with aac(6′)-Ib-cr), whereas no qnrA-like or qnrB-like genes were detected. Two qepA (1.9%) *E. coli* were isolated: 1 CTX-M14 and 1 CTX-M57, respectively alone and associated with aac(6′)-Ib-cr. Analysis of the gyrA chromosomal QRDR region revealed the presence of at least one mutation in all PMQR bacteria identified.

Conclusions: Our results show that isolates with aac(6′)-Ib-cr, often associated with the blaCTX-M-15 gene, are emerging among fluoroquinolone-resistant *E. coli* in the Saint-Etienne region, both in hospitals and in the community. Diffusion of other PMQR determinants such as qnr and qepA remains rare but is often associated with aac(6′)-Ib-cr, suggesting that plasmid diffusion of these resistance determinants may develop rapidly in the future.

P756 Additive effect of qnr genes, Ser83Leu substitution in DNA gyrase and Ser80Arg in topoisomerase IV on the fluoroquinolone resistance in *E. coli*

A. Briales, J.M. Rodriguez-Martinez, C. Velasco, P. Díaz de Alba, J. Pachón, J. Dominguez-Herrera, A. Pascual (Seville, ES)*

Objectives: The aim of this study was to evaluate the impact of qnrA, qnrB and qnrS in *E. coli* wild type strain and *E. coli* harbouring the most frequent substitutions in the QRDR regions of GyrA and ParC, against four different fluoroquinolones (FQ).

Methods: *E. coli* ATCC 25922 was used as a background strain. *E. coli* (GyrA Ser83Leu), *E. coli* (ParC Ser80Arg) and *E. coli* (GyrA Ser83Leu/ParC Ser80Arg) were obtained by gene replacement, and the respective derivatives carrying qnrA1, qnrB1 or qnrS1 into pBK-CMV vector by transfromation. In total twelve isogenic strains were investigated. MICs and MBCs of 4 fluoroquinolones were determined. Time killing curves were performed for each strain at 1 and 4 times MIC values, and at ciprofloxacin 1 and 2 mg/L.

Results: The effect on FQ resistance of the qnr genes was similar for CIP, LEV, MXF and NFX in the different genetic backgrounds. MICs of CIP against *E. coli* wild-type, *E. coli* (GyrA Ser83Leu) and *E. coli* (GyrA Ser83Leu/ParC Ser80Arg) were 0.002, 0.125, 0.25 mg/L, respectively. In presence of qnrA, qnrB or qnrS, MICs increased from 4 to 64-fold against this FQ. *E. coli* (gyrA Ser83Leu/parC Ser80Arg) harboring qnr genes showed MICs for CIP, LEV, MXF and NFX that ranging from 1–2, 1–4, 1–2 and 4–8 mg/L, respectively. MBCs of the four FQ were the same or 1–2 dilutions higher than the corresponding MICs in all the cases. In the time-killing assays, viable bacteria recovered for *E. coli* wild-type harbouring qnr genes were at least 100-fold higher compared to *E. coli* wild-type at 4xMIC of CIP at 24 h. In the time-killing assay at 1–2 mg/L of CIP, no viable bacteria were recovered for *E. coli* wild-type at 8 h, while 10^2–10^4 CFU/ml were recovered in presence of qnr. Those values were observed to 24 h for qnrA, qnrB and qnrS. At 1 mg/L of CIP, a marked re-growth is observed in *E. coli* (gyrA Ser83Leu) harbouring qnrA and qnrS. This re-growth was not observed for qnrB under the same conditions.

Conclusions: The additive effect of the qnr genes, Ser83Leu in the GyrA and Ser80Arg in ParC lead to intermediated susceptibility or resistance to FQs. Expression of qnr genes in Enterobacteriaceae, at least in *E. coli*, may have an important role to select one-step mutants with high level of FQ resistance with additional substitutions in the QRDR region of GyrA and ParC.

P757 AAC-(6′)-Ib-cr, QnrC and QnrS determinants among bacterial isolates from wild and food-producing animals in Portugal

E. Ferreira, L. Clemente, D. Jones-Dias, V. Manageiro, P. Themudo, T. Albuquerque, A. Amado, M. Caniça (Lisbon, PT)*

Objectives: Plasmid-mediated quinolone resistance (PMQR) is increasingly identified worldwide in Enterobacteriaceae of human origin. However, few reports describe their occurrence among bacteria from animal origin. The aim of this study was to evaluate the extension of PMQR in animals.
Methods: We screened two collections of Enterobacteriaceae isolates (n = 140) for the presence of PMQR determinants, identified at National Laboratory of Veterinary Research (LNIV, Lisbon), during a period of two years (2008–2009). A total of 51 *Salmonella* isolates from different serotypes were isolated from layers, and 89 *Escherichia coli* were isolated from farm animals, birds and mammals. PCR and nucleotide sequencing were used with specific primers to screen for the presence of the following genes: qnrA, qnrB, qnrC, qnrD, qnrS, aac-(6′)-Ib-cr and qepA genes. Susceptibility testing of isolates carrying PMQR determinants was performed against quinolones (nalidixic acid, ciprofloxacin, norfloxacin, pefloxacin, orbifloxacin, marbofloxacin and enrofloxacin) by disk diffusion and MIC methods, and interpreted according to SFM veterinary and EFSA guidelines, respectively.
Results: We identified a qnrC-positive *Salmonella enteritidis* isolated in a layer chicken. Three qnr genes were identified as qnrS1 and were detected in *E. coli* isolates from a broiler, a dog and a turtle-dove. The aac-(6′)-Ib-cr gene was detected in an *E. coli* isolated from a dolphin. None of the isolates, from both collections, carried qnrA, qnrB, qnrD or qepA genes. The isolates with aac-(6′)-Ib-cr and the other two QnrS1 determinants showed diminished susceptibility to all fluoroquinolones tested and to nalidixic acid but one of the isolates with QnrS1 determinant was susceptible to all antibiotics. Isolates with QnrC determinant showed susceptibility to pefloxacin and marbofloxacin.
Conclusions: This survey showed that PMQR determinants are present in animals from different environments in Portugal, including food-producing animals. To our knowledge this is the first report of a qnrC gene in isolates from animal origin. The study highlights the need of surveillance of this resistance mechanism and reinforces a more careful use of fluoroquinolones.

P758 Occurrence of plasmid-mediated quinolone resistance genes and extended-spectrum β-lactamases encoding genes among Gram-negative isolates from drinking and environmental waters

N. Mendonça, J. Ramalho, P. Vieira, A. Tavares, P. Pereira, G.J. da Silva (Coimbra, Leiria, PT)*

Background: Water constitutes a way of dissemination of antimicrobial resistance genes from bacteria of human and animal sources especially if they are inserted in mobile genetic elements.
Objective: The aim of the work was to search for plasmid-mediated quinolone resistance (qnr) genes and extended-spectrum β-lactamase (ESBL)-encoding genes among coliform and non-fermentative bacilli isolated from water of different origin.
Methods: Between May and October 2009, 187 isolates were recovered from drinking, recreational, superficial and underground waters. The antimicrobial susceptibilities were determined by the disc diffusion method. ESBL-phenotype was determined by double-disc synergy method with ceftazidime and cefotaxime. blaTEM and blaSHV genes were screened in ESBL-producers by PCR. Multiplex-PCR for the qnrA, qnrB and qnrS genes was performed in nalidixic acid resistant isolates. The amplicons detected were sequenced.
Results: Fifty three percent of the strains were isolated from underground water, 24% from drinking water, 14% from superficial water and 10% in recreational water. Among consumption and recreational water isolates, 2% were resistant to β-lactams. Furthermore, 3% of resistance to nalidixic acid was also detected among isolates from recreational water, where swimming pools were a major contributor. Underground water isolates showed higher level of resistance to amoxicillin/clavulanate (12%) and to nalidixic acid (7%) and a decrease of susceptibility to ciprofloxacin (1%). Imipenem and gentamicin showed good bacterial inhibitory activity with 99% and 79% of susceptibility, respectively. Among the 187 strains, 11% showed resistance to nalidixic acid of which only 15% presented qnrB. qnrB genes were found in a *Citrobacter* spp. isolated from water of an artesian well (33%) and *Escherichia coli* isolates from beach's waters (67%). It is important to highlight the levels of resistance detected to ceftazidime (4%) and cefotaxime (5%), mostly among the recreational waters. Although results from disc diffusion synergy method suggested that 3% of isolates were ESBL-producers, we didn't detect blaTEM or blaSHV genes by PCR.
Conclusions: The study showed the presence of antibiotic resistance in aquatic environment, mostly in consumption, recreational and underground waters and shows the presence of qnrB genes with potential lateral transfer which may act in synergy with other quinolone resistance mechanisms.

P759 Molecular analysis of fluoroquinolone-resistant extra-intestinal *Escherichia coli* isolates from dogs and humans in Australia

J.L. Platell, R.N. Cobbold, M.L. Menard, C.R. Clabots, M.A. Kuskowski, D.J. Trott, J.R. Johnson (Brisbane, AU; Minneapolis, US)*

Objectives: To assess the origins canine and human fluoroquinolone-resistant (FQr) phylogenetic group B2 FQr *E. coli* isolates in Australia.
Methods: Overall, 707 FQr extra-intestinal *E. coli* isolates (123 canine, 584 human) from Australian diagnostic laboratories (2007–2008) underwent PCR-based phylotyping. Group B2 isolates were selectively tested for single-nucleotide polymorphisms (SNPs) indicating sequence type ST131, sepsis-associated O types (rfb PCR), susceptibility to 19 antimicrobials, extended virulence genotypes (53 traits), pulsed-field gel electrophoresis (PFGE) profiles, and partial multi-locus sequence typing (MLST; fumC, gyrB, and recA).
Results: Group B2 accounted for 37% (263/707) of study isolates, including 11% (13/123) of canine but 43% (250/584) of human isolates ($P < 0.001$). 58% of group B2 isolates were multi-drug resistant (i.e., resistant to $\geqslant$4 antimicrobials), regardless of host. ST131 accounted for the majority of both human (205/250, 82%) and canine (9/13, 69%) group B2 isolates. PFGE analysis showed several instances of $\geqslant$94% similarity among ST131 isolates across host species both within Australia and compared with an international ST131 *E. coli* library. An O75:K+ clonal group (ST1193), which likewise included canine and human isolates, accounted for nearly 50% (24/49) of the non-ST131 group B2 isolates. Its two main pulsotypes were $\geqslant$90% similar; each exhibiting human-canine commonality. Group B2 isolates' virulence profiles differed significantly by clonal group, but not by host species.
Conclusions: Group B2 was significantly more prevalent among human than canine-source FQr *E. coli* in Australia, suggesting that dogs are a less important reservoir for such strains than are humans. Two clonal groups, ST131 (81%) and ST1193 (9%), accounted for 90% of FQr group B2 isolates. Across-species strain similarity (by ST, virulence genotype, and PFGE profile) supports human-dog exchange of FQr *E. coli* within Australia. Likewise, PFGE commonality with international ST131 isolates suggests that dogs and humans in Australia are participants in the intercontinental emergence and dissemination of ST131. This, plus Australia's unique banned use of FQs in production animals, argues against the local food supply as a source for Australian FQr group B2 strains.

P760 Antibiotic resistance of *Salmonella enterica* isolates from Greece mediated by tetA, qnrA and qnrB resistant genes

G. Filioussis, N. Tzimotoudis, E. Petridou, A. Zdragas, K. Papageorgiou, S.K. Kritas (Thessaloniki, GR)*

Objectives: The antibiotic resistance of fifty *Salmonella enterica* strains, isolated from animal and food sources in Greece and its association with resistance genes and plasmids were investigated.
Methods: Antibiotic susceptibility of the *Salmonella enterica* isolates was determined by dilution antimicrobial susceptibility tests in accordance to the M100-S16 method and the interpretive criteria of the Clinical and Laboratory Standards Insitute (CLSI, 2006). The MICs were determined as the lowest concentrations of antimicrobial agents that inhibited visible growth. PCR assays were performed to identify the presence of genes conferring resistances. Nucleotide sequences of PCR products were aligned and compared to GenBank homologue sequences (www.ncbi.nlm.nih.gov). The plasmid content of the resistant isolates was investigated by pulsed field gel electrophoresis (PFGE) after cleavage with Sau3AI restriction enzyme.
Results: Twelve isolates were resistant to tetracycline (MIC >128μg/ml), five to ampicillin (MIC >128μg/ml) and three to nalidixic acid (MIC >128μg/ml) and ciprofloxacin (MIC >128 μg/ml). The tetA gene was highly prevalent in *Salmonella* strains of our study, being detected in nine tetracycline-resistant isolates. Meanwhile qnrA and qnrB genes were detected in the three quinolone resistant strains. The extended sequence analysis of the products beard high identities when compared to GenBank sequences. Finally no specific bands in pulsed-field gel electrophoresis (PFGE) patterns could be attributed to plasmid DNA.
Conclusions: This is to the best of our knowledge the first report of tetA, qnrA and qnrB resistant genes in *Salmonella enterica* isolates in Greece. Plasmids and transposons carrying resistant genes have been isolated from salmonellas in many countries. As they seem to be more broadly distributed among enterobacteria than first assumed, our further work would be focused on the distribution of these DNA elements among resistant *Salmonella enterica* serovars.

P761 Plasmid-mediated quinolone resistance genes in Enterobacteriaceae isolates of human and animal origin in Lithuania

J. Povilonis, V. Seputiene, M. Jurenaite, M. Ruzauskas, R. Siugzdiniene, M. Virgailis, A. Pavilonis, E. Suziedeliene (Vilnius, Kaisiadorys, Kaunas, LT)*

Objectives: Plasmid-mediated quinolone resistance (PMQR) caused by qnr, qepA and aac(6′)-Ib-cr genes is emerging worldwide in Enterobacteriaceae. The aim of this study was to evaluate the prevalence of PMQR determinants in a collection of quinolone-resistant bacterial isolates of human and animal origin in Lithuania (2005–2008) and to investigate their association with extended-spectrum β-lactamase (ESBL)-producing isolates.
Methods: A total of 265 non-repetitive quinolone-resistant (Nal, Cip or Nor) bacterial isolates from human infections (n = 65 *E. coli*, n = 41 *K. pneumoniae*, n = 5 *K. oxytoca*) and from farm animals (n = 99 *E. coli*, n = 55 *S. enterica)* were studied. Screening of qnr (qnrA, qnrB, qnrS), qepA and aac(6′)-Ib-cr genes was carried out by multiplex and conventional PCR. Restriction analysis of PCR products was used to distinguish between aac(6′)-Ib-cr and aac(6′)-Ib alleles.
Results: Of 265 isolates, 61 (23%) harboured PMQR genes (clinical isolates: n = 30/65 (46%) *E. coli*, n = 18/41 (44%) *K. pneumoniae*, n = 2/5 (40%) *K. oxytoca*, animal isolates: n = 11/99 (11%) *E. coli*). The most prevalent was aac(6′)-Ib-cr gene, found in 57 (22%) isolates (clinical isolates: n = 29 *E. coli*, n = 17 *K. pneumoniae*, n = 1 *K. oxytoca*, animal isolates: n = 10 *E. coli*). The qnr genes were detected in 8 (3%) isolates and were represented by qnrB (clinical isolates: n = 1 *E. coli*, n = 1 *K. pneumoniae*, n = 1 *K. oxytoca*) and qnrS (clinical isolates: n = 1 *K. oxytoca*, animal isolates: n = 4 *E. coli*). qnr and aac(6′)-Ib-cr genes coexisted in three *E. coli* animal isolates and one *K. oxytoca* clinical isolate. No PMQR genes were observed in *S. enterica* animal isolates.
Among 71 *E. coli*, *K. pneumoniae* and *K. oxytoca* clinical isolates producing CTX-M type β-lactamases, four (5.6%) harboured qnr genes: qnrB (n = 1 CTX-M-3 *E. coli*, n = 1 CTX-M-15 *K. pneumoniae*, n = 1 CTX-M-15 *K. oxytoca*) and qnrS (n = 1 CTX-M-3 *K. oxytoca*). Thirty three (46%) isolates carried aac(6′)-Ib-cr gene (n = 14 CTX-M-15 *E. coli*, n = 18 CTX-M-15 *K. pneumoniae*, n = 1 *K. oxytoca*).
Conclusion: The aac(6′)-Ib-cr determinant is more prevalent than other PMQR genes ($P < 0.01$) in quinolone resistant *K. pneumoniae*, *K. oxytoca* and *E. coli* clinical isolates and *E. coli* animal isolates. A strong association ($P < 0.01$) between CTX-M15 and aac(6′)-Ib-cr determinants in CTX-M-producing *E. coli*, *K. pneumoniae* and *K. oxytoca* clinical isolates was determined.

P762 Prevalence of plasmid-mediated quinolone resistance determinants in Enterobacteriaceae strains isolated in north-eastern Italy

A. Mazzariol, R. Koncan, B. Kocsis, R. Aschbacher, P. Lanzafame, R. Fontana, M. Rassu, M. Schinella, G. Cornaglia (Verona, Bolzano, Trento, Vicenza, Rovereto, IT)*

Objectives: To investigate the prevalence of plasmid-mediated quinolone resistance determinants among Enterobacteriaceae isolated in North-East Italy.
Materials and Methods: We collected 756 Enterobacteriaceae, non-susceptible to fluoroquinolones and/or resistant to third-generation cephalosporins, isolated during 2007 and 2008 in five different microbiology laboratories (namely Verona, Vicenza, Bolzano, Trento and Rovereto). All isolates were screened by PCR for the presence of three qnr determinants – namely A, B, and S – for the qepA efflux pump and for aac(6′)-Ib-cr. These non-duplicate isolates included 497 *Escherichia coli*, 69 *Proteus* spp., 52 *Enterobacter* spp., 68 *Klebsiella* spp., 24 *Morganella morganii*, 18 *Citrobacter* spp, 21 *Providencia* spp, and 7 *Serratia* spp.
Results: Of 756 strains tested 36 (4.76%) presented either qnrS (28 strains) or qnrB (8 strains). No qnr A was detected. Of 497 *E. coli* only 12 (2.41%) carried a qnr determinant (namely qnrS). The prevalence of qnr determinants was higher in other species, namely 3.8% in *Enterobacter* spp. (two qnr B), 33.33% in *Citrobacter* spp. (six qnr B), and 22.05% in *Klebsiella* spp. (15 qnr S). The qep A gene was detected in four *K. oxytoca*. strains. (0.53% of the total isolates). The aac(6′)-Ib-cr determinant was detected in 66 strains (8.73% of the total), namely 56 *E. coli*, 8 *Klebsiella*, one *P. mirabilis* and one *M. morganii*. Five strains harboured more than one PMQR, i.e. four *E. coli* carried a qnrS plus aac(6′)-Ib-cr, and one *Klebsiella* harboured a qnrS plus a qepA). The ciprofloxacin resistance of strains carrying one of the plasmid-mediated determinants ranged between $\leqslant 0.25$ mg/L and 256 mg/L. Out of 108 strains carrying a PMQR, 67 produced at least one ESBL and 6 strains produced an MBL (VIM-1).
Conclusions: The prevalence of PMQR determinants in North-Eastern Italy proved low among *E. coli* but much higher in other Enterobacteriaceae genera such as *Klebsiella* and *Citrobacter*. The aac(6′)-Ib-cr variant was also frequently detected, whilst the prevalence of the qepA efflux pump is was very low. More often than not the PMQR determinants were found to be associated with ESBLs.

P763 Prevalance and diversity of plasmid-mediated quinolone resistance genes in extended-spectrum β-lactamase positive *Escherichia coli* and *Klebsiella pneumoniae*: first report of qepA from Turkey

Z. Gulay, M. Bicmen, D. Gur on behalf of the Hitit-2 Study Group*

Objective: Plasmid-mediated quinolone resistance (PMQR) seem to be emerging in Turkey as in the other parts of the world. However, previous studies have not included some of the newer members of the PMQR genes. In this study, we screened the PMQR determinants (qnr A, B, C, S, qepA and aac(6′)-Ib-cr) in ESBL-positive invasive *E. coli* (Ec) and *K. pneumoniae* (Kp) in order to update existing data.

Method: The isolates were collected from 12 centers in Turkey as a part of The Hitit-2 Surveillance Study. A total of 151 isolates (67 Ec, 84 Kp) were taken into the study. Of these, 105 (69.5%) were resistant to nalidixic acid (Nal) and 95 to both Nal and CIP. PMQR genes were detected by PCR. aac(6′)-Ib-cr variant is identified by digestion with BseG1. All positive results were confirmed by sequencing.
Results: Of the Nal(r) isolates 77 (73.3%) possessed one of the PMQR genes. Among these 13 (16.8%) carried more than one PMQR gene. The prevalance of PMQR genes was 12.5%-100% according to the centers. Qnr A(A1), B(B1, B2, B5, B6/9) and S (S1) were detected in 1.9%, 10.5% and 1.9% of the isolates respectively. These were colected in 5 different centers. None of the isolates carried qnrC. aac(6′)-Ib-cr was detected in 69.5% of the Nal(r) isolates and was found in all collaborating centers. 89% of aac(6′)-Ib-cr-positive isolates (43 Ec, 22 Kp) produced CTX-M-15 ESBL and of the Ec isolates 46.5% belonged to the ST131:O25b clone. PMQR genes were carried on plasmids with sizes 16–91 kb along with CTX-M β-lactamases in most cases. In two Ec isolates qepA gene is detected for the first time in Turkey. The isolates came from one center and also possessed aac(6′)-Ib-cr and qnrB2. They had identical ERIC-PCR patterns.
Conclusion: The results of this study show that both the prevalance and the diversity of PMQR genes are increasing in ESBL positive Enterobacteriaceae in Turkey. As a high number of isolates belong to the multiresistant ST 131:O25b clone which caused epidemics in the community, this study also indicates a potential risk of multiresistance in community acquired infections.

P764 ESBL carrying *Salmonella* isolates with reduced fluoroquinolone susceptibility in Finnish travellers returning from Asia

M. Gunell, P. Kotilainen, P. Huovinen, A-M. Rissanen, M. Korkeila, A. Siitonen, M. Österblad, J. Jalava, A. Hakanen (Turku, Kuopio, Lappeenranta, Helsinki, FI)*

Objectives: Fluoroquinolones and 3rd generation cephalosporins are drugs of choice to treat severe *Salmonella* infections. Reduced fluoroquinolone susceptibility among *Salmonella enterica* isolates has increased since late 1990's. Furthermore, proportion of ESBL producing strains of *Escherichia coli* and other species of Enterobacteriaceae has increased world wide. So far *Salmonella* isolates acquiring resistance determinant against both fluoroquinolones and 3rd generation cephalosporins have not been reported in Finland. This paper describes a series of ESBL carrying *Salmonella* isolates among travellers returning from Asia to Finland.
Methods: We included in this study four *S. enterica* isolates collected from two different hospitals in Finland during March and April 2009. The isolates were sent to the reference laboratory for ESBL gene determination. We performed an ESBL screening using a disk diffusion test with ampicillin (AMP) (10 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), cefotaxime/clavulanic acid (CD03) (30/10 μg) and ceftazidime/clavulanic acid (CD02) (30/10 μg) disks. These isolates were also screened for ESBL resistance genes of TEM-, SHV- and CTX-M families by PCR and sequencing. In addition, one strain from our earlier *Salmonella* collection showed ESBL phenotype and was taken into this study.
Results: All *Salmonella* isolates tested showed an ESBL phenotype: AMP disk 6 mm, CTX disk 6 mm, cefotaxime/clavulanic acid disk 25–29 mm, CAZ 6–16 mm and ceftazidime/clavulanic acid disk 23–28 mm. The one strain from the earlier collection had AMP MIC >128 mg/L, CTX MIC 16 mg/L, CAZ MIC >64 mg/L and cefuroxime MIC 32 mg/L. In addition, all of these isolates showed reduced ciprofloxacin susceptibility (MIC $\geqslant$ 0.125 mg/L). PCR results revealed that one of the isolates was simultaneously TEM-1 and CTX-M positive, three of the strains carried CTX-M-15 gene and the one strain from the earlier collection had TEM-1 gene.
Conclusions: To date, this is the first report on ESBL producing *Salmonella* strains in Finland. The rapid emergence of ESBL carrying *Salmonella* isolates with decreased ciprofloxacin susceptibility among travellers returning to Finland needs more attention. These findings suggest that ESBL screening is important not only with *E. coli* and *Klebsiella*, but also with *Salmonella* strains, especially with isolates showing the reduced fluoroquinolone susceptibility.

P765 Analysis of the mechanisms of fluoroquinolone resistance in clonal group ST 131 uropathogenic *Escherichia coli*

T. Gibreel, F. Bolton, J. Cheesbrough, M. Upton, A. Fox, A. Dodgson (Manchester, Preston, UK)*

Objectives: Recent studies have reported the widespread emergence of the O25:H4-ST131 clone of uropathogenic *Escherichia coli* (UPEC) which is characterized by CTX-M-15 ESBL production and has frequently been associated with fluoroquinolone resistance, this resistance has increasingly been traced to plasmid medicated factors such as the aac (6")-Ib-cr gene and different Qnr genes that offer topoisomerase protection. In this study we explore the prevalence of different quinolone resistance mechanisms among ST131 strains within a defined UPEC population.
Methods: 32 *E. coli* isolates were examined representing the total of ST 131 found among 250 non-duplicate isolates recovered from patients with urinary tract infection, in the northwest region of England between September 2007 and December 2008. All isolates were screened for mutations in quinolone resistance determinant regions (QRDRs) of gyrA and parC genes by sequencing the respective targets. A PCR-based restriction fragment length polymorphism (PCR-RFLP) assay was used to identify the aac (6')-lb-cr variants and screening for the three known qnr genes was carried out using Multiplex PCR.
Results: Of the 32 tested ST131 isolates 20 (62%) showed resistance to quinolones (nalidixic acid) and 19 (59%) expressed additional resistance to fluoroquinolones (ciprofloxacin). All ciprofloxacin resistant isolates had multiple mutations in both gyrA and parC genes while the one nalidixic acid resistant isolate that failed to show resistance to ciprofloxacin had a single gyrA mutation at codon 83 (Ser-Leu). On the other hand only nine isolates carried the aac (6')-lb-cr gene, which represents 50% of ciprofloxacin resistant isolates. Of the 20 isolates tested, none carried any of the targeted qnr genes.
Conclusion: The strong linkage between the ST131 clone and quinolone resistance has been suggested to be the result of the high prevalence of aac (6)-lb-cr among isolates of this clone. However, our study shows that fluoroquinolone resistance occurs in ST131 UPEC as a result of accumulated point mutations in both gyrA and parC genes, irrespective of their acquisition of any plasmid mediated resistance.

P766 Antimicrobial resistance of Gram-negative bacteria isolated from domestic and food-producing animals: potential reservoirs of plasmid-mediated quinolone resistance genes

N. Mendonça, P. Vieira, J. Ramalho, M.J. Saavedra, G.J. da Silva (Coimbra, Vila Real, PT)*

Objective: The aim of the study was to evaluate the antimicrobial resistance of domestic and food-producing animals' Gram negative bacteria and to screen for the presence of plasmid-mediated quinolone resistance genes (qnr) as few data on the prevalence of these determinants in strains from animals are available.
Methods: Gram negative bacteria were isolated from domestic (cat, dog and jennet) and food-producing (chicken, rabbit, trout and gilt-head bream) animals. Antimicrobial susceptibility was performed using the disc diffusion method according to CLSI guidelines. The presence of qnr genes was determined by Multiplex-PCR and DNA sequencing.
Results: Of the 53 strains studied, 22 were isolated from domestic animals (DA) and 31 from food-producing animals (FPA). The majority of isolates were Enterobacteria (70%) and 46% were *Escherichia coli*. Isolates of both groups showed high resistance to amoxicillin: 87% for FPA and 91% for DA, whose activity were recovered in the presence of clavulanic acid (26 and 67% of resistance, respectively). All FPA isolates were susceptible to ceftazidime, cefotaxime and imipenem. In contrast,

23%, 45% and 9% of DA isolates showed decrease susceptibility or resistance to these β-lactams, respectively. Isolates were more susceptible to gentamicin (100 and 91% for FPA and DA isolates, respectively) than to kanamycin (32 and 23% for FPA and DA isolates, respectively). For cloramphenicol and SXT, FPA and DA isolates showed 35% and 41% of resistance, respectively, while tetracycline resistance was higher for FPA (52%) than for DA group (41%). Both groups were more resistant to nalidixic acid (55% for FPA and 59% for DA) than to ciprofloxacin (19 for FPA and 27% for DA). In DA isolates were found 3 isolates with QnrB and 1 with QnrS, none susceptible to quinolones. One isolate with QnrB (quinolone resistant) and 6 with QnrS were found in FPA group, susceptible to quinolones. The six isolates were collected from rabbits.
Conclusion: This work shows differences in antibiotic resistance between isolates of DA and FPA. Noteworthy is the appearance of resistant isolates to broad spectrum β-lactams, including imipenem in isolates from DA group. qnr genes were detected in both groups, suggesting that animals may act as reservoirs of these genes, even in strains susceptible to nalidixic acid. Resistant bacteria present in DA and FPA can be transmitted to humans by contact or food-chain, an increasing risk for human health.

P767 Quinolone resistance mechanisms in *Escherichia coli* isolated from children (with or without diarrhoea) under 1 year in Lima, Peru

M.J. Pons, S.G. Mosquito, E. Mercado, P. Rivera, R. Maves, L.J. Del Valle, M. Vargas, T. Ochoa, J. Ruiz (Barcelona, ES; Lima, PE)*

Objectives: The objective was to analyse the quinolone resistance mechanisms (gyrA, parC, aac(6′)Ib-cr, qnrA,B,S) in *Escherichia coli* isolated from children (with or without diarrhoea) in a periurban area of Lima, Perú.
Method: Strains: A total of 85 *E. coli* isolates (11 Enteropathogenic – EPEC, 11 Diffussely adherent – DAEC, 27 Enteroaggregative – EAEC, 2 Enterotoxigenic – ETEC – and 34 non-diarrheogenic – non DEC) exhibiting resistance or diminished susceptibility to quinolones were analyzed. Antimicrobial susceptibility to nalidixic acid (NAL) and ciprofloxacin (CIP) was established by disk diffusion in accordance with CLSI. Presence of mutations in gyrA and parC genes was determined by PCR and sequencing.

Table 1. Quinolone resistance patterns

Nal	Cip	GyrA	parC	QnrB	AAC(6′)Ib-cr	NDEC	DEC
I	S	wt	wt	+	–	1	1
I	S	wt	wt	–	+	0	1
I	S	wt	wt	–	–	0	2
I	S	Leu 83	wt	–	–	0	2
R	S	wt	wt	+	–	2	1
R	S	wt	wt	–	–	1	5
R	S	wt	wt	–	+	0	7
R	S	Leu 83	wt	+	+	1	0
R	S	Leu 83	wt	–	+	2	11
R	S	Leu 83	wt	–	–	6	19
R	S	Leu 83	Ile 80	–	–	4	0
R	S	Leu 83	Ile 80	–	+	1*	1
R	S	Leu 83-Asn 87	Ile 80	–	–	1	0
R	S	Leu 83-Asn 87	Ile 80-Gly 84	–	–	1	0
R	R	Leu 83	Ile 80	–	–	1	0
R	R	Leu 83-Asn 87	Ile 80	–	–	8	0
R	R	Leu 83-Asn 87	Ile 80-Val 84	–	–	3	2

*This strain was PCR positive for QnrS.

Results: Attending to the resistance patterns and the mechanisms involved were described 18 resistance profiles (Table 1).
Among DEC isolates the most frequent resistance pattern was NalR, CipS followed by NalI CipS. No NalR CipR phenotype was detected among DEC isolates. Meanwhile NalR CipS phenotype was the most frequently detected in non-DEC isolates. All but 1 isolate, with one substitution in GyrA and one in ParC presents the phenotype NalR Cip S, while isolates with three or more substitutions in the targets were full resistant to both quinolones. The unusual phenotype NalI or NalR without target mutation was detected in 21 isolates, although in 13 cases presence of the searched plasmidic mechanisms was detected (QnrB in 3 cases and AAC(6′)Ib-cr in the remaining10).
Interesting the aac(6′)Ib-cr gene was mainly detected in DEC isolates that in non-DEC (20 vs 4 isolates).
Conclusions: A high frequency of isolates without identified mechanism of quinolone-resistance has been observed, suggesting an high relevance in the area of unusual mechanisms of resistance (such as hyperexpression of chromosomal efflux pumps or the plasmid encoded efflux pumps QepA or OqxAB).
Full characterization of these isolates is need to be performed in order to identify the involved mechanism of quinolone-resistance.

P768 Presence of different resistance determinants conferring plasmid-mediated quinolone resistance in *Salmonella* isolates from reptiles in Germany

B. Guerra, K. Thomas, J. Beutlich, S. Jahn, L. Cavaco, R. Helmuth, A. Schroeter, K. Veldman (Berlin, DE; Copenhagen, DK; Lelystad, DE)*

Objective: Molecular characterization of antimicrobial resistance (R) in *Salmonella* (S.) isolates originating from reptiles showing a plasmid-mediated quinolone resistance (PMQR)- phenotype, in Germany.
Methods: Among the *S. enterica* isolates from animal and food origin (2000–2008) obtained in the National Reference Laboratory for *Salmonella* (NRL-Salm) strain collection, all epidemiologically unrelated isolates showing a MIC for nalidixic acid = 8–32 mg/L and for ciprofloxacin 0.125–1 mg/L, were selected. Twelve isolates originated from reptiles. Molecular methods like PCR amplifications/sequencing, PFGE with XbaI, plasmid profile analysis and Southern-hybridization were used to characterize the resistance determinants and epidemiological relationship of the isolates.
Results: Nine isolates were positive for PMQR-genes. The qnrB19 gene was present in seven strains, two S. Urbana, two S. subsp. I rough, one S. subsp. II and two S. subsp. IV, isolated from different reptiles (i.e. iguana, bearded dragon, turtle, gecko, snake) in different German regions. The two S. Urbana and one of the rough isolates were isolated from different animals in the same zoo, and showed by XbaI-PFGE and plasmid analyses very similar patterns, suggesting the clonal spread of a qnrB19-postive strain. The qnrB6 and acc(6′)-Ib-cr were both found in one S. Lichtfield isolated from a turtle. The qnrS1 gene was found in a S. Pomona strain also isolated from a turtle in a different German region. Plasmid location of the genes was confirmed. No qnrA, qnrC, qnrD or qepA genes were detected among the isolates analysed.
Conclusions: Our results show that various determinants conferring PMQR are present in *Salmonella* isolates originating from different reptiles. These animals could be a reservoir for the further spread of these determinants, and thus they must be seen as a Public Health concern.

Pseudomonas

P769 A new pathway involved in cross-resistance to antibiotics in *Pseudomonas aeruginosa*

C. Muller, K. Jeannot, P. Plésiat (Besançon, FR)*

Objectives: Constitutive overproduction of the multidrug efflux system MexXY/OprM provides *P. aeruginosa* with a moderate resistance to fluoroquinolones, aminoglycosides, and cefepime. In mutants named agrZ, MexXY/OprM upregulation results from inactivation of gene mexZ, the product of which represses the meXY operon. However, other efflux mutants (agrW) have been reported among clinical strains, that exhibit an intact mexZ gene. This study investigates a subclass of this latter type of mutants, dubbed agrW2.
Methods: Spontaneous MexXY/OprM overproducing mutants from reference strain PAO1 were selected on amikacin *in vitro*. Their drug susceptibility profiles were determined with the conventional agar dilution method according to standard guidelines. Search for

mexZ mutations was performed by DNA sequencing and BLAST alignement analysis. Transript levels of genes mexY, mexZ, oprD, PA5470, and PA5471 together with production of transporter MexY were assessed by quantitative Real Time PCR (RT-qPCR) and immunoblotting, respectively. The point mutation in a selected agrW2 mutant was identified by whole genome sequencing (6.3 Mbp) by using an Illumina Genome Analyser.
Results: Analysis of spontaneous amikacin resistant clones from strain PAO1 led to identification of a new type of MexXY/OprM overproducing mutants (agrW2). Unlike previously reported agrW mutants, the agrW2 mutants exhibited increased resistance to carbapenems (imipenem 8 μg/mL) in addition to cefepime (16 μg/mL), ciprofloxacin (1 μg/mL), and aminoglycosides (amikacin 16–32 μg/mL). Upregulation of MexY gene (15–17 fold) and downregulation of porin OprD gene (0.06–0.07 fold) were in full agreement with this new resistance phenotype. Gene deletion experiments demonstrated that neither repressor gene mexZ nor the PA5471-PA5470 locus known as essential for mexXY drug induction were involved in MexXY/OprM derepression in the agrW2 mutants. Whole genome sequence analysis of a selected agrW2 clone revealed a single nucleotide change (G → A) inactivating a yet uncharacterized signal transduction system. Alteration of this system was recognized in clinical multidrug resistant strains.
Conclusion: This study provides new insight into the complex regulation of the MexXY/OprM pump. Importantly, it shows that aminoglycosides are able to select porin-deficient mutants of *P. aeruginosa* cross-resistant to carbapenems.

P770 Surveillance of *Pseudomonas aeruginosa* isolates resistant to carbapenems over a 7-year period (2003–2009) in Aveiro, Portugal

S. Ferreira, L. Tortas, E. Ramalheira, S. Mendo (Aveiro, PT)*

Background: *Pseudomonas aeruginosa* is an important pathogen responsible for nosocomial infections, mainly among immunocompromised patients. In the last years, several studies describing the occurrence of metalo-β-lactamases and their association with an increase on antibiotic resistance have been largely discussed. The aim of this work was to investigate the incidence and prevalence of carbapenem resistant *P. aeruginosa* since 2003, in a Hospital in the central region of Portugal (Hospital Infante D. Pedro, Aveiro).
Methods: *P. aeruginosa* isolates were recovered from patients of different wards admitted to the hospital during a 7 years period. The isolates were identified by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (bioMérieux, Marcy L'Étoile, France). The antibiotic susceptibilities were determined by disk diffusion according to CLSI guidelines. The detection of metallo-β-lactamases was determined by double disk test IMP/IMP+EDTA and confirmed by PCR followed by nucleotide sequence determination. Nucleotide and predicted aminoacid sequences were analysed by Clustal W and Blast programs and compared with others deposited in the databases.
Results: According to susceptibilities profiles, resistance to imipenem in the timeframe studied was higher than resistance to meropenem. During 2003 and until 2006 the number of resistance strains isolated was constant. Nevertheless resistance to both antibiotics has been markedly increasing in the last 3 years. The presence of blaVIM-2 emerged in 2007 as part of two distinct gene cassette arrays of class 1 integrons. Both integrons revealed to be present in the hospital since 2007 in non-related isolates and may be contributing for the increasing of resistance observed.
Conclusion: This study demonstrates the increase of carbapenem resistant strains in the last 3 years. These results may reflect the intensive use of carbapenems, namely imipenem in the recent years, thus compromising therapy of *P. aeruginosa* infections in this hospital.

P771 Molecular analysis and antimicrobial susceptibility of *Pseudomonas aeruginosa* clinical isolates producing metallo-β-lactamases

A. Bisiklis, C. Manolopoulos, E. Protonotariou, S. Arampatzi, E. Diza, F. Frantzidou (Thessaloniki, GR)*

Objectives: The aim of this study was to determine the prevalence of metallo-β-lactamases (MBLs) in clinical isolates of *P. aeruginosa*, exhibiting resistance to carbapenems in a tertiary care Greek hospital.
Materials and Methods: The period 2002–2005 a total of 101 non-repetitive clinical isolates of *P. aeruginosa* resistant to carbapenems (MIC > 16 μg/ml) recovered from clinical specimens were studied. Bacterial identification and susceptibility testing were performed using the VITEK2 automated system (bioMérieux, France). The MICs of imipenem and meropenem were determined by the E-test (AB Biodisk, Solna, Sweeden). All strains were screened for MBL production using the IMP-EDTA double disk synergy test. The isolates with positive the IMP-EDTA test were subjected to Real-time PCR (RT-PCR) using specific primers for the detection of blaVIM and blaIMP. Moreover, all MBL positive strains were studied for the presence of class 1 integron and sequencing analysis for the possible presence of other genes that coexist in the variable region of the integron.
Results: Among the 101 carbapenem-resistant *P. aeruginosa* isolates, 36 were positive by RT-PCR for blaVIM genes. Sequencing analysis revealed the exist of blaVIM-2 gene. Susceptibility testing showed that the isolates harboring blaVIM-2 had higher MICs in ceftazidime, cefepime, amikacin, tobramycin, ciprofloxacin, and lower MICs in pipercillin, piperacillin/tazobactam, aztreonam, gentamicin, netilmicin, and colistin than those that no gene was identified. Integron class 1 was found in 30 out of 36 strains tested. Sequencing analysis of the variable region of the integron 1 revealed that (a) 4 isolates harbored only blaVIM-2 and confered resistance to all b-lactams and aminoglycocides, (b) in 16 isolates blaVIM-2 coexisted with blaaacA29a which confers resistance to tobramycin and amikacin while (c) in 10 isolates blaVIM-2 coexisted with blaaadB which confer resistance to the aminoglycosides, gentamicin, tobramicin, netilmicin and amikacin, (d) the last 26 isolates did not confer resistance to all b-lactams.
Conclusions: (a) 35.6% of the studied *P. aeruginosa* carbapenem resistant isolates harbored a metallo-b-lactamase enzyme, VIM-2. (b) In a percentage of 86.1% blaVIM-2 was detected as a part of the gene cassette in the variable region of integron class 1. (c) In a percentage of 83.8%, blaVIM-2 coexisted with other gene cassettes (aminoglycosides) within the variable region of the integron 1.

P772 MBL's in *Enterobacter cloacae* and *Pseudomonas* spp. in a medical university hospital, Warsaw

J. Patzer, A. Rokosz, A. Sawicka-Grzelak, D. Dzierzanowska, T. Walsh, M. Toleman (Warsaw, PL; Cardiff, UK)*

Objectives: To analyse genetic structures containing metallo-β-lactamase (MBL) genes in 32 multiresistant isolates collected in the Warsaw Medical University Hospital, Poland during 2001–2005.
Methods: 25 *Pseudomonas aeruginosa*, five *P. putida,* one *P. stutzeri* and one *Enterobacter cloacae* isolates, positive by Etest MBL strips and carbapenem hydrolysis assays, were collected. The MBL positive strains were further tested by a combination of genetic techniques including PCR, RFLP of integron structures, sequencing, genomic location experiments using S1 and Spe-1 digests and sequencing.
Results: All isolates were positive by hybridization with the blaVIM-1 and blaVIM-2 radio-labelled probes. Digestion of the class-1 integron PCR products with HincII produced seven different integron RFLP types (Types A-G). Type A was found in six *P. aeruginosa*, four *P. putida* and one *P. stutzeri* strains and consisted of a class-1 integron harbouring the aacA4 and blaVIM-4 gene cassettes. Type B was found in 12 *P. aeruginosa* and one *E. cloacae* strains, harbouring an integron with the aacC4, blaVIM-4 gene cassettes and the insertion sequence ISpolnew. Seven strains harboured the blaVIM-2 gene cassette in several different

gene arrays and one strain of *P. aeruginosa* harboured the blaVIM-1 gene. Other genes cassettes commonly found are aadA6, aadA10 and aadB, conferring resistance to aminoglycosides. The blaVIM-4 genes were mostly located on chromosome whereas all blaVIM-2 genes were located on plasmids of various sizes ranging from 50kb to 350kb. There was also evidence of the blaVIM-2 gene on multiple plasmids in two of *P. aeruginosa* isolates.
Conclusions: The most commonly isolated MBL genes in Warsaw are the blaVIM-4 fused gene cassette and blaVIM-2 gene cassette. Of interest is the fact that blaVIM-2 gene is more often found on plasmids than on the chromosome which is the reverse for blaVIM-4. This observation may partly explain the fact that the blaVIM-2 gene is found more often than any other MBL gene.

P773 Rare occurrence of carbapenem-resistant *Pseudomonas aeruginosa* isolates carrying VIM-2 metallo-β-lactamase

S. Sardelic, B. Bedenic, C. Colinon, Z. Bosnjak, G.M. Rossolini, S. Kalenic (Split, Zagreb, HR; Villeurbanne, FR; Siena, IT)*

Objectives: Reports of resistance to carbapenems in *P. aeruginosa* caused by acquired metallo-β-lactamases (MBL) are increasing worldwide. Following the first report of two VIM-2-producing *P. aeruginosa* strains in Croatia, we determined the occurence of MBL-producing strains among imipenem resistant *P. aeruginosa* isolated from 2001 to 2007.
Methods: MICs to antipseudomonal antibiotics were determined by broth microdilution. O-serotypes were determined by slide-agglutination. Strain typing was achieved by PFGE of genomic DNA using the XbaI enzyme and analysis of electrophoretic profiles with GelCompare software. MBL genes were screened by PCR using specific primers amplifying VIM and IMP genes. Variable arrays of MBL-carrying integrons were determined by PCR-sequencing.
Results: Among 169 *P. aeruginosa* isolates studied, 138 (82%) were multiresistant, ie. resistant to at least three drugs of following classes of antibiotics: β-lactams, carbapenems, aminoglycosides, and fluoroquinolones. The most potent antibiotic was amikacin (83% susceptible isolates; MIC90 32 mg/L), followed by colistin (50% susceptible; MIC90 8 mg/L), and ceftazidime (31% susceptible; MIC90 64 mg/L), respectively. EDTA significantly lowered MICs to imipenem in 3.5% of isolates whereas boronic acid significantly lowered MICs to ceftazidime and imipenem in 60% of isolates. The most common serotype was O12 (59% of isolates) followed by O11 (17%) and O1 (4%). Most of O12 (85% of isolates) and O11 (69%) isolates were multiresistant. PFGE analysis determined 9 main clonal lineages. O12 isolates belonged mainly to predominant clone A and O11 isolates to the second most prevalent, clone B. Six strains (3.5% of strains) carried the blaVIM-2 MBL gene. blaVIM-2 was located within the first gene cassette of a novel class 1 integron sized 3.3 kb, followed by blaOXA-10, partial qacF, aacA4, and qacEdelta1 gene cassettes. All MBL positive strains except one (being O12) were O11. Four MBL positive strains belonged to the clone B, whilst remaining two were clonally unrelated.
Conclusion: Carbapenem resistance mediated by MBLs remains infrequent in Croatia. The blaVIM-2 genes were found located in a novel class 1 integron. Most of strains were significantly inhibited by boronic acid suggesting derepression of ampC enzyme as a main mechanism of resistance to β-lactams and possibly, to carbapenems.

P774 Nosocomial spread of multidrug-resistant *Pseudomonas aeruginosa* strain, producing the novel metallo-β-lactamase variant VIM-20, in a Spanish hospital

X. Mulet, M.D. Ocete, C. Juan, M.R. Guna, M. Jiménez, M. Chanzá, N. Tormo, J.L. Pérez, C. Gimeno, A. Oliver (Palma, Valencia, ES)*

Objectives: The increasing prevalence of infections by imipenem (IMP) and meropenem (MER) resistant *Pseudomonas aeruginosa* over the last years (2007–2009) in a Spanish hospital, prompted us to characterize the underlying resistance mechanisms and molecular epidemiology.
Methods: MicroScan panels were used for identification and initial susceptibility testing. MICs for selected strains were additionally determined by Etest. Etest MBL strips were used for metallo-β-lactamase (MBL) production screening, followed by confirmation through PCR and sequencing. Clonal relatedness was assessed by pulsed field gel electrophoresis (PFGE). blaVIM genes were cloned in plasmid pUCP24, and the conferred resistance profiles were determined after transformation into reference strain PAO1.
Results: The overall prevalence of infections by IMP and MER resistant isolates increased from 5% in 2007 to 22% in 2009. The increase was particularly noteworthy in the Intensive Care Unit (ICU), reaching 76% in 2009. Most of the isolates showed the same multidrug-resistance (MDR) phenotype, including ceftazidime (CAZ), cefepime (FEP), piperacillin–tazobactam (PTZ), ciprofloxacin (CIP), gentamicin (GEN), tobramycin (TOB), and amikacin (AMK), whereas aztreonam (ATM) susceptibility was variable. PFGE, performed in 6 representative isolates, confirmed the involvement of a single clone. All isolates yielded a positive result with Etest MBL screening and blaVIM-2 PCR. DNA sequencing of the PCR products revealed in all cases the presence of a new MBL variant, designated VIM-20, differing from VIM-2 in a single residue (H229R). blaVIM-2 and blaVIM-20 were cloned in parallel in pUCP24 and transformed into PAO1. The conferred resistance profiles were almost identical for both enzymes: MICs of IMP and MER >32 mg/L, MICs of CAZ, FEP, and PTZ 24–32 mg/L, suggesting similar hydrolytic properties that need to be confirmed through biochemical studies.
Conclusion: The increasing prevalence and diversity of MBLs produced by *P. aeruginosa* calls for active surveillance to control the dissemination of these highly concerning MDR determinants.

P775 Molecular characterization of genetic structures surrounding blaVIM-2 gene identified in *Pseudomonas aeruginosa* clinical isolates from Romania

M. Straut, S. Dinu, S. Cretoiu, B. Lixandru, M. Surdeanu (Bucuresti, RO)*

Objectives: Our laboratory has been monitoring the presence of metallo-β-lactamases (MBL) producing *Pseudomonas aeruginosa* (PAE) clinical isolates since 2001. In 2007 and 2008, the first five VIM producing strains, resistant to imipenem and meropenem, were identified in Bucharest. The aim of this study was to characterise the genetic structures associated with the blaVIM genes harboured by these strains.
Methods: The presence of MBL and MBL encoding genes was confirmed by analytical isoelectric focusing and by PCR with specific primers for blaVIM-like genes. Characterisation of the MBL gene and its genetic environment, including its association with integrons and transposons, was performed using PCR and DNA sequencing. The location of blaVIM genes was determined using the endonuclease I-CeuI technique. Serotyping was performed by 17 monovalent antisera against the O antigen according to the International Antigenic Typing Scheme. Clonal relationships were established by PFGE of SpeI-digested DNA and MLST.
Results: Five out of 84 multidrug resistant clinical isolates harboured blaVIM-2 gene. Four strains carried two blaVIM-2 gene copies, chromosomally located. One copy was identified on an unusual class 1 integron containing a complete tni module, similar to Tn402, at the 3′ end, and the gene cassette array: aacA7 –blaVIM-2 –dhfrB. The structure of this integron was similar to other TniC-like integrons described in several countries. Each of three strains harboured additionally a different standard class 1 integron. One integron of 4200 bp contained five antibiotic resistance genes, including blaVIM-2. MLST analysis showed that all these four strains belonged to sequence type ST233, which is a singleton. The remaining blaVIM-2 positive strain was assigned to the wide-spread sequence type ST235, which is the founder of an international clonal complex containing MBL isolates. In this particular strain, several blaVIM-2-carrying integrons were detected, with blaVIM-2 as the second gene cassette. For all five isolates, a

correlation between PFGE analysis profiles and serotyping results was noticed.
Conclusion: In Romania, the prevalence of MBL-producing PAE strains is still very low. This report is the first molecular characterisation of genetic environment of blaVIM-2 genes identified in PAE clinical isolates from Bucharest. It highlights the epidemiological importance of TniC-like integrons in connection with the global dissemination of blaVIM-2.

P776 Detection of metallo-β-lactamase blaVIM and blaIMP type genes in clinical isolates of *Pseudomonas aeruginosa* in north-western Iran

S. Farajnia, S. Yousefi, M.R. Nahaei, M.T. Akhi, R. Ghotasslou, M. Soroush (Tabriz, IR)*

Background: *Pseudomonas aeruginosa* is an important causative of nosocomial infections, particularly in intensive care units (ICUs) and burns units. The treatment of infections with this pathogen is considered a major clinical problem due to its high antimicrobial resistance. Production of metallo β-lactamases (MBLs) and deficiency of the outer-membrane protein OprD are basic mechanisms of resistance to imipenem in *Pseudomonas aeruginosa*. The acquired MBLs belong to two major types, IMP and VIM, each comprising multiple allelic variants. The aim of this study was to determine the prevalence of VIM-type and IMP type metallo β-lactamases among clinical isolates of *Pseudomonas* aeroginosa isolates.
Materials and Methods: A total of 324 non-duplicates, isolates of *Pseudomonas aeruginosa* were collected from two educational hospitals of Tabriz and Orumieh cities during September 2007 until September 2008. All isolates were identified as *Pseudomonas aeruginosa* by using conventional bacteriologic tests. The antimicrobial susceptibility test was carryed out by disc diffusion agar method. DNA extraction was conducted by SDS-proteinas K and CTAB method. Amplification of blaVIM and blaIMP type genes was performed by using specific primers in PCR reaction.
Results: Among 324 *Pseudomonas aeruginosa* isolates, 104(32.0%) isolates were non-susceptible to imipenem, where 89(27.47%) were resistant to imipenem and 15 (4.63%) isolates had intermediate resistance to imipenem. Genotypic experiments revealed that 18 (17.31%) of imipenem non-susceptible isolates had blaVIM type gene and 6 (5.77%) isolates had blaIMP type gene.
Conclusion: This study was the first report on isolation of MBLs producing *Pseudomonas aeruginosa* strains from North-west of Iran. The results of this study showed that both blaVIM and blaIMP type metallo β-lactamase producing *Pseudomonas aeruginosa* are present in study area. Therefore rapid and accurate detection of these isolates is vital for treatment and control the spread of such strains.

P777 Clonal dissemination of IMP-6 producing multidrug-resistant *Pseudomonas aeruginosa* in South Korea

J.S. Yoo, J. Byeon, J. Yang, J.I. Yoo, G. Chung, Y.S. Lee (Seoul, KR)*

Objectives: Carbapenem resistant *P. aeruginosa* (PA) has increased in Korea, among them, VIM, IMP types of metallo-β lactamase(MBL) were identified. Although increasing IMP types of MBL producing PA were recently reported in tertiary-care hospitals, there were few other reports. Thus we aim to describe the prevalence of MBLs and molecular epidemiology of MBL-producing PA isolated from various hospital settings.
Methods: We collected 851 non-duplicated PA isolates from non-tertiary hospitals and long-term care facilities from 2008 to 2009, and from tertiary-care hospitals from 2006 to 2007. Antimicrobial susceptibility tests were performed according to CLSI methods and an imipenem disk synergy test was performed on meropenem or imipenem non-susceptible PA. PFGE types were determined by Xba-I restriction and integron restriction types were analyzed by Mbo-I enzyme cutting.
Results: Carbapenem non-susceptible isolates were 310 (36.4%). MBLs were detected in 57 isolates (IMP-1(2), IMP-6(48), VIM-2(7)) and 48(84.2%) of them were isolated from urine. All of MBL producing isolates had class I integron, VIM-2 carrying integron of 2.5kb~7kb and IMP carrying integron of 4.5kb were also detected. The integron restriction profile of IMP types showed only two types, moreover 49 (98%) were of the same type (Type I) which includes blaIMP-6, qac, cmlA, blaoxa10, and aadA1. All IMP-6 producing PA were resistant to ceftazidime, cefepime, gentamycin, tobramycin, amikacin, and ciprofloxacin. PFGE analysis indicated all IMP-6 producing PA isolates had nearly identical patterns corresponding to >85% similarity, indicating clonality.
Conclusion: IMP-6 was the predominant type of MBL in this study, it was found in >80% of MBL in all hospital settings. It is suspected that IMP-6 producing PA which showed multi-drug resistant phenotypes has been disseminated in all kinds of hospitals in South Korea. Therefore, suitable intervention methods should be used to prevent the further spread of IMP-6 carrying PA.

P778 Characterization of carbapenem resistance mechanisms and integrons in *Pseudomonas aeruginosa* strains from a Spanish hospital

B. Rojo-Bezares, C. Seral, F.J. Castillo, S. Salvo, V. Estepa, M. de Toro, C. Torres, Y. Sáenz (Logrono, Zaragoza, ES)*

Objective: To characterize metallo-β-lactamases (MBLs) and integrons in imipenem-resistant *P. aeruginosa* (IRPA) strains from a Spanish hospital.
Methods: 42 IRPA isolates were recovered from different samples of 36 patients: surgical wound (14), urine (8), blood (7), bronchial aspirate (6), skin lesion (4), peritoneal fluid (2), and intraabdominal abscess (1) from June 2008 to June 2009. Susceptibility testing to 22 antibiotics was performed by WIDER microdilution system. MBL phenotype was determined by E-test and double disc method. Detection and characterization of MBLs and class 1 and 2 integrons were studied by PCR and sequencing. The oprD gene was analyzed by PCR and sequencing in non-producing MBL isolates, and mutations were determined by comparison with the sequence of control *P. aeruginosa* PAO1. Clonal relationship among IRPA was performed by PFGE using SpeI enzyme.
Results: A total of 32 indistinguishable PFGE patterns were detected among the 42 IRPA isolates. All IRPA showed a multirresistance phenotype, and the MBL phenotype was detected in 25 strains (60%) of 23 patients in which the blaVIM-2 gene was found. Class 1 integrons were detected in 32 IRPA (76%), 19 of them showed two different integrons, and another isolate amplified three class 1 variable regions. Class 2 integrons were not found in our strains. All 25 MBL-producing strains harboured the blaVIM-2 gene inside class 1 integrons, and in addition, 17 of them presented a second class 1 integron containing aminoglycoside resistance gene cassettes such as aadA1 and aac(3′)-I. Seven non-MBL-producing strains contained class 1 integrons that presented the following arrangements: aadB (2), aadA6+orfD (3), aadA7 (1), and aac(6′)-Ib+blaOXA-46 (1). Variations in oprD gene were detected in 14 of 17 non-MBL-producing strains that could have effect on the levels of carbapenem resistance.
Conclusions: High prevalence of MBL-producing *P. aeruginosa* (60%) has been found among the clinical IRPA. VIM-2 β-lactamase dissemination through mobile elements, like class 1 integrons, and the inclusion of other resistance genes is a cause of great concern because constitutes an effective way for dissemination of multiple antibiotic resistance. In addition, chromosomal variations of the oprD gene have been identified in our clinical IRPA.

P779 Prevalence of class A extended-spectrum β-lactamases in clinical isolates of *Pseudomonas aeruginosa* in Belgian hospitals

Y. Glupczynski, P. Bogaerts, A. Deplano, C. Berhin, H. Rodriguez-Villalobos (Yvoir, Brussels, BE)*

Objectives: Class A ESBLs are occasionally reported in non-fermenting genera but their real prevalence has only rarely been investigated.

The aims of this study were to display phenotypic evidence of ESBL production, to determine the prevalence of class A ESBLs and to characterize the genotypes of *P. aeruginosa* (PA) strains referred to two Belgian reference laboratories.
Methods: Antimicrobial susceptibility by disc diffusion and Etest MIC determination was performed on all PA clinical isolates referred from January 2005 to December 2008. PA isolates resistant to cefazidime (MIC > 8 mg/L) and with a positive double-disc synergy test were screened by PCR for the production of blaBEL, blaPER, blaGES and blaVEB alleles. Genes encoding metallo-β-lactamases were also sought in isolates with positive imipenem/EDTA synergy tests. Selected PCR products were sequenced. PFGE of SpeI-digested genomic DNA was used to compare isolates and selected isolates with defined resistance mechanisms were further characterized by MLST.
Results: 58 (2.7%) of 2150 PA isolates were considered potential ESBL producers, among these 48 were confirmed by molecular testing (39 blaBEL-1, 10 blaPER-1; and 2 isolates carried both genes). These PA originated from 16 Belgian hospitals located throughout the country. All but one of the 48 isolates clustered in one single major PFGE type (called ZT). The majority of the isolates were serotype O11, (serotype O15 and O4 were occasionally found). Five centers referred 15 BEL- or PER-positive *P. aeruginosa* strains which also co-produced VIM-2 or VIM-4 metallo-β-lactamases. MLST of seven representative PA isolates revealed the presence of the ST235 sequence type which belongs to the international clonal complex CC11.
Conclusion: Class A BEL-1 ESBL-producing PA isolates are now widespread in Belgian hospitals and their emergence apparently results from the clonal expansion and dissemination of the international ST235 clone. Of great concern was the finding that BEL and PER ESBLs often coexisted with VIM carbapenemases and caused outbreaks in four hospitals. Further surveillance studies are warranted since the spread of these pan-resistant PA strains may compromise the efficacy of current anti-PA regimens in Belgian hospitals.

P780 Influence of antibiotic treatments on gene expression of RND efflux pumps in successive isolates of *Pseudomonas aeruginosa* collected from patients with nosocomial pneumonia hospitalized in intensive care units from Belgian teaching hospitals

M. Riou, L. Avrain, F. El Garch, Y. Glupczynski, J.P. Pirnay, D. De Vos, P.M. Tulkens, F. Van Bambeke (Brussels, Gembloux, Yvoir, BE)*

Background: Emergence of resistance during therapy is a worrisome problem that must be addressed in life-threatening infections such as nosocomial pneumonia (NP). The contribution of efflux in this phenomenon remains largely undefined because undetected in routine. Our aim was to evaluate the impact of antibiotic treatment on the expression of genes coding for RND efflux pumps in *P. aeruginosa* (P.a) isolated from patients with NP.
Methods: Samples: 62 pairs of P.a. collected from NP pts in 5 ICUs (a) at the time of NP diagnosis (day 0) and (b) during treatment (day x). Analyses: clonality delineated by REP-PCR and phylogenetic analysis (Diversilab™; >95% identity). Gene expression evaluated by Real Time PCR (mex Q-Test Kit, Coris BioConcept) for mexA (constitutively expressed) and mexX (inducible with low expression level in WT strains), and by PCR on cDNA for mexC and mexE (repressed in WT strains). Antipseudomonal antibiotics prescribed: as from the clinical records of each patient.
Results: The table shows (a) a high prevalence of genes encoding RND efflux pumps in the initial isolates with mexA and mexX, alone or in combination, being most prevalent, (b) a further, highly significant increase of prevalence between day 0 and day x when considering all genes globally (Fisher's Exact Test 2-sided P = 0.0028) or mexA, mexX and mexA+mexX only (χ^2 Test for Independence P = 0.024). The most used antibiotics were piperacillin–tazobactam (26 pts), amikacin (22 pts), meropenem (20 pts), cefepime (19 pts), and ciprofloxacin (6 pts), but in combination for 69% of pts. No simple correlation could be established between overexpression of a specific gene pump and the corresponding antibiotics administered to the patient. However, the predominant overexpressions seen (mexA, mexX) matched well with the global use of the anti-pseudomonal antibiotics at the population level (see note in Table for substrate specificities).
Conclusion: The study points to a high initial prevalence of genes coding for efflux mechanisms towards the main antipseudomonal antibiotics in P.a. isolates from patients with NP and its further increase during treatment. An early detection of the genomic and functional overexpression of these efflux transporters may be useful for both epidemiological and therapeutic purposes. These observations also point to the interest of developing efflux inhibitors as complementary therapeutic agents.

Table. overexpression of genes coding for Mex pumps (total isolates: 62 pairs)

Day of collection	None	*mexA*	*mexX*	*mexA+mexX*	Others[a]
0[b]	37	7	6	6	6
x[c]	20	13	12	9	8

[a] *mexC*, *mexE*, *mexA+mexC*, *mexX+mexE, mexX+mexC+mexE*, *mexA+mexX+mexC+mexE*.
[b] Before initiation of the treatment.
[c] During treatment (last sample available; mean: 23 days; median: 17.5 days; extremes: 1–123 days).
Preferential substrates: MexAB-OprM: β-lactams and fluoroquinolones; MexXY-OprM: aminoglycosides, cefepime, fluoroquinolones.

P781 Prevalence of hypermutation in bloodstream isolates of multidrug-resistant *Pseudomonas aeruginosa*

V.H. Tam, C.G. Brioso, K.T. Chang, L.A. McCaskey, J.S. Weston (Houston, US)*

Objectives: Hypermutation is a well-recognized mechanism leading to multidrug resistance in *Pseudomonas aeruginosa* (PA) from cystic fibrosis patients. However, its prevalence is not well established in the non-cystic fibrosis cohort. We examined the prevalence of hypermutation in clinical isolates of multidrug resistant (MDR) PA obtained in our hospital.
Methods: All bloodstream PA isolates obtained from 2005 to 2008 were screened for multidrug resistance (defined as resistance to ⩾3 classes of antipseudomonal agents). The clonality of the MDR isolates was assessed by rep-PCR. The presence of hypermutation was assessed by quantitatively plating 10x serial dilutions of an overnight culture on media plates (with or without rifampin supplementation at 300 mg/l). Colony counts were numerated after up to 48 hours of incubation. The experiments were repeated at least once on a different day. Hypermutation was defined as ⩾20x increase in resistance mutation frequency, compared to wild types. In addition, the mutS genetic sequence was determined by PCR. For comparison, a standard wild type (PAO1) and an isogenic known hypermutable derivative (PAO1-delta-mutS) were used as negative and positive controls, respectively.
Results: Among 306 non-repeat PA isolates (from 254 episodes) screened, 40 isolates (from 26 episodes) were found to be MDR. None of the patient from which isolates were obtained had cystic fibrosis. Using the first available isolate for each bacteremia episode (n = 23), 17 distinct clones were revealed. Hypermutation was found in 3 isolates (prevalence rate = 1.2% of total and 13.0% of MDR isolates). Point mutations in mutS (D40N and G697S in 1 isolate, and M623K in another) were found in 2 isolates.
Conclusion: In contrast to cystic fibrosis patients, hypermutation was not a common mechanism leading to MDR PA. Understanding the prevalence and mechanism of resistance in PA may guide the choice of empiric therapy for nosocomial infections in our hospital.

P782 Susceptibility of bacteraemic *Pseudomonas aeruginosa*: a 55-country analysis from 2004 to 2008

D. Hoban, M. Hackel, M. Renteria, R. Badal, S. Bouchillon, B. Johnson, J. Johnson, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Background: *Pseudomonas aeruginosa* (PA) blood stream infections remain a therapeutic challenge worldwide and both empiric and directed therapy continues to evolve as antimicrobial resistance increases. The Tigecycline Evaluation Surveillance Trial (TEST) examines the susceptibility of pathogens isolated from patients in countries worldwide annually since 2004.
Methods: A large number of PA (2251) from blood culture from 1258 cumulative sites in various countries during 2004–2008 was tested. MICs were determined using supplied broth microdilution panels and interpreted according to FDA / CLSI guidelines.
Results: The % susceptible for those 2251 PA versus comparative antimicrobial agents is shown in the table.
Conclusions: Piperacillin–Tazobactam and amikacin were the most active agents overall during 2004–2008. However, both antimicrobials exhibited diminished activity in 2008 as compared to 2004. Resistance to meropenem, ceftazidime, cefepime and levofloxacin continued to increase. All studied antimicrobials displayed 7–20% less *in vitro* activity in 2008 as compared to 2004. The continued monitoring of susceptibility of PA globally is warranted as resistance continues to increase in all antimicrobial classes.

Drug	%Susceptible/year: 2004–2008 (N)				
	2004 (322)	2005 (341)	2006 (431)	2007 (621)	2008 (536)
Amikacin	97.2	92.7	89.8	89.4	83.6
Cefepime	79.2	72.7	76.6	76.2	68.5
Ceftazidime	82.6	78.0	77.7	76.5	63.8
Ceftriaxone	22.7	14.1	15.6	12.1	10.8
Levofloxacin	68.0	62.7	70.3	66.5	61.0
Meropenem	83.8	80.3	81.3	82.4	72.8
Pip-Tazo	90.1	86.8	88.2	87.1	75.9

P783 Activity of piperacillin/tazobactam and comparators against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from South Africa, 2004–2009

M. Renteria, S. Bouchillon, R. Badal, B. Johnson, J. Johnson, D. Hoban, M. Hackel, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Background: Piperacillin–tazobactam (PTZ) continues to be useful for the treatment of patients with polymicrobial infections caused by aerobic or anaerobic β-lactamase-producing bacteria. PTZ is indicated for severe community and hospital acquired infections including *Pseudomonas aeruginosa* as well as *Acinetobacter baumannii*. In the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), a comprehensive global surveillance study, we have monitored the *in vitro* activity of PTZ and comparators for 6 years.
Methods: We evaluated 435 *A. baumannii* (183) and *P. aeruginosa* (252) isolates from 21 cumulative sites in South Africa between 2004 and 2009. Isolates were identified at each study site and confirmed by the central lab following CLSI guidelines.
Results: The table shows MIC90 and % susceptible of PTZ and comparators against *P. aeruginosa* and *A. baumannii.*
Conclusions: Since the approval of PTZ in 1993, PTZ continues to demonstrate potent *in vitro* activity against *P. aeruginosa*. The activity of PTZ is comparable to that of amikacin, imipenem and meropenem. Only tigecycline demonstrated significant activity against *A. baumannii* with MIC90 values more than 8- to 128-fold lower than any other study drug.

	P. aeruginosa (n = 252)		*A. baumannii* (n = 183)	
	MIC_{90}	%S	MIC_{90}	%S
Piperacillin–Tazobactam	128	88	>128	28
Amikacin	64	85	>64	38
Cefepime	32	77	>32	24
Ceftazidime	16	84	>32	24
Ceftriaxone	>64	15	>64	15
Imipenem	8	86	>16	68
Meropenem	>16	80	>16	24
Levofloxacin	>8	64	>8	32
Tigecycline	>16	na	1	na

P784 *In vitro* activity of the ceftazidime/NXL104 combination against *Pseudomonas aeruginosa* clinical isolates

C. Miossec, A.M. Girard, A. Malavieille, M. Claudon, H. Goossens, M. Black, P. Levasseur (Romainville, FR; Antwerp, BE)*

Objectives: NXL104 is a new, non-β-lactam, β-lactamase inhibitor currently under Phase 2 clinical development in combination with ceftazidime (CAZ). NXL104 displays potent inhibition of both class A and class C enzymes, including CTX-M and KPC variants. CAZ/NXL104 activity has been demonstrated both *in vitro* and *in vivo* against CAZ-resistant Enterobacteriaceae species. The aim of the study was to evaluate the *in vitro* antibacterial activity of CAZ/NXL104 against *P. aeruginosa* clinical strains.
Methods: 126 *P. aeruginosa* strains were collected as consecutive isolates in a French hospital between December 2006 and April 2007. MIC determination was performed using NXL104 at 4 μg/mL fixed concentration, with variable concentrations of CAZ. Reference compounds included piperacillin/tazobactam (PIP/TZP), imipenem (IPM) and aztreonam (AZT). Clonal diversity of isolates was evaluated by Pulsed-Field Gel Electrophoresis (PFGE). Resistant strains were characterized according to presence / expression of β-lactamases, porins and efflux system components.
Results: The susceptibilities of the *P. aeruginosa* strains were: 65% to CAZ, 86% to IPM, 48% to AZT and 75% to PIP/TZP. The addition of NXL104 to CAZ resulted in lowering of the MICs to below susceptibility breakpoints in 86% of the resistant *P. aeruginosa* strains; NXL104 protection of CAZ rendered 94% of isolates of the whole panel susceptible to the combination. PFGE indicated high clonal diversity among the strains intermediate or resistant to CAZ (29 different pulsotypes in 44 strains). AmpC production was found to be the major cause of resistance in the strains non-susceptible to CAZ.
Conclusion: CAZ/NXL104 combination showed excellent *in vitro* activity against a recently collected panel of *P. aeruginosa* clinical isolates, reflecting the potent inhibitory activity of NXL104 against class C β-lactamases.

P785 *In vitro* activity of tigecycline against Enterobacteriaceae and *Acinetobacter baumannii*

V. Papaioannou, S. Tsiplakou, A. Stylianakis, P. Petraki, C. Karra, P. Papanikolaou, A. Panagopoulou, A. Koutsoukou (Athens, GR)*

Objectives: Tigecycline is a member of the glycylcycline class of antibiotics with a broad spectrum of activity which includes several Gram-positive and Gram-negative bacteria. The aim of this study was to evaluate the *in vitro* activity of this agent against Enterobacteriaceae and *Acinetobacter baumannii*.
Methods: A total of 1405 strains of Enterobacteriaceae (*K. pneumoniae* 442, *K. oxytoca* 25, *E. coli* 669, *E. cloacae* 165, *E. aerogenes* 22, *C. freundii* 27, *C. koseri* 15, *S. marcescens* 40), and of 347 strains of *A. baumannii* were studied. Isolates were recovered from blood cultures, body fluids, catheters tips, pus, bronchial secretions and urine samples in a one year period, from 01/11/08 to 31/10/09. The

identification and susceptibility testing was performed via the Vitek II system (Biomerieux, France), and when necessary susceptibility results were confirmed with the use of Etest strips (AB Biodisc, Sweden) according to CLSI guidelines. EUCAST Enterobacteriaceae breakpoints were used to interpret Tigecycline MIC results for Enterobacteriaceae and *A. baumannii*.

Results: Susceptibility testing revealed susceptibility to tigecycline at 83% for *K. pneumoniae*, 100% for *K. oxytoca,* 99.8% for *E. coli*, 94% for *E. cloacae*, 95% for *E. aerogenes,* 96% for *C. freundii,* 100% for *C. koseri,* 92% for *S. marcescens*, and 57% for *A. baumannii*. Susceptibility to amikacin for *K. pneumoniae* and *A. baumannii* was at 59% and 21%, to cefepime at 26% and 0.6%, to imipenem at 36% and 7.8% and to levofloxavin at 30% and 0.9% respectively. *K. pneumoniae* and *A. baumannii* were multidrug resistant to a great extend.

Conclusion: Tigecycline maintains potent *in vitro* activity against most strains of Enterobacteriaceae. Sensitivity percentages were similar to those noted to other European countries. As for *A. baumannii*, there is a decreased susceptibility but it may be an alternative treatment option when other agents are excluded due to multidrug resistance. Due to the possibility of gaining resistance, it is essential for a hospital to remain aware of the susceptibility patterns of this new agent.

Acinetobacter

P786 Genetic and functional variability of AmpC-type β-lactamase from *Acinetobacter baumannii*

J.M. Rodriguez-Martinez, L. Poirel, P. Nordmann (Le Kremlin Bicetre, FR)*

Objectives: AmpC cephalosporinases with broadened substrate activity (toward third/fourth-generation cephalosporins and/or carbapenems) have been reported in Enterobacteriaceae and *Pseudomonas aeruginosa*. Those "Extended-Spectrum AmpCs" (ESACs) have amino acids substitutions mostly located into four regions in the vicinity of the active site: the omega loop, the H-10 helix, the H-2 helix and the C-terminal end. The study was aimed to characterize the ampC genes from *Acinetobacter baumannii* isolates and to evaluate whether ESACs enzymes might be identified in that species and may contribute to resistance to broad-spectrum β-lactams.

Methods: Seventeen non-repetitive *A. baumannii* clinical isolates recovered in 2007–2008 from Bicetre Hospital and being resistant to ceftazidime (CAZ) were studied. MICs were determined by agar dilution and E-test. Pulsed-field gel electrophoresis was performed for molecular strain typing. PCR and sequencing were used to characterize the blaampC genes. Overexpression of AmpC was evaluated by using Mueller-Hinton agar plates containing 200 mg/L of cloxacillin (AmpC inhibitor). All the ADC variants were cloned in the pTOPO vector and expressed in *E. coli* TOP10 and *E. coli* HB4 (lacking OmpC and OmpF porins).

Results: Using cloxacillin containing plates, the susceptibility to ceftazidime was restored for 11 out of 17 isolates. Colinearity between the insertion sequence ISAba1 (providing strong promoter sequences) and the blaampC gene was observed in 10 out of those 11 isolates. Genotyping performed using PFGE revealed an important clonal diversity, with 7 distinct clones identified among the 17 isolates. Cloning and sequencing identified 5 distinct AmpC β-lactamase variants among the 17 isolates. Recombinant plasmids harbouring those different blaampC genes were transformed into *E. coli* TOP10 and *E. coli* HB4 recipient strains. A variability in the resistance to broad-spectrum cephalosporins (CAZ and CTX) was observed. Higher levels of resistance (4 to 64-fold) to CAZ and CTX were observed with recombinant *E. coli* strains expressing AmpC β-lactamases that had substitutions Val236Ala in the omega-loop or N311S in the helix H-10. These differences were observed both in *E. coli* Top10 or *E. coli* HB4.

Conclusions: Variants of *A. baumannii*-specific AmpC with extended activity torward ceftazidime and cefotaxime have been firstly identified. They might play a significant role in term of resistance to broad-spectrum cephalosporins.

P787 Extended-spectrum cephalosporinases hydrolyzing fourth-generation cephalosporins in *Acinetobacter baumannii*

J.M. Rodriguez-Martinez, P. Nordmann, L. Poirel (Le Kremlin Bicetre, FR)*

Objectives: AmpC cephalosporinases with broadened substrate activity (ESACs) have been reported in Enterobacteriaceae and *Pseudomonas aeruginosa*, which mainly present changes in four regions in the vicinity of the active site: the omega loop, the H-10 helix, the H-2 helix and the C-terminal end of the protein. Here, we characterized a new AmpC variant from *Acinetobacter baumannii* presenting a broadened substrate activity toward broad-spectrum cephalosporins.

Methods: Screening for ESAC production was performed using the Mueller-Hinton agar plates containing cloxacillin (AmpC inhibitor). MICs were determined by agar dilution and E-test techniques. PCR and sequencing were used to characterize the blaampC genes. The blaampC genes were cloned in vector pTOPO and expressed in *E. coli* TOP10 and *E. coli* HB4 (lacking OmpC and OmpF porins). Identified ESACs were purified and their Km and kcat values for β-lactams determined by UV spectrophotometry.

Results: One isolate was resistant to ticarcillin, piperacillin–tazobactam, ceftazidime, cefepime and aztreonam, and of intermediate susceptibility to imipenem. Using cloxacillin-containing plates, the susceptibility to ceftazidime and cefepime was restored, suggesting overproduction of an ESAC AmpC. Cloning and sequencing identified a new ADC AmpC β-lactamase variant presenting several amino acid substitutions, including the substitution Pro238Arg together with a duplication of the Ala residue at position 243 located inside the omega-loop. Recombinant plasmid harbouring this blaampC gene was transformed into *E. coli* TOP10 and *E. coli* HB4. A broadened hydrolytic activity was observed against ceftazidime, cefotaxime, cefepime, cefpirome and aztreonam, increasing the level of resistance of 8- to 512-fold, depending of the β-lactam molecule and the *E. coli* strain background. The catalytic efficiencies (kcat/Km) of the AmpC variants possessing these substitutions were increased against ceftazidime, cefotaxime, cefepime, cefpirome and against monobactams (aztreonam).

Conclusions: This is the first description of an ESAC-type β-lactamase from *A. baumannii*.

P788 Mechanisms of resistance to imipenem and extended-spectrum cephalosporins in *Acinetobacter baumannii* clinical isolates

H.Y. Lee, C.L. Chen, C.H. Chuang, T.Y. Lin, C. Wu, C. Chiu (Taoyuan, TW)*

Objectives: Nosocomial infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* have been increasing in recent years. In order to elucidate the current resistance mechanisms, 27 *A. baumannii* clinical isolates from a regional hospital in Taiwan in 2009 were examined in this study.

Methods: E-test analysis was employed to test the minimum inhibitory concentrations (MIC) to these isolates of 8 antibiotics. Primers specific for resistance genes (blaOXA-23, blaOXA-24, blaOXA-40, blaOXA-54, blaOXA-58, blaOXA-51, blaADC, blaIMP-1, blaVIM-1) were designed for PCR amplification and sequence identification. The upstream regions of the resistance genes were tested for ISAba1 as well.

Results: *A. baumannii* isolates showed the highest rate of susceptibility (100%) to colistin, followed by tigecycline (63.0%), ampicillin/sulbactam (55.6%), imipenem (51.9%), amikacin (30.0%), ceftazidime (22.2%), gentamicin (18.5%), and ceftriaxone (0%). PCR amplification and sequence analysis identified blaOXA-23 in 9 isolates, blaOXA-51-like in 22 isolates, blaOXA-58 in 2 isolates and blaADC-25 in all isolates. Most blaOXA-51-like resistance genes were similar to blaOXA-138. The upstream ISAba1 was found in 6 isolates with blaOXA-23 and 5 isolates with blaOXA-51-like and offered these 11 isolates with full resistance to imipenem (all MIC >32) (Table 1). The upstream ISAba1 was also found in 21 isolates with blaADC-25 and offered

these isolates with full resistance to both ceftriaxone and ceftazidime. In the opposite, all other 6 isolates carried blaADC-25 without upstream ISAba1 showed intermediately resistant to ceftriaxone but susceptible to ceftazidime. Isolates with combination of upstream ISAba1 and OXA-type b-lactamases or *Acinetobacter*-derived cephalosporinases had significant full resistant ability to imipenem or extended-spectrum cephalosporins than those without such combination (both $p < 0.001$). None of isolates carried blaIMP-1. Therefore, the resistant mechanisms were quite different from previous study in which the major resistant gene contributing to imipenem resistance in *A. baumannii* clinical isolates in 2006 was metallo-β-lactamase blaIMP-1.
Conclusion: Resistant mechanisms of *A. baumannii* changed rapidly. Current results showed the upstream ISAba1 combined with OXA-type b-lactamases or *Acinetobacter*-derived cephalosporinases offered *A. baumannii* isolates in 2009 with full resistance to imipenem or extended-spectrum cephalosporins, individually.

Table 1. Imipenem minimum inhibitory concentrations (MICs) of *A. baumannii* with different carbapenemase genes and insertion sequences

Carbapenemase genes and insertion sequences	Total number	No. of isolates MIC (μg/mL)		
		>32 R	8–16 I	0.25–4 S
IS*Aba1*–$bla_{\text{OXA-23}}$ + $bla_{\text{OXA-51-like}}$	5	5	0	0
IS*Aba1*–$bla_{\text{OXA-23}}$	1	1	0	0
IS*Aba1*–$bla_{\text{OXA-51-like}}$	5	5	0	0
$bla_{\text{OXA-23}}$ + $bla_{\text{OXA-51-like}}$	3	1	0	2
$bla_{\text{OXA-58}}$ + $bla_{\text{OXA-51-like}}$	2	0	1	1
$bla_{\text{OXA-51-like}}$ only	7	0	0	7
None	4	0	0	4

P789 OXA-58 in carbapenem-resistant *Acinetobacter baumannii*

Z. Aktas, M. Isin, C. Kayacan (Istanbul, TR)*

Objective: The aim of this study was to analyse the emergence of carbapenem resistance among hospital strains of *Acinetobacter baumannii*.
Methods: Twenty seven imipenem and meropenem non-susceptible clinical isolates of *A. baumannii* were identified using the API 20NE system. Antibiotic susceptibilities of these isolates were determined by disk diffusion; followed by imipenem, ertapenem and meropenem MICs by agar dilution, using CLSI interpretative criteria. Metallo-β-lactamase (MBL) production was tested phenotypically using MBL E-tests. Carbapenemase-encoding genes (blaIMP, blaVIM, blaOXA-48, blaOXA-23-like, blaOXA-24-like, blaOXA-58) were sought by PCR using consensus primers that were specific for each enzyme group. The isolates were also screened for intrinsic blaOXA-51-like carbapenemase gene. Sequencing reactions were performed with an automated sequencer (Beckman Coulter CEQ 8000 Genetic Analysis System). Isolates were genotyped by random amplified polymorphism (RAPD) DNA.
Results: Imipenem, meropenem and ertapenem MICs for the 27 clinical isolates ranged between 4 and 64 mg/L. MBL E-tests were positive for 11 of the 27 of isolates; however, PCRs revealed that all isolates were negative for the MBL genes (blaIMP and blaVIM, as well as for blaOXA-48, blaOXA-23-like and blaOXA-24-like genes. A blaOXA-58-like gene and *A. baumannii*-specific intrinsic blaOXA-51-like gene were amplified from all 27 clinical isolates. The results of RAPD analyses indicated that all isolates belonged to the same clone.
Conclusion: OXA-58 carbapenemase showed an intra-hospital spread in *A. baumannii* and its further spread has to be surveyed among carbapenem resistant strains.

P790 OXA-24-like carbapenemase genes among carbapenem-resistant *Acinetobacter baumannii* isolates from a clinical hospital centre in Zagreb, Croatia

I. Marekovic, L. Dijkshoorn, T. van der Reijden, A. Budimir, Z. Bosnjak, S. Kalenic (Zagreb, HR; Leiden, NL)*

Objectives: Carbapenem resistance in *Acinetobacter baumannii* has emerged worldwide as a significant problem leaving few therapeutic options for treatment of infections. A major problem regarding carbapenem resistance mechanisms are the acquired oxacillinases with carbapenemase activity (OXA enzymes). Recently, in the Clinical Hospital Centre Zagreb (Croatia) emergence of carbapenem-resistant *A. baumannii* isolates was noticed. The aim of the study was to investigate strain relatedness and carbapenemase encoding genes in carbapenem-resistant isolates.
Methods: Seventy *A. baumannii* isolates were collected from December 2008 to April 2009 in Clinical Hospital Centre Zagreb. Susceptibility tests were carried out by disc diffusion and E test. Carbapenems tested included meropenem and imipenem. Identification and genotypic analysis of the *Acinetobacter* isolates was assessed by AFLP genomic fingerprinting. Detection of genes coding for OXA-23-like, OXA-24-like, OXA-51-like and OXA-58-like carbapenemases was performed with multiplex PCR. The presence of the ISAba1 insertion sequence gene was also determined with PCR.
Results: All 70 isolates were identified as *A. baumannii* by AFLP analysis. Thirty-four (48.6%) isolates were either intermediate or resistant to at least one carbapenem. Intrinsic blaOXA-51-like gene was expectedly detected in all isolates. BlaOXA-24-like gene was detected in 30 (42.9%) isolates and blaOXA-58-like in one (1.4%) isolate. All blaOXA-24-like positive isolates were resistant to both carbapenems, while blaOXA-58-like positive isolate was intermediate. ISAbaI insertion sequence gene was detected in all but two isolates. These two were susceptible to carbapenems. With AFLP, 8 genotypes were distinguished. These were distributed over 2 single strains and 6 clusters with multiple isolates indicating the occurrence of multiple endemic strains. One major cluster (genotype 3) comprised 35 isolates most of which containing OXA-24-like carbapenemase genes and being carbapenem resistant. Genotype 6 (14 isolates), 7 (2 isolates) and 8 (one isolate) corresponded with EU clone I.
Conclusion: So far, the genes encoding OXA-24-like enzyme were confined to isolates from Spain and Portugal, and recently one isolate was reported from Czech Republic. Here we report occurrence of a major cluster of *A. baumannii* strains in our hospital in Zagreb in which carbapenem-resistance was associated with OXA-24-like carbapenemase genes.

P791 First report of OXA-72 – carbapenemase in clinical isolate of *Acinetobacter baumannii* in Colombia

S.Y. Saavedra, A.L. Leal, C.H. Saavedra, J.S. Castillo Londoño (Bogotá, CO)*

Objective: Characterization of a clinical isolate of *A. baumannii* resistant to carbapenems expressing OXA-72.
Methods: The isolate was recovered of a sample surgical wound site from a 47 years old male patient, hospitalized at Intensive Care Unit of a third level hospital in Bogota. The isolate was re-identified with API 20NE, the determination of antimicrobial susceptibility was performed by E-Test and the detection of genes encoding OXA carbapenemases was performed by PCR using specific primers, amplification products were evaluated by sequentiation. To determined the location of the OXA-72 gene, was analyzed genomic DNA digested with the endonuclease I-Ceu-I and plasmid DNA, which were separated by electrophoresis pulsed-field gel, followed by a Southerm blot and a double hybridization with probes 16S rRNA and blaOXA-24 for genomic DNA and with the latest probe to plasmid DNA. The probe was labeled by PCR with digoxigenin 11-dUTP (Roche), and detection was performed by immunodetection with an antibody anti-digoxigenin and was visualized

with a colorimetric substrate (NBT/BCIP). The transfer of resistance was evaluated by conjugation and electroporation using as recipient strain *E. coli* TOP10 the transconjugants and transformants were selected on Bertani Luria plates supplemented with streptomycin (50 mg/mL) and ampicillin (50 mg/mL).
Results: The isolate was resistant to meropenem, aztreonam and ceftriaxone with MIC > 32 mg/mL for each antimicrobial, showed intermediate resistance to imipenem with MIC 8 mg/mL and was sensitive to amikacin, ceftazidime, cefepime, ciprofloxacin, piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam and colistin. PCR gene detection from OXA carbapenems gene suggested presence from gene blaOXA-51-like and blaOXA-24-like, and the sequencing amplification products confirmed the presence from OXA-72 carbapenemase in the isolate. In studies of location of the genomic DNA only hybridized with probe for the 16S rRNA gene, while the plasmid DNA gave three hybridization signals with the blaOXA24 probe, demonstrating that the OXA-72 gene is on plasmid. Attempts to transfer resistance by conjugation or transformation of plasmid DNA were unsuccessful.
Conclusions: In isolation analyzed the results suggest that blaOXA-72 gene is located on plasmid, however it is not possible to establish if in multiple plasmids or a plasmid present in different forms.

P792 Frequent occurrence of genomic resistance islands among *Acinetobacter* spp.

L. Poirel, R. Bonnin, P. Nordmann (Le Kremlin Bicetre, FR)*

Objectives: Genomic islands have been recently identified in *Acinetobacter baumannii* (AbaR) from diverse isolates being either multidrug resistant or not. In *A. baumannii* strain AYE, the AbaR1 island was 86-kb in size, and harbored 45 resistance genes. Whole genome sequencing of a couple of *A. baumannii* strains from diverse geographical origins and distinct clonal lineages identified other AbaR elements, varying in size and content. Analysis of the AbaR surrounding sequences identified an hotspot for integration, corresponding to the ATPase encoding gene. AbaR elements are often bracketed by 5-bp duplications suggesting their acquisition through a transposition-based event. Our objective was to evaluate the distribution of AbaR-like islands among a collection of *A. baumannii* isolates from worldwide origin, to identify their content and their integration site. Additionally, transposition experiments were attempted to evaluate the mobility of one AbaR element.
Methods: Forty-seven clinical *Acinetobacter* spp. isolates were collected from worldwide origin, including 41 *A. baumannii*, 3 *A. junii,* 2 *A. johnsonii,* and 1 *A. lwoffii.* Detection of the AbaR elements was performed by PCR mapping followed by sequence analysis. Transposition/conjugation assays have been conducted with *A. baumannii* BM4547 (Rif-R) as recipient and *A. baumannii* B1190 possessing an AbaR10 element and harbouring a conjugative plasmid used as donor.
Results: Our collection consisted of strains belonging to the three different European clonal lineages (I, II, and III). 36 out of the 47 isolates possessed a truncated ATPase gene, being all *A. baumannii*. 30 out of those 36 isolates possessed the same ATPase-AbaR left-junction than that originally identified with AbaRI, and 28 possessed the same ATPase-AbaR right-junction. PCR mapping revealed that all the AbaR elements identified were heterogeneous in size and genetic content. Attempts to demonstrate the transposition of one AbaR from its original host failed. Attemps to demonstrate a circular form of those AbaR elements using an inverse-PCR approach did not identify any circular intermediate for all these structures.
Conclusion: This study showed that dissemination of AbaR elements among *A. baumannii* is widely spread, being identified among isolates from distinct clonal lineages. The variety of those AbaR elements suggests a capacity to evoluate and accumulate foreign genes, including resistance genes.

P793 A 63-kb genomic resistance island in an *Acinetobacter baumannii* isolate from 1977

L. Krizova, B. Berger-Bächi, A. Nemec (Prague, CZ; Zurich, CH)*

Objective: Multidrug resistance in *Acinetobacter baumannii* has been significantly associated with a few international clonal lineages. Multidrug-resistant (MDR) strains of two of these lineages (EU clones I and II) have occurred in European hospitals since at least the 1980s. Three large (86 kb, 63 kb and 56 kb), stucturally related genomic resistance islands (AbaR1, AbaR3 and AbaR5, respectively) integrated into the ATPase gene were recently found in EU clone I strains isolated between 1997 and 2004. The aim of this study was to assess whether a MDR outbreak strain of EU clone I isolated in 1977 harbours an AbaR-like resistance island.
Methods: Strain HK302 was isolated during a hospital outbreak in Switzerland in 1977 (Antimicrob Agents Chemother 1982; 22: 323) and later was allocated to EU clone I. Strain AYE harbouring AbaR1 was used as a positive control for the detection of AbaR1-associated genes and PCR mapping. Disruption of the ATPase gene was determined using PCR with primers derived from both ends of the gene. The presence of 29 AbaR1-associated genes was investigated by PCR using primers inferred from the sequence of AbaR1. PCR mapping using the Long Range PCR kit (Qiagen) was performed, followed by RFLP analysis of amplicons. The regions different from those of AbaR1 were sequenced.
Results: A genomic region inserted into the ATPase gene at the same position as known for AbaR1 was found in HK302. In addition, 18 AbaR1-associated genes were identified in this strain. PCR mapping combined with RFLP analysis revealed that HK302 harboured a 63-kb resistance island which shared regions of a total size of 57 kb with AbaR1. Two segments (sized 4 kb and 2 kb) different from those of AbaR1 were sequenced and found to correspond to the regions described in AbaR3, i.e. one comprising the topoisomerase topA gene and the other a transposon carrying blaTEM-1.
Conclusion: An AbaR3-like resistance island was identified in HK302, which indicates that highly complex resistance islands existed in EU clone I strains already in the late 1970s.
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P794 Multidrug resistance phenotype associated with presence of integrons and specific clonal types of *Acinetobacter* spp.

E.C. Clímaco, M.O. Gomes, A.L. Darini (Ribeirão Preto, Juiz de Fora, BR)*

Objective: The main objective of this study was to determinate the presence of class 1, 2 and 3 integrons in *Acinetobacter* spp. and the association of these elements with multidrug resistance phenotype and with clonal profile of the strains.
Methods: Firstly, 63 *Acinetobacter* spp., isolated from inpatients of University Hospital of Juiz de Fora, Minas Gerais State, Brazil, from 2006 to 2007, were classified as multidrug resistant (MDR) or non-multidrug resistant (n-MDR). Class 1, 2 and 3 integrons were investigated by specific PCR amplification of intI1, 2 and 3 genes fragments and by RFLP obtained by RsaI and HinfI digestion of intI1, 2 and 3 fragments, amplified with consensus primers. The prevalence of integrons were compared among MDR and n-MDR groups, and statistical significance was estimated applying Fisher's exact. PFGE was performed and types were defined by clusters formed at 85% Dice similarity cutoff on a dendrogram constructed by UPGMA.
Results: 48 (76.1%) out of 63 *Acinetobacter* spp. were considered MDR isolates. Class 1 integrons were detected in 11 (17.5%) *Acinetobacter* spp. isolates, by specific PCR and by RFLP, all of them were MDR. Class 2 integrons were found in 30 (47.6%) *Acinetobacter* spp. isolates, by RFLP and, in 23 of them, by specific PCR. 96.7% (29) of these isolates were MDR. Class 1 and 2 integrons relationship with MDR *Acinetobacter* spp. isolates was statistically significant. No class 3 integrons was found. PFGE analysis revealed

7 clonal types and two of them were prevalent. B type was prevalent among the strains isolated up to September 2006, and A type among the strains isolated after this period. 16 out of the 17 B type strains were MDR and 50% of them harboured class 1 integrons. All the 30 A type strains harboured class 2 integrons and 29, out of them, were considered MDR isolates.
Conclusions: The incidence of MDR phenotype was high among the studied strains. Class 2 integrons were more prevalent than class 1 ones in *Acinetobacter* spp. isolates. Although both classes of integrons seem to be associated with MDR isolates, this phenotype was statistically related with A and B clonal types and it might be consequence of accumulation of different resistance mechanisms in specifics clonal types.

P795 Aminoglycoside resistance in a clinical isolate of *Acinetobacter* genomic species 13TU is associated with the up-regulation of its AdeABC-like efflux system

A. Nemec, P.G. Higgins, P. Jezek, L. Krizova (Prague, CZ; Cologne, DE; Pribram, CZ)*

Objectives: *Acinetobacter* genomic species (GS) 13TU is a member of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. Although commonly isolated from hospitalized patients, this species, unlike *A. baumannii*, is usually well susceptible to antibiotics. In a Czech hospital, two isolates of a GS 13TU strain differing in their susceptibilities to aminoglycosides were obtained from the same patient. The aim of this study was to assess whether the difference is associated with the up-regulation of the GS 13TU efflux system related to the AdeABC system in *A. baumannii*.
Methods: The two isolates, NIPH 952 and NIPH 953, were obtained, respectively, from the sputum and gastric juice of an ICU patient. Compared to NIPH 952, NIPH 953 showed elevated MICs to aminoglycosides, in particular to gentamicin (1 versus 8 mg/l) and netilmicin (2 versus 16 mg/l). The presence of the genes encoding putative efflux components was tested by PCR using primers derived from the AdeABC genes. The ability of NIPH 952 to produce aminoglycoside-resistant variants was assessed by challenging it with 4 or 8 mg/l of gentamicin. The adeB gene was partially sequenced and its expression level was examined by real-time reverse transcription PCR (RT-PCR).
Results: PCR amplicons of expected sizes were obtained with primers targeting adeA, adeB and adeS in both NIPH 952 and NIPH 953. The sequences of the adeB-like amplicons were identical in both isolates and were 84–89% identical to the known adeB sequences in *A. baumannii*. Variants with gentamicin MICs of more than 4 mg/l were obtained from NIPH 952 at frequencies of $\sim 5 \times 10^{-9}$. Two of these variants were further investigated, i.e. NIPH 952-I (gentamicin MIC 8 mg/l) and NIPH 952-IV (gentamicin MIC 24 mg/l). Compared to NIPH 952, the susceptibility patterns of NIPH 952-I, NIPH 952-IV and NIPH 953 shared elevated MICs to aminoglycosides, tetracycline, tigecycline and ciprofloxacin, whereas no changes in MICs were observed for piperacillin, cefotaxime, sulphonamides or polymyxins. Consistently, RT-PCR identified 27-fold, 214-fold and 38-fold increases in mRNA transcripts for adeB in NIPH 952-I, NIPH 952-IV and NIPH 953, respectively, as compared to NIPH 952.
Conclusion: The aminoglycoside resistance of the gastric GS 13TU isolate is likely to result from the up-regulation of its efflux system homologous to the AdeABC system in *A. baumannii*.
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P796 Involvement of pmrA/B in colistin resistance in clinical isolates of *Acinetobacter baumannii*

A. Beceiro, M. Doumith, M. Hornsey, H. Dhanji, G. Bou, D. Livermore, N. Woodford (London, UK; A Coruna, ES)*

Objectives: Colistin (Col) resistance is rare in *A. baumannii* and little is known about its mechanism, although the pmrA/B genes which encode a two-component regulatory system have been implicated in laboratory mutants. We investigated the role of pmrA/B using a small panel of international clinical *A. baumannii* strains and laboratory mutants.
Methods: Clinical isolates included 5 that were Col-R (MIC 8–64 mg/L) and 5 Col-S (MIC $\leqslant$1 mg/L). We raised Col-R mutants of both ATCC 19606 and clinical isolate ABRIM (MICs for parent/mutant: 1 vs. 64 and 1 vs. 16, respectively), and also used a pair of clinical isolates, Ab133 and Ab132, belonging to a single strain and isolated from the same patient (MICs, 2 and 64 mg/L). Isolates were identified by phenotype and species-specific PCR. PFGE was used to determine relatedness. MICs were determined by agar dilution and Etest. Expression of pmrA/B was monitored by RT-PCR. Nucleotide sequences of pmrA/B were determined.
Results: Nucleotide sequences of pmrA/B were identical to reference sequences (e.g. CP000521) in all Col-S clinical isolates, whereas 4/5 Col-R clinical isolates harboured single mutations in PmrB (either S14L, L87F, M145K or P233S); the fifth had both F387Y and S403F. No mutations were found in PmrA. Single mutations in PmrB were also found in Col-R mutants of strains ATCC 19606 (A227V), ABRIM (N353Y) and in the Col-R clinical isolate Ab132 (L87F). RT-PCR identified mean 20.6 and 7.4-fold increases in pmrA and pmrB expression, respectively, in Col-R vs. Col-S isolates. Likewise, expression of pmrA was higher in the Col-R mutants of ATCC 19606, ABRIM and in isolate Ab132 (6.4, 19.5 and 4.2 fold, respectively), compared with their parents. Expression of pmrB was not elevated in the Col-R mutant of ATCC 19606 or in Ab132, but there was a 10.5 fold increase in the mutant of strain ABRIM.
Conclusions: Colistin is one of the few options for treating multi-resistant *A. baumannii* infections. Resistance requires at least two distinct genetic events: isolates must acquire at least one point mutation in PmrB (which are not localized to a specific domain) and up-regulation (at least) of pmrA. The functional significance of the individual PmrB mutations and the precise genetic events causing pmrAB up-regulation remain to be defined. Prompt detection and effective infection control measures are critical to prevent spread of resistant strains.

P797 Molecular characterization and outbreak analysis of multidrug-resistant *Acinetobacter baumannii* from German hospitals

Y. Pfeifer, S.H. Cho, P.G. Higgins, A.M. Fahr, T.A. Wichelhaus, K.P. Hunfeld, M. Martin, W. Witte (Wernigerode, DE; Seoul, KR; Cologne, Heidelberg, Frankfurt/M., Lubeck, DE)*

Objectives: Emergence and dissemination of multidrug-resistant *Acinetobacter baumannii* are of special concern because of limited therapeutic options and increased mortality. In many cases colistin is the only antimicrobial substance for an adequate treatment. In Germany *A. baumannii* outbreaks were observed mainly in the summer months. Here we report on molecular-epidemiological analysis of *A. baumannii* from different German hospitals.
Methods: The strain collection includes 22 isolates from two outbreaks in one hospital (A) in 2007 as well as 42 outbreak isolates collected in a surgical ICU (hospital B) in 2006–2008. Furthermore 32 isolates (2005–2009) from two diagnostic laboratories were analysed. Molecular typing by PFGE and sequence-based multiplex PCR to identify isolates belonging to members of the European clonal complexes I-III were performed. Relevant resistance genes (blaOXA, blaVIM and blaIMP) were identified by PCR and sequencing.
Results: All above mentioned isolates were resistant to fluoroquinolones, aminoglycosides, sulfonamides and β-lactams including carbapenems. PCR and sequencing of resistance genes revealed the presence of OXA-β-lactamases in all isolates, with OXA-23 (n = 45) and OXA-58 (n = 47) as the most prevalent types. In several outbreak-independent isolates the genes blaOXA-72 (n = 1), blaOXA-58+blaOXA-23 (n = 1) or blaOXA-66 + insertion sequence ISAba1 were identified. In one single carbapenem-resistant isolate no carbapenemase gene was found.
Multiplex-PCR analysis for identification of clonal lineages revealed that nearly all isolates are related to the European clones I (n = 19), II (n = 51)

and III (n = 2). Outbreak analysis of hospital A showed three different clones (PFGE-macrorestriction patterns) whereby eight identical isolates with OXA-23 are related to the European clone II (multiplex-typing). The index-patients were repatriated from hospitals in Turkey and Egypt. In hospital B the 27 isolates from 2006 and 2007 had identical PFGE patterns and contained OXA-58. However, in 2008 a new *A. baumannii* clone with OXA-23 was detected. Two patients died from an infection with this strain.

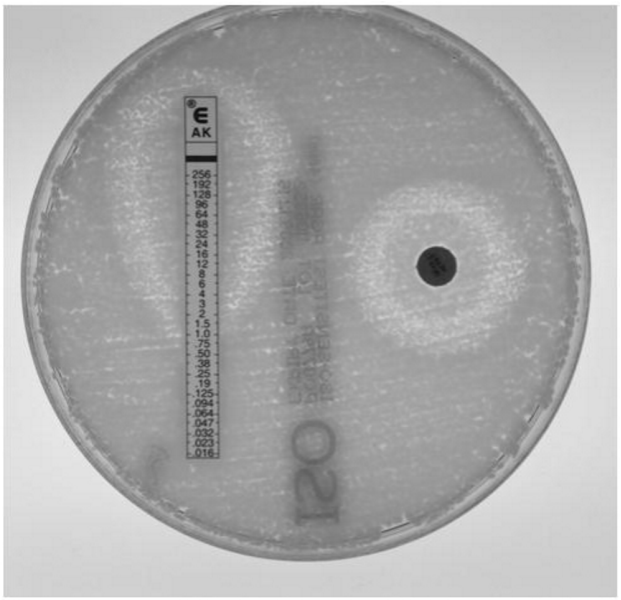
Conclusion: Infections with multidrug-resistant *A. baumannii* are still rare in Germany. The strains were imported by patients after return from abroad and further spread was probably due to transmission by staff or contaminated medical equipment. This highlights the need for the implementation of strict hygiene control measures or even isolation.

P798 Paradoxical amikacin susceptibility in a multidrug-resistant strain of *Acinetobacter baumannii*

D. Krahé, N. Gordon, B. David, D. Wareham (London, UK)*

Objectives: A multi-drug resistant isolate of *A. baumannii* recovered from a blood culture of a patient with multi-organ failure showed an 'Eagle'-like phenomenon in amikacin susceptibility testing, with growth inhibited at low concentrations but supported at concentrations above pharmacodynamic breakpoints. We investigated this finding and carried out molecular analysis of the strain.

Methods: The isolate was identified as *A. baumannii* by API 20NE and molecular detection of the blaOXA-51-like gene instrinsic to the species. Susceptibility to amikacin was carried out by disc diffusion testing according to BSAC methodology, by E-test and by determination of MICs by microtitre dilution. Multiplex PCRs were carried out to detect carriage of aminoglycoside acetyltransferases [aac(6′)-Ib, aac(6′)-Ih, aac(3)-II], nucleotidyltransferases [ant(2″)-I], phosphotransferases [aph(3′)-I, aph(3′)-VI, straA, straB] and 16S rDNA methyltransferases [armA, rmtA-D, npmA]. The effect of the generic RND efflux pump inhibitor PABA on the phenomenon was also assessed.



Results: In disc diffusion tests, growth was observed around 30 μg discs within a zone of bacterial inhibition. E-tests confirmed inhibition of growth between 0.5 and 2 mg/L of amikacin (Figure 1). In broth microtitre dilution tests – non turbid 'skipped' wells were observed in the dilution range 2–16 mg/L but only when an inoculum of 1 x 10(3) CFU / ml was used. PCR detected the carriage Of 3 aminoglycoside resistance genes, straB, aac(6′)-Ib and the armA methyltransferase known to mediate high level amikacin resistance. The addition of PABA at 50 mg/L did not influence the effects observed.

Conclusion: Treatment options for serious MDR *Acinetobacter* infections are extremely limited. We describe paradoxical susceptibility to amikacin analogous to that described by Eagle in Gram-positive bacteria in 1947 and by Lorian in the Enterobacteriaceae in 1979. Eagle demonstrated correlation of this effect *in vitro* with adverse effects *in vivo*. Further work is needed to characterize the exact mechanism, especially the regulation and contribution of ArmA, and to evaluate whether amikacin may have a role as a synergistic antibiotic at lower concentrations.

P799 Clinical and microbiological characterization of 20 carbapenem-resistant *Acinetobacter baumannii* bloodstream infections

J.Y. Song, H.J. Cheong, W. Choi, Y.M. Jo, I. Hwang, J. Heo, J. Noh, W.J. Kim (Seoul, KR)*

Objectives: The incidence of carbapenem-resistant *Acinetobacter baumannii* infection is increasing, which might be associated with increased morbidity and mortality among critically ill patients with limited therapeutic options. This study was conducted to evaluate the clinical and microbiological features of carbapenem-resistant *A. baumannii* bacteremia.

Methods: The medical records of 20 adult patients with carbapenem-resistant *A. baumannii* bacteremia admitted to Korea University Guro Hospital, from September 2005 through August 2009, were reviewed. With 20 bloodstream isolates, we intended to detect genes producing carbapenemases, and investigate inoculum effect on each antimicrobial agent (rifampicin, imipenem, colistin and tigecycline). With one blood isolate from patients with pneumonia, rifampicin-inducible resistance was examined using experimental mouse pneumonia model.

Results: Out of 20 carbapenem-resistant *A. baumannii* bloodstream infection (BI), most common primary focus was the catheter-related infection (45%), followed by pneumonia (25%), surgical site infection (15%), intra-abdominal infection (10%) and unknown sources (5%). Preceding colonization was found in just four patients (20%). Seventeen patients displayed BI by *A. baumannii* alone and three with polymicrobial BI. The 30-day overall mortality was 55%; inappropriate antimicrobial therapy (81.8% vs. 33.3%, p = 0.04) and high Pitt bacteremia score (p = 0.04) were statistically significant risk factors of mortality. All 20 isolates had blaOXA-51-like gene, two of which additionally had blaOXA-58-like gene and blaOXA-23-like gene respectively. Inoculum effect and rifampicin inducible resistance were not detected.

Conclusion: Low-level carbapenem-resistant *A. baumannii* strains with OXA-51-like carbapenemase were prevalent. Most BI by carbapenem-resistant *A. baumannii* strains developed in patients without known preceding colonization, resulting in delayed appropriate antibiotic therapy with high mortality rate.

P800 *In vitro* activity of biocides against predominant clones of *Acinetobacter baumannii* in Spain

M.A. Galán, F. Fernández Cuenca, S. Ballesta, M.C. Gómez-Sánchez, J. Vila, L. Martínez-Martínez, G. Bou, J. Rodríguez-Baño, A. Pascual (Badajoz, Seville, Barcelona, Santander, La Coruña, ES)*

Objectives: Reduced susceptibility to biocides may contribute to the persistence of some *Acinetobacter baumannii* (Ab) clones in hospitals. The aim of this study was to determinate the susceptibilities of prevalent clones of Ab to five biocides commony used in hospitals.

Methods: 8 clonally unrelated Ab with different antimicrobial susceptibility patterns corresponding to the most prevalent clones in Spain (Ab-GEIH), 1 pan-drug-resistant Ab (resistant to all commonly used antimicrobials: fluoroquinolones, aminoglycosides, carbapenems and other β-lactams, tygecycline and colistin) and 3 type strains (*A. baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213) were included. The biocides assayed were: domestic bleach (sodium hypochlorite), sterillium (propan-2-ol, propan-1-ol, mecetronium ethyl sulfate), ethanol, orsan (povidone-iodine) and clorhexidine digluconate. The antimicrobial activity of these

biocides was determined by microdilution and by disc diffusion. The maximum dilution of biocide at which there is no visible growth (MID, microdilution) and the diameter of growth inhibition (GID: disc diffusion) were determined.

Results: MID and GID results are presented in the table. By microdilution the Ab-GEIH clones were less susceptible to clorhexidine digluconate than pan-drug-resistant Ab clone, but more susceptible (6/8) than Ab ATCC. These Ab-GEIH clones were also slightly less susceptible to sterillium than pan-drug-resistant Ab clone and the ATCC strains. Five out of 8 Ab-GEIH clones were more susceptible to ethanol than pan-drug-resistant Ab clone. No significant differences were observed for domestic bleach and orsan when Ab-GEIH clones were compared with pan-drug-resistant Ab clone and the ATCC strains. Using the disc diffusion assay the Ab-GEIH clones were much less susceptible to clorhexidine digluconate than pan-drug-resistant Ab clone. The other biocides showed no significant differences in their activities when Ab-GEIH clones were compared with pan-drug-resistant Ab clone and the ATCC strains.

Conclusion: for sterillium and ethanol there was poor correlation between microdilution and disc diffusion. Susceptibility to biocides among Ab-GEIH clones was more variable for ethanol and clorhexidine digluconate than for domestic bleach, sterillium and orsan. The pan-drug-resistant Ab clone is more susceptible to clorhexidine digluconate than Ab-GEIH clones. New studies with more Ab clones are warranted.

Isolates or strains	Domestic bleach		Sterillium		Ethanol		Chlorhexidine digluconate		Orsan	
	MID	GID	MID	GID	MID	GID	MID	GID	MID	GID
Ab GEIH	1/64 1/128	37–42	1/32	9–11	1/128– 1/4096	6–8	1/8000– 1/120000	18–20	1/32	10–12
Ab PanR	1/64	40	1/128	10	1/128	6	1/240000	34	1/32	11
A. baumannii ATCC 19606	1/128	42	1/64	12	1/128	9	1/16000	18	1/16	11
P. aeruginosa ATCC 27853	1/32	30	1/64	11	1/4096	6	1/120000	20	1/16	8
S. aureus ATCC 29213	1/32	36	1/128	14	1/16384	7	<1/960000	34	1/32	11

P801 *Acinetobacter* spp. susceptibility to tigecycline determined by E-test and microdilution

L. Robles, J. Lozano, E. Riquelme, J.J. Blas, E. Simarro, M. Martínez, M.D. Crespo (Albacete, ES)*

Objective: To compare susceptibility to tigecycline through Etest and broth microdilution in *Acinetobacter baumannii* strains resistant to imipenem.

Methods: Isolates from patients admitted to our hospital between January 2007 and October 2009 were studied, considering only one isolate per patient. Identification and study of susceptibility were conducted with Vitek-2 system (Biomerieux®) and, in parallel, the susceptibility to tigecycline was studied with Etest® (AB BIODISK) in Mueller-Hinton agar (BioMedics). BSAC (British Society for Antimicrobial Chemoterapy) breakpoints were used – susceptible $\leqslant 1$, intermediate = 2, and resistant $\geqslant 2$ mg/l. The values read were rounded up to the upper dilution in base-2 system. We considered different MICs where variation existed in at least 1 dilution.

Results: 52 imipenem-resistant *A. baumannii* were isolated – 15 in 2007 (28.85%), 15 in 2008 (28.85%), and 22 in 2009 (42.3%). The allocation among services was 44.2% ICU, 23.1% Reanimation, 9.6% Internal Medicine, 7.7% Surgery, and 15.4% in other services. 36 (69.2%) isolates were susceptible to tigecycline by Etest (MIC: 0.5–1 mg/l) – amongst them, 13 (36.1%) shown a MIC value similar to the one obtained by microdilution, 14 (38.9%) a greater MIC value, and 9 (25%) a lesser value – 2 mg/l by microdilution. The remaining 16 (30.8%) strains shown an intermediate susceptibility to tigecycline (MIC=2 mg/l) by Etest. In 7 (43.75%) isolates, this value was similar to the one obtained by microdilution, whereas in 2 cases (12.5%) we obtained a greater MIC value by Etest – with the interpretation changing from intermediate to susceptible. We could not found any strain with MIC > 2 by Etest. A correlation between both methods was found ($r = 0.73$ $p \leqslant 0.05$). The percentages of intermediate or tigecycline-resistant strains were 30.8% by Etest and 27% by microdilution. As to the evolution in time, in 2007 a 33.3% of intermediate or resistant strains is found, 6.6% in 2008 and 45% in 2009.

Conclusions:

1. The greatest percentage of isolates was found in the critical care units.
2. Even though microdilution is the reference method, we realized that 17.3% strains found susceptible by Etest were intermediate by microdilution, which suggests that using Etest as the only study method for susceptibility might lead to mistaken interpretations and result in therapeutic failure.
3. We observed a significant increase of resistance to tigecycline in our hospital during the last year.

P802 *In vitro* activity of colistin sulfate in combination with other antimicrobials against *Acinetobacter baumannii*

M.E. Steed, K. Kaye, H. Salimnia, D. Marchaim, T. Chopra, M. Rybak (Detroit, US)*

Objective: Evaluate the synergy of colistin sulfate (CS) based combination regimens against multi-drug resistant (MDR) *Acinetobacter baumannii*.

Methods: The activity of CS, imipenem (IMP), ampicillin/sulbactam (AS), rifampin (RIF), and tobramycin (TOB) was evaluated against eight clonally unique strains of MDR *A. baumannii* from a multi-hospital system in Detroit, MI, USA. Minimum inhibitory concentrations were determined by broth microdilution according to CLSI guidelines. Time-kill experiments were performed with CS (1 and 0.5× MIC) alone and in combination with the other antimicrobials (0.5× MIC) at a starting inoculum of 10^6 CFU/mL and bacterial killing was evaluated at 4, 8, and 24 hours. Antimicrobial regimens included CS + IMP, CS + AS, CS + RIF, CS + TOB, CS + TOB + RIF, and IMP + AS. Synergy and bactericidal activity were defined as a $\geqslant 2$ log10 kill and a $\geqslant 3$ log10 kill, respectively.

Results: Colistin sulfate MICs ranged from 0.5 mg/L (7 strains) to 1 mg/L (1 strain). All strains were resistant to IMP and AS. Four strains were TOB susceptible (MIC 1–2 mg/L) and 4 strains were TOB resistant (MIC 16 to $\geqslant$2038 mg/L). RIF MICs ranged from 2 to $\geqslant$128 mg/L. For CS at 1x MIC, synergy and bactericidal activity was observed for CS + RIF (1 strain), CS + TOB (1 strain), COL + IMP + RIF (1 strain), and COL + TOB + RIF (2 strains). At 0.5× the MIC for all antimicrobials tested, synergistic and bactericidal activity was observed for COL + IMP (7 & 5 strains), COL + AS (6 & 5 strains), COL + RIF (7 & 4 strains), COL + TOB (5 & 4 strains), and COL + TOB + RIF (8 & 6 strains). The combination of IMP + AS was synergistic against 7 strains.

Conclusions: Synergistic activity was observed for all combinations against some of the isolates tested. The combination of COL + IMP and COL + TOB + RIF displayed the most consistent synergistic and bactericidal activity against the tested strains.

P803 Evaluation of antibiotic synergy against multidrug-resistant *Acinetobacter baumannii*: comparison of three methods

T.Y. Tan, T.P. Lim, W. Lee, L. Ng, A.L. Kwa (Singapore, SG)*

Objectives: *Acinetobacter baumannii* has emerged as a pathogen with the potential to develop multi-drug resistance (MDR), leaving few therapeutic options. Combination antibiotic therapy may be considered for infections with MDR strains of *A. baumannii*. However, there is scanty data on *in vitro* testing for antibiotic combinations, and the most appropriate antibiotics to use in combination. This study evaluated two antibiotic combinations for MDR *A. baumannii*, using three different testing methods.

Methods: Twelve strains of MDR *A. baumannii* were collected from five hospitals. Minimum inhibitory concentrations (MIC) to polymyxin B, rifampicin and tigecycline were determined by microbroth dilution, and clonal relatedness was determined by molecular strain typing. All strains were tested for the presence of *in vitro* synergy to polymyxin B-rifampicin and polymyxin B-tigeycline combinations by time-kill assays, Etest and chequerboard methods. The definition of

synergy for time-kill assays used was ⩾2log10 decrease in cfu/ml for the antibiotic combination compared with its more active constituent. The definition of synergy for Etest and chequerboard methods was a fractional inhibitory concentration index of ⩽0.5.
Results: All isolates were susceptible to polymyxin B (MIC=0.5 mg/l), while the distribution of MIC was more variable for rifampicin (2–32 mg/l) and tigecycline (2–>256 mg/l). All study isolates were shown to be unrelated by molecular strain typing. *In vitro* synergy was demonstrated in 7 (58%) isolates to polymyxin B-rifampicin and polymyxin B-tigecycline combinations, when tested by time-kill assay. None of the isolates demonstrated *in vitro* synergy against the same antibiotic combinations when synergy testing was performed by Etest. Only one isolate demonstrated *in vitro* synergy to polymyxin B-tigecycline when tested by chequerboard assay.
Conclusion: The results of *in vitro* antibiotic synergy testing for combinations of polymyxin B, tigecycline and rifampicin against *A. baumannii* vary according to the testing method used. Time-kill assays demonstrated *in vitro* synergy in over half of the study isolates, but equivalent results were not obtained by the other two methods. Further work is required to clarify the role of *in vitro* synergy testing, in particular, by correlation with clinical outcome measures.

P804 Multidrug-resistant *Acinetobacter baumannii* blood isolates: *in vitro* activity of colistin and tigecycline, existence of metallo-β-lactamase production and heteroresistance

A. Ergin, O. Koseoglu Eser, G. Hasçelik (Ankara, TR)*

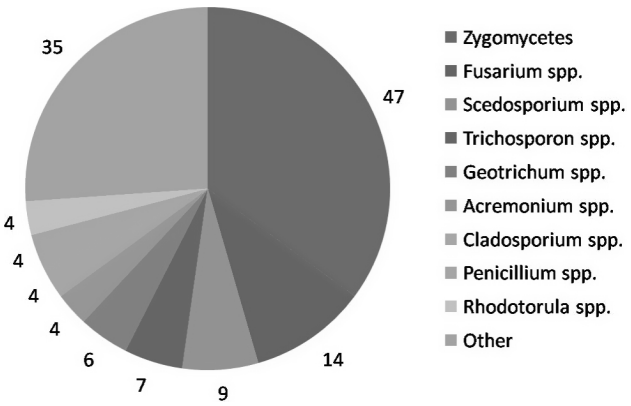
Objectives: The aims of the study were to determine the *in vitro* susceptibility of colistin and tigecycline, metallo β-lactamase(MBL) production and heteroresistance among multi-drug resistant(MDR) *Acinetobacter baumannii* strains isolated from blood samples between 2004–2009.
Methods: Sixty MDR *A. baumannii* blood isolates that were fully resistant to piperacillin (PIP), amikacin (AN), gentamicin (GM), imipenem (IMP), meropenem (MER), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), levofloxacin (LEV) detected by BD Phoenix and were tested for colistin (CL) and tigecycline (TGC) susceptibility by E-test. Heteroresistant colonies searched by E-test for colistin and by disk diffusion test for IMP. MBL production was determined by the combined disk test with IMP-0.1 M EDTA and IMP-0.5M EDTA. An increase in zone diameter of ⩾4 for 0.1M IMP-EDTA disks and ⩾7 mm for 0.5 M IMP-EDTA disks was considered positive for MBL production.
Results: Thirty-two (53.3%) of the invasive isolates were from intensive care units (ICUs). MIC90 values for the isolates were >64μg/ml for PIP, >32μg/ml for AN, >8μg/ml for GM, >8μg/ml for IMP, >8μg/ml for MER, >32μg/ml for CTX, >16 μg/ml for CAZ, >2 μg/ml for CIP, >4μg/ml for LEV, respectively. Resistance rate for colistin was 1.6%. There were two separate probable heteroresistant isolates for colistin. The MIC values for these heteroresistant strains were 8 and 24 μg/ml, respectively. There was 12 fold increase in MICs among heteroresistant isolates compared to the original MIC (0.094 and 0.25 μg/ml) levels. Tigecycline resistance rate was 6.7%. Sixteen (26.6%) of the isolates were susceptible to IMP. No heteroresistant strains were determined among IMP susceptible isolates. Among 60 *A. baumannii* isolates, 18 (30%) yielded positive results by 0.1M IMP-EDTA disk test whereas 46(76.6%) of the isolates were positive by 0.5M IMP-EDTA disk test. None of the imipenem susceptible strains gave positive MBL results by both of the disk tests.
Conclusion: In this study, randomly selection of invasive MDR *A. baumannii* isolates gave us an excellent view of colistin and tigecycline, appearing as the only susceptible antimicrobials in five years time but their limited usage in the clinical setting, existence of resistant and heteroresistant isolates indicate that new antimicrobials should be investigated against this important pathogen.

Miscellaneous fungal infections

P805 Fungiscope – a global database for rare fungal infections

M. Rüping, A. Kindo, W. Heinz, A. Hamprecht, G. Fischer, O.A. Cornely, J.J. Vehreschild on behalf of the ISHAM Working Group Fungiscope*

Objectives: We are coordinating a global registry for cases of rare invasive fungal diseases (IFD). Our objective is to broaden the knowledge on epidemiology, to determine the clinical pattern of disease, to describe and improve diagnostic procedures and therapeutic regimens, as well as to facilitate exchange of clinical isolates among the contributors.
Methods: Fungiscope™ – A Global Rare Fungal Infection Registry is an international university-based case registry that collects data of patients with rare IFD, using a web-based electronic case form at www.fungiscope.net. For inclusion in the registry cases have to have positive cultures or histopathological, antigen or molecular genetic evidence of IFD and the associated clinical symptoms and signs of invasive infection. The data that are entered onto the registry include demographics, underlying conditions, neutrophil count, concomitant immunosuppressive medications, clinical signs and symptoms of IFD, site of infection, diagnostic tests performed, pathogen identification, antifungal treatment, surgical procedures performed, response to treatment, overall survival and attributable mortality.
Results: Overall, 134 cases have been completed. Results from 17 additional cases are pending. Chemotherapy or allogeneic stem cell transplantation for a haematological malignancy was the most predominant risk factor (n=47; 35%), as well as diabetes mellitus (n=36; 27%), stay at an intensive care unit (n=22; 16%) and chronic renal failure (n=17; 13%). In 47 patients (35%) the lung was the organ of first diagnosis, followed by the sino-nasal region in 22 (16%) and deep soft tissue infections in 20 (15%) patients. For 74 (55%) patients, a favourable outcome, defined as a complete or partial response to treatment of IFD was documented. Overall mortality and mortality attributable to IFD was 38% (n=51) and 23% (n=31), respectively.
Conclusion: The clinical relevance of rare IFD is increasing steadily. In a short period of time, a wide varietyof cases from Europe, Asia and South America could be documented. Further investigators and coordinators are cordially invited to contribute to Fungiscope.



P806 Multicentre prospective monitoring for empirical treatment of invasive fungal infection using high negative predictive value diagnostic strategies

L. Hasseine, P. Marty, S. Cassaing, F. Robert-Gangneux, L. Gaudron, R. Knecht, L. Bonesso, M-D. Linas, A. Berry, J-P. Gangneux (Nice, Toulouse, Rennes, FR)*

Objectives: In order to overcome moderate sensitivity of various diagnostic tests during invasive fungal infections (IFI), early treatment strategies such as empirical antifungal therapy (EAT) have been assessed. EAT showed efficacy in improving the outcome of infected patients

but remains costly. The aim of this work was to standardize in 3 teaching hospitals the radiological and biological monitoring of EAT, among which a new panfungal PCR assay (PF-PCR), and to evaluate prospectively their negative predictive value (NPV).
Methods: 108 patients from Nice, Toulouse and Rennes University hospitals (France) were enrolled at day 0 of their EAT prescription. They benefited from a radiological and biological monitoring to confirm or exclude an IFI. The biological screening consisted of mycological analysis of various samples (blood culture, bronchoalveolar fluid, ...), galactomannan (GM) antigen assay in blood samples twice a week, and a weekly whole blood PF-PCR assay that was developed for this study.
Results: Among the 108 patients treated, 53 were considered non infected (according to EORTC/MSG 2008 criteria) and 55 patients were infected: 37 invasive aspergillosis (5 proven, 28 probable and 4 possible), 16 candidemia, 1 fusariosis and 1 cryptococcosis.
(i) We first evaluated the performance of our PF-PCR assay on the whole population: SEN = 65%, SPE=83%, PPV=80% and NPV=70%. Our results associated to other recently published data underlined the limited sensitivity of PCR for the diagnosis of IFI and incite to optimize the design and the standardization of molecular tools.
(ii) We then focused on filamentous IFI in order to evaluate the NPV of different diagnostic strategies. From our results, we can draw that a combination of 2 negative diagnostic tests allows the exclusion of most IFI with a NPV of (i) 96% when combining negative CT scan and 2 consecutive PF-PCR; (ii) 90% when combining 2 consecutive PF-PCR and 2 consecutive GM; (iii) 88% when combining negative CT scan and 2 consecutive GM.
Conclusion: Efforts must still be done to increase the sensitivity of the diagnosis of IFI, and early therapeutic strategies such as EAT remain useful. But in parallel, radiological and biological results should rapidly allow the exclusion of non-infected patients in order to reduce its cost. In this work, we show that the combination of a negative CT scan with a negative PF-PCR detection has the highest NPV and allows the stop of EAT within the first fifteen days.

P807 Impact of a multidisciplinary approach of invasive fungal infections from diagnosis to treatment

F. Lieutier, V. Mondain, M. Gari-Toussaint, L. Hasseine, A. Sirvent, M. Poirée, T. Dantin, N. Mounier, A. Mousnier (Nice, FR)*

Invasive fungal infections (IFI) are severe diseases affecting immunocompromised patients. In France, since 2007, the cost of expensive recommended antifungal treatments (AFT) is not included in the normal per-case payment and each suspected IFI must be systematically documented during interdisciplinary team meetings (ITM) including an infectious disease specialist, a haematologist, a mycologist and a pharmacist, when AFT are discussed.
Objective: to evaluate the impact of our ITM.
Methods: in 2008, 179 advices were provided in real time to prescribing doctors concerning 109 patients receiving AFT. This was done during 31 ITM. From January to September 2009, 192 advices were provided during 27 ITM, Each patient's condition was documented according to clinical presentation including emergency high resolution computed tomography (CT) of the chest and abdomen and microbiological data as *Aspergillus* galactomannan antigen assay twice a week, pan-fungal PCR, and mycological analysis of various samples (Bronchoalveolar fluid, blood culture ...).
Results: in 2008, 8 cases of probable and 2 of possible invasive aspergillosis (IA) were diagnosed (all in a haematology department, with one paediatric case). Four patients died: in one case IA may have been a causative factor. Incidence rate for IA was 4.7% among patients with acute leukaemia and stem-cell transplantation, which seems low compared to the literature. Advices were followed in 90% of cases. In 2008 and 2009 (until september), 95% of treatments involving added costs, were in line with provisions for marketing authorization.
Number of empirical AFT:
First half the year 2003 19/41 (46%)
First half the year 2008 7/23 (30%)
First half the year 2009 9/39 (23%)
To conclude, currently, 100% of patients benefit from a radiological and biological monitoring, in order to optimize the diagnosis and AFT. Thus, the empirical's antifungal (AF) number has been reduced from 46 to 23%. Indeed, we observed a 17% drop in added AF costs and 12% for other systemic AF (2007 versus 2008). A good compliance with the suggested advices shows that prescribing doctors are in favour of the scheme. The consumption of AF expressed in Daily Defined Dose will allow further assessment of practice trends.

P808 Real-life comparison of empirical and prophylactic antifungal strategies in high-risk AML patients at a tertiary cancer centre

D. Gutmann, J. Kersten, P. Schafhausen, T. Brümmendorf, M. Trepel, C. Bokemeyer, J. Panse (Hamburg, Aachen, DE)*

Objectives: Invasive fungal infections (IFI) remain a major threat for patients with acute myelogenious leukemia (AML) during induction chemotherapy neutropenia $\geqslant$10 days. Two studies demonstrating reduction in morbidity and mortality through prophylaxis with posaconazole lead to an AI recommendation in various guidelines. However, prompt empirical or pre-emptive therapies are still used alternatives as real life data about posaconazole prophylaxis are scarce and overtreatment and increasing costs are of concern. We compared clinical and economic parameters of empirical and prophylactic strategies at a tertiary cancer center during intensive building (re-) construction.
Methods: Retrospective chart review study of 104 patients with primary or secondary AML treated between January 2005 and February 2009. Each induction chemotherapy or consolidation therapy with neutropenia $\geqslant$10 days was counted as an episode (n = 222). Patients were stratified according to their antifungal approach: primary prophylaxis (n = 35, 51 episodes, group 1), empirical therapy (n = 63, 111 indices, group 2), secondary prophylaxis (n = 41, 60 episodes, group 3, comprised of patients from groups 1 and 2).
Results: Demographics, AML subtypes, co-morbidities and neutropenic days (median = 13) were comparable in all three groups; no patient received G-CSF support. Logistic regression analysis revealed days of neutropenia, performance status, use of antibiotics and secondary AML as risk factors for development of IFI, while primary prophylaxis reduced the risk for possible/probable/proven IFI by 86.7% compared to empirical therapy ($p = 0.001$). Incidences of probable/proven IFI were 3% in group 1, 29% in group 2 and 7% in group 3 ($p = 0.001$) and 17%, 69% and 37% ($p < 0.001$) when possible IFIs were included. Mortality during observation period was similar (4%, 6.4%, 7%, NS). Also similar were isolated pathogens, additional antifungals, change of antibiotics, days at intensive care unit (ICU), bacterial infections except for enterocolitis (45% group 2 vs 18% and 28% in groups 1 and 3). Side effects were slightly higher in group 2 (8% vs 2% and 5%, NS). Days of hospitalization, antifungal, antibiotic, and antiviral costs were comparable, while imaging costs were significantly higher in group 2 ($p = 0.006$).
Conclusion: Posaconazole prophylaxis significantly reduces incidences of IFI in high risk AML patients, while mortality rates, days of hospitalization, anti-infective and overall costs are not reduced.

P809 Prophylaxis drug monitoring of itraconazole and voriconazole in the paediatric onco-haematological setting

F.G. De Rosa, L. Baietto, A. D'Avolio, G. Ventimiglia, M. Siccardi, E. Biasin, F. Carraro, F. Nesi, M. Simiele, M. Sciandra, G. Di Perri (Turin, IT)*

Objectives: There is a growing knowledge of the clinical importance of therapeutic drug monitoring of azoles such as itraconazole, voriconazole in the treatment of invasive fungal disease (IFI) due to variations in absorption, metabolism and individual factors such as heterogeneity of hepatic enzymes. In this paper we report our data based on a regional-referral laboratory for the measurement of plasma concentration of azoles in a variety of patients to illustrate the usefulness of Prophylaxis Drug Monitoring (PDM).

Methods: Paediatric patients with onco-haematological diseases in oral antifungal prophylaxis for more than five days with Itraconazole (ITC) (oral solution) or voriconazole (VRC), were included in the study. Blood samples were collected at steady state, immediately before drug administration (Ctrough). Plasma drug measurement was performed by HPLC-MS validated method. The evaluated Ctrough were compared with previously reported concentrations effective in the prevention or treatment of IFI: 250 ng/mL for ITC and 1000 ng/mL for VRC.
Results: 10 patients received ITC (mean age 15.4±5.8 years old; mean body weight 59.3±27.9 Kg) and 11 patients were treated with VRC (mean age 14.9±6.4 years old, mean body weight 51±24.8 Kg). ITC was administered daily at 4 mg/Kg in 5 patients, 5 mg/Kg in 3 patients and at lower dosages in the others. VRC was administered at a dosage ranging from 2 to 14 mg/Kg daily. Mean Ctrough values were 158.2±131.5 ng/mL and 2855.8±2068.3 ng/mL for ITC and VRC, respectively.
The percentage of patients receiving ITC having a Ctrough ⩾250 ng/ml was 20%. The percentage of patients receiving VRC having a Ctrough ⩾1000 ng/ml was 91%. There was a significant correlation ($p = 0.025$) between plasma Ctrough and VRC dosage (mg/Kg) but not for ITC.
Conclusion: Our data show that the administration of ITC as antifungal prophylaxis for IFI is almost always associated with reduced plasma concentrations, with reported ineffectiveness in the prevention of IFI. The administration of VRC is more frequently associated with therapeutic drug levels. In the setting of antifungal prophylaxis, VRC should be preferred to ITC. Efforts should be made to pursue PDM when prophylaxis with ITC is chosen in high-risk patients. Future studies are needed to illustrate the value of PDM of azoles to ensure that optimal plasma levels are achieved in at-risk patients.

P810 Distribution of zygomycetes species in France, 2003–2008

E. Dannaoui, D. Garcia-Hermoso, K. Sitbon, D. Hoinard, O. Lortholary, F. Dromer for the French Mycoses Study Group*

Objectives: We have recently shown an increased incidence of zygomycosis over a 10-year period in France (Bitar et al. Emerg Infect Dis 2009). Here, we describe the diversity of zygomycetes species responsible for invasive fungal infection in France (2003–2008) and their relationship with infection characteristics and outcome.
Methods: Cases were recorded during the nationwide survey of "emerging" mycoses through the electronic secured database called RESOMYC. The causative species were identified at the NRCMA by morphology and by sequencing the ITS region.
Results: Among the 110 cases (65% males, mean age±SD=47±21 yrs), the distribution of the species was as follow: 36 (37%) Lichtheimia spp. (formerly *Absidia* spp.), 25 (21%) *Rhizopus oryzae*, 12 *Rhizomucor pusillus*, 13 *Rhizopus microsporus*, 6 *Mucor circinelloides*, 5 *Saksenaea vasiformis*, and 13 isolates accounting for 8 other species.
The main characteristics of the cases involving the 5 major species are summarized in Table 1.

	Lichtheimia spp.	*R. oryzae*	*Rhizomucor pusillus*	*R. microsporus*	*M. circinelloides*
Underlying diseases*					
Hematol. malignancies	14/23 (61%)	8/20	7/10 (70%)	5/9	0/6
Diabetes	0/23	10/20 (50%)	2/10	1/9	0/6
Solid organ transplant	5/23	0/20	1/10	2/9	0/6
Polytraumatisms	4/23	2/20	0/10	1/9	6/6 (100%)
Infections					
Rhinocerebral*	5/32	14/25 (56%)	0/10	1/13	0/6
Pulmonary*	14/33	6/23	11/12 (92%)	10/13 (77%)	0/6
Cutaneous*	16/33 (49%)	5/24	2/11	1/13	4/6
Disseminated**	6/36	2/25	2/12	0/13	0/6
Death at 90 days**	10/27	6/17	6/12	6/11	0/5

*P < 0.01; **NS.
Denominators correspond to cases with available information.

Conclusion: The distribution of the main species involved in France differs from that reported in other countries/geographical areas. In particular, a high proportion of cases were due to Lichtheimia spp. Since the proportions of underlying factors, infection localizations and outcome differ according to zygomycetes species, accurate identification is important for a better understanding of the epidemiology and pathophysiology of these infections.

P811 Estimation of the number of mucormycosis infections in France through combined datasets, 2005–2007: preliminary results

D. Van Cauteren, G. Morizot, F. Lanternier, E. Dannaoui, F. Dromer, O. Lortholary, D. Bitar (Paris, FR)*

Introduction: A retrospective study of mucormycosis cases (Retrozygo) is undergoing in order to describe these severe infections and their outcome in France. Cases were identified through 2 sources: the French hospital information system (PMSI) and the National Reference Center for Mycoses and Antifungals (NRCMA). The PMSI is a managerial tool based on the systematic collection of information for any new hospital admission and the NRCMA coordinates a recently established voluntary network of French mycologists. Using these two sources of data we applied a capture-recapture approach to estimate the number of mucormycosis cases in France between 2005 and 2007.
Methods: Cases identified through the datasets were included in the Retrozygo study after verification of their clinical symptoms and biological confirmation. Under the hypothesis of independence between sources and equal catchability in each source, estimates of the total expected numbers were calculated through a capture-recapture method. By matching several sources of information coming from the same population, the capture-recapture method allows to estimate the number of cases not identified by any of the sources, the total number of cases (N) in the population and the sensitivity of each source.
Preliminary results: Among 175 mucormycosis cases identified by the two sources, 94 cases were excluded and 87 confirmed: 31 cases were identified in both sources, 28 were reported through the NRCMA only and 22 through the PMSI only.
After capture-recapture analysis, the total number of expected cases was 100.9 (95% Confidence interval: 0–228). Based on these results, the sensitivity is 52% for PMSI, 58% for NRCMA and 87% when both data are combined together.
Discussion: As zygomycosis is a rare disease, the wide confidence intervals obtained around (N) may be due to the small numbers of cases in each source of data. The respective yield of each dataset is fairly satisfactory and the combination of both sources provides a good sensitivity. We may therefore consider that the present Retrozygo study will capture the majority of cases which occured in France within the study period, providing an accurate description of cases and their outcome.

P812 Fungal rhinosinusitis in Kuwait: a 14-year retrospective analysis of the aetiologic spectrum

Z. Khan, S. Ahmad, R. Chandy, L. Joseph, D. Farhat, S. Al-Hajri (Safat, Jabriya, Jabrya, KW)*

Objective: Fungal rhinosinusitis, once considered an uncommon disorder, is now being increasingly recognized worldwide. In the present study, we have analyzed the etiologic spectrum of fungi isolated from clinical material obtained from patients with rhinosinusitis over a 14-year period (January 1996 – October 2009) in Kuwait.
Methods: All the clinical specimens (nasal tissues, curettages/washings and swabs) received from different hospitals in Kuwait were cultured on Sabouraud dextrose agar supplemented with chloramphenicol. The identity of the isolates was established by typical phenotypic characteristics following the standard procedures. The molecular identification of the isolates was carried out by PCR amplification using panfungal primers and sequencing of the amplified products. Repeated isolations of the same fungus from an individual patient were excluded from the analysis.
Results: Fungal isolates from 83 patients with rhinosinusitis were analyzed. The age of these patients ranged between 3–70-years, 7% of them were <12 years of age. Forty (48%) of the patients were males and 63% of them were Kuwaiti nationals. Of the 83 fungal isolates, 36 (43%) were identified as *Aspergillus flavus*, 30 (36%) as *Bipolaris hawaiensis*, 6 as *Aspergillus niger*, 4 as *Aspergillus terreus*, 3 as *Aspergillus fumigatus*, and one each as *Bipolaris specifera*, *Paecilomyces*

lilacinus and Alternaria alterans. Of the total 30 isolates of *B. hawaiensis,* 26 (87%) came from Kuwaiti patients. The distribution of 36 *A. flavus* isolates between Kuwaiti and non-Kuwaiti patient was 13 (36%) and 23 (64%), respectively.
Conclusions: The findings suggest that *A. flavus* and *B. hawaiensis* are the two major species associated with the etiology of fungal rhinosinusitis in Kuwait. The isolation rate of *A. flavus* from Kuwaiti patients was only 25% (13 of 52), but much higher from non-Kuwaiti patients (23 of 31, 74%). Furthermore, the isolation of *B. hawaiensis* predominantly from the Kuwaiti patients is also an interesting observation warranting further studies on aerobiological and immunological aspects of rhinosinusitis in Kuwait.

P814 A retrospective evaluation of the efficacy of voriconazole in 24 ocular *Fusarium* infections

G. Obenga, T. Gaujoux, O. Lortholary, P.F. Troke for the French Mycosis Study Group*

Objective: *Fusarium* spp. cause problematic ophthalmic infections as they have low susceptibility to many antifungal agents and are, thus, difficult to treat. We examined the potential efficacy of voriconazole (VCZ) for therapy of *Fusarium* infections of the eye.
Methods: VCZ-treated patients (pts) with proven or probable keratitis or endophthalmitis were identified from the Pfizer VCZ database (9 pts) and 6 French ophthalmology departments (15 pts). Sociodemographic features, predisposing factors, history of corneal trauma, associated ocular conditions, other systemic diseases and prior therapies were analysed. Investigator-determined success was defined as infection resolution and/or ulcer healing. Failure was no response or persistent infection. In some pts adjunctive surgery was also required (penetrating keratoplasty, vitrectomy in emergency, evisceration) to resolve the infection.
Results: Most pts were Caucasian (83%) and male (71%). The site of infection was keratitis (63%) or endophthalmitis (37%) with proven infection in 23 (96%). Prior therapy was topical and/or systemic amphotericin (46%), fluconazole (17%) or others (33%), often in combination. Fungal pathogens isolated were *F. solani* (14, 58%), *F. moniliforme* (1), *F. oxysporum* (1) and *Fusarium* spp (8). VCZ was administered systemically, topically and/or by intraocular injection, with 16 pts (66%) receiving VCZ as salvage and 8 as primary therapy.
Response by site of infection was 73% for keratitis and 66% for endophthalmitis. Overall response was 71% and 7pts required adjunctive surgery. However, response was 88% for 8 primary VCZ therapy pts and 64% for 16 salvage therapy pts. Response by species was, *F. solani* 9/14 (55%) and all others 8/10 (80%). In 13 pts (77%) VCZ was used in combination (response 77% Vs 64% alone) with topical [amphotericin B 10/24 (42%) caspofungin 5 (21%), natamycin 1 (4%)] and systemic agents [caspofungin 3 (13%), amphotericin 2 (8%)].
Conclusions: Topical and systemic VCZ is effective alone or in combination with other antifungal agents for therapy of *Fusarium* keratitis or endophthalmitis. This therapy appears promising and a larger, prospective study is warranted.

Underlying condition (n)	Response by site n/N (%)		Median VCZ therapy (days)
	Keratitis	Endophthalmitis	
Steroids (2)	0/1 (0)	1/1 (100)	43–110
Surgery, trauma, burns (16)	9/11 (82)	4/5 (80)	47 (7–213)
Immune normal/unknown	2/3 (66)	1/3 (33)	92 (11–145)
Total (24)	11/15 (73)	6/9 (66)	54 (7–213)

P815 Fatal mycotic aneurysms due to *Scedosporium apiospermum*

A.T. Ong, R. Bency, C.C. Blyth, M. Vicaretti, A. Harun, W. Meyer, N. Gilroy, S.C. Chen (Westmead, AU)*

Objectives: *Scedosporium* are emergent pathogens in both immunocompromised and immunocompetent patients that are difficult to treat. Mycotic aneurysms due to *Scedosporium* have been infrequently reported. Two cases are presented and the literature reviewed.
Results: Case 1: A 48-yr-old diabetic male developed headache, left-sided visual loss and cranial nerve palsies 3 weeks post left partial mastoidectomy for cholesteatoma. Magnetic resonance imaging (MRI) showed extensive skull base osteomyelitis. Intravenous antibacterial agents were commenced and functional endoscopic sinus surgery performed. *Scedosporium apiospermum* was cultured. The patient received 12 weeks of antibacterials and voriconazole with clinical response. Repeat imaging was performed two weeks post ceasing therapy due to worsening headache. This demonstrated persistent osteomyelitis with extension into the right petrous temporal bone and a superior cerebellar artery aneurysm. Despite surgical clipping, he died from aneurysmal rupture and massive cerebellar infarction.
Case 2: A 55-year-old living-related renal transplant recipient developed an index finger lesion. Fungal culture grew *S. apiospermum.* Voriconazole was given for 3 months with apparent complete response. He represented three months later with bilateral flank and lower back pain. MRI of the spine, abdomen and pelvis showed inflammatory aortitis with lumbar osteomyelitis and a paravertebral abscess. A subcutaneous nodule became evident on the right wrist. *S. apiospermum* was cultured from both the paravertebral abscess and subcutaneous nodule. Voriconazole treatment was initiated. Rapid progressive dilatation of the aorta prompted surgical resection and replacement with a polytetrafluoroethylene graft. Histopathology of the aorta revealed granulomatous mycotic vasculitis and culture yielded *S. apiospermum.* The patient initially clinically responded to surgery/voriconazole but four months post surgery, developed refractory abdominal pain from an occluded superior mesenteric artery graft and died.
Conclusions: Angioinvasive complications following *Scedosporium* infection are rare. Eight cases have been described with a mortality of 88%. Early diagnosis is essential for optimal management which requires aggressive surgical resection and prolonged antifungal therapy. High risk patients with localized infection should be carefully monitored for delayed complications.

P816 Performance of the Vitek 2 yeast antifungal susceptibility card compared to broth microdilution for *Candida* and *Cryptococcus* sp.

W.W. Chan, C. Sand, B. Jansen, R. Rennie, J. Fuller (Edmonton, CA)*

Objectives: Standardized broth microdilution (BMD) for yeast susceptibility testing (CLSI M27) is a well-characterized reference assay but is reliant on visual growth endpoint determination. The AST-YS01 card for the VITEK 2 platform (bioMérieux, Marcy l'Etoile, France) provides an automated susceptibility test for yeasts that determines growth spectrophotometrically. We compared the performance of the AST-YS01 to BMD for *Candida* spp. and *Cryptococcus* spp. using stringent agreement criteria.
Methods: Testing was performed on 172 archived clinical isolates of *Candida* spp. (70 *C. albicans*, 49 *C. glabrata,* 17 *C. krusei,* 12 *C. tropicalis,* 11 *C. parapsilosis,* 13 others) and 37 isolates of *Cryptococcus* spp. (32 *C. neoformans,* 4 *C. gattii,* 1 *C. albidus)*, including 44 resistant strains. MIC values for all isolates were determined for fluconazole (FLUC), voriconazole (VORI), amphotericin B (AMB), and 5-flucytosine (5FC) using BMD (CLSI M27-A3) at 24 & 48 hours of incubation. Except for *Cryptococcus* spp. with VORI, AST-YS01 MIC values of all four drugs were determined for all isolates. Essential agreement (EA) was calculated using± 1 and± 2 log2 dilution boundary limits relative to the BMD MIC. Categorical agreement was determined using available CLSI interpretive breakpoints and $\leqslant 2$ for AMB. Discordant MIC pairs (>2 $\log_2$ dilution discrepancy) were retested with both methods.
Results: EA exceeded 90% in all scenarios when using $\pm 2 \log_2$ dilution boundary limits, which has been previously reported. The AST-YS01 card showed better EA for all drugs to the 24 h BMD MIC, except for 5FC. However, VORI versus *Candida* spp. was the only test scenario that achieved an EA value >90% using ± 1 dilution threshold.

Notwithstanding, EA for 5FC against *Candida* and *Cryptococcus*, and AMB against *Candida* were 88.6%, 89.8%, and 88.2%, respectively. Categorical agreement was >90% for all drugs except FLUC against *Candida*.

Conclusion: The VITEK 2 AST-YS01 yeast susceptibility test is a standardized and reproducible system. However, its performance compared to the reference BMD (CLSI) is suboptimal when we apply the same EA criteria used routinely for antibacterial test performance studies (±1 dilution). Unlike EUCAST, CLSI BMD methods do not use spectrophotometry for endpoint determination. In the future, this level of standardization may be all that is required to improve test performance interpretations, while maintaining stringent criteria for test agreement.

Table 1. Essential agreement (EA) between VITEK 2 Yeast Card & broth microdilution

Genus	Drug	EA at 24 h (%)		EA at 48 h (%)		CA at 48 h (%)
		+/−1 dil	+/−2 dil	+/−1 dil	+/−2 dil	
Candida	5FC	88.6	94.0	93.4	**98.2**	95.2
	AMB	89.8	98.8	80.1	**98.2**	98.8
	FLUC	86.2	93.4	83.8	**93.4**	86.8
	VORI	91.0	95.8	83.1	**91.6**	93.4
Cryptococcus	5FC	88.2	97.1	79.4	**97.1**	100
	AMB	85.3	100	76.5	**100**	100
	FLUC	82.4	97.1	73.5	**97.1**	100

dil: dilution.

P817 Risk factors for mortality of central nervous system cryptococcosis in patients with AIDS

A. Cabello, C. Isea, M. Arsuaga, M. Górgolas, M. Fernández Guerrero (Madrid, ES)*

To determine prognosis and risk factors for therapeutic failure in the short term(mortality and relapses)in HIV-positive patients with CNS cryptococcosis.

Methods: retrospective review of consecutive cases of CNS cryptococcosis complicating HIV-infection seen in a single institution since 1996. Patients with CNS involvement and isolation of *C. neoformans* from CSF and/or blood cultures were analyzed. Medical records and microbiologic charts were reviewed according to a defined protocol.

Results: 26 (1.4%) patients from a cohort of 1,800 patients with an AIDS-defining condition patients were assessed. Cryptococcosis was the AIDS-defining condition in 20 of these cases (77%). The mean age was 34.4±9.7 years; 92% were men and 73% were MSM. Most patients followed a subacute course (<4 weeks) characterized by fever (70%), headache (60%) and lethargy (30%). The mean CD4+ T cell count was 61.6±65 (median 38.5). Brain CT-scans showed hypodense lesions (cryptococcomas)in 4 cases. India ink stain showed yeast in 23 cases (88%) and they were considered abundant in 16(66.6%). CSF cultures were positive in 24 (92%) and fungemia was determined in 15 out of 19 patients in whom blood cultures were taken(79%). Crypto antigen in CSF was positive 23 patients (88%) and the levels were >1024 in 18 (78%). Patients with crypto antigen titers >1024 had CD4+ T cell counts significantly lower than their counterparts with titers <1024 (43±38 vs 115±102; $p < 0.05$). Patients were treated with liposomal anfotericin B with or without 5-flucytosine for a mean of 2.7±1.3 weeks generally followed by fluconazole for long periods. Failures were observed in 7 patients (31%): 2 patients died and 6 had a clinical relapse within 3 months after diagnosis. Both patients who died and 5 out of 6 relapses were patients with crypto antigen titers >1024 and low CD4+ T cell counts.

Conclusions: Although rare, CNS cryptococcosis continues to produce significant morbidity and mortality in the HAART's era. Profound immunosuppression and high titers of crypto antigen in CSF are associated with greater risk of mortality and treatment failures.

P818 Clinical epidemiology of cryptococcal infections in Singapore

M. Chan, D. Lye, A. Chow, Y.S. Leo, T. Barkham (Singapore, SG)*

Objective: Cryptococcosis has been reported in Singapore however prevalence of subtypes, difference in clinical presentation, underlying host status and treatment outcome are unknown.

Methods: All patients with laboratory confirmed cryptococcal infections admitted from June 1999 to March 2007 to Tan Tock Seng Hospital, a 1200 bed tertiary care centre in Singapore were retrospectively reviewed. Stored isolates were cultured on canavanine-glycine-bromothymol blue agar and subtyping was done by polymerase chain reaction restriction fragment length polymorphism. Demographic, clinical, laboratory and treatment outcome were analysed.

Results: Of 62 patients with cryptococcosis, only 3 (5%) were infected with *Cryptococcus gattii* and all were immunocompetent. In contrast, *Cryptococcus neoformans* infected mainly immunocompromised hosts (91%) with AIDS being the underlying risk factor in 80%. Median age was 39 years and 79% were male. Median duration of symptoms was 14 days with main complaints of fever (79%), headache (71%) and cough (45%). Abnormal findings of meningism occurred in 19%, cranial neuropathy 10% and papilloedema 6%. Chest radiographs were abnormal in one third. Brain imaging showed meningeal enhancement in 13% and hydrocephalus in 6%. Cerebrospinal fluid india ink smears and fungal cultures were positive in 73% and 85% respectively. Median CSF cryptococcal antigen titre was 512 compared to 2048 in serum. Blood cultures were positive in 47%. Total duration of treatment was 15 months with median 14 days initial treatment with intravenous amphotericin B. Neurosurgical shunt insertion was required in 15%. Mortality at 30 days was 15%. Patients with *Cryptococcus gattii* were more likely to have pneumonia, pulmonary cryptococcoma and were all immunocompetent. Patients with *Cryptococcus neoformans* were younger and likely to have AIDS. Clinical features and outcome were otherwise similar.

Conclusions: *Cryptococcus neoformans* was the predominant subtype in Singapore and infected younger, mainly immunocompromised hosts with AIDS. *Cryptococcus gattii* was uncommon, causing pulmonary manifestations in older, immunocompetent patients.

P819 Serotypes distribution and antifungal susceptibility of *Cryptococcus neoformans* in Spain

A. Gomez-Lopez, O. Zaragoza, M.J. Buitrago, J.L. Rodriguez-Tudela, M. Cuenca-Estrella (Majadahonda, ES)*

The aetiological agent of cryptococcosis is classified in two species; *Cryptococcus gattii* and *C. neoformans*. This species includes two varieties: var. *grubii* (serotype A) and var. *neoformans* (serotype D), as well as an AD hybrid.

Objective: The aim of this study was to evaluate the distribution and susceptibility profile of varieties of *C. neoformans* in a collection of clinical isolates, to provide insights on the epidemiology and clinical management.

Methods: A total of 45 isolates received in our institution was evaluated. The isolates were firstly identified as *Cryptococcus neoformans* by routine morphological and physiological tests. A multiplex PCR (pending of patent) to identify both *Cryptococcus neoformans* varieties and *C. gattii,* was used for serotyping. MICs for amphotericin B (AMB), 5-flucytosine (5FC), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), ravuconazole (RVC) and posaconazole (POS) were determined according to the recommendations proposed by the European Committee on Antifungal Susceptibility Testing (EUCAST-definitive document), with minor modification to improve the growth of the strains.

Results: *C. neoformans* var. *grubii* (serotype A) was the predominant (31/45, 69%) variety, following by *C. neoformans* var. *neoformans* (serotype D, 7/45, 15.5%). The remaining 7 isolates were identified as AD hybrids. The table displays susceptibility results.

Conclusions: (i) Our findings are consistent with previous studies, which indicate that var. *grubii* is the most prevalent cryptococcal isolate. (ii) The new multiplex PCR assay was useful for typing. (iii) The

prevalence of AD hybrids in clinical isolates may be higher than the current available estimations due to improper identification of this serotype. AD hybrids may be more common in clinical sources than was previously thought (iv) *C. neoformans* AD hybrid showed significantly lower MICs to 5FC (ANOVA, $p < 0.01$).

Antifungal agent	*C. neoformans* var. *grubii*, serotype A (n = 31)		*C. neoformans* var. *neoformans*, serotype D (n = 7)		*C. neoformans* AD hybrid (n = 7)	
	GM (range)	MIC_{90}	GM (range)	MIC_{90}	GM (range)	MIC_{90}
AMB	0.18 (0.03–4)	0.5	0.09 (0.03–0.25)	0.25	0.07 (0.03–0.25)	0.25
5FC	14.3 (4–64)	32	9.7 (1–32)	32	1.4 (0.5–4)	4
FLC	18.3 (2–>64)	>64	9.7 (0.5–>64)	>64	6.5 (1–16)	16
ITC	0.19 (0.03–>8)	1	0.25 (0.06–2)	2	0.10 (0.03–0.5)	0.5
VRC	0.18 (0.02–>8)	0.5	0.25 (0.13–1)	1	0.22 (0.03–4)	4
RVC	0.14 (0.03–0.5)	0.5	0.27 (0.06–2)	2	0.12 (0.02–1)	1
POS	0.17 (0.03–8)	0.5	0.22 (0.13–0.5)	0.5	0.11 (0.02–1)	1

P820 A 12-year review of *Candida* spp. meningitis in patients following neurosurgery

D. O'Brien, D. Hughes, N. Stevens, F. Fitzpatrick, E. Smyth, H. Humphreys (Dublin, IE)*

Objective: To review all cases of *Candida* spp. meningitis in patients following a neurosurgical procedure over a 12 year period in a tertiary care hospital in Dublin, Ireland.
Methods: From September 1998 to July 2009 all cases of *Candida* spp. meningitis in patients following a neurosurgical procedure were identified using a computer search. A retrospective chart review was performed to analyse patient demographics, outcome, nature of neurosurgical procedure, underlying co-morbidities, species of *Candida* implicated and type of antifungal agent used for treatment. In addition, time to removal of any neurosurgical device and resolution of infection following isolation of *Candida* spp. from the cerebrospinal fluid (CSF) was recorded.
Results: Twelve cases of *Candida* spp. meningitis were identified. The majority of isolates were associated with external ventricular drains (EVDs), one was associated with a lumbar drain and one case followed insertion of Gliadel wafers (a bioplymer wafer to deliver chemotherapy). *Candida albicans* was the predominant species recovered and amphotericin B the antifungal agent most commonly prescribed. Infection was associated with patients who were on treatment for antecendent bacterial CNS infection and those who underwent multiple EVD or ventriculo-peritoneal shunt revisions. All patients had received broad spectrum antibiotics prior to *Candida* spp. meningitis. An increase in mortality was seen in those patients who had an underlying malignancy.
Conclusion: *Candida* spp. neurosurgical infection remains a rare occurrence. Prior antibiotic use, recent or current bacterial CNS infection and multiple neurosurgical device revisions are associated with *Candida* app. meningitis. An awareness ot these factors may lead to improved management and better outcome in these cases.

P821 Searching risk factors for developing serologically proven candidiasis in ICU patients

J. Pemán, R. Zaragoza, G. Quindós, M. Alkorta, M.S. Cuétara, J. Camarena, P. Ramírez, M.J. Gimenez, E. Martín-Mazuelos, M. Linares-Sicilia, J. Pontón for the Candida albicans Germ Tube Antibody Detection in Critically Ill Patients (CAGTAUCI) Study Group*

Objectives: A prospective, cohort, observational multicentre study was carried out in six intensive care units (ICU) of tertiary-care Spanish hospitals to identify the patients who could develop serologically proven candidiasis in critical care setting.
Methods: *Candida albicans* Germ Tube Antibody (CAGTA) test was performed twice a week if predetermined risk factors were present, and serologically demonstrated candidiasis was considered if the testing serum dilution was $\geqslant$1:160 in at least one sample.
Results: Fifty-three critically ill non-neutropenic patients were included. Twenty-two patients (41.5%) had CAGTA-positive results. Neither corrected colonization index nor antifungal treatment had influence on CAGTA results. No statistical diferences were found in the rate of highly colonized patients between positive- and negative-CAGTA results at the end of the study (77.2% vs. 89.6%, respectively; $p = 0.26$). The presence of acute renal failure at the beginning of the study was more frequent in CAGTA-negative patients ($p = 0.02$). Previous surgery was more frequent in CAGTA-positive patients ($p = 0.03$).
Conclusions: This study identified previous surgery patients as the principal risk factor associated with serologically proven candidiasis. The use of CAGTA technique should be mandatory in surgical patient admitted in ICU. This technique could help to identify occult invasive *Candida* infection in ICU population.

P822 A number of diverse human pathogenic fungi produce proteases that can degrade immune proteins in the central nervous system

G. Rambach, I. Mohsenipour, C. Lass-Flörl, J. Rainer, R. Würzner, C. Speth (Innsbruck, AT)*

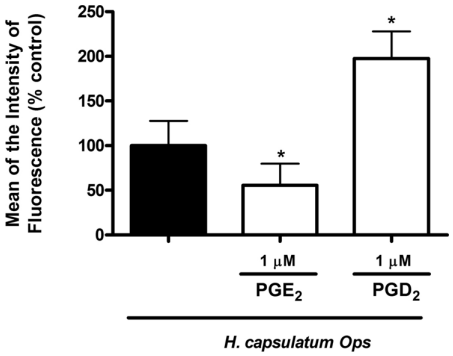
Objectives: In invasive fungal infections, the high lethality rate of more than 90% in cases of the affection of the central nervous system (CNS) indicates an insufficiency of the local immunity. We studied the capacity of diverse pathogenic fungi to degrade components of the complement system and functional surface proteins of immune cells in the CNS.
Methods: Pathogenic species of *Aspergillus*, the *Pseudallescheria*/*Scedosporium* cluster and some members of Zygomycetes were grown in medium or cerebrospinal fluid (CSF), with or without supplements. Degradation of soluble complement proteins was evaluated by Western Blot. Hyphal opsonization was examined by immunofluorescence; cellular expression of surface proteins was quantified by FACS.
Results: The growth of *Aspergillus* spp in CSF resulted in secretion of proteolytic factors which degraded various complement proteins. The extent of the proteolysis was dependent on the time period of fungal growth and the *Aspergillus* species. *A. fumigatus*, the predominant cause of cerebral aspergillosis, induced a rather quick and strong degradation. The fungal secretion of proteases correlated with a diminished opsonization of *Aspergillus* hyphae by complement proteins and proteolytic destruction of complement receptor CR3 (CD11b/CD18) on the surface of immune cells. Both opsonization of pathogens and recognition of deposited complement proteins by receptors are crucial for an efficient antifungal attack.
Other *Aspergillus* species and further pathogenic fungi could also be shown to secrete proteolytic factors. Compared to environmental isolates, patient isolates of *Aspergillus terreus* seemed to destroy complement more rapid. Within the Pseudallescheria/*Scedosporium* cluster, the asexual form *Scedosporium* generally appears to be more active in this regard than the perfect stadium Pseudallescheria. Among Zygomycetes, we found complement-degrading isolates of *Rhizomucor pusillus* and *Rhizopus microsporus*. *Candida* spp. and *Cryptococcus neoformans* will be tested next.
Conclusions: *Aspergillus* spp and other pathogenic fungi secrete proteases which can efficiently degrade complement proteins. This may represent a pivotal evasion mechanism especially in the CNS that is separated from the peripheral immune weapons by the blood-brain-barrier. On the other hand, these proteases represent an interesting therapeutic target to decrease the lethality of cerebral fungal infections.

P823 Prostaglandins as modulation of phagocitosis by *Histoplasma capsulatum*-infected alveolar macrophages

P. Pereira, C.S. Bitencourt, D.F. Santos, R. Nicolete, L. Faccioli (Ribeirão Preto, BR)*

Introduction: Prostaglandins (PGs) are importants mediators of the inflammation and immune response, beyond participating as immunomodulators in some experimental models. Recents studies have demonstrated the role of the PGE(2) on the inhibition of Fcγ-receptor-mediated phagocytosis and microbial killing by alveolar macrophages

(AMs). However, nothing is known about the effect of PGD(2) in mediating phagocytosis. In histoplasmosis, the pulmonary injury occurs after inhalation of *Histoplasma capsulatum* (Hc), In this context, AMs are the first cells to encounter and phagocyte Hc in the host.
Objectives: To investigated the effects of exogenous and endogenous PGE(2) and PGD(2) on FcγRI-mediated phagocytosis.
Methods and Results: Mice were infected (i.t.) with 1×10^6 yeast/animal (lethal inoculum) and daily treated or not with celecoxib (0.5, 1 or 5 mg/kg). The administration of celecoxib reduced 30-days mortality from 100% to 20% (0.5 or 1 mg/kg) to 58% (5 mg/kg) in lethal Hc-infected mice. The mouse resident AMs were collected from BALF. The counts of the number of phagocited yeasts were conducted as phagocytic index in Hc-infected AMs using or not the inhibitors of PGs. To verify which prostaglandin it could be involved in the suppression of the phagocitosis, AMs rats were obtained via *ex vivo* lung lavage and ressuspended in RPMI to a final concentration of 2×10^6 cells/ml. After the incubations the cells had following pretreatment for 30 min inhibitor of PGs (celecoxib and indomethacin), and then for 5 min PGE(2) or PGD(2). The adherent cells were infected *in vitro* with FITC labeled-Hc IgG opsonized (Hc-ops) or not (Hc-non). Phagocytosis was determined 2 h after, as established: at a ratio of 1:1 (macrophages/ yeast), using celecoxib and indomethacin (10uM) or PGD(2) and PGE(2) (1uM). Our findings showed that PGD(2) induced greater phagocytic of Hc-ops than PGE(2).
Conclusion: The results suggest an antagonistic effect elicited by the two evaluated prostaglandins during phagocytosis process. Further studies will be necessary in order to understand the mechanisms involved in the phagocytosis of Hc yeasts.
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P825 Voriconazole serum levels measured by high-performance liquid chromatography: a single-centre study in 14 patients

A. Gomez-Lopez, E. Cendejas, J. Garcia Rodriguez, A. Gutierrez-Altes, J.L. Rodriguez-Tudela, M. Cuenca-Estrella (Majadahonda, Madrid, ES)*

Background: Recent data have suggested that efficacy and tolerance of Voriconazole (VRC) could be increased with therapeutic drug monitoring (TDM).
Objective: We show results of a retrospective study involving patients receiving VRC for fungal infection treatment whose through serum levels were monitored.
Method: A simple/fast high-permormance liquid chromatography (HPLC) assay for VRC measurement in serum samples was used.
Results: A total of 14 patients were included, 10 men and 4 women with a mean age of 46.8 years (4–87). The most frequent underlying condition was malignant haematological disease (6/14, 43%) following by solid organ transplantation (4/14, 29%), EPOC (2/14, 14%) and others (2/14, 14%). Among patients evaluated, 8 (57%) were treated for aspergillosis (1 proven, 6 probable, and 1 possible), 2 (14%) were treated for *Aspergillus* infection in a prosthesis, 2 (14%) for *Scedosporium* infection (1 proven arthritis, 1 probable pulmonary infection), 1 (7%) for proven invasive fusariosis, and 1 (7%) for an open wound infection caused by *A. fumigatus*. A total of 59 through serum levels were performed, with a median number of 4.2 per patient (1–15 determinations). Median VRC through levels was 1.54 (range 0.0–6.4 mg/L). Twelve patients had more than one drug concentration determination. Low/high VRC serum levels was defined according to a recent paper by Pascual et al. (1–5.5 mg/L). Twenty five out of 59 (42%) of measurements from 9 different patients were below 1 mg/L, range 0.0–0.91 mg/L. In 6 out of these 9 patients VRC serum levels reached the therapeutic range (1–5.5 mg/L) after the first monitoring. Most of them (5 out of 6) experienced microbiological response (described as proven or presumed eradication of the fungal pathogen). The remaining 3 patients had persistent VRC levels below 1 mg/L (2 out of them had experienced a hepatic transplantation). Two of them experienced a positive microbiological response. In the other hand, 3 patients had VRC levels persistently into the proposed therapeutic range (media 2.6 mg/L, range 1.3–6.4 mg/L). All of them had complete resolution of their fungal infection episode. Only one patient had VRC measurement higher than 5.5 mg/L, but no toxicity was reported.
Conclusions: (i) Lack of response to therapy was more frequent in patients with VRC levels persistently lower than 1 mg/L (ii) Detection of VOR trough levels outside the therapeutic interval may lead to adjust dosage and improve patient's outcome. (iii) Prospective trials including VRC TDM are needed.

P826 Onychomycosis by *Paecilomyces lilacinus* in a patient with leukonychia

P. Innocenti, E. Pagani, D. Vigl, V. Pasquetto, C. Cemin, K. Scalzo, H.P. Huemer, C. Larcher (Bolzano, IT; Innsbruck, AT)*

Introduction: *Paecilomyces lilacinus* (P.I.) is a filamentous fungus living saprophytic in the environment. Very rarely it causes infection in immunocompetent humans, mostly ocular infections (after lens implantation) or cutaneous and sub-cutaneous infections. P.I. isolates usually show low susceptibility to conventional antifungal drugs *in vitro*, and variable susceptibility to novel triazoles.
Methods: During a period of 2 years 4 cultures of nail fragments were grown from a 41 y old female on modified Sabouraud/Dextrose agar (CAF bioMérieux, CAF+CEX Biolife). Cultures were continued in suspension and the hyphae stained with lactophenole blue analysed microscopically. 2 samples were tested with molecular methods, i.e. automated DNA extraction with easyMAG (bioMérieux), amplification of the fungal internal transcribed spacer (ITS) region by PCR and automated DNA sequencing of the ITS 1 and 2 regions (ABI3130). The sequence results were evaluated using TraceEdit Pro (RIDOM Bioinformatics) and a validated reference database for humanpathogenic fungi. Additionally NIH database GenBank and the BLAST algorithm was used.
Results: Growth of a filamentous hyaline fungus with white colonies with lila shade developing in 4–5 days was observed in all samples. The presence of Penicillium like conidiophores with divergent long terminal phialides was seen with little elliptical conidia. DNA sequencing confirmed the genus Paecilomyces determining the species as *P. lilacinus* with 99% homology of the ITS1 and 2 regions with GenBank isolate accession number AY213667 and other P.I. entries.
Conclusion: P.I. infection of the nails is a very rare event in healthy, not immunocompromised persons and it is unclear whether the precedent pregnancy of the patient had triggered the onset. The infection of the right hallux did not improve by systemic (Lamisil/terbinafine) and local treatment (Locetar/amorolfin nail lacquer). Also change to Sporanox/itraconazol only led to deterioriation, so finally the extraction of the nail had to be performed given the known difficulties with treatment against P.I. described in the literature. This case highlights the usefulness of molecular techniques, especially use of the highly discriminative ITS region for fungal typing.

P827 Comparison of diagnostic methods in the evaluation of onychomycosis in patients referred to a hospital and dermatology outpatients clinics in Sari, Iran

T. Shokohi, Z. Haj Heydari, I. Haghani, A. Khalilian, R. Agilli, S. Mayahi (Sari, IR)*

Objectives: Onychomycosis is common nail problem, accounting for up to half of all nail diseases. Onychomycosis requires administration of antifungal agents for long periods. Nail disorders may mimic to onychomycosis clinically. Therefore sensitive, quick and inexpensive test is essential for screening nail specimens. Recently, It has been reported that KONCPA (KOH treated Nail clipping + PAS) test might be a useful complementary method with high positive rate and excellent view for making the diagnosis of onychomycosis. The aims of this study were to compare culture, KOH preparation, calcofluor white stain (CFW), and KONCPA in the diagnosis of onychomycosis and to determine their sensitivity, specificity, positive predictive value and negative predictive value.
Methods: In the present study, nail specimens were collected from 101 patients suspected to onychomycosis during 14 months. Nail specimens were examined by potassium hydroxide 20%, KONCPA, calcofluor white stain and culturing on Sabouraud's dextrose agar containing chloramphenicol (SC), Sabouraud's dextrose agar containing cyclohexamide and chloramphenicol (SCC). Culture was chosen as the gold standard for statistical analysis.
Results: Of the patients, 100 had at least 1 of the 4 diagnostic methods positive for the presence of organisms. The following were calculated for each test: sensitivity; specificity; positive predictive value; and negative predictive value. The positive rates of fungal culture, KOH preparation, CFW and KONCPA were 74.2%, 85.1%, 91.09%, and 99.01%, respectively. The sensitivities of each of the techniques were as follows: KOH 92%; CFW 96%; and KONCPA 100%. KONCPA method were more sensitive than KOH preparation ($P = 0.00002$) and CFW ($P = 0.0008$). The specificities were as follows: KOH 38%; CFW 23%; and KONCPA 3.8%. The positive predictive value calculated for the different techniques were: KOH 81%; CFW 78%; and KONCPA 75%. In terms of negative predictive value, the results were: KOH 66%; CFW 66%; and KONCPA 100%.
Conclusion: KONCPA was the most sensitive among the tests. It is also superior to the other methods in its negative predictive value. It was easy to perform, rapid, gave significantly higher rates of detection of onychomycosis compared to the standard methods, namely KOH mount and mycological culture and potentially is the single method of choice for the evaluation of onychomycosis.

P828 Detection of dermatophyte infections in nail samples by PCR targeting the DNA topoisomerase II genes

M. Hui, M. Eastel, D. Jin, L.H. Fu, N.M. Luk, A.K. Chan, K.K. Lo, C.Y. Chan (Shatin, Hong Kong, HK)*

Objectives: Dermatophytic infections of nails can be caused by Trichophyton species, Epidermophyton species and Microsporum species. However, establishment of the etiological agents are often difficult due to the lack of sensitivity with traditional microscopic and culture techniques. Therefore, we aimed to develop a direct detection method by PCR targeting the dermatophyte DNA topoisomerase II genes.
Methods: Primers pair targeting the dermatophytes DNA topoisomerase II genes TopII-F (5'-GAGTTGATGGAGTATAGAGAGGT-3') and TopII-R (5'-GCTTTGGTGTACATTTCCATGT-3') was designed. The amplified DNA product size was approximately 690bp. Type strains of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*, 2 *Aspergillus* species and 5 *Candida* species were tested for sensitivity and specificity. Clinical nail samples were collected from patients suspected to have dermatophytic nail infections. These samples were subjected to microscopic examination for fungal filaments, culture for dermatophytes, and PCR detection. DNA was extracted from 30–50 mg nail pieces by incubating in extraction buffer for 10min at 95ºC. The reaction mixtures comprised of 1uL of DNA, 2.5uL of 10X buffer, 0.375uL of dNTPs, 1uL of each primer, and 0.125uL Taq polymerase. The PCR cycles comprised of denaturation at 95ºC for 10min, followed by 35 cycles of 30s at 94ºC, 30s at 58ºC and 30s at 72ºC. The PCR products were visualised by gel electropheresis on 1.5% agarose gel.
Results: A total of 112 nail samples were collected. Microscopic examination was positive for fungal filaments in 13 (11.6%) samples. Culture was positive for dermatophytes in 14 (12.5%) samples. Ten of which were *Trichophyton rubrum*, 2 were *Trichophyton mentagrophytes*, 1 was Trichophyton verrucosum and another one was *Trichophyton tonsurans*. PCR detection were positive in 42 (37.5%) samples. Four culture positive cases were negative for PCR detection. 28 PCR positive cases were microscopy- and culture-negative.
Conclusion: Traditional diagnostic methods by microscopy and culture were technically simpler than molecular detection method. However, the former method was time-consuming and crippled with low recovery rate. Direct detection of targeted DNA topoisomerase II genes was rapid and sensitive. Improved detection of dermatophytes was observed with the additional use of PCR technique.

Antifungal resistance

P829 Dihydropteroate synthase-gene mutations decreased in *Pneumocystis jirovecii* isolated from Spanish patients

R. Morilla, C. De La Horra, V. Friaza, M.A. Montes-Cano, E. Campano, L. Rivero, S. Gutierrez, N. Respaldiza, J. Medrano, J.M. Varela, E. Calderon (Seville, ES)*

Background: Sulfa drugs, trimethoprim-sulfamethoxazole and dapsone, are mainstays for prophylaxis and treatment of *Pneumocystis* pneumonia (PcP), a life-threatening disease in immunosuppressed patients. The inability to culture *Pneumocystis* has led to develop molecular techniques based on identification of punctual mutations on the Dihidropteroate Synthase gene (DHPS) that cause sulfa resistance in other microorganisms. A key issue is whether the emergence of DHPS mutations is result of transmission between patients or arises from selection by the pressure of sulfa drugs, two possibilities are not mutually exclusive. The role of *Pneumocystis* colonized subjects in transmission of DHPS mutations still unknown. The aim is to provide epidemiological data of *P. jirovecii* DHPS mutations among PcP patients and immunocompetent colonized subjects.
Methods: The study included 47 PcP patients and 75 *Pneumocystis* colonized subjects during 2001–2007 identified by nested PCR at mtLSUrRNA gene. DHPS mutations were studied by Restriction Fragment Length Polymorphism using Acc I and Hae III at nucleotide positions 165 and 171 respectively.
Results: The analysis showed a 19.7% prevalence of DHPS gene mutations in the overall population. All possible polymorphisms described were identified. There were not difference between the frequency of DHPS mutations in PcP patients and colonized subjects (23.4% vs 17.3%; $p = 0.75$). A trend towards decreased frequency was observed during this period in the Spanish population (from 31.3% of DHPS-mutations during 2001 to 11.6% at 2007). The use of sulfamethoxazole was also measure in this area showing a 41% of decreasing.
Conclusions: Similar DHPS pattern was observed in PcP patients and immunocompetent colonized subjects suggests that both group could share a common transmission cycles of mutated strains. This arise question about the role that colonized subjects could represent as reservoir for DHPS mutations with ability to transmit them to immunocompromised hosts susceptible to PCP. The reduction of DHPS-mutations could be due to the diminuish of trimethoprim-sulfamethoxazole comsum.

P830 Comparison of EUCAST and CLSI M27-A3 broth microdilution methods for testing susceptibilities of 144 *Candida* spp. to anidulafungin

S. Algarate, C. Seral, J. Gil, R. Benito, F.J. Castillo, C. Rubio-Calvo (Zaragoza, ES)*

Objective: The aim of this study was to compare MICs of anidulafungin obtained by the European Committee on Antibiotic Susceptibility Testing (EUCAST) and CLSI (formely NCCLS) methods for 53 *C. albicans*, 32 *C. glabrata,* 20 *C. parapsilosis,* 18 *C. tropicalis,* 11 *C. krusei,* 7 *C. famata,* 2 *C. lusitaniae* and 1 *C. guilliermondii* isolates obtained from clinical samples.
Methods: A total of 144 isolates of *Candida* spp. included 53 *C. albicans* (36.81%), 32 *C. glabrata* (22.22%), 20 *C. parapsilosis* (13.89%), 18 *C. tropicalis* (12.5%), 11 *C. krusei* (7.64%), 7 *C. famata* (4.86%), 2 *C. lusitaniae* (1.39%) y 1 *C. guilliermondii* (0.69%) isolates identified by Chromagar medium and API® 20C AUX system were tested. These isolates were recovered from blood culture (38), catheter (16), respiratory sources (27), urines (29), biological fluids (17) and other sources (17) of different hospitalized patients. MICs of anidulafungin were determined by broth microdilution methods following the recommendations of EUCAST and CLSI-M27-A2. In addition, correlation between the methods was determined.
Results: Table lists the MIC50, MIC90 and range (mg/L) of anidulafungin determined by the two reference methods.
All the isolates but *C. parapsilosis* showed a high intrinsic activity (MIC90, mg/L) against anidulafungin, and the rank order of activity was 0.125 for *C. albicans* and *C. krusei,* 0.25 for *C. glabrata* and *C. famata* by the two methods tested. In *C. tropicalis* was 0.25 by CLSI and 0.125 by EUCAST method. The percentages of correlation: CLSI/ EUCAST±2 dilutions were between 82.88–100%, according to the species. Six *C. parapsilosis* isolates showed the higher MICs of anidulafungin, 8 mg/L (1 strain (5%)) y 4 mg/L (5 strains, 20%) by the two methods.
Conclusions: Anidulafungin showed a high intrinsic activity against *Candida* spp. being lower to *C. parapsilosis.* Our prospective surveillance reveals no evidence of emerging anidulafungin resistance among *C. albicans*, the most frecuent specie isolated. The variability observed (MICs range 0.03–8 mg/L) in *C. parapsilosis* could be influenced by the lack of discrimination of species within the complex. Comparison of both methods indicates that EUCAST and CLSI standards for yeast result in essentially equivalent MICs of anidulafungin.

Microorganism	Anidulafungin (CLSI-M27-A3)			Anidulafungin (EUCAST)		
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
C. albicans (53)	0.06	0.125	<0.015–2	0.03	0.125	<0.015–1
C. glabrata (32)	0.125	0.25	<0.015–1	0.125	0.25	<0.015–1
C. parapsilosis (20)	1	4	0.03–8	1	4	<0.015–8
C. tropicalis (18)	0.06	0.25	<0.015–0.5	0.03	0.125	<0.015–0.5
C. krusei (11)	0.125	0.125	<0.015–0.25	0.06	0.125	<0.015–0.125
C. famata (7)	0.125	0.25	0.03–0.5	0.06	0.25	<0.015–0.25
C. lusitaniae (2)	–	–	0.03–0.06	–	–	<0.015
C. guilliermondii (1)	–	–	1	–	–	1

P831 The increase of chitin content in the cell wall accounts for the reduced echinocandin susceptibility displayed by clinical isolates of *C. parapsilosis*

A. Silva, S. Costa-de-Oliveira, M.M. Azevedo, I. Miranda, R. Rocha, R. Silva, C. Pina-Vaz, A.G. Rodrigues (Porto, Vila Nova de Famalicão, PT)*

Objectives: Given the potential for decreased susceptibility of *C. parapsilosis* to echinocandins, it is advisable to gather additional information regarding this opportunistic fungal pathogen. Thus, the objective of this study was to determine the mechanism of the reduced echinocandin susceptibility showed by *C. parapsilosis*.
Methods: Ten *C. parapsilosis* clinical isolates were included in this study. The susceptibility profile to three echinocandins (caspofungin, micafungin and anidulafungin) was determined according to CLSI M27-A3 protocol. Comparative DNA sequence analysis of the hot spot 1 (HS1) region of FKS1 gene was also performed. For this, the FKS1 HS1 was amplified by PCR and sequenced in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Additionally, the amount of chitin was measured using two methodologies: classical, through quantification of glucosamine release by acid hydrolysis of purified cell walls; and a flow cytometry (FC) method, using a specific fluorescent chitin marker, calcofluor white (CFW). Blastonidia suspensions (106 cells/mL) in sterile water were stained with 2.5 μg/mL CFW; the intensity of fluorescence of cells was evaluated by FC in a BD FACSCANTO II (Becton Dickiinson, Madrid, Spain). The fluorescence emitted by fungal cells is directly proportional to chitin amount.
Results: Four isolates were considered susceptible to echincandins and other six were non-susceptible to at least one of the three antifungals tested. Alignment of the deduced amino acid HS1 sequences from susceptible and non-susceptible *C. parapsilosis* isolates did not reveal any polymorphism involving amino acid changes, neither among the *C. parapsilosis* studied nor with the previously reported sequence. The amount of chitin in the cell wall was much higher on non-susceptible strains by both methods.
Conclusion: Overall, this study provides evidence that no sense-to-sense mutations are present in FKS1 HS1 gene of these isolates and that the increase in MIC values to echinocandin drugs may be due to an increase of the chitin cell wall content. Flow cytometric method described seems to be a new and excellent toll for chitin content determination.

P832 Two different mechanisms for reduced *C. glabrata* susceptibility to echinocandins after anidulafungin treatment

S. Costa-de-Oliveira, A. Silva, M.M. Azevedo, I. Miranda, R. Rocha, R. Silva, E. Ricardo, A.G. Rodrigues, C. Pina-Vaz (Porto, Vila Nova de Famalicão, PT)*

Objectives: Echinocandin resistance in candidiasis has been rare, however only caspofungin has been extensively used. Some case reports described FKS gene point mutation as responsible for acquired echinocandin resistance during treatment. Further than glucan synthesis, the increase in chitin content also plays a role in maintaining fungal cell integrity against echinocandins. This is a case report of a 71 year old female that was admitted in the Intensive Care Unit with a diagnostic of pancreatitis. Her hospital course was complicated by multiple *Candida* infections. She was treated with anidulafungin during 27 days. *Candida glabrata* isolates from blood and other body sites were recovered before and after anidulafungin treatment. To clarify the underlined resistance mechanisms the chitin content and the hot spot region 1 (HS1) of the FKS1 gene were assessed.
Methods: Antifungal susceptibility tests to azoles, amphotericin B (AMB), caspofungin (CAS) and anidulafungin (AND) were performed, according to CLSI M27 A3 protocol. Chitin content was determined by measuring the glucosamine release by acid hydrolysis of purified cell walls. Calcofluor white (CFW) resistance was determined by inoculating serial cell dilutions in YPD + CFW and AND. After DNA extraction a random amplification of polymorphic DNA was performed using primers OPE-18 and OPA-18. The HS1 of the FKS1 gene was amplified from *Candida glabrata* isolates and sequenced in an ABI PRISM 3130 Genetic Analyzer.
Results: The isolates recovered at the beginning of the AND treatment were S to all antifungals tested. However the isolates recovered during treatment (from blood and peritoneal fluid) showed a non susceptible (NS) pattern to echinocandins. Pre and postreatment isolates were confirmed to be isogenic. In the NS blood isolate the chitin content was 5 times higher than the peritoneal fluid isolate and in susceptible strains. A C-T mutation of FKS1 gene was found in the isolate recovered from peritoneal fluid (NS isolate), at position 1975, which results in the substitution of serine by proline in the 659 position of the amino acid sequence. This was the only isolate that was able to grow on the CFW + 2μg/ml AND YPD plates.
Conclusions: Point mutations in the HS1 of the FSK1 gene of *C. glabrata* peritoneal fluid isolates or and an increased in the chitin

cell wall content on *C. glabrata* blood isolate were responsible for the development of echinocandin resistance during anidulafungin therapy.

P833 Echinocandin E-test endpoint for *Aspergillus* shows poor essential agreement with the reference minimum effective concentration

J. Fuller, A. Schofield, S. Jiwa, B. Jansen, C. Sand, R. Rennie (Edmonton, CA)*

Objective: The Clinical and Laboratory Standards Institute (CLSI) M38-A2 broth microdilution (BMD) method for antifungal susceptibility testing of filamentous fungi now includes guidelines for testing echinocandin activity using the minimum effective concentration (MEC) as the endpoint measurement. The objective of this study was to determine if the caspofungin Etest could accurately and reliably measure the MEC of a large collection of clinical *Aspergillus* isolates.
Methods: Caspofungin activity against *Aspergillus* isolates was tested by BMD (CLSI M38-A2) for MEC (visual and microscopic) determination and by Etest for MIC using both a RPMI agar and Mueller-Hinton agar (supplemented with glucose and methylene blue [MGM]). Etest was performed using a modification of the CLSI M44-A disk diffusion method and the inocula prepared for BMD. All tests were incubated for 24 h. Etest MICs were recorded as the lowest concentration of caspofungin where the zone of growth inhibition intersected with the Etest strip. MICs were rounded up to the next even log2 concentration for the evaluation. Etest values + 1 log2 dilution of the reference BMD MEC were considered to be in essential agreement (EA).
Results: BMD MEC was determined for 345 clinical *Aspergillus* isolates, including 105 *A. fumigatus*, 62 *A. flavus*, 104 *A. niger*, 50 *A. nidulans,* and 24 *A. terreus*. Regardless of the *Aspergillus* species or agar medium tested, the caspofungin Etest consistently produced a markedly lower growth inhibition endpoint (MIC). The EA of the Etest on MGM and RPMI to BMD MEC was 18% and 26%, respectively. The geometric mean values for BMD MEC vs MGM Etest were 0.137 μg/ml and 0.024 μg/ml, respectively, and the geometric mean values for BMD vs RPMI were 0.128 μg/ml and 0.031 μg/ml, respectively. Comparatively, 91% of paired MGM and RPMI Etest results were within 2 log2 dilutions of each other and consistently produced clearly defined endpoints.
Conclusion: BMD for filamentous fungi is not an ideal test format for many clinical laboratories and the simplicity of the Etest may make it a more viable option. Although the results from this study demonstrate a clear discordance between the absolute values generated by the caspofungin BMD and Etest, it is difficult to dismiss the consistent performance of the Etest. An expanded optimization of test conditions will hopefully improve Etest concordance with BMD MEC without compromising consistency.

P834 A characterization of mean inhibitory concentrations and patient outcomes in patients failing to respond to fluconazole treatment of severe infections caused by *Candida* sp.

B. Footer, S. Monte, L. Podkulski, G. Prescott, C. Rotstein, J. Brown, J. Paladino, J. Schentag (Buffalo, US; Toronto, CA)*

Objectives: The goal of this study was to determine if fluconazole failures are more frequently associated with fluconazole-resistant or fluconazole-susceptible strains of *Candida*, and to compare the calculated AUIC values of patients with fluconazole-susceptible verses fluconazole-resistant isolates.
Methods: This study was a retrospective, case series of adult patients (>18 years) with an index *Candida* bloodstream infection and sufficient demographic, laboratory and microbiologic data to calculate serial AUICs and determine clinical and microbiologic outcome. Patients had to have received at least three days of fluconazole and organism susceptibility had to be available prior to enrolment. Although all had to initially fail fluconazole, both failures and successes were enrolled and analysed.
Results: 97 cases were enrolled with 52 (54%) being culture positive for *Candida albicans*. Although 98% of *C. albicans* isolates were susceptible, clinical success was achieved in only 58% of cases and the organism persisted at day 10 in 27% of the cases. 8 cases (15%) were found to have an AUIC < 100. The AUIC < 100 group tended to have a higher median MIC compared to the AUIC > 100 (0.56 vs. 0.19, $p = 0.47$). Compared to *C. albicans*, more intermediate and resistant organisms were observed in the non-*albicans* species group as 80% of isolates were susceptible ($p = 0.005$). Clinical success rates differed significantly between susceptible and non-susceptible isolates (53% vs. 11%, $p = 0.039$), while bacterial eradication tended to occur less often in nonsusceptible compared to susceptible organisms (44% vs. 61%, $p = 0.461$). A total of 18 cases (40%) had AUICs <100 with median MICs in those cases being higher compared to cases with AUIC > 100 (2.26 vs. 0.29, $p = 0.058$).
Conclusion: Fluconazole failure is almost always associated with susceptible organisms, particularly for *C. albicans*. For non-*albicans* species more intermediate or resistant organisms were observed, and the overall MIC was higher than for *C. albicans*. Failure in susceptible organisms occurs because fluconazole dosing does not achieve a blood concentration high enough to eradicated organisms with susceptible but somewhat higher MICs. Lowering the breakpoint would rationalize failure in some but not all of these patients. If the breakpoints are not lowered, further research is needed to determine the fluconazole dosing necessary to achieve success in those patients with susceptible but higher MICs.

P835 Fungaemia by *Candida parapsilosis*: *in vivo* induction of azole resistance due to prolonged therapeutic exposure

A. Silva, S. Costa-de-Oliveira, I. Miranda, C. Pina-Vaz, A.G. Rodrigues (Porto, PT)*

Objectives: A patient with *C. parapsilosis* fungaemia was submitted to fluconazole during eleven days, after which he died. Several *C. parapsilosis* isolates had been collected before, during and after initiation of fluconazole therapeutics. A microbiological characterization of the first and the last isolate was performed.
Methods: *In vitro* antifungal susceptibility testing to four azoles (fluconazole, itraconazole, voriconazole and posaconazole) was performed according to CLSI protocols. Molecular typing of the *C. parapsilosis* isolates was carried out by RAPD. The stability of resistant phenotype (acquired *in vivo*) was assessed *in vitro* through repeated incubation of the isolate without antifungal. The role of active efflux pumps in drug resistance was evaluated using flow cytometric analysis with rhodamine 6G (Rh-6G), a specific substrate for efflux pumps.
Results: The initial isolate was susceptible to all azoles, while the last recovered isolate was resistant to all four tested azoles, displaying high MIC values. Molecular typing of isolates demonstrated that the last isolate and pre-treatment isolate were isogenic. Therefore, we report, the *in vivo C. parapsilosis* acquisition of antifungal resistance, which maintained stable after incubation without antifungal. No difference in Rh-6G staining was found between the susceptible and the resistant isolate, suggesting that *in vivo* azole resistance did not involve increase in efflux pumps activity.
Conclusion: We report a clinical case of fungaemia by *C. parapsilosis* which acquired resistance to azoles after exposure to fluconazole. The resistance was characterized by an increase of fluconazole, itraconazole, voriconazole and posaconazole MIC values, which kept stable. Interestingly, azole resistance due to overexpression of efflux pumps was not confirmed. Continued fluconazole pressure was associated with the development of multi-resistance.

P836 *In vitro* susceptibilities of invasive fungi isolated from Austrian patients against echinocandins and commonly used azoles

B. Willinger, L. Sittenthaler, E. Steiner, A.M. Hirschl, C. Lass-Flörl (Vienna, Innsbruck, AT)*

Objective: The aim of the study was to determine the *in vitro* susceptibilities of invasive fungal isolates collected since June 2008 by

20 different Austrian medical centres against commonly used azoles and the three available echinocandins.
Methods: The clinical samples yielding the growth of fungi included blood cultures and other sterile specimens such as intravascular catheters, biopsies or aspirates, samples from the lower respiratory tract. Antifungal susceptibility for yeasts was assessed using a broth microdilution method following the Clinical Laboratory Standards Institute M27-A3 guidelines whereas moulds were tested as described by the European Committee on Antimicrobial Susceptibility Testing. For moulds also amphotericin B was tested.
Results: So far 552 yeast isolates have been collected. *C. albicans*, followed by *C. glabrata*, *C. parapsilosis* and *C. tropicalis* were the most common isolated species. In addition we tested 35 strains of filamentous fungi including 26 strains of different *Aspergillus* species and 5 strains of zygomycetes.
For *Candida* species, the MIC50 and MIC90 were 0.5 mg/l and 8 mg/l for fluconazole, 0.03 mg/l and 0.25 mg/l for voriconazole, 0.25 mg/l and 1.0 mg/l for caspofungin, 0.03 mg/l and 0.25 mg/l for anidulafungin, 0.25 mg/l and 0.5 mg/l for micafungin, respectively. Thus, the agent with the best *in vitro* activities for the azoles was voriconazole and for the echinocandins anidulafungin. Only 4.6% of *Candida* isolates were resistent to fluconazole, 2.0% to voriconazole, 0.5% to anidulafungin, 0.4% to caspofungin and 0.7% to micafungin respectively. 15.5% of *C. glabrata* strains were resistant to fluconazole, and 10.9% to voriconazole. Concerning the echinocandins elevated MICs were observed for *C. glabrata*, *C. parapsilosis*, *C. guilliermondii* and *C. lusitaniae*. For *Aspergillus* species, the MIC50 and MIC90 were 1.0 mg/l and 8 mg/l for amphotericin B, 0.5 mg/l and 2 mg/l for voriconazole, 0.5 mg/l and 1.0 mg/l for posaconzole. Concerning the echinocandins, only five strains showed MICs below 8 mg/l. For the zygomycetes, the MICs were within the expected range.
Conclusion: Up to date our multicenter study reveals no evidence of emerging azole or echinocandin resistance among invasive clinical isolates of *Candida* spp. The MICs assessed for mould were within the expected range.

P837 Susceptibility testing of anidulafungin against clinical isolates of *Candida parapsilosis* in comparison with caspofungin and micafungin

M. Ghannoum, J. Herbert, N. Isham (Cleveland, US)*

Objectives: Anidulafungin may have better *in vitro* activity against *C. parapsilosis* compared to the other echinocandins, as suggested by published case reports. *C. parapsilosis* isolates from burn unit patients who failed caspofungin were found to have elevated minimum inhibitory concentration (MIC) to caspofungin and micafungin but not to anidulafungin. To expand upon this observation, we conducted a comparative study of the *in vitro* activity of anidulafungin, caspofungin, and micafungin against a large number of *C. parapsilosis* clinical isolates.
Methods: MIC's for anidulafungin, caspofungin and micafungin for 100 clinical isolates of *C. parapsilosis* were determined, following the CLSI M27-A3 standard for the susceptibility testing of yeasts. Minimum fungicidal concentration (MFC) determinations were also determined by subculturing the total contents of each clear well from the MIC assay for colony count.
Results: The MIC range and MIC90 (defined as the lowest concentration to inhibit 90% of isolates tested) were 0.06–4.0 and 2.0 micrograms/ml, respectively, for all three echinocandins. In contrast, the MFC90 of anidulafungin was 8.0 micrograms/ml, compared to >64 micrograms/ml for both caspofungin and micafungin.
Conclusion: Although there were no differences in MIC across the three echinocandins, anidulafungin had more potent cidal activity compared to caspofungin and micafungin against a large number of clinical isolates of *C. parapsilosis*. This observation suggests that anidulafungin may be more effective in eradicating *C. parapsilosis* in clinical infections. Comparative trials are needed to confirm this observation.

P838 Antifungal susceptibility of yeasts isolated from patients with fungaemia: comparison of the E-test on direct blood samples and CLSI M27-A3

J. Guinea, S. Recio, P. Escribano, T. Peláez, M. Torres-Narbona, M. Rodríguez-Créixems, C. Sánchez-Carrillo, E. Bouza (Madrid, ES)*

Objectives: We prospectively performed antifungal susceptibility testing using the E-test (ETdir) on direct blood samples from 163 patients (225 strains), and on 63 strains artificially inoculated in BACTEC. ETdir MICs (24 h of incubation) of amphotericin B, fluconazole, voriconazole, posaconazole, isavuconazole, and caspofungin were compared with results using CLSI M27-A3.
Methods: Strains were from highly fluconazole-susceptible species (FLUCO-S: 93 *C. albicans*, 77 *C. parapsilosis*, 18 *C. tropicalis*, 6 *C. dubliniensis,* 1 *C. neoformans,* 1 *C. kefyr)*, or less fluconazole-susceptible species (FLUCO R: 52 *C. glabrata*, 29 *C. krusei,* 8 *C. guilliermondii,* 2 *R. mucilaginosa,* 1 *T. mucoides)*. Voriconazole breakpoints were used for posaconazole and isavuconazole (R $\geqslant$4 μg/mL). Strains were classified as susceptible (S) or resistant (R) following the CLSI procedure. The percentage of strains correctly classified by ETdir was calculated.
Results: Major discrepancies (M: R by ETdir and S by CLSI) were found mostly for *C. glabrata*, *C. krusei* and the triazoles. Very major discrepancies (VM: S by ETdir and R by CLSI) were found for fluconazole in *C. krusei* (n = 2), and for voriconazole in *C. glabrata* (n = 1) and *T. mucoides* (n = 1) (see table).
Conclusions: ETdir in yeast-positive blood cultures is rapid (24 h) and easy, and reliably determines the antifungal susceptibility to triazoles and caspofungin of yeasts (especially highly fluconazole-susceptible species) from fungemic patients.
J. Guinea (CA08/00384) and M. Torres-Narbona (CM08/00277) are contracted by FIS. This study received financial support from Basilea Pharmaceutica.

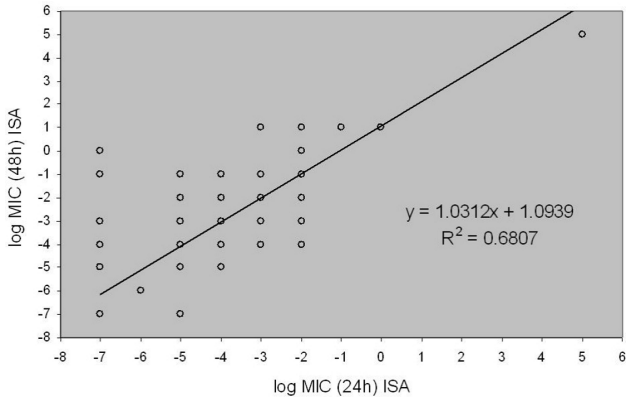
	Overall (correct/VM/M)	FLUCO-S (correct/VM/M)	FLUCO-R (correct/VM/M)	% of strains with MIC discrepancies $\leqslant\pm$2-fold dilutions
Fluconazole	90.9/0.7/8.4	100/0/0	71.7/2.2/26.1	78.8
Voriconazole	98.3/0.7/1	100/0/0	94.6/2.2/3.3	83.7
Posaconazole	88.5/0/11.5	100/0/0	64.1/0/35.9	63.5
Isavuconazole	96.5/0/3.5	99.5/0/0.5	90.2/0/9.8	71.9
Amphotericin B	31.7/66.5/1.8	25.3/73.7/1	45.5/51.1/3.4	62
Caspofungin	100/0/0	100/0/0	100/0/0	88.2

P839 Can isavuconazole MICs for yeasts be read after 24 hours of incubation?

J. Guinea, P. Escribano, S. Recio, T. Peláez, E. Bouza (Madrid, ES)*

Objectives: CLSI M27-A3 has mostly been validated for determination of the MIC after 48 hours of incubation, except in the case of fluconazole (24 h). The activity of isavuconazole has mostly been determined against yeast isolates after 48 h of incubation. Using the M27-A3 methodology, we studied the activity of isavuconazole against *Candida* spp and other yeast isolates and determined the correlation between MICs after 24 and 48 h.
Methods: A total of 297 recent clinical yeast blood isolates (n = 193) or other clinical specimen sites (n = 104) were studied: C albicans (n = 82); C glabrata (n = 67); C krusei (n = 31); C parapsilosis (n = 63); C tropicalis (n = 17); *Candida* spp (n = 7); and other yeast isolates (n = 30). The antifungal activity of isavuconazole was determined according to M27-A3. MIC (50% inhibition of growth) was read after 24 h and 48 h incubation. The MICs at 24 h and 48 h were considered to agree (essential agreement) when the value recorded after each incubation time was within $\pm$2 or fewer 2-fold dilutions of the other, considering 48 h as the gold standard. In the absence of breakpoints for isavuconazole, we chose voriconazole breakpoints ($\leqslant$1 μg/mL, susceptible; 2 μg/mL susceptible–dose dependent; $\geqslant$4 μg/mL, resistant) to evaluate the categorical agreement between MICs determined at 24 h and 48 h.
Results: MICs after 24 h of incubation were not determined for 6/297 (2%) isolates (1 C krusei and 5 other) due to poor growth. Overall, the

antifungal activity (in μg/mL) of isavuconazole obtained after 24/48 h of incubation was: MIC50 (⩽0.015/0.031), MIC90 (0.125/0.25), and MIC range (⩽0.015–>16). Although the overall essential agreement between both procedures was 87.6% (Figure), inter-specific differences were noted with C parapsilosis and C tropicalis showing the highest agreement (100%) and C glabrata the lowest (67.2%). Strains were classified (after 24/48 h of incubation) as susceptible (99%/97.9%), susceptible–dose dependent (0.3%/1.4%), and resistant (0.7%, C tropicalis). Categorical agreement was 98.9%, and only 3 strains (2 C glabrata and 1 C tropicalis) showed minor errors.
Conclusions: The antifungal activity of isavuconazole against *Candida* and other yeasts can be obtained after 24 hours of incubation with no major or very major discrepancies. Validation of this procedure in future studies including a higher number of fluconazole/isavuconazole-resistant isolates is warranted. J. Guinea (CA08/00384) is contracted by FIS.



P840 *In vitro* activity of isavuconazole against clinical non-*Candida* yeast isolates determined by E-test and CLSI M27-A3

J. Guinea, S. Recio, P. Escribano, T. Peláez, E. Bouza (Madrid, ES)*

Objectives: Isavuconazole is a new triazole undergoing phase III study for the treatment of patients with fungemia. The antifungal activity of isavuconazole against non-*Candida* yeast isolates causing fungemia has only been assessed for a limited number of isolates. We determined the antifungal activity of isavuconazole against rare non-*Candida* yeast isolates and compared it with that of other marketed triazoles.
Methods: We studied 29 clinical non-*Candida* strains: Dipodascus capitatus (n = 9; 31%), Rhodotorula mucilaginosa (n = 2; 6.9%), *Saccharomyces cerevisiae* (n = 15; 51.7%), and Trichosporon spp. (n = 3; 10.3%). All isolates were identified by amplifying and sequencing the ITS1-5.8S–ITS2 region (White et al). The antifungal activity of fluconazole, voriconazole, posaconazole, and isavuconazole was determined using the CLSI M27-A3 microdilution procedure. That of isavuconazole was also determined using the E-test. Each CLSI and corrected (two-fold scale) E-test MIC was further converted to a log2 MIC. The methods agreed when the log2 MIC measured by each was within ±2 or fewer twofold dilutions of the other.
Results: The MIC50, MIC90, and range of MICs (in μg/ml) for each antifungal were as follows: posaconazole (0.5/2/0.062–2); fluconazole (4/32/1–>128); voriconazole (0.125/0.5/0.031–1); and isavuconazole (CLSI: 0.125/0.5/<0.015–1; E-test: 0.125–1/0.031–2). For isavuconazole, CLSI M27-A3 and the E-test agreed for 51.7% (±1 twofold dilutions) and 75.8% (±2 twofold dilutions) of strains. According to the breakpoints suggested, the strains were classified as follows: fluconazole (susceptible, 72.4%; susceptible-dose dependent, 20.6%; resistant, 6.9%) and voriconazole (susceptible, 100%). The only two strains of *R. mucilaginosa* showing fluconazole resistance (>128 μg/ml) were voriconazole-susceptible and had MICs for isavuconazole ⩽0.125 μg/ml.
Conclusions: The non-*Candida* isolates of Dipodascus capitatus, Rhodotorula mucilaginosa, *Saccharomyces cerevisiae*, and Trichosporon spp. studied were highly susceptible to isavuconazole (MICs ⩽1 μg/ml), even the fluconazole-resistant strains. The E-test is an alternative means of determining the antifungal activity of isavuconazole against non-*Candida* isolates. J. Guinea (CA08/00384) is contracted by FIS.

P841 Detection of amphotericin B resistance in *Candida haemulonii* and closely related species, using Etests, Vitek-2 Yeast Susceptibility Test, and CLSI M27 broth microdilution

J.H. Shin, S.H. Kim, B.J. Oh, M.N. Kim, S.J. Jang, M.G. Shin, S.P. Suh, D.W. Ryang (Gwangju, Seoul, KR)*

Objectives: The emerging fungal pathogen *Candida haemulonii* and closely related species often show high-level resistance to amphotericin B (AMB). This study compared the utility of four antifungal susceptibility testing methods in the detection of AMB-resistant isolates of these fungi.
Methods: A total of 51 clinical isolates (eight isolates of *C. haemulonii,* 10 isolates of *Candida* pseudohaemulonii, and 33 isolates of *Candida* auris) were obtained from eight Korean hospitals during 2004–2009. For each isolate, AMB susceptibility Etests (AB Biodisk, Solna, Sweden) were performed on Mueller-Hinton agar supplemented with glucose and methylene blue (Etest-MH), and on RPMI agar supplemented with glucose (Etest-RPMI). Isolates were also examined in Vitek-2 yeast susceptibility tests, and by the CLSI M27 broth microdilution method (CLSI-BMD).
Results: Of the four methods, Etest-MH generated the broadest distribution of AMB minimum inhibitory concentrations (MICs), ranging from 0.125 to 32 ug/ml for all 51 isolates. MICs ranged from 4 to 32 ug/ml in the 18 isolates of *C. haemulonii* and *C. pseudohaemulonii,* which we categorized as resistant. All 33 isolates of *C. auris* were susceptible, with MICs ranging from 0.125 to 0.38 ug/ml. When AMB MIC values from the other three test methods were compared to those produced by Etest-MH, the essential agreements (within 2 dilutions) of Etest-RPMI, Vitek-2, and CLSI-BMD were 88.2%, 92.2%, and 72.5%, respectively. Etest-RPMI and CLSI-BMD produced two very major errors (5.9%), while Vitek-2 produced no major errors, but two minor errors (5.9%).
Conclusion: Of the four methods evaluated, Etest-MH appears to provide the best discrimination between AMB-resistant and susceptible isolates of *C. haemulonii* and closely related species; Vitek 2 can be a useful screening method for detecting AMB resistance in these isolates.

P842 Investigation of efflux pumps and ERG11 gene expression levels in *Candida albicans* isolates by semi-quantitative reverse transcription-polymerase chain reaction

O. Irmak, M. Doluca Yücesoy (Izmir, TR)*

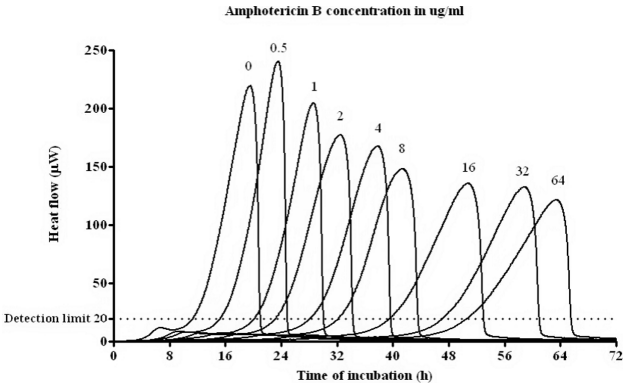
Objective: Widespread and repeated use of fluconazole resulted in resistance problem in *Candida albicans* strains. In this study, investigation of the expression of efflux pump encoding genes, CDR1, CDR2, and MDR1 and lanosterol 14 α-demethylase enzyme encoding gene, ERG11 in fluconazole (Flu) susceptible (S)/resistant(R)/dose dependent susceptible (DDS) *C. albicans* isolates was aimed in order to determine the role of these two mechanisms in fluconazole resistance.
Methods: Four Flu R/DDS, seven Flu S and three tailing effect showing S *C. albicans* strains which were isolated from clinical specimens in three University hospitals in Turkey were included. Relative expression of CDR1, CDR2, MDR1 and ERG11 transcripts were determined by semi-quantitative reverse transcription polymerase chain reaction and compared with drug susceptible *C. albicans* ATCC 14053 strain. The expression of these genes was normalized with their ACT1 expression levels.
Results: CDR1 and CDR2 transcripts were 3.12–7.16 and 1.83–18.0 fold upregulated, respectively in Flu R/DDS *C. albicans* isolates when compared to *C. albicans* ATCC strain. Trailing isolates showed 0.64–2.06 and 0.9–3.1 fold CDR1 and CDR2 expression while the sensitive isolates expressed low levels of these genes or didn't seem to express at all. When MDR1 and ERG11 expressions were examined, it was

observed that three and all Flu R/DDS as well as six and four Flu S isolates expressed these two genes at various levels, respectively.
Conclusion: As a result, it can be concluded that overexpression of efflux pumps especially CDR1 and CDR2 can be important mechanisms of Flu resistance in our *C. albicans* isolates.

P844 Growth-related heat production of *Aspergillus fumigatus* in presence of liposomal amphotericin B – a novel rapid real-time method for evaluation of antifungals

U. Furustrand, E. Seiler, A. Trampuz (Lausanne, CH)*

Objectives: Current detection and susceptibility testing of moulds have insufficient sensitivity, require up to 72 h of incubation and carries considerable risk of cross-contamination. We evaluated a closed-system method for real-time detection of growth-related heat production of *Aspergillus fumigatus*. Measurements were performed with liposomal amphotericin B (AMB) (AmBisome) to evaluate the potential of microcalorimetry for rapid, accurate and easy-to-perform susceptibility testing of moulds.
Methods: *A. fumigatus* strain ATCC 204305 was used. Microcalorimetry was performed in duplicate by adding 0.05 ml of *A. fumigatus* suspension containing 10^4 cells (determined in a haematocytometer by microscopy) in 2.95 ml Sabouraud Dextrose Broth containing serial 2-fold dilutions of AMB. Heat production was measured at 37ºC under static conditions and detection time (in h), peak heat flow (in microwatt) and total heat produced (in Joules) were determined. The lower detection limit of heat production was defined at 20 microW. The minimal heat inhibition concentration (MHIC) was defined as the lowest antifungal concentration inhibiting heat production during 24 h of incubation.
Results: Heat production of *A. fumigatus* without AMB (positive control) was detected after 11.3 h, with a peak of 220 microW and a total heat of 5 J. Growth media with AMB but without *A. fumigatus* (negative control) showed no detectable heat production. With increasing AMB concentration, the detection time was proportionally delayed (Figure) by 3.9 h (at 0.5 ug/ml), 8.8 h (at 1 ug/ml), 11.8 h (at 2 ug/ml), 16.8 h (at 4 ug/ml), 20.8 h (at 8 ug/ml), 28.2 h (at 16 ug/ml), 35.7 h (at 32 ug/ml) and 39.2 h (at 64 ug/ml). The MHIC of AMB was 4 ug/ml. The peak of heat flow gradually decreased from 220 microW (without AMB) to 120 microW (with 64 ug/ml AMB), while the total heat produced remained similar at about 5 J.
Conclusion: Microcalorimetry allowed evaluation of activity of liposomal AMB against *A. fumigatus* by inhibiting its growth-related heat production. This method has the potential for rapid (12–24 h), accurate and real-time susceptibility testing of various moulds and for evaluation of new antifungals. In further steps, systematic optimization and standardization of calorimetric conditions are needed.



Candida – infections

P845 *Candida* colonization among paediatric cancer patients and its impact on (1–3)-β-D-glucan, C. mannan and *Candida* DNA detection in serum samples

E. Mokaddas, M. Burhama, Z. Khan, S. Ahmed (Dasma, KW)*

Objective: An 18-month prospective surveillance study was undertaken to determine the extent of *Candida* colonization among pediatric cancer patients and its possible impact on detection of (1–3)-b-D-glucan (BDG), *Candida* mannan and *Candida* DNA in serum samples.
Methods: A total of 972 swabs originating from throat (n = 294), nostrils (n = 600), anus (n = 28), and groin (n = 50) from 63 pediatric cancer patients were cultured on Sabouraud dextrose agar supplemented with chloramphenicol. Serum samples were collected from the patients colonized with *Candida* species at a single or multiple sites for the detection of BDG by Fungitell kit (Associates of Cape Cod Inc., USA), *Candida* mannan by Platelia *Candida* Ag (BioRad, Marnes La Coquette, France) and *Candida* DNA by using panfungal and/or species-specific primers and DNA sequencing.
Results: Seventy-four (7.6%) swab cultures from 35 (55.5%) patients yielded *Candida* species. The isolates included 61 of *C. albicans*, 8 of *C. dubliniensis,* 2 each of *C. glabrata* and *C. tropicalis* and one of *C. krusei.* Eleven patients were colonized at three or more sites. None of the serum samples from the colonized patients yielded BDG and/or *Candida* mannan levels higher than the currently recommended cut-off values of >80 pg/ml and >0.5 ng/ml used for the two diagnostic tests, respectively. The geometric means of BDG and *Candida* mannan levels were 31.6 + 12.98 pg/ml and 0.17 + 0.04 ng/ml, respectively. Likewise, none of the serum samples (n = 36) of patients who were colonized with *Candida* species yielded a positive PCR test for *Candida* DNA. During the study period, only two colonized patients subsequently developed candidemia due to *C. tropicalis*. Besides positive blood cultures, *C. tropicalis* DNA, BDG (119.3 and 203 pg/ml), and *Candida* mannan (0.77 and 0.28 ng/ml) were also detected in serum samples during the candidemic episode.
Conclusions: The present study demonstrates that while mucosal colonization with *Candida* species in cancer patients is not uncommon, it may not give rise to diagnostically significant levels of BDG, *Candida* mannan or to *Candida* DNA in serum specimens. The study reinforces the diagnostic utility of these markers in the diagnosis of invasive candidiasis.

P846 *Candida albicans* interferes with galactomannan antigenaemia in human bronchoalveolar lavage fluids

F. Persat, P. Vanon, S. Ranque, S. Picot (Lyon, Marseille, FR)*

Objective: Galactomannan (GM) detection in bronchoalveolar lavage (BAL) is often described as an adjunctive diagnostic tool for the diagnosis of aspergillosis. *Candida albicans* infections are not a recognized cause of false-positive GM assay when performed on serum. However yeasts can be present in much larger amounts in BAL fluid than in serum. This study aimed at testing whether the presence of *C. albicans* would influence the GM assay results in patient BAL fluid.
Methods: 70 BAL samples, originating from 66 patients hospitalized in various wards (haematology, intensive care units, pneumology), were routinely analyzed with direct microscopic examination (ME) by Giemsa and Gomori-Grocott stainings and culture. Platelia®-*Aspergillus* (BioRad) GM ELISA assay was performed retrospectively. BALs were splitted into four groups: (1) ME negative and culture negative (n = 20); (2) ME with pseudomycelia-yeasts and pure *C. albicans* culture (n = 23); (3) ME with hyphae and pure *Aspergillus fumigatus* culture (n = 10); (4) ME negative and *A. fumigatus* pure culture (n = 17). Platelia®-*Aspergillus* was performed as indicated by the manufacturer; the results were index values, a 0.5 positivity threshold was used as recommended in serum. Data were then analysed using increasing positivity threshold values.

Results: Index values significantly differed among the four groups ($p < 0.05$, Kruskall-Wallis test). In the group 3 (hyphae and *A. fumigatus* culture) 9 BAL index values were >1.5, and 6 (60.0%) were >8. In the group 4 (negative ME and *A. fumigatus* culture), 12 (70.6%) were >0.5. In the control groups 1 & 2, 15 were >0.5; 13 (86.6%) of those belonged to group 2 (pseudomycelia-yeasts and *C. albicans*).
Conclusions: The presence of numerous pseudomycelia and yeasts in ME was associated with increased GM index values that were similar to those obtained for BALs with negative ME and *A. fumigatus* culture. In this case, GM results in BAL should be more cautiously interpreted. Whether a specific positivity threshold should be used when numerous yeasts and pseudomycelia are detected in the BAL fluid remains to be addressed.

BAL groups	N	ELISA GM index positivity thresholds Number of positive (%)			
		⩾0.5	⩾0.7	⩾1.0	⩾1.5
ME with hyphae + *A. fumigatus* culture	10	9 (90.0)	9 (90.0)	9 (90.0)	9 (90.0)
Negative ME + *A. fumigatus* culture	17	12 (70.6)	9 (52.9)	8 (47.1)	6 (35.3)
ME with pseudomycelia and yeasts + *C. albicans* culture	23	13 (56.5)	11 (47.8)	8 (34.8)	5 (21.7)
Negative ME + sterile culture	20	2 (10.0)	2 (10.0)	1 (5.0)	1 (5.0)

P847 Effects of three different topical antimicrobial dressings on *Candida albicans*-contaminated full-thickness burns in rats

A. Acar, F. Uygur, H. Diktas, R. Evinc, E. Ulkur, O. Oncul, L. Gorenek (Istanbul, TR)*

Background: In this experimental animal study, the effects of three different topical antimicrobial dressing on *Candida albicans* contaminated full-thickness burn in rats were analyzed.
Methods: Totally 32 adult Wistar rats (body weight 200–220 gr) were used. Acticoat TM® (Smith and Nephew, Istanbul, Turkey), chlorhexidine acetate 0.5% (Bactrigrass® and Mycostatine (Nystatin®) were compared to assess the antifungal effect of a once-daily application on experimental rat 15% full-skin thickness burn wound seeded 24 hours earlier with a 108 CFU/mL standard strain of *Candida albicans* ATCC 90028. All the animals were scarified at post burn day 7. The quantitative counts of seeded organism in burn eschar and subjacent muscle were determined, beside the cultures of left ventricle blood and lung biopsies.
Results: While there were significant differences between Acticoat TM® group (4±1x102) and control group (5±6x104), and between Mycostatin group (4±4x102) and control group ($P = 0.01$, $P = 0.01$), there were no significant differences between chlorhexidine acetate 0.5% group (2±3x104) and control group ($P = 0.7$) respectively. Acticoat TM® and Mycostatin were sufficient to prevent to *C. albicans* from invading to the muscle and from causing systemic infection.
Conclusions: The animal data suggest that Mycostatin is the most effective agents in the treatment of *C. albicans*-contaminated burn wounds, and Acticoat TM® is a choice of treatment on fungal burn wound infection with antibacterial effect and the particular advantage of limiting the frequency of replacement of the dressing.

P848 Prevalence and antimycotic susceptibility of *Candida* sp. causing vulvovaginitis

E. Krstevska-Kelepurovska, E. Krsteva, I. Kelepurovski, O. Jankova, A. Delova (Bitola, MK)*

Objectives: The aim of this study was to ascertain the prevalence and species representation of pathogenic yeasts in patients with clinical symptoms of vulvovaginitis. Determination of *in vitro* susceptibility of yeast isolates to clinically used antimycotic agents.
Methods: This study was conducted in six-months period and included 1058 sexually active women with clinical symptoms of vulvovaginitis. Identification of the yeast isolates was performed using Chromatic *Candida* agar (Liofilhem). Speciation of the yeasts was done using a standardized biochemical commercial system API ID 32 C (bioMérieux, France). *In vitro* susceptibilities were determined using API ATB FUNGUS 3 (bioMérieux, France).
Results: A total of 1058 women were included in the study and in 181 (17.1%) yeasts were isolated. *Candida albicans* was the most common species among the isolates 94 (51.9%) followed by *C. glabrata* 39 (21.5%). Other *Candida* spp. isolated were *C. kefyr* 17 (9.4%), *C. tropicalis*, *C. krusei* and *Sach. cerevisiae* 9 (5.0%) each, *C. sache* and *C. parapsilosis* 2 (1.1%) each. Thus the overall prevalence of non-*albicans Candida* species was 87 (48.1%). Resistance to Amphotericin B was detected in 42 (23.2%) of all analysed strains (in 16% of *C. albicans*, 25.6% – *C. glabrata*, 17.6% – *C. kefyr)*. Resistance to Fluconazole was seen in 25 (13.8%) of strains (in 18% strains of *C. albicans*, 11.8% – *C. glabrata*). Resistance to Itraconazole was detected in 59 (32.6%) isolates (in 18% strains of *C. albicans*, 76.9% – *C. glabrata*, 11.8% – *C. kefyr)*. Resistance to Voriconazole was identified in 15 (8.3%) strains (in 7.4% – *C. albicans*, 12.8% – *C. glabrata*, 11.8% – *C. kefyr)*. We also found 7 (3.9%) strains resistant to Flucitosine (*C. albicans*, *C. tropicalis*, *Sach. cerevisiae*).
Conclusion: In this study, we observed that *C. albicans* (51.9%) was the most frequent etiologic agent associated with mycotic vulvovaginitis. Thus, in recent years, there has been a significant increase of infections caused by non-*albicans* species of *Candida*, particularly *C. glabrata* (21.5%) and *C. kefyr* (9.4%). Most non-*albicans Candida* species have higher percent of resistance to the antifungal agents, and infections they cause are difficult to treat. This phenomenon emphases the importance of identification and surveillance of the *Candida* species in the clinical settings.

P849 Distribution of *Candida* sp. of colonized patients in intensive care units

F. Ergin, M. Yetkin, C. Bulut, B. Oral, G. Ertem, N. Tulek, A.P. Demiroz (Ankara, TR)*

Objectives: *Candida* infections mainly evolve from endogenous colonization. *Candida albicans* remains the most frequent cause of candida colonization. In this study we evaluated the epidemiology of candida species of colonizated patients in intensive care unit.
Methods: The study was carried out between September 2008 and February 2009 with the patients older than 18 years of age in a medical/surgical intensive care unit. Throat, nose, skin (axilla), urine and rectal swab cultures were taken at admission and weekly from each patient. *Candida* colonies were identified by using germ tube test, CHROMagar *Candida* Medium (Becton Dickinson, Germany) and ID 32 C (BioMeriux, France) yeast identification kit.
Results: A total of 1691 samples were taken over the study period. 815 samples were belonging to the colonized patients. Of the 815 samples collected from 42 patients, 143 (18%) yielded *Candida* species. These included 66 (46%) *C. albicans*, 39 (27%) *C. glabrata*, 11 (8%) *C. kefyr,* 10 (7%) *C. tropicalis*, 8 (6%) *C. krusei,* 4 (3%) *C. lusitaniae*, 3 (%2) *C. sake* and 2 (%1) *C. holmii* isolates.
Candida colonization was found with the highest percentage from throat, urine and rectal swab samples. *C. albicans* and non-*albicans Candida* species are similar rate in throat and urine samples, but non-*albicans Candida* species are most common in rectal samples.
Of these colonized patients, invazive candida infection developed in nine patients; five of them candidemia and four of them urinary tract infection. *C. albicans* (3) and *C. glabrata* (2) were the cause of candidemia. Urinary tract infections were developed by *C. albicans, C. glabrata, C. tropicalis* and *C. krusei.*
Conclusion: *Candida* species are increasingly causing nosocomial infections in intensive care units. Although *Candida albicans* is still major pathogen, rate of non albicans *Candida* species are increasing also.

P850 Antifungal susceptibility patterns of *Candida* isolates originated from invasive candidiasis

A. Stylianakis, V. Papaioannou, S. Tsiplakou, P. Papanikolaou, K. Tsopelas, A. Panagopoulou, C. Karra, A. Koutsoukou (Athens, GR)*

Objectives: The aim of our study was to evaluate the antifungal profiles, against commonly used and newer antifungal agents of *Candida* isolates, causing invasive candidiasis.
Methods: During one year period (11/2008–10/2009) we examined forty three *Candida* isolates responsible for thirty seven distinct cases of candidaemia and six other invasive candidiasis. The latter isolates derived from surgical wounds (n = 3), lower respiratory tract specimens (n = 2) and normally sterile samples (n = 1, ascitic fluid) each of them represented a unique infectious episode. The fungal identification procedure to species level included germ tube production and the use of the automated compact system VITEK 2 (Biomerieux). The antifungal susceptibility testing was performed by using E-test strips (Biomerieux) and RPMI 1640 agar with 2% glucose according to the manufacturer's instructions. MIC was evaluated according to CLSI criteria for 5-flucytocine (FC), fluconazole (FL), voriconazole (VO), micafungin (MCF) and anidulafungin (AND) while a provisional susceptibility breakpoint of $\leqslant$1 mg/L was used for amphotericin B (AP) and posaconazole (POS).
Results: *C. parapsilosis* was the predominant species (n = 31, 72%), followed by *C. albicans* (n = 12, 28%). All isolates were inhibited at MICs $\leqslant$1 mg/L of amphotericin B, posaconazole and micafungin. Only 5 out of 31 (17%) *C. parapsilosis* isolates were susceptible to AND while all *C. albicans* isolates were susceptible to the same agent. 37 isolates were examined and found susceptible to the new echinocandins AND, MCF, and to triazoles POS and VO. The susceptibility percentage of the examined *C. parapsilosis* / *C. albicans* isolates to the rest antifungal agents, was 88.4% / 83.7% for FC against *C. parapsilosis* / *C. albicans*, and 34.9% / 30.2% for FL against the same isolates, respectively.
Conclusion: *C. parapsilosis* prevails among the isolates responsible to invasive candidiasis, in our hospital. The more recent antifungal agents POS and MCF as well as AP, VO were active against all *Candida* isolates tested, FC possessed a satisfactory antifungal activity and AND was active against all the *C. albicans* isolates. The low degree of susceptibility to FL and AND observed against some *Candida* isolates highlights the necessity of administrating antifungal agents in accordance to the susceptibility test of the responsible isolates.

P851 Epidemiological trends in *albicans* and non-*albicans* nosocomial candidaemia

J. Esperalba, I. Rebollo, M.P. Mendaza, M.L. Gómez-Lus, S. Alcántara, M.F. Portero, D. Dámaso (Majadahonda, Madrid, ES)*

Objectives: To evaluate the incidence, risk factors and mortality rate of candidemia, as well as, determine differences of *albicans* and non-*albicans* candidemia.
Methods: Retrospective and observational study conducted from June 2008 to May 2009 at the Puerta de Hierro Majadahonda University Hospital in Madrid, Spain. It is a 613-bed hospital that covers a population of approximately 5 hundred thousand people from 20 towns in the northwest of Madrid. Candidemia was defined as being found in at least one positive blood culture yielding *Candida*. For the isolation and identification of *Candida* we used the routine methods: the BACTEC 9240 system and CHROMagar *Candida* Medium, Auxacolor or Api 20 C Aux systems. For each episode of candidemia, once we had identified the specie, we collected clinical and demographic data, predisposing disease and risk factors according to the scientific knowledge reviewed. We used either the χ^2-test or the Fisher Exact-test to compare categorical variables and to observe the factors associated with albicans and non-*albicans* candidemias.
Results: A total of 28 cases of candidemia were documented, ranging 1.6 cases per 1.000 admissions. Overall, 36.7% of the episodes were due to *C. albicans*, followed by *C. parapsilosis* (30%), *C. tropicalis* (13.33%), *C. krusei* (10%) and *C. glabrata*, *C. dublinensis* and *C. lusitaniae* with one isolate each (3.33% each). Candidemia was predominant (35.7%) in the intensive care unit (ICU). The ICU patients had a significant higher risk to suffer a non-*albicans* than albicans candidemia (p = 0.057). The rest of demographic data, risk factors, underline diseases and outcome rates between the two groups did not reach statistical significance (table). Mostly, we identified that the risk factors more frequently associated were intravascular catheter management, previous antimicrobial therapy and being in an immunosuppressive situation. The attributed outcome rate was 44.4%.
Conclusions: Candidemia is an important infection in terms of incidence and mortality that occurs mainly in critical care and haematology units. Although *Candida albicans* was still the predominant species, we observed a high rate of non-*albicans* strains that currently are less susceptible to fluconazole. The results of this survey help us to establish the pertinent epidemiological, diagnostic and therapeutic measures for the control of this nosocomial illness.

Table. Candidemia in Puerta de Hierro Majadahonda University Hospital, Madrid

Characteristic	Total patients (27)	*C. albicans* (11)	*Candida* non-*albicans* (19)	p value
Male (%)	15 (55.5)	5 (45.5)	12 (63.2)	
Female (%)	12 (44.4)	6 (54.5)	7 (36.8)	
Age (years), mean	63 (22–83)	59.45 (38–81)	65.4 (22–83)	0.282
Neoplasia (%)	13 (48.2)	7 (63.6)	7 (36.8)	0.389
HIV infection (%)	1 (3.7)	1 (9.1)	0	
PDA (%)	1 (3.7)	1 (9.1)	0	
COPD (%)	0	0	0	
Diabetes mellitus (%)	6 (22.2)	2 (18.2)	4 (21.1)	1
Ischemic cardiopathy (%)	4 (14.8)	2 (18.2)	2 (10.5)	0.970
Cardiovascular accident (%)	1 (3.7)	1 (9.1)	0	
Central venous catheter (%)	20 (74.1)	7 (63.6)	15 (78.9)	0.627
Urinary catheter (%)	16 (59.3)	4 (36.4)	12 (63.2)	0.299
Endotracheal intubation (%)	6 (22.2)	2 (18.2)	4 (21.1)	1
Nasogastric intubation (%)	4 (14.8)	1 (9.1)	3 (15.8)	1
Corticosteroid therapy (%)	12 (44.4)	4 (36.4)	8 (42.1)	1
Immunosuppression (%)	19 (70.4)	7 (63.6)	14 (73.7)	0.869
Antimicrobial therapy (%)	24 (88.9)	9 (81.8)	17 (89.5)	0.970
Surgery (%)	19 (70.4)	7 (63.6)	12 (63.1)	1
ICU (%)	13 (48.2)	2 (18.2)	11 (57.9)	**0.057**
Transplant (%)	5 (18.5)	0	5 (26.3)	
Mortality (%)	12 (44.4)	5 (45.5)	7 (36.8)	0.712

HIV: Human immunodeficiency virus; PDA: Parenteral drug addicts; COPD: Chronic obstructive pulmonary disease; ICU: Intensive care unit.

P852 Risk factors and the incidence of candidaemia in the intensive care unit: a 5-year cross-sectional study

N. Yapar, M. Akan, V. Aokan-Oguz, C. Ergon, M. Hancer, M. Doluca Yücesoy (Izmir, TR)*

Objective: In recent years, an increase in the frequency of candidemia has been observed. In this study we aimed to investigate the incidence of candidemia and risk factors associated with this infection in our Intensive Care Unit (ICU).
Methods: A cross sectional study in the ICU of a university hospital was conducted from 1 January 2004 to 31 December 2008. Candidemia was defined as isolation of any species of *Candida* from at least one blood culture of patients. For all patients demographic and clinical data were collected from medical reports and medical microbiology laboratory retrospectively. All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS, Version 15.0). For the variables that had significance in the χ^2 tests, logistic regression analysis was performed. P values of <0.05 were considered as statistically significant.
Results: In five years period, 66 candidemia cases were identified among 1076 cases. Overall incidence of candidemia was 3.38 per 1000 patient

days. Of the cases 35 (53%) were male and 31 (47%) were female. Mean age was 54.39±23.89 in case group and 53.25±23.01 in control group. Risk factors of candidemia were given in the table.

Conclusion: In this study, we observed that important risk factors for candidemia were red blood cell (RBC) transfusion, total parenteral nutrition (TPN) and previous usage of antibiotics and antifungals. Important risk factors such as central catheterization and mechanical ventilation were not found significant in this study, because of very commonly implementation of those procedures in the ICU. We concluded that a great number of candida blood stream infections were preventable by reducing of unnecessary invasive procedures or antimicrobials.

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Table. Risk factors of candidemia

Risk factor	P value	Logistic regression analysis	
	(χ^2 tests)	P value	OR (95% CI)
Gender	0.103	0.02	1.83 (1.07–3.12)
Tracheostomy	<0.01		
RBC transfusion	<0.01	0.05	1.86 (0.99–3.50)
Abdominal surgery	0.02		
TPN	<0.01	0.09	1.63 (0.92–2.87)
CVC (Lower extremity)	0.01		
Antibiotic use	<0.01		
Piperacillin–tazobactam	<0.01		
Glycopeptides	<0.001	<0.001	4.10 (2.27–7.41)
Aminoglycosides	<0.001	<0.01	2.20 (1.26–3.82)
Carbapenems	<0.001	0.02	1.94 (1.06–3.54)
Antifungal use	0.46	0.06	0.40 (0.15–1.04)

P853 Adult and neonatal intensive care unit candidaemia: retrospective analysis

A. Spiliopoulou, S. Vamvakopoulou, C. Bartzavali, G. Dimitracopoulos, E.D. Anastassiou, M. Christofidou (Patras, GR)*

Objectives: To investigate the isolation and distribution rate of *Candida* spp. in blood cultures and to evaluate antifungal susceptibility during a 12-year period (1997–2009) in a tertiary care hospital's adult (AICU) and neonatal (NICU) intensive care units.

Methods: Positive blood cultures (BacT/Alert, Organon Teknika) were examined microscopically directly for yeasts or pseudohyphae and subcultured on Sabouraud dextrose agar (Difco). *Candida* isolates were screened by germ tubes test and identified using API 20CAUX and automated VITEK system (both Biomerieux). Antifungal susceptibility was carried out by E-test (AB Biodisk) on RPMI -2% glucose agar. MIC was evaluated according to CLSI criteria for the following antifungal agents: amphotericin B (AM), 5-fluorocytocin (FC), ketoconazole (KE), itraconazole (IT), fluconazole (FL), voriconazole (VO), posaconazole (PO) and caspofungin (CF). According to European Organization for Research and Treatment of Cancer, an episode of candidaemia was defined as one or more positive blood cultures for *Candida* species isolated from patients with clinical signs of infection. Subsequent positive cultures were defined as new episode, only if there was an interval of at least 12 weeks between the two episodes.

Results: During the study period there were 285 candidemia cases. Thirty three (33) cases were identified in AICU and sixty five (65) cases in NICU The causative species in AICU / NICU were: *C. albicans* 22 strains (67%) / 39 strains (60%), *C. parapsilosis* 6 (18%)/18(27.7%), *C. glabrata* 3 (9%)/5 (7.7%), *C. tropicalis* 2 (6%)/2 (3.1%) and *C. guilliermondii* 0 (0%)/1 (1.5%). In terms of susceptibility to antifungals, *C. albicans* isolates demonstrated excellent sensitivity rates to AM, FC and CF, whereas the highest resistance rates were observed to azoles, especially IT (27% and 16% in AICU and NICU respectively), FL (18% and 8%) and KE (14% and 16%). *C. parapsilosis* isolates were sensitive to all antifungals with the exception of IT (11% resistance in NICU). *C. glabrata* and *C. tropicalis* demonstrated the higher resistance rates to azoles among all *Candida*.

Conclusions: ICU Candidemia is predominantly caused by *C. albicans.* Parenteral alimentation and use of central venous catheters seem to be associated with high incidence of *C. parapsilosis*, which is the second predominant specie in both ICUs. *C. glabrata* is the third more often isolate and its identification is clinically important due to high resistance to azoles.

P854 A 9-year species and antifungal susceptibility study of bloodstream *Candida* isolates in a Greek trauma hospital

A. Stylianakis, S. Tsiplakou, V. Papaioannou, K. Tsopelas, P. Petraki, A. Panagopoulou, K. Ntetsika, A. Koutsoukou (Athens, GR)*

Objectives: To study the epidemiology of candidaemias, the species distribution among *Candida* isolates recovered from blood cultures and their antifungal susceptibility profiles in a trauma Hospital in Greece, during a nine year time period (11/2000–10/2009).

Methods: During the examined period 316 candidaemias were studied, corresponding to equal numbered patients. The blood cultures were incubated in the automated blood culture system BACTEC9240(Becton & Dickinson). The fungal identification procedure to species level included microscopic examination of the yeast colonies, germ-tube test, assimilation test of carbohydrates by API20AUX(Biomerieux) and/or using the automated system VITEK2(Biomerieux). The antifungal susceptibility testing (MIC) was performed by E-test strips(AB, Biodisk) according to the manufacturer's instructions. MIC was evaluated according to CLSI criteria for 5-flucytocine(FC), fluconazole(FL), ketokonazole(KE), itraconazole(IT), voriconazole(VO). As susceptible to amphotericin B(AP) considered the isolates possessing MIC values of $\leqslant$1 mg/L.

Results: The 66.1%(209/316) of candidaemias occurred in the ICUs, the 23.4%(74/316) in the surgical wards and the rest 10.4%(33/316) in the medical wards. The crude fatal rate was 34.5%. All candidemia episodes were caused by a single *Candida* species. The species distribution was *C. parapsilosis* (n = 159, 50.3%), *C. albicans* (n = 67, 21.2%), *C. glabrata* (n = 24, 7.6%), *C. tropicalis* (n = 20, 6.3%), *C. lusitaniae* (n = 14, 4.4%), *C. famata* (n = 14, 4.4%), *C. guilliermondii* (n = 11, 3.5%), *C. intermedie* (n = 4, 1.3%) and *C. utilis* (n = 3, 1.0%). All isolates were susceptible to AP. A decreased susceptibility (DD-S) to VO was found in two *C. parapsilosis* isolates and in one *C. albicans* isolate while two *C. albicans* isolates were resistant to the same agent. The number and the susceptibility percentage of the two most common, *C. parapsilosis*(n)/*C. albicans*(n1) isolates to the rest antifungal agents were: n = 152, 95.4%/n1=62, 91.9% for FC, n = 79, 68.4%/n1=33, 60.6% for FL, n = 63, 84.1%/n1=33, 30.3% for KE, n = 62, 64.5%/n1=23, 43.5% for IT, respectively.

Conclusion: *C. parapsilosis* was the predominant cause of candidaemias in our Hospital. The crude mortality rate was high. The low susceptibility to triazoles and the dose-dependent susceptibility or resistance emerged to VO, weak the armamentarium against this serious infection. Present study shows that AP remains the drug of choice against bloodstream *Candida* isolates.

P855 The characteristics of candidaemia at a tertiary care cancer centre in Japan, with special interest in the type of malignancies

N. Kishida, Y. Gu, F. Takahiro, K. Okinaka, T. Sato, N. Ohmagari (Sunto-gun, Tokyo, JP)*

Objectives: One of the most important risk factors for candidemia is malignancy, especially in the gastrointestinal tract. However, the details of the type of gastrointestinal tract malignancies or the anatomic site of origin of the malignancies associated with this risk are not well documented. Also reports on the characteristics of candidemia among cancer patients in Japan are scarce.

Methods: The records of all consecutive patients with candidemia diagnosed between January 2006 and December 2008 at the Shizuoka

cancer centre, a 557-bedded tertiary care cancer centre in Japan, were reviewed.

Results: There were 85 episodes of candidemia during the study period. Fifty-four episodes (64%) involved male patients, and 31 (36%) involved female patients. The median of age of the patients was 62 years (range, 40–83). Eighty-three episodes (98%) occurred in patients with solid tumours, and only 2 in patients with haematological malignancies. Of the 85 episodes, 62 (73%) occurred in patients with upper gastrointestinal tract malignancies, including pharyngeal, oesophageal, gastric or hepato-biliary-pancreatic cancer, while the remaining occurred in the patients with other malignancies. The leading type of malignancy is gastric cancer (25 out of 85 episodes; 29%). Fifty-two out of the 85 episodes (61%), occurred in patients with a history of steroid use, and 49 (58%) occurred in patients with peritoneal metastasis. Seventy-seven (91%) of the episodes occurred in patients with insertion of a central venous catheter or other port devices. Of the patients with a central venous catheter or other port devices, 73 (95%) episodes had hyperalimentation. Only seven of the episodes (8.2%) occurred in patients with documented neutropenia. The 30-day mortality was 29%.

The distribution of the candida species was: 32%, *Candida albicans*; 31%, *Candida* grabrata; 22%, *Candida parapsilosis*; 7%, *Candida tropicalis*; 1%, *Candida krusei*.

Conclusion: These data suggests that gastric cancer may be one of the most important risk factors for candidemia. In addition, other upper gastrointestinal tract malignancies are also potential risk factors for candidemia. Therefore, one of typical clinical pictures of candidemia in Japan would be a non-neutropenic and non-haematological patient who has upper gastrointestinal tract malignancies especially gastric cancer with peritoneal metastasis, using a central venous catheter or other port devices for hyperalimentation.

P856 Prospective diagnostic performance of the (1–3)-Beta-D-glucan assay in haematological patients with invasive candidiasis

A. Alhambra, M.S. Cuétara, M. Catalán, M.E. Alvarez, M.D. Moragues, J. Pontón, A. del Palacio (Madrid, Leganés, Bilbao, ES)*

Objectives: Diagnosis of invasive candidiasis (IC) is challenging. Invasive diagnostic procedures are precluded in haematological patients and traditional microbiologic techniques have low sensitivity. (1–3)-β-D-glucan (BG) assay is a non-invasive diagnostic tool that may increase the diagnosis of IC.

Methods: BG detection was performed at the discretion of the clinical haematological team. We prospectively assessed the serum levels of BG assay in patients with a compatible clinical syndrome or either at risk for IC. Conventional microbiologic methods, tissue biopsies, blood cultures and necropsies together with the assessment of risk factors, signs, symptoms and radiologic imaging were used for the diagnosis of IC as defined by De Pauw et al (Clin Infect Dis 2008; 46: 1813). Patients were stratified as proposed by Prentice et al (Br J Haematol 2000; 110: 273). ROC curves were used for defining the best BG cutoff.

Results: 98 patients (181 episodes) were included in the study. The distribution of episodes in relationship with the risk group was: high risk (n = 72); intermediate high (n = 21); intermediate low (n = 71) and low (n = 17). The prevalence of IC was 5.10%. With a BG cutoff of 80 pg/mL the sensitivity, specificity, positive and negative predictive values were 80, 64.52, 10.81 and 98.36% respectively.

Conclusions: The high negative predictive value of BG can exclude reasonably IC in haematological patients even though 80% of patients in our cohort were treated prophylactically with antifungals. In our population the best cutoff for the diagnosis of IC was 80 pg/mL.

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Characteristics of 5 patients with proven IC

No.	Risk group	Underlying disease[1]	Site of isolation	*Candida* spp.	Prophylaxis	No. of serum samples	No. of +ve sera	Highest BG level
1	High intermediate	AML	Blood	*C. albicans*	Fluconazole	23	1	80
2	High	NHL	Blood	*C. albicans*	Fluconazole	5	2	134
3	High	BL	Blood	*C. krusei*	Itraconazole	16	6	158
4	Low intermediate	CLL	Blood/liver	*C. parapsilosis*	None	5	0	45
5	Low	CLS	Blood	*C. albicans*	None	4	4	1216

[1] AML: Acute myeloid leukemia; NHL: Non-Hodgkin's lymphoma; BL: Burkitt lymphoma; CLL: Chronic lymphocytic leukemia; CLS: Chronic lymphocytic syndrome.

P857 Trends in frequency, species distribution and antifungal susceptibility of fungal bloodstream isolates of paediatric patients, 2000–2009

E. Romanowska, P. Nadkowska, B. Garczewska, D. Dzierzanowska (Warsaw, PL)*

Objectives: In the last decade we have observed a decrease of infections caused by *Candida albicans*, and a shift towards non albicans species and occurrence of other fungal genus. The aim of this study was to determine the aetiology, frequency and antifungal susceptibility of clinical fungal strains isolated from blood samples of paediatric patients hospitalized in The Children's Memorial Health Institute.

During a 10-year period time from 2000 to 20 November 2009 were analized 158 isolates obtained from positive blood cultures of 127 patients.

Methods: The clinical material was peripheral blood directly incubated in automated Bact/Alert system (bioMérieux). After receiving positive cultures of blood specimens, fungal isolates were identified by their morphological and biochemical features by using ID 32C (bioMérieux) and microscoping (moulds). Antifungal susceptibility was assessed using ATB Fungus tests (bioMérieux).

Results: Mycological assays confirmed in 158 cases candidiases with fungemia. The highest number of these infections occurred in the oncological ward (27.2%), nutrition and paediatrics ward (19.5%), intensive care unit (18.3%), transplantology and surgery ward (9.4% and 8.2% respectively). Other wards with a lower than 3% occurrence of fungemia were: cardiology, immunology, urology, neonates and intensive care neonates ward. The main aetiological factor was *Candida parapsilosis* (40%) followed by *C. albicans* (35.5%), *C. tropicalis* (5.7%), *C. glabrata* (3.8%) and *Saccharomyces cerevisiae* (3.8%). Other identified fungal species: *C. famata, C. intermedia, C. lusitaniae*, *C. guilliermondii, C. kefyr, C. krusei, C. dubliniensis, Trichosporon asahii*, *Fusarium* chlamydosporum. Majority of *C. albicans* strains were sensitive to amphotericin B (100%) and fluconazole (96.4%). Among non-*albicans* strains increasing resistance to fluconazole was found: *C. parapsilosis* (11.1%) and *C. tropicalis* (22%). One from 150 clinical *Candida* spp strains was resistant to voriconazole (*C. glabrata*).

Conclusions:

- *Candida albicans* was a predominant etiological factor of fungemia till year 2005.
- Increasing prevalence of non albicans candidiemias was observed with a benefit towards *C. parapsilosis*.
- Received isolates show increasing occurrence of *Candida* species with genetical resistance to fluconazole and amphotericin B and appearance of other fungal genus: Saccharomyces, Trichosporon, *Fusarium*.
- *Candida* blood infections showed increasing resistance to fluconazole and itraconazole.

P858 Epidemiology of candidaemia in an Italian tertiary hospital during a 5-year period: January 2004-December 2008

A.M. Azzini, A. Tedesco, G. Lo Cascio, F. Soldani, P. Sette, M. Cordioli, E. Concia (Verona, San Bonifacio, IT)*

Objective: To evaluate trends in candidaemia (CA) between 2004 and 2008.

Methods: All CA episodes were retrospectively identified and demographic/clinical characteristics, data about systemic antifungal/antibacterial drugs and others predisposing factors within the 2

weeks before its onset were collected, including catheter-related CA. The antifungal susceptibility patterns of isolated species were collected.
Results: 170 episodes of CA were identified, 91.2% hospital acquired. The average incidence of CA was 1.72/10000 patient-days, ranging from 1.71 in 2004 to 1.31 in 2008, with a peak of 2.53 in 2005. 29.4% of CA occurred in ICUs, followed by surgical and medical wards (both 28.2%) and neonatal intensive care unit (NICU) (14.1%). Table 1 shows demographic characteristics, comorbidities and predisposing factors of the studied population. *C. parapsilosis* was isolated in 41.8% of cases, followed by *C. albicans* (34.1%), *C. glabrata* (14.7%), *C. tropicalis* (2.9%) and *C. krusei* (1.8%). Non-albicans species exceeded *C. albicans* during the entire study period.
29.6% of CA was persistent. CVC-related CA were 31.2%, in 74.6% of them CVC was removed but in 26.2% later than 48 hours from the diagnosis.
84.4% of patients received antifungal therapy: in 59.2% of cases it started within 48 hours from the diagnosis. It was adequate in 62.5% for posology and in 56.6% for the susceptibility pattern of the isolated mycete. Fluconazole was employed in 67.8%, followed by any amphotericin B lipid-formulation (44.8%) and caspofungin (13.2%). No azole-resistant *C. albicans* was isolated, 2.8% of *C. parapsilosis* was fluconazole-resistant. The 30-day crude mortality rate was 34.6%.

Table 1

Characteristics	Ward				
	Total (n = 170)	NICU (n = 24)	ICU (n = 50)	Surgical (n = 48)	Medical (n = 48)
Age (range)	53.9 (0–92)	20.3 (7–74)[a]	63.6 (17–86)	63.9 (21–92)	60.5 (7–92)
Sex (male)	105 (61.8)	12 (50.0)	28 (56.0)	33 (68.7)	32 (66.7)
Illness severity					
McCabe 1	40 (23.5)	3 (12.5)	9 (18.0)	12 (25.0)	16 (34.8)
McCabe 2	98 (57.6)	5 (20.8)	28 (58.0)	35 (72.9)	27 (58.7)
McCabe 3	32 (18.8)	16 (66.7)	12 (24.0)	1 (2.1)	3 (6.5)
Severe sepsis/shock	34 (20.0)	4 (16.6)	22 (44.0)	5 (10.4)	8 (6.3)
WBC/neutrophils	11587/9073	15265/–	12522/10629	11213/9307	8939/6870
Comorbidities	143 (84.1)	24 (100.0)	43 (86.0)	40 (83.4)	38 (79.2)
Diabetes	27 (15.9)	0 (0.0)	12 (24.0)	9 (18.8)	6 (12.5)
Solid neoplasm	63 (37.1)	0 (0.0)	19 (38.0)	32 (66.7)	12 (25.0)
Haematological neoplasm	18 (10.6)	0 (0.0)	5 (10.0)	1 (2.1)	8 (16.7)
Transplant	1 (0.6)	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
HIV/AIDS	5 (2.9)	0 (0.0)	1 (2.0)	0 (0.0)	4 (8.3)
Rheumatic disease	10 (5.9)	0 (0.0)	4 (8.0)	1 (2.1)	5 (10.4)
VLBW	19 (11.2)	19 (79.1)[b]	0 (0.0)	0 (0.0)	0 (0.0)
Chronic liver disease/ chronic renal failure/COPD	81 (47.6)	5 (20.8)	35 (70.0)	19 (39.6)	25 (52.1)
Risk factors	170 (100.0)	24 (100.0)	50 (100.0)	48 (100.0)	48 (100.0)
TPN	119 (70.0)	23 (95.8)	28 (56.0)	33 (68.7)	35 (72.9)
CVC	154 (90.6)	24 (100.0)	48 (96.0)	38 (79.1)	44 (91.7)
CVC non tunnelled	119 (70.0)	6 (25.0)	46 (92.0)	34 (70.8)	35 (72.9)
CVC tunnelled	16 (9.4)	1 (4.2)	7 (14.0)	5 (10.4)	11 (22.9)
PICC	12 (7.1)	12 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
umbilical	17 (10.0)	17 (70.8)	0 (0.0)	0 (0.0)	0 (0.0)
Urethral catheter	109 (64.1)	3 (12.5)	47 (94.0)	32 (66.7)	27 (56.2)
VM	81 (47.6)	21 (87.5)	38 (76.0)	13 (27.0)	9 (18.7)
Neutropenia	10 (5.8)	0 (0.0)	3 (6.0)	0 (0.0)	7 (14.6)
Steroids	36 (21.2)	0 (0.0)	11 (22.0)	8 (16.7)	17 (35.4)
Chemotherapy	11 (6.5)	0 (0.0)	3 (6.0)	0 (0.0)	8 (16.7)
Immunosuppressors	7 (4.1)	0 (0.0)	1 (2.0)	3 (6.2)	3 (6.3)
GVHD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Transplantation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dialysis	9 (5.2)	0 (0.0)	8 (16.0)	0 (0.0)	1 (2.1)
Surgery	93 (54.7)	4 (16.7)	32 (64.0)	40 (83.3)	18 (37.5)
abdominal	72 (42.3)	3 (12.5)	26 (52.0)	34 (70.8)	10 (20.8)
pancreatic	23 (13.5)	0 (0.0)	9 (18.0)	22 (45.8)	1 (2.1)
Re-intervention	29 (17.1)	1 (4.2)	14 (28.0)	9 (18.7)	4 (8.3)
Stay in intensive care	98 (57.6)	24 (100.0)	50 (100.0)	16 (33.3)	13 (27.0)
Length of stay (range)[a]	17.6 (1–84)	20.2 (7–74)	20.2 (1–74)	9.1 (1–39)	14.1 (1–84)
⩾3 days	87 (51.2)	24 (100.0)	44 (88.0)	14 (29.1)	10 (20.8)
Antibiotic therapy[c]	150 (88.2)	22 (91.6)	43 (86.0)	44 (91.6)	41 (85.4)
Antimycotic prophylaxis[c]	43 (25.3)	6 (33.3)	14 (28.0)	9 (18.7)	14 (29.1)
With azolics	34 (20.0)	4 (16.6)	13 (26.0)	8 (16.7)	13 (27.0)

WBC, white blood cell; TPN, total parenteral nutrition; CVC, central venous catheter; PICC, peripheral inserted central catheter; VM, mechanical ventilation; GVHD, Graft versus host diseases; NICU, neonatal intensive care unit; ICU, intensive care unit.
[a]In days. [b]18 (75.0%) premature infants with ELBW (extremely low birth weight). [c]During previous 2 weeks for at least 3 consecutive days.

Conclusions: The annual incidence of CA was high but in decline after a peak in 2005. CA was more frequent in ICU and NICU (24.7 and 6.5/10000 patient-days), followed by surgical and medical wards. Incidence rates in surgical wards was slightly higher than that in medical ones: in the first ones there was a greater frequency of abdominal and pancreatic surgery, but in the second a wider use of CVC, TPN and steroids. CVC related CA was about 30% (44.2% by *C. albicans;*37.2% by *C. parapsilosis)*despite of the use of this device. Of interest is the high frequency of non albicans species due to a relative greater number of *C. parapsilosis* and *C. glabrata.* Worrisome is their frequent resistance or S-DD susceptibility to fluconazole, related to a wider use of the drug the year before the peak in reduced susceptibility.

P859 Should fungi be monitored in hospital-related drinking-water?

M.L. Pedro-Botet, I. Sanchez, M. Batlle, L. Mateu, C. Barrenetxea, A. Sanchez, R. Duarte, M. Rovira, J. Estella, A. Llorente, D. Gallardo, I. Jarque, D. Serrano, J. Sanchez de Toledo, L. Vazquez, M. Sabria for the Proifi Study Group CIBERes), Spain*

Drinking-water, although potable, may contain a range of microorganisms including non-fermentors gramnegative bacilli, *Mycobacterium* spp. and fungi not suitable for some patients within health care facilities. Specific regulation on hospital potable water does not exist.
Objective: To investigate fungi in drinking-water of Departments of Hematology (DH).
Methods: Annual fungal investigation (since July 2008) of cold (CW) and hot (HW) water from DH and 3-yr surveillance of hospital-acquired invasive fungal infection (IFI) in patients undergoing hematopoietic progenitor transplantation (HPT) or leukemic patients on intensive chemotherapy (LPIC).
Results: 12 DH were included. The mean follow-up was 10.5 months (6–16m). Fungi were isolated in 119 (54%) of 220 water samples, cold water samples (62/118;74%) were colonized significantly more frequently than hot water samples (45/102; 44.1%). Analysis of different types of fungi showed that septated molds were the most prevalent (73, 33.1%) with Penicilium spp., Cladosporium spp. and Alternaria spp. being of note. *Aspergillus* and *Fusarium* were found in 2 (1 in hot and 1 in cold) and 6 (2 in hot and 4 in cold) water samples, respectively. Zygomycetes were isolated in 10 (2 hot and 8 cold) water samples with *Rhizopus* spp. being the most prevalent. The prevalence of yeasts was 16.4% (36/220). Only 2 cases of hospital-acquired probable invasive aspergillosis were detected. *Aspergillus* was not recovered in patient-related water samples.
Conclusions: Fungal colonization amongst water samples in DH is high (54%). CW in DH is colonized significantly more frequently than HW. Regulations on hospital potable water are warranted.

Pulmonary mycoses

P860 The diagnosis of pulmonary mycoses: histomorphological criteria contra immunohistochemical reactivity

H.E. Jensen (Frederiksberg, DK)*

Objectives: As a consequence of even suspecting the presence of pulmonary mycoses clinically, cases are often not diagnosed until histopathology is applied. Distinctive morphological details may provide a tentative identification, but the appearance of fungi in tissue sections is affected by steric orientation and age of the fungus. Moreover, sharing of morphological similarities and the presence of sparse and/or atypical fungal elements will hamper a clear-cut diagnosis. In the present study, the histomorphological diagnosis in 55 cases of pulmonary mycoses was compared with the diagnosis obtained immunohistochemically.
Methods: In order to obtain a second opinion, a total of 55 pulmonary mycosis cases were received from 1996 to 2009. The suspected diagnoses were as follows: aspergillosis: 29; aspergillosis and candidosis: 6; zygomycosis: 8; and unidentified: 12. All cases were immunohistochemically screened with three commercial available antibodies from AbD Serotec reacting specifically with agents of aspergillosis (MCA 2576), candidosis (1750–5557), and zygomycosis (MCA 2577). Difficulties for obtaining a correct histomorphological diagnosis were evaluated, too.
Results: A correct diagnosis of aspergillosis and zygomycosis was upheld in 76% and 88%, respectively. The suspicion of a dual infection of aspergillosis and candidosis was only confirmed in 1 case, whereas aspergillosis and candidosis alone was diagnosed in 4 and 1 cases, respectively. Aspergillosis was confused with zygomycosis in

4 cases, and aspergillosis and candidosis in 2 cases. Only 1 case of zygomycosis was misdiagnosed as aspergillosis. Unidentified cases were identified as aspergillosis (n = 4). Zygomycosis (n = 1), aspergillosis and candidosis (n = 1), candidosis (n = 2). Four cases remained unidentified together with one suspected aspergillosis case, which failed to react immunohistochemically. Diagnostic histomorphological criteria were especially challenged in chronic lesions which contained massive necrosis, and when only few fungal elements were present.
Conclusion: The application of immunohistochemistry changes the histomorphological diagnoses in 28% of the cases. Moreover, the technique diagnosed 67% of 12 unidentified causes of pulmonary mycoses. Therefore, the application of the present series of antibodies in immunohistochemical techniques will be advantageous for achieving a more complete identification of fungi, which is mandatory for an optimal treatment strategy.

P861 Yeasts and mycelial fungi in lung cavities of tuberculosis patients

A. Kulko, P. Dreval (Moscow, RU)*

Objective: To analyze the outcomes of seven-year mycology studies of lung cavities contents of pulmonary tuberculosis (TB) patients.
Methods: Microscopy and culture (Sabouraud medium with chloramphenicol) of samples from lung cavities (aspirates, materials of biopsies and resections); species identification of isolated strains of yeasts (morphological properties on chromogenic media: CandiSelect® 4, Bio-Rad Labs. and chromID® *Candida*, bioMérieux; biochemical properties: Auxacolor® 2 test-system, Bio-Rad Labs.) and mycelial fungi (conventional methods, identification media: Czapek-Dox and potato dextrose agar).
Results: In 2002–2009 we studied samples from lung cavities (caverns, tuberculomas, cysts, aspergillomas) of 264 pulmonary TB patients. Cultural studies revealed fungi in 59 patients. The isolated strains belonged to 18 species of 9 genera: yeasts – *Candida* (4 species), *Geotrichum* (1); mycelial fungi – *Alternaria* (1), *Aspergillus* (6), *Cladosporium* (1), *Fusarium* (1), *Paecilomyces* (1), *Penicillium* (2), *Rhizopus* (1). The fungi from the genus *Aspergillus* were found in lung cavities of 36 patients: *A. fumigatus* – in 23 patients, *A. flavus* – in 3, *A. niger* – in 4, *A. restrictus* – in 2, *A. terreus* – in 3, *A. versicolor* – in 1. The fungi from the genus *Candida* were found in 10 patients: *C. albicans* – in 5 patients, *C. glabrata* – in 3, *C. kefyr* – in 1, *C. tropicalis* – in 1. It is important to note, that the fungi isolated from lung cavities of 21 out of 59 (35.6%) patients were also revealed from bronchoalveolar lavage fluid (BAL) and/or sputum.
Conclusions: Lung cavities in TB patients represent an essential risk factor for opportunistic pulmonary mycosis. Development of secondary pulmonary aspergillosis (aspergilloma, other forms of pulmonary aspergillosis) is very likely in such patients. If fungi are found in a lung cavity, it is advisable to perform additional mycology studies of sputum and BAL.

P862 First quantitative data on *Pneumocystis jirovecii* spread in the air surrounding PCP patients

F. Choukri, J. Menotti, C. Sarfati, J.C. Lucet, G. Nevez, Y. Garin, F. Derouin, A. Totet (Paris, Brest, Amiens, FR)*

Objectives: Airborne transmission of *Pneumocystis* has been demonstrated in animal models and is strongly suspected between humans. However, information concerning the fungal burden of *Pneumocystis* in the environment is still lacking. Our objectives were to quantify *P. jirovecii* (the human-derived *Pneumocystis* species) and estimate its diffusion in the air surrounding patients with *Pneumocystis* pneumonia (PCP), using a high-volume sampler combined with a real-time PCR assay for *P. jirovecii* detection and quantification.
Methods: Hospitalized patients with confirmed PCP were prospectively enrolled from January 2008 to July 2009. As early as possible after diagnosis of PCP, air samples were collected at different distances from PCP patients' heads (1, 3, 5 and 8 m) i.e. inside and outside the patient room. Control samples were collected away from patient wards and outdoors. At each place, 1,500 L of air were collected using the Coriolis air sampler (Bertin technologies) which finally delivers a 10 mL liquid sample. Detection and quantification of *P. jirovecii* DNA in the collected liquid medium were performed using a real-time PCR assay targeting the mitochondrial large subunit rRNA gene of *P. jirovecii.* Plasmid suspensions were used as standards for quantification, giving the correspondence between threshold cycle (Ct) values and the copy number per microL of extracted DNA. The detection limit was estimated at 7 copies/microL of extracted DNA.
Results: Air samples were collected in the environment of 19 PCP patients. At a 1-meter distance from patients' heads, *P. jirovecii* DNA was detected in 15/19 (79.8%) cases, with fungal burdens ranging between $7.5\,10^3$ and $4.5\,10^6$ gene copies/m^3. These levels decreased with distance from the patients ($p < 0.002$), but 4/12 (33.3%) of the samples taken at 8 meters (i.e. in the corridor adjacent to their room) were positive. The forty control samples were PCR negative for *P. jirovecii* detection.
Conclusion: Our method is efficient to quantify *P. jirovecii* fungal burdens in the air and to estimate its diffusion in the environment of patients with PCP. This study provides the first quantitative data on the spread of *P. jirovecii* in the air, and supports the risk of direct transmission in the proximity of patients with PCP. These results make it possible to estimate hazard exposure, and open the field for PCP microbial quantitative risk assessment.

P863 Risk factors for *Pneumocystis jirovecii* pneumonia in kidney transplant recipients and appraisal of strategies for selective use of prophylaxis

M.G. de Boer, F.P. Kroon, S. le Cessie, J.W. de Fijter, J.T. van Dissel (Leiden, NL)*

Selective use of Trimethoprim-Sulfamethoxazole (TMP-SMX) or alternative chemoprophylaxis for *Pneumocystis* pneumonia (PcP) in kidney transplant recipients based on risk factor analysis is not a universally adapted strategy and evidence based sources are limited. In a large retrospective case–controlled study, potential risk factors for PcP in kidney transplant recipients were investigated to define parameters for use in clinical prediction rules and guidelines that could optimize PcP prophylaxis in this population. Fifty-two cases were identified between January 1983 and September 2008; PcP incidence was 2.7% per 1000 transplantations and developed between 60 days and 24 months post transplantation in 85% of cases. For each case two time-matched controls were enrolled. The complete analysis was performed in a time-matched statistical model (using STATA) to prevent periodical bias. At baseline, age >55 years and use of basiliximab were predictive for development of PcP (adjusted odds ratio's 2.7 and 0.2). CMV infection within 3 months prior to PcP (OR 3.0 95% CI 1.2–7.8) and treatment for rejection (OR 5.8 95% CI 1.9–17.5) were independently associated with PcP in a multivariate analysis. Increasing numbers of rejection treatments correlated with higher risks and shorter times between transplantation and PcP. A prediction model derived from the data indicated that incidences between 1.0% and 5.0% and low mortality, allow a choice for selective prophylaxis targeting the first 6 months for all- and the first year post transplantation for patients >55 years of age and those treated for rejection to avoid unneeded TMP-SMX side effects while achieving a PcP incidence <1.0%. Furthermore, a strategy of not prescribing prophylaxis, except to those at extremer risk (e.g. after 3 or more treatments for rejection), can be pursued when PcP incidence is <1.0% over a prolonged period of time. Caveats however still exist: in case of sudden rises in incidence or 'outbreak', this strategy should be readily abandoned and replaced by more intense use of prophylaxis. Current guidelines may be refined accordingly although validation of selective risk based strategies for PcP prophylaxis requires prospective evaluation.

P864 Different protein expression pattern induced by *Pneumocystis jirovecii* colonization in idiopathic interstitial pneumonia patients

V. Friaza, R. Morilla, C. De La Horra, N. Respaldiza, M.A. Montes-Cano, S. Gutierrez, J. Martin Juan, J.M. Varela, J. Medrano, E. Calderon (Seville, ES)*

Background: It has been describe the presence of *Pneumocystis* jirovecci colonization in Idiopathic Interstitial Pneumonia (IIP) patients. However the role of *Pneumocystis* play in the physiopathology of these diseases remains still unknown. *Pneumocystis* colonization could induce changes on the protein expression in its host. To identify these alterations could help to understand the role of *Pneumocystis* in IIP.
Aim: To determinate difference induced by *Pneumocystis* colonization in protein expression in IIP patients.
Methods: BALF from 10 well characterized NII patiens (5 colonizated and 5 non-colonized by P jirovecii) were collected and centrifuged to 600xg during ten minutes and supernatant were pooled separately. Supernatant pooled were concentrated with Microcon Ultracel YM-3 (Millipore) and 200 ug of total proteins was Albumin and IgG depleted using the Depletion Spin Trap Kit of GE Healthcare. Resultant proteins were methanol:chloroform precipitated and following resuspended in 0.1% SDS to proceed trypsin digest, ITRAQ Labelling and LC-MS/MS analysis.
Results: 136 different proteins were detected in BAL fluid. High confidence sequence information and expression level for 65 polypeptides, including metabolic enzymes, immunity-related proteins, surfactant associated protein, calcium-binding proteins, redox protein, peptidases and proteases. Thirty-four proteins displayed higher levels of expression in colonized patients and twenty-eight presented lower levels in colonized patients.
Conclusion: There are difference protein expression pattern probably related with *P. jirovecii* colonization in IIP patients. These data deserve further researches for unravelling the role of *P. jirovecii* colonization.
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Molecular mycology

P865 Preliminary study on antifungal susceptibility and molecular relatedness of *Candida glabrata* in intensive care units

M.E. Fadda, M. Deplano, M.B. Pisano, R. Podda, G.S. Podda, S. Cosentino (Monserrato, Cagliari, IT)*

Objectives: *Candida glabrata* is now emerging as an important human pathogen responsible for candidemia. The prevalence of *C. glabrata* has increased in the last decade and this species has recently emerged as significant pathogen in critically ill patients in Intensive Cure Units (ICU).
The objective of this preliminary study was to define antifungal susceptibility and molecular relatedness of *C. glabrata* isolates recovered from colonizing and invasive sites of patients hospitalized in the ICUs of two hospitals during a 12 month period.
Methods: Antifungal susceptibility profile to amphotericin B, fluconazole, itraconazole, ketoconazole, voriconazole, and caspofungine was determined by disk diffusion method. Polymerase Chain Reaction (PCR) fingerprinting with the oligonucleotide 5′-GAGGGTGGCGGTTCT-3′ (M13) as single primer was used for a rapid molecular genotyping. A total of 53 *C. glabrata* isolates from 22 patients were considered.
Results: Most of *C. glabrata* isolates were observed in pharyngeal swabs and in feces or rectal swabs. A good sensibility to amphotericin B was observed among isolates from both ICUs. All the strains were resistant or DDS to itraconazole and ketoconazole. Among the 31 *C. glabrata* isolates that were resistant or SDD to fluconazole, 18 (58%) had a low sensibility to voriconazole. At a 60% similarity the isolates were grouped in two clusters. Several patients were colonized by similar isolates in different anatomic sites supporting an endogenous transmission. Similar RAPD-PCR profiles found from isolates from different patients suggesting an exogenous acquisition.
Conclusions: Our preliminary study has contributed to monitor trends of resistance to antifungal drugs. Caution is advised when considering voriconazole therapy for *C. glabrata* candidemia in setting that predict "older" (fluconazole, itraconazole, ketoconazole) azoles resistance. RAPD with the M13 primer was effective for *C. glabrata* typing as it differentiated multiple isolates from different patients and was able to find colonization with similar isolates in different anatomic sites of the same patient. Our results suggest a possible cross transmission of *C. glabrata* strains and support the importance of a continued epidemiological surveillance, particularly in the ICUs.

P866 Identification of *Candida* by the PCR and microarray-based Prove-it™ Sepsis assay

A. Aittakorpi, P. Kuusela, P. Koukila-Kähkölä, M. Vaara, M. Petrou, V. Gant, M. Mäki (Helsinki, FI; London, UK)*

Objective: Prove-it™ Sepsis assay is a rapid, broad-range PCR and microarray-based assay designed to identify the majority of sepsis-causing bacteria. The pathogen panel covers 50 Gram-negative and Gram-positive bacterial species. It also reports meticillin resistance by detecting the mecA gene. The assay has been clinically validated using positive blood cultures (specificity 99%, sensitivity 95%). After the validation, the test has been further developed to identify 8 *Candida* spp. This study aims to evaluate the performance of yeast identification of the Prove-it™ Sepsis assay.
Methods: 3318 blood samples from patients with suspected sepsis were collected in Helsinki University Central Hospital Laboratory (HUSLAB; Finland) and in University College London Hospital (UK). Blood culture bottles of BacT/ALERT 3D (bioMérieux) and BACTEC 924 (Becton Dickinson) were incubated for a total of 6 days or until flagged as positive. DNA was extracted from blood cultures using the DNA extraction instrument easyMAG (bioMérieux) and stored at –20ºC prior to the Prove-it™ Sepsis assay. Conventional identification was done for all positive samples and DNA extracts of positive yeast samples were selected for the Prove-it™ Sepsis analysis of 3 hours assay time.
Results: Of the analyzed 3318 blood cultures, 2107 were positive by conventional identification methods. Of these, yeast was detected in 44 (2.1%) samples. Prove-it™ Sepsis assay identified *Candida* species from 43 samples, having yeast coverage of 97.8%. Prevalence of identified *Candida* spp. were: 73% *C. albicans*, 14% *C. glabrata*, 5% *C. tropicalis*, 5% *C. lusitaniae*, and 2% *C. parapsilosis*. From two samples containing two different *Candida* species, only one of them was identified. Otherwise there was no discordance between identification results obtained by conventional methods and the Prove-it™ Sepsis assay. No cross-hybridizations with bacterial capture oligonucleotides of the assay, leading to false bacterial identification, were observed.
Conclusions: The rapid and accurate *Candida* identification from positive blood cultures by modified Prove-it™ Sepsis provides a novel tool for a faster and more evidence-based patient management which is known to contribute to a positive outcome. Currently, the evaluation of Prove-it™ Sepsis assay is continued blindfold with higher amount of clinical yeast samples.

P867 The challenges and feasibility of validating molecular diagnostic kits on multiple real-time PCR platforms

S.A. Follett, R. O'Neill, K. Radej, A. Moody (Manchester, UK)*

Purpose: The MycAssay family of molecular diagnostic real-time PCR assays are designed to enhance the speed, sensitivity and specificity of fungal diagnosis and are initially validated on the Cepheid SmartCycler. However, for broad application across the fungal community, they need to run on multiple platforms, thus offering a commercial, standardised assay suitable for all potential users with their existing laboratory equipment. Although the chemistry of the assays are designed to work with the

majority of real-time PCR instruments, the reality of conducting platform transfer studies for an *in vitro* diagnostic assay is not simple.
Methods: Common commercially available real-time PCR platforms were assessed and prioritised for platform transfer. The MycAssay *Aspergillus* assay was tested on a number of different platforms during assay optimisation. Each platform was assessed for suitability by determining efficiency, as well as the ability to reliably distinguish negative samples. Replicate runs were performed to check that threshold and other analysis settings were robust. Platforms satisfying these criteria were then used for validation studies to satisfy the requirements for *in vitro* diagnostic devices.
Results: The list of PCR platforms that can support the MycAssay family of products includes; AB7500, RotorGene Q, Stratagene MX3005P, Roche LightCycler 2.0 and Bio-Rad CFX96. Initial formulation testing with the MycAssay *Aspergillus* product showed acceptable results for both the *Aspergillus* and internal amplification control reactions on all platforms, allowing use in the research setting. However, the ability to consistently reproduce results, as required for a CE-marked diagnostic, varied between platforms. The source of this variation appears to be in the platform specific interpretative software. In addition, while very similar, Ct (or equivalents) results were not 100% comparable across platforms.
Conclusions: Initial tests with molecular diagnostic assays may indicate suitability on multiple real-time PCR platforms. However, the work required to ensure long-term robustness can be far more complex than expected. While raw data can look normal, analysis algorithms within each manufacturer's software programs can cause the generation of discrepant amplification curves and false positive results. Therefore, before a molecular diagnostic kit can reliably be used on multiple platforms it must undergo the meticulous testing required for CE-marking standards.

P868 Purification of cell-bound and free circulating bacterial, fungal and viral nucleic acids from large blood volumes using the QIAamp® pure pathogen nucleic acid kit

T. Doedt, S. Martsch, D. Heckel (Hilden, DE)*

Objective: The objective of this study was to develop a chemistry and protocol for copurification of bacterial, fungal and viral nucleic acids from large volumes of whole blood ($\geqslant$5 ml) with the capability to elute in small volumes ($\leqslant$100 μl). To obtain maximal sensitivity, microbial cell-bound as well as free circulating nucleic acids should be purified with parallel depletion of the human DNA background.
Method: Human whole blood was spiked with various viruses, bacterial and fungal cells as well as purified microbial DNA to mimic free circulating DNA. The protocol was evaluated by determination of pathogen nucleic acids by quantitative real time (rt)PCR, as well as total DNA by UV/Vis spectroscopy. Performance was further compared to commercially available reference kits.
Results: The QIAamp® Pure Pathogen Nucleic Acid Kit showed highest yield of cell-bound and free circulating bacterial, fungal and viral nucleic acids when compared to reference kits, which were either limited in blood input volume or not capable of depleting the human DNA background. These results could be obtained by splitting the blood sample into plasma and a cellular fraction. The latter is partially lysed to release the human genomic DNA, which in a subsequent centrifugation is separated from the non-lysed microbial cells. The microbial nucleic acids are released in a second, mechanical lysis step and combined with the previously collected plasma, which is then subjected to a high-volume silica-membrane based sample preparation. The QIAamp® Pure Pathogen Nucleic Acid Kit was the only kit in this study, which was able to deplete the human DNA background ($\geqslant$90%) while concentrating viral and free circulating nucleic acids in addition to cell-bound microbial nucleic acids.
Conclusions:

- The QIAamp® Pure Pathogen Nucleic Acid Kit is the first published protocol enabling highly sensitive purification of bacterial, fungal and viral nucleic acids with parallel depletion of human DNA.
- The silica membrane based protocols enable high input volumes (3–8 ml whole blood) and elution in volumes as small as 20μl allowing enrichment of pathogen nucleic acids by a factor of 400.
- The copurification of microbial cell-bound and free circulating nucleic acids allow for maximal sensitivity.
- The protocol provides nucleic acids that perform well in sensitive downstream applications such as real-time PCR and RT-PCR.

P869 Species identification of non-sporulating moulds characterized as *Mycelia sterilia* isolated from clinical specimens by molecular methods

D.W. Santos, V.R. Azevedo, S.S. Gonçalves, S. Mesquita, A.S. Melo, A.L. Colombo (Sao Paulo, BR)*

In the last years, phaeohyphomycosis are increasing as subcutaneous infections, with potential for blood and visceral dissemination. Some dark moulds isolates cannot growth and sporulate on different media, making impossible the identification by conventional methods.
Objective: Our goal was to identify the species of isolates characterized as *Mycelia sterilia* obtained from clinical samples using ITS region sequencing.
Methods: Clinical samples were obtained from 9 patients: eight kidney recipient patients who developed subcutaneous fungal infections and one case of keratitis. All fungal infections had the diagnosis confirmed by histopathology using specific dyes for fungal detection (Grocott and Fontana-Masson) and conventional culture (growth of *Mycelia sterilia*). Since the histopathology showed septated demaceatious hyphae and the culture identified nonsporulating fungi for all of these clinical samples, the mycelia of these cultures were submitted to DNA extraction using PrepMan Ultra kit. PCR assays were run to amplify the ITS region of rDNA gene and the amplicons were sequenced. The sequences were submitted for comparison on genebanks (National Center for Biotechnology Information and Centraalbureau voor Schimmelcultures) for species identification.
Results: All culture results exhibited nonsporulating mycelia despite using different specific media for inducing sporulation. After ITS region amplification and sequencing, the amplicon sequences were submitted to the genebank sites for comparison to the databases. The basic alignment search tool demonstrated high identity values (>98%) for all the isolates tested. We obtained the following species identification: Alternaria alternata (2), Alternaria tenuissima (1), Cochliobolus lunatus (2), Cochliobolus australiensis (1), Botryosphaeria dothidea (1), Ochroconis gallopava (1) and Ascomycetes sp (1).
Conclusions: *Mycelia sterilia* isolates were frequently found in culture recovered from kidney recipient patients under immunosupression with calcineurin inhibitors. It is not clear whether calcineurin could have any impact on inhibiting sporulation of filamentous fungi. ITS region amplification and sequencing was a helpful tool for species identification of *Mycelia sterilia* isolates and could allow defining the best treatment for the patients. One isolate was not identified at the species level, possibly it may represent a species which ITS reference sequence is not deposited in genebanks.

P870 Multilocus sequence typing of 156 bloodstream isolates of *Candida albicans* from 10 university hospitals in Korea

J.H. Shin, S. Kim, S.H. Park, M.N. Kim, H.S. Lee, Y. Uh, C.J. Moon, M. Joo, M.G. Shin, S.P. Suh, D.W. Ryang (Gwangju, Seoul, Jeonju, Wonju, KR)*

Objectives: This study was performed to investigate the clonality of *Candida albicans* bloodstream isolates from 10 Korean hospitals, and to validate Multilocus Sequence Typing (MLST) against pulsed-field gel electrophoresis (PFGE) in the genotyping of *C. albicans* strains of multi-institutional origin.
Methods: A total of 156 bloodstream isolates from 156 patients were obtained from a Korean nationwide surveillance study, conducted between September 2006 and August 2007. Partial internal sequences of

seven housekeeping genes (AAT1a, ACC1, ADP1, MPIb, SYA1, VPS13, and ZWF1b) were determined. PFGE typing was performed as part of restriction endonuclease analysis of genomic DNAs using BssHII (REAG-B). Clonal strains were defined by the isolation of more than two strains of the same diploid sequence type (DST), with the same or similar REAG-B pattern.
Results: The 156 isolates yielded 114 unique DSTs by MLST. Ninety-six isolates belonged to new sequence types (89 distinct DSTs), whereas 60 isolates belonged to previously described types (25 DSTs). MLST revealed that 58 isolates (37.2%) shared 16 distinct DSTs. When the MLST results were combined with REAG-B, 35 (22.4%) isolates shared 11 genotypes, suggesting clonal strains. Among the 35 clonal isolates, 8 (two isolates from each of four genotypes) came from the same hospitals, but other genotype isolates came from different hospitals.
Conclusion: The present study shows that *C. albicans* candidemia in Korea may frequently involve clonal strains. Additionally, MLST is needed to clarify genotyping by REAG in clonality analysis of *C. albicans* strains of multi-institutional origin.

P871 *Lodderomyces elongisporus*: first report on its isolation and characterization from clinical specimens in Kuwait

Z. Khan, S. Ahmad (Safat, KW)*

Objective: *Lodderomyces elongisporus*, is a recently recognized bloodstream pathogen with phenotypic similarities with *Candida parapsilosis* and other members of *C. parapsilosis* complex. So far, only little information is available about the geographic distribution of this species. The aim of this study was to determine its occurrence among phenotypically identified *C. parapsilosis* strains isolated from a variety of clinical specimens in Kuwait.
Methods: A total of 165 strains identified as *C. parapsilosis* by Vitek2 yeast identification system were studied. All the isolates were cultured on BBLTM CHROMagar *Candida* for development of turquoise blue color colonies for provisional identification of *L. elongisporus.* The identity of the isolates was further studied on BBLTM Corn Meal agar for the formation of ascospores. The unequivocal identification of *L. elongisporus* was confirmed by direct DNA sequencing of internally transcribed spacer (ITS)-1 and ITS-2 regions of rDNA.
Results: Of the 165 isolates cultured on Chromagar *Candida*, two strains produced turquoise blue color colonies and also formed ascospores on Corn Meal agar as well as on Malt extract agar. They were tentatively identified as *L. elongisporus.* The isolates originated from the tip of central venous catheter and sputum. Direct DNA sequencing of the ITS region of rDNA confirmed the identification of both the isolates as *L. elongisporus.* Of the remaining isolates, 158 and five strains were identified as *C. parapsilosis* and *C. orthopsilosis,* respectively. The rDNA sequence data of all five *C. orthopsilosis* and four randomly selected *C. parapsilosis* strains were consistent with their species-specific identification.
Conclusions: The isolation of *L. elongisporus* has been reported from the Middle East for the first time. The identity of the two isolates was confirmed by phenotypic characteristics and DNA sequencing of rDNA. This report suggests that *L. elongisporus* may be masquerading as *C. parapsilosis* in some cases of invasive candidiasis.
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P872 Rapid detection and identification of fungal pathogens by rolling circle amplification using Fonsecaea as a model

M. Najafzadeh, J. Sun, V. Vicente, S. de Hoog (Utrecht, NL; Parana, BR)*

We describe a rapid and sensitive assay for detection and identification of pathogenic fungi without sequencing. The method is presented with species of Fonsecaea, agents of human chromoblastomycosis, as a model. The ITS rDNA region of 103 Fonsecaea strains were sequenced and aligned in view of designing three specific padlock probes to be used for the detection of single nucleotide polymorphisms (SNPs) of Fonsecaea species. The 38 strains included to test the specificity of rolling circle amplification (RCA) comprised 17 isolates of Fonsecaea pedrosoi, 13 of *F. monophora* and 8 of *F. nubica.* The assay proved to successfully amplify DNA of the target fungi at the level of species; while no cross reactivity was observed. The amplification product was visualized on a 1% agarose gel to verify the specificity of probe-template binding. Amounts of reagents were minimized to avoid the generation of false positive results. The simplicity, sensitivity, robustness and low costs provide RCA a distinct position as highly practical method among isothermal techniques for DNA diagnostics.

P873 Prevalence of *C. albicans* and dermatophytes in onychomycosis

G.J. Wisselink, E. van Zanten, A.M. Kooistra-Smid (Groningen, NL)*

Objectives: Onychomycosis is predominantly caused by dermatophytes with *Trichophyton rubrum* being the primary causative organism. However, recent studies report yeast and molds not only as contaminants, but are increasingly reporting them as pathogens in onychomycosis. *Candida albicans* is the most frequently reported cause of candidal onychomycosis. The aim of this study was to determine the prevalences of *C. albicans* and dermatophytes in finger- and toenail onychomycosis.
Methods: Sixty-three finger- and 68 toenail clippings were collected from patients suspected of onychomycosis in our mycology department. As a control group, 86 finger- and toenail clippings were collected from people not suspected of suffering onychomycosis. Culture for *C. albicans* was only performed on the nail clippings collected from the patient group. On all specimens EasyMag DNA extraction (Biomerieux, Boxtel, The Netherlands) was performed, using overnight pre-lysis. Internallly controlled multiplex real-time PCR was performed for the following targets: *T. rubrum, T. interdigitale* and *C. albicans*.
Results: Culture for *C. albicans* yielded 13% and 0% positives in samples of finger- and toenail samples respectively.
Fingernails: Of all 63 patient samples real-time PCR yielded 60% *C. albicans*, 27% *T. rubrum* and 3% *T. interdigitale.* Of all 86 samples of the control group real-time PCR yielded *C. albicans*, *T. rubrum* and *T. interdigitale* in 16%, 4% and 2% respectively.
Toenails: In all 68 samples in the patient group real-time PCR detected *C. albicans*, *T. rubrum* and *T. interdigitale* in 21%, 53% and 24% respectively. Using real-time PCR, the samples in the control group yielded *C. albicans*, *T. rubrum* and *T. interdigitale* in 13%, 8% and 14% respectively.
Double infections: Double infections of a dermatophyte and *C. albicans* in the patient group detected by real-time PCR were observed in 13% of the samples of the toenails and in 18% of the samples of the fingernails.
Conclusion: *C. albicans* is significantly more prevalent in fingernails of the patient group compared to the control group, odds ratio: 7.82 ($p = 5 \times 10^{-8}$), suggesting that *C. albicans* plays an important role in fingernail onychomycosis. *T. rubrum* is significantly more prevalent in both finger- and toenails of the patient group compared to the control group, odds ratios: 12.70 ($p < 10^{-8}$) and 10.22 ($p = 7.2 \times 10^{-5}$) respectively.
Furthermore, this study showed a large increase in sensitivity using PCR for the detection of *C. albicans* over culture.

P874 The impact of bronchoalveolar lavage fluid sampling analyzed by molecular methods for the diagnosis of invasive fungal infections

N. Kokturk, A. Kalkanci, S.Z. Aki, Z.A. Yegin, Z.N. Ozkurt, O. Tunccan, S. Ozyegen Aslan, B. Yalcin, E. Senol, G. Turkoz Sucak, M. Yagci, S. Kustimur (Ankara, TR)*

Objectives: Polymerase chain reaction (PCR) from bronchoalveolar lavage (BAL) clinical samples has been used to assist in the diagnosis of invasive fungal infections (IFI). Culture and histopathological examination of BAL fluid are useful but have suboptimal sensitivity and in the case of culture may require several days for fungal growth to be evident. As a part of the diagnostic procedure for suspected pulmonary

infections in 30 patients with haematological malignancy, bronchoscopy with BAL were performed.
Methods: Between January and November 2009, we prospectively followed up 30 patients with pulmonary infiltrates suggestive of pneumonia. Fungal stains, cultures, galactomannan detection and PCR for *Aspergillus*, *Candida* and *Pneumocystis* were performed on all patients. All patients had fever and pulmonary infiltrates on the chest roentgenogram on the day that the BAL fluid was obtained. Galactomannan antigen detection was also performed with serum samples.
Results: The 30 patients consisted of 6 patients with probable IFI (microbiologic culture or galactomannan), 24 patients with possible IFI (consistent clinical and radiologic findings). The sensitivity, specificity, positive predictive value, and negative predictive value of PCR were 93%, 100%, 38%, and 99%, respectively, for probable IFI, and 64%, 93%, 52%, and 96%, respectively, for possible IFI.
Asergillus DNA was detected by real time PCR or *Aspergillus* galactomannan ELISA were positive with BAL fluid or blood samples from three of 24 patients who had radiological evidence of aspergillosis. *Candida* DNA was detected with BAL or blood samples from five of 30 patients, while *Pneumocystis* DNA was not detected in patients who had radiological evidence of pulmonary infection.
Patients diagnosed with probable IFI according to traditional microbiological methods had significantly higher mortality rates compared to their counterparts who had in addition a PCR-based diagnosis (80 vs 30%, $p < 0.003$).
Conclusion: This study demonstrates that PCR-based DNA detection for a diagnosis of IFI from BAL fluid has a significant effect on the outcome of patients with fungal infections, probably related to earlier diagnosis. The use of bronchoscopy with BAL allowed correct identification of seven microorganisms involved. Four patients' pneumonias had a multiple etiology. The bronchoscopy methods used were well tolerated even by patients whose condition was poor.

P875 Usefulness of real-time PCR and direct sequencing in the diagnosis of invasive mycoses – in the case of six leukaemic patients with proven/probable aspergillosis

U. Nawrot, M. Ussowicz, K. Wlodarczyk, M. Pajaczkowska, K. Kalwak, A. Chybicka (Wroclaw, PL)*

The development of the new diagnostics methods enabling early diagnosis of invasive mycoses (IFI) and early, targeting antifungal therapy represent a task of primer importance.
In this paper we present the results of retrospectively performed molecular examinations of blood and tissue samples collected from six patients with either proven (1 patients) or probable (5) IFI. The patients were treated in the Dept of Pediatric Hematology and Oncology, BMT Unit of Wroclaw Medical University, in years 2006–2009. Investigated material included a series of weakly taken blood samples (6–20 samples per patient), which were available for four patients, and biopsy from lungs (2 patients), liver (1 patient), and gallbladder (1 patient). All patients were previously tested with Platelia *Aspergillus* tests (Bio Rad) for *Aspergillus* galactomannan and showed at last two positive results. No fungi were cultured from tested biopsies, however microscopic examination revealed presence of fungal elements, except of gallbladder, which were negative. DNA from clinical samples was extracted with the use of QIAmpDNA Mini kit (Qiagen) and mechanical cells disruption by beads beating (blood, tissues). qPCR was performed on Rotor-Gene 6000 (Corbett Life Science) system using either *Aspergillus*-specific primers and hydrolysis probe ASP 28P (Williamson 2000, White 2006), targeting the 28S rRNA gene, or panfungal primers Fungi 5.8f and ITS4 (Bergman 2007, White 1990), amplifying the ITS2 region of rDNA, and the intercalating dye SYBR-Green I. Selected amplicons were sequenced on both strands and obtained sequences were analysed using the Blast software and Genbank-EMBL database.
The *Aspergillus*-specific qPCR correlated good with the Platelia *Aspergillus* tests. A total of 16 GM-positive blood samples were positive in qPCR, however among 22 GM-negative samples 6 were positive in qPCR. The amplicons obtained from 8 blood samples (2 samples per patient) and two lung biopsies showed 98–99% sequence similarity to *A. fumigatus*, thus *A. fumigatus* can be regarded as likely causative agent of infection. Surprisingly, the liver sample from patient with suspected reactivation of aspergillosis revealed the presence of DNA of *C. dubliniensis.* The sequence analysis indicated that PCR product obtained with DNA from gallbladder was probably a result of contamination with Cladosporium.
The DNA-based methods seems to be valuable tool for improving mycological diagnosis in immunocompromised patients.

P876 Molecular approaches to fungal infections in high-risk haematology patients

A. Kalkanci, S.Z. Aki, Z.A. Yegin, E. Suyani, O. Tunccan, N. Kokturk, B. Yalcin, S. Ozyegen Aslan, E. Senol, G. Turkoz Sucak, S. Kustimur (Ankara, TR)*

Objectives: In high-risk patient cohorts, such as patients after allogeneic stem-cell transplantation, or patients with acute leukaemia, early diagnosis of invasive fungal infections (IFIs) is essential, as delayed or missing diagnosis of IFI results in increasing rates of mortality. Current non-culture based, diagnostic tests for systemic fungal infections include measurement of *Aspergillus* galactomannan (GM) and polymerase chain reaction (PCR) assays for *Pneumocystis*, *Aspergillus* or *Candida* DNA. The aim of this study was to investigate the diagnostic utility of both the aspergillus galactomannan (GM) antigen and the fungal PCR assays in the diagnosis of IFI in high risk febrile neutropenic cancer patients.
Methods: During one year period, 40 febrile neutropenic (FN) cases at high risk for developing IFI while receiving chemotherapy were investigated at Gazi University Faculty of Medicine. These patients were subjected to clinical evaluation, chest CT scan, conventional blood cultures for bacterial and fungal pathogens, aspergillus GM antigen detection and PCR assay utilizing *Pneumocystis*, *Candida* and *Aspergillus* primers. Patients were screened twice a week by PCR and antigen testing during fever and were followed-up for a minimum of 1 year.
Of the 40 cases, 3 were proven IFI; whereas 7 cases were probable and 30 cases were possible IFI.
Results: *Aspergillus* antigen test showed a sensitivity of 75%, specificity of 60%, positive and negative predictive values of 50% and 85%; respectively. Based on positive results for proven/probable IFI and compared to culture results, *Candida*, *Aspergillus* and *Pneumocystis* PCR assays showed sensitivity, specificity, positive and negative predictive values of between 70–75%, 90–92%, 80–84% and 80–87%; respectively.
Conclusions: The introduction of a comprehensive diagnostic strategy to exclude invasive fungal infection in high-risk patients with haematological malignancy can result in improvements in clinical management. These molecular assays provide high potential in terms of sensitivity and specificity, but vary widely in their feasibility and up to now have not been standardised. The results of PCR assay was reasonably specific but not very sensitive and had a chance of missing the diagnosis of IFI. The PCR assay seems a promising test for objectively defining IFI, but is not recommended as the only tool for diagnosing IFI. Combining microscopy, culture, and PCR may improve the diagnostic outcome.

P877 The protein "mycoarray": a novel immunoassay for the serological diagnosis of primitive invasive mycoses

A. Ardizzoni, M.C. Baschieri, L. Manca, C. Farina, C. Cermelli, M. Meacci, C. Venturelli, E. Blasi (Modena, Milan, IT)*

Objectives: Invasive fungal infections are an important cause of morbidity and mortality in an increasingly higher number of patients, also because of difficulties in providing a rapid and appropriate diagnosis. In some cases, detection of a specific antibody response is a crucial diagnostic tool; however, the available serological assays often provide qualitative results only, their sensitivity and specificity are poor and long time procedures are required. In addition, patients who suffer from

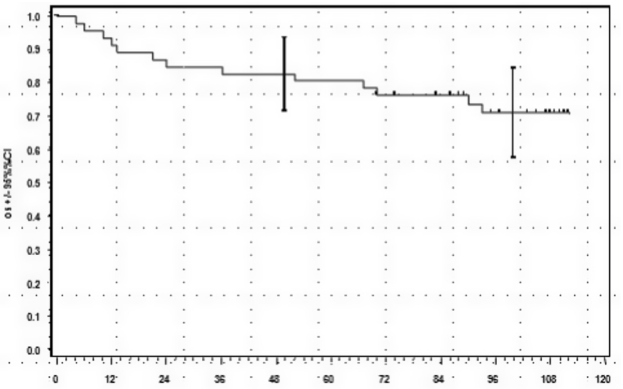
an invasive mycosis may have multiple infections likely underestimated by conventional diagnostic approaches. In order to couple the serology of primitive invasive mycoses to the protein microarray technology, a "mycoarray" assay has been designed and set up.
Methods: Four antigen extracts (histoplasmin, coccidioidin, Coccidioides "TP" antigen and aspergillin) and the appropriate controls were spotted in various conditions onto a restricted area of a microscope slide. The arrays were then incubated with immune sera produced in goat against each single antigen or, subsequently, with human sera (6 from patients affected by primitive invasive mycoses and 7 from healthy individuals). The occurring immunocomplexes were detected by indirect immunofluorescence.
Results: The pilot experiments, conducted using the goat immune sera, allowed to establish the optimal spotting conditions for each antigen in terms of both spotting buffer and extracts' dilution. The "mycoarrays", obtained by spotting each of the fungal antigens in its best condition, were then processed with sera either from patients or control subjects. In all the cases, the serological reactivity detected by the arrays processed with the patients' samples was in agreement with the clinical and microbiological diagnosis; no reactivity was ever observed in the arrays processed with the negative control sera.
Conclusions: The "protein mycoarray" is sensitive enough to discriminate between healthy individuals and patients affected by histoplasmosis or coccidioidomycosis. Because of its intrinsic features, miniaturization and multiparametricity, this novel diagnostic tool allows to cut out costs and to shorten times-to-results. It follows that the "mycoarray" has all the potentialities to be included in the daily clinical practice in the near future.

Aspergillosis

P878 A phase II dose escalation study of caspofungin for invasive aspergillosis

O.A. Cornely, J.J. Vehreschild, M. Rüping, D. Arenz, S. Schwartz, C.P. Heussel, G. Silling, M. Mahne, J. Franklin, U. Harnischmacher, A. Wilkens, F. Farowski, M. Karthaus, T. Lehrnbecher, A.J. Ullmann, M. Hallek, A. Groll (Cologne, Berlin, Heidelberg, Münster, Munich, Frankfurt, Mainz, DE)*

Objectives: Treatment of invasive aspergillosis (IA) fails in up to 50% and mortality is at least 30%. Antifungal combination treatment has not been proven to be beneficial and dose escalation with liposomal amphotericin B did not improve outcome. New approaches are needed.
Methods: High dose caspofungin was investigated in an escalating Fibonacci approach in IA defined according to modified EORTC/MSG criteria. In cohorts at 70 mg, 100 mg, 150 mg, or 200 mg QD, 8 patients each were to receive caspofungin first-line treatment for proven/probable IA for up to 28 days. Dose limiting toxicity was defined as 2 of 8 patients in the same cohort with the same grade $\geqslant$4 non-haematological treatment-related adverse event (TRAE), or 4 of 8 patients with a grade $\geqslant$3 non-haematological TRAE. If no dose-limiting toxicity was reached, 12 additional patients were enrolled in the 200 mg cohort. Patients unevaluable for toxicity or pharmacokinetic analysis were replaced.
Results: A total of 46 patients were treated in the 4 cohorts (9, 8, 9, 20 pts). IA was proven in 2.2% and probable in 97.8%. Patient characteristics were as follows: Median age 61 years (min 18.3, max 73.7); 21/46 (45.7%) female. Underlying diseases distribution was: AML 50%, ALL 8.7%, lymphoma 19.6%, chronic lymphocytic leukaemia 10.9%, other 10.9%. Median duration of treatment was 24.5 days. Two (4.3%) patients with treatment durations $\leqslant$5 days were replaced for pharmacokinetic analysis, but evaluated for safety and efficacy. No dose-limiting toxicity was found by investigator or DSMB assessment. At end of treatment (EOT) complete plus partial response, was achieved in the 4 cohorts in 4/9, 3/8, 6/9, 12/20 patients, i.e. 25/46 (54.3%) of the total population. Stable disease was achieved in 4 patients (8.7%), 17 (37%) patients failed treatment. Overall survival at 12 weeks was 76.1%. After a 12 week follow-up attributable mortality was 8.7%. Death due to malignancy occurred in 10.9%, to sepsis in 8.7%.
Conclusions: In the first-line treatment of proven or probable invasive aspergillosis no dose-limiting toxicity of caspofungin at doses up to 200 mg QD was found. Complete plus partial response rates at EOT were 54.3% after dose-escalated caspofungin treatment, and thus in the range of the success rates previously reported with voriconazole and liposomal amphotericin B. Twelve weeks after start of treatment the 23.9% overall mortality was lower than found in the literature.



Caspofungin MTD: Overall survival with 95% confidence intervals. Censored at 112 days (= 28 days treatment + 12 weeks follow-up).

P879 Invasive aspergillosis increases the risk of death in acute myeloid leukaemia patients

M. Michallet, S. Kraghel, T. Bénet, M. Sobh, N. Voirin, M. El Hamri, G. Cannas, F. Nicolini, E. Nicolas-Virelizier, H. Labussière, S. Ducastelle, R. Crocchiolo, F. Barraco, X. Thomas, Y. Chelghoum, M. Nicolle, A. Bienvenu, F. Persat, F. de Monbrison, N. Fanari, S. Madene, P. Vanhems (Lyon, FR)*

Objectives: To study the impact of invasive aspergillosis (IA) incidence during induction therapy for acute myeloid leukemia (AML) patients on short and long term overall survival.
Methods: A retrospective cohort study was performed in the Hematology department of the Edouard Herriot Hospital, Lyon (France). Patients with newly diagnosed AML between 01/01/2004 and 31/12/2007 were retrospectively included and the follow-up was censored at 30/06/2009. Data were extracted from medical charts and from the prospective surveillance of IA (EORTC diagnosis criteria). The patients with IA after post induction evaluation were excluded (N = 5). A Cox proportional hazard model with diagnosis of IA and post induction evaluation (complete remission [CR] of AML or failure of chemotherapy) as the main exposure besides age, year of inclusion, WHO status, cytogenetic group, kind of induction chemotherapy, and hematopoietic stem cell transplantation, was fitted.
Results: Overall, 262 patients counting for 149 370 patient-days were analysed, the median age at diagnosis was 56.6 years (47.9–64.2 years), and 196 (75%) had CR. There were 58 (22%) IA cases with a median interval between induction and IA of 30 days (range, 16–27 days); 29 (50%) IA were possible, 24 (41%) probable, and 5 (9%) proven. At the last follow-up, 165 (63%) patients died with a median overall survival of 18 months (95% confidence interval [95% CI] 14–23 months). The 4 year-survival of patients having had IA was 14%, and without IA 32% (P = 0.01). The 2 year-survival of patients achieving of CR was 54% vs. 5% for patients with failure of chemotherapy was 5% (P < 0.001). Cox multivariate analysis (cf. Table) showed that patients in CR with IA presented a higher risk of death compared to patients in CR without IA (Hazard ratio=1.66, 95% CI 1.05–2.65, P = 0.031). In addition, IA was associated with a higher risk of death in patients with failure of chemotherapy compared to patients in CR without IA (Hazard ratio=6.43, 95% CI 3.72–11.10, P < 0.001). The WHO status, cytogenetic group and kind of induction chemotherapy were associated with lower survival.

Conclusion: IA was associated with a high risk of death in AML patients whether they were in CR or failure after induction chemotherapy. Cytogenetic group or WHO status are not modifiable risk factors for death in this population while prevention of IA with environmental procedures or using individual prophylaxis will improve survival outcome.

Table. Factors independently associated with the survival in acute myeloid leukemia patients in induction chemotherapy, multivariate Cox proportional hazard model

Characteristics	Hazard ratio	95% CI	P
Invasive aspergillosis and post-induction evaluation			
No invasive aspergillosis, and CR	1.00 (Ref.)	–	–
Invasive aspergillosis, and CR	1.66	(1.05–2.65)	0.031
No invasive aspergillosis, and failure	6.19	(4.11–9.29)	$<10^{-3}$
Invasive aspergillosis, and failure	6.43	(3.72–11.10)	$<10^{-3}$
WHO status			
<2	1.00 (Ref.)	–	–
⩾2	1.84	(1.30–2.60)	0.001
Cytogenetic group			
Favorable	1.00 (Ref.)	–	–
Intermediary	4.52	(1.62–12.62)	0.004
Unfavorable	5.46	(1.98–15.07)	0.001
Not possible to classify or not done	8.14	(2.82–23.50)	$<10^{-3}$
Induction chemotherapy			
Intensive	1.00 (Ref.)	–	–
Standard	2.04	(1.40–2.99)	$<10^{-3}$

CR, complete remission.

P880 Five-year surveillance of invasive aspergillosis in a German tertiary care hospital

K. Graf, S. Mohammad Khani, E. Ott, Z. Demir, F. Mattner, P. Gastmeier, S. Ziesing, I. Chaberny on behalf of the Institute for Medical Microbiology and Hospital Epidemiology*

Objectives: Invasive aspergillus infections (IA) are a severe complication in immuncompromised, especially transplantated, patients. Unfortunately diagnosis can be difficult in multimorbide patients and one must assume that several patients die failing diagnosis and special treatment. The aim of this study was to detect high risk patients to introduce special preventive measures and to get information about quick diagnosis of infection.
Methods: After implementation of a surveillance protocol in 2003, we surveyed all hospitalized patients by positive microbiological cultures, pathologic diagnoses and/ or pharmacologic informations about antifungal treatment in 2003–2007: Cases were reviewed and were classified according to international consensus criteria (EORTC). Possible nosocomial acquisition was determined.
Results: Among the 704 alerts, 214 IA cases were observed: 56 proven cases (26%), 25 (12%) probable cases and 133 (62%) possible cases. The incidence was 1.6%. Among the proven and probable IA cases, we observed 56% nosocomial cases (45/81) and 21% cases of undetermined origin (17/81). Many patients (37/81) had organ transplantation, followed by bone marrow transplantated patients (8/81), but we also found a high number of other patient groups. We detected 10 malignancy tumor patients and 26 other patients with chronical organ diseases. Among the proven and probable cases we detected 53 patients undergoing immunsuppressive therapy. 45% of the proven patients died during their hospital stay. 11 of the proven patients did not get any antifungal treatment, seven of those patients died. Overall 32 patients (57%) were diagnosed by pathologic results, 16 patients were diagnosed by autopsy and seven patients (13%) would not have been diagnosed without autopsy.
Conclusion: High risk patients are not only patients undergoing transplantation, but all patients undergoing immunsuppressive therapy. Diagnosis is difficult and 13% of all cases are diagnosed by autopsy only. Thus, this study shows the importance of IA surveillance in all high-risk units to improve infection prevention measures.

P881 Invasive infections caused by non-*Aspergillus* moulds identified by sequencing analysis at a tertiary care hospital in Taiwan, 2000–2008

M.S. Hsu, Y.T. Huang, P.R. Hsueh (Panchiao, Taipei City, TW)*

Objectives: The purpose of this study is to describe the clinical and microbiological characteristics of 103 patients with cultures positive for non-*Aspergillus* molds from 2000 to 2009 were described.
Methods: Invasive mold infections were defined in accordance with the EORTC/MSG criteria. The mold isolates were identified to the species level by sequence comparison analysis of the internal transcribed spacer (ITS) regions using primers ITS1 and ITS4. For *Fusarium* species, sequence analysis of the translation elongation factor 1 α (EF1 α) region was also performed with primers EF1 and EF2.
Results: Among the 103 patients, 27 cases (26.2%) met the criteria for proven or probable IMI. The etiologic pathogens were *Fusarium* species in 12 (44.4%) patients, Paecilomyces species in 7 (25.9%), Zygomycetes in 5 (18.5%), and Scedopsorium species in 3 (11.1%) (Table 1). Predisposing factors included hematological malignancies (48%), steroid use (40%), solid organ tumor (12%), hematopoietic stem cell (4%) or solid organ transplantation (12%), AIDS (8%), diabetes mellitus (8%) and myelodysplastic syndrome (4%). The lung was the most common main infection site (33%), followed by orbito-sinus-facial (18.5%), skin and soft tissue (18.5%), blood (11.1%), and bile (7.4%). Mortality was highest in patients with zygomycosis (4 out of 5 patients died [80%]), followed by fusariosis (41.7%). 100% of *Fusarium* were inhibited by ⩽2 umg/ml of amphotericin B. Paecilomyces isolates showed different susceptibility patterns among species. For the Zygomycetes, amphotericin B was the most active agent. *Scedosporium apiospermum* was relatively susceptible to the newer azoles.
Conclusions: Non-*Aspergillus* invasive mold infections occurred predominantly in immunocompromised patients. Patients with underlying hematological disorders were associated with increased risk of disseminated disease and high mortality. Antifungal susceptibility varied considerably between these species. This emphasized the importance of identifying infecting mold organisms to the species level, which is seldom achieved at standard clinical laboratories.

P882 Burden of invasive aspergillosis in COPD non-traditional host: a hospital database analysis

D. Patel, X. Gao, J. Stephens, M. Forshag, M. Tarallo (Bethesda, New York, US)*

Objectives: Although invasive aspergillosis (IA) occurs predominantly in immunocompromised hosts, it has been reported in patients with chronic obstructive pulmonary disease (COPD). The aim of this study was to estimate the excess clinical and economic burden of IA in COPD patients compared to COPD patients without IA using a US inpatient claims database (Premier Perspective™ Comparative Database).
Methods: Adults diagnosed with COPD (ICD-9 code = 491, 492, 493.2x, 496) + aspergillosis (117.3x) + pneumonia (484.1, 484.6, 484.7, 487.0) were identified in the Premier database(2005 to 2008). Patients were excluded if ICD-9 codes were present for malignancies, HIV, or conditions that would put them at risk for IA aside from COPD. Patients with a charge for antifungal therapy during the admission were included. To estimate the excess resource utilization and mortality, COPD+IA patients (Cases) were compared to the matched COPD patients without aspergillosis (Controls) by univariate analyses. Cases and controls were matched (1:2) based on age, gender, race, payer, hospital characteristics, geographical area, comorbidity index, and disease severity. Mortality, length of stay (LOS), and costs were compared between groups.
Results: We identified 475 COPD+IA patients, with a mean age of 69 years, 50% male and 76% Caucasian. 85% patients were on a high dose corticosteroid therapy, with 55% exposed to this therapy for more than 14 days. On average, antifungal therapy was initiated on the 6th day of hospital stay, with a mean length of therapy being 15 days. 35% of patients required mechanical ventilation, 56% had some ICU stay,

and 31% were in the ICU when their antifungal therapy was initiated. Occurrence and mortality rates were higher among COPD inpatients with an ICU stay compared to those without an ICU stay (1.2% vs.0.7% and 45.2% vs. 29%, respectively). Compared to controls, COPD+IA cases incurred significantly higher costs ($48,163 vs. $30,210, $p < 0.001$), longer LOS (23 vs. 14 days, $p < 0.001$), longer ICU stay (14 vs. 7 days, $p < 0.001$), and higher mortality (29 vs. 23%, $p = 0.016$). Findings in subgroup analyses were consistent.
Conclusion: COPD+IA patients have significantly higher resource utilization, costs and mortality compared to COPD patients without IA. Consideration of IA as a cause of infection in hospitalized COPD patients, followed by appropriate treatment, has the potential to impact important clinical and economic outcomes.

P883 Is liver cirrhosis a predisposing condition to invasive aspergillosis?

J. Guinea, M. Torres-Narbona, P. Muñoz, R. Bañares, M. Giannella, M. Valerio, E. Bouza (Madrid, ES)*

Objectives: Liver cirrhosis has recently been described as a risk factor for invasive aspergillosis, particularly in critically ill patients. We determined the clinical presentation and outcome of invasive aspergillosis in patients with liver cirrhosis.
Methods: From January 1999 to March 2009, we recorded all proven or probable cases of culture-documented invasive aspergillosis (EORTC criteria) in patients with liver cirrhosis. Patients with malignancy, solid organ transplantation and chronic obstructive pulmonary disease were excluded.
Results: We documented 122 cases of proven or probable invasive aspergillosis and 9 (7%) had of liver cirrhosis. Liver cirrhosis was due to idiopathic autoimmune hepatitis (n = 7), alcoholism (n = 1), and HCV and alcoholism (n = 1). The mean age of the patients was 53.8 y (range 30–79 y) and 6 were male. Overall, 7 patients required admission to intensive care units when invasive aspergillosis was diagnosed. Three patients developed acute liver failure before diagnosis. All patients were receiving corticosteroids and broad-spectrum antibiotics on diagnosis. The disease was always nosocomially acquired and the lung was involved in all patients (one patient also had probable brain involvement, and another probable ocular involvement) with nonspecific radiological findings and bilateral infiltrates. *Aspergillus fumigatus* was isolated in sputum (n = 2), bronchial aspirate (n = 8), and bonchoalveolar lavage (n = 3). Galactomannan determination was performed in 5 patients and was positive in 4 ($\geqslant$1 ng/mL). Eight patients received antifungal therapy [amphotericin B (n = 2), voriconazole + caspofungin (n = 4), amphotericin B + caspofungin (n = 2)], but only 2 survived (voriconazole + caspofungin). Overall mortality was 77.7% (n = 7).
Conclusions: Cirrhotic patients receiving corticosteroids and broad-spectrum antimicrobial agents should be considered potential invasive aspergillosis patients (7% of all cases of invasive aspergillosis in our institution). Lung and extra-pulmonary structures could be involved. The yield of galactomannan was very high. M. Torres-Narbona is contracted by Fondo de Investigación Sanitaria (FIS) CM08/00277. Jesús Guinea is contracted by FIS (CA08/00384).

P884 Retrospective analysis of invasive aspergillosis in haemato-oncological department, 2005–2008

B. Weinbergerova, Z. Racil, I. Kocmanova, E. Sevcikova, M. Toskova, J. Winterova, M. Lengerova, J. Mayer (Brno, CZ)*

Background: Invasive aspergillosis (IA) is a serious infectious complication in immunocompromised patients (pts.). Particularly pts. with hematological malignancies are at high risk of IA.
Methods: The goal of our retrospective analysis was to evaluate incidence, early diagnostic possibilities and effect of antifungal therapy in proven and probable IA in immunocompromised pts. in our institution during 2005–2008. We followed EORTC/MSG criteria in evaluation of IA diagnosis (2002) and therapy response (2008).
Results: In the mentioned period we documented 54 probable and 7 proven isolated pulmonary IA. Prolonged profound neutropenia (56%) and long-term use of corticosteroids (34%) were identified as the major risk factors of IA. 20 cases (33%) had normal finding on pulmonary X-ray. 54 cases (89%) had abnormality on pulmonary CT with "halo" sign as the most frequent finding (34%). 93% pts. had consecutive positivity of serum-galactomannan (S-GM). In 38 cases (62%) consecutive positivity of S-GM occured before (3 days in avg.) pathologic scan. These drugs or combination were used as primary therapy: 41% voriconazole (VORI), 7% itraconazole (ITRA), 5% caspofungin (CASPO), 38% VORI+CASPO, 3% amphotericine B deoxycholate (AMB) and 6% none. Overall RR to primary therapy was 56% (VORI 60%, VORI+CASPO 78%, ITRA 25%, CASPO 0% and AMB 0%). In 9 cases we have to use salvage therapy (VORI+CASPO in 7 pts., posaconazole+CASPO in 1 pt. and VORI in 1 pt.). Overall RR to salvage therapy was 78%. The overall mortality rate was 44%, with 11% attributable to IA.
Conclusions: On the basis of our analysis we confirm common risk factors of IA and major role of S-GM and CT for early diagnosis and prompt start of antifungal therapy of IA. We reached good results in RR with using both VORI and combination of VORI+CASPO in primary therapy of IA. Antifungal combination had excellent effect also if used as salvage treatment of this infection.
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P885 Value of combined use of bronchoalveolar lavage and galactomannan antigen in the management of pneumonias in haematological patients

L. Verga, F. Farina, F. Rossini, I. Casaroli, E. Pogliani (Monza, IT)*

Objectives: In our retrospective study we investigated the optimal timing of bronchoalveolar lavage (BAL) and the comparison of galactomannan antigen (GM) determined on BAL and serum in patients (pts) with pneumonia.
Methods: From January 2007 to June 2009, 130 consecutive pts with haematological disease were investigated for the presence of 175 pneumonias in our Unit.
At the onset of fever, blood cultures and assay GM were perfomed for 3 days. A chest computerized tomography (CT) was performed if pt was still febrile after 4 days. Pts began antifungal drugs if there was a possible or probable fungal infection. The decision to perform a BAL was made on the basis of CT images and response to therapy (tp). Pts characteristics and results are summarized in Table 1.
Results: The time between the onset of neutropenia and fever, the onset of fever and CT, the CT and BAL and between the start of antifungal tp and the BAL was 8, 5.5, 5, 5.38 days respectively. Among 148 serum GM, 31 were positive with a mean value of 1.35 (range 0.6–4.8). We performed 75 BAL (average 11 days after the onset of fever). At the time of procedure, 25 pts were already receiving broad-spectrum antibiotic tp and 32 antibiotic and antifungal tp at therapeutic doses (76% patients were in tp). 25 BAL demonstrated a pathogen by culture, immunofluorescence or RT-PCR or by direct microscopic examination. GM in BAL was performed in 52 samples. GM was positive on BAL in 15 pts: mean value of 2.06 (range 0.54–4.47); in 4 cultures were positive for fungus. 9/15 were on antifungal tp from an average of 7 days. In 9/15 also serum GM was positive at the time of BAL. Of the 31 events with positive serum GM, BAL was performed in 16: among them, 9 confirmed GM BAL positivity, while 3 were negative for GM. 2 showed *Aspergillus* culture positive without GM. We found 6 pts with BAL GM positive and serum GM negative. 36 BAL have provided an etiological diagnosis. The diagnostic power was 48%. We changed tp in 38% of patients thanks to BAL. However, there was no difference in mortality rate between patients with a positive and negative BAL.
Conclusion: In our experience, when BAL is used late, pt's clinical conditions and the great number of antibacterial and antifungal therapies may negatively influence its results. We noticed that if we perform it early during the course of disease we could improve the outcome and avoid

unnecessary therapies. We must keep on comparing the sensibility of GM on serum and on BAL.

Table 1: Characteristics of patients in study

	No. of patients (%)
Age (years)	
⩽65	98 (75%)
>65	32 (25%)
Gender	
Male	72 (55%)
Female	58 (45%)
Haematological disease	
AML	69 (54%)
ALL	11 (8%)
Lymphoproliferative disorders	37 (28%)
Myeloproliferative disorders	7 (5%)
Others	6 (5%)
State of illness during the event	
Onset	66 (39%)
RC	45 (25%)
RP	25 (14%)
PR	35 (20%)
Not classifiable	4 (2%)
Antifungal prophylaxis	
Yes	54 (31%)
No	121 (69%)
Neutropenia	
Yes	115 (66%)
No	60 (34%)
Hyperpyrexia	
Yes	159 (91%)
No	16 (9%)
Chest CT	
Yes	161 (92%)
No (only chest X-ray)	14 (8%)
BAL	
Yes	75 (43%)
No	100 (57%)
Died:	
Yes	36 (28%)
No	94 (72%)

P886 Invasive aspergillosis in haemato-oncological patients – 40 cases from a single centre

V. Kaplanova, M. Krizo, M. Kouba, P. Cetkovsky for CELL, the Czech Leukemia Study Group for Life*

Objectives: Invasive aspergillosis (IA) is a major opportunistic infection in patients with haematological malignancies. The incidence of IA has increased significantly in the past years. The high mortality reflects both the invasivity of the pathogen and the profound immunosuppression of the host organism. There has been a major development in diagnostic and treatment options for IA within the past few years.
Methods: We retrospectively searched documentation of the patiens treated in our tertiary haematlogical centre in the period 2005–2009 to identify the cases of IA and describe their pattern of diagnosis, clinical picture, treatment and outcome.
Results: We identified 40 cases (average age 53 years) of IA (according to EORTC-MSG criteria: 3 proven, 31 probable and 6 possible but clinically apparent cases). 38 patients suffered from pulmonary IA, 1 patient had blood stream infection and one patient had IA of the paranasal sinuses. In one patient was the diagnosis of IA based on autopsy, 39 patients were diagnosed to have IA while alive. The underlying diagnosis was acute leukemia in 32 (80%) patients, 22 (55%) cases developed IA after remission induction therapy for acute leukemia, 7 (18%) after allogeneic haematopoietic stem cell transplantation (aloSCT). The microbiological criterion for diagnosis was galactomannan detection in 36 of 37 probable and possible cases. 32 patients (80%) died and 8 patients (20%) are alive at follow-up (9/2009). In 22 (55%) patients IA was either direct cause of death in 13 (32%) or it was an important factor contributing to death in 9 (23%). Of the surviving patients 4 were initially treated by voriconazole, 2 by combination of voriconazole and echinocandin and two (low risk) by itraconazole. Of the 10 patients initially treated by different amphotericin B formulations only 1 patient reached partial remission, all the others were switched to other therapy due tu ineffectivity or died.
Conclusions: Our analysis shows that IA is an extremely severe complication of treatment of acute leukemias and aloSCT with high mortality. Galactomannan assay has a central role in diagnosis of IA. Voriconazole, a current therapy of choice, was more efficacious than amphotericin B formulations in our analysis.

P887 Compartmental pharmacokinetics and pharmacodynamics of amphotericin B in an *in vitro* model of invasive pulmonary aspergillosis

J.M. Lestner, S.J. Howard, J. Goodwin, L. Gregson, J. Majithiya, T.J. Walsh, G.M. Jensen, W.W. Hope (Manchester, UK; Maryland, San Dimas, US)*

Objectives: To define the extent of tissue penetration and antifungal effect of three clinical formulations of amphotericin B (AmB) in cellular and fluid compartments of the lung using an *in vitro* model of invasive pulmonary aspergillosis (IPA).
Methods: An *in vitro* model, which utilised a cellular bilayer and air-fluid interface, was developed to examine the PK-PD of AmB. Lipid formulations (liposomal (LAmB) and lipid-complex (ABLC)) were compared with AmB deoxycholate (DAmB). Concentrations of galactomannan (GM) were used to define PD relationships in endovascular and alveolar compartments, and concentrations of AmB within cellular and fluid compartments were measured by HPLC. A mathematical model was used to calculate the area under the concentration time curve (AUC) within compartments and estimate the extent of drug penetration. Fungal biomass and drug-hyphal-host cell interactions were visualised by confocal microscopy using an *A. fumigatus* transformant expressing green fluorescent protein and sulforhodamine-labelled liposomes.
Results: In the absence of treatment hyphal penetration occurred at 14–16 hours post-inoculation, mirrored by a progressive rise in GM. The presence of AmB significantly altered fungal growth; DAmB induced a steep exposure-response relationship in alveolar and endovascular compartments; whereas LAmB and ABLC induced more languid concentration-response relationships, with incomplete suppression of galactomannan in the alveolar compartment. Concentrations of AmB progressively declined in the endovascular fluid as the drug distributed throughout the compartments. High concentrations of AmB were observed in the cellular bilayer: AUCs for AmB were 10–300 times higher within the cells compared to those within endovascular fluid. The E50 in the endovascular compartment was 0.12, 1.03, and 4.41 mg/L for DAmB, LAmB and ABLC, respectively; and in the alveolar compartment was 0.17, 7.76 and 39.34 mg/L, respectively. Confocal microscopy demonstrated that liposomes engaged directly with hyphae and host cells.
Conclusion: The PK-PD relationships of 3 clinical formulations of AmB differ markedly within this model of IPA. Drug distribution within fluid and cellular sub-compartments within target tissues is of importance in understanding pharmacological effect.

P888 *Aspergillus*-derived metabolites contribute to tissue damage in cerebral aspergillosis

C. Speth, M. Hagleitner, G. Rambach, C. Lass-Flörl, K. Pfaller, I. Mohsenipour (Innsbruck, AT)*

Objective: The pathogenesis of cerebral aspergillosis is as yet insufficiently clarified and urgently needs further examination to develop

adequate counterstrategies. Since neural damage occurs not only in close proximity to the fungal hyphae our studies concentrate on a putative contribution of fungal metabolites to the induction of tissue lesions. Application of reducing agents was tested as therapeutic approach as the biochemical structure of the toxins indicate a putative neutralization of the toxins by these compounds.

Methods: Astrozytic and neuronal cell lines as well as primary microglia were incubated with various fungal toxins, including gliotoxin, patulin and citrinin. Toxicity was controlled by measuring mitochondrial activity (MTS test), immunological competence was quantified as phagocytic activity. Electron microscopy studies completed the examination of toxic effects.

Results: The fungal metabolites gliotoxin, patulin and citrinin were all able to affect the viability of astrocytes, neurons and microglia in a dose-dependent manner. Gliotoxin was the most potent toxin, damaging the cells already in nanomolar concentrations. Electron microscopy studies showed changes in morphology and explicit signs of apoptosis for gliotoxin-treated cells compared to mock-treated control cells. Staining with annexin and propidium iodide confirmed the dose-dependent induction of the apoptotic program by gliotoxin as well as by patulin. Furthermore, phagocytic activity of the cells incubated with gliotoxin and patulin was depressed already in subtoxic concentrations.

The reducing components glutathione and DTT were both capable to neutralize the deleterious effect of gliotoxin and patulin. All cells survived otherwise toxic concentrations of the metabolites in the presence of those substances and retained their phagocytic capacity.

Conclusions: Fungal metabolites were identified as putatively potent inducers of tissue damage in cerebral aspergillosis since they significantly interfere with viability of all brain cell types tested. Being secreted by *Aspergillus* they might affect extended regions of brain tissue. Beside direct damage the metabolites reduce the immunologic capacity of the cells by reducing their phagocytic activity. Both effects might act synergistically and contribute to the pathogenesis of cerebral aspergillosis. Reducing agents constitute promising therapeutic components as they neutralize the toxic effect of at least some of the metabolites.

P889 Effect of amphotericin B on *Aspergillus terreus* conidia and hyphae of different growth stages using growth curves analysis

K. Krompa, L. Zerva, J. Meletiadis (Athens, GR)*

Background: Delayed administration and suboptimal exposure to antifungal drugs may be detrimental for infections caused by *A. terreus*. Therefore, we studed the pharmacodynamic characteristics of supra and sub MIC (minimal inhibitory concentrations) concentrations of amphotericin B to *A. terreus* conidia and hyphae of different growth stages using growth curves analysis.

Methods: Inocula of 104CFU/ml from three clinical isolates of *A. terreus* were prepared in 200μl RPMI1640+0.165M MOPS and incubated in a 96-well flatbottom microplates at 37ºC for 96 h. The MIC of AMB was 1–2 mg/l for all isolates. AMB was added into corresponding wells at final concentrations of 0.25×, 1×, and 4× MIC after 0 h, 24 h, 48 h and 72 h of incubation. *A. terreus* was in form of conidia at 0 h, of hyphae at 24 h and of mycelia at 48 h and 72 h. The turbidity of each well was monitored continuously by measuring the optical density at 630nm every 15min. Growth curves were constructed and fungal growth before and after addition of the drugs was compared at each time point and for each concentration. Reduction of fungal growth was calculated as 100%x(ODo-ODf)/ODo where ODo and ODf are the initial and final optical densities after drug addition, respectively. Time to regrowth after drug addition was also determined. All tests were performed in duplicate.

Results: AMB completely inhibited fungal growth at 4× MIC when added at 0 h for all three stains. When 1×MIC and 0.25×MIC of AMB was added at 0 h there was complete inhibition of growth only for 51 h (43–58 h) (median and range) and 32 h (21–39 h), respectively for all strains. When 4×MIC of AMB was added at 24 h, 48 h and 72 h, fungal growth was reduced by 19% (17–20%), 27% (25–29%) and 31% (27–35%), respectively without regrowth being observed. When 1×MIC of AMB was added at 24 h, 48 h and 72 h, fungal growth was reduced by 18% (14–20%), 30% (21–31%) and 23% (22–30%) with subsequent regrowth being observed after 8–28 h, 4–6 h, and 3–6 h, respectively. When 0.25×MIC of AMB was added at 24 h, 48 h and 72 h, fungal growth was reduced by 14% (14–15%), 25% (25–26%) and 21% (21–22%) with subsequent regrowth being observed after 8–12 h, 4–8 h, and 3–7 h, respectively.

Conclusion: AMB reduced fungal growth at supra and sub MIC concentrations but regrowth being observed at 1× and 0.25× but not at 4×MIC. Growth curves analysis revealed important concentration and time dependent pharmacodynamic characteristics of AMB against *A. terreus*.

Resistance in fastidious bacteria

P890 Investigation of prevalence of major antibiotic resistance and enterotoxin genes among clinical *Bacteroides* strains from a European antibiotic susceptibility survey

J. Sóki, Z. Eitel, E. Urbán, G. Terhes, E. Nagy on behalf of the ESGARAB*

Objective: Our aims were to examine the prevalence of common antibiotic resistance genes of *Bacteroides* in a large set of isolates, to compare their genotypes with their resistance patterns and to gain information on the potential of the emergence of multiply resistant strains.

Methods: Antibiotic susceptibility was previously measured by agar dilution. The genotypes for the cepA, cfxA, cfiA, ermF, nim, tetQ and bft genes were examined by RealTime PCR using 96 well plate platform and fluorescent dye (SybrGreen) detection. The cepA, cfxA, ermF and tetQ patterns were detected for 184 strains while the presence of the cfiA, nim and bft genes were recorded for 693 strains.

Results: The most common gene was the tetracycline resistance, tetQ gene with the prevalence 78.5 and 85% among *B. fragilis* and non-*fragilis Bacteroides* (NFB) strains, respectively. Similar high frequency was found for the cepA gene with 74.3% among *B. fragilis* and with 57.5% among NFB strains. The presence of cepA and ermF genes was in the same range as phenotypic resistance for cefoxitin and clindamycine, respectively, but also differentially distributed between *B. fragilis* and NFB strains. The differences of prevalence were statistically significant. The presence of cfiA and bft genes among *B. fragilis* strains were 8.7 and 14.2%, respectively. However, some (7) bft genes were detected among NFB strains, and the coincidence of cfiA and bft genes were found among *B. fragilis* (4) strains. The statistical analysis confirmed the independent occurrence of the bft and cfiA genes in the *B. fragilis* strains. As regards the four other genes examined, a similar analysis demonstrated that the occurrence of the cepA and cfiA genes negatively correlate with a high level of significance among *B. fragilis* strains. For cfxA and ermF a clear conclusion could not be drawn because of the low number of strains involved, and for tetQ the test was not significant. Out of the 693 *Bacteroides* strains examined only two harboured nim genes.

Conclusion: The analysis of a high number of *Bacteroides* strains for antibiotic and virulence genes opened an unseen view of complexity of the interactions of these genes and taxa. An alarming coincidence of bft and cfiA genes, previously regarded as nonexistent, was observed. Further investigations should reveal the factors behind the differential occurrence of the genes between *B. fragilis* and between *B. fragilis* and NFB species.

P891 ACI-1 β-lactamase of *Acidaminococcus intestini* is carried in a highly modular mobile genetic island

M. Rodríguez-Alcayna, G. D'Auria, A. Latorre, R. Cantón, J.C. Galán, F. Baquero (Madrid, Valencia, ES)*

Objective: To characterize the genetic environment of blaACI-1 gene present in the chromosome of human *Acidaminococcus intestini* and the possible events involved in its horizontal gene transfer.
Methods: β-lactam resistant RYC-MR95, RYC-MR02, RYC-MR88, RYC-MR24, RYC-MR37 strains of *A. intestini* were collected from clinical samples at our Hospital. Genome extraction, pyrosequenciation and inverse PCR plus sequenciation were used in the characterization process. Bioinformatics analysis included BLAST, Artemis, database search and neighbour-joining (NJ) for phylogenetic trees.
Results: A 55,797 bp DNA sequence flanked by 67 bp direct repeats (DRs), carrying blaACI-1 gene and presenting two large serine-recombinases was detected in RYC-MR95. This sequence constitutes the core element. Such element was found to be inserted into a greater one (100,778 bp), also flanked by 54 bp DRs, carrying a single recombinase. Southern blot assays using several orfs as probes revealed the presence of 2 copies of the core element in *A. intestini* chromosome. The second copy, of 55.8 Kb, lacking the blaACI-1 gene, had an insertion of 55,131 bp, and the complex was flanked by the same 67 bp DRs than the first core element. The Acidaminococcus sp. D21 complete genome sequence (EMBL), has only one copy of the core element, which was inserted in the same site that the second copy in *A. intestini* RYC-MR95, whereas the specific site of insertion for the first copy was empty in Acidaminococcus sp D21. These results suggest horizontal gene transfer events. To confirm this point several housekeeping and ACI-1 element genes were sequenced in all the strains of the study, belonging to different Acidaminococcus species. The phylogenetic tree obtained by NJ discriminated the branches for different species, while the phylogenetic trees obtained using genes from core element showed a mixture between branches, suggesting horizontal gene transfer. Such element shows modules from different origins, including genes of five different functional groups: recombinases, polymerases and endonucleases, virulence, transcriptional regulation and methylases and phage origin genes.
Conclusions: A mobile sequence of 55.8 Kb was found in two sites in the chromosome of *A. intestini* RYC-MR95. According to known sequence in Acidaminococcus sp. D21 these sites are site-specific suggesting mobilization by site-specific recombinases. Different functional modules from different origins suggest a modular evolution.

P892 Prevalence of primary clarithromycin resistance in *Helicobacter pylori* strains over a 15-year period in Spain

S. Agudo, T. Alarcón, P. Urruzuno, Á. Somodevilla, M. López-Brea (Madrid, ES)*

Introduction: Clarithromycin is one of the most important antibiotics for *H. pylori* treatment and it is the most important factor to predict eradication failure. This study will analyze the progression of clarithromycin resistance and the most common point of mutations over a 15 year period in Spain.
Methods: We studied 64 biopsies from *H. pylori*-positive patients from four different regions from Spain in 1994 and 118 biopsies between June 2008 to January 2009 from Madrid. Clarithromycin resistance was determined by E-test, strains were considered resistant if MIC > 1 mg/L. DNA extraction was carried out with phenol/chloroform/isoamil alcohol (25:24:1) in 1994 and by the NucliSens easyMAG platform with the NucliSens magnetic extraction reagents (bioMérieux). Clarithromycin resistant and sensitive strains were by PCR and DNA sequencing for mutations in the 23S rRNA gene.
Results: In the 2008 period, we found 42 out of 118 (35.6%) strains resistant to clarithromycin by E-test. E-test results were confirmed for the presence of point mutation in 34 (88.1%) of these strains. There were 8 *H. pylori* strains resistant to clarithromycin by E-test but without point of mutation in the 23S rRNA gene. Point mutation A2143G was found in 85.3% of the strains, A2142G was found in 8.8% of the strains and T2182C was found in 5.9% of the strains. Dual mutations were found in 8.8% of the strains. On the other hand in 1994, 10 out of 64 (15.6%) strains were resistant to clarithromycin by E-test and molecular biology. 7 out of 10 (70%) resistant strains were from Madrid patients. 9 strains had the point of mutation in the position A2143G and one in the position A2142G.
Conclusions: Clarithromycin resistance was higher in Madrid than in the rest of Spain in 1994. However, in Madrid there has been the same level of resistance to clarithromycin and the same type of mutation in the last fifteen years.

P893 Mutant ribosomal protein S5 discovered in spectinomycin-resistant *Neisseria gonorrhoeae*

E. Ilina, N. Oparina, M. Malakhova, A. Borovskaya, I. Demina, M. Serebryakova, M. Rogova, V. Govorun (Moscow, RU)*

Objectives: Spectinomycin (SPC) is an antibiotic recommended for gonorrhea treatment. The only mechanism described for the *Neisseria gonorrhoeae* (NG) resistance to SPC are mutations in 16S rRNA which are rare in clinical population. The other probable ways for SPC resistance formation was investigated by *in vitro* selection of two SPC resistant strains from different NG serovars. The possibility of SPC resistance in NG due to isolated mutation T24P in ribosomal protein (RP) S5 was demonstrated.
Methods: Two resistant NG strains were obtained from parent susceptible ones by *in vitro* selection on GC agar supplemented with 1% IsoVitaleX and increasing concentrations of SPC (32 mkg/ml – 256 mkg/ml). Sequence analysis of 16S rRNA as well as RPs S5, S4 and S8 were performed with dedicated primers using the BigDye Terminator kit and the ABI Prism 3100 Genetic Analyser (Applera, USA). The 2D electrophoresis of CHAPS soluble fraction of NG and protein identification were done as described earlier [Demina I. A., 2009].
Results: SPC-resistant mutants were obtained from parent strains belonged to P1A and P1B serovars after a month of selection. No substitutions presumably associated with SPC resistance were found in four copies of 16S rRNA genes analyzed independently. RPs S5, S4 and S8 located nearby the SPC binding site were studied too. The only mutation Thr24Pro was observed in RP S5 for both mutants. In accordance with alignment this position matched to Ser22 of *E. coli* RP S5, whose mutations were previously reported to result in SPC resistance [Itoh, 1973].
Comparative proteomics of mutant strains vs. parent ones demonstrated the multiple changes in the mobility of the same proteins. Several genes encoding such proteins were sequenced, and no shift-explanating substitutions were found. Such effect may be explained by disorders in the protein synthesis by mutant ribosome because of similar influence of RP S5 mutations was also found for *E. coli* [Kirthi, 2006].
Conclusion: For the first we have shown that the SPC resistance of NG could be generated by RPs5 mutation. Basing on proteomic research data we have suggested that SPC-resistant NGs are rare due to disorders in translation related to such phenotype.

P894 Molecular characterization of penicillin-binding-protein-3 in clinical isolates of *Haemophilus influenzae* with reduced susceptibility to cefotaxime, taken from the British Society for Antimicrobial Chemotherapy respiratory resistance surveillance study 20

D. Knight, K. Maher, J. Blackman Northwood, R. Reynolds, I. Morrissey (Fordham, Birmingham, UK)*

Objective: The British Society for Antimicrobial Chemotherapy (BSAC) respiratory resistance surveillance study monitors resistance in community-acquired lower respiratory infections in the United Kingdom and Ireland. The objective of this study was to identify the mechanism of

cefotaxime non-susceptibility in three non-β-lactamase producing strains of *Haemophilus influenzae* collected from 2004/05–2007/08.
Methods: MIC determination was achieved by using the BSAC agar dilution susceptibility testing method. To determine the mechanism of cefotaxime non-susceptibility, penicillin-binding-protein 3 (PBP3) sequencing was performed as previously described (Straker et al, J.Antimicrob. Chemotherapy 51:523. 2003).
Results: Minimum inhibitory concentration (MIC) was determined for 3740 clinical isolates of *H. influenzae* collected as part of the BSAC Respiratory Resistance Surveillance Study 2004/05–2007/08. Of 3740 clinical *H. influenzae* isolates, 3205 (86%) were β-lactamase negative. Of these, 3115 (97%) had cefotaxime MICs of ⩽0.06 mg/L, 66 (2.1%) had MICs of 0.12 mg/L and 19 (0.6%) were resistant to cefotaxime with MICs of 0.25 mg/L. We identified two isolates (HI040610 and HI062032) with a cefotaxime MIC of 0.5 mg/L and one (HI072003) with MIC of 1 mg/L. The table shows the amino acid substitutions found in the PBP3 of these three isolates, with two highly susceptible isolates and two reference strains for comparison.
Conclusion: Reduced susceptibility to cefotaxime, requiring MICs >0.25 mg/L is rare in non-β-lactamase producing strains of *H. influenzae*. We have shown that the presence of amino acid substitutions within the region adjacent to the PBP3 active site appear to cause raised cefotaxime MIC. A substitution at amino acid 350 may cause a moderate increase in cefotaxime MIC (0.12 mg/L), but a combination of one or more mutations between amino acid positions 490 and 517 confer isolates with the highest cefotaxime MIC (⩾0.5 mg/L).

Isolate	Cefotaxime MIC	PBP3 Amino Acid positions					
	(mg/L)	350	490	502	511	517	526
HI072031	0.008	D	G	A	V	R	N
H1072049	0.008	D	G	A	V	R	N
ATCC 49247	0.015	D	G	A	V	R	**K**
NCTC 11931	0.12	**N**	G	A	V	R	N
HI040610	0.5	D	**E**	**V**	V	R	**K**
HI062032	0.5	D	G	A	V	**H**	N
HI072003	1	D	G	A	**A**	**H**	N

P895 High-level trimethoprim-resistant *Haemophilus influenzae* is due to multiple changes in the dfr gene and its promoter plus the possession of plasmidic dfrA17 gene

B. Aracil, S. García-Cobos, M. Pérez-Vázquez, F. Román, J. Oteo, V. Bautista, J. Campos (Madrid, ES)*

Objective: *Haemophilus influenzae* (Hi) is an important human pathogen. Cotrimoxazol has been used for the treatment of respiratory and urinary infections caused by this and other pathogens and is still being used in many countries; however, although trimethoprim (T) resistance is broadly distributed in Hi, only limited data are available on the mechanisms responsible of T resistance. Therefore, the aim of study was to describe the modifications found in the constitutive dihydrofolate reductase gene (dfr) and to study plasmidic dihydrofolate reductase genes (dfrA) associated with highly T resistant Hi.
Methods: We studied 58 clinical Hi strains (21 T susceptible and 37 T highly resistant, 42 of them were capsulated of types b, e and f, and 16 were non-capsulated. A DNA fragment of 535 bp containing the dfr gene and its promoter region, was amplified by PCR and sequenced. An *in vitro* mutation model was also developed. Single nucleotide polymorphisms (SNPs) of dfr and its promoter were analysed in the same bacterial collection and *in vitro* T resistant mutants. In addition, the plasmidic genes: dfrA7, dfrA9, dfrA12, dfrA13, dfrA14, dfrA17 were identically amplified and sequenced.
Results: No aminoacid changes were detected in the dfr protein of 12 T susceptible strains (7 clinical isolates and 5 susceptible controls); 9 additional Ts isolates had 1 to 4 changes. T resistant Hi strains had an average of 6 amino acid substitutions (range 1–13). The two most frequent substitutions in T resistant strains were Lys107 to Gln (56.7%), and Met21 Ile (48.6%). In addition, all T resistant strains had changes in the dfr promoter region while none of the T susceptible strains did. SNPs analysis showed that T resistant strains were highly polymorphic (average of SNPs patterns 12, range 0–24) in comparison with T susceptible ones (average of SNPs patterns 1, range 0–3). T resistant isogenic mutants reproduced in part the aminoacid changes found in the wild T resistant strains. Besides we found the dfrA17 plasmidic gen in all T resistant and none T susceptible strains. Another plasmidic dfr genes studied, were not found.
Conclusions: (1) Highly T resistant in Hi is due to multiple aminoacid substitutions in the dfr enzyme plus the possession of dfrA17 gene. (2) Resistant strains also show nucleotide changes in the promoter region of dfr.(3) Dfr gene is genetically much more diverse in resistant strains.

P896 Expression of *Moraxella catarrhalis* outer membrane porin M35 – regulation by exposure to aminopenicillins and cold shock

M. Jetter, R. Troller, C. Aebi (Berne, CH)*

Objectives: The outer membrane protein M35 of *Moraxella catarrhalis* is an antigenically highly conserved porin and involved in the uptake of essential nutrients, but also in the susceptibility to aminopenicillins. M35 knock-out mutants show a significantly higher MIC (minimal inhibition concentration) for aminopenicillins than their respective wild-types. The aim of this study is to determine the biological mechanism of this potentially new antimicrobial resistance mechanism of *M. catarrhalis* and the possibility of M35 as a cold shock regulated protein.
Methods: PCR using m35 specific primers was used to detect the m35 gene in clinical isolates. The m35 mRNA expression of the strains 300, O35E and 415 after exposure to amoxicillin or cold shock treatment was measured by real time PCR and normalized to their 16S mRNA expression. The expression of the M35 protein after growth with amoxicillin respectively at 26ºC vs. 37ºC was analyzed by SDS-PAGE and Western Blot.
Results: The screening of 52 clinical middle ear isolates resulted in positive PCR products for all tested isolates. The analysis of the m35 mRNA expression after amoxicillin treatment showed an up to 80% down regulation compared to the amoxicillin-free control in all three strains tested. To demonstrate that this down regulation is not only a transcriptional phenomenon, OMP of all three strains were isolated and protein expression was analyzed by Western Blot. In-deed, all three strains showed a lower M35 expression after growth with amoxicillin than without amoxicillin. The cold shock analysis resulted in the same effect as amoxicillin treatment. All three strains grown at 26ºC showed an up to 80% down regulation of m35 mRNA expression compared to 37ºC and protein expression of M35 after cold shock treatment was reduced as well.
Conclusion: The altered expression of M35 after aminopenicillin exposure indicates that this is a new resistance mechanism against aminopenicillins in *M. catarrhalis*. In addition to the production of β-lactamases, down regulation of porin expression is a well known resistance mechanism in other bacteria. The differences in the expression after cold shock treatment demonstrates that M35 is an additional cold shock-regulated protein of *Moraxella catarrhalis* whose biological function needs further investigation.

P897 Frequency and molecular characterization of macrolide and fluoroquinolone resistance in *Helicobacter pylori* isolates identified at a university hospital, Karachi, Pakistan

S. Rajper, E. Khan, Z. Ahmad, Z. Alam, R. Hasan (Karachi, PK)*

Background: *Helicobacter pylori* (*H. pylori*) is the major infectious cause for gastritis, peptic ulcer disease, gastric MALTomas and gastric adenocarcinoma. Prevalence of *H. pylori* in Pakistan is upto 80% in adult population alone and antibiotic susceptibility data for *H. pylori* in Pakistan is virually non-existant. Treatment is based empirically following international guidelines. In this study we determined the

susceptibility of *H. pylori* against two frequently used antibiotics i.e clarithromycin and fluoroquinolones in biopsy samples by Reverse Hybridization. To the best of our knowledge this is the first study reporting resistance pattern and molecular characterization of *H. pylori* isolates from Pakistan.

Objectives: To determine the frequency and molecular characterization of clarithromycin and fluoroquinolones resistance in *H. pylori* isolates at Aga Khan University Hospital, Karachi.

Materials and Methods: gastric antral biopsies(n = 115) positive for *H. pylori* on histological findings were included in this study. DNA extraction was done from paraffin embedded tissue biopsy samples followed by amplification and hybridization. Resistance to antibiotics was determined by detecting mutations in the known genomic sites. Fluoroquinolone resistance was determined by detecting mutations in gyrA while clarithromycin resistance was detected by 23S rRNA mutation on hybridization strip.

Results: Of the 115 isolates processed, fluoroquinolone resistance was noted in 49.13%(57/115) with GyrA 87 mutation being the most common, where as clarithromycin resistance was noted in 34.78% (40/115) with A2147G being the commonest mutation observed. Overall 25.21% (29/115) showed mutations suggestive of resistance to both drugs. Heteroresistance was also observed in 36.52%(42/115) isolates.

Conclusion: Resistance to fluoroquinolone and clarithromycin is common in *H. pylori* isolates yielded at Aga Khan University. This alerts towards the risk of treatment failure in patients who are treated empirically. Susceptibility tests are needed to improve patient care by providing targeted therapy especially in Pakistan where burden of disease is high and no data is available to guide clinicians to choose appropriate therapy.

P898 Mutations of gyrA and parC in *Neisseria gonorrhoeae* clinical isolates in Hong Kong

L.K. Chan, M. Hui, Z.K. Liu, L.Y. Lam, N.M. Luk, A.K. Chan, K.K. Lo, C.Y. Chan (Hong Kong, CN)*

Objective: The antimicrobial susceptibility of *Neisseria gonorrhoeae* against fluoroquniolones has shown regional differentiation with regard to the mutations in the gyrA and parC subunits. With different mutation sites in gyrA and parC reported worldwide, we studied the phenotypic and genotypic resistance to fluoroquinolones in *Neisseria gonorrhoeae* in Hong Kong and compared that with other regions.

Methods: 86 clinical isolates of *Neisseria gonorrhoeae* had been collected from the Yaumatei Social Hygiene Clinic in Hong Kong. Detection of mutations in the gyrA and parC genes was performed by sequence analysis. MIC values were determined by agar dilution method according to the NCCLS.

Results: None of the strains were susceptible to fluoroquiniolones. 95% of the clinical isolates demonstrated resistance to ciprofloxacin and had MIC $\geqslant$1 mg/L. Four of the isolates fell into the intermediate range with MIC of 0.12–0.25 mg/L.

The result of sequence analysis showed that 98.8% of the isolates had mutations due to amino acid changes both at position 91 and 95 in the gyrA gene. All isolates demonstrated a change of Ser-91 $\rightarrow$ Phe, 23 isolates (26.7%) showed a change of Asp-95 $\rightarrow$ Gly, 56 isolates (65.1%) showed a change of Asp-95 $\rightarrow$ Ala and 6 isolates (7%) showed a change of Asp-95 $\rightarrow$ Asn. Only one isolate showed mutations at Ser-91 $\rightarrow$ Phe and Ala-92 $\rightarrow$ Pro with no mutation at position 95. Mutation in parC gene mainly appeared at amino acid positions 86 to 91: 6 isolates (7%) had mutation at Asp-86, 58 isolates (67.4%) had mutation at Ser-87 and 10 isolates (11.6%) had mutation at Glu-91. The 84 strains were categorized into 22 types as shown in the table. Our results concurred with most of the findings reported in other regions worldwide and in addition we had found 3 new mutation sites of Gly-35 $\rightarrow$ Asp, Asp-86 $\rightarrow$ Gly and Glu-91 $\rightarrow$ Ala in the parC gene. One silent mutation in gyrA and four silent mutations in parC were found which gave no amino acid change.

Conclusion: The multiple mutation sites found in the Quinolone-resistant determining regions (QRDR) suggest that they are important in the development of quinolone resistance. Most of the mutation profiles were consistent with previously reported categories. Accumulation of mutation in gyrA and parC is now of common occurrence.

Table. Amino acid substitutions in *gyrA* and *parC* genes in relation to ciprofloxacin MICs

Mutations								No. of strains	MIC (mg/L)
gyrA			*parC*						
Ser-91	Ala-92	Asp-95	Gly-35	Asp-86	Ser-87	Ser-88	Glu-91		
Phe	Pro							1	1
Phe		Gly						1	2
Phe		Gly		Asn				4	4–16
Phe		Gly		Gly				1	4
Phe		Gly			Arg			11	1–32*
Phe		Gly					Ala	1	2
Phe		Gly					Gly	2	4–16
Phe		Gly	Asp		Arg			1	32
Phe		Gly			Arg	Pro		1	32
Phe		Gly			Asn		Gln	1	4
Phe		Ala						10	0.25–4**
Phe		Ala					Gly	1	4
Phe		Ala			Arg			26	1–32
Phe		Ala			Asn			12	0.12–16***
Phe		Ala			Cys			1	4
Phe		Ala			Ile			1	32
Phe		Ala				Pro		1	4
Phe		Ala					Gly	2	4–16
Phe		Ala					Ala	1	4
Phe		Ala			Arg	Pro		1	8
Phe		Asn			Ile			3	32
Phe		Asn					Gly	3	1

*Only one isolate exhibited a MIC of 1 mg/L; all others had MIC $\geqslant$4 mg/L.
**Only two isolates exhibited MICs of 0.25 mg/L; all others had MIC $\geqslant$1 mg/L.
***One isolate exhibited MIC of 0.12 mg/L and one exhibited MIC of 0.25 mg/L; all the others had MICs $\geqslant$2 mg/L.

New antimicrobials: oxazolidinones, fluoroketolides and pleuromutilins

P899 PNU-100480, PNU-101244 and PNU-101603: preliminary broth microdilution quality control ranges versus bacterial reference strains and *in vitro* antibacterial effect studies

S. McCurdy, M. Huband (Groton, US)*

Objective: PNU-100480 is an orally active oxazolidinone being developed for the treatment of susceptible, multi-drug and extensively drug resistant strains of *Mycobacterium tuberculosis*. PNU-101603 and PNU-101244 are primary metabolites of PNU-100480. This study was undertaken to determine preliminary broth microdilution quality control ranges for these compounds against Clinical and Laboratory Standards Institute (CLSI) bacterial reference strains. In addition, the effects of various experimental parameters on MICs were determined including: inoculum size, pH, cation concentration, temperature, incubation atmosphere, and human serum (*in vitro* effects).

Methods: PNU-100480, PNU-101244 and PNU-101603 broth microdilution plates were prepared and tested according to CLSI guidelines and included linezolid as a control. Two lots of cation-adjusted Mueller-Hinton broth [CAMHB] (BD and Remel) and two lots of CAMHB +3% lysed horse blood (Remel) were included. On separate days, each reference strain and lot of media was tested. At the study completion, the MIC for each organism/compound and lot of medium was determined and preliminary QC ranges proposed and *in vitro* effects determined.

CLSI reference strain	QC range MIC, μg/mL	Preliminary distribution of MICs
PNU-100480		
Staphylococcus aureus ATCC 29213	2–4	2 (18)[a]; 4 (10)
Enterococcus faecalis ATCC 29212	2	2 (28)
Streptococcus pneumoniae ATCC 49619	1	1 (14)
PNU-101244		
Staphylococcus aureus ATCC 29213	2–4	2 (26); 4 (2)
Enterococcus faecalis ATCC 29212	1–2	1 (23); 2 (5)
Streptococcus pneumoniae ATCC 49619	0.5	0.5 (14)
PNU-101603		
Staphylococcus aureus ATCC 29213	4	4 (28)
Enterococcus faecalis ATCC 29212	2–4	2 (27)
Streptococcus pneumoniae ATCC 49619	0.5–1	0.5 (11); 1 (3)

[a]Number of tests with this MIC.

Conclusions: Preliminary QC ranges for PNU-100480, PNU-101244 and PNU-101603 were determined and *in vitro* effects were examined. No significant lot to lot variability was observed for compound MICs in CAMHB or CAMHB +3% LHB and no *in vitro* effects were found.

P900 *In vitro* and *in vivo* activity of PF-03315011: a new oxazolidinone antibacterial versus recent bacterial clinical isolates

M. Huband, L.M. Mullins, P.J. Pagano, J.B. Penzien, S. McCurdy, M.M. Lemmon, G.W. Gibson, K. Leach, L. Qiu, J. Vara Prasad (Groton, Ann Arbor, US)*

Objective: Antibacterial resistance in Gram-positive bacterial species continues to develop in both nosocomial and community settings (multi-drug resistant *Streptococcus pneumoniae*, MRSA {USA 100, USA 300, cfr}, vancomycin-intermediate *Staphylococcus aureus*). PF-03315011 is a new oxazolidinone being developed to treat infections caused by susceptible and multi-drug resistant Gram-positive bacteria. This study investigated the *in vitro* antibacterial activity of PF-03315011 and comparator compounds by MIC, MBC, time-kill, and *in vitro* toxicity assays. *In vivo* testing (PD50 determinations) was performed in CD1 female mice using protocols approved by the Pfizer Animal Care and Use Committee in compliance with National Institute of Health guidelines for proper care and use of laboratory animals.
Methods: MICs, MBCs, and time-kill assays followed Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: In a *Staphylococcus aureus* (MRSA) acute systemic murine infection model, PD50s (oral dosing, QD) for PF-03315011 and linezolid were 0.52 and 1.52 mg/kg, respectively; similarly, in a pneumococcal pneumonia mouse model, (oral dosing, BID), PF-03315011 and linezolid PD50s were 2.0 and 2.6 mg/kg, respectively.
Conclusions: This study confirms the *in vitro* antibacterial activity and *in vivo* potency of PF-03315011 against clinically significant Gram-positive bacteria.

Organism (n)	MIC, μg/mL		
	PF-03315011	Linezolid	Levofloxacin
Staphylococcus aureus oxacillin-S (20)	4	4	0.25
Staphylococcus aureus MRSA (118)[a]	4	4	32
Staphylococcus epidermidis (36)[b]	2	2	64
Coagulase negative staphylococci (54)[c]	4	4	32
Streptococcus pyogenes (28)	4	2	2
Streptococcus agalactiae (21)	4	2	1
Streptococcus pneumoniae (116)[d]	2	2	16

[a]Includes 47 MRSA, 33 community acquired MRSA, 34 levofloxacin-R MRSA, and 4 VISA.
[b]Includes 13 oxacillin-resistant *S. epidermidis* and 23 oxacillin-susceptible *S. epidermidis* strains.
[c]Includes 18 *Staphylococcus haemolyticus*, 13 *S. lugdunensis*, 10 *S. saprophyticus*, and 13 *S. warneri*.
[d]Includes penicillin-susceptible, -intermediate, -resistant, and levofloxacin-R *Streptococcus pneumoniae* strains.

P901 CEM-101, a novel fluoroketolide, tested against European clinical isolates from 2009 (first-year surveillance results)

R. Jones, D. Farrell, H. Sader, M. Stilwell, M. Castanheira (North Liberty, US)*

Objectives: To determine the potency and spectrum of CEM-101, a new fluoroketolide, tested against contemporary (2009) European (EU) pathogens. Preliminary results suggest that CEM-101 has expanded activity against multidrug-resistant (MDR) pathogens associated with community-acquired bacterial pneumonia (CABP), and skin and skin structure infections (SSSI) when compared to macrolides (erythromycin [ER], azithromycin [AZ], clarithromycin [CL]), clindamycin (CC) and telithromycin (TE).
Methods: EU CEM-101 surveillance study collected 3,531 strains, as following: *S. aureus* (SA; 1,398), coagulase-negative staphylococci (CoNS; 454), enterococci (ENT; 613), *S. pneumoniae* (SPN; 485), viridans group (VGS; 98) and β-haemolytic (BHS; 212) streptococci, *H. influenzae* (HI; 242) and *M. catarrhalis* (MCAT; 29). These consecutive strains were susceptibility (S) tested by CLSI methods and results were interpreted by EUCAST breakpoints; TE interpretive criteria were applied to CEM-101 for comparison purposes only. Thirteen countries and nearly 30 medical centers were sampled.
Results: CEM-101 was very active against SPN (MIC90, ⩽0.06 mg/L), VGS and BHS (MIC90, ⩽0.03 mg/L) with 100.0 and 98.1–100.0% of isolates inhibited at ⩽1 and ⩽0.25 mg/L, respectively. This potency was ⩾ two fold greater than TE, and CEM-101 inhibited at 3.7% more SPN at ⩽0.25 mg/L. The tested SPN was only 72.0, 74.2 and 82.1% S to penicillin (PEN), ER and CC, respectively. Against HI and MCAT, CEM-101 was quite active (MIC90/% inhibited at ⩽4 mg/L): 2/99.6 and 0.06/100.0, respectively (two-fold more active than TE). This activity against Gram-negative CABP pathogens was most like AZ. SA and CoNS (MIC50, 0.06 mg/L for both) were generally S to CEM-101 (92.1 and 71.2% S versus 90.5 and 70.5% S for TE). ENT was only moderately S to CEM-101 (MIC50/90, 1/2 mg/L), but was two-fold more potent than TE. *E. faecalis* (EF) isolates were usually more S (MIC50 at 0.25 mg/L) than other ENT. The EU collection sampled had 22.7% MRSA, 82.8% MRCoNS, 1.7% vancomycin-resistant (VR) EF, 36.8% VR *E. faecium*, 41% PEN-R VGS, 4.8% TE-R *S. pyogenes* and 16.9% ampicillin-R HI.
Conclusions: CEM-101 clearly exhibited greater potency than currently available MLSB agents (including TE, another ketolide) against potentially indicated pathogens causing CABP or SSSI. Expanded clinical investigations of CEM-101 appear warranted for oral and parenteral route coverage of emerging MDR strains.

Organism (no.)	% occurrences at CEM-101 MIC (mg/L):								
	⩽0.03	0.06	0.12	0.25	0.5	1	2	4	>4
S. aureus (1,398)	16.0	71.7	**3.5**	0.4	0.3	0.3	<0.1	<0.1	7.7
CoNS (454)	31.7	28.0	9.9	0.9	0.7	–	–	0.2	**28.6**
Enterococci (613)	32.6	2.3	1.8	1.6	8.7	20.1	**28.2**	4.6	0.2
S. pneumoniae (485)	**92.8**	2.5	1.4	2.7	0.4	0.2	–	–	–
VGS (98)	**93.9**	6.1	–	–	–	–	–	–	–
BHS (212)	**90.6**	3.8	2.4	1.4	1.9	–	–	–	–
H. influenzae (242)	–	–	0.4	1.2	23.6	61.6	**12.4**	0.4	0.4
M. catarrhalis (29)	20.7	**72.4**	6.9	–	–	–	–	–	–

[a] – = no occurrences, 0.0%; boldface value at MIC_{90}.

P902 Multiple dose pharmacokinetics and safety of CEM-101, a new fluoroketolide, in healthy subjects

J. Still, K. Clark, T. Degenhardt, D. Scott, P. Fernandes, S. Sharples (Chapel Hill, Tempe, US)*

Objectives: CEM-101 is a potent new fluoroketolide under development for treatment of bacterial respiratory tract and other infections. CEM-101 pharmacokinetics (PK) and safety at single doses to 1600 mg have been reported previously. Safety and PK following escalating multiple oral doses were investigated in this study.
Methods: This was a randomized, double-blind, placebo-controlled, dose escalation study. Escalating doses (200, 400, and 600 mg) were administered once daily for 7 days to healthy adult subjects (5:2, active:placebo; 200 mg [n = 7], 400 mg [n = 14] and 600 mg [n = 14]). Physical examinations, vital signs, ECGs, clinical laboratory tests, and adverse events were monitored throughout the study. Blood samples for CEM-101 concentrations and PK assessments were collected pre-dose and frequently to 24 h post-dose on Day 1 and to 72 h post-dose on Day 7.
Results: CEM-101 mean Cmax values on Days 1 and 7 were 0.113 and 0.248 mcg/mL for the 200 mg group, 0.579 and 1.09 mcg/mL for the 400 mg group, and 0.862 and 1.50 mcg/mL for the 600 mg group. Corresponding AUC(0–24) values were 0.888 and 2.31 mcg·h/mL, 4.85 and 13.30 mcg·h/mL, and 7.64 and 18.40 mcg·h/mL on Days 1 and 7. Increases in Cmax and AUC(0–24) were more than dose proportional from 200 to 400 mg and then approximately dose proportional from 400 to 600 mg. At all doses CEM-101 exposures were higher on Day 7 than

Day 1, indicating that accumulation occurred over the dosing period. Across the dose range the mean Tmax increased from 3.0 to 4.0 hours on Day 1 and from 3.5 to 4.0 hours on Day 7 and the mean T1/2 increased from 3.64 to 5.06 hours on Day 1 and from 5.39 to 7.64 hours on Day 7. All doses of CEM-101 were safe and generally well tolerated. Gastrointestinal AEs, mostly mild, occurred in each dose group. ALT or AST increases occurred in 4 of 10 subjects that received 600 mg CEM-101; all were low-level, transient, reversible, and not associated with signs or symptoms of toxicity.

Conclusion: Over the 200 to 600 mg dose range, multiple doses of CEM-101 were safe and generally well tolerated in healthy male and female subjects. Cmax and AUC0-inf increases were more than dose proportional across the dose range and moderate accumulation of CEM-101 was noted after 7 days of administration.

P903 CEM-101, a novel ketolide; *in vitro* activity against *Legionella pneumophila*

J. Dubois, P. Fernandes (Sherbrooke, CA; Chapel Hill, US)*

Objective: CEM-101 is a new fluoroketolide that has potent activity against respiratory tract pathogens. The activity against a variety of *Legionella pneumophila* serogroup was investigated.

Methods: The *in vitro* activity of CEM-101 was compared with that of telithromycin, azithromycin, erythromycin, levofloxacin and doxycycline against a total of 300 *Legionella pneumophila* by a standard agar dilution procedure using buffered yeast extract agar. The species tested included *L. pneumophila* serogroup 1 (125 isolates), serogroup 2 (28), 3 (25), 4 (36), 5 (25), 6 (50) and serogroup 7, 8, 9, 12 (11).

Results: CEM-101 (MIC90 0.016 mg/L) was as active as levofloxacin (MIC90 0.016 mg/L) against *L. pneumophila* and was more active than telithromycin (MIC90 0.06 mg/L), azithromycin (MIC90 0.25 mg/L), erythromycin (MIC90 1 mg/L) and doxycycline (MIC90 1 mg/L). Against the most frequent *L. pneumophila* such as serogroup 1, the MIC90 of CEM-101 (0.03 mg/L) was superior to telithromycin (0.06 mg/L), azithromycin (0.5 mg/L), erythromycin (1 mg/L) and doxycycline (1 mg/L). Against *L. pneumophila* serogroup 1, the MIC90 of CEM-101 (0.03 mg/L) was similar to levofloxacin (0.016 mg/L). CEM-101 was less active against *L. pneumophila* serogroup 1, 2, 3, 4, 5, and 6 strains (MIC90 0.016 mg/L) than *L. pneumophila* serogroup 7, 8, 9 and 12 (MIC90 0.008 mg/L).

Conclusions: These data confirm the interesting activity of this new fluoroketolide CEM-101 against *Legionella pneumophila*.

P904 CEM-101, a novel ketolide: *in vitro* activity against resistant strains of *Streptococcus pneumoniae* and *Haemophilus influenzae*

J. Dubois, P. Fernandes (Sherbrooke, CA; Chapel Hill, US)*

Objective: CEM-101 is a promising fluoroketolide that has potent activity against respiratory tract pathogens resistant to other macrolide agents. Its activity against a variety of resistant strains of *Streptococcus pneumoniae* and *Haemophilus influenzae* was investigated.

Methods: The *in vitro* activity of CEM-101 was compared with that of telithromycin, azithromycin, erythromycin, levofloxacin and doxycycline against a total of 199 resistant *S. pneumoniae* and 191 resistant *H. influenzae* by agar dilution procedures (CLSI, M7-A7, M100-S18). The tested strains included *S. pneumoniae* erythromycin-resistant (erm B genotype; 107 isolates and mefE genotype; 54) and ciprofloxacin-resistant (gyrA and parC genotype; 38) and also *H. influenzae* ery-resistant (ermA,B,C genotype; 138) and cipro-resistant (gyrA and parC genotype; 53).

Results: Against *S. pneumoniae* ery-resistant strains (ermB genotype), the activity of CEM-101 (MIC90 1 mg/L) and levofloxacin (MIC90 2 mg/L) was superior to the macrolides tested: telithromycin (MIC90 4 mg/L), azithromycin (MIC90 ⩾64 mg/L), erythromycin (MIC90 ⩾64 mg/L)) and doxycycline (MIC90 32 mg/L). Against *S. pneumoniae* ery-resistant (mefE genotype) group, CEM-101 (MIC90 0.25 mg/L) was the most active agent followed by levofloxacin (MIC90 2 mg/L), telithromycin (MIC90 8 mg/L), doxycycline (MIC90 16 mg/L), azithromycin (MIC90 ⩾64 mg/L) and erythromycin (MIC90 ⩾64 mg/L). Against *S. pneumoniae* cipro-resistant (gyrA and parC genotype) group, CEM-101 (MIC90 0.25 mg/L) was also the most active agent tested followed by telithromycin (MIC90 1 mg/L), levofloxacin (MIC90 2 mg/L), doxycycline (MIC90 16 mg/L), azithromycin (MIC90 ⩾64 mg/L) and erythromycin (MIC90 ⩾64 mg/L). Against *H. influenzae* ery-resistant (ermA,B,C genotype) strains, CEM-101 (MIC90 4 mg/L) was the most active macrolide tested followed by telithromycin (MIC90 16 mg/L), azithromycin (MIC90 16 mg/L) and erythromycin (MIC90 ⩾64 mg/L). Against *H. influenzae* cipro-resistant (gyrA and parC genotype) group, CEM-101 (MIC90 2 mg/L) was slightly more active than telithromycin (MIC90 4 mg/L) and levofloxacin (MIC90 4 mg/L).

Conclusions: These data confirm the interesting activity of the new fluoroketolide CEM-101 against resistant *Streptococcus pneumoniae* and *Haemophilus influenzae*.

P905 Expanded studies of CEM-101, a novel fluoroketolide, tested against invasive isolates of *N. meningitidis,* including fluoroquinolone-non-susceptible resistant strains

R. Jones, D. Biedenbach, L. Woosley, G. Gerken, M. Castanheira (North Liberty, Iowa City, US)*

Objectives: To evaluate the potential role/potency of CEM-101 tested against *N. meningitidis* (NM) as a decolonizing regimen. More than 100 invasive clinical isolates were screened, including three cases of ciprofloxacin-non-susceptible NM (C-NSNM) provided by the CDC (Drs. H. Wu and M. Warton) occurring in North Dakota and California (2007–2008).

Methods: 62 isolates were previously studied and that collection was expanded to 103, including the C-NSNM (ciprofloxacin MICs, 0.06–0.25 mg/L). The strains (>90% blood cultures) were collected in 1997–2009 from 43 medical centers in North (58 strains) and South (13) America, Europe (31) and Asia-Pacific (1). Strains were tested by CLSI broth microdilution methods for susceptibility (S) to CEM-101 and 11 comparators, including β-lactams, fluoroquinolones (FQs), macrolides and three other classes. Serological identification was performed for serogroups (SGs) B, C, Y and W135. NM displaying elevated ciprofloxacin MIC values (⩾0.06 mg/L) were evaluated for mutations on gyrA or B and parC or E, and in the efflux pump gene mtrR.

Antimicrobial agent	MIC (mg/L)			%S[a]	%R[a]
	50%	90%	Range		
CEM-101	⩽0.015	⩽0.015	⩽0.015–0.06	–[b]	–
Telithromycin	⩽0.015	0.03	⩽0.015–0.12	–	–
Azithromycin	0.06	0.12	⩽0.015–0.25	100.0	–
Clarithromycin	0.03	0.12	⩽0.015–0.25	–	–
Erythromycin	0.12	0.25	0.03–0.5	–	–
Penicillin	0.03	0.12	⩽0.015–0.25	84.5	0.0
Ceftriaxone	⩽0.015	⩽0.015	⩽0.015	100.0	–
Ciprofloxacin	⩽0.008	⩽0.008	⩽0.008–0.25	97.1	1.9
Levofloxacin	⩽0.008	⩽0.008	⩽0.008–0.25	97.1	1.9
Minocycline	0.12	0.25	⩽0.008–0.25	100.0	–
Rifampin	0.015	0.03	⩽0.008–0.12	100.0	0.0
Trimethoprim/sulfamethoxazole	0.12	2	⩽0.06–4	50.5	47.6

[a] Susceptibility criteria based on CLSI M100-S19 (2009).

[b] – No susceptibility criteria have been proposed, but azithromycin at ⩽2 mg/L has been considered susceptible (active); CLSI M100-S19.

Results: Penicillin-S was 84.5% with no resistant (R) strains detected. All isolates were S to ceftriaxone, azithromycin, minocycline and rifampin. 97.1% of NM were S to ciprofloxacin and levofloxacin (⩽0.015 mg/L); however, about one-quarter of the strains had reduced S to nalidixic acid (MIC, ⩾8 mg/L), which can correlate with diminished S to FQs, and 3 isolates (CDC strains) were frankly non-S. R to trimethoprim/sulfamethoxazole (T/S) was 47.6%. Of MLSB agents, CEM-101 was the most active (MIC90, ⩽0.015 mg/L) compared to

telithromycin (0.03 mg/L), azithromycin and clarithromycin (0.12 mg/L) and erythromycin (0.25 mg/L). The prevalence rates (%) of SGs were: B (41.7), C (37.9), Y (12.6) and W135 (2.9); only 5 were not typeable. Three strains with ciprofloxacin MIC values at $\geqslant$0.06 mg/L harbored gyrA mutations that generated the amino acid substitution T91l. These strains carried no alterations on the remaining tested genes (gyrB, parC, parE and mtrR).
Conclusions: CEM-101 was the most active MLSB agent tested against NM strains (all MICs $\leqslant$0.06 mg/L), with a potency two-to $\geqslant$16 fold greater than any other in class drug. CEM-101 was active against NM isolates non-S to β-lactams, T/S and especially FQs. Further studies should determine if CEM-101 can eradicate NM from the nasopharynx of at-risk patients in cases where R to other potential decolonizing agents has emerged.

P906 Safety, tolerance and pharmacokinetics of single and repeat doses of BC-3781, a novel antimicrobial

W.T. Prince, W.W. Wicha, F. Obermayr, V. Moschetti, R. Novak (Vienna, AT; Dusseldorf, DE)*

Background: BC-3781 is a new pleuromutilin which is in clinical development for the treatment of skin and skin structure infections and pneumonia. BC-3781 shows excellent antimicrobial activity against relevant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Reported here are the results of the first two studies investigating the safety, tolerance and pharmacokinetics (PK) of BC-3781.
Methods: In a double-blind, placebo randomized, single intravenous (i.v.) dose escalation study BC-3781 was given to 2 cohorts of 6 healthy subjects each. Each subject in Cohort 1 received 25, 50 and 100 mg BC-3781 and placebo and in cohort 2, 200, 300 and 400 mg BC-3781 and placebo. In a second double-blind, placebo-controlled, randomized study 2 cohorts of 12 subjects were given single and repeat doses of BC-3781 or placebo. Cohort 1 received 75 mg BC-3781 or placebo: Day 1 single dose, days 3–14 twice daily (q12) and day 15 single dose. Cohort 2 received 150 mg BC-3781 or placebo. In both studies vital signs, laboratory safety parameters, adverse events and ECG were recorded and samples taken for PK.
Results: No adverse events of clinical concern were reported. Also, there were no clinically significant changes in vital signs or safety laboratory parameters in any subject at any session. At expected therapeutic doses BC-3781 was well tolerated but at the highest single doses tested (300 and 400 mg) some subjects showed signs of local intolerance. The plasma concentration of BC-3781 after i.v. administration shows a multi-phasic decline. After single doses the terminal half life is 8.6–12.0 h and AUC increases linearly with dose supporting q12 dosing. After repeat dosing of BC-3781 to steady state the AUC increased and no significant increase of Cmax was seen. The large volume of distribution indicates BC-3781 is well distributed.
Conclusions: BC-3781 was safe and well tolerated up to the maximum doses tested, 400 mg single dose and 150 mg q12. The PK of BC-3781 show that it behaves in a predictable manner in humans and the plasma levels indicate it has therapeutic potential for the treatment of Gram positive infections. BC-3781 is being progressed into phase 2 studies.

P907 *In vivo* pharmacodynamic activity of BC-3781

W.A. Craig, D. Andes, Z. Ivezic-Schoenfeld, W.W. Wicha, R. Novak (Madison, US; Vienna, AT)*

Background: BC-3781 is an antimicrobial agent of the pleuromutilin class inhibiting the prokaryotic protein synthesis. BC-3781 is in clinical development for intravenous and oral treatment of skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). We examined the pharmacodynamics of BC-3781 against clinical isolates of *Staphylococcus aureus* (including methicillin-resistant *S. aureus*) and *Streptococcus pneumoniae* (including penicillin-resistant *S. pneumoniae*) in a neutropenic murine thigh and lung infection model.
Methods: Animals were treated either with single doses of 5 to 160 mg/kg or alternatively fractionated doses of 2, 4 or 8 applications per day were given to determine the PK/PD index most relevant for efficacy. To investigate *in vivo* killing and post-antibiotic effects, single doses of 10, 20 and 40 mg/kg were administered subcutaneously. At start of therapy CFU/thigh or lung of either SA or SP in infected mice varied from 10^5 to 10^7. The 12 hour dose required for a net bacteriostatic effect was determined using the Emax dose-response model. Plasma protein binding was determined using equilibrium dialysis.
Results: BC-3781 exhibited time-dependent killing with moderate post-antibiotic effects (PAE) of about 3.5 hours for *S. pneumoniae* and around 1.5 hours for *S. aureus*. The 24 h AUC/MIC and Time>MIC were identified as the PK/PD indices most important for efficacy. There was only a slightly enhanced activity of the drug in the presence of white blood cells. The drug appeared to have 3- to 6-fold higher potency in the lung than the thigh. The *in vitro* plasma protein binding in mice was 80%.
Conclusions: The bacteriostatic free 24 h AUC/MIC ratio required for a selection of clinical isolates of *S. aureus* and *S. pneumoniae* strains ranged from 8.04 to 16.50. This range provides the basis for the 24 h fAUC/MIC target ratio required for efficacy in SSSI and CAP patients.

P908 Pharmacokinetic, mass balance and tissue distribution of [14C]-BC-3205 in non-pigmented rats

W.W. Wicha, Z. Ivezic-Schoenfeld, R. Novak (Vienna, AT)*

Background: BC-3205, a pleuromutilin antibiotic, is a new oral development compound for human use. It has finished four Phase I studies demonstrating good oral bioavailability in combination with a favorable safety and tolerability profile. Current clinical development targets the indications skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). BC-3205 has demonstrated both *in vitro* activity and *in vivo* efficacy in animal models against a broad range of bacteria often encountered in SSSI and CAP. Target pathogens include among others methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Streptococcus pneumoniae*, atypicals and *Haemophilus influenzae*.
Methods: [14C]-labeled BC-3205 was administered to non-pigmented male and female rats as single oral dose of 30 mg/kg via gavage. The substance-associated radioactivity of whole blood, plasma, expired air, urine, feces, and carcasses was detected using liquid scintillation counting techniques. The tissue distribution was investigated by quantitative whole-body autoradiography (QWBA).
Results: Following a single oral administration of [14C]-BC-3205 to male and female rats Cmax was reached within 3 h after administration. QWBA showed rapid and uniform tissue distribution with peak tissue concentrations around Cmax. Within the investigated time all intra-organ radioactivites approached the lower limit of quantification, indicating a total elimination of the BC-3205 associated radioactivity. This was in line with the results of the excretion balance, showing 96% total recovery of radioactivity after 7 days in males and females. No differences in gender could be observed.
Conclusions: BC-3205 showed a rapid and homogeneous tissue distribution of radioactivity from blood to tissues. The excretion of BC-3205 related radioactivity was rapid with 92.5% of material being excreted within 48 h. Following an oral dose of [14C]-BC-3205, the majority of the radioactivity was excreted via the fecal route (93.1%).

P909 Pharmacokinetic, mass balance and tissue distribution of [14C]-BC-3781 in non-pigmented rats

W.W. Wicha, Z. Ivezic-Schoenfeld, R. Novak (Vienna, AT)*

Background: BC-3781 is a new antimicrobial agent of the pleuromutilin class. BC-3781 finished successfully four Phase I studies demonstrating a favorable safety and tolerability profile. Current clinical development targets the indications skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP) for the oral and intravenous

route of application. BC-3781 exhibits excellent antimicrobial activity against an entire set of different bacterial pathogens often encountered in skin and skin-structure and respiratory tract infections. Target pathogens include among others methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Streptococcus pneumoniae*, atypicals and *Haemophilus influenzae*. In various murine efficacy models BC-3781 demonstrated very good potency characterized by a concentration-dependent killing and a moderate post-antibiotic effect.

Methods: [14C]-labeled BC-3781 was administered to non-pigmented male and female rats as single i.v. dose of 10 mg/kg. The substance-associated radioactivity of whole blood, plasma, expired air, urine, feces, and carcasses was detected using liquid scintillation counting techniques. The tissue distribution was investigated by quantitative whole-body autoradiography (QWBA).

Results: The plasma concentration-time curve of i.v. administered [14C]-BC-3781 described a multi-phasic decline, with a rapid initial distributional phase followed by a terminal elimination phase. Drug concentrations measured in the majority of tissues including skin, soft tissues and lungs were substantially higher compared to plasma levels. Within the investigated time all intra-organ concentrations of BC-3781 approached the lower limit of quantification, being in line with a 96% total recovery of radioactivity after 7 days. No differences in gender could be determined.

Conclusions: BC-3781 showed a high tissue affinity and a rapid and homogeneous distribution of radioactivity from blood to tissues. The excretion of BC-3781 and/or its metabolites was rapid with 92% of the material being excreted within 48 h via the urinary (13%) and fecal route (79%).

P910 Antimicrobial activity of the investigational pleuromutilin compound BC-3781 against Gram-positive organisms commonly associated with cutaneous infections

S. Putnam, D. Biedenbach, H. Sader, Z. Ivezic-Schoenfeld, S. Paukner, R. Novak, R. Jones (North Liberty, US; Vienna, AT)*

Objective: To determine the antimicrobial activity of BC-3781 against recent clinical isolates of Gram-positive cocci. BC-3781 is an investigational semi-synthetic pleuromutilin derivative, which inhibits ribosomal protein synthesis. BC-3781 binds to 50S ribosomal subunit and cross resistance with other antimicrobial classes is very uncommon.

Methods: Antimicrobial activity of BC-3718 and comparator agents was determined against 827 non-duplicate Gram-positive organisms, including staphylococci (413), streptococci (302) and enterococci (112). Susceptibility (S) testing used the CLSI reference broth microdilution method (M07-A8, 2009) for MIC determinations with BC-3781 and comparator drugs (azithromycin [AZ], clindamycin [CL] and linezolid [LZ]). Interpretation of MIC values were based on CLSI (M100-S19, 2009) and EUCAST (2009) S criteria.

Results: Staphylococcal isolates were very S to BC-3781 with highest MIC value being 0.25 and 1 mg/L for *S. aureus* (SA) and coagulase-negative staphylococci (CoNS), respectively. BC-3781 was eight- to 16-fold more potent than LZ and also showed greater potency compared to AZ and CL when tested against staphylococci (Table). Methicillin-S and -resistant (R) staphylococci showed similar BC-3781 MIC distributions. Highest BC-3781 MIC value among β-haemolytic streptococci (BHS) was 0.12 mg/L. BC-3781 was slightly more active than CL and eight- to 16-fold more active than LZ when tested against BHS. Viridans group streptococci (VGS) was also BC-3781-S, but MIC values were slightly elevated compared to BHS. BC-3781 showed potent activity against vancomycin-S and -R *E. faecium* (EFM), and it was significantly more active than comparators against EFM. However, 28.6% of EFM exhibited higher (⩾2 mg/L) BC-3781 MIC values (mechanism unknown).

Conclusions: BC-3781 was very active against a contemporary (2008–2009) collection of staphylococci, streptococci and enterococci, organisms commonly associated with cutaneous infections. BC-3781 activity was not adversely influenced by R to methicillin among staphylococci or vancomycin among enterococci. Further studies are warrant to determine the role of this novel pleuromutilin for the treatment skin and skin structure infections.

Table. Activity of BC-3781 and comparator agents tested against selected Gram-positive cocci

Organism	BC-3781		Azithromycin		Clindamycin		Linezolid	
(no. tested)	$MIC_{50/90}$	%S[a]	$MIC_{50/90}$	%S[a]	$MIC_{50/90}$	%S[a]	$MIC_{50/90}$	%S[a]
SA (314)	0.12/0.12	100.0[a]	16/>16	34.1	0.12/>16	77.1	2/2	100.0
CoNS (99)	0.06/0.12	100.0[a]	>16/>16	47.5	0.12/>16	76.8	1/1	99.0
BHS (202)	0.03/0.06	100.0[a]	0.12/8	84.7	0.06/0.12	94.1	1/1	100.0
VGS (100)	0.12/0.5	98.0[a]	0.25/8	– [a]	0.03/0.12	91.0	1/1	– [a]
EFM (112)	0.12/16	71.4[a]	>16/>16	– [a]	>16/>16	– [a]	2/2	97.3

[a]An ECV of ⩽1 mg/L was applied for comparison purposes only; breakpoint criteria published by EUCAST; and – = no breakpoint has been established.

P911 Activity of BC-3205 when tested against Gram-positive organisms responsible from skin and skin structure infections

S. Putnam, D. Biedenbach, D. Farrell, R. Novak, S. Paukner, Z. Ivezic-Schoenfeld, H. Sader, R. Jones (North Liberty, US; Vienna, AT)*

Objective: To assess the *in vitro* activity for BC-3205, a novel semi-synthetic pleuromutilin, against a contemporary collection of Gram-positive cocci. Pleuromutilins inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. BC-3205 is in early stage clinical development for oral treatment of multidrug-resistant (R) skin and skin structure infections (SSSI).

Methods: Over 800 unique contemporary clinical isolates of Gram-positive organisms tested for susceptibility (S) by broth microdilution methods (CLSI, M07-A8) to BC-3205 and comparator agents, including azithromycin (AZ), clindamycin (CL) and linezolid (LZ). The organisms tested were *S. aureus* (SA; 314), coagulase-negative staphylococci (CoNS; 99), *E. faecium* (EFM; 112), β-haemolytic streptococci (BHS; 202) and viridans group streptococci (VGS; 100). Interpretation of results was guided by CLSI (M100-S19, 2009) and EUCAST criteria.

Results: BC-3205 was very active against SA (MIC50/90, 0.12/0.12 mg/L) and showed similar potency against methicillin-S (MSSA) and -R (MRSA) strains. Against MRSA, BC-3205 (MIC50/90, 0.06/0.12 mg/L) showed significantly greater activity than CL (MIC50/90, 0.12/>16 mg/L), LZ (MIC50/90, 2/2 mg/L) and AZ (MIC50/90, >16/>16 mg/L). Methicillin-S (MSCoNS) and -R CoNS (MRCoNS) were also very S to BC-3205 (see Table). LZ (MIC50/90, 1/1 mg/L) was also very potent against CoNS, but eight- to 16-fold less active than BC-3205. CL and AZ showed more limited activity against CoNS, especially MRCoNS strains (MIC50/90, 0.12/>16 and >16/>16 mg/L, respectively). BC-3205 was highly active against vancomycin-S (VS) and vancomycin-R (VR) EFM (MIC50, 0.12 mg/L for both). BHS strains were very S to BC-3205 (MIC50/90, 0.06/0.06 mg/L). BC-3205 (MIC50/90, 0.06/0.12 mg/L) was four- and 16-fold more active than AZ (MIC50/90, 0.25/8 mg/L) and LZ (MIC50/90, 1/1 mg/L); but slightly less potent than CL (MIC50/90, 0.03/0.12 mg/L), when tested against VGS.

Conclusions: BC-3205 was very active against a representative collection of contemporary pathogens associated with SSSI. Pending further pharmacokinetic/pharmacodynamic and early clinical trial studies, BC-3205 appears to be a promising treatment for cutaneous infections caused by R Gram-positive organisms.

Organism	Number (cumulative percentage) of strains inhibited at BC-3205 MIC (mg/L) of:									
(no. tested)	⩽0.03	0.06	0.12	0.25	0.5	1	2	4	8	⩾16
MSSA (102)	2 (2.0)	14 (15.7)	81 (95.1)	5 (100.0)						
MRSA (212)	18 (8.5)	106 (58.5)	88 (100)							
MSCoNS (50)	1 (2.0)	25 (52.0)	21 (94.0)	1 (96.0)	1 (98.0)	1 (100.0)				
MRCoNS (49)	3 (5.9)	11 (28.6)	29 (87.8)	6 (100)						
VS-EFM (78)	1 (1.3)	20 (26.9)	18 (50.0)	9 (61.5)	1 (62.8)	1 (64.1)	1 (65.4)	6 (73.1)	3 (76.9)	18 (100.0)
VR-EFM (34)	1(2.9)	13 (41.2)	10 (70.6)	5 (85.3)	1 (88.2)	1 (88.2)	1 (88.2)	2 (94.1)	2 (94.1)	2 (100.0)
BHS (202)	99 (49.0)	98 (97.5)	5 (100.0)							
VGS (100)	37 (37.0)	41 (78.0)	16 (94.0)	4 (98.0)	2 (100.0)					

P912 Disc diffusion and MIC quality control ranges for BC-3205, a novel pleuromutilin class antimicrobial

J. Ross, S. Putnam, R. Novak, S. Paukner, Z. Ivezic-Schoenfeld, H. Sader, R. Jones (North Liberty, US; Vienna, AT)*

Objectives: To establish the disk diffusion (DD) and MIC quality control (QC) ranges for BC-3205, a novel semi-synthetic pleuromutilin derivative in the early stage clinical development for oral treatment of skin and skin structure infections (SSSI).
Methods: These QC studies for the 20-mcg BC-3205 disk and broth microdilution method follow the CLSI M02-A10 (2009), M07-A8 (2009) and M23-A3 (2008) document using eight laboratories, two lots of BC-3205 disks, three or more different medium lots. The results are presented as proposed QC ranges for four ATCC strains: *S. aureus* ATCC 25923, *H. influenzae* ATCC 49247, *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 (MIC only). BC-3205 DD and MIC QC ranges were established per a CLSI M23-A3 study design. Ten replicates with each of 3 QC strains produced 1,440 zone diameters for two disk lots of BC-3205 (20-mcg disk) provided by the Mast Group. Clindamycin (CL; 2-mcg disk), azithromycin (AZ; 15-mcg disk) and linezolid (LZ; 30-mcg disk) were utilized as control agents for DD, while retapamulin, AZ and levofloxacin were the control agents for MIC testing.
Results: Proposed QC ranges are listed in the Table. No significant differences (>1 mm) were noted between media or disk lots when testing either BC-3205 or control agents. *S. aureus* ATCC 25923 produced larger variations between laboratories with the most extreme laboratory modes having a 7 mm difference. One laboratory submitted significantly larger zone diameters for *H. influenzae* ATCC 49247 and was excluded from evaluation leaving seven laboratories for a valid CLSI QC study. MIC values for *H. influenzae* 49247 showed trailing endpoints of at least one dilution step. One laboratory was excluded from analysis due to outlier values. A 7 mm zone diameter is proposed for *S. pneumoniae* ATCC 49619 which includes 99.7% of all results. Excluding one aberrant laboratory from the MIC testing analysis produced all seven participant results within the proposed 0.06–0.25 mg/L range. All but one MIC result for the control agents were within expected ranges, when applicable. The control disks (LZ, AZ, CL) provided a valid internal control for the study, with 97.7 to 100.0% of zones within CLSI published QC ranges.
Conclusions: An acceptable QC range was established for the four QC organisms that will guide clinical and reference laboratories involved in clinical trials and facilitate the regulatory review process of BC-3205.

QC organism	MIC/Disk diffusion zone diameters for BC-3205:	
	Proposed range (mg/L / mm)	% in range
S. aureus ATCC 29213/25923	0.03–0.25 / 25–33	100.0 / 95.4
H. influenzae ATCC 49247	1–4 / 18–24	81.8 / 91.6
Excludes one participant	1–4 / 18–24	87.6[a] / 100.0[a]
S. pneumoniae ATCC 49619	0.06–0.25 / 20–26	100.0[a] / 99.7

[a]One laboratory was excluded from evaluation, *H. influenzae* QC range for MICs was not acceptable.

P913 Correlation of *in vitro* susceptibility testing results for an investigational pleuromutilin (BC-3781) using MIC and disc diffusion methods against Gram-positive pathogens

D. Biedenbach, H. Sader, S. Putnam, S. Paukner, R. Novak, Z. Ivezic-Schoenfeld, R. Jones (North Liberty, US; Vienna, AT)*

Objective: To establish the intermethod agreements for testing BC-3781 using the CLSI reference broth microdilution (BMD) MIC and disk diffusion (DD) tests for staphylococci, streptococci and enterococci. This pleuromutilin agent has a unique mode of action which interferes with bacterial protein synthesis without cross-resistance (R) to other antimicrobial classes.
Methods: Strains with pleuromutilin-R phenotypes were included to define the epidemiologic cutoff value (ECV)/susceptibility (S) breakpoints for this agent. These strains included 11 non-wildtype (WT) staphylococcal isolates that carried either cfr or vga(A). The enterococcal population also included strains with non-WT BC-3781 MICs and vancomycin-R isolates. The overall collection was dominated by recent (2008–2009) clinical isolates of staphylococci (316), streptococci (302) and *E. faecium* (EFM; 112). Isolates were tested by BMD and DD, using a 20-mcg disk concentration and CLSI methods (M07-A8, 2009; M02-A10, 2009). Media used were Mueller-Hinton broth or agar supplemented with blood products (horse or sheep blood). Comparator agents included azithromycin, linezolid and clindamycin.
Results: Using an ECV MIC of ⩽1 mg/L and a correlate zone diameter at ⩾20 mm for the BC-3781 DD, resulted in no intermethod discords for the staphylococci. One organism was noted at a BC-3781 MIC of 1 mg/L (methicillin-S CoNS; zone diameter at 24 mm) and one strain with a MIC of 2 mg/L (methicillin-R *S. aureus*; zone diameter at 15 mm). All non-WT strains had zone diameters around the 20-mcg BC-3781 disk of 6–17 mm. The WT population of EFM contained MIC values at ⩽1 mg/L and zone diameters at ⩾21 mm and excellent separation between WT and non-WT enterococci was observed. All streptococci would be considered S or WT at MICs of ⩽1 mg/L and zone diameters of ⩾20 mm, except for two isolates in the *S. bovis* group (2 of 10 strains tested; 20%). Cross-R or -S with other agents (macrolides, oxazolidnones, lincosamides) was not demonstrated.
Conclusions: The proposed/tentative ECV breakpoints (⩽1 mg/L and ⩾20 mm) proposed were without intermethod error for any organism group. If an intermediate category (⩽2 mg/L, 17–19 mm) was applied, intermethod correlations would also be very acceptable with extremely rare (0.4%) minor error. This study demonstrates that accurate breakpoints can be selected and should be considered for use in the early clinical trials for BC-3781, a promising agent for the cutaneous infections.

Organism/MIC interpretation[a] (no.)	No. at DD interpretation:	
	S or WT (⩾20 mm)	R or non-WT (⩽19 mm)
Staphylococcus spp. (316)		
S or WT (⩽1 mg/L)	305	0
R or non-WT (⩾2 mg/L)	0	11
Enterococcus faecium (112)		
S or WT (⩽1 mg/L)	80	0
R or non-WT (⩾2 mg/L)	0	32
Streptococcus spp. (302)		
S or WT (⩽1 mg/L)	300	0
R or non-WT (⩾2 mg/L)	0	2

P914 Correlations of broth microdilution MIC and disc diffusion results for an investigational agent, BC-3205 among potentially indicated species

D. Biedenbach, H. Sader, Z. Ivezic-Schoenfeld, R. Novak, S. Paukner, S. Putnam, R. Jones (North Liberty, US; Vienna, AT)*

Objective: To determine the correlation between the CLSI reference broth microdilution (BMD) MIC (mg/L) and the zone diameter diffusion (mm) results obtained for BC-3205 when tested against targeted Gram-positive pathogens. BC-3205 is semi-synthetic pleuromutilin derivative that interferes with bacterial protein synthesis. Cross resistance with other antimicrobial classes has not been observed with BC-3205 and it is being developed for the treatment of skin and skin structure infections (SSSI) including multidrug-resistant (R) species.
Methods: Recent (2006–2009) clinical isolates of *S. aureus* (214), coagulase-negative staphylococci. (102), *E. faecium* (EFM; 112), β-haemolytic streptococci (202) and viridans group streptococci (100) were tested by CLSI BMD and disk diffusion (20-mcg) using reference methods and appropriate media (M07-A8, 2009; M02-A10, 2009). Staphylococcal strains with non-wildtype (WT; ⩾2 mg/L) MIC values for other pleuromutilin compounds (carrying vga[A] or cfr) were included in the study to determine tentative epidemiologic cutoff values

(ECV) for MIC and disk diffusion tests with BC-3205. Comparator agents included azithromycin (AZ), linezolid (LZ) and clindamycin (CL).
Results: Using tentative ECV breakpoints (⩽1 mg/L and ⩾20 mm) BC-3205 produced rare intermethod errors (see Figure). Excellent discrimination by BMD between WT and non-WT populations were evident among staphylococci, regardless of methicillin susceptibility. A single non-WT MRSA strain (MIC, 1 mg/L) fell below the proposed ECV (⩽1 mg/L) introducing a major interpretive error (0.1%). The WT EFM population contained MIC values at ⩽1 mg/L and zone diameters ⩾20 mm with no zones between 16–19 mm, enabling excellent separation between WT and non-WT EFM (MICs, ⩾2 mg/L). BC-3205 scattergrams for the streptococci demonstrated dominant susceptibility (MICs, ⩽0.5 mg/L and zone diameters at ⩾21 mm). Intermethod interpretive agreement for the control agents ranged from 98.6 (AZ, CL) to 100.0% (LZ).
Conclusions: Correlations of 20-mcg BC-3205 zone diameters with the CLSI reference MIC values were excellent and extremely rare intermethod error (0.1%) was noted when using a susceptible or ECV breakpoints of ⩽1 mg/L and ⩾20 mm. These tentative criteria should be considered for use in the early clinical trials. Significant cross-R or -susceptibility with other agents (macrolides, oxazolidinones, lincosamides) was not observed.

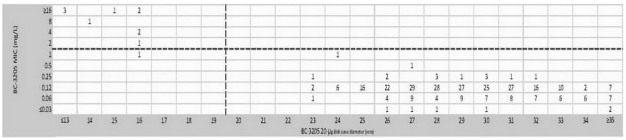


Table. BC3205 scattergram comparing MIC and zone diameter results for all *Staphylococcus* spp.[a] (316 strains). [a]Includes *Staphylococcus aureus* (214) and coagulase-negative staphylococci (102).

Miscellaneous antibiotic resistance

P915 High prevalence of amikacin susceptibility in aac(6′)-Ib-positive *Enterobacter cloacae*

S.Y. Kim, Y.J. Park, J.K. Yu (Suwon, Seoul, KR)*

Objectives: The aac(6′)-Ib gene encodes aminoglycoside 6′-N-acetyltransferase. This gene confers resistance to amikacin, tobramycin, kanamycin, netilmicin but not to gentamicin. The EUCAST recommends reporting amikacin as intermediate for Enterobacteriaceae which is intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin, because production of acquired aac(6′)-I may not confer phenotypic amikacin resistance. The single amino-acid substitution in aac(6′)-Ib (Leu119Ser) results in decrease in amikacin MIC and increase in gentamicin MIC. We investigated correlation between the aac(6′)-Ib and aminoglycoside MICs.
Methods: This study included 73 aac(6′)-Ib-positive *E. cloacae* isolates from 12 laboratories between March and July 2005. All isolates had been screened previously by PCR for the presence of aac(6′)-Ib. Susceptibility to amikacin (2–256 mg/L), kanamycin (2–256 mg/L), tobramycin (0.5–64 mg/L), and gentamicin (0.5–64 mg/L) were determined by an agar dilution method. To determine the presence of Leu119Ser mutation, sequencing was performed in isolates with amikacin MIC ⩽16 mg/L.
Results: Of 73 aac(6′)-Ib-positive isolates, 64 (87.7%) were susceptible to amikacin, 16 (21.9%) to kanamycin, 17 (23.3%) to tobramycin, and 32 (43.8%) to gentamicin by the CLSI guideline; 47 (64.4%) were susceptible to amikacin, 16 (21.9%) to tobramycin, and 25 (34.2%) to gentamicin by the EUCAST. Of amikacin-susceptible isolates, 15 of 64 (23.4%) and 6 of 47 (12.8%) were non-susceptible to tobramycin and susceptible to gentamicin by the CLSI and EUCAST, respectively. Only 4 of 64 isolates with amikacin MIC ⩽16 mg/L had single amino-acid susbstitution on aac(6′)-Ib (Leu119Ser); three isolates had amikacin MICs ⩽2 mg/L and one an MIC of 4 mg/L.
Conclusion: Although the EUCAST breakpoint instead of the CLSI breakpoint was used, many *E. cloacae* isolates with the aac(6′)-Ib were susceptible to amikacin. Further investigations are required to determine whether amikacin-susceptible isolates carrying the aac(6′)-Ib are associated with therapeutic failure.

P916 Loop-mediated isothermal amplification method for rapid and simple detection of 16S rRNA methylase genes, rmtA, rmtB and armA, and detection situation in Japan

M. Nagasawa, M. Kaku, K. Yamaguchi, K. Kamachi, J. Wachino, K. Yamane, Y. Arakawa (Miyagi, Tokyo, JP)*

Background: Recently, a series of special methylases that protect microbial 16S rRNA, the main target of aminoglycosides, was identified in nosocomial pathogens, e.g., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia marcescens*. To prevent and control the spread of such resistant bacteria in hospitals, there is a need for a rapid and simple detection method for the aminoglycoside-resistance genes. Moreover, no exact isolation frequency of such microbes was determined in Japan after the report of Yamane et al., EID, 13:642–646, 2007.
Methods: For specific detection of 16S rRNA methylase genes, rmtA, rmtB and armA, a set of LAMP primes (F3, B3, FIP, BIP, LF and LB) was designed for each gene by targeting their specific region using Primer Explorer V4 software, respectively. The length of targeting rmtA, rmtB and armA genes was 211, 226, and 259bp, respectively. The LAMP reactions were carried out with the Loopamp DNA amplification kit (Eiken Co. Ltd., Japan) at 65ºC, and directly detected with the naked eye using Loopamp fluorescent detection reagent. The template DNA was extracted from each aminoglycoside-resistant isolate (12 bacterial species, 68 isolates) by a simple boiling method, and subjected to the LAMP methods. Strains used were collected from 33 nationwide hospitals.
Results: The rmtA-LAMP specifically detected all *P. aeruginosa* isolates harboring rmtA in a 40-min reaction, but no amplification was found in strains harboring rmtB or armA. Similarly, the rmtB- and armA-LAMP exclusively detected strains harboring only rmtB and armA, respectively. The 16S rRNA methylase genes were found in various bacterial species. The detection frequency in each species was from 0.07 to 3.51% of all the clinical isolates tested and from 3.8 to 66% of the aminoglycoside-resintant isolates.
Conclusions: The rmtA-, rmtB-, and armA-LAMP assay would promise rapid and simple identification of 16S rRNA methylase-producing strains among aminoglycoside-resistant clinical isolates in ordinary microbiology laboratories without using any specific and expensive equipments.

P917 Molecular characterization and antimicrobial susceptibility of *Escherichia coli* isolates from faeces of healthy humans

M. Citar, B. Jesenko, Z. Petkovsek, S. Markovic, M. Kuzmic, V. Krizan-Hergouth, D. Zgur-Bertok, M. Starcic Erjavec (Ljubljana, SI)*

Objectives: The bacterium *Escherichia coli* (*E. coli*) is the prototypic commensal species of the facultative anaerobic microflora in the lower intestine of healthy warm-blooded organisms. Usually, *E. coli* and its host coexist in mutual benefit but there are several highly adapted *E. coli* strains that have acquired specific virulence factors and can cause an impressive variety of different types of intestinal and extraintestinal diseases. In order to screen for the prevalence of several virulence (related) genes, a collection of 90 commensal *E. coli* strains isolated from feces of healthy humans was studied.
Methods: Isolates were collected at the Department of Biology, Biotechnical Faculty, University of Ljubljana, during the time period from 01.03 to 04.09.2009. Strains were isolated from feces of healthy men and women of different ages that were not any antimicrobial medicines for therapeutic and prophylactic purposes.
Using the method of PCR amplification with specific primers all isolates were screened for the following virulence related genes: cnf1 (cytotoxic

necrotising factor 1), hlyA (haemolysin), papGII (class II PapG adhesin), papGIII (class III PapG adhesin), sfaDE (S fimbriae), afa/draBC (Afa/Dr adhesins), iucD (aerobactin), usp (uropathogenic-specific protein), tcpC (a new Toll/interleukin-1 receptor domain), traJ (transfer region), and rmoA (modulator of gene expression); two plasmid replication regions, RepFIB and RepFIIA; and phylogenetic groups (A1, B1, B2 and D). Further, the strains were tested for their susceptibility to three widely used antibiotics: tetracycline, ciprofloxacine and ampicillin.

Results: Our results showed that 33% of the studied *E. coli* isolates belonged to the B2 group, 30% to the D group, 22% to the A group, and 14% to the B1 group. The cnf1 gene sequence was detected in 6% of the strains, hlyA in 8%, papGII in 8%, papGIII in 3%, sfaDE in 17%, afa/draBC in 4%, iucD in 39%, usp in 24%, tcpC in 8%, traJ in 26%, rmoA in 27%, RepFIB in 33% and RepFIIA in 37% of the tested isolates.

Of all the tested strains 73% were susceptible to ampicillin, 98% to ciprofloxacine and 82% to tetracycline.

Conclusion: Our results indicate that among the gut microbiota individual commensal *E. coli* strains exhibit the virulence potential to instigate extraintestinal infections.

	Prevalence	
	no. of strains	(%)
Phylogenetic group		
A	20	(22)
B1	13	(14)
B2	30	(33)
D	27	(30)
Virulence factor		
cnfl	5	(6)
hlyA	7	(8)
papGII	7	(8)
PapGIII	3	(3)
sfaDE	15	(17)
afa/draBC	4	(4)
tucD	35	(39)
usp	22	(24)
tcpC	7	(8)
traJ	23	(26)
rmoA	24	(27)
RepFIB	30	(33)
RepFIIA	33	(37)
Antibiotic resistance		
ampicillin	66	(73)
tetracycline	74	(32)
ciprofloxacin	88	(98)

P918 Faecal carriage of antibiotic-resistant *Escherichia coli* in healthy children and home-raised chickens: a household study in a resource-limited setting

E. Riccobono, A. Mantella, F. Bartalesi, H. Gamboa, A. Bartoloni, G.M. Rossolini, L. Pallecchi (Siena, Florence, IT; Camiri, BO)*

Objective: In resource-limited countries (RLCs), home-raised domestic animals live in close contact with household members, and exposure to animal intestinal flora is favored by condition of poor sanitation. In a previous study carried out with healthy children in urban areas of Bolivia and Peru, we evidenced high rates of acquired resistance in commensal *E. coli*, including to drugs not routinely used in childhood (e.g. tetracycline and quinolones). Here we studied resistant commensal *E. coli* from healthy children and home-raised chickens in households from one of the previously surveyed urban area.

Methods: Fecal carriage of resistant *E. coli* was studied in 21 healthy children aged 6–72 months and 21 home-raised chickens, from 12 households in Villa Montes (Tarija Dept., Bolivia). Molecular analysis (population structure of resistant bacteria, nature of acquired resistance genes and their mobile genetic supports) was performed with isolates exhibiting resistance to tetracycline-TET (n = 66) or nalidixic acid-NAL (n = 30), collected from 6 children and 6 chickens from 3 selected households.

Results: Except for resistance to (fluoro)quinolones, which was higher in chickens ($P < 0.05$), resistance rates in children and chickens were overall high and comparable. TET- and NAL-resistant isolates belonged to phylogenetic group A (67%) (significantly more prevalent in children), B1 (18%) (only in chickens) and D (15%) (equally distributed). Identical RAPD-types were found in children or chickens living in the same household, but in neither case human-animal sharing of the same resistant clone was observed. TET-resistant isolates carried tet(A) or tet(B) genes (equally distributed in isolates of human and animal origin), often located on conjugative plasmids with different replicon combinations. 2 NAL-resistant clones from children carried the qnrB19 gene, located on small non transferable ColE-type plasmids. Plasmid transfer in different clones was never observed.

Conclusions: Household animals-humans transmission of resistant bacteria in RLCs might not represent a major risk factor for fecal carriage of resistant bacteria.

P919 Prevalence of sul genes in *Escherichia coli* causing urinary tract infection in the community

R. Diaz, S. Ferreira, A. Freitas, S. Mendo (Aveiro, PT)*

Objectives: Urinary tract infections (UTI) are increasing significantly among the community. *Escherichia coli* is the main microorganism responsible for urinary infections (UTI) and shows a significant percentage of resistance to Sulfamethoxazole + Trimethoprim (SxT). Antimicrobial resistance is a major problem in the clinical context and it represents a significant threat for the general healthy public. The aim of the present study was to evaluate the prevalence of antibiotic resistant strains, namely to SxT, collected in a health care unit in Aveiro, central region of Portugal.

Methods: 547 *E. coli* isolates were collected, between October and December 2008, from community patients with suspected UTI. From those, 25 were resistant to SxT and therefore selected for the study. The presence of int1, sul1, sul2, sul3 genes and dfr variants was investigated by PCR. Nucleotide sequence of the amplicon products was determined in both strands and compared to others deposited in the Genebank databases.

Results: Antibiotic resistance profile of the 25 isolates, revealed a high rate of resistance to Amoxicilin, Cefatrizin, Norfloxacin and SxT.

Nineteen isolates were positive for the presence of the int1 gene, indicating the presence of class 1 integron(s); 13 were positive for sul1, 16 for sul2 and 2 for the sul3 gene. Variants of the dfr genes were predominant in the amplified variable regions.

sul3 gene was found associated with class1 integron which is quite unusual.

Conclusions: The percentage of resistance to SxT in the *E. coli* strains was high. The presence of class 1 integrons in a high rate was also surprising considering a community population. These results are of major concern since they can compromise the use of antibiotics usually employed in the treatment of UTI, namely the empiric treatment with SxT.

P920 Plasmid dissemination of sul2 among non-typhoidal *Salmonella* isolates in Portugal

T. Curiao, P. Antunes, R. Matias, L. Peixe, T.M. Coque (Madrid, ES; Porto, PT)*

Objectives: Sulfonamide resistance is increasingly reported among *Salmonella* isolates from different European countries, sul2 being the most widespread sul gene. Our aim was to characterize the plasmids and the genetic environment associated to sul2 among Portuguese multidrug-resistant *Salmonella* isolates.

Methods: We studied 22 sul2-*Salmonella* isolates representatives for different sources (hospital, food, piggeries), serotypes (Typhimurium,

Enteritidis, Brikama, Saintpaul) and PFGE-clones (2002–2007). Identification of sul genes (sul1, sul2, sul3) and sequences related to their platforms (ISCR2, repCpRSF1010, strAB) were done by PCR. Genetic environment was characterized by PCR mapping, long-PCR-RFLP and sequencing. Conjugation assays were performed by standard procedure. Plasmid analysis included size determination (S1 nuclease), incompatibility grouping (PCR, hybridization and sequencing) and comparison of RFLP patterns (PstI and ClaI).
Results: Most sul2 isolates were resistant to ampicilin, streptomycin, tetracycline and trimethoprim and they also contained either sul1 (n = 10) or sul3 (n = 2). Conjugative transfer of sul2 was achieved for 50% of the isolates. sul2-plasmids ranged from 7 to 250 Kb, some containing two copies of the gene. Conjugative plasmids corresponded to IncI1 (n = 3, same RFLP type), IncFIIa (n = 2), IncN (n = 3, same RFLP type), IncA/C, (n = 2), or IncHI1 (n = 1). IncI1, IncN, IncA/C and IncFII plasmids were recovered from food and clinical isolates in different years. The sul2 gene was associated with strAB (n = 14) and also with ISCR2 (n = 6, ISCR2-strB-strA-sul2, both platforms being located in a diverse range of plasmids. Some plasmids were untypeble by the method used (n = 7).
Conclusions: sul2 is located in a diversity of plasmids among *Salmonella* from Portugal. The identification of widespread elements in both clinical and community settings is of concern since they might fuel the dissemination of antibiotic resistance in other hosts.

P921 Co-dispersion of copper and antibiotic resistance genes among enterococci from different ecological niches, Portugal

E. Silveira, A.R. Freitas, T.M. Coque, L. Peixe, C. Novais (Porto, PT; Madrid, ES)*

Objectives: In Europe, copper compounds are used in industry, agriculture and animal production. Evidences concerning copper effect on the selection of antibiotic (AB) resistant strains are scarce and limited to enterococci from the animal production setting. Our goal was to analyze the occurrence of tcrB among enterococci from different ecological niches and to assess the co-transference of tcrB with several AB resistance genes.
Methods: Representative enterococci from hospitalized patients (H, n = 90), healthy humans (HV, n = 92), poultry (P, n = 127), piggeries-animals/environment (PE, n = 197); sewage/river (SR, n = 43) collected from North, Centre and South Portugal (1996–2007) were analyzed. They belonged to different species and presented diverse antibiotic resistant patterns. tcrB was searched by PCR as described. Mating assays using *E. faecium* GE1/BM4105RF or *E. faecalis* JH2–2 as recipients were accomplished for representative tcrB+ isolates (n = 47) using BHI supplemented with rifampicin, fusidic acid plus tetracycline (Te), erythromycin (Er), vancomycin (Vc) or gentamicin (Gm). Susceptibility to nine AB was determined by disk diffusion (CLSI). Presence of genes coding for antibiotic resistance (tetM,L; ermB, vanA, aac6-aph2) was screened by PCR.
Results: tcrB was detected in 20% (109/549) of the isolates studied including PE (26%; n = 52/197), H (21%-n = 19/90), SR (21%; n = 9/43), HV (20%; n = 18/92) and P (9%; n = 11/127)]. They correspond to *E. faecium* (25%, 56/224), *E. faecalis* (16%, 30/183) and *Enterococcus* spp (24%, 24/142). tcrB+ transconjugants were selected in plates supplemented with Er, Te, Vc (93%, 25/27 of positive matings; 53%, 8/15, 67%, 2/3, respectively) and co-transferred with variable AB resistance patterns: Er-80% (20/25, ermB), Te-64% (16/25, 5 tetM, 9 tetML; 2 tetL), Vc-28% (7/25, vanA) high level of resistance (HLR) streptomycin-20% (5/25), cloramphenicol-8% (2/25) or HLR-Gm-4% (1/25; aac6-aph2).
Conclusions: Several Portuguese settings seem to be reservoir of enterococci harbouring tcrB and AB resistance genes, both located in common platforms or co-transferred during the same genetic event. Horizontal dissemination of AB resistance mobile elements might be favoured by the selective pressures exercised by the use of both AB and copper compounds.

New resistance mechanisms

P922 Respective contribution of PatA/PatB and PmrA in fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*

A. Lismond, M.I. Garvey, F. El Garch, S. Delvigne, P.M. Tulkens, L.J. Piddock, F. Van Bambeke (Brussels, BE; Birmingham, UK)*

Background: Two efflux systems have been identified so far for fluoroquinolones (FQ) in *S. pneumoniae* (SP), namely PmrA (MFS superfamily), and PatA/PatB (ABC transporters). Previous studies suggest a predominant role of PatA/PatB in FQ resistance of laboratory strains. The aim of the present study was to determine which of these two systems could be primarily involved in the resistance of clinical isolates to FQ.
Methods: 5 strains showing a phenotype suggestive of efflux were selected from a large collection of SP isolates obtained from CAP patients, and compared to ATCC49619. MICs were measured in Mueller Hinton II agar supplemented with 5% defibrinated horse blood (±reserpine [efflux inhibitor]). Expression levels of patA, patB, and pmrA genes were evaluated by real-time PCR. Gene inactivation was obtained by transformation using genomic DNA of ATCC49619 disrupted in one of the genes under study (spectinomycin-resistance cassette inserted in the middle of the gene of interest).
Results: Reserpine or gene disruption had no effect in ATCC49619. Disruption of patA or patB was as effective as reserpine to reduce FQ MIC in all clinical isolates, irrespective of the pump(s) overexpressed (SP13 did not revert to wild-type MIC because of the presence of target mutations). Disruption of pmrA had only a modest effect on NOR MIC in SP257.
Conclusions: Disruption of patA or patB is sufficient to reduce MIC to the value measured in the presence of reserpine. PatA/PatB, even when expressed at a basal level, contribute to resistance to FQ in the clinical isolates analyzed while PmrA has almost no impact.

Strains	Gene expression[a]			CIP MIC (mg/L)[b,c]					NOR MIC (mg/L)[b,c]				
	patA	*patB*	*pmrA*	w/o R	with R	*patA–*	*patB–*	*pmrA–*	w/o R	with R	*patA–*	*patB–*	*pmrA–*
ATCC 49619	basal	basal	basal	0.5	0.5	**0.5**	**0.5**	**0.5**	4	2	**4**	**2**	**4**
SP13	+	+	+	16	4	**2**	**2**	16	256	16	**16**	**16**	128
SP207	+	+	+	4	0.5	**1**	**0.5**	no[d]	32	2	**4**	**2**	no[d]
SP295	+	+	basal	2	0.5	**0.5**	**0.5**	1	16	2	**2**	**2**	8
SP257	basal	basal	+	1	0.5	**0.5**	**0.5**	1	8	2	**2**	**2**	**4**
SP298	basal	basal	+	2	0.5	**0.5**	**1**	2	8	2	**4**	**4**	16

[a] + denotes a value >2-fold that measured in ATCC49619 (basal level).
[b] In control conditions (without reserpine ([w/o R]); + reserpine 10 mg/L [with R]; in strain disrupted for *patA* [*patA–*], *patB* [*patB–*], or *pmrA* [*pmrA–*].
[c] Figures in bold denote MICs brought back to those measured with reserpine (+/– 1 dilution).
[d] Disruptant non obtained so far (no).

P923 Development and stability of non-susceptibility to vancomycin in methicillin-resistant *Staphylococcus aureus*

V. Doddangoudar, M. Boost, M. O'Donoghue, P. Appelbaum (Kowloon, HK; Hershey, US)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) with non-susceptibility to vancomycin (VISA) was first documented in 1997, but the underlying mechanisms remain unclear. Reduced susceptibility is typically reported in isolates from patients who have received vancomycin therapy, usually lasting several weeks. We induced and tracked hVISA/VISA development in MRSA clinical isolates by exposure to sub-inhibitory levels of vancomycin and determined the stability of these non-susceptible phenotypes.
Method: 30 clinical isolates of MRSA were subjected to increasing concentrations of vancomycin based on their initial and subsequent MIC to vancomycin for 60 days. Strains were incubated with vancomycin at 50% MIC for 48 h at 370C. The MICs were then determined by standard agar dilution and spiral gradient endpoint (SGE) methods and strains passaged to fresh media. After 60 days, stability of non-susceptibility was determined by transferring the strains to fresh drug-free broth every 48 h and determining MIC weekly for a further 60 days. Development and loss of resistance patterns were plotted.

Results: All isolates developed non-susceptibility, with final MICs by 60 days ranging from 6–20μg/ml (mean 10μg/ml, MIC90 20μg/ml). Time required for the VISA development varied from 24–54 days and several distinct patterns of MIC increase were observed. Removal of drug pressure led to loss of non-susceptibility (mean MIC of 2.1μg/ml (range 0.5–5)) in all but one strain (20μg/ml) after 4 weeks.
Conclusion: It is important to recognize the strong potential for MRSA strains to become vancomycin non-susceptible, as currently there is general belief that hVISA/VISA development are rare events. All isolates became vancomycin non-susceptible by 60 days. Of 6 strains reaching an MIC 20μg/ml, equivalent to CLSI resistance definition, one converted to a stable phenotype. Current terminology may need to be re-addressed, as hVISA/VISA can reach 20μg/ml though not having vanA. Whilst non-susceptibility is clearly linked to selective pressure, the rapid development within the period of regular therapy demonstrates that treatment failure could easily occur. Use of SGE provided exact MICs rather than the large stepwise increments of doubling dilutions, allowing patterns of non-susceptibility development to be determined. Further work on non-susceptibility development is continuing, examining the mutations in regulator genes during development and loss of non-susceptibility.

P924 Total genome sequencing and complete genetic analysis of all SNP-linked phenotypes reveals novel clues to the emergence of low-level glycopeptide intermediate resistance in *Staphylococcus aureus*

A. Renzoni, D. Andrey, C. Barras, A. Monod, P. Vaudaux, D. Lew, W. Kelley (Geneva, CH)*

The precise mechanisms leading to the emergence of low level glycopeptide resistance in *S. aureus* are poorly understood. In this study, we used high throughput whole genome sequencing to detect differences between two isogenic strains: a parental strain (ISP794) and a stable derivative (AR376) selected for growth on 4 μg/ml teicoplanin. Surprisingly, we uncovered only three single nucleotide polymorphisms (SNPs) mapping to three separate genes which could account for the observed alteration in antibiotic sensitivity. Nonsense mutations occurred in stp1 encoding a serine/threonine phosphatase and in yjbH, encoding a putative negative regulator of the redox sensor and global transcriptional regulator, Spx. A missense mutation in vraS resulted in the non-conservative amino acid change G45R in the histidine kinase sensor of cell wall stress, VraS. Using genetic methods, all single, pairwise, and reconstructed triple SNP combinations were subsequently evaluated for their contribution to emergence of low level glycopeptide resistance. We found that the mutations within stp1 and vraS contributed partially alone and acted together synergistically to evoke reduced teicoplanin sensitivity. Mutation within yjbH was found to have no detectable effect on glycopeptide resistance under our experimental conditions. Our results are the first to report both the combined complete genome sequencing and complete genetic analysis leading to enhanced understanding of antibiotic resistance emergence in this major human pathogen. The significantly low cost and high benefit of applying high throughput sequencing to obtain clues to genetic changes underlying drug resistance argues for the broader application of this method in the near future. Previous work in our laboratory (Renzoni, A et al 2009 AAC 53:903–911) using the reduced glycopeptide sensitivity strain AR376 revealed that disruption of either of two previously uncharacterized adjacent genes named trfA and trfB (for teicoplanin resistance factor) could restore sensitivity to teicoplanin in this background. The mechanisms of action of trfA and trfB remain to be determined, but our present study now indicates they must function to disrupt the effects of dual phosphosignalling pathways.
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P925 VanM gene cluster – a new glycopeptide resistance gene cluster found in a clinical isolate of *Enterococcus faecium*

X. Xu, D. Lin, Y. Guo, S. Wu, X. Ye, D. Zhu, M. Wang (Shanghai, CN)*

Objectives: Since the glycopeptide-resistant clinical isolates of *Enterococcus* species were reported in 1988, glycopeptide-resistant Enterococci (GRE) are now encountered in hospitals in most countries. Seven types of gene clusters (vanA, vanB, vanC, vanD, vanE, vanG and vanL) contribute to glycopeptide resistance in *Enterococcus* spp. worldwide. A novel glycopeptide-resistant determinant, vanM gene cluster, was cloned from a clinical isolate of *E. faecium*.
Methods: Antimicrobial susceptibility testing and conjugation experiment were carried out in a glycopeptide-resistant clinical isolate from a teaching hospital in Shanghai, China. By PCR amplification, walking sequencing, and restriction fragment cloning, the glycopeptide-resistant gene and its flanking sequences were determined. The obtained sequence was compared with known glycopeptide resistance gene clusters for sequence identity by clustal W method.
Results: A clinical strain of *E. faecium* Efm-HS0661 was isolated from an inpatient with an intra-abdominal infection and was resistant to most antimicrobials, including vancomycin and teicoplanin. Its glycopeptides resistance could be transferred to *E. faecium* BM4105RF by conjugation. The donor and its transconjugant were negative by PCR for the known van genes. With cloning method and primer walk sequencing, we found a novel gene cluster, designated vanM gene cluster. The D-Ala:D-Lac gene, vanM, was 1032bp in length and encoded a 343-amino-acid protein, which shared 79.9%, 70.8%, 66.3%, and 78.8% amino acid identity with VanA, VanB, VanD, and VanF, respectively. Though vanM DNA sequence was most close to vanA, the organization of vanM gene cluster was most similar to vanD gene cluster. Upstream of vanM gene cluster there was an IS1216-like element, which may play an important role in the dissemination of resistance determinant. Of 10 GRE strains isolated from the teaching hospital from 2005 to 2008, 6 strains had vanM genotype and 4 strains were vanA genotype.
Conclusion: A new glycopeptide resistance determinant, vanM gene cluster, was characterized from a clinical isolate of glycopeptide-resistant *E. faecium*. The vanM gene cluster was more common than vanA gene cluster at a teaching hospital in Shanghai.

P926 VanN, a novel type of transferable vancomycin resistance in *Enterococcus faecium* 08-174

F. Lebreton, N. Bourdon, P. Berger, M. Fines-Guyon, R. Leclercq, V. Cattoir (Caen, Marseille, FR)*

Objectives: Vancomycin-resistant enterococci are recognized as a major cause of nosocomial infections. To date, seven vancomycin resistance determinants have been characterized: vanA, vanB, vanC1/C2, vanD, vanE, vanG and vanL genes. The aim of this study was to investigate the mechanism of vancomycin resistance in *Enterococcus faecium* 08-174, a clinical isolate devoid of known vancomycin resistance gene.
Methods: Vancomycin-resistant *E. faecium* 08-174 was isolated from a rectal swab in 2008 in Marseille, France. DNA amplification of a putative van gene was carried out by using degenerated primers V1 and V2 designed to amplify an internal fragment of ligase genes. The PCR product was then cloned and sequenced. Thermal asymmetric interlaced PCR (TAIL-PCR) assays were performed to determine the entire sequence of the novel van gene. Transfer of vancomycin resistance from *E. faecium* 08-174 to *E. faecium* BM4107 and *Enterococcus faecalis* JH2–2 was attempted by filter mating experiments. Plasmid analysis was performed by using a modified Kieser technique.
Results: *E. faecium* 08-174 displayed low-level resistance to vancomycin (MIC, 12 mg/L) and susceptibility to teicoplanin (MIC, 2 mg/L). A ca. 600-bp PCR product was obtained with V1 and V2 primers and sequence analysis revealed significant similarities with van genes. TAIL-PCR allowed us to determine the entire sequence of this novel ligase gene designated vanN. The deduced protein shared the highest amino acid identity with the D-Ala-D-Ser VanL, VanC2, VanC1 and VanE

ligases (65%, 56%, 55% and 53%, respectively). Attempts to transfer vancomycin resistance from *E. faecium* 08-174 to *E. faecalis* JH2–2 were unsuccessful whereas a transconjugant was obtained with *E. faecium* BM4107 used as a recipient. vanN sequence could be amplified by PCR from the transconjugant. Analysis of plasmid content in *E. faecium* 08-174 and its transconjugant revealed the presence of a ca. 75-kb plasmid.

Conclusion: We identified a previously unknown type of glycopeptide resistance and demonstrated its transferability to another *E. faecium* strain. Phenotypic (low-level resistance to vancomycin) and genotypic (homology with VanL, VanC and VanE) traits suggest that VanN may be a novel D-Ala-D-Ser ligase. Further investigations are being conducted to confirm this hypothesis. This would constitute the first case of transferable D-Ala-D-Ser type resistance and would increase the burden of transferable vancomycin resistance.

Staphylococcus

P927 Vancomycin minimum bactericidal concentration analysis of methicillin-resistant *Staphylococcus aureus* bacteraemia isolates and correlation with daptomycin and vancomycin minimum inhibitory concentrations

C. Doern, H. Honda, W.M. Dunne Jr., D. Warren* (St. Louis, US)*

Objectives: The objective of this study was to analyze the relationship between vancomycin minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for methicillin-resistant S aureus (MRSA) bacteraemia isolates collected in a U.S. academic hospital from 2005 to 2007. We also assessed the correlation between vancomycin MIC/MBCs and daptomycin MIC.

Methods: MICs were determined for MRSA bacteraemia isolates using the MicroScan – Pos MIC Panel Type 26. MicroScan panels were inoculated from overnight growth on blood agar plates and incubated at 35 degrees Celsius for 24 hr. Vancomycin MBCs were calculated by plating 20 ul (in duplicate) of broth from the vancomycin MIC well through the vancomycin 16 ug/ml well. The MBC was defined as 99.9% killing of the initial inoculum at 24 hr. Two-tailed Spearman's rho was used to calculate correlation coefficients.

Results: 174 MRSA isolates were tested. The MIC-50 of daptomycin and vancomycin were 0.5 (range $\leqslant$0.25–4 ug/ml) and 2.0 ug/ml (range 0.5–4 ug/ml), respectively. The MIC-90 of daptomycin was 1.0 ug/ml and the MIC-90 of vancomycin was 2.0 ug/ml. The MBC-50 of vancomycin was 2.0 ug/ml and the MBC-90 was 8.0 ug/ml. Increased vancomycin MIC correlated with increased daptomycin MIC among isolates in our cohort ($r = 0.334$; $p < 0.001$) Increased vancomycin MBC also correlated with increased daptomycin MIC ($r = 0.366$; $p < 0.001$). Vancomycin MIC and MBC were highly correlated ($r = 0.714$; $p < 0.001$); in 124/174 (71%) of isolates tested the vancomycin MBC was the same as the MIC. 12 (6.9%) MRSA isolates were vancomycin-intermediate *S. aureus* (VISA) and 5 (2.9%) were non-susceptible to daptomycin (defined as MIC > 1 ug/ml). VISA strains were more likely to be non-susceptible to daptomycin [2/12 (17%) of VISA vs. 3/162 (2%) non-VISA isolates; $p = 0.039$].

Conclusions: In this cohort of MRSA bacteraemia specimens the vancomycin MBC and MIC were identical in almost three-quarters of isolates. Decreased susceptibility to vancomycin correlated to decreased susceptibility to daptomycin.

P928 Activity profile of tigecycline and comparator agents against skin and wound isolates collected across Europe, 2006–2009

N.P. Brown, C.M. Pillar, C. Thornsberry, D.F. Sahm, M. Dowzicky (Chantilly, Collegeville, US)*

Objective: TIG was approved in 2006 for the treatment of complicated skin and skin structure infections. The spectrum of activity of TIG includes both Gram-positive and Gram-negative pathogens, including those with commonly encountered resistance phenotypes. It is important to continue to monitor the activity of commonly utilized agents, in particular recently approved agents, for resistance among targeted pathogens. This study reports the current activity profile of TIG and comparators against recent Gram-positive and Gram-negative pathogens collected in Europe focusing on skin and wound isolates.

Methods: 955 skin/wound and 2299 non-skin/wound Gram-positive and Gram-negative organisms collected from 2006–2009 were centrally tested by broth microdilution (CLSI M7-A8). Isolates were obtained from sites distributed across 12 European countries. EUCAST breakpoints (BP) were used to interpret TIG MIC results and CLSI (M100-S19) BP were used to interpret all other agents.

Results: Against Gram-positive skin/wound isolates consisting of *S. aureus* (including MRSA), group A streptococci (GAS), and vancomycin susceptible *E. faecalis* (VSE), TIG displayed potent *in vitro* activity with MIC50s of 0.03 mg/L (GAS) and 0.12 mg/L (MSSA/MRSA/VSE) and MIC90s of 0.06 mg/L (GAS) and 0.25 mg/L (MSSA/MRSA/VSE), several fold lower than evaluated comparators. Over 99% of Gram-positive skin and wound isolates were susceptible to TIG and to other Gram-positive agents (linezolid, daptomycin, vancomycin). TIG was also potent against Gram-negative enteric skin/wound isolates, with MIC50s ranging from 0.12 to 0.5 mg/L and MIC90s from 0.5 to 1 mg/L. No TIG resistance was detected among skin/wound isolates across the evaluated Enterobacteriaceae spp., as was the case with imipenem. In contrast, resistance was readily detected at varying levels among the evaluated cephalosporins, gentamicin, ciprofloxacin, and piperacillin/tazobactam. There was no notable difference in TIG activity profile between that observed with skin/wound isolates and non-skin/wound isolates.

Conclusions: TIG was potent against both Gram-positive and Gram-negative skin/wound pathogens in Europe. Excluding *S. aureus* where <1% of isolates were TIG resistant, no TIG was observed among the evaluated isolates. The broad spectrum potency and overall lack of resistance highlights the therapeutic potential of TIG, however continued surveillance is warranted to monitor for emerging resistance among primary pathogens.

P929 CEM-102 (fusidic acid) *in vitro* activity and evaluation of molecular resistance mechanisms among European Gram-positive isolates, 2008–2009

M. Castanheira, D. Farrell, M. Janechek, R. Jones (North Liberty, US)*

Objectives: To evaluate the activity of fusidic acid (FA) among Gram-positive bacteria collected in European medical centers in the 2008–2009 period and to analyze the prevalence of FA resistance (R) mechanisms among staphylococci (2008).

Methods: A total of 7,504 strains collected from 29 European (EU) medical sites located in 13 countries were susceptibility (S) tested by CLSI reference broth microdilution against FA and comparator agents. 336 *Staphylococcus* spp. (2008 only) displaying FA MIC at $\geqslant$2 mg/L were tested for the presence of fusB, fusC and fusD and mutations on fusA and fusE (FA primary and secondary active site).

Results: FA was very active against all staphylococci displaying a MIC50 of 0.12 mg/L regardless of methicillin-resistant (MR) profile. Applying EUCAST breakpoints (none available for CLSI), 90.7% of *S. aureus* (SA) strains were S to FA, with lower rates observed among MRSA (77.9%). Coagulase-negative staphylococci (CoNS) demonstrated 36.7% R against FA (14/867 *S. saprophyticus* with intrinsically elevated FA MIC). MRCoNS displayed 40.5% of FA-R. FA demonstrated marginal activity against enterococci and streptococci, with MIC50 values for β-haemolytic, group A, B and viridians group streptococci, *S. pneumoniae* and enterococci ranging from 4 to 8 mg/L. Among 336 staphylococci (FA MIC, $\geqslant$2 mg/L), the presence of acquired FA-R genes was detected in 64.9% of the strains (36.6% fusB and 28.3% fusC). fusB and fusC rates among FA-R strains were 10.4 and 17.3% for SA and 26.1 and 11.3% for CoNS, respectively. fusA mutations were detected in 57 of 62 FA-R SA, most common being aminoacid alterations on position 461 (Leu to Lys/Ser). One SA showed a mutation on fusE (Q140L). Ireland and Greece showed the highest SA FA-R rates with high prevalence of L461K fusA mutation (clinical outbreaks). Low staphylococci FA-R rates (1.4–3.1%) were observed in Israel, Italy, Poland, Spain and Sweden.

Conclusions: FA appears to be a valuable alternative to other anti-MRSA oral agents in the treatment of serious staphylococci infections. Despite the long term of FA clinical use in European countries, staphylococci R rates are still remarkably low except in clonal occurrences in a minority of institutions.

Organism (no. tested)	MIC (mg/L)		EUCAST[a]	
	50%	90%	S%	R%
S. aureus (3,898)	0.12	0.5	90.7	9.3
MSSA (2,894)	0.12	0.25	95.1	4.9
MRSA (1,004)	0.12	>8	77.9	22.1
CONS (867)	0.12	>8	63.3	36.7
MSCoNS (176)	0.12	8	78.4	21.6
MRCoNS (691)	0.25	>8	59.5	40.5
Beta-haemolytic streptococci (374)	8	>8	–	–
Group A streptococci (137)	4	8	–	–
Group B streptococci (160)	8	>8	–	–
Viridans Group streptococci (167)	>8	>8	–	–
S. pneumoniae (930)	8	>8	–	–
Enterococcus spp. (1,268)	4	4	–	–

[a] –: no interpretative criteria have been established.

P930 Antimicrobial activity of ceftaroline tested against *Staphylococcus aureus* from the United States and Europe, 2008–2009

H. Sader, G. Moet, R. Mendes, R. Jones (North Liberty, US)*

Objectives: To evaluate the potency and spectrum of ceftaroline against contemporary *S. aureus* strains and the correlation between SCCmec type and ceftaroline MICs. Ceftaroline is a novel, parenteral cephalosporin in late-stage clinical development for treatment of community-acquired pneumonia and complicated skin and skin structure infections. Ceftaroline exhibits broad-spectrum activity against Gram-negative and -positive organisms, including methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. pneumoniae*.

Methods: A total of 8742 unique clinical *S. aureus* strains were consecutively collected in 2008–2009 from 29 hospitals located in Europe (EU; 3899 strains) and 27 in the USA (4843 strains). Strains were tested for susceptibility (S) to ceftaroline and numerous comparators by CLSI broth microdilution methods. Additionally, 100 strains for which SCCmec types had been previously characterized by PCR were tested for S to ceftaroline and selected β-lactams.

Results: MRSA rates were 55.2% in the USA and 25.8% in EU, ranging from 0.8% in Sweden to 59.5% in Greece. Ceftaroline (MIC50, 0.25 mg/L) was 16-fold more active than ceftriaxone (MIC50, 4 mg/L) against methicillin-S *S. aureus* (MSSA) and showed potent activity against MRSA (MIC50 1 mg/L and MIC90, 1–2 mg/L; see Table). All MRSA were inhibited at ⩽2 mg/L of ceftaroline except 4 clonally related strains from a Greek hospital and one strain from Italy (MIC 4 mg/L for all). In EU and the USA, 37.1% and 34.6% of MRSA strains were resistant (R) to clindamycin, and 85.9% and 65.9% were R to levofloxacin, respectively. In contrast, trimethoprim/sulfamethoxazole (98.4–98.6% S), linezolid (100.0% S), and vancomycin (100.0% S) remained very active against MRSA. Ceftaroline MIC values were lowest among MRSA strains with SCCmec type IV, followed by those with SCCmec types II, III, and I.

Organism (no. of strains)	Cumulative % inhibited at ceftaroline MIC (mg/L) of:						
	⩽0.06	0.12	0.25	0.5	1	2	4
MSSA							
Europe (2895)	0.9	5.9	88.3	99.9	100.0	–	–
USA (2169)	0.7	4.5	88.4	99.9	100.0	–	–
MRSA							
Europe (1004)	0.0	0.0	2.2	31.6	83.9	99.5	100.0[a]
USA (2674)	0.0	0.1	1.1	34.3	94.7	100.0	–

[a] Only 5 strains (Greece and Italy).

Conclusions: Ceftaroline was very active against a large collection of MSSA and MRSA strains recently (2008–2009) isolated in EU and USA hospitals. Based on its favourable antimicrobial profile, ceftaroline is a very promising anti-staphylococcal therapeutic option widely applicable to EU and the USA. MRSA isolates with SCCmec type IV, which are found in USA pandemic clone USA300 and several clones circulating in EU, exhibited the lowest ceftaroline MIC results.

P931 Activity of oritavancin and comparators *in vitro* against standard and high inocula of *Staphylococcus aureus*

F. Arhin, I. Sarmiento, T. Parr Jr., G. Moeck (Ville Saint Laurent, CA)*

Objective: Inoculum size-dependent increases in minimum inhibitory concentrations (MICs) of some antibiotics have been linked with therapeutic failures. We studied the impact of inoculum size on the growth-inhibitory and killing activities of oritavancin (ORI) and comparators *in vitro* against *S. aureus* by broth microdilution and time-kill assays at clinically-relevant concentrations of drugs.

Methods: MIC determinations and time-kill assays followed CLSI guidelines. Tests were performed at standard inocula (10^5 CFU/mL) and high inocula (10^7 CFU/mL). Drugs tested were ORI, vancomycin (VAN), daptomycin (DAP) and linezolid. In time-kill assays, drugs were tested at static concentrations approximating their free peak (fCmax) and free trough (fCmin) in plasma when administered at approved doses for complicated skin and skin structure infections. ORI fCmax and fCmin were based on a single 1200 mg dose. The *S. aureus* (SA) strains used were ATCC 29213 (VAN susceptible), ATCC 43300 (methicillin-resistant SA [MRSA]), ATCC 700699 (VAN-intermediate SA [VISA]) and NRS 402 (VISA).

Results: ORI MICs were 16-fold higher for all strains at the higher inoculum relative to standard inoculum. MICs of comparators were 2- to 8-fold higher when tested at the higher inoculum. In time-kill assays, when tested at its fCmin and fCmax, ORI was bactericidal against ATCC 29213 and ATCC 43300 at standard and high inocula. At its fCmax, ORI was bactericidal at standard inoculum but had no effect at the higher inoculum against both VISA strains. At fCmin, ORI had no effect on the VISA strains at either inoculum size. VAN was bactericidal against ATCC 29213 and ATCC 43300 at both inoculum sizes at both fCmax and fCmin. VAN had no effect on the VISA strains at both inoculum sizes at its fCmin but was bactericidal and bacteriostatic at fCmax against both strains at standard and high inocula, respectively. DAP had no effect on all strains at its fCmin at both inoculum sizes. At its fCmax, DAP was bactericidal against ATCC 29213 and ATCC 43300 at both inoculum sizes. Against the VISA strains, DAP at both fCmax and fCmin had no effect at both inoculum sizes.

Conclusions: ORI activity was diminished against all *S. aureus* strains at high inoculum size. The activity of comparator antibiotics was also diminished butto a lesser extent. In infections with a high anticipated bacterial burden, alternative dosing of oritavancin or synergistic combinations with additional agents may be warranted.

Agent	Fold increase in MIC at higher inoculum relative to lower inoculum			
	ATCC 29213	ATCC 43300	ATCC 700699	NRS 402
Oritavancin	16	16	16	16
Vancomycin	2	2	2	2
Daptomycin	8	8	2	2
Linezolid	2	2	2	4

P932 Antimicrobial spectrum of activity of telavancin and comparator agents tested against methicillin-resistant *Staphylococcus aureus* recovered from United States and European hospitals over a 3-year sampling period (2007–2009)

R. Mendes, H. Sader, M. Janechek, R. Jones (North Liberty, US)*

Objectives: To monitor the activities of telavancin (TLV) and comparators against methicillin-resistant *S. aureus* (MRSA) collected from the United States (US) and Europe (EU) including Turkey, over a three year period (2007–2009). TLV was recently approved in the US and Canada for the treatment of adult patients with complicated skin and skin structure infections (cSSSI) caused by susceptible Gram-positive pathogens. This new lipoglycopeptide has shown potent *in vitro* activity against staphylococci, including multidrug-resistant (MDR) strains.
Methods: A total of 4,077 and 1,334 MRSA isolates were collected respectively from 42 and 29 hospitals in the US and EU (13 different countries). All isolates were submitted to a coordinator laboratory. Species identifications were confirmed by standard algorithms and, when necessary, by Vitek 2. Antimicrobial susceptibility testing was performed by CLSI methods (M07-A8, 2009). Interpretive criteria were those from EUCAST (2009), except for TLV where the susceptibility breakpoint approved by the US-FDA for *S. aureus* (⩽1 mg/L) was applied.
Results: MRSA were recovered from bacteremia (38.5%), pneumonia (18.0%), SSSI (36.2%) and other infection sites (7.3%). MRSA rates ranged from 56.2% in 2007 to 52.0% in 2009 in the US and from 28.3% in 2007 to 22.7% in 2008 in EU. Among EU countries, the MRSA rates varied considerably, ranging from 0.6% in Sweden to 64.7% in Greece. Overall TLV was consistently active against MRSA over the study period (MIC90, 0.25 mg/L; 100.0% susceptible; see table). Resistance to teicoplanin was noted among isolates from both regions, and resistance rates were highest in Turkey (19.5%) and Italy (4.1%). Daptomycin (DAP; MIC90, 0.5 mg/L), linezolid (LZD; MIC90, 2 mg/L) and trimethoprim/sulfamethoxazole (TMP/SMX; MIC90, ⩽0.5 mg/L) were also active against MRSA. Quinupristin/dalfopristin (Q/D) MIC90 values increased one doubling dilution among US and EU MRSA. Susceptibility rates to gentamicin were higher in the US (⩾95.6%) compared to EU (80.8%-86.9%).
Conclusions: Over a three year surveillance period TLV exhibited sustained potency against MRSA isolates from the US and EU. While vancomycin, DAP, LZD and Q/D were also active against these strains, overall TLV MIC90 values were at least 2-fold lower when compared to these agents. These data emphasize the importance of continued longitudinal surveillance to monitor the activities of marketed antimicrobial agents, mainly against MDR strains.

Antimicrobial agent	MIC_{90} (mg/L) / % susceptible[a]						
	US (no. tested by year)			Europe (no. tested by year)			Overall
	2007 (1,560)	2008 (1,277)	2009 (1,240)	2007 (366)	2008 (650)	2009 (318)	(5,411)
Telavancin	0.25/100.0	0.25/100.0	0.25/100.0	0.25/100.0	0.25/100.0	0.25/100.0	0.25/100.0
Vancomycin	1/>99.9	1/100.0	1/100.0	1/100.0	1/100.0	1/100.0	1/>99.9
Teicoplanin	⩽2/99.6	⩽2/99.6	⩽2/99.6	⩽2/98.6	⩽2/98.1	⩽2/97.8	⩽2/99.3
Daptomycin	0.5/99.9	0.5/99.6	0.5/100.0	0.5/100.0	0.5/100.0	0.5/100.0	0.5/99.9
Linezolid	2/99.9	2/100.0	2/99.8	2/100.0	2/100.0	2/100.0	2/99.9
Q/D	0.5/99.8	0.5/99.4	1/99.6	0.5/98.9	1/98.9	1/99.7	0.5/99.5
TMP/SMX	⩽0.5/97.4	⩽0.5/99.0	⩽0.5/98.3	⩽0.5/99.5	⩽0.5/98.9	⩽0.5/98.1	⩽0.5/98.3
Clindamycin	>2/63.0	>2/62.3	>2/67.3	>2/72.1	>2/62.9	>2/69.5	>2/64.8
Gentamicin	⩽2/95.6	⩽2/97.8	⩽2/97.4	>8/86.9	>8/80.8	>8/86.8	⩽2/93.6
Tetracycline	⩽2/93.9	⩽2/94.9	⩽2/93.3	⩽2/92.9	⩽2/80.9	⩽2/93.4	⩽2/92.3

[a] Interpretive criteria were those from EUCAST (2009), except for telavancin where the susceptibility breakpoint approved by the US-FDA for *S. aureus* (⩽1 mg/L) was applied.

P933 Increased vancomycin MIC among MRSA isolates in Malaysia: truth or myth?

N. Ahmad, S. Nawi, G. Rajasekaran, A. Husin, N. Rahman, N. Maning, M. Aziz (Kuala Lumpur, Selangor, Johor, Sabah, Penang, Kelantan, MY)*

Vancomycin has been the drug of choice for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA). Susceptible *Staphylococcus aureus* strains normally have vancomycin MIC values that ranged between 0.5 and 2 μg/mL. Even though methicillin-resistant *Staphylococcus aureus* (MRSA) with vancomycin MIC of 2 μg/mL is considered susceptible to vancomycin, treatment failures for strains with this MIC have been reported. The prevalence of *Staphylococcus aureus* clinical strains with high MICs is not known in Malaysian hospitals.
Objective: This study was conducted to determine the vancomycin MIC of *Staphylococcus aureus* isolates obtained from clinical samples from 6 major Malaysian hospitals.
Methodology: Methicillin-susceptible and methicillin resistant *Staphylococcus aureus* (MSSA and MRSA) strains isolated from invasive infections and non-colonizers were included in this study. The strains were collected sequentially between March and July 2009 from 6 major Malaysian hospitals. Vancomycin MICs were determined by broth microdilution method (CLSI) and E-test method.
Result: A total of 600 strains were included in this study, in which 300 were MSSA and 300 were MRSA. By both microdilution method, 87% of the MSSA isolates have vancomycin MIC of 0.5 μg/mL and 13% with MIC 1 μg/mL. No isolates showed MIC value of 2 μg/mL. Using E-test method, the vancomycin MIC of the MSSA ranged from 0.75 to 2 μg/mL, where 16% of the isolates have vancomycin MIC of 2 μg/mL. For MRSA isolates, using broth microdilution, the vancomycin MIC ranged from 0.25 to 1 μg/mL where 41% of the isolates showed MIC of 1 μg/mL. Using E-test method, the vancomycin MIC ranged from 0.5 to 2 μg/mL, where 31% of the strains showed MIC of 2 μg/mL.
Conclusion: Using broth microdilution test, all the MSSA and MRSA strains in this study showed MIC values of ⩽1 μg/mL. However, the percentage of isolates with vancomycin MIC 1 μg/mL was higher among MRSA compared to MSSA isolates. Using E-test method, two-fold higher vancomycin MIC values was observed, where MIC of 2 μg/mL was detected in both the MSSA and MRSA strains. However, the strains with MIC 2 μg/mL were much higher among MRSA compared to the MSSA strains. The clinical implication of dilution difference between these two methods is unknown. Further studies need to be carried out to determine which method can be relied on when making therapeutic recommendations.

P934 Is there a relationship between oritavancin *in vitro* microbiological parameters and clinical outcome in a 2007–2008 phase 2 study of complicated skin and skin-structure infections?

A. Belley, T. McClure, I. Sarmiento, F. Arhin, T. Parr Jr., G. Moeck (St. Laurent, CA)*

Objective: Oritavancin (ORI) is an investigational lipoglycopeptide in clinical development for complicated skin and skin structure infections (cSSSI) caused by Gram-positive organisms. A previous study has demonstrated a relationship between vancomycin minimal inhibitory concentration (MIC) or bactericidal activity with clinical outcome in the treatment of *Staphylococcus aureus* bacteraemia (J Clin Micro 2004; 42:2398–2402). We explored whether ORI MIC or a microbiological parameter from *in vitro* time-kill studies could distinguish *S. aureus* clinical isolates from patient cure or failure outcome groups in a recent Phase 2 study assessing ORI efficacy in cSSSI.
Methods: ORI MIC determinations and time-kill studies were performed on *S. aureus* isolates (n = 87) from the clinically-evaluable population of patients enrolled in a Phase 2, multicenter, double-blind, randomized study of ORI treatment of cSSSI. Time-kill kinetics were determined at 0.12 and 0.5 mg/L ORI to monitor rebound growth characteristics and assess killing at a bactericidal concentration, respectively. Logistic regression modeling (outcome = microbiological parameter) was performed on the following microbiological parameters: area under the bactericidal curve (AUBC) of log-transformed cfu/ml from 0 to 4 h ($AUBC_{0–4\,h}$) or 4 to 24 h ($AUBC_{4–24\,h}$), rate of kill between 0 and 4 h ($Slope_{0–4\,h}$) or mean of log-transformed cfu/ml at 0.5 h ($CFU_{0.5\,h}$) or 12 h ($CFU_{12\,h}$) to determine if isolates from the cure and failure clinical outcome groups demonstrated significantly different results. Mean values from isolates of each clinical outcome group are presented with standard deviation (SD).

Results: *S. aureus* isolates from both clinical cure and failure groups had ORI MIC distributions that were not significantly different using Fisher's exact test. Logistic regression modeling revealed that the $AUBC_{0-4\,h}$, $AUBC_{4-24\,h}$, $Slope_{0-4\,h}$, $CFU_{0.5\,h}$ and $CFU_{12\,h}$ derived from time-kill studies using either 0.12 mg/L or 0.5 mg/L ORI were not significantly different between these groups.
Conclusions: *In vitro* microbiological parameters derived from MIC and time-kill studies could not distinguish between isolates of *S. aureus* from the cure and failure clinical outcome groups in a recent cSSSI study. We propose that in the absence of resistance to ORI, underlying patient heterogeneity likely accounts for the therapeutic failure to ORI observed in the Phase 2 study.

Microbiological parameter derived from time-kill studies	Concentration of oritavancin used (mg/L)	Clinical outcome, mean (SD)	
		Cure, n = 43	Failure, n = 44
$AUBC_{0-4\,h}$ (log cfu/ml)·h	0.12	4.8 (1.3)	4.6 (1.3)
$AUBC_{4-24\,h}$ (log cfu/ml)·h	0.12	51.2 (19.8)	43.7 (25.7)
$CFU_{12\,h}$ (log cfu/ml)	0.12	4.2 (0.6)	4.2 (0.5)
$AUBC_{0-4\,h}$ (log cfu/ml)·h	0.5	1.8 (0.6)	1.9 (0.6)
$Slope_{0-4\,h}$ (log cfu/ml)/h	0.5	–3.4 (1.4)	–3.4 (1.5)
$CFU_{0.5\,h}$ (log cfu/ml)	0.5	4.3 (1.5)	4.3 (1.5)

P935 Antimicrobial susceptibility of *Staphylococcus capitis* from two Scandinavian hospitals

L. Buarø, T. Monsen, J. Afset, K. Bergh (Trondheim, NO; Umeå, SE)*

Objective: The aim of the study was to examine the antimicrobial susceptibility of clinical *Staphylococcus capitis* isolates from one Norwegian and one Swedish hospital.
Methods: A total of 54 clinical *S. capitis* isolates, 22 Norwegian and 32 Swedish, were identified by standard methods and examined for susceptibility to 15 antibiotics using agar disk diffusion (n = 11) and Etest (n = 4). Breakpoints applied were defined by the Norwegian Working Group for Antibiotics except for vancomycin (VA) and teicoplanin (TP) for which EUCAST breakpoints were used. Inducible clindamycin (CM) resistance was examined by double disk diffusion, and oxacillin (OX) resistance by mecA PCR. Screening for glycopeptide resistance was done by Etest macro method, brain heart infusion agar supplemented with VA 6 mg/L or TP 5 mg/L and Mueller-Hinton agar with TP 5 mg/L. Molecular typing was done by PFGE.
Results: Rates of reduced susceptibility among the Norwegian isolates were: chloramphenicol 4.5%, CM 36.4%, erythromycin 36.4%, fusidic acid 13.6%, gentamicin (GM) 40.9%, norfloxacin 31.8%, OX 50%, tetracycline 13.6%, co-trimoxazole 9.1%, and VA 13.6%. In the Swedish isolates reduced susceptibility was detected to the following antibiotics: GM 100%, OX 100%, TP 75%, and VA 90.6%. All isolates from both hospitals were susceptible to linezolid, rifampicin, tigecycline and quinupristin-dalfopristin. A positive glycopeptide resistance screening result was obtained in 78.1%-100% of the Swedish and 0–45.5% of the Norwegian isolates depending on the method used. Divergent results were observed between different glycopeptide resistance screening methods in 16 of the isolates. The Norwegian isolates showed considerable heterogeneity both with respect to antimicrobial susceptibility (16 different profiles) and genotype (16 PFGE patterns). In contrast, a high degree of homogeneity was observed with only three different susceptibility profiles and PFGE patterns in the Swedish collection of isolates.
Conclusion: The *S. capitis* strains studied showed considerable heterogeneity in antimicrobial susceptibility, but were uniformly susceptible to four of the drugs tested. Reduced susceptibility to vancomycin was detected in isolates from both hospitals. Discrepant results were observed between different glycopeptide resistance screening methods. Species specific antimicrobial susceptibility data for a larger collection of epidemiologically unrelated *S. capitis* strains are warranted.

P936 Comparison of oritavancin activity against *Staphylococcus aureus* isolates from a 2007–2008 phase 2 study and from 1999–2002 phase 3 studies of complicated skin and skin structure infection

G. Moeck, F. Arhin, J. Milata, T. McClure, K.J. Walker, T. Parr Jr. (Saint-Laurent, CA; Indianapolis, US)*

Objective: Oritavancin is an investigational lipoglycopeptide under study for treatment of serious Gram-positive infections. Its spectrum of activity includes methicillin-resistant *S. aureus* (MRSA). We compared oritavancin susceptibilities of *Staphylococcus aureus* from a phase 2 complicated skin and skin structure infection (cSSSI) study (single/infrequent oritavancin dosing; conducted between 2007–2008) to those from two phase 3 cSSSI studies (once-daily oritavancin dosing for 3–7 days; conducted between 1999–2002) to assess whether oritavancin potency was affected by concurrent changes in prevalence and epidemiology of MRSA between the studies.
Methods: Pathogens were isolated locally from patient specimens. A single isolate with the highest oritavancin minimum inhibitory concentration (MIC) per patient was studied, yielding a total of 682 strains (252 MRSA, 430 methicillin-susceptible *S. aureus* [MSSA]). Susceptibilities to oritavancin, vancomycin, teicoplanin, daptomycin, linezolid, oxacillin, quinupristin-dalfopristin, tetracycline, tigecycline, and trimethoprim-sulfamethoxazole were tested centrally by broth microdilution following CLSI M7-A7 guidelines. Oritavancin assays used 0.002% polysorbate-80 (as per 2009 CLSI M7-A8 guidelines).
Results: In the microbiologic intent-to-treat populations, MRSA incidence increased dramatically over time, from 30% in the phase 3 studies conducted in 1999–2002 to 57% in the phase 2 study conducted in 2007–2008. Oritavancin MIC90 against MRSA from the phase 2 study (0.12 mg/L; n = 103) was 2-fold lower than that from the phase 3 studies (0.25 mg/L; n = 149); oritavancin MIC90 values against MSSA (0.12 mg/L) were unchanged over time. Oritavancin potency was identical against MRSA and MSSA isolates from the Phase 2 study, with an MIC90 of 0.12 mg/L for both phenotypes.
Conclusion: Whereas the incidence of MRSA nearly doubled between 1999–2002 and 2007–2008 in studies of cSSSI, oritavancin exhibited equivalent or greater *in vitro* potency against recent isolates, regardless of methicillin resistance phenotype. This finding provides evidence of sustained anti-MRSA activity of oritavancin over time and suggests a potential role for oritavancin in treating serious MRSA infections in a contemporary hospital setting.

Study (years)	Organism (n)	MIC_{90} (mg/L)	Cumulative % inhibited at oritavancin MIC (mg/L)						
			⩽0.03	0.06	0.12	0.25	0.5	1	2
Phase 3 (1999–2002)	MRSA (149)	0.25	14.8	48.3	82.6	99.3	99.3	99.3	100
	MSSA (352)	0.12	18.5	57.7	91.2	100			
Phase 2 (2007–2008)	MRSA (103)	0.12	58.2	83.5	97.1	100			
	MSSA (78)	0.12	51.3	85.9	97.4	98.7	100		

P937 Evaluation of oritavancin and comparators against *Staphylococcus aureus* with varying susceptibility to vancomycin

C. Vidaillac, J. Parra-Ruiz, M. Rybak (Detroit, US)*

Objectives: Oritavancin (ORI) is a novel lipoglycopeptide antibiotic with activity against Gram-positive pathogens including glycopeptide-resistant isolates. In an effort to understand whether ORI could represent a therapeutic option for infections caused by resistant strains, we assessed the *in vitro* activity of ORI and relevant comparator agents against a collection of clinical isolates of heterogeneous vancomycin intermediate *S. aureus* (hVISA) and vancomycin resistant *S. aureus* (VRSA).
Methods: A collection of non-redundant clinical isolates of hVISA (n = 100) and VRSA (n = 10) strains were evaluated in this study. All hVISA isolates were previously characterized by population analysis and Macro Etest, as previously recommended by Wootton et al. All VRSA were selected from the NARSA collection. Susceptibility testing was performed in duplicate by broth microdilution following CLSI M7-A8 guidelines using vancomycin (VAN), ORI, daptomycin (DAP),

teicoplanin (TEC), cefoxitin (CFX) and oxacillin (OXA). Quality control was performed using *S. aureus* ATCC 29213. Four isolates, including 1 vancomycin intermediate *S. aureus* (VISA), 1 VRSA, 1 hVISA and 1 methicillin-resistant *S. aureus* DAP non susceptible were evaluated by time kill experiments (TK) against VAN, DAP, TEC and ORI at 4 and 8× MIC using a starting inoculum of 5–6 log CFU/mL. Bactericidal activity was defined as ⩾3 log10 kill compared to the starting inoculum.
Results: Susceptibility results are reported in Table 1. Overall, ORI MIC ranges were lower than other lipo/glycopeptide comparators. MIC values of the 4 isolates used in TK ranged between 0.12 to 1 mg/L for ORI, 1 to 64 mg/L for VAN, 2 to 8 mg/L for TEC and 0.25 to 2 mg/L for DAP. DAP and ORI were the most effective drugs against all tested isolates, exhibiting cidal activity within 8 h at either 4× and 8× the MIC. In contrast, neither TEC nor VAN were cidal against the VRSA and the MRSA daptomycin non susceptible isolates at 24 h. VAN was bacteriostatic at either 4× and 8× MIC against the hVISA strain. All tested antimicrobials exhibited cidal activity at 24 h against the VISA isolate.
Conclusions: Both ORI and DAP exhibited potent *in vitro* activity against isolates with reduced susceptibility to VAN. In TK, ORI and DAP at 4× and 8× MIC demonstrated rapid cidal activity against all tested isolates. Further investigations are warranted to better define the therapeutic value of ORI in the treatment of multi-drug resistant *S. aureus* infections.

Table 1. *In vitro* susceptibility of isolates in a large collection of hVISA/VRSA to oritavancin and comparators

Antimicrobials		Susceptibility values (in mg/L) of	
		hVISA (n = 100)	VRSA (n = 10)
ORI	MIC_{50}	0.25	0.5
	MIC_{90}	0.5	1
	Range	0.03–1	0.25–2
VAN	MIC_{50}	1	512
	MIC_{90}	2	512
	Range	0.5–2	64–1024
DAP	MIC_{50}	0.25	0.25
	MIC_{90}	0.5	1
	Range	0.12–1	0.12–2
TEC	MIC_{50}	1	64
	MIC_{90}	2	512
	Range	0.12–16	16–1024
OXA	MIC_{50}	128	64
	MIC_{90}	512	128
	Range	4–512	64–256
CFX	MIC_{50}	128	64
	MIC_{90}	256	256
	Range	8–>512	32–256

P938 Activity of ceftaroline against selected clinical isolates of *Staphylococcus aureus* from the Canadian Bacterial Sentinel Network

D. Farrell, A. McGeer, K. Green, D. Low (Toronto, CA)*

Objective: To evaluate the activities of ceftaroline against contemporary clinical isolates of *Staphylococcus aureus* collected in Canadian medical centres as part of the Canadian Bacterial Surveillance Network (CBSN). Ceftaroline is a novel, parenteral cephalosporin exhibiting bactericidal activity against Gram-positive organisms, including methicillin-susceptible and -resistant *S. aureus* (MSSA and MRSA), and common Gram-negative pathogens. CBSN has conducted cross-Canada *S. aureus* surveillance since 1987.
Methods: Susceptibility testing for ceftaroline and comparator antimicrobials was performed using CLSI broth microdilution methods on 591 isolates obtained from the CBSN (1999–2009) supplemented with 42 strains from the Network of Antimicrobial Resistance in *S. aureus* (NARSA) program. Community-acquired (CA) and hospital-acquired (HA) MRSA, MSSA, and vancomycin-intermediate *S. aureus* (VISA) isolates were included. Where possible, the most recent CBSN isolates were selected; number of isolates per year (year/n): 1999/1; 2002/92; 2003/62; 2004/40; 2005/56; 2006/25; 2007/195; 2008/110; 2009/10.
Results: Ceftaroline exhibited activity against all 633 strains and all MRSA, MSSA, and VISA subgroups (highest ceftaroline MIC, 2 mg/L) (Table). Ceftaroline had a lower MIC90 against MSSA strains (n = 105; MIC90, 0.25 mg/L) than against MRSA strains (n = 528, MIC90, 1 mg/L). Resistance to mupirocin, tetracycline, gentamicin, fusidic acid, erythromycin, or cotrimoxazole, and decreased susceptibility to vancomycin, did not affect ceftaroline activity against *S. aureus*.
Conclusions: Ceftaroline demonstrated potent *in vitro* activity against selected contemporary CBSN and NARSA *S. aureus* isolates, including strains with resistance to methicillin and/or reduced susceptibility to vancomycin. *S. aureus* resistance to commonly used antimicrobials did not adversely affect ceftaroline activity. These data highlight the potential utility of ceftaroline in treating *S. aureus* infections, including MSSA, MRSA, and VISA, in both community and hospital settings.

	n	Cumulative % at ceftaroline MIC (mg/L)					MIC (mg/L)	
		0.12	0.25	0.5	1	2	MIC_{50}	MIC_{90}
All	633	2.1	17.9	75.8	95.1	100	0.5	1
CA-MRSA	214	–	1.8	89.7	99.5	100	0.5	1
CA-MSSA	86	14	98.8	100	–	–	0.25	0.25
HA-MRSA	275	–	2.2	60.4	90.2	100	0.5	1
HA-MSSA	14	7.1	85.7	100	–	–	0.25	0.25
MR-VISA	39	–	2.6	43.6	92.3	100	1	1
MS-VISA	5	–	100	–	–	–	–	–

MR = methicillin resistant; MS = methicillin susceptible.

P939 Trends in ceftobiprole susceptibility among 2001–2008 consecutive methicillin-resistant *Staphylococcus aureus* blood isolates

Y. Golan, L. McDermott, D.R. Snydman (Boston, US)*

Objective: Treatment options for MRSA blood-stream infections remain limited. Resistance to the macrolides, fluoroquinolones, and tetracyclins is common and a recent MIC creep to vancomycin has been described. Ceftobiprole is an investigational cephalosporin with extended activity against MRSA. We describe trends in ceftobiprole susceptibility among consecutive blood isolates of MRSA from a tertiary-care hospital.
Methods: All 2001 through 2008 MRSA blood isolates were saved. Susceptibility to vancomycin and ceftobiprole was tested in duplicate using broth microdilution according to recommended CLSI methodology. CLSI reference strains *S. aureus* 29213 and *E. faecalis* 29212 served as quality controls. Ceftobiprole powder was supplied by Johnson & Johnson. Only the first isolate from each bacteremia episode was tested.
Results: A total of 385 MRSA isolates were included of which 32% were isolated from ICU patients and 68% from ward patients. Culture sources included peripheral blood (71%), central vascular catheter (25%) and arterial line (4%). Ceftobiprole MIC distribution was narrow with 99% of isolates exhibiting an MIC of 0.5–2μg/ml. Ceftobiprole MIC range, MIC50, and MIC 90 were 0.25–4μg/ml, 1micog/ml, and 2μg/ml, respectively. Ceftobiprole geometric mean MIC decreased during the study period from 1.02μg/ml in 2001–4 to 0.84μg/ml in 2005–8. In comparison, vancomycin geometric mean MIC has increased from 1.10μg/ml in 2001–4 to 1.22μg/ml in 2004–8. Higher vancomycin MICs were not associated with higher Ceftobiprole MICs. Ceftobiprole geometric mean MICs for isolates with a vancomycin MIC of ⩽1 μg/ml and 2 μg/ml were 0.93μg/ml and 0.97μg/ml, respectively. Ceftobiprole geometric MICs were higher among isolates from ICU patients as compared to ward patients (1.15μg/ml vs 0.94μg/ml, respectively) and similar among isolates from peripheral blood and central lines (1.00μg/ml and 0.97μg/ml, respectively).

Conclusions: Ceftobiprole susceptibility among MRSA blood isolates is within a tight MIC range and has not increased from 2001 to 2008. Ceftobiprole's activity is not affected by vancomycin MIC and it remains as active against isolates with a vancomycin MIC of 2μg/ml. Slightly higher Ceftobiprole MICs were observed in isolates from patients in the ICU.

P940 Effect of tigecycline against methicillin-resistant coagulase-negative staphylococci, with reduced susceptibility to glycopeptides, growing as planktonic and adherent cells

A. Grossato, C. Boldrin, A. Di Gabriele (Padua, IT)*

Objectives: In the present study the activity of tigecycline (Wyeth), a recently introduced drug, was investigated against a significant number of methicillin-resistant coagulase-negative staphylococci (CoNS-MR), belonging to different species: 58 *S. epidermidis*, 30 *S. haemolyticus,* 8 *S. hominis*. The majority of them showed reduced susceptibility to glycopeptides (2 *S. haemolyticus* were resistant to teicoplanin) and showed ability to produce biofilm (BF).
Methods: Non repetitive isolates of CoNS-MR, collected from high-risk units (Neonatal ICU, Surgery's Units, Burns Unit etc) and obtained from clinically significant specimens, were identified with API ID32 (Bio-Mérieux). PCR tests were performed to confirm methicillin-resistance (mecA) and to examine the presence of ica gene cluster, often found in adhesive strains. MICs and MBCs were determined traditionally by broth micro-dilutuion method. The MICs of BF forming bacteria were tested in a pre-formed 24hBF, using the static microtiter model. To quantify BF, before and after treatment with tigecycline, OD570 of stained BF were determined colorimetrically and the number of bacteria estimated by classical plate counting.
Results: All strains were mecA positive and their methicillin MIC range was 512–64 mg/L. The presence of ica locus was demonstrated in 87% of *S. epidermidis* and 40% of *S. haemolyticus.* As to BF formation all ica-positive strains were BF strains. MICs and MBCs of glycopeptide and tigecycline were summarized in Table 1 and in Table 2.
Conclusion: Tigecycline showed a good *in vitro* activity on CoNS-MR, including glycopeptide-intermediate-resistant isolates. MBCs against BF producing bacteria are similar to those determined against planktonic bacteria. These previous results indicate tigecycline as a likely drug for the therapy of BF-associated infections.

Table 1. *In vitro* activity of glycopeptides and tigecycline against planktonic CoNS-MR

Strains	MIC (mg/L)					
	Vancomycin		Teicoplanin		Tigecycline	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
S. epidermidis	2	16	2	4	0.06	1
S. haemolyticus	4	8	2	128	0.06	0.5
S. hominis	2	4	4	8	0.06	0.25

Table 2. MBCs of tigecycline against planktonic and BF-CoNS-MR

Strains	MBC (mg/L)			
	Non-BF CoNS MR		BF CoNS-MR	
	MBC_{50}	MBC_{90}	MBC_{50}	MBC_{90}
S. epidermidis	2	16	4	16
S. haemolyticus	0.5	8	1	8
S. hominis	1	4	2	8

Streptococci and pneumococci

P941 Antimicrobial susceptibility of Gram-positive non-urinary isolates to fosfomycin

M. Falagas, S. Maraki, D. Karageorgopoulos, A. Kastoris, A. Kapaskelis, G. Samonis (Athens, Heraklion, GR)*

Objective: We aimed to evaluate the antimicrobial activity of fosfomycin against Gram-positive non-urinary isolates collected at the microbiological laboratory of the University Hospital of Heraklion, Crete, Greece, in 2008.
Methods: We included in our study all Gram-positive clinical isolates originating from sites other than the urinary tract that were collected over a 1-year period (January to December 2008) at the microbiological laboratory of the 700-bed, University Hospital of Heraklion, Heraklion, Crete, Greece.
Results: Susceptibility testing was performed with the disk diffusion method for a total of 1846 isolates; 1275 (69.1%) were susceptible to fosfomycin. Specifically, 416/419 (99.3%) *Staphylococcus aureus* isolates [including 129/130 methicillin resistant *Staphylococcus aureus* (MRSA) isolates], and 745/961 (77.5%) coagulase-negative staphylococcal isolates were susceptible to fosfomycin. Among 42 *Streptococcus pneumoniae*, 64 *Streptococcus pyogenes*, and 93 other streptococcal isolates, 61.9%, 40.6%, and 48.4%, respectively, were susceptible. Fosfomycin was inactive against the 166 enterococcal isolates tested.
Conclusions: This old antibiotic may deserve consideration for further studies and use in clinical practice, especially for *Staphylococcus aureus* (including MRSA) infections.

P942 Spectrum of activity of oritavancin and comparator agents tested against subsets of vancomycin-resistant enterococci from the United States and Europe

R. Mendes, S. Putnam, H. Sader, R. Jones (North Liberty, US)*

Objectives: To evaluate the antimicrobial activities of oritavancin (ORI) and comparators tested against subsets of vancomycin-resistant enterococci (VRE) recovered from hospitalized patients in the United States (USA) and Europe (EU) through the International ORI Surveillance Program (2008–2009), which is part of the worldwide SENTRY Antimicrobial Surveillance Program.
Methods: Enterococci (2,841) were consecutively collected from the USA (28 sites) and EU (28 sites). Isolates were submitted to a monitoring laboratory where identifications were confirmed by standard algorithms and Vitek 2. Isolates were tested for susceptibility (S) by reference CLSI methods (M07-A8, 2009). Interpretive criteria were those from CLSI (M100-S19, 2009). The VanA phenotype was characterized by non-S to vancomycin (VAN) and teicoplanin (TEC), while the VanB phenotype was non-S to VAN and S to TEC. The VanC phenotype was based on species identification *(E. casseliflavus* and *E. gallinarum)*.
Results: Isolates were dominantly from bacteremia (61.5%), skin and skin structure (14.2%) and urinary tract infections (12.5%). 24.8% of strains were non-S to VAN, comprising 88.5% VanA, 6.7% VanB and 4.8% VanC phenotypes. 76.7% of VAN-non-S were from the USA. *E. faecium* (EFM) represented 92.4% and 58.3% of strains with VanA and VanB phenotypes, respectively. ORI was $\geqslant$4-fold more active against VAN-S EFM (MIC50, $\leqslant$0.004 mg/L) than VAN-S *E. faecalis* (EF; MIC50, 0.015 mg/L; see table). ORI activity against isolates displaying a VanB phenotype was similar to that against VAN-S isolates from the same species. ORI MIC90 values against VanA species were 16-fold higher than their respective S strains. Ampicillin (MIC90, 2 mg/L; 97.8% S), daptomycin (DAP; MIC90, 1 mg/L; 100.0% S) and linezolid (LZD; MIC90, 2 mg/L; 100.0% S) were also active against EF displaying a VanA phenotype, while DAP (MIC90, 2 mg/L; 99.7% S), LZD (MIC90, 2 mg/L; 98.8% S) and quinupristin/dalfopristin (MIC90, 1 mg/L; 95.2% S) were active against EFM with a VanA phenotype.
Conclusions: ORI exhibited very potent *in vitro* activity against this contemporary VRE collection from two regions. ORI MIC values were

elevated when tested against isolates displaying a VanA phenotype relative to VAN-S and VanB strains; however, all enterococci were inhibited by ORI at only 1 mg/L. In addition, ORI was at least 2- and 8-fold more active than the comparators when tested against EF and EFM with a VanA phenotype, respectively.

Organism phenotype	MIC (mg/L)		Number (cumulative %) of isolates inhibited by oritavancin MIC (mg/L) of:							
(no. Tested)	50%	90%	⩽0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5
E. faecalis										
VAN-S[a] (1,709)	0.015	0.03	35 (2.0)	443 (28.0)	767 (72.8)	379 (95.0)	75 (99.4)	7 (99.8)	2 (99.9)	1 (100.0)
VanA (46)	0.25	0.5	1 (2.2)	0 (2.2)	1 (4.3)	1 (6.5)	2 (10.9)	5 (21.7)	17 (58.7)	17 (95.7)
VanB (20)	0.015	0.03	–	7 (35.0)	9 (80.0)	4 (100.0)	–	–	–	–
E. faecium										
VAN-S[a] (421)	0.004	0.008	222 (52.7)	190 (97.9)	8 (99.8)	1 (100.0)	–	–	–	–
VanA (583)	0.03	0.12	11 (1.9)	42 (9.1)	56 (18.7)	200 (53.0)	208 (88.7)	58 (98.6)	6 (99.7)	2 (100.0)
VanB (28)	0.008	0.008	13 (46.4)	14 (96.4)	0 (96.4)	1 (100.0)	–	–	–	–
VanC (34)[b]	0.008	0.015	4 (11.8)	25 (85.3)	4 (97.1)	1 (100.0)	–	–	–	–

[a] VAN-S = vancomycin-susceptible. [b] Includes *E. casseliflavus* (11 isolates) and *E. gallinarum* (23 isolates).

P943 *In vitro* activity of retapamulin and 16 other antimicrobial agents against *Streptococcus pyogenes* clinical isolates

E. Tamayo, M. Montes, V. Iriarte, J.M. Garcia-Arenzana, E. Pérez-Trallero (San Sebastian, Tres Cantos, ES)*

Retapamulin is a novel semi-synthetic antimicrobial agent approved for topical treatment of impetigo and infected small lacerations, abrasions or sutured wounds in humans. It selectively inhibits bacterial protein synthesis through a novel mechanism of action. *Streptococcus pyogenes* and *Staphylococcus aureus* are the main pathogens isolated from skin infections.

Objectives: To evaluate *in vitro* activity of retapamulin and other 16 antibiotics used for skin and soft tissue infections, either of topical (fusidic acid, mupirocin, neomycin, bacitracin) or systemic (amoxicillin, erythromycin, cloxacillin, clindamycin, tetracycline) use against *Streptococcus pyogenes*.

Methods: Four hundred *Streptococcus pyogenes* clinical isolates obtained between January 2007 and December 2008 at Hospital Donostia were tested by broth microdilution test (Sensititre, Trek Diagnostics Systems, UK) performed and interpreted according to CLSI M100-S19 guidelines. A retapamulin breakpoint MIC of ⩽0.25μg/mL was used (CLSI has not interpretive standards for susceptibility).

Clinical isolates were obtained from skin lesions (n = 144) and other body sites (n = 256). Samples of resistant isolates (R) were included: Erythromycin-R (n = 187), clindamicin-R (n = 103), bacitracin-R (n = 45) tetracycline-R (n = 99), and fluoroquinolone-R (n = 59).

Results: All isolates were retapamulin susceptible with MICs ranging from 0.015μg/mL to 0.12 μg/mL, being the MIC_{50} = 0.03μg/mL and MIC_{90} = 0.06 μg/mL. Retapamulin had a higher intrinsic activity than that obtained for all other tested topical antibiotics. The only antimicrobial agents with an MIC_{90} ⩽ 0.12 μg/mL were the penicillins and retapamulin. MIC results of all tested antimicrobial agents are summarized in table1.

Conclusions: Retapamulin showed an excellent *in vitro* activity against *Streptococcus pyogenes* isolates obtained from different body sites including resistant and multiresistant isolates.

Table 1. MIC_{50} and MIC_{90} of retapamulin and other 16 antimicrobial agents against 400 *Streptococcus pyogenes* isolates

	MIC (μg/mL) skin isolates (n = 144)			MIC (μg/mL) isolates from other body sites (n = 256)			Total (n = 400)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Mupirocin	<0.12	0.25	<0.12–1	<0.12	0.25	<0.12–1	<0.12	0.25	<0.12–1
Bacitracin	1	2	<0.25–>32	1	32	<0.25–>32	1	32	<0.25–>32
Fusidic acid	4	8	2–16	4	8	0.25–32	4	8	0.25–32
Neomycin	32	>64	<8–>64	32	>64	<8–>64	32	>64	<8–>64
Gentamicin	4	4	<1–32	4	4	<1–>32	4	4	<1–>32
Erythromycin	<0.25	>32	<0.25–>32	8	>32	<0.25–>32	<0.25	>32	<0.25–>32
Josamycin base	<0.5	>4	<0.5–>4	<0.5	>4	<0.5–>4	<0.5	>4	<0.5–>4
Clindamicin	<0.25	>1	<0.25–>1	<0.25	>1	<0.25–>1	<0.25	>1	<0.25–>1
Lincomycin	<0.5	>4	<0.5–>4	<0.5	>4	<0.5–>4	<0.5	>4	<0.5–>4
Norfloxacin	<4	<4	<4–>32	<4	16	<4–>32	<4	16	<4–>32
Ciprofloxacin	<1	<1	<1–32	<1	2	<1–>4	<1	2	<1–32
Tetracycline	<2	>8	<2–>8	<2	>8	<2–>8	<2	>8	<2–>8
Tigecycline	<0.5	<0.5	–	<0.5	<0.5	–	<0.5	<0.5	–
Penicillin	<0.015	<0.015	<0.015–0.06	<0.015	<0.015	<0.015–0.03	<0.015	<0.015	<0.015–0.06
Amoxicillin	<0.03	<0.03	<0.03–0.12	<0.03	<0.03	<0.03–0.06	<0.03	<0.03	<0.03–0.12
Cloxacillin	0.12	0.12	<0.12–>0.5	0.12	0.12	<0.06–>0.5	0.12	0.12	<0.06–>0.5
Retapamulin	0.03	0.06	0.015–0.12	0.03	0.06	0.015–0.12	0.03	0.06	0.015–0.12

P944 *In vitro* susceptibility of *Streptococcus agalactiae*: results of the Tigecycline Evaluation Surveillance Trial (TEST), 2004–2008

D. Hoban, M. Hackel, M. Renteria, R. Badal, S. Bouchillon, B. Johnson, J. Johnson, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Background: *Streptococcus agalactiae* (GBS) is a major cause of neonatal and perinatal disease as well as a causative pathogen of bacteremia and occasional skin and skin structure infections and urinary tract infections. Although all GBS remain susceptible to penicillin, resistance to erythromycin and clindamycin has been documented. The Tigecycline Evaluation Surveillance Trial (TEST) examines the susceptibility of pathogens isolated from patients in countries worldwide.

Methods: Clinically significant GBS were obtained from the following infection sites: blood, urine, respiratory, skin and skin structure, intra-abdominal and gastrointestinal. MICs were determined for 6,428 isolates of GBS isolated from a cumulative total of 1258 sites in 55 countries during 2004–2008 using supplied broth microdilution panels. Results were interpreted according to FDA / CLSI guidelines.

Results: The MIC50 and MIC90 (mg/L) for 6,428 GBS versus comparative antimicrobial agents is shown in the table.

Conclusions: The MIC frequency distribution did not increase for any studied antimicrobial over the 5 year study period. Global GBS isolated from a variety of clinical specimens continued to demonstrate low MIC50 and MIC90 including ampicillin.

Drug	*S. agalactiae* – MIC_{50}/MIC_{90} (mg/L)				
	2004	2005	2006	2007	2008
Ampicillin	<0.06/0.12	0.12/0.12	0.12/0.12	0.12/0.12	<0.06/0.12
Ceftriaxone	0.06/0.12	0.06/0.12	0.06/0.12	0.06/0.12	0.06/0.12
Meropenem	⩽0.12/⩽0.12	⩽0.12/⩽0.12	⩽0.12/⩽0.12	⩽0.12/⩽0.12	⩽0.12/⩽0.12
Tigecycline	0.03/0.25	0.03/0.06	0.03/0.12	0.03/0.12	0.06/0.12
Vancomycin	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5
N=	926	1036	1442	1858	1166

P945 Susceptibility of *Streptococcus agalactiae* strains to lactic acid bacteria activity

M. Bodaszewska, T. Gosiewski, M. Brzychczy-Wloch, P. Adamski, M. Strus, P.B. Heczko (Cracow, PL)*

Objectives: The vagina of healthy women is a balanced ecosystem in which lactobacilli (Lactic Acid Bacteria; LAB) are predominant. *Streptococcus agalactiae* (Group B streptococci; GBS) is one of coexistent components of vaginal microflora. Antagonistic metabolites of LAB protect against multiplication of pathogenic microorganisms as well as GBS. Therefore, the aim of the study was the assessment of sensibility of GBS strains (in relation to capsular polysaccharides of GBS) to antagonistic activity of chosen species of LAB.

Materials and Methods: In the antagonism study 26 strains of GBS isolated from the vagina of pregnant and nonpregnant women (n = 18) and from newborns (n = 8) were tested. Characteristics of capsular polysaccharides were tested using serological kit and multiplex PCR method. Investigated GBS strains belonged to Ia, Ib, II, III or V serotypes. The antagonistic properties of LAB were tested by 4 species originating from the vagina of healthy women: *L. plantarum* (n = 3), *L. fermentum* (n = 2), *L. gasserii* (n = 2) and *L. rhamnosus* (n = 2) and 1 standard strain. Antagonism between LAB and GBS was tested in a mixture of fluid 24 hrs cultures and the results were determined quantitatively by serial dilutions in 3 time intervals (2 hrs, 4 hrs, 6 hrs).

Results:

1. Some strains of LAB have an ability to kill GBS already after 2 hrs.
2. Between isolates originating from women and newborns there were no statistically significant dependences in susceptibility of GBS strains to LAB activity in general. However, statistically significant dependence was found in susceptibility of both groups of GBS strains to *L. plantarum* (p = 0.019). GBS strains isolated from women were more susceptible to LAB than GBS isolates from newborns.

3. Statistically significant dependence was confirmed for sensitivity of GBS serotypes to LAB effect ($p = 0.0052$). GBS strains with serotype III were the least sensitive to LAB activity as opposed to serotype V ($p < 0.05$).
4. LAB were insensitive to GBS activity. Their number did not change during the study.

Conclusions: Sensitivities of GBS are not species attributes so they change among strains and depend on GBS serotype. Probably the ability of LAB species to rapidly kill GBS even after 2 hrs has resulted from a mixture of 24 hrs fluid cultures and activity of bacterial cells or bacterial metabolites. More studies are needed to elucidate the antibacterial property of *L. plantarum* and opportunity of its application as probiotic preparation.

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P946 Incidence and antibiotic sensitivity of *Streptococcus agalactiae* isolated from the genital tract of sexually active individuals

E. Ostorházi, B. Farkas, I. Bencze-Györi, B. Tóth, S. Kárpáti, F. Rozgonyi (Budapest, HU)*

Objectives: The aim of this study was to examine the frequency and antibiotic susceptibility of *Streptococcus agalactiae* cultured from genital discharges of sexually active individuals attended to our STD outpatient service.

Methods: Sample was taken with universal swab into transport medium then cultured on CROMagar and bood agar simultaneously in 5% CO2 thermostate at 35 Co for 24 h. *S. agalactiae* identification was made with conventional tests. Disc diffusion method was used for the determination of antibiotic sensitivity. Only one isolate one patient was considered.

Results: Between May 2008 and October 2009 a total of 416 *S. agalactiae* strains was obtained from urethra (164), vagina (173), glans-wound (97), ejaculate (14), urine (27) and anus (3). Resistance to tetracycline (89%), erythromycin (51%) and clindamycin (52%) proved to be extraordinary high. 45% of the strains was simultaneously resistant to tetracycline-macrolide-lincosamide combination, therefore, administering ex juvantibus any of them can select the cross-resistant strains to all these three classes of antibiotics. 97–96% of the strains were susceptible to all β-lactams and moxifloxacin, however, penicillin-resistant *S. agalactiae* appeared in Hungary in our patients, too.

Conclusions: Successful treatment of colonisation, carrying or manifest infection caused by *S. agalactiae* needs not only cultivation but also a prior antibiotic resistance testing since near half of the strains is multidrug resistant. In case of mix infections with *S. agalactiae* and *Chlamydia* or Ureaplasma, moxifloxacin seems to be the drug of choice since it is effective against all of them.

P947 *In vitro* anti-bacterial activity against *Streptococcus pneumoniae* strains isolated from respiratory infections in adults in France since 2002: annual analyses using new EUCAST breakpoints

H. Drugeon, A. Michaud-Nerard (Nantes, Beaucouze, FR)*

Objective: The objective of this pneumococci resistance survey is to assess the susceptibility to a range of antibiotics usually prescribed/recommended of *Streptococcus pneumoniae* (SP) strains isolated from adult respiratory tract infections in French hospital microbiology laboratories (36–42).

Methods: During each campaign, participating laboratories had to include 5 SP strains monthly from October to March. Minimal inhibitory concentrations were determined in a central laboratory (Drug R&D, Beaucouzé), by using a microdilution method against the following antibiotics: penicillin (PEN), erythromycin (ERY), amoxicillin (AMX), cefuroxime (CXM), telithromycin (TEL), pristinamycin (PRI) et levofloxacin (LVX). The rates of susceptible (S), intermediate (I) and resistant (R) strains were calculated in accordance with 2009 EUCAST recommendations (with French Microbiology Society –antiogram committee guidelines for PRI).

Results: 7074 strains have been studied. The PEN decreased susceptible SP strains rates decreased from 2002–2003 (53.9%) to 2005–2006 (42.1%) and then stabilised. The high level of AMX resistance was stable around 3%. Macrolides resistance was constantly decreasing from 54.2% to 35.5%. Resistance to LVX was currently less than <1%. In 2008–2009, TEL was active on 97.2% of strains and PRI on 99.9% respectively.

S/R percents are shown in the Table.

Conclusion: macrolides resistance rate is decreasing significantly whereas the PEN diminished resistance is stable around 42% and the diminished resistance to AMX 28%. LVX, PRI and TEL remain active on SP.

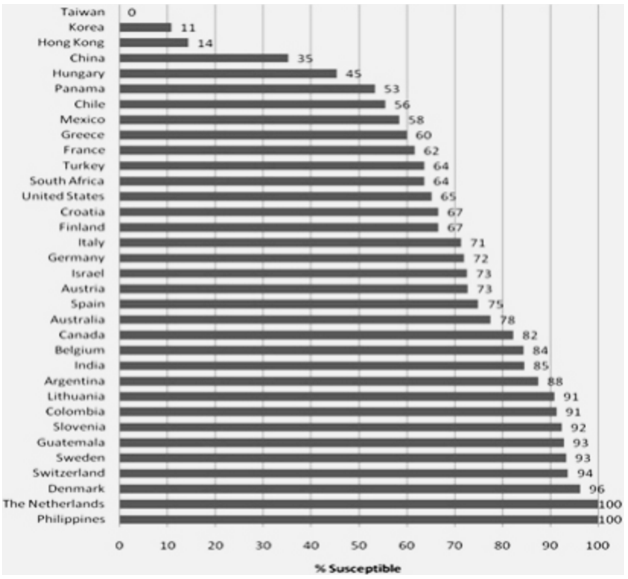
Campaign	n	Susceptible/Resistant (%)						
		PEN	AMX	CFM	ERY	TEL	LVX	PRI
2002–2003	991	46.1/3.8	63.4/3.6	53.3/44.7	45.8/53.4	95.6/2.4	98.6/1.4	100/0
2003–2004	1003	49.5/6.7	67.3/3.0	58.7/39.9	48.1/51.8	94.9/2.6	99.0/1.0	100/0
2004–2005	1022	50.6/1.2	69.4/2.5	59.3/38.0	49.5/50.2	95.9/1.6	99.1/0.9	99.6/0.1
2005–2006	1027	57.9/7.2	72.8/4.5	65.9/32.0	57/42.7	95.3/2.6	99.1/0.9	99.9/0
2006–2007	1024	55.8/5.6	68.3/2.8	63.9/34.2	54.5/45.3	93.1/3.8	98.9/1.1	99.9/0
2007–2008	1025	58.6/5.4	72.8/2.5	65.2/32.9	57/42.3	94.2/3.9	99.5/0.5	99.8/0
2008–2009	979	57.7/4.2	74.5/3.1	67.0/31.5	64.5/35.1	97.2/1.5	99.6/0.4	99.9/0
Total	7074	53.8/4.9	69.8/3.2	61.9/36.1	53.7/45.9	95.2/2.6	99.1/0.9	99.9/0

P948 Surveillance of macrolide activity against *Streptococcus pneumoniae* in a multi-country analysis – TEST 2007–2009

S. Bouchillon, J. Johnson, R. Badal, S. Hawser, M. Hackel, B. Johnson, D. Hoban, M. Renteria, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Objective: Macrolide susceptibility rates (%S) against *Streptococcus pneumoniae* (Spn) have remained relatively stable in the past decade between 65−70%. However, macrolide %S varies widely from country-to-country and region-to-region. The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) is an ongoing global surveillance designed to follow trends in antimicrobial activity. This report evaluates macrolide activity globally against *S. pneumoniae* during the years 2007 through 2009.

Methods: 2286 clinical isolates were collected from 424 cumulative investigative sites in 48 countries. Clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI and FDA (tigecycline) guidelines.



Results: Macrolide %S by country is presented in the graph.

Conclusions: The lowest macrolide susceptibility rates were seen in four Asian countries with %S ranging from 0% (Taiwan) to 35% (China). The highest macrolide %S rates were seen in Europe but rates there still ranged widely from 45% to 100%.

P949 Differences between adult and paediatric serotypes and activity of ceftaroline for recent US isolates of *Streptococcus pneumoniae*

M.R. Jacobs, S. Bajaksouzian, A.R. Windau, C.E. Good, D. Biek, I. Critchley, H. Sader, R. Jones (Cleveland, Oakland, North Liberty, US)*

Objectives: The activity of ceftaroline, an investigational parenteral cephalosporin, was studied against prevailing US adult and pediatric serotypes of *Streptococcus pneumoniae*. Ceftaroline has broad-spectrum activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *S. pneumoniae* (MDRSP), and common Gram-negative pathogens.
Methods: *S. pneumoniae* isolates were received in 2008 from 22 cities in 19 states throughout the US. Serotyping was performed by capsular swelling with commercial antisera. Serotype 6C was identified by PCR. MICs of ceftaroline, penicillin, amoxicillin, and ceftriaxone were determined by CLSI broth microdilution method.
Results: A total of 891 isolates were tested. Isolates came from all body sites, with distribution as follows: lower respiratory tract 58.9%, blood 15.6%, upper respiratory tract 8.4%, middle ear 7.4%, and other sites 9.7%. Serotype distribution by age for 859 strains with age data available is shown in the Table.
Ceftaroline was the most active of the tested agents against *S. pneumoniae*, with MICs ranging from ⩽0.008 to 0.5 mg/L and MIC90s of 0.12 mg/L for adult isolates and 0.25 mg/L for pediatric isolates. MIC90s were 2 and 4 mg/L for penicillin, 4 and 8 mg/L for amoxicillin, and 1 and 2 mg/L for ceftriaxone, with pediatric isolates less susceptible than adult isolates.
Conclusion: Pediatric and adult pneumococcal isolates differed widely in serotype distribution, with serotype 19A providing one third of isolates among children. Vaccine serotypes were uncommon in both age groups. Pediatric isolates were less susceptible to penicillin, amoxicillin, and ceftriaxone than were adult isolates. Ceftaroline was more active against *S. pneumoniae* than any other tested agent.

Serotype (%)	Adult isolates (N = 574)	Pediatric isolates (N = 285)	All isolates (N = 859)
Vaccine*	5.9	7.0	6.3
19A	15.0	32.6	20.8
3	10.5	6.3	9.1
35B	7.5	5.6	6.9
7F	7.0	3.9	5.9
11A	5.7	4.6	5.4
6C	4.5	4.9	4.7
15A	4.9	3.2	4.3
22F	4.9	2.5	4.1
23A	3.8	3.9	3.8
23B	3.8	3.5	3.7
Other	26.5	22.1	25.0

*7-valent conjugate vaccine serotypes.

Staphylococcus – resistance

P950 Impact of ciprofloxacin pre-exposure on emergence of rifampin resistance mutations in *Staphylococcus aureus*

P. Vaudaux, R.A. Villet, J.P. Didier, E. Huggler, D. Lew (Geneva, CH)*

Objectives: The high prevalence of nosocomial methicillin-resistant *S. aureus* (MRSA) isolates displaying widespread resistance to several antibiotic classes is explained by the strong selective pressure of widely used antimicrobial agents. In addition, DNA-damaging agents such as fluoroquinolones (FQ) might directly promote resistance-conferring mutations by activating error-prone, bacterial DNA repair pathways. This study evaluated the contribution of ciprofloxacin (CPX)-triggered, DNA repair systems, in promoting rifampin (RIF) resistance-conferring mutations in *S. aureus*.
Methods: FQ-susceptible and FQ-resistant, laboratory or clinical *S. aureus* isolates were exposed to sub-inhibitory (ranging from 1/2 to 1/8 MIC) concentrations of CPX at different growth phases in liquid cultures. Frequencies of RIF-resistant mutants in CPX-exposed compared to CPX-unexposed organisms were determined by selective plating on RIF (0.25 mg/L)-supplemented agar. MICs of individual, RIF-resistant mutants were determined by macrodilution, and correlated with the resistance-conferring mutations identified by RNA polymerase B subunit (rpoB) gene sequencing. Fitness costs of the different RIF-resistant mutants were evaluated by competitive *in vitro* growth with respective RIF-susceptible parents. CPX-triggered, key components of DNA repair pathways were monitored by real-time RT-PCR.
Results: The frequency of RIF-resistant clones on CPX-free pre-cultures of FQ-resistant and FQ-susceptible strains was ca. 10^{-7}, but increased dose-dependently by ca. 3–10 fold after exposure to sub-MICs of CPX. *In vitro* fitness costs of RIF-resistant mutants, as well as their increased RIF MICs, were strongly influenced by the nature of each rpoB mutation. rpoB sequencing in >200 RIF-resistant colonies revealed a wide diversity of RIF resistance-conferring point mutations. In addition, some sub-MICs of CPX triggered a high frequency of chromosomal insertions or deletions in rpoB, which led to low-level RIF-resistant mutants. In contrast, >10-fold lower frequencies of RIF-resistant mutants occurred in a recA-null mutant grown in the presence or absence of CPX.
Conclusion: While exerting a moderate quantitative increase on rates of RIF resistance, sub-MICs of CPX may also trigger substantial, unanticipated qualitative changes in *S. aureus* genomic composition, which may be due to the SOS error-prone response, and potentially other, currently studied DNA repair systems.

P951 Differential expression of proteins in MRSA-15 after treatment with manuka honey investigated by 2D electrophoresis

R. Jenkins, R. Cooper, N. Burton (Cardiff, UK)*

Objectives: To use 2-D electrophoresis coupled with MS/MS MALDI-TOF to investigate changes in protein expression of meticillin resistant *Staphylococcus aureus* 15 (MRSA-15) cells treated with manuka honey.
Methods: EMRSA-15 (NCTC 13142) was cultivated at 37ºC for 4 hours in TSB with and without 10% (w/v) manuka honey, and in TSB with 10% (w/v) artificial honey (to determine if results seen were due to sugars). Cells were harvested, treated in a bead beater and cell free extracts containing 150 μg of protein were separated by 2D electrophoresis using pH 3–10 IPG strips for isoelectric focussing followed by 4–12% polyacrylamide gel electrophoresis in the second dimension. After staining, images were recorded using a UVP AutoChemi gel doc system and analysed using PDQuest software. Triplicate samples were analysed. The spot showing highest differential expression (down regulation) was then analysed by MALDI-TOF MS/MS.
Results: Spot patterns from honey treated cells were distinct from those of untreated cells and cells treated with artificial honey. Compared to untreated samples, 82% and 70% of proteins matched for artificial honey and manuka honey, respectively. The protein which was most highly down regulated after honey treatment compared to untreated and artificial treatments was identified as universal stress protein A (uspA), by combined PMF and MS/MS queries on the MASCOT database search engine embedded into the Global Proteome Server explorer software on the Swiss-Prot database. Six peptides were identified ($E < 0.05$).
Conclusion: Changes in protein expression in manuka honey treated MRSA were not caused by sugars alone. The uspA protein confers a general stress endurance activity on cells and its down regulation could indicate a potential mode of for manuka honey. The differential expression of other proteins must be further investigated by MALDI-TOF MS/MS.

P952 Impact of daptomycin resistance on the susceptibility of methicillin-resistant *Staphylococcus aureus* to gentamicin, rifampicin and vancomycin

J.M. Entenza, M. Giddey, J. Vouillamoz, P. Moreillon (Lausanne, CH)*

Objectives: Several studies have shown that decreased daptomycin (DAP) susceptibility in *Staphylococcus aureus* strains is correlated with alterations in a few loci, including dlt (responsible for D-alanylation of lipoteichoic acids), mprF (responsible for the addition of lysine to phosphatidylglycerol in the plasma membrane), yycG (encoding a sensor histidine kinase involved in both cell wall and cell membrane metabolism) and mutations in rpoB/C subunits of the RNA polymerase, identified in later stages of DAP resistance. Modifications in both dlt and mprF of DAP-resistant (DAP-R) derivatives enhance the positive charge of the cell envelope and could also affect the activity of positively-charged molecules such as gentamicin (GEN). Likewise, mutations at specific sites of rpoB are associated with rifampicin (RIF) resistance, and thus DAP-R related mutations in this locus (and maybe rpoC) might affect RIF susceptibility as well. Since GEN and RIF are maybe used as partner drugs in combination therapy against methicillin-resistant *S. aureus* (MRSA) infections, we determined whether the *in vitro* development of DAP resistance in MRSA could have an impact on the susceptibility of GEN and RIF. VAN, for which a decreased susceptibility was previously observed in *S. aureus* with decreased DAP susceptibility, was used as control.
Methods: Four MRSA clinical isolates were selected for DAP-resistance by *in vitro* serial passage (for 7 days) on increasing concentrations of the drug. The MIC of DAP was recorded after each passage, and the MICs of VAN, GEN and RIF were recorded in DAP-R derivatives at the end of the selection process.
Results: MICs at baseline and after *in vitro* serial DAP exposures are presented in the Table. As previously shown, selection for DAP-R was paralleled by an increase in VAN MIC, resulting in VAN-intermediate *S. aureus* (VISA). Likewise, all four DAP-R MRSA derivatives displayed consistent 2- to 4-fold increase in GEN MIC. In contrast, RIF MICs did not change for two isolates, and increased only by 2-fold in the two other strains.
Conclusions: Development of DAP-R in *S. aureus* is readily associated with decreased susceptibility to VAN and GEN, but is less likely to affect susceptibility to RIF.

	MICs (mg/L) for DAP-susceptible/DAP-resistant MRSA			
	MRSA-1	MRSA-2	MRSA-3	MRSA-4
DAP	0.5/8	0.25/8	0.5/8	0.5/4
VAN	1/4	1/4	1/4	2/4
GEN	16/32	1/4	1/4	0.25/0.5
RIF	0.008/0.016	0.008/0.008	0.004/0.008	0.016/0.016

P953 Reversal of methicillin resistance in *Staphylococcus aureus* by thioridzine: a universal phenomenon?

M. Poulsen, J.K. Klitgaard, E.M. Lillebæk, M. Skov, B.H. Kallipolitis, H.J. Kolmos (Odense, DK)*

Objectives: Thioridazine, a phenothiazine agent, has been shown to reverse oxacillin (methicillin) resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro*. The molecular mechanism underlying this effect appears to involve a reduced transcription of the mecA gene, resulting in a reduced protein level of PBP2a. So far this has been shown with only one clinical MRSA isolate. The aim of this study was to determine if resistance reversal with thioridazine could be reproduced in a broader selection of clinical isolates, and if the molecular mechanism was the same in all isolates. To this end, we tested the effect of thioridazine on all major epidemic clones of MRSA and Staphylococcal cassette chromosome mec (SCCmec) types.
Methods: Growth of MRSA isolates was examined in liquid media in the presence of the methicillin analogue oxacillin and the non-antibiotic thioridazine alone and in combination. Furthermore, the transcription of mecA was analyzed by Primer extension under the same conditions.
Results: We observed an increased susceptibility of MRSA towards oxacillin in the presence of oxacillin and thioridazine compared to bacteria grown with oxacillin or thioridazine alone. Transcription of mecA was reduced with increasing concentrations of thioridazine in the presence of oxacillin compared to bacteria grown with oxacillin or thioridazine alone. However, in a few isolates mecA was transcribed constitutively and did not display a reduced transcription of mecA with increasing concentrations of thioridazine in the presence of oxacillin. Thioridazine itself did not affect the growth of MRSA or the level of mecA mRNA.
Conclusion: Results from the present study indicate that the reversal effect of thioridazine on oxacillin resistance is universal for clinical MRSA isolates of various epidemiological origins. Furthermore, the data indicate that for most of the tested isolates, reversal of methicillin resistance by thioridazine in MRSA may be explained by a reduced transcription of the methicillin resistance gene, mecA. However, for a few isolates, the reversal effect could not be explained by a reduction in the level of mecA mRNA, indicating that the molecular mechanism behind the reversal effect of thioridazine is far more complex than first anticipated.

P954 Site-specific mutation of *Staphylococcus aureus* VraS reveals a crucial role for the VraR-VraS sensor in the emergence of glycopeptide resistance

E. Galbusera, A. Renzoni, D. Andrey, A. Monod, C. Barras, P. Tortora, A. Polissi, W. Kelley (Milan, IT; Geneva, CH)*

An initial response of *Staphylococcus aureus* to encounter with cell wall-active antibiotics occurs by transmembrane signaling systems which orchestrate changes in gene expression to promote survival. Histidine kinase two-component sensor-response regulators such as VraRS contribute to this response. In this study, we have focused on elucidating molecular details of the VraS membrane sensor phosphotransfer signal transduction. We have used *in vitro* autophosphorylation assay with purified VraS[64–347] lacking its transmembrane anchor region and tested site-specific kinase domain histidine mutants. We have identified VraS H156 as the probable site of autophosphorylation. Using phosphotransfer assay, we have shown 32P transfer *in vitro* from VraS to purified VraR response regulator, but not to a mutant VraR (D55A) lacking the phosphate acceptor aspartate. To examine the functional consequences of the specific loss of VraS-VraR signaling, we site-specifically engineered a *S. aureus* chromosomal mutation, vraS H156A. Comparison of the mutant H156A strain and its isogenic vraS wild type parent by northern blot and qRT-PCR following exposure to cell wall active antibiotics revealed a pronounced reduction, but not complete abrogation, of transcriptional induction of the vraRS operon promoter. This result suggests that additional inducing signals, independent of VraS-driven phosphotransfer, exist for this promoter. Genetic studies show that the vraSH156 strain is severely impaired in its ability to generate first step reduced susceptibility teicoplanin and vancomycin mutants. We also extended our analysis to derivatives of *S. aureus* strains Newman and COL constructed by bacteriophage-mediated transduction of the vraS H156A allele. Collectively, our results reveal important details of the VraRS TCS signaling system and reinforce the prediction that blockade of sensor kinase activity will have profound effects on the emergence of cell wall active antibiotic resistance in this organism.

P955 Importance of efflux systems on the resistance to fluoroquinolones in *Staphylococcus aureus*

S.S. Costa, C. Falcão, M. Viveiros, J. Melo-Cristino, L. Amaral, I. Couto (Lisbon, PT)*

Objectives: To study the role played by efflux pumps in the resistance towards fluoroquinolones in clinical isolates of *Staphylococcus aureus*.

Methods: The efflux pump activity of 52 *S. aureus* clinical isolates from a Portuguese Hospital showing fluoroquinolone resistance was evaluated by the Ethidium Bromide-Agar Cartwheel Method. All efflux-positive and a group of efflux-negative isolates was selected for further characterization by PFGE and minimum inhibitory concentration determination to different efflux pumps substrates (fluoroquinolones, dyes and biocides), in the presence and absence of several efflux pump inhibitors (EPIs – thioridazine, chlorpromazine, verapamil, reserpine, carbonyl cyanide m-chlorophenylhydrazone). The presence and expression of the genes coding for the most important *S. aureus* efflux pumps (norA, norB, norC, mepA, mdeA, sepA, qacA/B, and smr) were assessed by PCR and qRT-PCR, respectively. The variability of the norA gene and its promoter region was studied by DNA sequencing. Mutations that confer resistance to fluoroquinolones were screened by sequencing the quinolones-resistance determining regions (QRDR) of grlA and gyrA genes.
Results: The application of these different approaches detected increased efflux pump activity on 12 out of the 52 clinical isolates tested and correlated this activity with increased resistance to fluoroquinolones. The inhibition of the active efflux systems did not result in reversion of the resistance phenotype to susceptibility, yet it implied a significant decrease in the resistance levels to these antibiotics, regardless of the type(s) of mutation(s) found in grlA and/or gyrA genes.
Among the efflux-positive isolates, over-expression of mepA, mdeA and norB/norC was detected. Two norA alleles were found (norAI and norAII), of which norAI was the prevalent one. However, no direct correlation could be established between the type of norA allele and the susceptibility profile towards the different efflux pump substrates tested.
Conclusions: The results obtained in this work underline the contribution of efflux systems for the emergence of high-level resistance to fluoroquinolone and multidrug resistant *S. aureus* in hospitals and reveal that pumps other than NorA are involved in this type of resistance.

P956 Interactions of oritavancin with Lipid II and interpeptide bridge-containing Lipid II variants

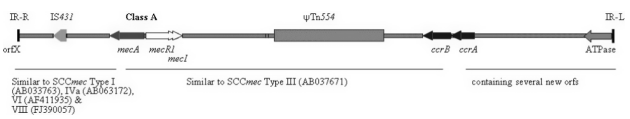
T. Schneider, A. Müller, A. Far, G. Moeck, H.G. Sahl (Bonn, DE; St Laurent, CA)*

Objectives: Oritavancin is a semi synthetic derivative of the glycopeptide chloroeremomycin with activity against Gram-positive pathogens, including vancomycin-resistant staphylococci and enterococci. Contrary to vancomycin, binding of oritavancin (ORI) to the cell wall precursor Lipid II appears to involve, in addition to the D-Ala-D-Ala terminus, the interpeptide crossbridges, as seen by NMR. We studied the impact of ORI and of its des-N-methylleucyl variant (des-ORI), which is unable to bind to the D-Ala-D-Ala terminus, on staphylococcal interpeptide bridge formation and Lipid II transglycosylation/transpeptidation.
Methods: The impact of ORI and des-ORI on pentaglycine interpeptide bridge formation and on polymerisation of Lipid II and Gly1–5 containing Lipid II variants was studied *in vitro* using recombinant FemXAB and PBP2 from *S. aureus* (Schneider et al., AAC 2009; 53: 1610). The glycopeptides were tested in molar ratios ranging from 0.25 to 4 with respect to radiolabelled Lipid II. Reaction products were extracted and analysed by thin layer chromatography and subsequent phosphoimaging. Binding constants of ORI to the different Lipid II species were determined using liposomes doped with the Lipid II variants and [14C]-labelled ORI.
Results: All Fem enzymes and PBP2 were inhibited by ORI in a dose-dependent fashion. Similarly, des-ORI showed inhibitory activity in the FemXAB-catalyzed synthesis of Lipid II-Gly1–5 (8% residual Lipid II-Gly5 formation with ORI, as compared to 12% residual production with des-ORI; antibiotic:Lipid II ratio of 2:1). Moreover, des-ORI was inhibitory in the PBP2 transglycosylation/transpeptidation assays. ORI caused cell wall precursor accumulation in VanA type enterococci prompting additional studies with lipid II variants carrying a D-Ala-D-Lac terminus and a pentaglycine-bridge attached.
Conclusions: The data verify the additional binding interactions between ORI and Lipid II and provide further evidence that binding of Lipid II involves both the D-Ala-D-Ala terminus and the pentaglycine interpeptide bridge. Multiple interactions between ORI and cell wall precursors, as demonstrated here, predict a reduced propensity for target pathogens to develop mutational resistance to ORI.These studies offer further experimental support to the observations that ORI inhibits transglycosylation/transpeptidation and causes accumulation of cell wall precursors even in VanA type vancomycin resistant organisms.

P957 A new SCCmec element in a *Staphylococcus cohnii* clinical isolate might be an ancestral structure of type III SCCmec

Z. Zong, Y. Gao, X. Lv (Chengdu, CN)*

Objective: To characterize a "non-typeable" SCCmec in a *Staphylococcus cohnii* clinical isolate.
Methods: The species identification for methicillin-resistant *S. cohnii* clinical isolate WC28 was performed by sequencing the 16s rDNA gene. The detection of mecA gene and typing for SCCmec were carried out using multiplex PCR (mPCR) as described previously (J Clin Microbiol 2005; 43: 5026–33.). Universal primers for amplifying ccrA and ccrB genes were designed on the basis of the sequence alignment of all known variants, which were retrieved from GenBank. A universal primer for left side inverted repeat (IR-L) of SCCmec was deigned according to known variants of SCCmec. The whole SCCmec was obtained by three long-range PCR (Fermentas, Burlington, ON, Canada) amplifying regions from mecA to orfX, from ccrA/B to mecA and from IR-L to ccrA/B and sequencing. The sequence close to IR-L was identified using inverse PCR and HindIII-restricted WC28 genomic DNA was self-ligated and used as template.
Results: WC28 can grow on agar plates containing 4 mg/L cefoxitin and has a class A mecA complex (mecI-mecR1-mecA). However, SCCmec of WC28 can not be typed by the mPCR method used, suggesting that WC28 might harbor a new SCCmec element. The ccrA and ccrB genes of WC28 were obtained using the newly-designed universal primers and displayed the highest similarity (89%) with ccrASHP (GenBank accession no. EU934095) of *Staphylococcus haemolyticus* and ccrB5 (AM904731) of *Staphylococcus pseudintermedius*, respectively. The SCCmec of WC28 was fully characterized, ca. 35-kb in length, flanked by 8-bp perfect IR and generating 12-bp direct target repeat. Several new ORFs including a putative AAA-ATPase-encoding gene were identified in the J1 region (from IR-L to ccr). The J2 region (from ccr to mec) is almost identical to the corresponding part of SCCmec III (AB037671) and has a PsiTn554 transposon, while the J3 region (from mecA to orfX) is the same as that in SCCmec type IV (AB063172) containing a single copy of IS431.
Conclusions: WC28 has a new SCCmec element sharing similar features with several known SCCmec including Type III and IV. As the structure downstream of mecA in WC28 is also seen in a few different types of SCCmec and type III SCCmec appears to be a hybrid of two different SCC elements, the SCCmec of WC28 might be an ancestral structure of the widespread type III SCCmec in the methicillin-resistant *Staphylococcus aureus*.



P958 Endemy of methicillin-resistant *Staphylococcus capitis* in French neonatal intensive care units

F. Laurent, O. Raulin, D. Fournier, H. Salord, M. Bonfils, M. Bes, C. Roure-Sobas, J. Grando, J. Picaud, F. Vandenesch, S. Tigaud (Lyon, FR)*

Coagulase negative staphylococci (CoNS) are frequently isolated from blood culture in critically ill neonates. *Staphylococcus capitis* has not been reported as the predominant species responsible for such infections.

However, from January 2004 to June 2009 at the neonatal ward of the Northern Hospital Group in Lyon, a total of 386 CoNS isolates were detected in 478 positive blood cultures (81%), including 209 *S. capitis* in 136 neonates, i.e., 54% of all CoNS positive cultures. Almost all isolates (99%) were resistant to methicillin and gentamicin. Seventeen of these methicillin resistant *S. capitis* (MRSC), randomly chosen from blood of neonates over the 2005 to 2009 period, were compared using pulsed-field gel electrophoresis (PFGE) with 33 *S. capitis* strains collected from 6 other French hopsitals: i) 22 MRSC isolated from blood of neonates collected in six French hospitals; ii) 11 MRSC and 3 MSSC isolated from adults and children (more than 2 years old).
Whereas all isolates from adults revealed unrelated PFGE profiles, the PFGE profiles of the 39 MRSC from neonates clustered, showing more than 80% of homology. Likewise, 15 MRSC isolates were studied by analysing SCCmec: the eight isolates from neonates (from 6 different hospitals) revealed SCCmec type V whereas the seven MRSC epidemiologically unrelated had different ones.
Our data highlight the fact that a single clone is circulating in different neonatal wards in France. At our knowledge, it is the first nationwide endemic spread of MRSC. Investigations are underway to i) identify risk factors associated to MRSC infections in neonates ii) identify the sources and/or reservoir of this clone; iii) investigate the potential spread of this clone in other European countries.

P959 Genetic relationship between methicillin-resistant and methicillin-susceptible *Staphylococcus pseudintermedius* isolates

J. Mikalsen, J. Slettemeås, H. Tharaldsen, M. Sunde (Oslo, NO)*

Objectives: In 2008, 200 β-haemolysin-producing staphylococcal isolates from canine infections were included in the Norwegian monitoring programme for antimicrobial resistance in the veterinary sector (NORM-VET). The isolates originated from clinical submissions from dogs in five geographically distinct regions of Norway. This strain collection was supplemented with methicillin resistant isolates (MRSP) strains from the national diagnostic reference laboratory. Little is known about spread of methicillin susceptible *S. pseudintermedius* (MSSP) in the dog population, and knowledge about the phylogentic relation between MSSP and MRSP isolates is sparse. To investigate this further we have applied molecular typing to a selection of MSSP and MRSP strains for comparison of resolution and identification of successful lineages in the Norwegian dog population.
Methods: Susceptibility was determined with a broth microdilution method (VetMIC™). Identification of *S. pseudintermedius* was performed by pta amplification/MboI restriction, detection of mecA was performed by PCR. Selected isolates were screened for reduced susceptibility to disinfecting agents. Molecular typing was carried out using Multi Locus Sequence Typing (MLST) and Pulsed Field Gel Electrophoresis (PFGE).
Results: A large proportion (92%) of the isolates included in the surveillance program was identified as *S. pseudintermedius.* Resistance to oxacillin was not detected among these strains, having oxacillin minimum inhibitory concentrations (MICs) of 0.125 mg/L to 1 mg/L. The MRSP strains from the diagnostic laboratory had oxacillin MICs from 1 to 32 mg/L. None of the investigated isolates exhibited increased tolerance to disinfecting agents.
Preliminary typing results indicate presence of at least two successful MSSP clusters containing strains isolates from dogs in distinct geographical areas. Moreover, our data indicates presence of related strains, with and without the mecA gene, within the same linage.
Conclusion: The investigated MRSP strains exhibit overlapping oxacillin MIC distribution to the wild type distribution, this represents a challenge for identification of true MRSP isolates based on phenotypical criteria. New methodologies with different resolution indicate the existence of some clusters representing possible successful lineages. However, more cost efficient typing methods are needed for implementation for epidemiological surveillance and risk assessments.

P960 Correlation between superantigenic toxin gene profiles with molecular types of 157 methicillin-resistant *Staphylococcus aureus* isolates from Taiwan

W. Wang, J. Lu, J. Sun (Miao-Li, Taichung, Taipei City, TW)*

Background: *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is a major cause of bacteraemia and sepsis. It also produces various exotoxins that cause food poisoning and toxic shock syndrome with high morbidity and mortality.
Objectives: The goals of this study were to delineate the superantigenic toxin gene profiles and to correlate them with molecular types of clinical MRSA isolates from Taiwan.
Methods: One hundred and fifty-seven MRSA isolates from patients with bacteraemia were obtained from the collection of Study Monitoring Antimicrobial Resistance Trends (SMART). Eighteen superantigen toxin genes were examined by multiplex PCR. MRSA isolates were typed by PCR based on polymorphisms in accessory gene regulator (agr) and staphylococcal cassette chromosome mecA (SCCmec). Correlations between toxin gene profiles and molecular types were determined by Fisher's exact test.
Results: Sixteen superantigenic toxin gene profiles were found in 157 non-duplicate MRSA isolates with the mecA gene. Of these, 95 (60.5%) isolates possessed sea-selk-selq, 17 (10.8%) isolates had seb-selk-selq, and 12 (7.6%) isolates contained selk-selq. Significant associations were found between sea-selk-selq and SCCmec III-agr I, seb-selk-selq and SCCmec IV- and VT-agr I, and selk and SCCmec III-agr I ($p < 0.001$). By comparing molecular types with each toxin gene, sea, selk, and selq were found to be associated with SCCmec III and agr I, and seb, sec, seg, sei, selm, seln, selo, selp, and tst1 were associated with SCCmec II, IV, and agr II ($p < 0.001$). No isolates harbored see, seh, selj, or selr.
Conclusions: Limited superantigenic toxin gene profiles were identified in MRSA isolates from Taiwan. Significant associations between toxin gene profiles and particular molecular types were noted.

Table 1. Association of superantigenic toxin gene profiles with molecular types

Superantigenic toxin gene profiles	SCC*mec* type	*agr* type	No. (%)
None	V_T	I	3 (1.9)
selk-selq	III	I	12 (7.6)
sed-seg-sei-sell-selm-seln-selo-selp-tst1	II	II	1 (0.6)
sec-seg-sell-selm-seln-selo	IV	I	1 (0.6)
sec-seg-sei-sell-selm-selo	IV	II	2 (1.3)
sec-seg-sei-sell-selm-selo-selp-tst1	II	II	3 (1.9)
sec-seg-sei-sell-selm-selo-selp-tst1	II	I	1 (0.6)
sec-seg-sei-sell-selm-seln-selo	IV	II	1 (0.6)
sec-seg-sei-sell-selm-seln-selo-selp-tst1	II	I	1 (0.6)
sec-seg-sei-sell-selm-seln-selo-selp-tst1	II	II	1 (0.6)
sec-seg-sei-sell-selm-seln-selo-selp-tst1	IV	I	1 (0.6)
seb-selk-selq	IV	I	8 (5.1)
seb-selk-selq	V_T	I	8 (5.1)
seb-selk-selq	IV	II	1 (0.6)
seb-selk-selp-selq	IV	I	6 (3.8)
seb-sei-selk-selq	IV	I	2 (1.3)
sea	III	I	7 (4.5)
sea-selk-selq	III	I	95 (60.5)
sea-selk-sell	V	I	1 (0.6)
sea-sec-seg-sei-sell-selm-selo-selp-tst1	IV	II	1 (0.6)
sea-seb-selk-selq	III	I	1 (0.6)
Total			157 (100.0)

P961 Correlation between molecular types and *in vitro* susceptibility to vancomycin and daptomycin in 158 methicillin-resistant *Staphylococcus aureus* blood isolates from Taiwan

W. Wang, T. Chiueh, J. Lu (Miao-Li, Taipei, Taichung, TW)*

Background: *Staphylococcus aureus* is a major cause of bacteraemia and sepsis with an extremely high mortality. Methicillin-resistant *S. aureus* (MRSA) has emerged since 1960s with a prevalence of 70–80%

in Taiwan, but limited antibiotic regimens are available for MRSA infections with occasional treatment failures.
Objectives: The purpose of this study was to correlate molecular types with *in vitro* antibiotic susceptibility of MRSA isolates obtained from patients with MRSA infections in Taiwan.
Methods: MRSA isolates from patients with bacteraemia were obtained from the collection of Study Monitoring Antimicrobial Resistance Trends (SMART). Minimal inhibitory concentrations (MICs) of isolates to vancomycin (VA) and daptomycin (DPC) were determined by the E-tests according to the manufacture's instructions. MRSA isolates were also typed by specific PCR based on polymorphisms in accessory gene regulator (agr) and staphylococcal cassette chromosome mecA (SCCmec). Correlation between MICs and molecular types was determined by Pearson's and Mann–Whitney U (M-W-U) tests.
Results: A total of 158 non-duplicate MRSA isolates with mecA gene were selected. Of these, 7 (4.4%) were SCCmec type II; 116 (73.4%) type III, 23 (14.6%) type IV, 1 (0.6%) type V, and 11 (7.0%) type VT. One hundred forty-eight isolates (93.7%) belonged to agr I, and the other 10 belonged to agr II (6.3%). The mean MICs of the isolates for VA and DPC were 1.67±0.34 ug/ml and 0.25±0.12 ug/ml, respectively. Correlation factor (r) between MICs for VA and DPC was 0.47. MIC for VA of SCCmec III isolates was higher than those of types IV and VT isolates ($p < 0.001$). MICs for DPC of SCCmec II and III isolates were higher than those of types IV and VT isolates ($p < 0.001$).
Conclusions: Two major clones of MRSA blood isolates with molecular types of SCCmecIII-agrI and SCCmecIV-agrI were predominant in Taiwan. The VA MICs of MRSA isolates correlated with those of DPC. Higher VA and DPC MICs by E-test were seen in molecularly defined hospital-associated MRSA strains (SCCmec II and III) than those in community-associated MRSA strains (SCCmec IV and VT).

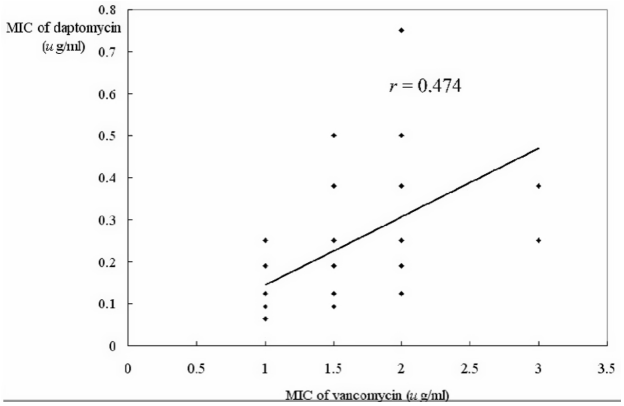


Figure. Correlation between MICs to vancomycin (VA) and daptomycin (DPC) in 158 MRSA blood isolates.

P962 Hyperproduction of norA by (methicillin-resistant) *Staphylococcus aureus* is associated with its genetic background

P. Wolffs, S. Croes, M. Luijten, R. Deurenberg, E. Stobberingh (Maastricht, NL)*

Objectives: NorA is a chromosomally encoded efflux pump of *S. aureus*, which is known to be involved in the efflux of fluoroquinolones, such as norfloxacin, as well as ethidium bromide and disinfectants, such as quaternary ammonium compounds. In literature, mutations in the promoter of norA have been described to lead to overexpression of the pump and consequently lead to increased resistance of *S. aureus* to the different substrates. So far, no large studies have focused on determining the prevalence of norA hyperproducing *S. aureus*, nor have looked at the relationship between genetic background and norA hyperproduction. Our study is aimed to provide answer to these questions.
Methods: A collection of *S. aureus* isolates (community- and hospital-associated) was compiled to cover both methicillin resistant and methicillin susceptible strains and a range of different clonal complexes (CCs) as determined by multilocus sequence typing (MLST). Antibiotic susceptibility profiles were determined by microbroth dilution. The norA promoter was sequenced to determine the presence of mutations leading to hyperproduction.
Results: In total 228 *S. aureus* strains were characterised and 71 of these strains harboured mutations leading to overexpression of norA. In strains resistant to fluoroquinolones, a larger percentage (43%) of hyperproducers was found as compared to sensitive strains (28%). For other antibiotics no clear relations were found so far. For example, there was no significant difference between the percentage of hyperproducers in methicillin resistant or susceptible strains. However, a very clear relation was found with the genetic background the strains associated with. CC30 (n = 25), CC45 (n = 26) and CC121 (n = 15) were found to consist solely of hyperproducers where in other CCs, a maximum of 7% of hyperproducers was found, with most CCs containing no hyperproducers at all. In addition to the mutations found which are known to lead to hyperproduction of norA, also other novel mutations were found.
Conclusion: Hyperproduction of norA is correlated to certain clonal complexes. Work is currently ongoing to determine the impact of novel mutations on the expression of norA and correlated to that, on resistance to different norA substrates.

P963 Characterization of lytic bacteriophages and co-treatment with azithromycin to methicillin-resistant *Staphylococcus aureus*

S. Kim, M. Rahman, S.M. Kim, S.Y. Seol, D.T. Cho, J. Kim (Daegu, KR)*

Objectives: Recently, lytic bacteriophages (phages) have come into spotlight as an alternative therapeutic agent for the treatment of infections caused by multi-drug resistant (MDR) bacteria. In this study, we characterized newly isolated lytic phages able to kill methicillin-resistant *Staphylococcus aureus* (MRSA) and determined the bactericidal efficacy of the phages alone or phage-azithromycin mixture for MRSA.
Methods: Phages were isolated from several nosocomial strains of MRSA by induction with mitomycin C. The genomic DNA, protein profiles, and morphologies of the phages were characterized by restriction endonucleases digestion, SDS-PAGE, and transmission electron microscope, respectively. Lytic activities of the phages against MRSA were assessed by the plaque assay with 102 strains of MRSA belong to four different clonal types (MLST 1, 5, 72 and 239). Bactericidal efficacy of the phage-azithromycin mixture for MRSA was also assessed with bactericidal experiment.

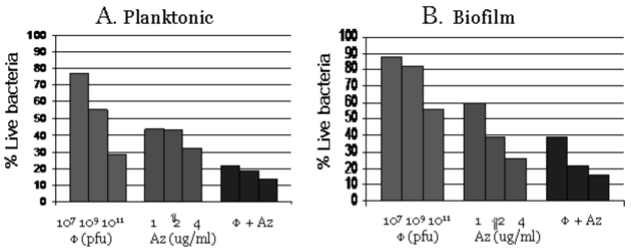


Figure. Bactericidal efficacy of the phage-azithromycin mixtures for MRSA. MRSA planktonic cells (A) and biofilm cells (B) were killed by SAP26 alone (green) and SAP26–azithromycin mixtures (blue) in dose-dependent manner. SAP26–azithromycin mixtures were 10^7 pfu + 1 ug/mg, 10^9 pfu + 2 ug/mg, and 10^{11} pfu + 4 ug/mg, from left to right bar.

Results: Five lytic phages which showed a different susceptibility to 102 MRSA isolates were isolated. One phage was unique in band patterns according to the results of restriction mapping and SDS-PAGE. The others were indistinguishable in band patterns but their host ranges were different. Among them, SAP26 phage showing the broadest MRSA host range (101 lysed/102 hosts, 99%) was selected to characterize the

morphologic and genotypic features. SAP26 was a kind of siphoviridae having a double stranded DNA genome, an icosahedral head and a long tail. SAP26 of 1011 pfu could kill approx. 70% of 1010 cfu planktonic cells of MRSA and 45% of 1-day old biofilm cells of MRSA for 24 hrs comparing with untreated control. However, SAP26 of 1011 pfu-4 ug/ml azithromycin mixture could kill approx. 97% of 1010 cfu planktonic bacteria and 95% of 1-day old biofilm bacteria for 24 hrs comparing with untreated control. These bactericidal effects of SAP26 and the co-treatment with azithromycin for MRSA showed dose-dependent manner, respectively.
Conclusion: This study suggests that the mixture of SAP26 phage and azithromycin would be a good therapeutic agent for the treatment of MRSA infections and biofilm-associated MRSA infection.

MRSA detection

P964 Evaluation of GeneXpert MRSA data: proposal to introduce a 'grey zone' for interpretation of the results

P. Vandecandelaere, R. Joseph, C. De Ridder (Ypres, BE)*

Objective: In order to avoid reporting false positive and false negative results by the GeneXpert Xpert MRSA real-time PCR assay (Cepheid) we evaluated if implementation of grey zone interpretation criteria based on SCC-Ct value and SCC-endpoint value, could improve the performance characteristics of the test.
Methods: Screening swabs of nose and throat, perineum and wounds were sampled with eSwab (Copan), specimens of the same patient were pooled for analysis. The specimens were tested by Xpert MRSA assay and by broth-enriched culture (Trypticase soy broth with 5% sodium chloride) followed by plating onto a chromogenic medium (MRSA-Select, BioRad). A grey zone interpretation was implemented for Xpert-MRSA negative results with SCC-Ct value >0 and for Xpert-MRSA positive results with SCC-endpoint <150 or SCC-Ct value >34.
Results: Among 659 specimens tested, 112 (17%) yielded a positive MRSA culture.
When Xpert MRSA results are compared with broth-enriched chromogenic medium culture as reference method, the sensitivity was 78.3%, the specificity was 95.9%, the positive predictive value (PPV) was 84.2% and the negative predictive value (NPV) was 94.1%.
Forty-two of the 659 specimens (6.5%) comply with the proposed grey zone criteria.
Among the Xpert MRSA negative specimens 14 had a SCC-Ct value >0: compared with broth-enriched chromogenic culture, 8 of these results (57%) were false negative.
Among the Xpert MRSA positive specimens 28 had SCC-endpoint <150, SCC-Ct value >34, or both: compared with broth-enriched chromogenic culture, 14 of these results (50%) were false positive.
Excluding these 'grey zone' specimens, the Xpert MRSA assay has a sensitivity of 81%, specificity of 98.6%, PPV of 93.3% and NPV of 95.5%.
Conclusions: By implementing a grey zone for negative Xpert MRSA results with SCC-Ct value >0 and for positive Xpert MRSA results with SCC-endpoint <150, SCC-Ct value >34, or both, we can improve PPV of the Xpert MRSA assay from 84.2% to 93.3% and NPV from 94.1% to 95.5%.

P965 Is there a need for a 'grey zone' for Xpert MRSA rapid testing?

K. Van Vaerenbergh, A. Boel, R. Cartuyvels, G. Coppens, J. Frans, A-M. Van den Abeele, H. De Beenhouwer for the BILULU Study Group*

Objectives: Xpert MRSA (GX) testing was introduced in routine screening for MRSA because of the high negative predictive value (NPV). In this setting the potential benefit of the use of an equivocal interpretative category (grey zone) was evaluated in order to ameliorate the reliability of a fast screening pathway.
Methods: Among five microbiology laboratories the MRSA screening procedure was standardized for pooled samples. Indications for GX screening were defined as: previously being MRSA positive, recent hospitalisation, resident in a chronic care facility and room mate of a MRSA positive patient. The follow-up of patients in decolonisation was done by culture on chromogenic media after non-selective enrichment. GX gives a positive/negative result based on a cut-off Ct value of 36 cycli. Ct values of all negative GX tests were reanalyzed and any sample with a $Ct > 36$ was cultured.
Results: During a first extensive phase of evaluation all samples were cultured and GX tested. This evaluation showed a high NPV (99.3%) and an acceptable sensitivity (>95%) of GX in our patient populations, permitting the use of GX to give fast and reliable exclusion of MRSA positivity. Because of the rather low positive predictive value (80.3%), positive results of GX needed to be confirmed by culture. GX was introduced in routine since January 2009. Until September 2009, 1536 samples were analysed with GX. For 1331 samples, GX was negative. Culture was positive for 167 of the 205 positive GX samples, giving a PPV of 81.5% for GX and a NPV of 99.3%, confirming our first results. 42 samples had a negative GX result with a Ct above 36. Of these 9 were positive with culture. Thus considering an equivocal interpretative category 5% more positive samples were recovered.
Conclusion: A 'fast-screening' pathway based on Xpert MRSA gives rapid and reliable exclusion of MRSA. However in our patient population 5% of the positive samples were GX negative but with a Ct above 36. In order to increase the sensitivity of the fast screening pathway, introduction of an equivocal interpretative category – to be confirmed by culture – seems mandatory.

P966 Performance characteristics and utility of the cepheid MRSA-Xpert for screening for MRSA from direct patient's samples at a tertiary care centre

S. Al Johani, J. Akhter (Riyadh, SA)*

Background: Methicillin-resistant *Staphylococcus aureus* is a well-recognized pathogen responsible for nosocomial infections. More than 10% of bloodstream infections in hospitals are due to MRSA, which associated with worse outcomes than those with methicillin-sensitive S aureus. These encourage scientists to provide rapid screening tool for high risk patients.
Objective: To evaluate the performance and utility of the Cepheid Xpert MRSA test for rapid screening of high risk patients at a 1000 beds tertiary care Hospital.
Materials and Methods: A total of 7394 high risk patients at King Abdulaziz Medical City were sampled and screened for MRSA from November 2008 – August 2009. Of those 324 were excluded by the system, and from the remaining 7070 specimens there were 6630 negative specimens and 440 positive specimens. We evaluated the 440 positive specimens further to determine the number of true positives and any false positives using two methods, first by culture confirmations and second by graphic analysis of the real time PCR and melting curve analysis on the screen.
Results: We find that out of the 440 positive samples by GeneXpert system, there were 235 (53.40%) culture confirmed positive and 205 (46.59%) culture negative for MRSA, there were 3 samples reported negative by PCR and turned to be culture positive. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the test as follow: 98.74%, 96.98%, 52.93% and 99.95% respectively. We then report the results based on the real time PCR graphic analysis, regardless to the reported result by the system. Then out of the 440 positive samples, there were 282 tests compatible with true positive and the rest looks negative. The statistical analysis with the second method does not affect our sensitivity but it improves the specificity and PPV to 99.31% and 83.33% respectively.
Conclusion: We conclude that the Xpert MRSA assay is a simple, rapid, and accurate method for performing active surveillance for MRSA in a variety of health care populations, a critical graphic analysis of the real time PCR is essential to avoid overcalling MRSA cases.

P967 Evaluation of the performance characteristics of the new NucliSens® EasyQ MRSA for detection of methicillin-resistant *Staphylococcus aureus* in nasal swabs

L. Cavalié, O. Baron, M. Archambaud, R. Bauriaud, D. Clavé, M.F. Prere, C. Jay, N. Fortin, S. Tierrie, C. Croguennec, S. Grosz, V. Maisonniac, R. Cottarel, N. Marty (Toulouse, Grenoble, FR)*

Objectives: To prevent and minimize the spread of MRSA in our hospital, patient screening is performed on admission to intensive care units and during prolong stays in the geriatric department according to the "search and isolate" principle.
The aim of this study was to compare 3 screening techniques: the new NucliSENS EasyQ® MRSA assay (NEQ), the BD GeneOhm MRSA assay (BD) and culture on chromID® MRSA.
Methods: Sampling was conducted by swabbing patients with a 552C dry flocked swab (for NEQ) and a 141C non-flocked swab (for culture and BD) in the right and then in the left nostril of patients. A total of 340 samples were analyzed with the 3 methods. The BD technique is a real time PCR technique detecting the SCCmec-orfX junction. NEQ is a real-time NASBA DNA-based technique performed on the NucliSENS® EasyQ platform and targeting both SSCmec cassette junction and mecA gene.
Results: Using culture as the reference technique, results obtained for NEQ showed a sensitivity of 93.55% (29/31), a specificity of 91.45% (278/304), a negative predictive value (NPV) of 99.3% and a positive predictive value (PPV) of 52.72%. For BD, results showed a sensitivity of 96.77% (30/31), a specificity of 88.54% (255/288), a NPV of 99.6% and a PPV of 47.6%. The number of invalid tests was 7/340, i.e. 2.06%, for NEQ and 32/340 i.e. 9.41% for BD.
Conclusion: The low PPV, close to 50%, for both BD and NEQ, requires a control with culture in case of positive result. The good NPV (>99%) for these 2 techniques enables a negative result to be given for patients screened as negative with a very low risk of error (less than 1%). Screening of negative patients is therefore ensured by these 2 techniques in about 3 hours, compared with culture which requires 48 hours. This can reduce the duration of isolation on hospital wards and thereby also decrease the extra costs. Finally, the low rate of invalids obtained with NEQ is a main advantage that leads to time and cost savings. According to the performance obtained, NucliSENS EasyQ MRSA is a new assay adapted to an efficient MRSA screening in a cost effective way.

P968 Comparison of two rapid molecular methods: GeneOhm MRSA and NucliSens EasyQ MRSA with the chromID MRSA chromogenic medium for detection of methicillin-resistant *Staphylococcus aureus* in 2,003 nasal swabs

V. Vernet-Garnier, C. Jay, J. Madoux, S. Gicquel, N. Fortin, C. de Champs, O. Bajolet (Reims, Grenoble, FR)*

Objectives: Hospital acquired infections with Methicillin Resistant *Staphylococcus aureus* (MRSA) pose a major problem threat to hospitalized patients, increasing morbidity and mortality. Screening for MRSA colonization in patients is a procedure carried out by the hospital infection control to identify carriers at an early stage. We compared a new NASBA method targeting both SCCmec cassette junction and mecA gene (NucliSENS EasyQ® MRSA, bioMérieux – NEQ) and a PCR method targeting the SCCmec junction only (BD GeneOhm MRSA – BD), with a selective chromogenic MRSA agar (chromID® MRSA, bioMérieux).
Methods: A prospective trial including a total of 2003 fresh nasal specimens were collected from 1521 patients hospitalized in 12 units. Testing required 2 swabs per patient: a dried flocked swab (Copan 552C) for NEQ and a non-flocked swab for BD and chromID MRSA. In case of suspected colonies on chromID MRSA, biochemical identification and methicillin resistance confirmation were performed by using VITEK® 2 (bioMérieux) after subculture on blood agar. Frozen lysates were repeated in case of invalid results with NEQ or BD.
Results: Both molecular methods presented high sensitivities (NEQ 92.9%, BD 92.8%) and specificities (NEQ 97.5%, BD 96.7%). Agreement between NEQ and BD on chromID NEG samples is 96.4%. Of 41 positive (POS) with BD and NEG with NEQ, 8 (19.5%) patients were known to be colonized with MRSA according to patients' follow-up. Of 27 POS with NEQ and NEG with BD, 9 (33.3%) patients were known to be colonized with MRSA. This suggests a higher rate of false positives using BD compared to NEQ.
21 were found NEG by chromID MRSA but POS by NEQ and BD showing that molecular biology is likely to be more sensitive than culture. The percentage of invalid rate was 1.0% with NEQ and 3.5% with BD.
Conclusions: Both molecular assays are useful methods for providing MRSA results within a very short period of time, compared to culture (results between 48 and 72 h). Time to result was 3 hours maximum from swabs to result dispatch using 44 tests per run for NEQ versus 14 tests for BD only. NEQ will give a MRSA positive result only if both targets SCCmec cassette junction and mecA gene are detected, reducing therefore the risk of false positive. This advantage combined with the low rate of invalid results provide a better cost management and an improved productivity. In conclusion, the new NASBA NEQ MRSA assay demonstrates higher performance than BD.

P969 Nasal swabs alone are inadequate for optimum detection of methicillin-resistant *Staphylococcus aureus* colonization using the GeneOhm MRSA assay

K. Williams, E. James (London, UK)*

Objective: To determine the proportion of cases of MRSA colonisation that are not identified if the perineum is not sampled when screening patients using the GeneOhm MRSA assay.
Methods: Routine MRSA screening was performed using double-headed swabs taken from nose and perineum sites. Swab heads from each site were processed together in a single extraction step. An aliquot from each of the nose/perineum extractions was then amplified by GeneOhm MRSA reaction in a Smartcycler. Samples from newly identified MRSA cases were cultured and the second head of each swab extracted and processed individually by GeneOhm MRSA.
Results: Of 127 samples positive in combined nose and perineum reactions, 20 (16%) were positive by PCR in the perineum site alone when tested individually. Of 83 culture positive cases, 12 (14%) were not detected by PCR of the nose sample when tested individually. Compared to direct culture on chromogenic media the positive predictive values of nose and combined nose/perineum methods were 67% and 65% respectively.
Conclusion: Using nasal swabs alone when screening patients using the IDI-MRSA will miss a significant proportion of MRSA carriers. Combining nose and perineum samples does not affect the positive predictive value of the assay.

P970 The prevalence of the Panton-Valentine leukocidin-positive, community-acquired methicillin-resistant *Staphylococcus aureus* isolates in Queensland, Australia

M. Costello, F. Huygens, R. Vohra (Brisbane, AU)*

Objective: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections are emerging worldwide. The epidemiology of MRSA has changed recently with new strains causing infections in the community. In addition to novel methicillin resistance gene cassettes, these harbor the Panton-Valentine leukocidin genes (PVL). In Queensland, PVL-positive MRSA belonging to multilocus sequence type (ST) 93 has spread and caused severe infections including deaths. The aim of this project was to determine the prevalence of PVL positive MRSA strains in the community by Real-Time PCR, and determine the dominant genotype using Single Nucleotide Polymorphisms (SNPs).
Methods: 222 CA-MRSA isolates from specimens including swabs collected from patients in the community in the period from May 2008 to May 2009 were analysed. Isolates were confirmed as *Staphylococcus*

aureus by phenotypic methods and confirmed as CA-MRSA by the antibiogram i.e. resistant to one or more non β-lactam antibitiocs. PCR was performed for nuc and mecA genes, and confirmed for the presence of PVL gene by PCR. All isolates were then typed by SNP typing to determine their genotype.
Results: Of the 222 CA-MRSA isolates, 207 (77%) of the isolates were positive for the PVL gene. 16 different ST were detected, of which 88 (39%) of the 222 were found to be ST93, 20 (9%) were found to be ST30, 12 (5.2%) were ST22 and 8 (3.2%) were ST1. 100% of ST93 isolates were PVL positive. The median age distribution of PVL positive ST93 isolates was 45 years with 49% in the age group between 0 and 29 years. 90% of ST93 isolates were from patients in Southeast Queensland.
Conclusions: 77% of CA-MRSA were found to be PVL positive. Of the 16 CA-MRSA clones that were characterized, ST93 and ST30 accounted for half. In addition, PVL was detected in EMRSA-15 (ST22) isolates; a hospital associated MRSA clone that is known to be highly transmissible in the healthcare setting. An international PVL-positive CA-MRSA clone USA300 MRSA (ST8) was also seen in this study. The presence of ST8 suggests that these strains have been introduced from the USA and even though their numbers are small and widely dispersed in Queensland they are of concern as local transmission may be occurring. This study has shown that all ST93 strains from the community were positive for PVL. The implications are that they are less likely to be treated appropriately and more likely to cause severe infections leading to hospitalizations.

P971 Real-time PCR demonstrates the inhibitory activity of probiotic soluble fractions on *Staphylococcus aureus* quorum-sensing genes expression

A. Cotar, V. Lazar, S. Dinu, C. Bleotu, D. Pelinescu, M.C. Chifiriuc Balotescu (Bucharest, RO)*

Objectives: The purpose of this study was to demonstrate by real-time PCR the inhibitory activity of the soluble fraction of *Lactobacillus paracasei* subsp. *paracasei* probiotic strain culture on the expression level of quorum-sensing agr genes, involved in the regulation of coordinated virulence factors expression in *Staphylococcus* (S.) aureus strains.
Methods: The study was performed on 20 *S. aureus* strains isolated from cardiovascular devices associated infections. PCR multiplex assay of the hypervariable domain of the agr locus was used to identify the accessory gene regulator (agr) specificity groups (I to IV) of the tested strains. The *S. aureus* strains were previously grown in the presence of subinhibitory concentrations of sterile supernatants of probiotic culture and subsequently investigated for the expression of different phenotypic features: growth curve, adherence to inert substrate (slime test), ability to ferment mannitol and haemolysins production. Total RNA was extracted from control as well as from bacterial cultures grown in the presence of probiotic soluble fraction. Random hexamers primers were used for cDNA synthesis. For the quantification of agr gene expression there was used real-time qRT-PCR with minor groove binder probes with a non-fluorescent quencher bound to the 3 end (Applied Biosystems). The 16S rRNA reference gene was used for normalization of the results.
Results: The soluble fraction of *Lactobacillus paracasei* subsp. *paracasei* probiotic strain culture inhibited the adherence to inert substratum, the ability to ferment mannitol and the expression of haemolysins in the tested strains. The *S. aureus* strains belonged to agr I (6 strains) and agr III (14 strains) specificity groups. The results qRT-PCR showed that the expression of agr I decreased with 42% and that of agr III with 34% in the probiotic treated cultures comparatively with control cultures expression.
Conclusion: The results of comparative quantification of agr expression in *S. aureus* strains grown in the presence of soluble fraction from probiotic cultures showed an important inhibitory effect of the probiotic soluble molecules on the agr expression. The inhibition of this important regulatory mechanism of *S. aureus* virulence factors expression could represent a possible new strategy for the attenuation of pathogenicity and virulence in *S. aureus* strains isolated from cardiovascular devices associated infections.

P972 Livestock-associated MRSA CC398 strains and human epidemic MRSA strains are distinguished by a novel single-nucleotide polymorphism in the *Staphylococcus aureus* SCCmec-orfX junction

U. Reischl, T. Holzmann, J. Frick, S. Hörmansdorfer, K. Becker, R. Köck, C. Tuschak, A. Sing (Regensburg, Oberschleißheim, Münster, DE)*

Objectives: Although classically considered as a nosocomial pathogen, reports of methicillin resistant *Staphylococcus aureus* (MRSA) carriage or infection in the community have become increasingly common during the past decade. Recent studies in many European countries demonstrated that especially swine, swine farmers or other persons in contact with livestock are colonized with MRSA. Thus, livestock animals may represent a new, separate reservoir for MRSA, which are now termed livestock-associated MRSA (LA-MRSA). Molecular characterization revealed that a distinct subgroup, the MRSA clonal complex 398 (CC398), is predominant within this collective.
Methods: Different real-time PCR assays for direct detection of MRSA in clinical specimens targeting staphylococcal cassette chromosome mec (SCCmec) right extremity sequences and the *S. aureus* chromosomal orfX gene (BD GeneOhm MRSA, Cepheid Xpert MRSA, and the Roche LightCycler® MRSA Advanced test) were evaluated for their performance to cover LA-MRSA strains of the most prevalent CC398 by testing a collection of 184 MRSA strains of human and animal origin obtained from five European countries.
Results: With the exception of 6 MRSA strains harboring "uncommon" SCCmec elements (IVa, n = 1; VII, n = 1; untypeable, n = 5), all LA-MRSA strains tested were covered by the investigated PCR assays. Sequencing of the region around the *S. aureus* SCCmec integration site revealed a characteristic single-nucleotide polymorphisms (SNP) in the SCCmec-orfX junction of MRSA CC398. Within a total assay time of 60 min plus 10 min for subsequent melting curve analysis, MRSA CC398 isolates of our study were clearly distinguished from human epidemic MRSA strains by a characteristic SNP-induced shift in the Tm value as observed in the Roche LightCycler® MRSA Advanced test. Melting curve analysis data were not accessible with the other evaluated real-time PCR assays.
Conclusions: Since MRSA carriage in livestock animals may give rise to transmission and infection in humans, the development of rapid molecular methods for screening and identification of LA-MRSA CC398 will have important implications for surveillance, epidemiology and infection control initiatives. Here, the novel SNP may serve as a suitable CC398 marker for screening and diagnostic purposes. Studies to confirm the diagnostic accuracy of the presented SNP-based strain identification concept with a larger collection of clinical and animal-associated MRSA strains are ongoing.

P973 Characterization of the microbiocoenosis of the human anterior nares

D.H. Pieper, M.L. Wos-Oxley, I. Plumeier, S. Taudien, M. Platzer, G. Peters, C. von Eiff, R. Vilchez-Vargas, K. Becker (Braunschweig, Jena, Muenster, DE)*

Objectives: *Staphylococcus aureus* colonizes the anterior nares of about 20–30% of humans persistently and the other intermittently. Nasal carriage by this opportunistic pathogen plays a crucial role as a source of invasive infections. While differences in colonization by *S. aureus* have been attributed to host factors, also microbial inter-species interactions may also influence colonization. However, the composition of the microbial flora inhabiting the anterior nares is recorded only in part because respective studies were traditionally performed using culture-dependent methods. In this study, the bacterial diversity of the anterior nares was analyzed by the means of both cultivation and culture-independent tools.
Methods: The nasal microbial communities of 40 healthy volunteers from two locations in Germany were studied using single strand chain

polymorphism (SSCP) analysis of 16S rRNA gene fragments including a subset of 20 volunteers comprising nasal *S. aureus* carriers (n = 10) and non-carriers (n = 10) that was additionally applied for cultivation purposes. Furthermore, specimens of six representative individuals were analyzed by 454-pyrosequencing.
Results: The results of the cultivation approach were largely confirmed by the 16S rRNA gene fingerprinting tools, however, the molecular approach revealed much more phylotypes comprising a total of 30 bacterial phylotypes belonging to 4 bacterial phyla and 13 families including putative novel taxa. Beside *Propionibacterium acnes*, the most frequently occurring phylotypes across all volunteers were those related to corynebacterial and staphylococcal species observed in 85–95% of volunteers followed by phylotypes related to species of the genera Dolosigranulum, *Finegoldia* and Peptoniphilus (in 40–60% of volunteers). While *Finegoldia magna* and Peptoniphilus spp. showed a statistically significant co-occurrence indicating a putative positive (symbiotic) interaction, a substantially different distribution pattern was found e.g. for *Corynebacterium* pseudodiphtheriticum and *S. aureus* showing significant avoidance to each other. A statistically significant converse association was also detected between *S. aureus* and *F. magna.*
Conclusion: In summary, applying the molecular approach, the core nasal community was identified and indexed. Knowledge of the precise composition of nasal flora may contribute to develop strategies in order to better control or eradicate *S. aureus*.

P974 False negative *S. aureus* test results in the Slidex Staph Plus agglutination test is mainly caused by spa-type t001 and t001 related strains

F. Szabados, J. Woloszyn, M. Kaase, S. Gatermann (Bochum, DE)*

Objectives: The sensitivity of commercial agglutination kits for fast identification of *S. aureus* is well below 98%. In this study we reevaluated the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination kit with three sets of molecular characterized *S. aureus* strains.
Methods: In this study 363 molecularly defined clinical non-copy strains of methillin-susceptible *S. aureus* (MSSA) and 240 molecularly defined clinical non-copy strains of methillin-resistant *S. aureus* (MRSA) were included. Additionally, a collection of 104 molecularly defined, pulsed field gel electrophoresis (PFGE) divergent MRSA strains were tested, to compare the sensitivity of agglutination based identification to matrix assisted laser desorption / ionisation time of flight mass spectrometry (MALDI-TOF MS) based identification using the Biotyper 2.0 database.
Results: The sensitivity of MALDI-TOF MS based *S. aureus* identification using the Biotyper 2.0 database was 100% (603 of 603).
Sensitivity of Slidex Staph Plus (bioMérieux) kit was higher in methicillin-susceptible *S. aureus* (MSSA) strains (356 of 363 strains = 98%) than in methicillin-resistant *S. aureus* (MRSA) strains (227 of 240 strains = 94.5%, p = 0.0035). Two of seven MSSA agglutination negative MSSA strains were spa-type t001(28.5%). Ten of thirteen agglutination negative MRSA were spa-type t001(76.9%).
To rule out predominance of local MRSA-strains, the sensitivity was additionally tested on 104 PFGE divergent MRSA-strains.
In the PFGE divergent MRSA collection, the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination kit was unexpected low (70.1%). Interestingly, strains tested false negative by the Slidex Staph Plus (bioMérieux) agglutination method in the PFGE divergent strain collection were spa-type t001(24 of 32) and t001 related (7 of 32) strains.
To rule out a problem specific to the Slidex Staph Plus kit, the sensitivity of two additional agglutination kits, the Pasteurex (Biorad; 82.7%) and Staphytect Kit (Oxoid; 78.8) were tested in the PFGE divergent MRSA strain collection (table 1).
Conclusion: The sensitivity of *S. aureus* identification by MALDI-TOF MS was superior (100%) to the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination test in MRSA. The MALDI-TOF MS based identification could be a substitution in agglutination test negative but suspected *S. aureus* isolates, especially if the local prevalence of t001and t001related strains is high.

Table 1

Strain	Species	PFGE-group	sub-group	*spa*-type	Slidex Staph plus	Staphytect Plus	Pastorex Staph Plus
1497	MRSA	01	2	001	neg	neg	p
2397	MRSA	02	1	001	neg	neg	neg
1352	MRSA	02	4	001	neg	neg	neg
2387	MRSA	02	7	001	neg	neg	neg
1583	MRSA	02	8	480	neg	neg	neg
2052	MRSA	02	9	480	neg	w	neg
1867	MRSA	02		001	neg	w	p
2615	MRSA	03	1	001	neg	neg	w
1874	MRSA	03		013	neg	neg	neg
0680	MRSA	05		001	neg	neg	w
2021	MRSA	06		001	neg	neg	p
2682	MRSA	07	10	001	neg	neg	w
2029	MRSA	07	11	001	neg	w	neg
1827	MRSA	07	3	001	neg	w	p
1655	MRSA	07	4	001	neg	neg	neg
2131	MRSA	07	6	001	neg	p	neg
1343	MRSA	07	7	001	neg	neg	neg
2610	MRSA	07		001	neg	w	p
2770	MRSA	09		001	neg	neg	w
2293	MRSA	10		001	w	w	w
1496	MRSA	11	1	001	neg	w	p
1565	MRSA	13	3	008	w	neg	neg
1651	MRSA	13	9	064	neg	neg	neg
1578	MRSA	14	3	109	neg	neg	w
1321	MRSA	22	2	001	neg	neg	neg
2239	MRSA	22	3	001	w	w	neg
0673	MRSA	29		480	neg	w	w
1518	MRSA	34		480	neg	neg	p
2450	MRSA	34		001	neg	neg	neg
2487	MRSA	35	13	032	neg	neg	neg
1452	MRSA	36		001	neg	neg	neg
0992	MRSA	43		5712	neg	neg	neg
1367	MRSA	45		001	neg	w	w
2351	MRSA	34		001	neg	w	w

Resistance to linezolid and mupirocin

P975 Effect of sub-minimum inhibitory concentrations of mupirocin on growth of high-level mupirocin-resistant *Staphylococcus aureus*

E. Pérez Roth, E. Armas González, J. Alcoba Florez, S. Kwong, N. Firth, S. Méndez Álvarez (La Laguna, Santa Cruz de Tenerife, ES; Sydney, AU)*

Objectives: Mupirocin is used to control the prevalence of methicillin-resistant *Staphylococcus aureus*. Conjugative plasmids harboring the ileS2 gene play a key role in the dissemination of high-level mupirocin resistance. Here we have investigated the effects of sub-minimum inhibitory concentrations (sub-MICs) of mupirocin on growth kinetics of *S. aureus* strains carrying different ileS2-encoding plasmids.
Methods: Wild-type *S. aureus* strains carrying ileS2-encoding plasmids belonging to four different structural groups (i.e., S1 to S4) were included. In addition, *S. aureus* NRS107 strain harboring pGO400, a well known ileS2-carrying plasmid, was used. Transconjugants were generated by filter mating experiments in order to compare the growth of isogenic strains carrying the different plasmids. To analyze the influence of mupirocin on growth rates of resistant cells, strains were cultured at 37ºC either in the absence or presence of sub-lethal concentrations of mupirocin and bacterial densities were monitored turbidimetrically (OD600) at 30 min intervals for 3.5-h. Each data point was the mean of at least three independent experiments.
Results: Growth experiments revealed that in the absence of mupirocin there was no significant difference between the growth rates of all

wild-type and transconjugant strains. When mupirocin was added to the medium the most common effect on growth was an incremental lag compared with antibiotic free controls. This effect was observed even at low concentrations of mupirocin. The curves highlighted a more extended delay in the growth of strains carrying plasmids belonging to the S3 and S4 groups, which include the sequenced pPR9 plasmid, in comparison to the other strains. However, some strains showed no lag whatsoever in comparison with the controls when grown in mupirocin. We confirmed the nucleotide identity of the ileS2 gene from each plasmid, ruling out ileS2 mutations as the cause of the differences.
Conclusion: The results indicate that ileS2-carrying plasmids that encode high-level mupirocin resistance differ significantly in their effectiveness at enabling growth of bacterial hosts in the presence of sub-MIC levels of mupirocin. This suggests that some plasmids confer a greater evolutionary advantage than others, which will likely have ramifications for the ongoing development of resistance. The molecular basis of the observed variation in mupirocin resistance phenotype is under investigation.

P976 Characterization and comparative analysis of pPR9, a staphylococcal conjugative plasmid encoding high-level mupirocin resistance

E. Pérez Roth, S. Kwong, J. Alcoba Florez, N. Firth, S. Méndez Álvarez (Santa Cruz de Tenerife, ES; Sydney, AU)*

Objectives: High-level mupirocin resistance has been acquired by different methicillin-resistant *Staphylococcus aureus* (MRSA) clones due to the dissemination of plasmids bearing the ileS2 resistance gene. To gain insights into the evolution of these medically important plasmids and mupirocin resistance we have undertaken a comprehensive comparison between the conjugative multiresistance plasmid pPR9 and other characterized ileS2-carrying plasmids.
Methods: pPR9 was sequenced using a shotgun strategy and probable genes were annotated. The predicted proteins were searched, using the BLAST algorithm, against a nonredundant database at the National Center for Biotechnology Information. Potential protein functional domains were identified by searching against the NCBI conserved-domain and Pfam databases. Comparative analyses were undertaken with the other available completely sequenced ileS2-containing plasmids pUSA03 and pV030–8, and the partially characterized plasmid pGO400. Pairwise nucleotide comparisons were generated using JDotter and GATA. Multiple sequence alignments were carried out with ClustalW.
Results: We have determined and analyzed the complete sequence of pPR9, a 41.7-kb pSK41-like conjugative ileS2-carrying plasmid recovered from a MRSA strain belonging to the ST36-SCCmecII pandemic genotype. Unlike previously described pSK41-like plasmids, the conjugative transfer region of pPR9 is not flanked by copies of IS257, and the plasmid was found to utilize a novel chimeric replication initiation protein. Intriguingly, BLAST searches revealed that the N-terminal 174 amino acids of pPR9 Rep share significant similarity to the N-terminal ends of phage proteins that are annotated as putative replication proteins. Accessory regions encoding antibiotic resistance also differed in pPR9, which also contains a second resistance determinant in the form of a complete copy of a Tn552-like β-lactamase transposon. Significantly, comparative analysis revealed that the insertion sites of the ileS2 gene in pPR9 and each of the three other plasmids are distinct, suggesting that these plasmids have each arisen from independent acquisition events associated with insertion sequence IS257. In several cases, this element appears likely to influences the level of transcription of the ileS2 gene.
Conclusion: Our findings highlight the continuing genetic flexibility of staphylococcal conjugative plasmids and the key role of IS257 in mediating resistance gene capture.

P977 The resistance mechanism mediated by the cfr gene is predominant in clinical isolates of *Staphylococcus epidermidis* linezolid-resistant obtained in a Spanish hospital

E. Baos, G. Morales, J. Picazo (Madrid, ES)*

Objectives: To determine the presence of the cfr gene in *S. epidermidis* linezolid-resistant isolates obtained from clinical specimens of patients admitted to our hospital in one year.
Methods: We collected all *S. epidermidis* linezolid-resistant identified in all type of biological specimens received during the period of one year. Identification and determination of antibiotic susceptibility were performed using automated methods as Vitek 2 and Wider. Resistance to linezolid was confirmed using E-test. DNA of linezolid-resistant strains was obtained by the triton-boiling method and the presence of the cfr gene was determined by PCR and analyzed using agarose gel electrophoresis.
Results: From April to December 2008 a total of 28 *S. epidermidis* linezolid-resistant strains were isolated. 15 of them carried the cfr gene, 54% of all strains. 29.6% of the strains were obtained from blood cultures, 37% from catheter (central and peripheral) and 33.4% from other samples (aspirate, surgical wounds . . .).
The hospital wards where *S. epidermidis* linezolid-resistant strains were more frequently isolated were the ICU (66.6%) and traumatology and nephrology (11.1%).
From January to May 2009 we isolated 15 *S. epidermidis* linezolid-resistant strains, 12 of them carried the cfr gene, representing 80%. 26.3% of the strains were isolated from blood cultures, 36.8% from catheters and 36, 9% from other samples.
Again, the hospital wards where *S. epidermidis* linezolid-resistant strains were more frequently isolated were the ICU (52.6%) and traumatology (21%).
In one year period 43 *S. epidermidis* linezolid-resistant isolates were analyzed and we found that in 62.8% of them the resistance to linezolid was mediated by the cfr gene.
Conclusions: In our hospital the predominant mechanism for linezolid-resistance in *S. epidermidis* is the presence of cfr gene.
These data rise concern about the possibility of horizontal transmission of this gene.

P978 Molecular characterization of linezolid-resistant *Staphylococcus epidermidis*

I. Spiliopoulou, A. Damani, S. Schoina, A. Liakopoulos, M. Marangos, E. Malli, L. Leonidou, V. Maroulis, D. Velissaris, F. Fligou, K. Filos, E.D. Anastassiou, E. Petinaki (Patras, Larissa, GR)*

Objectives: To determine the molecular mechanism of resistance to linezolid and set up the genetic relationship among *Staphylococcus epidermidis* isolates in Greece.
Methods: A total of 22 linezolid-resistant *S. epidermidis* (MIC > 8 mg/L) isolated between February to August 2009 from blood and intravenous catheter cultures of individual patients in two Greek University Hospitals (from Patras and Larissa) were investigated. Bacterial DNA was firstly tested for the presence of the most common resistance mechanisms, i.e. the presence of G2576T mutation by PCR followed by NheI digestion and the presence of cfr gene also by PCR. In addition, in order to detect other than G2576T mutations, the region V of 23S rRNA gene was sequenced and resulting data were analyzed by the use of CHROMAS. Clonal relatedness of all isolates was investigated by MLST. For each patient, demographic data (age, gender, source of specimen and antibiotic therapy) were included.
Results: According to MIC values isolates were classified into two categories: the first included ten isolates with MIC ranges between 8 to 32 mg/L, whereas, the second twelve with MICs greater than 256 mg/L. Among the first group, three carried the C2534T mutation, while seven isolates carried the G2576T one. All isolates with MICs greater than 256 mg/L carried the recently reported T2504A mutation. MLST analysis revealed the presence of two clones: clone ST22, including strains that

carried both T2504A and C2534T mutations, and clone ST2, including strains with G2576T mutation. The ST22 clone is closely related to ST2 with a difference of the tpiI allele. The majority of patients were treated with linezolid prior to isolation of the specific strain.
Conclusion: Dissemination of common clones of linezolid-resistant *S. epidermidis* among hospitals located in different areas of the country emphasizes the need for strict control measures application, such as isolation of infected patients, increased environmental cleaning, hand hygiene and antibiotic policy.

P979 Emergence of linezolid-resistant Gram-positive clinical isolates due to cfr-methylase gene production associated with G2576T mutation in the 23S rRNA

E. Cercenado, M. Marín, R. Insa, E. Bouza (Madrid, ES)*

Objectives: To characterize a total of 46 linezolid-resistant (LNZ-R) clinical isolates of grampositive cocci recovered in our laboratory from January 2009 to October 2009.
Methods: MICs were determined by the broth microdilution method using commercialized MicroScan panels (Siemens). CLSI guidelines (2009) were followed for determination of breakpoints. LNZ-R isolates were confirmed by E-test. Detection of the G2576T mutation was performed by PCR and sequenciation of the 23S rRNA gene. Presence of the cfr-methylase gene was determined by PCR and sequencing as previously described. *S. aureus* ATCC 29213 was used as linezolid-susceptible control strain.
Results: The 46 LNZ-R clinical isolates recovered over the period of study were *S. epidermidis* (39 isolates), methicillin-resistant *S. aureus* (MRSA) (4 isolates), *E. faecium* (2) and *E. faecalis* (1) corresponding to 40 patients. The origin of the isolates was IV catheter (14 isolates), wound/abscess (14), blood (5), pleural fluid (4), tracheal aspirates (4), CSF (3), and peritoneal fluid (2). The LNZ-MICs against the isolates ranged from 8 to >256 mg/L. The patients were hospitalized in 14 different wards, but 3 wards included 15 patients. Not all the patients received linezolid previous to the isolation of the LNZ-R isolates. A total of 20 isolates produced only the cfr gene (4 MRSA isolates from patients isolated in different wards at different times, 1 *E. faecalis*, 1 *E. faecium*, and 14 *S. epidermidis*); 19 had the G2576T mutation in addition to the cfr gene, and 7 isolates (all *S. epidermidis*) presented only the G2576T mutation.
Conclusions: To our knowledge this is the first description in which LNZ resistance in different species of grampositive clinical isolates is due to the concomitant production of the cfr-methylase gene and the G2576T mutation. We also describe for the first time the cfr production in clinical isolates of *E. faecium* and *E. faecalis*, and the spread of the cfr gene among MRSA isolates from non-related patients.

P980 Nosocomial outbreak of methicillin and linezolid-resistant *Staphylococcus epidermidis* associated with catheter-related bloodstream infections in intensive care unit patients

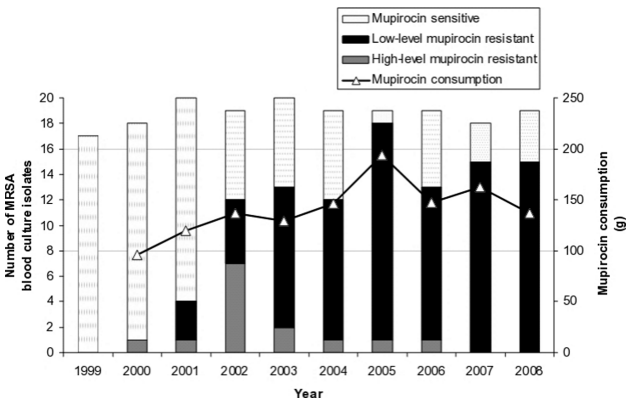
C. Seral, Y. Sáenz, S. Algarate, E. Durán, P. Luque, C. Rubio-Calvo, C. Torres, F.J. Castillo (Zaragoza, Logroño, ES)*

Objective: To investigate the clonality of the strains and the mechanisms of resistance to linezolid and other antibiotics in a nosocomial outbreak of meticillin and linezolid-resistant *S. epidermidis* (MLzRSE) associated with catheter-related bloodstream infection (CR-BSI) in an ICU.
Methods: From March 2008 to August 2009, 27 MLzRSE out of 128 coagulase-negative staphylococci (21.09%) associated with CR-BSI in the ICU of our hospital were recovered from blood cultures (14) and catheter (13) of 21 patients. Thirteen patients (61.90%) had received linezolid during the three months preceding isolation of the MLzRSE. Susceptibility testing to 22 antibiotics was performed by WIDER microdilution system. Linezolid, daptomycin and tigecyclin MICs were determined by E-test and susceptibility to MLS, aminoglycosides, chloramphenicol and tetracycline antibiotics by disk diffusion. Detection and characterization of meticillin [mecA], linezolid [23S rRNA mutations and cfr], aminoglycosides [ant(6)-Ia, aac(6′)-Ie-aph(2″)-Ia, aph(3′)-IIIa and ant(4′)] and chloramphenicol [catA] resistance genes were studied by PCR and sequencing. Clonal relationship was performed by PFGE using SmaI enzyme and by MLST typing. The SCCmec type, the ica gene, IS256 and Tn4001 transposon were also studied by PCR.
Results: Three closely related pulsotypes were identified among the 27 studied MLzRSE that belonged to the sequence type ST2 and harboured the mecA gene and the SCCmec III type. Analysis inside the amplified fragment of 23S rRNA region determined that the strains recovered from patients 1 to 4 (pulsotype P1a) showed the nucleotide mutation G2474T and carried the aac(6′)-Ie-aph(2″)-Ia, ant(4′) and catA genes, whereas the strains from patients 5 to 21 (pulsotype P1b and P1c) showed the mutation G2603T in the 23S rRNA and carried the aac(6′)-Ie-aph(2″)-Ia gene. None strains amplified the cfr gene. The amplification of the ica gene and the IS256 were positive in all 27 MLzRSE strains.
Conclusions: The emergence of two new epidemic linezolid resistant *S. epidermidis* strains, nearly coincident in time and genetically related, with two different mutations in the 23S rRNA (G2474T and G2603T) non-previously described before, are reported in this study as cause of a nosocomial outbreak.

P981 Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* and mupirocin consumption over 10 years in a tertiary hospital

A. Lee, P. François, G. Renzi, M. Macedo, N. Vernaz, J. Schrenzel, D. Pittet, S. Harbarth (Geneva, CH)*

Objectives: Topical mupirocin is widely used to eradicate carriage and prevent infection with methicillin-resistant *Staphylococcus aureus* (MRSA). However, resistance to this antibiotic is emerging and may be the result of selective pressure from mupirocin use. Low-level mupirocin resistance (L-MuR) is associated with native tRNA synthetase mutations while high-level resistance (H-MuR) is usually due to plasmid encoded mupA (novel tRNA synthetase). This study aimed to determine the prevalence and molecular mechanisms of mupirocin resistance in MRSA blood culture isolates in our institution. We also aimed to correlate mupirocin use and prevalence of resistance over time.
Methods: The first 20 non-duplicate MRSA blood culture isolates per year collected and stored at our institution between 1999 and 2008 were selected for the study. Non-viable and contaminated isolates were excluded. The remaining isolates were screened for mupirocin resistance using a 0.5 McFarland suspension on Mueller-Hinton agar with a 5 microgram disk. Resistant isolates had MIC determinations with Etests, an allelic discrimination assay to detect the V588F point mutation and MupA PCR. Mupirocin consumption data between 2000 and 2008 were obtained.



Mupirocin resistance in MRSA blood cultures and mupirocin consumption, 1999 to 2008.

Results: 200 MRSA isolates were retrieved. Of these, 9 were excluded due to contamination and 3 were non-viable. The remaining 188 isolates

were further evaluated. Resistance to mupirocin was found in 0 of 17 isolates in 1999, increased to 18 of 19 isolates (95%) in 2005 and then decreased to 15 of 19 isolates (79%) in 2008 (Figure). The majority of resistant isolates had L-MuR (89 of 103 isolates, 86%). The V588F point mutation was found in all isolates with L-MuR. However, it was also present in 12 of 14 (86%) isolates with H-MuR. The mupA gene was found in all isolates with H-MuR. The trend in the consumption of mupirocin paralleled the prevalence of resistance during this period, with an increase from 96g to 194g between 2000 and 2005, then decrease to 137g in 2008 ($r = 0.87$, $p = 0.002$).

Conclusions: The prevalence of mupirocin resistance in our institution is increasing and predominantly consists of L-MuR characterized by the V588F mutation. This correlates with an increase in mupirocin use over this period. The therapeutic implications of L-MuR remain to be determined.

P982 Comparison of prevalence of antiseptic resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci from nurses and the general population in Hong Kong

M. Zhang, M. Boost, M. O'Donoghue, E. Tong, K. Hiramatsu (Kowloon, HK; Tokyo, JP)*

Objective: Widespread use of antiseptic agents has led to the emergence of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) with decreased antiseptic susceptibility. This has been associated with the presence of several genes including qacA/B and smr. This study determined and compared the prevalence of antiseptic resistance genes in *S. aureus* and CNS from nurses and the general population and investigated risk factors for carriage by nurses of qacA/B or smr positive *S. aureus* and CNS.

Methods: Nasal swabs were collected from 235 nurses and cultured for the presence *S. aureus* and CNS. The nurses completed a questionnaire providing demographics and data on antiseptic use, contact with MRSA positive patients, and recent antibiotic therapy. 200 recent carriage isolates of *S. aureus* and CNS from the general population were used for comparison. PCR was used to detect mecA, qacA/B and smr genes. Univariate and multivariate analysis were used to investigate associations between presence of antiseptic resistance genes and potential risk factors.

Results: A significant difference was noted in the prevalence from nurses and the general public of both qacA/B and smr in CNS isolates and of qacA/B in *S. aureus* (see table). Overall MRSA carriage was low (3% nurses; 0.5% general public). There was a significantly higher prevalence of qacA/B in MRCNS strains than in MSCNS isolates ($p < 0.001$), but, whilst the rate was higher in MRSA than MSSA, this did not reach significance.

Contact with MRSA patients increased risk of carriage of strains with antiseptic resistance genes (OR 2.26; $p = 0.031$). Presence of mecA in an isolate increased the risk of harbouring antiseptic resistance genes (OR 2.78; $p < 0.001$).

Table. Prevalence of antiseptic resistance genes in staphylococcal isolates from nurses and the general population

	Gene	Nurses (%)	General population (%)	OR	p
S. aureus	*qacA/B*	52.8	11.3	8.8	<0.001
	smr	5.4	5.6	na	ns
CNS	*qacA/B*	43.3	13.5	8.4	<0.001
	smr	18.5	8.5	2.4	0.002

Conclusions: The increased prevalence of antiseptic resistance genes in isolates from nurses indicates that the hospital environment exerts selective pressure for carriage of these strains. The increased proportion of antiseptic resistance gene positivity in mecA positive isolates suggests co-selection of these genes, as does the increased risk of carriage of antiseptic resistance gene positive strains by those in recent contact with MRSA positive patients. Although qacA/B and smr do not confer antiseptic resistance at concentrations used, they may pose an infection control risk by persisting in areas with low level antiseptic residues. The association between mecA and qacA/B/smr may contribute to survival of MRSA in the hospital environment.

Nosocomial MRSA surveillance and control

P983 Significant decrease in the prevalence of MRSA – another inspiring tendency in Bulgaria

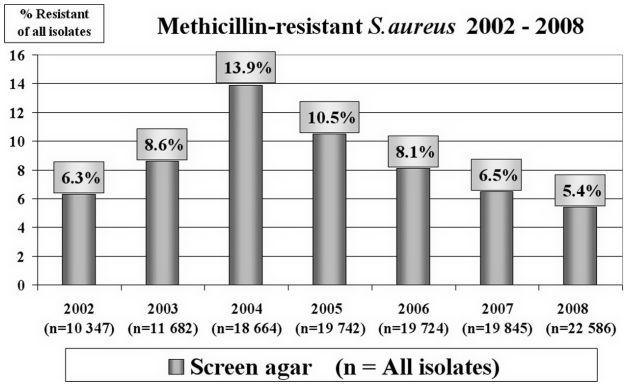
M. Petrov, T. Velinov, V. Dimitrova, T. Kantardjiev (Sofia, BG)*

Objectives: Investigation of the resistance levels and trends for MRSA isolated in Bulgaria, from 2000 to 2008, as part of the Bulgarian Surveillance Tracking Antimicrobial Resistance – BulSTAR.

Methods: BulSTAR monitors the isolation and antimicrobial susceptibility of all clinically significant microorganisms isolated from blood cultures and other important sources in the participating microbiology laboratories since 1997. One hundred and sixty-eight microbiology laboratories from all regions of Bulgaria participated in the surveillance in 2008. The number of investigated *S. aureus* strains varied between 10 000 and 22 000 per year. The participating laboratories identified all microorganisms and performed susceptibility testing in accordance with the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS). MRSA were confirmed on Screen-agar or other relevant CLSI methods like detection of PBP2′ or mecA gene. Each year an increasing number of laboratories ($n = 133$ in 2008) have been using Laboratory Information Systems (LIS) like WHONET or the local software National Information System "Clinical Microbiology and Infections Surveillance" for laboratory data management. According to the recommendations they report only the first isolate per patient for the year, though a part of the participants lacking LIS are still reporting multiple isolates ($n = 35$ in 2008).

Results: Data from BulSTAR show an inspiring tendency. The overall percentage of MRSA among *Staphylococcus aureus* isolates is decreasing in Bulgaria from 13.9% in 2002 to 5.4% in 2008 (Figure 1). This is mainly due to raised awareness on the problems with MRSA, increased efforts on infection control and hand hygiene, rather then to altered antibiotic policy in hospitals in the country.

Conclusions: The overall levels of MRSA in Bulgaria are decreasing since 2002. The EARSS Annual Report 2007 indicated that seven Member States report significantly decreasing percentages of MRSA among *Staphylococcus aureus* from bloodstream infections. This is likely due to increased measures on infection control, hand hygiene and antibiotic policy in hospitals in these countries as demonstrated by national data from, e.g. Slovenia, France and UK. Further investigations will determine that Bulgaria can be added to those seven countries (M. Petrov and co-workers, unpublished data).



P984 Hospital outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new, possibly hyperepidemic variant from a previously sporadic strain

O. Clerc, L. Senn, C. Bellini, I. Nahimana Tessemo, D.S. Blanc, G. Zanetti (Lausanne, CH)*

Objective: To describe an ongoing outbreak that tripled the annual detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in a tertiary care hospital.
Methods: Active surveillance of MRSA is performed since 20 years in our hospital. Our protocol includes screening of patients transferred from high-incidence health-care institutions or countries, roommates of new MRSA cases, and wards where $\geqslant 2$ patients acquired MRSA during the same week. Contact precautions are used for known carriers. PFGE was used for molecular typing until 2004, and was then replaced by Double-Locus Sequence Typing (DLST).
Results: A median yearly incidence of 173 new carriers of MRSA was observed from 2002 to 2007. Since September 2008, an increasing number of new cases were observed, mainly as successive clusters limited to distinct wards, reaching a total of 398 until October 2009. The yearly incidence of new cases rose to 275 in 2008 and 613 in 2009. 60% of the cases were due to one strain: DLST 4–4, ST 228, SCCmecI. The incidence of new cases due to the previously predominant strains remained unchanged. The epidemic strain corresponded to a new variant of a clone responsible for a previous outbreak in 2001, and only sporadically isolated (mean of 20 cases/year) since then. A case–control study documented a significant association between acquisition of the epidemic strain and a stay in intensive and intermediary care units, a highest number of internal transfers, but did not identify a point source of transmission. Infection control practices and antibiotic policy had remained unchanged for several years. Compliance with hand-hygiene as monitored yearly was on the rise. Screening of 313 healthcare workers only found one carrier of the epidemic strain lately in the outbreak. Additional infection control measures were enforced, including screening at ICU admission and discharge with PCR-based rapid test, routine screening for all patients leaving epidemic wards, introduction of PCR-based rapid test for contact tracing, additional working forces for environmental disinfection, and hospital-wide education of healthcare workers. However, the outbreak was still ongoing after 5 months.
Conclusions: Factors linked to the dissemination of this new variant in our institution remain undetermined. This unresolved outbreak suggests that this new variant acquired hyperepidemic properties, which calls for further investigations.

P985 Nosocomial infections by methicillin-resistant (MRSA) and methicillin-sensitive *Staphylococcus aureus*: further exploring the role of prior antibiotic usage as a predictor of MRSA infection

I.G. Baraboutis, E.P. Tsagalou, E. Mylona, V. Papastamopoulos, M. Samarkos, C. Gogos, A. Skoutelis, S.B. Johnson (Athens, Patra, GR; Chicago, US)*

Objectives: Both total antimicrobial use as well as specific antimicrobial classes have been implicated as risk factors for nosocomially-acquired MRSA (NA-MRSA) infection. The aims of the study were: 1.to explore predictors of a new NA-MRSA infection in comparison with a new nosocomially-acquired MSSA (NA-MSSA) infection, 2.to thoroughly assess recent antibiotic use (within the last 30 days of the positive culture date) qualitatively and quantitatively.
Methods: The time-period for our study was from October 1997 through September 2001. From the infection control records, we identified two groups of inpatients, one with a new positive MRSA culture and one with a new positive MSSA culture. We considered the infection acquired in-hospital if (1) the culture was taken more than 48 hours after admission and (2) if it was taken less than 48 hours but the patient had been hospitalized within the last month. We only included patients with adequate clinical and/or laboratory indications for active infection and not just colonization. We recorded data pertinent to widely accepted risk factors for *Staphylococcus aureus* colonization and infection considering events up to 30 days before the positive culture date. We used the electronic pharmacy records to obtain detailed data on intravenous antibiotic use during the month before the culture date.
Results: We identified 127 patients with a new NA-MRSA infection and 70 patients with a new NA-MSSA infection eligible for further analysis. In univariate analysis, significant differences were noted in rates of hemodialysis, recent invasive procedures and major surgery, intubation, indwelling urinary catheter placement, use of at least 3 antibiotics, use of penicillins, clindamycin, aminoglycosides, fluoroquinolones (FQs), and quantitative use of total antibiotics, β-lactams, penicillins, β-lactam/inhibitor combinations, FQs and aminoglycosides. In multivariate analysis, no single factor stood out when including only qualitative antibiotic use while total antibiotic use was the only independent predictor of NA-MRSA infection when including only quantitative data in the model. No siginificant differences in outcome were noted.
Conclusion: From the analysis of these strictly defined groups deriving from a highly homogeneous population, we conclude that quantitative antibiotic usage as a whole was the strongest predictor of NA-MRSA infection, more than individual antibiotic usage or other traditional risk factors for NA-MRSA infection.

P986 Effect of introduction of new antibiotic guidelines on undetected methicillin-resistant *Staphylococcus aureus* carrier rate on elderly care wards

F.M. Awadel-Kariem, R.N. Manzoor, H. O'Connor, S. Khan (Stevenage, UK)*

MRSA is endemic in elderly care (EC) wards. This represents a hidden threat which puts these patients at risk of serious systemic infection and can be a source of infection to other hospitalised patients.
Objectives: To establish the impact of changing Antibiotic Guidelines on the MRSA carrier rates on EC wards in a district general hospital.
Methods: The study was carried out in two phases. An audit was conducted in 2007 prior to the introduction of antimicrobial guidelines that restricted the use of cephalosporins (CEP) and fluoroquinolones (FQ). A follow-up audit was conducted in 2008 after the successful implementation of the guidelines. In both audits, all patients in two EC wards were screened for asymptomatic MRSA colonisation at multiple sites including nose, throat and groin. Swabs were processed for detection of MRSA using two protocols: a) 3M BacLite method; and b) MRSA Chromogenic Agar method. Positive patients were then notified to the infection control team and to the doctors looking after them to start the eradication protocol.
Results: In the first audit, 48 patients in 2 EC wards were screened. Of these, 10 were found to be positive for MRSA in one or more sites. Out of the 10 MRSA colonised patients 5 had confirmed *Clostridium difficile* Associated Disease (CDAD). The new guidelines, restricting the use of CEP and FQ, were then introduced. Several prescribing audits confirmed that changes in antibiotic prescribing habits had been effected. In the follow-up audit, 51 patients were screened. Of these only 3 were found to be positive for MRSA in 1 or more sites. In this audit, no patients developed CDAD. There is a considerable shift in the MRSA rates between 2007 and 2008 audits (20.8% and 5.9%; $P = 0.028$). A similar shift is also seen in the CDAD rates (10.4% and 0.0%; $P = 0.018$).
Conclusion: The linkage of CDAD and MRSA colonisation rates provides further evidence that they share similar risk factors (including overuse of CEP and FQ). It is well-established that antibiotic stewardship can influence the rates of CDAD and of MRSA bacteraemia and severe disease. This study shows that it can also influence the rate of MRSA colonisation on EC wards. As most health-care associated infections (HCAI) are associated with misuse of antibiotics, it is not surprising that this study also showed reduction in CDAD. Rates of other HCAI (such as multi-resistant Gram-negative organisms) may also be reduced at the same time.

P987 Risk factors for MRSA carriage at admission to rehabilitation centres

Y. Lerman, W. Naamana, J. Vidal Samso, J. Fierro Banzo, R. Formisano, A. Salvia, A. Rossini, M.P. Balice, J. Salomon, C. Lawrence, J. Lasley, J. Hart, M. Paul, E. Isakov, S. Lidji, M. Hochman, M. Kazma, A. Klein, H. Mishali, Y. Carmeli on behalf of the MOSAR WP-5*

Background: Identification of MRSA carriers upon admission to rehabilitation center may be important in order to install preventive measures. We aimed to investigate the prevalence and predictors of MRSA carriage in this setting.
Methods: A multicenter European study on prevention of spread of resistance is conducted as part of MOSAR in 8 rehabilitation wards. A prospective case–control study performed between Oct 08 and September 09. 742 patients to were screened for nasal carriage of MRSA on admission to the study wards. Swabs were plated on BBLII CHROMagar plates, and suspected colonies which were coagulase positive were identified as MRSA. Demographic and clinical data were collected from patient's records. Risk factor univariate and multivariate analysis were performed using SPSS software.
Results: 742 patients (age 72, 44.5% male) were screened, 64 (8.6%) were found as MRSA carriers. Carriage varied between the centers (7.4%-14.6%), MRSA positive cases where compared to MRSA negative controls: cases were more frequently younger than 65 (38% vs. 26%), female sex (74% vs. 58%), admitted after >2 weeks acute care hospital stay (58% vs. 40%), admitted with an infection or colonization (30% vs. 19%), admitted after staying in other long term care facility (30% vs. 13%), had peripheral vascular disease (16% vs. 6%), and had been treated with BL/BLI antibiotic (18% vs. 6%) or antibiotics classified as other (32% vs. 15%(in the past month. Multivariate model identified the following independent predictors for MRSA carriage; female sex ($p < 0.001$); admitted after staying in other long term care facility ($p = 0.003$), BL/BLI antibiotic treatment ($p = 0.008$); treatment with antibiotics classified as "Other" ($p = 0.017$).
Conclusions: MRSA carriage on admission to rehabilitation center was found commonly, but prevalence varied widely. Predictors identified in this study are not specific enough to direct infection control measures.
Acknowledgement: supported by the European commission grant (FP6): European Network for Mastering Hospital Antimicrobial Resistance and its Spread into the Community (MOSAR; LSHPCT- 2007-037941).

P988 Prevalence and risk factors of methicillin-resistant *Staphylococcus aureus* colonization among residents living in long-term care facilities

J.A. García-García, J. Santos-Morano, J.E. Corzo-Delgado, M.L. Martín-Ponce, L.M. Martín-Rodríguez, S. Vergara-López, C. Castro, A. Mateos-Gómez, E. Bayoll-Serradilla, F. Duarte-Sánchez, E. León-Jiménez, J. de la Cueva-Rubio, J.M. Gómez-Mateos (Seville, Osuna, ES)*

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) asymptomatic colonization predominantly affect individuals with co-morbidity or other specific risk factors, such as prolonged hospital stay and long-term care facilities. However, there is scarce information about risk factors associated with MRSA colonization in these centers.
Objective: The objective of our study was to determine the prevalence and factors associated with MRSA nasal colonization among subjects living in long-term care facilities in southern Spain.
Patients and Methods: During the period from 31st March to 31st October 2009, all subjects living in ten long-term care facilities of our area were included in a cross-sectional study. Patients were screened using nasal swabs. Residents were classified as nasal MRSA carriers, methicillin-susceptible *S. aureus* carriers and non-carriers. The following data were obtained for all residents: age, sex, time of living in residence, number of residents, comorbidities (Charlson index), functional status (Barthel index), previous antibiotic treatment in the last month, prior MRSA isolation, presence of decubitus ulcers, use of invasive devices and invasive procedures (surgery, endoscopies) in the last year. Data were analyzed using SPSS version 15.
Results: Three hundred and seventy-two subjects were included. Male sex were 136 (37%). The median (Q1-Q3) age of our population was 81 (75–86) years. The median (Q1-Q3) time of living in residence was 20 (10–35) months. Barthel index lower than 40 occurred in 228 (62%). Thirty-one (8%) subjects were taken antibiotic treatment in the last month. The prevalence of *S. aureus* and MRSA nasal colonization was 24% and 12%, respectively. Significant risk factors, identified by multivariate analysis, were recent antibiotic use ($p = 0.027$), long time of living in a long-term care facility ($p = 0.019$) and recent invasive procedure ($p = 0.014$).
Conclusions: The prevalence of MRSA nasal colonization in the long term care facilities of our area is high. In these centers, subjects under recent antibiotic use, long time of living in the residence and recent contact with hospitals are more susceptibility to be colonized.

P989 Cost analysis of rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* in ICUs in a low endemic setting

M.W. Wassenberg, A. Troelstra, J. Kluytmans, M. Bonten on behalf of the Dutch Rapid MRSA Diagnostics Study Group*

Objectives: Pre-emptive isolation of high risk patients is a cornerstone of the Dutch MRSA control strategy. However, most high risk patients are not colonized and remain in isolation for 3 to 5 days awaiting conventional culture results. We determined cost-efficacy of adding rapid diagnostic testing to the current MRSA control policy in intensive care units (ICUs).
Methods: A prospective multi-center study was performed in 12 Dutch hospitals between 12/05 and 06/08. Patients at risk of MRSA colonization and fulfilling the criteria for pre-emptive isolation according to Dutch guidelines were eligible. In addition to the standard set of conventional broth-enriched cultures, BD GeneOhmTM MRSA PCR ('IDI') (BD Diagnostics) or the Xpert MRSA assay ('GeneXpert') (Cepheid) was performed directly on patient material. Costs of IDI and GeneXpert PCR were €56.22 and €69.62 per test, respectively. Conventional culture results were used as reference to determine sensitivity (Sens), specificity (Spec), and positive/negative predictive values (PPV/NPV).
Results: 170 ICU patients (mean 52 years and 58% male) were enrolled. 89 (52%) using IDI and 81 (48%) using GeneXpert PCR, yielding 519 IDI PCRs and 288 GeneXpert PCRs (4.7 per patient including nose, throat, perineum; with GeneXpert four or more sites were pooled). MRSA prevalence was 2.9% and important risk categories were contact screening related to MRSA positive patients 1.0% (1/97), hospitalization abroad 2.0% (1/51) and contact with pigs 27.3% (3/11). Compared to the reference Spec and NPV of IDI PCR (no MRSA positive patients) was 93.3% and 100%, and Sens, Spec, PPV and NPV of GeneXpert PCR were 100%, 96.1%, 62.5% and 100%, respectively. In 105 (62%) patients pre-emptive isolation was discontinued upon negative PCR results (study protocol did not permit discontinuation of isolation in all hospitals). Median time between start of isolation and discontinuation of isolation in these patients (TOT) was 24.5 hours (27.6 and 22.1 hours for IDI and GeneXpert PCR, respectively). Median time between start of pre-emptive isolation and notification of culture results was 91.9 hours. Average screening costs per patient were €327.84 and €247.54 for IDI and GeneXpert, respectively.
Conclusion: In a low endemicity setting, guiding of pre-emptive isolation upon MRSA PCR on ICUs is safe and reduces pre-emptive isolation time to 24.5 hours at the costs of €247.54 (GeneXpert PCR) or €327.84 (IDI PCR) per patient screened.

P990 ***Staphylococcus aureus* reservoirs and transmission routes in a neonatal intensive care unit: healthcare workers, mothers' nipples and clinical folders**

M. Aires-de-Sousa, T. Conceição, M. Miragaia, E. Paulino, R. Barroso, M.J. Brito, L. Sancho, T. Sardinha, H. Carreiro, G. de Sousa, M.C. Machado, H. de Lencastre (Oeiras, Amadora, PT)*

Objectives: *Staphylococcus aureus* is a major human pathogen associated with a high mortality rate in neonatal intensive care units (NICU). The aim of the present study was to identify reservoirs and routes of transmission of *S. aureus* isolates responsible for infection in the NICU of Hospital Fernando da Fonseca, a large (670 beds) tertiary hospital located in the suburbs of Lisbon, Portugal.
Methods: Between July 2005 and December 2007, the presence of *S. aureus* was evaluated in all infection episodes that occurred in the NICU. Samples were recovered from the infected babies (hemoculture, catheters, umbilical and intestinal exudates and urine), from nasal swabs of the parents and health care workers (HCW) that had contact with the babies, from the mothers' nipples in case of breast feeding, and from the environment of the NICU. All isolates were tested for the presence of mecA and virulence determinants including the Panton-Valentine leukocidin (PVL), staphylococcal leukotoxins LukM and LukE/D, staphylococcal exfoliative toxins A, B and D, hemolysins γ, γ-variant and β, staphylococcal enterotoxins A-E, G-J, L and P, and the toxic shock syndrome toxin-1. Moreover, isolates were characterized by pulsed-field gel electrophoresis, spa typing, and multilocus sequence typing.
Results: During the study period, 12 infection episodes occurred in this NICU from which 54 *S. aureus* isolates were recovered. The molecular characterization of the isolates revealed that the majority (70%, n = 38) belonged to three main clones recovered during unrelated episodes: PFGE type A, spa type t1228, ST5 (17 isolates); PFGE type B, spa type t012, ST30 (12 isolates), and PFGE type C, spa type t922, ST1 (9 isolates). None of the isolates was resistant to methicillin nor carried the PVL genes. However, leukotoxin LukE-LukD and hemolysin γ-variant were detected in all isolates other than clones B and J. The transmission routes could be identified in three episodes and involved: (i) one baby and three HCW; (ii) one baby and one HCW; (iii) a baby and the mother's nipples. Strains responsible for infection were detected in unrelated episodes among different HCW and on a clinical folder.
Conclusions: Clonal types A, B and C are endemic in this unit. HCW, clinical folders and the mothers' nipples were identified as possible reservoirs and vehicles of dissemination of *S. aureus* strains. Additional infection control measures are warranted in this NICU.

P991 **Bacteria, bacteria, everywhere: the role of gloves, handwashing and environment in a microbiology laboratory**

L. Ng, S. Tng, S.K. Ng, L.C. Eng, B.C. Ng, S. Chan, T.Y. Tan (Singapore, SG)*

Objectives: This study was performed in a microbiology laboratory to: (a) investigate the effect of glove usage on hand contamination (b) document the effectiveness of hand hygiene (c) document the extent of bacterial contamination on commonly used laboratory surfaces and (d) differentiate contamination by commensal bacteria and potential bacterial pathogens.
Methods: Bacterial culture was performed from the hands of 12 laboratory technologists prior to commencing work, after working with bacterial cultures for 2.5 hours, and after standard handwashing. Staff were divided into two groups: those who used gloves routinely during work, and those who did not. Commonly touched surfaces in the laboratory were also sampled by swabbing a defined surface area. Bacterial culture was performed semi-quantitatively on chromogenic agar for presumptive identification of methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella* spp., Enterobacteriaceae and *Pseudomonas aeruginosa*. Total viable counts were performed on non-selective blood agar plates. MRSA and *P. aeruginosa* were confirmed by standard laboratory methods.
Results: Technologists working without gloves had a significantly increased risk of hand contamination with MRSA (risk ratio 15.3), but not with Enterobacteriaceae. *P. aeruginosa* or *Salmonella* spp. were not isolated from any hand. Handwashing with chlorhexidine-based solutions was effective at removing both pathogens and commensal skin bacteria. Bacterial density was highest on tap handles, followed by telephone handsets. Of potential pathogens, only MRSA was commonly detected (highest density on telephone surfaces, followed by computer keyboards). *P. aeruginosa* and Enterobacteriaceae were occasionally isolated from environmental surfaces, but *Salmonella* spp. was not encountered. MRSA was detected on 70% of laboratory surfaces sampled.
Conclusions: The use of gloves in microbiology laboratories is protective against MRSA. MRSA is also the most common potential pathogen isolated from laboratory surfaces.

Antibiotic resistance in streptococci

P992 **Characterization of a high-level telithromycin-resistant clinical isolate of *Streptococcus pneumoniae* collected from the PROTEKT US surveillance study (years 1–6)**

D. Knight, J. Blackman Northwood, S. Brown, C. Couturier, I. Morrissey (Fordham, UK; Portland, US; Paris, FR)*

Background: PROTEKT US investigated the susceptibility of key respiratory isolates collected from the USA between 2000 and 2006. Of the 55729 *Streptococcus pneumoniae* (SP) from PROTEKT US years 1–6 only 22 (0.04%) were telithromycin-resistant (TelR). Of these isolates all except one (PU6080005) had a Tel MIC of 4 or 8 mg/L. PU6080005 had a Tel MIC of ⩾256 mg/L and in addition to macrolide MIC values of ⩾256 mg/L the isolate was also resistant to tetracycline but susceptible to β-lactams and fluoroquinolones.
Methods: The presence of ermB was determined by PCR. Segments of the L4 & L22 riboprotein genes, the ermB gene and the 4 copies of the 23S rRNA gene were sequenced using previously published methods.
Results: All TelR SP contained ermB with a stop codon at position 28 of their upstream control peptides. In addition, isolate PU6080005 possessed a novel U754A point mutation (*E. coli* numbering) at all four alleles of domain II in the 23S rRNA. Riboprotein sequencing showed wildtype L4 and L22 alleles.
Conclusion: We present the first recording of a clinical strain of SP with a Tel MIC of ⩾256 mg/L. The presence of ermB and the truncation of its upstream control peptide confer low level resistance to telithromycin. Riboprotein L4 and L22 were of wildtype in consequence we attribute the very high Tel MIC to a U754A point mutation in domain II of the 23S RNA at all four alleles never before reported in SP. Indeed it has been demonstrated in *E. coli*, that the proximity of hairpin 35 (residues 738–759) within domain II of 23S rRNA is close to the peptidyl transferase centre and thus a constituent part of ribosomal function and macrolide and ketolide binding affinity. The mutation U754A indentified in *E. coli* conferred low level TelR in addition to erythromycin resistance. We propose that the U754A mutation at all four alleles can cause high level TelR in SP, although this is extremely rare (1 in 55729).

P993 **Prevalence of first-step parC mutants with amino acid alterations at resistance hot spots S79 and D83 among *Streptococcus pneumoniae* isolates in Germany**

B. Körber-Irrgang, A. Röhrig, M. Pletz, M. Kresken (Rheinbach, Hanover, DE)*

Objectives: Resistance (R) of *S. pneumoniae* (SPN) to fluoroquinolones (FQ) is mainly associated with mutations in the genes parC and gyrA encoding for the ParC subunit of topoisomerase IV and the GyrA subunit of DNA gyrase, respectively. Complete R to anti-pneumococcal FQs such as levofloxacin (LVX) requires alterations in both enzymes, while single mutations (first-step mutants) are often associated with susceptibility

to LVX. However, a first-step mutation increases the likelihood for the acquisition of a second mutation resulting in complete R. Among first-step mutants, those with an alteration in ParC predominate. The aim of this study was to evaluate the prevalence of isolates with a first step mutation at the resistance hot spots S79 or D83 in parC among SPN recovered from outpatients with respiratory tract infections (RTIs).
Methods: 296 isolates consecutively collected in 29 laboratories between Jan-Apr 2007 were studied. MICs of LVX, ciprofloxacin (CIP), moxifloxacin (MFX) and norfloxacin (NOR) were determined by the broth microdilution method according the standard ISO 20776–1:2006. EUCAST clinical breakpoints were applied to interpret MICs of LVX, CIP and MFX. PCR-RFLP analyses were performed to screen the isolates for mutations at amino acid positions S79 and D83 of parC. Isolates harbouring mutations at the respective positions were further analysed by sequencing.
Results: All isolates were susceptible to LVX and MFX, while 18/296 (6.1%) were R to CIP. The peak MIC of NOR was 8 mg/L. Isolates exhibiting NOR MICs of >8 mg/L (n = 72) were included in the PCR-RFLP analyses. RFLP screening indicated that 10/72 isolates harboured a mutation in parC. In nine isolates only synonymous nucleotide exchanges at D83 were found. MICs of NOR, CIP, LVX and MFX for these isolates were 16–32, 2–4, 1–2 and 0.125–0.25 mg/L, respectively. One isolate, with MICs of 64, 4, 2 and 0.25 mg/l for NOR, CIP, LVX and MFX, respectively, had an amino acid exchange at position 79 (serine to phenylalanine). This resulted in an overall prevalence of first-step par C mutants with exchanges at amino acid positions S79 or D83 of 0.3% (1/296).
Conclusion: Based on our data, first step par C mutants with alterations at amino acid positions S79 and D83 are rare among SPN isolates recovered from outpatients with RTIs in Germany. Regular screening for such mutants, however, is important because they are precursors of complete FQ-resistant isolates.

P994 *Streptococcus pneumoniae* invasive isolates of serotypes 19A and 24F after pneumococcal conjugate vaccine implementation in Italy

M. Del Grosso, G. Gherardi, R. Camilli, F. D'Ambrosio, M. Monaco, G. Petrucci, G. Dicuonzo, A. Pantosti (Rome, IT)*

Objectives: Introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) has caused a decrease of pneumococcal infections due to vaccine serotypes and an increase of infections due to non-vaccine serotypes. In Italy, in the periods 2001–2003 and 2006-June 2009 an increased proportion of serotypes 19A (from 3.9 to 12.1%) and 24F (from 0.23 to 3.2%) has been observed in *Streptococcus pneumoniae* isolates from invasive diseases. Aim of this study was to characterize isolates belonging to serotypes (st) 19A and 24F collected after PCV7 implementation in Italy.
Methods: All invasive *S. pneumoniae* st19A (79) and st24F isolates (21), recovered from 2006 to June 2009, were studied. Antibiotic susceptibilities were determined by Etest. Resistance genes erm(B), mef(A), mef(E), tet(M), cat, were detected by PCR assay. To define clonal groups, the isolates were examined by PFGE and representative isolates by MLST.
Results: Of the 79 st19A *S. pneumoniae* isolates, 15 (18.9%) were penicillin (PEN) non susceptible (PNSSP); 47 (59.5%) were resistant to erythromycin (ERY), 44 (55.7%) to clindamycin (CLI), 44 (55.7%) to tetracycline, 1 to chloramphenicol. By PFGE, the large majority of the 64 PEN susceptible (PSSP) isolates clustered in PFGE type 44, corresponding to the clonal complex (CC) 199, which is related to the clone Netherlands15B-37. 57.1% of CC199 isolates carried erm(B) and tet(M). The majority of PNSSP isolates clustered in PFGE type 2, corresponding to CC63, related to Sweden15A-25. The majority of CC63 isolates carried erm(B) and tet(M). One PNSSP isolate carrying erm(B), mef(E) and tet(M), with a unique PFGE profile, was found to correspond to CC271, related to the multidrug-resistant clone Taiwan19F-14. Of the 21 st24F isolates, 10 (47.6%) were PNSSP, all were ERY and CLI resistant and carried erm(B), 7 of which carried also tet(M). They clustered in the PFGE type 3 corresponding to CC230, related to the Denmark14–32 clone. Among the PSSP, fully susceptible to the antibiotics tested, two different PFGE types were observed: type 1, corresponding to CC156, related to Spain9V-3, and type 84 corresponding to CC72.
Conclusions: In Italy, the increase of st19A and st24F isolates was mainly due to clonal spread. In both serotypes, PSSP and PNSSP isolates are associated with different and specific clones, e.g. CC199 with st19A PSSP isolates. Studies to define the resistance elements associated to these clones are under way.

P995 Worldwide prevalence of macrolide resistance genotypes among *Streptococcus pneumoniae* from 2004 to 2008

C. Lascols, M. Hackel, A. Wierzbowski, D. Hoban, S. Bouchillon, B. Johnson, J. Johnson, M. Dowzicky (Schaumburg, US; Winnipeg, CA; Collegeville, US)*

Background: *S. pneumoniae* is a key causative pathogen of community-acquired respiratory tract infections. The clinical management of these infections is complicated by the worldwide emergence and spread of resistance to commonly used antibiotics. Resistance to macrolides in *S. pneumoniae* is mediated by two major mechanisms: methylation of the ribosomal macrolide target site, encoded by ermB or drug efflux, encoded by mefA or mefE. Erm(B) typically confers high-level resistance to macrolides, lincosamides and streptogramin B, whereas Mef(A) or (E) confers low-level resistance to macrolides only. The aim of this study was to determine the prevalence of such mechanisms of resistance in various geographical regions.
Methods: A total of 648 macrolide-resistant (erythromycin MIC ⩾1 mg/L) *S. pneumoniae* clinical isolates collected through the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) from 2004 to 2008 were tested. Detection of genes conferring macrolide resistance (ermB, mefE and mefA) was performed by multiplex PCR.
Results: Among these 648 isolates, 58.6% were isolated in North America, 24.7% in Europe, 7.1% in Asia, 6.5% in Latin America, 1.5% in Africa and 0.8% in Middle East and South Pacific.
Of the 648 isolates studied, 35.2% were ermB-positive, 32.9% were mefE-positive, 25% were positive for both ermB and mef and 4.5% were mefA-positive. 2.5% of the isolates were negative for any of these mechanisms. mefE was the most common gene identified in North America (41.6%) while in Europe the most frequent was ermB (67.5%). A high prevalence of ermB/mefE genes (25%) can be highlighted in North America.
Conclusions: The worldwide distribution of pneumococcal macrolide resistance is an important phenomenon because of the diversity of prevalence by region. A rapid increase in isolates containing both ermB and mefE has been noted in the last several years in the United States.

Region	Macrolide resistance mechanism (number of strains)					
	ermB	*mefE*	*mefA*	*ermB+mefE*	Other	Total
North America	84	158	3	126	9	380
Europe	108	17	16	13	6	160
Asia	20	13	–	13	–	46
Latin America	8	20	10	3	1	42
Africa	2	1	–	7	–	10
Middle East	3	2	–	–	–	5
South Pacific	3	2	–	–	–	5
Total	228	213	29	162	16	648

P996 Macrolide resistance mechanisms in serotypes of *Streptococcus pneumoniae*

C. Lascols, M. Hackel, K. Nichol, A. Wierzbowski, D. Hoban, S. Bouchillon, J. Johnson, M. Dowzicky (Schaumburg, US; Winnipeg, CA; Collegeville, US)*

Background: *Streptococcus pneumoniae* is an important pathogen that causes severe life-threatening illnesses in the elderly and children. Increases in macrolide resistance in *S. pneumoniae* clinical isolates have significant clinical implications. The most common mechanisms of macrolide resistance are methylation of the ribosomal target site (encoded by the ermB gene) and drug efflux (encoded by the mefA or the mefE gene). The aim of this study was to characterize these mechanisms in various serotypes of *S. pneumoniae* in order to understand the relationship between macrolide resistance and capsular serotypes.
Methods: 465 macrolide-resistant (erythromycin MIC $\geqslant$1 mg/L) clinical *S. pneumoniae* isolates, collected through the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), were evaluated. Detection of genes involved in macrolide resistance (ermB, mefA and mefE) and serotyping were performed by multiplex-PCR.
Results: 54% of the isolates tested were from North America, 27% from Europe with the remainder (19%) from other continents. Among these 465 strains, the most prevalent pneumococcal macrolide resistance genotypes were mefE/A (42%) and ermB (37%) followed by ermB+mefE (18%) and then other mechanisms (3%). The most prevalent serogroup was 19 (34%) and 6 (17%).
Among ermB+mefE-positive isolates, 84% were serogroup 19 while only 21% were serogroup 19 among ermB-positive isolates. Among mefE/A-positive isolates, only 25% were serogroup 19 and the remaining were other serotypes. High-level resistance (MLSB phenotype) was associated with serogroup 19 while lower-level resistance (M phenotype) was associated with other serotypes.
Conclusions: This study confirms that certain capsular serotypes are associated with macrolide resistance and confirms also the predominance of high-level macrolide resistance among serogroup 19. These findings emphasize the need for continuous worldwide monitoring of macrolide-resistance and serotypes among *S. pneumoniae*.

Serotype	Macrolide resistance mechanism (number of strains)				
	ermB	*mefE/A*	*ermB+mefE*	Other	Total
19A/F	36	48	71	2	157
6A/B	32	46	1	1	80
Other	105	99	13	11	228
Total	173	193	85	14	465

P997 Genetic elements carrying either mef(E) or erm(B)+mef(E) genes within pre-PCV-7 non-invasive Spanish *Streptococcus pneumoniae* isolates belonging to PCV-7 serotypes

E. Gomez, T.M. Coque, F. Baquero, R. Canton (Madrid, ES)*

Objectives: After the introduction of the heptavalent conjugate vaccine (PCV-7), serotypes included in the vaccine (4, 6B, 9V, 14, 18C, 19F and 23F) have decreased worldwide. In Spain, macrolide resistance was maintained high after PCV7. To explain this, clonality and genetic elements carrying mef(E) or erm(B)+mef(E) genes within pre-PCV-7 isolates belonging to these serotypes were studied.
Methods: 28 *S. pneumoniae* isolates belonging to serotypes 6B(n = 8), 9V(n = 3), 14(n = 10), 19F(n = 5) and 23F(n = 2) carrying mef (n = 11) or erm(B)+mef(E) (cMLSB phenotype, n = 15 and iMLSB, n = 2) recovered from 1999 to 2003 were studied. Population structure was analyzed by MLST. Antibiotic susceptibility was determined by microdilution and interpreted with EUCAST criteria. Genetic elements associated with macrolide resistance were determined by screening of sequences related to MEGA, MAS (Macrolide-Aminoglycoside-Streptothricin), Tn917 and Tn916 derivatives (intTn916, xisTn916, tnpATn917, tnpRTn917, tetM, sat4, aphA-3) by multiplex PCR. Linkage among sequences was established by overlapping PCR based on known platforms (Tn6003, Tn6002, Tn3872) and sequencing.
Results: Isolates carrying mef genes were grouped in the Spain9V-3 clonal complex (n = 8) and 3 singleton clones (n = 3). Resistance rates for penicillin, cefotaxime and tetracycline were 91% (10/11), 45% (5/11) and 27% (3/11) respectively. All contained the MEGA element, except one that harboured the mef(A) gene. intTn916, xisTn916, and tetM genes were found in 3 isolates. No linkage between MEGA and Tn916 was detected. Isolates containing erm(B)+mef(E) genes were grouped in 2 clonal complexes (n = 6) and 8 singleton clones (n = 11). Resistance rate for penicillin was 88% (15/17), cefotaxime 41% (7/17) and tetracycline 88% (15/17). intTn916, xisTn916, and tetM genes as well as the MEGA element were detected in 100% of the isolates. Sequencing of different modules of Tn916 including an insertion of 13,000bp fragment in the orf19-orf20 region (partial sequence compatible with a related MAS element) indicates the presence of a Tn916 derivative similar to Tn6002-Tn6003. They also contained MEGA not linked to Tn916.
Conclusions: The association of different transposable elements carrying macrolide resistance genes and a polyclonal structure within pre-PCV-7 non-invasive Spanish *S. pneumoniae* isolates belonging to PCV-7 serotypes might have contributed to the high macrolide resistance rates in our country after the PCV-7 introduction.

P998 Molecular identification of macrolide-resistant *Streptococcus pyogenes* clones in central Greece

E. Tatsidou, E. Malli, A. Damani, K. Pantelidi, E. Drougka, I. Spiliopoulou, E. Petinaki (Larissa, Patra, GR)*

Objectives: To investigate possible changes of the percentage and the type of macrolide resistance among *Streptococcus pyogenes* in Central Greece during a three year period.
Methods: A total of 495 *S. pyogenes* collected from clinical specimens during 2006–2008 were tested for their resistance to macrolide by disk diffusion method in combination with DD test. The ermA (TR), ermB and mef genes were detected by PCR. In addition, all macrolide-resistant isolates were characterized by multi-locus sequencing typing (MLST).
Results: The percentage of macrolide-resistant isolates was 24% and remained stable during the studied period. Among the resistant strains, 17% expressed the M phenotype and 83% expressed the MLS(B) phenotype. All strains of MLS(B) phenotype carried the ermA(TR) gene, while the expression of M phenotype was correlated with the presence of mef gene. Two major clones were identified among M isolates, characterized as sequence types ST39 and ST28, whereas clones ST39, ST63, ST550 and ST89 dominated among MLS(B) isolates.
Conclusions: Although the overall percentage of macrolide-resistant *S. pyogenes* strains in Central Greece has increased since 2001 (19.5%), an inversion in the dominant phenotypes has been identified, with an increase in the MLS(B) phenotype combined with a decrease of the M phenotype, that predominated until 2005 in our area. The presence of ST39 and ST28 clones has been reported previously in other European countries, suggesting a Europe-wide dissemination of few macrolide-resistant lineages.

P999 Molecular epidemiology of macrolide- and telithromycin-resistant group A *Streptococcus* and correlation with antibiotic use in Belgium

L. Van Heirstraeten, J. Vervoort, C. Lammens, S. Coenen, N. Hens, M. De Falleur, H. Goossens, S. Malhotra-Kumar (Wilrijk, Brussels, BE)*

Objectives: We assessed the evolution of macrolide-(MAC-R) and telithromycin-resistant (TEL-R) Group A *Streptococcus* (GAS) during 2004–2008 and also examined the link between resistance and use of macrolides, lincosamides, and streptogramins (MLS) in Belgium.
Methods: A total of 6673 confirmed GAS isolates recovered from tonsillopharyngitis patients in Belgium during 2004–2008 were phenotyped for macrolide resistance based on double disc diffusion and MICs

to erythromycin, clindamycin, and telithromycin. MAC-R GAS were classified into known phenotypes (constitutive, cMLS; inducible, iMLS; and efflux, M). MAC-R genes (erm(A), erm(B), mef(A)) were detected by PCR. Resistant GAS negative for MAC-R genes were analyzed by sequencing L4, L22 and 23S rRNA genes. Emm typing was done by PCR-sequencing. MLS use data for 2003–2008, collected by the Belgian Institute of Health and Disability Insurance (WHO ATC DDD, 2009), was correlated to TEL-R GAS data for 2004–2008 utilizing linear regression.

Results: Overall, 298 (4.5%) MAC-R and 87 (1.3%) TEL-R GAS (TEL MIC $\geqslant$4μg/ml) were identified during 2004–2008 (Table). Prevalence of MAC-R GAS decreased significantly from 2004 to 2005 (χ^2 test; $P < 0.01$) and remained steadily low for the next 3 years ($P \geqslant 0.29$). Prevalence of TEL-R GAS was low during all 5 years. cMLS phenotype was identified in 159 (53.4%), M phenotype in 92 (30.9%) and iMLS phenotype in 47 (15.8%) MAC-R GAS. Majority of the 298 MAC-R isolates carried erm(B) (170, 57.1%), followed by mef(A) (96, 32.2%), and erm(A) (30, 10.1%). Five (1.7%) MAC-R GAS did not show presence of MAC-R genes; 2 harboured the A2059G mutation. TEL-R GAS (TEL MICs 4–32 μg/ml) generally harboured erm(B) alone or with mef(A). One TEL-R isolate each harboured mef(A) and a constitutively expressed erm(A) gene. MAC-R GAS were distributed into 8 major emm types (emm11, 26.2%; emm28, 19.5%; emm12, 15.8%; emm4, 9.7%; emm77, 6.0%; emm1, 3.7%; emm75, 3.0%; emm89, 3.0%). TEL-R GAS predominantly belonged to 3 emm types (emm11, 48.3%; emm28, 18.4%; emm12, 9.2%). MLS use declined from 2003 to 2004 and remained at lower levels till 2008 (Table). Use of a million DDDs of MLS in the preceding year was associated with a 0.82% (95% CI, 0.05–1.59; $P = 0.042$) increase in TEL-R GAS.

Conclusions: Prevalence of MAC-R GAS decreased in Belgium during 2004–2008. TEL-R GAS were restricted to a few emm types. An overall decline in MLS use seems to be a factor in sustaining low levels of TEL-R GAS in Belgium.

Table. Yearly prevalence of macrolide- and telithromycin-resistant Group A *Streptococcus* during 2004–2008 and consumption of macrolides, lincosamides, and streptogramins (MLS) during 2003–2008 in Belgium

Year	No. of GAS isolates			Proportions of MAC-R phenotypes			MLS (J01F) use*
	Screened	MAC-R (%)	TEL-R (%)	cMLS	iMLS	M	
2003	–	–	–	–	–	–	11.2
2004	1553	114 (7.3%)	38 (2.5%)	72 (63.2%)	4 (3.5%)	38 (33.3%)	8.8
2005	1439	64 (4.4%)	3 (0.2%)	33 (51.6%)	7 (10.9%)	24 (37.5%)	9.5
2006	1603	53 (3.3%)	22 (1.4%)	24 (45.3%)	14 (26.4%)	15 (28.3%)	9.4
2007	1175	35 (3.0%)	15 (1.3%)	21 (60.0%)	6 (17.1%)	8 (22.9%)	10.0
2008	903	32 (3.5%)	9 (1.0%)	9 (28.1%)	16 (50.0%)	7 (21.9%)	10.8

*MLS (J01F) use da a expressed in million DDDs.

P1000 Erythromycin-resistance decrease in group A streptococci from acute pharyngitis attributable to change of emm distribution over a 7-year period

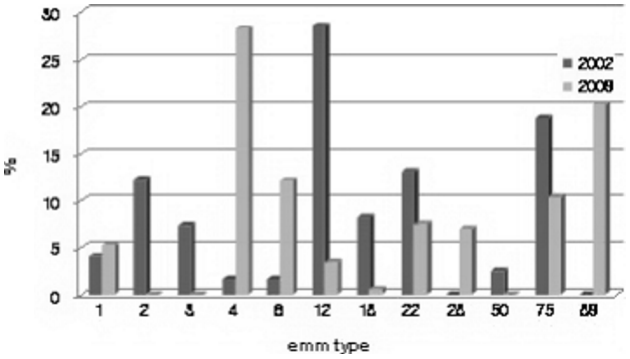
S. Kim, E. Koh, I. Bae, N.Y. Lee, H. Jung (Jinju, Seoul, Masan, KR)*

Objectives: Group A Streptococci (GAS) are the major pathogens associated with bacterial pharyngitis in children. With the increasing resistance of GAS to macrolides in some countries (including Korea), the erythromycin (EM) resistance rates and emm genotypes of GAS were compared by study period.

Method: Throat cultures were taken from 499 patients with acute pharyngitis (two years to 18 years old) at three pediatric clinics in Jinju, Korea from September, 2008 to February, 2009. A total of 174 strains of GAS were isolated and antimicrobial susceptibility testing was performed using the disk diffusion method. The phenotypes of macrolide resistance were evaluated, and the frequency of ermB and mefA genes was determined by PCR. The emm genotype was identified with PCR and sequencing.

Results: The resistance rates of GAS to EM, clindamycin and tetracycline were 4.6%, 2.9% and 2.3%, respectively. Constitutive resistance with the ermB gene was 62.5%, whereas M phenotype with the mefA gene was 37.5%. The emm4 was most frequent (28.2%) and emm89 (20.1%) was next common.

Conclusion: The EM resistance rate of GAS isolated from acute pharyngitis in 2009 was 4.6% which was dramatically decreased from 44.8% in 2002. The emm4 was most frequent (28.2%) and most of EM resistant strains were emm28. A significant decrease of emm12 strains (3.4%) among total isolates also may have contributed to a rapid decrease of EM resistance compared to 2002.



P1001 Assessment of macrolide and tetracycline resistance phenotypes/genotypes among streptococci isolates from bovine subclinical mastitis

M. Rato, R. Bexiga, S.F. Nunes, L. Cavaco, C.L. Vilela, I. Santos-Sanches (Caparica, Lisbon, PT; Cambridge, UK; Copenhagen, DK)*

Objectives: To identify macrolide/tetracycline phenotypes and genotypes among streptococcal species from bovine subclinical mastitis, valued in therapeutic policies and to evaluate the routes of gene dissemination.

Methods: A total of 32 *Streptococcus agalactiae* (Group B *Streptococcus*-GBS), 18 *S. dysgalactiae* subsp. *dysgalactiae* (Group C *Streptococcus*-GCS) and 30 *S. uberis,* from 11 farms, were included in the study. Resistance to penicillin-P, ampicillin-AMP, erythromycin-E, pirlimycin-PRL, tetracycline-T, and the constitutive macrolide-lincosamide resistance phenotype (cMLS) was evaluated by disk diffusion. Resistance genes (mefA/ermA/ermB/linB/tetM/tetO/tetT/tetS/tetQ/tetK/tetW/tetL) were PCR screened. Clonality was assessed by $\geqslant$80% similarity in dendrograms (BioNumerics v. 4.0 software, Applied Maths) using SmaI-pulsed field gel electrophoresis (PFGE) patterns. Cfr9I digestion was used in one SmaI-resistant GCS isolate. Presence of the composite transposon Tn1207.3/phi10394.4 encoding a methyltransferase responsible for nontypeability using SmaI, was PCR tested in all GCS isolates. This element is carried by the human pathogen *S. pyogenes* (Group A *Streptococcus*-GAS).

Results: Co-resistance to macrolides-E and lincosamides-PRL (18–27%) and T-resistance (60–100%) was observed in all species. Resistance to PRL and susceptibility to E (LSA phenotype) was found in GCS and *S. uberis.* Diverse genotypes were found: ermB/tetO/tetK in GBS; ermB/tetO or linB/tetM in GCS; ermB/tetO or linB/tetS in *S. uberis.* A total of three PFGE clusters comprised 72% of the GBS and four PFGE clusters comprised 53% of the *S. uberis* and all were found to be herd-specific. Among four PFGE clusters comprising 56% of the GCS, three included strains from different farms. Amplification of Tn1207.3/phi10394.4 left junction showed an amplicon size different from what's described for GAS and no amplification of the right junction was observed.

Conclusions: Decreased susceptibility to P and AMP was noted in all species, in contrast to other studies. Amplification of Tn1207.3/phi10394.4 left junction (despite variable size) in bovine GCS suggests that this mobile element may be inserted in the comEC locus as mapped for GAS. The linB gene, known to be carried by a large conjugative plasmid was found in *S. uberis* and GCS indicating a possible horizontal gene transfer event. The putative linkage of several erm/tet genes in unique strains and clones suggests vertical and horizontal gene dissemination.

P1002 Molecular characterization of levofloxacin resistance in *Streptococcus dysgalactiae* ssp. *equisimilis* isolates from Portugal

M.D. Pinho, M. Ramirez, J. Melo-Cristino on behalf of the Portuguese study group for streptococcal infections*

Objectives: A very high proportion of *S. dysgalactiae* subsp. *equisimilis* levofloxacin resistant isolates (12.1%) was found among a collection of β-haemolytic group C and G streptococci from Portugal (n = 315). To gain further insights into the resistance mechanisms involved and the clonal structure of the resistant population, we undertook molecular characterization and evaluated the occurrence of recombination with *Streptococcus pyogenes* (GAS) DNA.
Methods: Antimicrobial susceptibility testing was determined by disk diffusion and MIC testing was done for all the levofloxacin nonsusceptible isolates. Pulsed-field gel electrophoresis (PFGE) and emm typing were used to characterize the population. Sequence analysis of the quinolone-resistance determining region (QRDR) of gyrA and parC was carried out in 56 *S. dysgalactiae* subsp. *equisimilis* isolates, including representatives of the resistant, intermediate and susceptible populations. Phylogenetic and recombination analysis were performed with MEGA 4 and RDP3 software, respectively.
Results: A total of 38 isolates were resistant to levofloxacin (MIC range, 6 to >32 ug/mL), and 4 expressed intermediate resistance (MIC range, 3 to 4 ug/mL). Among resistant isolates, 11 distinct emm types were found distributed in 9 PFGE clusters that overlapped with the main clusters detected in the population. The emm types stG166b (n = 12) and stG6792 (n = 8) accounted for more than half of resistant isolates. Most resistant isolates were found dispersed in the population but stG166b was distributed in two main clusters, one comprising only levofloxacin resistant isolates and the other only susceptible ones. Mutations in both gyrA and parC QRDR were necessary for high level resistance to levofloxacin while single mutations in each gene resulted in slightly increased MICs. A much greater diversity was found among parC alleles than among gyrA. Evidence for recombination with GAS was found in some parC alleles of both resistant and susceptible isolates.
Conclusion: Levofloxacin resistance had a polyclonal origin, with resistance emerging among most susceptible clones. Our data supports the existence of a common reservoir of genes conferring quinolone resistance shared between *S. dysgalactiae* subsp. *equisimilis* and GAS. The ocurrence of recombination with foreign DNA is not strictly associated with resistance in *S. dysgalactiae* subsp. *equisimilis*.

P1003 Distribution of serotypes, antimicrobial resistance and virulence-related genes among group B streptococci of vaginal origin isolated in Romania

M. Straut, S. Cretoiu, V. Ungureanu, A.M. Petrini, R. Georgescu, L. Grigore, C.R. Usein (Bucuresti, RO)*

Objectives: The first regional surveillance of group B streptococci (GBS) of vaginal origin isolated from healthy women has been organized within a national research project. The aim of this prospective study is to characterise GBS isolates using phenotypic and molecular methods.
Methods: Ninety-six GBS vaginal isolates were examined by phenotypic and genotypic techniques. All isolates were characterized by latex agglutination serotyping, susceptibility to antimicrobial agents and, when appropriate, tetracycline resistance genes (tetM, tetO), tetM gene association with conjugative elements of the Tn916 family, macrolide resistance phenotypes and genes (ermA, ermB, mefA) were searched. Molecular typing included capsular type assigning by multiplex PCR assay and PCR screening for five major surface protein antigen genes. Clonal relationships among epidemiologically unrelated isolates were established by PFGE analysis of SmaI macrorestriction patterns.
Results: The isolates studied were fully susceptible to penicillin, ampicillin, and cefuroxime, but the rate of tetracycline resistance was high, accounting for 95% of them. tetM was the most prevalent gene. tetO gene was detected in association with tetM in 7 resistant strains. The int-Tn gene, encoding the integrase of Tn916, was found in 75% of the strains harbouring tetM. Twenty percent of the strains studied exhibited decreased susceptibility to macrolide and were positive for ermB, constitutive MLS being the dominant phenotype. The isolates belonged to every serotype from Ia, Ib to V and no non-typeable cases were encountered. Serotypes III and II have been the most prevalent, confirming previous findings. The presence of one of the following surface protein genes encoding A-C, Alp1, Rib, Alp2/3, and Alp4 protein was documented in all the isolates tested. The association of serotypes III and II with Rib protein encoding gene, and of serotype V with AC was found.
Conclusion: Although testing for GBS carriage is mandatory during pregnancy, the laboratory data regarding characteristics of GBS circulating strains among Romanian women population is scarce. This report is the first molecular approach in characterisation of native GBS isolates. Our results may provide useful additional information regarding the reservoir of strains for a better understanding of the epidemiology of GBS infections in Romania.

P1004 Diversity of CTn6000 among enterococci from different ecological niches

C. Novais, A. Freitas, F. Baquero, L. Peixe, A.P. Roberts, T.M. Coque (Porto, PT; Madrid, ES; London, UK)*

Objectives: Conjugative transposons (CTn) have contributed to the spread of tetracycline resistance by encoding tetM (Tn916, Tn5397, Tn5801) and less frequently, tetS (Tn6000, Tn916S). CTn6000 (formerly EfcTn1) was initially identified in an *Enterococcus casseliflavus* strain from a primate (J. Bacteriol 2006;188:4356) but it seems to be spread among enterococcal species from different ecological niches (19th ECCMID; P947). The presence and diversity of Tn6000 was analyzed among tetracycline resistant enterococci from diverse origins.
Methods: Preliminary detection of CTn6000 was carried out by screening for the presence of integrase (intCTn6000) and excisionase (xisCTn6000) genes among enterococci from animals (n = 164), healthy/hospitalized human (n = 227) and environment (n = 126) (1996–2008) by PCR and sequencing. Further analysis of CTn6000 backbone was accomplished by long-PCR mapping based on the 33kb CTn6000 sequence (A.P. Roberts, unpublished). Amplicons were digested (TaqI/AluI/EcoRI/DdeI) and representatives of different restriction profiles were sequenced. Analysis of the chromosomal insertion site of CTn6000 and mating assays were carried out as previously described. Clonal relationship among strains was evaluated by PFGE and MLST.
Results: CTn6000 was found in 2% of human (n = 5/227) and environmental (n = 3/126) isolates each. They include 5 *E. faecalis* (4 healthy humans faeces, ST55), 2 *E. faecium* (environmental farm sample and human drinking water-ST32, CC22) and 1 *Enterococcus* spp (farm waste lagoon). Two CTn6000 variants designated as types B and C were identified. They differ from the original backbone (type A) by the absence of a group II intron inserted in orf14 (CTn6000 type C) and the presence of ISEfm2 in the intergenic region of orf13tetS (CTn6000-B). All variants were inserted within the gene coding a L31 ribosomal protein. Transfer of CTn6000 was only achieved for the CTn6000-A carrying strain.
Conclusion: CTn6000 is a site-specific integrative element widely disseminated among different enterococcal species from community origin. The diversity of backbones identified in this highly modular platform (CTn6000 contains sequences from mobile elements of different Gram-positive bacteria) highlights the plasticity of CTns and the interactions of bacteria from these ecological niches.

Vancomycin-resistant enterococci (VRE)

P1005 Risk factors for the acquisition of VRE in a German university hospital

A. Kola, I. Hoffmann, D. Sohr, I. Chaberny, S. Suerbaum, P. Gastmeier (Berlin, Hanover, DE)*

Objective: To analyse an increase of vancomycin-resistant *E. faecium* (VREfm) at Hanover Medical School (MHH), a 1 400 bed university hospital with 40 000 admitted patients per year. The proportion of VREfm rose from 1.2% in 2004 to 20.5% in 2006.
Methods: Genotyping of VREfm was performed by pulsed field gel elecrophoresis (PFGE): SmaI-restriction profiles yielding a similarity of >80% using the Dice coefficient (<4 different fragments) were considered as clonally related. Typing results and conventional epidemiology were applied for transmission analysis. For risk factor analysis, a case–control study was carried out: Cases were inpatients with VREfm acquisition from January 2005 through December 2006, controls patients with vancomycin-susceptible *E. faecium*. Case and control patients were matched 1:1 on date and site of isolation.
Results: PFGE was performed on 171 isolates of 166 patients hospitalized on 30 wards and revealed 57 different genotypes (36 unique and 21 in clusters of 2–31 isolates belonging to the same type). Sixty-one patients (37%) with VREfm were in the general surgery unit and 38 patients (23%) in the haematological oncology unit. In these units, 30% of VREfm were due to patient-to-patient transmissions. Multivariate logistic regression analysis of 142 cases and 142 controls identified the presence of neutropenic days (OR 7.7, CI95 1.5–39.8, $p < 0.05$) and a hospital admission within the previous 12 months (OR 5.3, CI95 2.5–11.3, $p < 0.05$) as risk factors for acquisition of VREfm. Additional independent risk factors were use of glycopeptides antibiotics (OR 3.7, CI95 1.6–8.7, $p < 0.05$), mechanical ventilation (OR 3.4, CI95 1.1–10.1, $p < 0.05$) and a history of additional multidrug-resistant organisms (OR 3.4, CI95 1.4–7.9, $p < 0.05$).
Conclusion: Risk factors like immunosupression, previous hospitalization and mechanical ventilation indicate that patients with severe underlying diseases were mainly affected by VREfm. In these patients, the use of glycopeptide antibiotics was a main risk factor for VREfm acquisition. A substantial proportion of VREfm acquisition was due to transmission.

P1006 Financial burden of nosocomial infections caused by vancomycin-resistant *Enterococcus*

E. Ott, L. Schwadtke, K. Graf, F. Schwab, F. Bange, I. Chaberny (Hanover, Berlin, DE)*

Background: Nosocomial infections due to vancomycin-resistant *Enterococcus* (VRE) increased considerably during the last years. The purpose of this study was to investigate the costs for nosocomial VRE-infections inside the German DRG-System.
Methods: A case–control study was performed comparing patients with nosocomial infections caused by VRE and by vancomycin-susceptible *Enterococcus* (VSE) between January 2005 and December 2008. 47 nosocomial cases with VRE-infections were matched to 47 controls with VSE-infections for admission and discharge in the same year, the minimum length of stay corresponding to time at risk of the case, Charlson comorbidity-Index ±1, stay on intensive care units (ICU) and non-intensive-care units as well as for the type of infection, which were defined using the CDC criteria.
Results: The median overall costs for cases with nosocomial VRE-infection were significant higher than for control patients (€ 57,817 vs. € 38,334; $p = 0.045$). The median attributable costs for VRE-infections were € 12,979 ($p = 0.058$). Furthermore, the multivariate analysis showed that cost were driven by VRE-infection (multiplicative effect (ME) = 1.4; $p < 0.042$), ventilation >500 h (ME = 3.3; $p < 0.001$), and stay on ICU (ME = 2.4; $p = 0.002$). In contrast age over 60 years was a predictor for decreased costs (ME = 0.7; $p = 0.034$).
Conclusion: This analysis revealed that nosocomial infections due to VRE are associated with high costs for healthcare systems compared with VSE-infections. Therefore hospital personal should implement control measures to prevent the transmission of VRE.

P1007 Characterization of vancomycin-resistant *Enterococcus* isolates recovered in a Spanish hospital, 2003–2008

M. Lopez, E. Cercenado, E. Bouza, F. Ruiz-Larrea, Y. Sáenz, C. Torres (Logroño, Madrid, ES)*

Objective: To study and characterize the resistance phenotype and genotype, the mechanism of vancomycin resistance and the virulence genes, in vancomycin-resistant enterococci (VRE) isolated in a Spanish hospital.
Methods: Fifty VRE were recovered at the Gregorio Marañón Hospital, Madrid, during a period of six years (2003–2008) from a total of 10,381 enterococci (0.48%). A species-specific PCR was used to identify these isolates. Antimicrobial susceptibility to 11 antibiotics was determined by disk diffusion and by broth microdilution methods. Vancomycin resistance genotype was determined by PCR and sequencing of vanA, vanB, vanC-1, vanC-2/3 and vanD genes. The presence of the resistance erm(B), tet(M), tet(L), ant(6)-Ia, aac(6′)-aph(2″), aph(3′)-IIIa, catA and the virulence esp, hyl genes was determined by PCR. Tn5382 and Tn1546 structures were characterized in vanB and vanA isolates, respectively.
Results: Twenty two of the VRE isolates were *E. faecalis*, 13 *E. faecium*, 13 *E. gallinarum,* 1 *E. avium* and 1 *Enterococcus* spp. The most prevalent vancomycin genotype was vanB (46%), followed by vanA (28%) and vanC1 (26%). No vanD gene was detected in our isolates. Almost all VRE isolates showed a multiresistance phenotype and harboured different resistance genes (number of isolates): erm(B) (45), tet(M) (37), tet(L) (31), ant(6)-Ia (28), aac(6′)-aph(2″) (21), aph(3′)-IIIa (41) y catA (1). The analysis of the virulence genes esp and hyl showed the following results (number of isolates): esp and hyl (20), esp (10), hyl (13), none of these genes (7).
All of the vanB isolates presented the vanB operon included in the Tn5382 and none of them contained IS150, ISEnfa110 or ISEnfa200 insertion sequences. In six vanB strains the linkage pbp5-Tn5382 was evidenced. Study of the vanA operon, included in Tn1546, revealed 8 different structures, one of them identical to the prototype (GenBank accession no. M97297), detected in two isolates, and the remaining seven, showed different insertion sequences or deletions.
Conclusions: The incidence of VRE was low. Vancomycin-resistance is more frequent among *E. faecium*, and the most prevalent genotype is vanB2. vanA-containing enterococci showed a high variability in Tn1546 structure.

P1008 Diversity of hyl plasmids among international CC17 *Enterococcus faecium* strains, 1992–2009

A.R. Freitas, A. Tedim-Pedrosa, C. Novais, L. Peixe, F. Baquero, T.M. Coque (Porto, PT; Madrid, ES)*

Objectives: To analyze the location and diversity of hyl genetic context among epidemiological relevant *Enterococcus faecium* (Efm) strains recovered from hospitalized patients of different countries over the last two decades.
Methods: We analyzed 145 vancomycin-resistant (VREfm; 125 vanA, 20 vanB) isolates causing documented hospital outbreaks in 23 countries (1986–2009) and 83 VSEfm [52 ampicillin-resistant (AREfm) and 31 ampicillin-susceptible ASEfm] bloodstream isolates from Spain (Madrid; 1995–2008). Clonality was established by PFGE and MLST. The hylEfm gene was detected by PCR, sequencing and S1/I-CeuI PFGE hybridization. Analysis of Hyl plasmids included determination of size and the detection of relaxases (rel) and rep initiator proteins (rep) by multiplex PCR typing schemes, designed to identify 100 different Gram-positive plasmids, hybridization and sequencing.
Results: Hyl was identified among CC17-VREfm (28/145, 19%, 9STs) recovered from 15 countries (Spain, Portugal, The Netherlands,

Germany, Poland, Hungary, Serbia, Italy, Greece, USA, Canada, Brazil, Australia, Tunisia, South Arabia) of 5 continents (1992–2009) and among CC17-AREfm isolates (17/52, 33%; 6 STs) from Spain (1997–2007). None of the ASEfm (n = 31; most CC9 and CC22) analyzed contained hyl. All sequenced hyl genes were identical to genes encoding putative hyaluronidase proteins from different *E. faecium* genomes. Hyl was consistently located on large plasmids ranging from 170 kb to 375 kb. Although most of them did not contain any of the rep and rel sequences analyzed, we identified relpEF1 (n = 15), relpAD1 (n = 2) or both (n = 3) in some plasmids <300kb. Under antibiotic selection with vancomycin, some relpEF1-Hyl+ plasmids (n = 4/15) were cotransferred with vanA plasmids (3 plasmids of 50–60kb) or vanB-CTn (n = 1). van and hyl genes were not identified in the same platform in any isolate.

Conclusions: Hyl is located on a diversity of large plasmids recovered from isolates of different countries since early 90's. The wide distribution of these hyl-megaplasmids among VREfm and AREfm belonging to CC17 might enhance adhesion and invasiveness abilities of CC17 as suggested (Arias et al. AAC 2009; 53:4240) and thus, to have contributed to the recent success of this lineage as leading nosocomial pathogen. The possibility of cotransfer with plasmids encoding resistance to vancomycin is of concern since it would further facilitate the success of CC17 in hospitals.

P1009 Plasmid analysis of vancomycin-resistant enterococci isolates from Portuguese poultry and healthy humans

A.R. Freitas, C. Novais, A. Cunha, E. Silveira, L. Peixe, T.M. Coque (Porto, PT; Madrid, ES)*

Objectives: Despite growth promoter ban in Europe in 1997, vancomycin-resistant enterococci (VRE) are still recovered from poultry (P) and healthy humans (H) in Portugal. We analyzed the genetic context of VRE strains to better understand the plasmid ecology and the maintenance of community VRE in this country.

Methods: Fifty vanA enterococci from P carcasses (26 *E. faecium* (Efm), 7 *Enterococcus* spp. (Es), 5 *E. hirae* (Eh), 4 *E. durans* (Ed); 1 *E. faecalis* (Efc); 9 commercial brands) and faecal samples of HV (7 Efm) were studied. Clonality (PFGE, MLST), conjugation, antibiotic susceptibility (CLSI) were performed by standard procedures. Plasmid analysis included determination of size, content and location of vanA (S1-PFGE, hybridization); comparison of RFLP patterns; and detection of relaxases (rel), replication proteins (rep) and toxin-antitoxin systems (specific PCR typing, sequencing).

Results: We identified 30 PFGE types: 25 among P (6 within Efm-CC9 and 1 was Efc-ST16) and 5 among H (2 within Efm-CC17 and 1 singleton) multidrug resistant isolates, some persisting over years. Eight Tn1546 types, mostly prototype A (30%), were located on large plasmids (50–165kb). vanA was transferred to Efm GE-1 or 64/3 and Efc JH2–2 recipients (n = 24/50, 48%; 16Efm, 4Eh, 3Es, 1Ed), frequently associated with resistance to erythromycin (46%) and/or streptomycin (21%). Plasmids contained rel/rep sequences homologous to pEF1 (60%/40%), theta replicating pB82/p200B (36%/50%), rolling-circle-replicating pEFNP1 or pJS42/pRI1 (10%/14%), Inc18 (0%/6%), pheromone-responsive pAD1 or pAM373 (34%/15%), pHTβ/pMG1 (32%/14%) and mosaic as pVEF1/2 (0%/22%) or pRUM (6%/16%); and the toxin-antitoxin omega-epsilon-zeta system (26%). vanA plasmids often contained rel-pEF1 and rep-pEF1 although we did not identify any rel in some cases. Rep-pRUM was confined to plasmids from H. Three vanA plasmids from P were identified among isolates of different species, commercial brands and years i) type A, 60–80kb (4 Efm, 2 Eh clones, 3 brands, 1999–2000); ii) type B, 50kb (2 Efm clones; 2 brands; 2001) and iii) type C, 75kb (Efm, Ed; 1999).

Conclusion: Besides clonal spread, different plasmid types seem to have contributed to the maintenance and spread of VRE in the community setting of our country. Further analyses are required to complete the genetic backbone of vanA plasmids and their association with particular Tn1546 types.

P1010 Vancomycin-resistant enterococci in Finland, 2006–2009

M. Karden-Lilja, J. Vuopio-Varkila, S. Salmenlinna (Helsinki, FI)*

Objectives: Vancomycin-resistant enterococci (VRE) are an emerging cause of hospital-acquired infections. Especially one genetic lineage of *Enterococcus faecium*, designated as clonal complex 17 (CC17) based on multilocus sequence typing (MLST), ampicillin resistance and presence of esp gene, has spread worldwide. In Finland, the first VRE epidemics were reported in the mid 1990s; since then eight epidemic strains have been recognised. The aim of this study was to characterise the recent Finnish VRE isolates by molecular means.

Methods: All VRE findings are reported to the National Infectious Diseases Register and the corresponding isolates (1 per person) are sent to National Institute for Health and Welfare for typing. All isolates from 2006–2009 were analysed by pulsed-field gel electrophoresis (PFGE). A strain was considered as epidemic when a PFGE profile with <7 band differences was encountered from at least five persons. MLST was performed to selected strains of *E. faecium* representing different PFGE types from years 2006–2009 and to the Finnish epidemic VRE strains from previous years. The presence of van genes was verified by PCR.

Results: In 2006–2009, VRE was isolated from 132 persons. The annual number of strains varied between 58 in 2007 and 7 in 2009 and the majority (117/132, 89%) came from two of the 21 health-care districts. Thirteen isolates (10%) were from clinical infections, including three from blood. *E. faecium* accounted for 90% (119/132) of the isolates and 92% of these carried vanB resistance gene (110/119).

In total, 49 PFGE types were encountered; two types, VRE IV and VRE VII, accounted for 46% (61 isolates) and 15% (20 isolates) of the isolates, respectively. A sporadic PFGE type was found from 44 (33%) isolates.

Among 30 strains selected for MLST, 11 sequence types (ST) were found. The STs of the two most prevalent epidemic strains were ST18 (VRE IV) and ST343 (VRE VII). Other STs among epidemic strains were: ST16, ST17 and ST252. Of the 13 sporadic strains selected for MLST, 8 had a different ST (ST275, ST78, ST412, ST192 and two new STs) from STs of any of the epidemic strains.

Conclusions: VRE infections are rare in Finland. Most VRE strains are vanB positive *E. faecium* and based on MLST, belong to CC17. Uneven geographical distribution suggests differences in VRE diagnostic and/or surveillance.

P1011 Epidemiology of vancomycin-resistant enterococci in an Irish tertiary care hospital

C. Wrenn, J. Kavanagh, N. O'Flaherty, L. Fenelon, K. Schaffer (Dublin, IE)*

Background: A significant increase of infections with vancomycin resistant enterococci (VRE) was noted among haematology, liver transplant patients and general surgical patients of a large tertiary care hospital in Ireland during 2006/7. Only patients on high risk ward areas (haematology, liver transplantation and intensive care) had been screened weekly for VRE carriage. Pulsed field gel electrophoresis (PFGE) identified two major outbreak strains circulating in the hospital (EC4 and EC5). Objective: To investigate the VRE epidemiology in the hospital in an attempt to contain VRE cross infections.

Methods: An environmental screen for VRE was performed in the high risk ward areas and on the general surgical wards. In addition 115 faecal specimen taken from the general in-patient population from all wards and 100 specimens referred from the community were anonymously investigated for VRE. Faecal specimens were cultured on chromID VRE. After biochemical identification and antibiotic susceptibility testing clonal relationships were investigated by PFGE.

Results: The haematology and liver transplant unit are cleaned daily with hypo-chlorite solution. From these wards only 1% of environmental specimens were found to be positive. In contrast 19% of surfaces screened in the surgical ward area which is not routinely cleaned with hypo-chlorite solution yielded positive cultures for VRE. Screening of

115 faeces samples from the general in-patient population revealed a VRE prevalence rate of 38.3%. However VRE was found in 1 of 100 samples referred from the community. Pulsed field gel electrophoresis demonstrated that 66.7% of the isolates recovered from the in-patient population belonged to the outbreak clusters EC4 and EC5.
Conclusion: VRE colonization is endemic in the endemic in the hospital. Two dominant clonal strains are circulating in the hospital suggesting that the main mode of transmission is nosocomial and environmental screening suggests that contaminated surfaces are potential sources of cross infection.

P1012 Expansion of CC2 *Enterococcus faecalis* lineage in a Spanish tertiary hospital and its surrounding area

P. Ruiz-Garbajosa, A. Tedim-Pedrosa, C. Rodríguez, R. Cantón, F. Baquero, T.M. Coque (Madrid, ES)*

Background: The *Enterococcus faecalis* (Efc) population structure comprises widespread clonal complexes (CC2, CC9 and CC87) over-represented among hospitalized patients with heterogeneous distribution in different countries. Vancomycin resistant Efc is mostly linked to CC2 lineage. The aim of this study is to assess the population structure of Efc isolates in a geographic area with low occurrence of vancomycin resistance.
Material and Methods: We analyzed 54 Efc isolates recovered from hospitalized and community-based patients (one per patient) during 2009. The collection included 20 invasive clinical isolates (blood isolates); 20 non-invasive clinical isolates (13 urines and 9 wounds) and 14 faecal isolates. Population structure was analyzed by MLST (www.mlst.net). Antibiotic susceptibility was determined by microdilution (CLSI). Presence of asa1, cyl, esp, and gelE virulence genes were tested by a multiplex PCR.
Results: Isolates were resistant to erythromycin (Er, 69%), levofloxacin (Lvx, 35%), minocicline (Mn, 42%) and high levels of resistance (HLR) to streptomycin (HLR-Sm, 50%) and gentamicin (HLR-Gm, 38%). None of the isolates were resistant to ampicillin, vancomycin, teicoplanin, linezolid or daptomycin. The presence of gelE, asa1, esp and cylA genes were detected in the 87%, 63%, 15% and 18% of the isolates, respectively. MLST analysis revealed 27 STs, being ST6-CC2 (n = 16, 29%) and ST16 (n = 5, 9%) the most prevalent lineages. Remaining Efc isolates (n = 33) were distributed in 25 STs (1 CC4, 1 CC9, 2 CC21, 1 CC25, 4 CC30, 2 CC55, 3 CC72) and 17 singletons (19). ST6 comprises isolates from blood (50%), urine and wounds (38%) and feces (12%) from patients at different hospital wards and community-health care centers. Most of ST6 isolates contained gelE and asa1 (69%) and a high percentage (81%) were resistant to Er, Mn, Lvx with HLR-Gm and HLR-Sm. Only 3 isolates were cylA positive. ST16 isolates were recovered from non-invasive sources and were HLR-Gm. Other STs (25 STs/33 isolates) corresponded to isolates resistant to less than two antibiotics (29/33; 88%). They contained either gelE alone (12/33; 36%) or in combination with asa1 (14/33; 42%).
Conclusions: We describe the predominance of the globally spread CC2 lineage among Efc isolates from our area, many of them causing serious infections. Expansion of this lineage is of concern since it can serve as substrate for vancomycin resistance as reported in other European countries.

P1013 Prevalence of faecal carriage of vancomycin- and ampicillin-resistant enterococci among healthy people in Cantabria, Spain

P. Goicoechea, M. Romo, J.I. Soto, J. Calvo, M.G. Gonzalez, L. Martínez-Martínez, M.V. Francia (Santander, Noja, ES)*

Objectives: During the last decade, the spread of vancomycin-resistant (VRE) and ampicillin-resistant (ARE) enterococci has greatly increased in our hospital. We aimed to determine the prevalence of VRE and ARE faecal colonization among nonhospitalized individuals living in our community. Antibiotic susceptibility, clonal relationship and the presence of virulence determinants were specifically determined.
Methods: From March 2008 to August 2009, rectal swabs from 595 nonhospitalized subjects were inoculated onto azide bile-esculin agar plates with ampicillin (16 mg/L) or vancomycin (6 mg/L) for 48 h. Identification and susceptibility testing were done with the Walk-Away System and confirmed by 16S DNA sequencing and E-test method, respectively. Clonal relatedness was assayed by PFGE and MLST. Specific virulence determinants were tested by PCR as previously described.
Results: 5 strains were isolated from the vancomycin agar plates: 1 *E. faecium*, 1 *E. gallinarum* and 3 *E. casseliflavus.* 12 individuals were ARE carriers (2%). All ARE isolates were *E. faecium*. The percentages of resistance of the 13 *E. faecium* isolates recovered were: ampicillin (92), ciprofloxacin (69), erythromycin (61), high-level resistance streptomycin (84), high-level resistance gentamicin (0), tetracycline (53), vancomycin (0), chloramphenicol (46), linezolid (0), quinupristin/dalfopristin (53). Vancomycin MIC for the one *E. faecium* isolate recovered after vancomycin selection was 6 mg/L. The isolates were classified into 6 PFGE types, highlighting the presence of two clones containing 6 and 3 isolates, respectively. MLST analysis displayed 4 known sequence types (STs), ST117 (n = 6), ST264 (n = 3), ST265 (n = 1), ST5 (n = 1) and 2 new STs (n = 2). Only the CC17 isolates (n = 6) were positive for esp acm and entA virulence genes.
Conclusions: ARE faecal carriage was 2% among healthy individuals in our community. As many as 46% of the ARE isolates belonged to CC17 clonal complex suggesting a possible hospital acquisition of such strains. Our results indicate absence of VRE colonization outside hospitals in Cantabria in contrast to the findings in other European countries.

P1014 Prevalence of colonization with vancomycin-resistant and ampicillin-resistant *Enterococcus faecium* in a university hospital in northern Spain

M. Romo, P. Goicoechea, J. Calvo, M. de la Cal, M. Gozalo, M. Alvarez, M.C. Fariñas, L. Martínez-Martínez, M.V. Francia (Santander, ES)*

Objectives: CC17 *Enterococcus faecium* has increasingly been reported as a nosocomial pathogen worldwide. We aimed to investigate vancomycin-resistant (VRE) and ampicillin-resistant (ARE) *E. faecium* intestinal colonization among inpatients at our hospital. Antibiotic susceptibility, clonal relationship and the presence of virulence determinants or mobile genetic elements were specifically determined.
Methods: From February to August 2009, 576 patients admitted to several units at our hospital (and without hospital stays during the previous year) were screened for VRE or ARE faecal carriage. Rectal swabs (collected on admission and weekly until the patients' discharge) were inoculated onto azide bile-esculin agar plates with ampicillin (16 mg/L) or vancomycin (6 mg/L) for 48 h. Identification and susceptibility testing were done with the Walk-Away System and confirmed by 16S DNA sequencing and E-test method, respectively. Clonal relatedness was assayed by PFGE and MLST. Specific virulence determinants and plasmid content were tested by PCR as previously described.
Results: On admission, 24 patients were colonized with ARE and 1 with VRE. 9 patients were ARE colonized after a hospital stay >48 h. The percentages of resistance of the 34 *E. faecium* isolates recovered were: ampicillin (97), ciprofloxacin (91), erythromycin (85), high-level resistance streptomycin (82), high-level resistance gentamicin (8), tetracycline (32), vancomycin (2), teicoplanin (2), chloramphenicol (26), linezolid (0), quinupristin/dalfopristin (29). The isolates were classified into 14 PFGE types, highlighting the presence of 1 major clone containing 15 isolates. MLST analysis displayed 9 known sequence types (STs), ST117, ST18, ST17, ST192, ST375, ST262, ST264, ST265, ST123 and 2 new STs. Most of the isolates belonged to CC17 (76%). The percentages of virulence genes were as follows: esp (55), hyl (17), acm (44) and entA (88). Isolates encoded a variety of plasmid modules, being pRE25, pEF1, pCIZ2, pAD1 and pEFNP1 the predominant ones.
Conclusions: 1 of every 25 patients was ARE colonized on admission at our hospital. As CC17 represents a clonal complex usually associated to hospital settings and rarely found in the community, CC17 carriage

on admission might be associated with previous hospital stays. CC17 ARE intestinal persistence times might then be longer than 1year, facilitating dissemination of these strains. VRE was nearly absent among hospitalized patients at our hospital.

P1015 Phenotypic and genotypic traits of vancomycin-resistant enterococci in a teaching hospital, Ankara, Turkey: the first vanB-positive *Enterococcus faecium* isolates

F.A. Coskun, I. Mumcuoglu, N. Balaban, Z.C. Karahan, E. Us, A. Tekeli, I. Baran, S. Kursun (Ankara, TR)*

Objectives: Since the first VRE isolation in our country in 1998, various hospitals have been reporting increasing number of VRE isolations. In this study, our objective was to determine genetic relationships and resistance genotypes of VRE strains which were isolated from surveillance cultures of patients mostly staying in ICUs and cultures of various clinical specimens.
Methods: From January 2007 to March 2008 a total of 777 rectal swab from 520 patients staying mostly in ICUs and some other clinics, 460 environmental specimens and 121 rectal swab specimens from hospital staff were obtained. VITEK-2 system (bioMérieux, France) was used for identification and antimicrobial susceptibility test of enterococci. MICs of vancomycin and teicoplanin were also analyzed by broth microdilution method according to CLSI guidelines. PFGE was used to analyze clonal relationships and PCR was used for determination of resistance genes.
Results: Forty-six VREs were isolated from 42 patients. VRE colonization rate in our hospital was found 8.07%. No VRE was isolated from environmental cultures and rectal swab specimens of hospital staff. All isolates were identified as *E. faecium*. PFGE data of 8 VRE isolates were undetermined so remaining 38 strains were included to study. These strains were isolated from 33 rectal swab specimens and 5 clinical materials. Strains were mostly isolated from ICUs and hematology clinics as expected. As a result of broth microdilution method 30 strains were found VanA phenotype, 8 strains were found Van B phenotype. vanA resistance gene was determined by PCR in all 30 isolates that had VanA phenotype. vanB resistance gene was determined in all 8 strains that had VanB phenotype. Thirty-eight VRE strains formed 6 different band models (A, B, C, D, E, and F) at PFGE. This band models contain 8, 10, 3, 5, 3, 3 isolates, respectively. All isolates with VanB type resistance were in group A. Patients in this group were all hemodialysis patients from different clinics.
Conclusion: This is the first report of vanB resistance gene *E. faecium* strains in our country. This emphasizes that VRE resistance diversity as well as isolation rate is increasing. VRE isolation rates in our hospital are gradually increasing since the first isolation of VRE which is a great concern. Effective surveillance studies should be done and source of infection should be identified and expansion should be limited in order to eliminate the spread of disease.

P1016 Polyclonal outbreak in a low-endemic area of CC17 genogroup vancomycin-resistant *Enterococcus faecium* containing vanB2-Tn5382 and axe-txe plasmid addiction system on pRUM-like transferable plasmid

E.K. Bjørkeng, G. Rasmussen, A. Sundsfjord, L. Sjöberg, K. Hegstad, B. Söderquist (Tromsø, NO; Örebro, SE)*

Objectives: To characterize an outbreak of vancomycin resistant *Enterococcus faecium* (VREfm) with 17 isolates from 12 infected and 3 colonized patients in a University hospital in Sweden between 2002–2004. All patients had underlying disorders and had received antimicrobial therapy during the last 3 months.
Methods and Results: Pulsed field gel electrophoresis (PFGE) revealed that 14 isolates belonged to 3 different PFGE types and in addition 3 isolates had unique patterns. All isolates had multi locus sequence types (ST17 (n = 5); ST18 (n = 3); ST125 (n = 7); ST262 (n = 1); ST460 (n = 1)) belonging to the successful hospital adapted CC17 genogroup and harboured 3–5 virulence genes associated with CC17. The VREfm isolates were shown to be susceptible to teicoplanin by Etest which was in accordance with their vanB2 genotype. The first isolate had a unique PFGE type and Southern hybridizations of S1 nuclease digested genomic DNA separated by PFGE suggested a chromosomally located vanB2 integrated in a transferable genetic element (Tn5382), while the other five PFGE types had Tn5382 located on a pRUM-like replicon containing a plasmid addiction system (axe-txe). These resistance (R)-plasmids supported intraspecies transfer by filter mating. In patient 6 both PFGE type III ST17 and later PFGE type I ST125 were isolated. R-plasmid transfer in this patient seems to have resulted in a more successful clone (PFGE type I ST125).
Conclusion: VanB-type VREfm can spread in a low-endemic area through successful clones and plasmids with stability functions.

P1017 Bacteraemia caused by non-*faecalis*, non-*faecium* *Enterococcus* sp. at a medical centre in Taiwan, 2000–2008

Y.T. Huang, C.C. Lai, C. Wang, C.H. Liao, P.R. Hsueh (Taipei City, TW)*

Objectives: Bactermia caused by non-*faecalis* and non-*faecium Enterococcus* species are emerging. Nevertheless, the knowledge about infections caused by these enterocci is limited.
Methods: We retrospectively review the computerized database of the bacteriology laboratory from January 2000 through December 2008 at National Taiwan University Hospital to identify patients with non-*faecalis* and non-*faecium* enteroccal bacteremia. Demographic data, underlying diseases, clinical, microbiological, treatment and outcome were collected. All the isolates were identified by the Rapid ID 32 STREP system (bioMérieux Vitek, Marcy l'Etoile, France), VITEK II (bioMérieux Vitek) and confirmed by sequencing 16S rRNA gene. Isolates with discordant identification results were further analyzed by sequencing groESLgenes and spacer region.
Results: During the study period, 1887 patients had enterococcal bacteremia and 182 (9.6%) of them were caused by non-*faecalis* and non-*faecium* enterococcal. These included *E. casseliflavus* (n = 59, 3.1%), *E. gallinarum* (n = 58, 3.0%), *E. avium* (n = 45, 2.4%), *E. hirae* (n = 9, 0.5%), *E. raffinosus* (n = 9, 0.5%), *E. durans* (n = 2, 0.1%), *E. cecorum* (n = 2, 0.1%), and *E. canintestini* (n = 1, 0.5%). Among the 182 patients, 74 (40.7%) had catheter-related bloodstream infection and 69 (37.9%) presented with biliary tract infection. Ninety-nine (54.4%) episodes of bacteremia acquired in the hospital and 106 (58.2%) episodes were caused by polymicrobial pathogens. All cause mortality rate was 42.0% (76/181) and 51 (28.7%) deaths were considered to be related to bacteremia. The attributed mortality was significant higher among patients with immunocompromised status, hospital acquired bacteremia, prior hospitalization and ICU admission.
Conclusions: Non-*faecalis* and non-*faecium Enterococcus* species is not rare in Taiwan. These unusual pathogens can cause protean manifestations which varied with the infecting *Enterococcus* species. The mortality of bacteremia remains high, especially for immunocompromised patients and patient with hospital-acquired infection.

P1018 Outcome of enterococcal bacteraemia in relation to start of therapy

M. Pinholt, N. Frimodt-Møller, J. Dahl Knudsen (Hvidovre, Copenhagen, DK)*

Objectives: To compare the mortality and the effect of delayed therapy on mortality associated with *Enterococcus faecalis* and *Enterococcus faecium* bacteraemia.
Methods: We performed a retrospective study of patients diagnosed with enterococcal bacteraemia in the department of Clinical Microbiology at Hvidovre University Hospital, serving five public hospitals (2200 somatic beds) in the Copenhagen City area (630,000 inhabitants). All episodes of enterococcal bacteraemia from January 1, 2006 to July 31, 2009 were included. If a patient had more than one episode, only the first episode was included. Patients with cases of polymicrobial bacteraemia were excluded. Data regarding the time to administration of appropriate

therapy were obtained from medical records. Appropriate therapy was for *E. faecalis* ampicillin, benzyl-penicillin, piperacillin, vancomycin or linezolid, for *E. faecium* vancomycin or linezolid, if susceptible. Thirty-day mortality was the main outcome measurement.
Results: A total of 322 patients with enterococcal bacteraemia were recorded (*E. faecalis*, N = 209 and *E. faecium*, N = 113). Polymicrobial cases were excluded (N = 117), why 205 patients were included in the study (*E. faecalis*, N = 125 and *E. faecium*, N = 80). Two isolates were vancomycin resistant. *E. faecalis* and *E. faecium* bacteraemia were community-acquired in 68.8% and 22.5%, respectively. The *E. faecalis* and *E. faecium* 30-day mortalities were 24.8% and 37.5%, respectively (survival curves, log rank test, p = 0.04). For both species, start of appropriate therapy more than 48 h after sampling was associated with decreased cumulative survival rates and with no difference between the two species. The average time to appropriate therapy was significantly shorter for *E. faecalis* (mean: 1.5 days, median 1) than for *E. faecium* (mean 1.8 days, median 2), (p = 0.03).
Conclusions: Mortality after *E. faecalis* and *E. faecium* bacteraemia increased significantly with increasing delay of start of appropriate antibiotic therapy. The 30-day mortality was higher for *E. faecium* as compared to *E. faecalis*. The cumulative survival rates in relation to start of therapy were not different. Delay of appropriate therapy was more frequent for *E. faecium* compared to *E. faecalis*, which could be the explanation for the higher 30-day mortality associated with *E. faecium*.

P1019 A sustained hospital outbreak of VRE bacteraemia due to the emergence of *Enterococcus faecium* ST 203

P.D. Johnson, S.A. Ballard, E.A. Grabsch, T.P. Stinear, T. Seeman, H. Young, M.L. Grayson, B.P. Howden (Melbourne, AU)*

Objectives: Improved infection control at our health service has been associated with a progressive reduction in MRSA bacteraemia but a paradoxical rise in vancomycin resistant *Enterococcus faecium* bacteraemia. We hypothesised that a new hospital-adapted strain of *Enterococcus faecium* (Efm) was responsible for this paradox.
Methods: We reviewed all patient episodes of Efm bacteraemia (both vancomycin-sensitive [VSEfm] and vancomycin-resistant [VREfm]) over an 11.5-year period (1998–2009). The first available isolate from each patient with confirmed VSEfm or VREfm bacteraemia was typed using multi-locus sequence typing (MLST). We also performed optical mapping and whole genome sequencing on representative isolates from the two most common sequence types (STs), one from early in the study period (October 1998, AUS0004, ST 17) and the other more recent (February 2009, AUS0085, ST 203).
Results: 85 isolates (51 VSEfm, 33 VREfm vanB genotype and 1 VREfm vanA genotype) obtained from patients with Efm bacteraemia were typed by MLST. Seventeen different STs were identified. From 1998 to 2006, bacteraemia rates remained stable and the most common blood culture isolate was Efm ST 17 (21/45 [47%]). Efm ST 203 was not isolated. In late November 2005 Efm ST 203 was detected for the first time as VSEfm in a patient's blood culture. The first VREfm ST 203 was isolated in a blood culture obtained in March 2007. Since 2007, coinciding with the emergence of VREfm ST 203, the rate of VREfm bacteraemia has increased exponentially with ST 203 accounting for 19/25 (76%) of VREfm blood culture isolates, while ST 17 only accounted for 4/25 (16%) in the same period. During 2009, 10/14, (71.4%) of all Efm bacteraemia isolates were ST 203. Although Efm ST 203 is only a double locus variant of Efm ST 17, comparative genomics revealed more than 500 kb of sequence difference between AUS0004 (ST 17) and AUS0085 (ST 203), suggesting Efm ST 203 was introduced recently from outside our hospital rather than developing locally from an endemic ST 17 strain.
Conclusions: The initial detection of VSEfm ST 203 was followed within 15 months by the emergence of VREfm vanB ST 203 that is now causing a sustained outbreak of VRE bacteraemia. The dominance of Efm ST 203, compared with other Efm-STs, suggests enhanced survival and/or virulence under hospital conditions, but the determinants of this adaptation/virulence are not yet known.

Community-acquired MRSA and MSSA

P1020 Molecular characterization of Panton-Valentine leukocidin positive methicillin-resistant *Staphylococcus aureus* isolates in Serbia

I. Cirkovic, M. Svabic Vlahovic, S. Stepanovic (Belgrade, RS)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of both nosocomial and community-acquired infections. Severe MRSA infections, including necrotizing pneumonia, bacteraemia and skin and soft tissue infections (SSTIs) have been associated with the virulence factor Panton-Valentine leukocidin (PVL). The aim of this study was to investigate the presence of PVL genes, clonality and their susceptibility patterns in MRSA isolates collected from patients in Serbia.
Methods: MRSA strains were collected from January to July 2007 in 30 clinical microbiology laboratory in Serbia. The identification of MRSA was confirmed by detection of nuc gene and mecA gene by PCR. The presence of genes encoding PVL was examined by PCR. The phenotypic and genotypic characteristics of the strains were determined including antibiotic susceptibility, SCCmec and agr types. Clonality of the MRSA isolates was determined by pulsed field gel electrophoresis (PFGE).
Results: During the six months period 162 MRSA isolates were collected. Presence of PVL was demonstrated in 4 (2.5%) MRSA strains. Three (75%) PVL positive MRSA strains had SCCmec V and agr type 1, were susceptible to all non β-lactam antibiotics exept gentamicin, kanamycin and tobramycin; and 2 of these strains were clonally related according to PFGE analysis. The remaining PVL positive strain, had SCCmec IV and agr type 3, were susceptible to all non-lactam antibiotics exept kanamycin, tetracycline and intermediate resistant to fusidic acid; and was not clonally related to any other strain.
Conclusion: PVL positive MRSA strains are an increasing problem due to its involvement in SSTIs. In our country these isolates represent 2.5% of MRSA strains. A half of these PVL positive MRSA isolates were clonaly related.

P1021 High diversity of PVL-positive methicillin-resistant *Staphylococcus aureus* in western Switzerland, 2005–2009

L. Senn, P. Basset, C. Bellini, G. Zanetti, D.S. Blanc (Lausanne, CH)*

Objective: To describe the molecular epidemiology of Panton Valentin Leukocidin (PVL)-positive MRSA among newly diagnosed carriers in an area of low MRSA prevalence over a five-year period (2005–2009).
Methods: All MRSA isolates (one per patient) recovered in the Vaud canton of Switzerland were analyzed by the Double Locus Sequence Typing (DLST) method (based on clfB and spa loci). The SCCmec type of each isolate was determined with the first two PCRs of the Kondo scheme. PVL-encoding genes lukS-PV and lukF-PV were co-amplified by PCR reactions.
Results: From January 2005 to October 2009, 2290 MRSA isolates (1 per patient) were analysed. Presence of PVL was detected in 115 (5%) isolates with a mean number of cases of 23 per year (range 14 to 36). Most isolates were recovered in patients with skin infections (70%) and no outbreak was observed.
These isolates were grouped into 38 different DLST types. From DLST and SCCmec type, it can be inferred that 42 cases (37%) belonged to ST8-SCCmecIV, 31 cases (27%) to ST80-SCCmecIV (European CA-MRSA clone), and 42 cases to others clones (e.g. ST5-SCCmecV, ST152-SCCmecV, and ST30-SCCmecIV). Interestingly, only about half of isolates of ST8-SCCmecIV showed the presence of the ACME region that is specific to the USA300 clone. The other isolates belonged to the Hospital Associated (HA) clone of Lyon and have acquired the PVL genes.
Conclusion: The high diversity of DLST genotypes and the absence of outbreak showed that local transmission of PVL-positive MRSA was not frequent in our region.

P1022 Widespread dissemination of the Panton-Valentine leucocidin positive ST93-MRSA-IV clone in the Australian community

G.W. Coombs, J. Pearson, G. Nimmo, K. Christiansen on behalf of the Australian Group for Antimicrobial Resistance*

Objectives: To characterise and describe the epidemiology of Panton-Valentine leucocidin (PVL) positive community-associated MRSA (CA-MRSA) strains circulating in the Australian community.
Methods: In 2008, the 5th Biennial Community S aureus Surveillance Programme was performed by the Australian Group for Antimicrobial Resistance (AGAR). Up to 100 clinically significant consecutively isolated S aureus from 100 different outpatients were collected by 31 laboratories located throughout Australia. MRSA were referred to the Western Australian Gram-positive Bacteria Typing and Research Unit for typing. Isolates were characterised by pulsed-field gel electrophoresis (PFGE) and clonality determined by MLST/SCCmec typing. The presence of PVL determinants was detected by PCR.
Results: 18% (553) of the 3,075 S aureus isolated were identified as MRSA (this represents a significant increase [<0.0001] when compared to the 2000 AGAR survey). Molecular typing was performed on 98.9% (547) of MRSA of which 62.2% (340) were identified as CA-MRSA strains (20 MLST/SCCmec types from 22 PFGE pulostypes). Of these 64.7% (220) were PVL positive. ST93-MRSA-IV (colloquially known as Queensland CA-MRSA) accounted for 67.7% (149) of the PVL-positive CA-MRSA and 44.1% (150) of all CA-MRSA strains. Six international PVL-positive CA-MRSA were identified (ST30-MRSA-IV {Oceanic CA-MRSA} [46], ST8-MRSA-IV {USA300} [10], ST1-MRSA-IV (USA400) [9], ST80-MRSA-IV {European CA-MRSA} [2] and ST59-MRSA-VT {Taiwan CA-MRSA}[1]). The remaining three PVL-positive CA-MRSA were identified as ST5-MRSA-IV, ST78-MRSA-IV and ST88-MRSA-IV. A significant difference ($p < 0000.1$) in the age of PVL-positive CA-MRSA (mean 33y, median 30y) and PVL-negative CA-MRSA infected patients (mean 53y, median 56y) was identified. Although isolated in a range of specimens PVL-positive CA-MRSA were predominantly associated with skin and soft tissue infections (SSTI).
Conclusions: In earlier AGAR Community S aureus Surveillance Programmes CA-MRSA infections in Australia were primarily caused by PVL-negative strains. Due to the emergence and wide spread dissemination of ST93-MRSA-IV and the introduction of several international PVL-positive CA-MRSA strains a significant increase in the number of CA-MRSA infections occurring in the Australian community has been identified. This increase has primarily been associated with younger Australians with SSTI that often require hospitalisation.

P1023 Molecular epidemiology and mechanisms of resistance of Panton-Valentine leukocidin positive methicillin-resistant *Staphylococcus aureus* strains

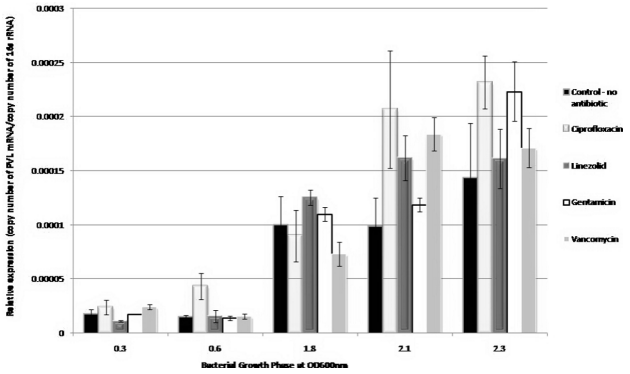
C. Pitart, C. Lozano, E. Gómez-Sanz, N. Cobos, J. Mensa, A. Soriano, C. Torres, J. Vila, M.T. Jiménez de Anta, F. Marco (Barcelona, Logroño, ES)*

Objectives: Panton-Valentine leukocidin positive methicillin-resistant *Staphylococcus aureus* (PVL (+) MRSA) isolates have increasingly been noted worldwide due to its involvement in severe infections. In our institution these isolates represent 11.5% of all MRSA strains. In this study, we investigated the molecular epidemiology of PVL (+) MRSA isolates and their antibiotic (ATB) resistance mechanisms.
Methods: 33 PVL (+) MRSA strains were collected from patients (one for patient) seen in our institution (October 07-May 09). All strains were genotyped by pulsed field gel electrophoresis (PFGE) after digestion with SmaI. Only one isolate of each PFGE subtype and same susceptibility pattern was further studied for molecular characterization. spa and SCCmec typing, agr polymorphism and testing for ACME-arcA genes was performed to 15 strains. MLST typing was done to isolates showing different spa types and to two invasive strains. Isolates were screened for the presence of resistance genes: erythromycin (ER) and clindamycin (CL) (ermA, ermB, ermC and msrA), gentamicin (GE) and tobramicin (TO) (aac(6′)-aph(2″), ant(4′)), mupirocin (MU) (mupA) and tetracycline (TE) (tetK, tetM, tetL). Mutations in grlA and gyrA were characterized to study quinolone (FQ) resistance. NorA efflux mediated resistance was also studied performing norfloxacin (NOR) MICs in presence of reserpine.
Results: PVL positive MRSA strains belonged to four PFGE types (20 isolates A, 11 B, 1 C and 1 D) and 9 subtypes. All isolates were susceptible to cotrimoxazole, rifampin, vancomycin, teicoplanin, daptomycin, fusidic acid and linezolid. Resistance to ATB was as follows: ER 51% (msrA), ER and CL MLSB constitutive phenotype 10% (msrA+ermC), TE 12% (tetK), GE and TO 6% (one isolate aac(6′)-aph(2″) and the other aac(6′)-aph(2″)+ ant(4′)-Ia). Two isolates showed high level resistance to mupirocin due to plasmid mediated mupA. 21 isolates showed FQ resistance (63%). Amino acid substitutions observed were: grlA Ser80Tyr (17), grlA Ser80Phe (4) and gyrA Ser84Leu (21). One fold NOR MIC decrease was seen in 20 isolates in presence of reserpine. The dominant clone (ST8/SCCmecIVa/t008/agr1/ACME+/msrA+) represented 57% of strains. Other clones observed were ST8/SCCmecIVa/t4816/agr1/ACME+/msrA+ (1); ST8/SCCmecIVc/t008/agr1 (11); ST146/SCCmecIV/t002/agr2 (2) and ST72/SCCmecIV/t791/agr1 (1).
Conclusion: A major clone (57%) exhibited ST8, spa t008, agr1, SCCmecIVa, presence of ACME and msrA. This clone related to USA300 lineage included one isolate causing bloodstream infection and meningitis.

P1024 Modulatory effects of antimicrobials on the expression of Panton-Valentine leucocidin gene in community-acquired methicillin-resistant *Staphylococcus aureus*

C.K. Wong, E.T. Leung, S.S. Chau, L. Zheng, K.F. To, M. Ip (Hong Kong, HK)*

Objective: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have emerged as a major health concern worldwide. The possession of panton-valentine leucocidin (PVL) in CA-MRSA has been associated with recurrent soft tissue infections and necrotizing pneumonia, amongst others. Studies showed that expression of virulence genes by *Staphylococcus aureus* is under tight regulatory control and depends on strain types and could be modulated by external stimuli, including drugs such as antibiotics. This study investigates the effects of commonly used antimicrobial agents at sub-inhibitory level on PVL expression in CA-MRSA *in vitro*.
Methods: A representative strain of CA-MRSA of the Southwest Pacific clone, ST30 (spa t019), was used in the experiments. CA-MRSA was cultured to late log phase in liquid medium. Sub-inhibitory concentration, equivalent to 1/8th MIC of the respective antimicrobial (vancomycin, linezolid, gentamicin and ciprofloxacin), was added to the broth culture at early log phase (time point at OD600nm0.3). Aliquots of the broth pre- and post antibiotics were collected at various time points (at OD600nm 0.6, 1.8, 2.1, 2.3) representing different phases of bacterial growth for mRNA measurements. Total RNA was extracted and reverse transcribed to cDNA for quantitative analysis of mRNA transcripts by RT-qPCR. PVL expression levels were normalized against endogenous 16sRNA levels and calculated as relative expression (ratio of copy numbers of PVL against copy numbers of 16sRNA).
Results: PVL expression was up-regulated *in vitro* to different extent by different classes of antimicrobials, as compared to the control group ($p < 0.05$) (see figure). The effects are most marked with fluoroquinolone, ciprofloxacin.
Conclusions: Expression of staphylococcal virulence genes eg PVL in CA-MRSA could be affected by the use of different classes of antimicrobials. Regimens that minimize release of PVL and other virulent toxins production may reduce severity of disease and improve treatment outcome in CA-MRSA infections.



Effect of antimicrobials on PVL expression.

P1025 Quantification of PVL production by PVL-MRSA isolated in England and Wales

E. Boakes, A.M. Kearns, C. Badiou, C. Ratat, M. Leportier, G. Lina, R. Hill, M.J. Ellington (London, UK; Lyon, FR)*

Objectives: PVL production is often linked with some of the most successful lineages of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). We sought to quantify PVL production amongst dominant clones of PVL-MRSA in England and Wales and to identify associations between the levels of PVL production, the type of bacteriophage encoding the PVL genes and/or the genetic lineage of the PVL-MRSA isolates tested.
Methods: We examined PVL production from 113 PVL-MRSA isolates, representing USA300 (CC8); South West Pacific clone (SWP) (CC30) and the European clone (CC80). In addition we analysed PVL-MRSA from CCs 1, 5, 22, 59, 88 and ST93. Culture supernatant from standardised growth in CCY broth was taken in triplicate and PVL was quantified using a solid-phase sandwich ELISA as described previously. The PVL-phages were detected by PCR, as described previously.
Results: The UK PVL-MRSA tested produced 2.5–178.1 ng/ml of PVL. Within MLST lineages, isolates harboured different PVL phages and produced varying levels of PVL. CC8 isolates (n = 12) harboured Sa2USA and produced relatively high levels of PVL (39.61–178.13 ng/ml, mean 89.75 ng/ml). In contrast some lineages produced low levels of PVL ranging between 2.5–77.63ng/ml (mean = 33.72); these included CC22 isolates (n = 47) which had 1 / 3 different PVL phages (108PVL, PVL and icosahedral phage), CC5 isolates (n = 9) had 1 / 2 different PVL phages (Sa2USA or unknown phage) and CC88 isolates (n = 5) which all had an unknown elongated phage. All other lineages; CC1, 30, 59, 80 and 93 (with phages Sa2USA, Sa2MW, Sa2958-like phage and unknown PVL phage/s) produced PVL concentrations between 6.27–95.81ng/ml (mean = 47.94). Multiple examples of highly related isolates that produced substantially different amounts of PVL were noted (CC8, CC22, CC93).
Conclusion: Among PVL-MRSA found in the UK, the level of PVL produced *in vitro* varied between lineages and also varied substantially from isolate to isolate within the same lineage. There does not appear to be a clear association between genetic lineage and the level of PVL produced, nor is there any obvious association with the amount of PVL produced and the type of PVL encoding phage. Notwithstanding the variance observed amongst highly related isolates in this study suggests mechanisms of modulation of production exist. Other factors effecting PVL production, including host factors, warrant further investigation.

P1026 Unravelling Panton-Valentine leucocidin positive methicillin-resistant *Staphylococcus aureus* transmission in North Staffordshire, United Kingdom, using variable-number tandem-repeat typing

K. Hardy, P. Hawkey, C. McMurray, S. Shabir, A.M. Kearns, J. Orendi (Birmingham, London, Stoke-on-Trent, UK)*

Objectives: To understand the detailed molecular epidemiology of PVL-positive community-associated MRSA (CA-MRSA) in North Staffordshire, UK.
Methods: All MRSA isolates from an acute hospital and the community (March 2006 to June 2009) that were either ciprofloxacin sensitive/intermediate or clinical information suggested PVL-MRSA were confirmed by multiplex PCR. All confirmed PVL-positive MRSA isolates were epidemiologically typed using staphylococcal interspersed repeat unit (SIRU) typing. Seven variable number tandem repeat loci located around the genome were amplified and a 7 digit profile recorded. The household contacts of PVL-MRSA cases were screened and epidemiological information regarding contacts recorded.
Results: A total of 63 patients were identified as being colonised or infected with PVL-MRSA strains. Fifty four were designated as community acquired, 7 hospital acquired and the origin of acquisition could not be determined for two. Of 18 different SIRU profiles, four were predominant. Of these four, three were closely related (1–2-2–2-9–5-2, 1–2-1–2-9–5-2, 1–2-2–2-9–2-1) and belonged to the ST30-IV CA-MRSA lineage, with the two most predominant profiles differing by only one repeat. There were multiple PVL-MRSA cases in 14 households and on all but one occasion, the SIRU profiles were indistinguishable from other cases within the same household. Nine SIRU profiles were associated with household or hospital transmission and nine SIRU profiles with sporadic cases. Of the 24 index cases, 10 had no significant history of travel outside the UK in the previous two years.
Conclusions: Multiple PVL-MRSA clones are circulating within North Staffordshire, with four strains predominating. SIRU demonstrates good utility in being able to demonstrate cases of cross transmission within and between households that have epidemiological links.

P1027 Panton-Valentine leukocidin production and association with SCCmec types in MRSA in Turkey: is PVL still the marker of SCCmec type IV?

Z. Gulay, M. Bagsever, D. Gur, C. Kayacan, G. Soyletir (Izmir, Ankara, Istanbul, TR)*

Objectives: To investigate the association of Panton-Valentine Leukocidin(PVL) and SCCmec type IV in MRSA isolates which were collected from clinical samples between 2006–2009 in three different cities (Izmir, Istanbul and Ankara) in Turkey.
Methods: A total of 203 MRSA isolates were taken into the study. SCCmec typing and the presence of luk S-PV and luk F-PV genes were studied by multiplex polimerase chain reaction (PCR).
Results: SCCmec type IV/IV E was detected in 6.3%, 9.6% and 14.6% of MRSA isolated in Izmir, Ankara and Istanbul respectively. The prevalence increased from 0.0% in 2006 to 4.8% in 2007 and to 23% in 2008 and then decreased to 14% in 2009. Among 19 isolates bearing a type IV SCCmec, only four strains were PVL positive. PVL positive isolates were collected either in Ankara or Izmir in 2008. Nevertheless, PVL genes was only detected in isolates carrying SCCmec type IV.
Conclusion: This study shows that in Turkey 79% of MRSA isolates bearing a type IV/IVE SCCmec which is usually associated with community acquired MRSA (CA-MRSA), do not carry the PVL gene. This indicates that detecting PVL gene is not a reliable marker for SCCmec type IV and CA-MRSA. Other interesting findings of this study is the increase in the prevalence of MRSA/SCCmec type IV in Turkey and the probable association of this SCCmec type with hospital acquired MRSA(HA-MRSA).

P1028 Variability of fusidic acid-resistant methicillin-sensitive *Staphylococcus aureus* isolates in Casablanca, Morocco

M. Elazhari, K. Zerouali, N. Dersi, R. Saile, M. Timinouni, M. Hassar (Casablanca, MA)*

Objectives: The aim of the present study is to determine the prevalence of fusidic acid-resistant methicillin-susceptible *Staphylococcus aureus* (FAR-MSSA) isolated from community infections, and to define whether there are links between the antibiotic susceptibility of these strains, their origin and accessory gene regulator (agr) groups, and to correlate them with their toxic gene profile.
Methods: Isolates were collected from clinical laboratories located in Casablanca, from 1st January 2007 to 31st October 2008. They were screened for susceptibility to fusidic acid by using a disc diffusion method. MICs of fusidic acid for FAR-MSSA, their agr group, the presence of toxins genes and pulsed-field gel electrophoresis (PFGE) patterns were investigated.
Results: Of 140 *S. aureus* isolates, 18 (12.9%) exhibited resistance to fusidic acid. The "seh " toxin gene was found in all 14 strains whose harboured an agr group III, 9 of them were found with "sek" plus "seq" toxin genes, witch 6 isolates, generally isolated from pus, and were multidrug-resistant, shared an identical PFGE pattern, and possessed the leukocidins (luk S-PV, luk F-PV) genes.
Conclusions: In conclusion, we found a close relationship between the presence of the seh gene and the possession of agr group III in fusidic acid-resistant methicillin-sensitive *S. aureus* (FAR-MSSA) strains. In addition, our results indicate a relationship between 6 FAR-MSSA strains belonging to the same pulsotype and harbouring agr group III with luk-PV toxin genes.

P1029 Community-acquired methicillin-resistant Stapylococcus aureus carrying the Panton-Valentine genes in invasive infections in Algiers

N. Ramdani-Bouguessa, K. Antri, H. Ziane, F. Djennane, M. Bes, G. Lina, M. Tazir, J. Etienne (Algiers, DZ; Lyon, FR)*

Objectives: Community acquired methicillin-resistant *S. aureus* (C-MRSA) are spreading worldwide. They are responsible for lifethreatning infections such as, necrotizing pneumonia and osteomyelitis. Panton-Valentine leukocidin (PVL), a virulent factor, plays a role in the pathophysiology of these infections. In Algeria, a high prevalence of CA-MRSA-PVL+ has already been reported, however, the frequency of C-MRSA-PVL+ in invasive infections is underestimated. The aim of this study is to evaluate the frequency of invasive infections due to CA-MRSA-PVL+.
Methods: A prospective study was conducted from january 2008 to october 2009. *S. aureus* was identified by coagulase and latex agglutination. Antibiotic susceptibilities were determined by disk diffusion according to CLSI recommendations. *S. aureus* identification was based on colony morphology, microscopic examination, results of coagulase rabbit plasma and Staphyslide agglutination tests (bioMérieux). The detection of mecA gene, PVL-genes (LukS-PV, lukF-PV) and accessory gene regulator alleles (agr types 1 to 4) was performed by multiplex PCR for 45 of the 51 invasive *S. aureus* isolates.
Results: A total of 695 *S. aureus* strains has been collected, 356 (51%) were responsible for community-acquired infections. Of them, 57 were responsible for invasive infections: pneumonia (n = 26), bone infections (n = 19), bacteraemia (n = 9), central nervous infection (n = 3), endocarditis (n = 2) and peritonitis (n = 1). Thirty-two (56%) of these infections were from children. C-MRSA infections were detected in 18 patients. PVL-genes were detected in 20 (44.4%) isolates: bone infections (n = 10), pneumonia (n = 7), bacteraemia (n = 1) and 2 endocarditis. These isolates harboured the following profiles: agr3,mecA+,pvl+ (n = 13), agr3,mecA–,pvl+ (n = 4) and agr4,mecA–,pvl+ (n = 3). Three patients died, all had C-MRSA-PVL+ infections, two pneumonia, one bacteraemia and one endocarditis.
Conclusions: A high prevalence of C-MRSA-PVL+ severe infections is observed, physician should reconsider the empiric antibiotic treatment of these infections.

P1030 Community-associated methicillin-resistant *Staphylococcus aureus* as the cause of healthcare-associated infection: experience from an Indian tertiary hospital

B. Dhawan, R. Gadepalli, C. Rao, V. Sreenivas, A. Kapil (New Delhi, IN)*

Objective: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is an emerging threat worldwide. These strains now cause serious infections in hospitalized patients. We conducted a prospective study to evaluate the frequency of infections due to CA-MRSA strains among hospitalized patients with HA-MRSA skin and soft tissue infections (SSTIs). We also analysed the patients demographic characteristics, clinical outcome and antimicrobial susceptibility patterns associated with CA-MRSA infections acquired in the health care settings compared with infection caused by HA-MRSA strains.
Methods: Fifty-six MRSA isolates obtained from patients admitted to an Indian tertiary hospital from September 2008 through August 2009 with HA-MRSA SSTIs as per established definitions were included for analysis in the study. Strains were tested for staphylococcal chromosomal cassette mec (SCCmec) type I, II, III, IV & V and presence of Panton-Valentine Leukocidin (PVL) genes by polymerase chain reaction (PCR) methods. Susceptibility to ten antimicrobials was determined by the disk diffusion method. Bacterial isolates were independently classified as either CA-MRSA strains or healthcare-associated MRSA (HA-MRSA) strains according to established molecular definition of CA-MRSA and HA-MRSA.
Results: Twenty-five (44.6%) of the 56 MRSA isolates had a genetic type characteristic of CA-MRSA strains; eighteen (32.1%) were SCCmec type V and seven (12.5%) were SCCmec type IV. Ten of the 25 CA-MRSA isolates (40%) carried PVL genes. The remaining 31 (56.4%) isolates belongs to the SCCmec type I/III group. None of the isolates were SCCmec type II. No differences were noted between the patients in the SCCmec-I/II/III group and the patients in the SCCmec-IV/V group with respect to the age; sex; medical service; underlying co-morbidities or clinical outcome. Except for amikacin, whose susceptibility rates were significantly more for CA-MRSA strains, there was no statistical difference in the susceptibility pattern of CA-MRSA and HA-MRSA strains to ciprofloxacin, erythromycin, clindamycin, tetracycline, co-trimoxazole and rifampicin. No resistance to vancomycin, teicoplanin and linezolid was seen in both CA-MRSA and HA-MRSA strains.
Conclusion: MRSA strains with molecular characteristics of CA-MRSA strains have emerged as an important cause of health-care-associated SSTIs in our hospital. Further research is warranted to detect the impact of CA-MRSA strains in Indian hospitals.

P1031 Changing proportions of clonal lineages of CA-MRSA in Germany, 2006–2009

W. Witte, C. Cuny, F. Layer (Wernigerode, DE)*

Objective: To follow the proportion of clonal lineages of CA-MRSA in samples of MRSA isolates from all Germany sent to a national reference centre for molecular typing.
Methods: Typing my means of spa-typing, MLST, grouping of SCCmec elements, AST by microbroth dilution assay, PCR demonstration genes associated with virulence (luk-S, lukF, arcA, seh, etd) and resistance.
Results: Altogether the proportion of PVL-pos. MRSA of community origin in the sample rose from 3.2% in 2005 to 6.2% in 2009. Within this group the frequency of CA-MRSA ST8 decreased from 71% to 32% whereas that of CA-MRSA ST8 ("USA300") increased from 12% to 53%. The proportion of other clonal lineages such as ST1(IV), ST5(V), ST22(IV), ST30(IV), ST152(V)did not exceed 5–10%. The frequency of Livestock associated, PVL-neg. CC398(V) from severe skin/soft tissue infections (SSTI) in humans exposed to livestock was

11%.One PVL-pos. MRSA attributed to CC45(IV) was observed for the first time. About 4% of the isolates (attributed to ST1, ST5, ST8, ST22, ST152) had acquired aminoglycoside resistance (aph2"aac6'). Furthermore tetracycline resistance emerged in a variety of clonal lineages besides ST8..

Conclusion: The increasing proportion of CA-MRSAST8 and the emergence of LA-MRSA CC398 as cause of SSTI need particular attention in surveillance. Less broad resistance. Phenotypes are no longer a characteristic of CA-MRSA.

P1032 Community-acquired methicillin-resistant *Staphylococcus aureus* in the Tyrol (Austria) – a prevalence survey with genetical characterization

M. Berktold, D. Orth, K. Grif, M. Mäser, R. Würzner (Innsbruck, AT)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) are one of the most important human pathogens causing invasive infections of the skin and soft tissue. Recently, certain strains among MRSA developed which affected not only hospitalized patients, but also healthy outpatients. These strains are called community-acquired MRSA (caMRSA) and they typically possess the lukS-lukF gene, coding for Panton-Valentine-Leukocidin.

The aim of this study was to investigate the occurrence and characteristics of caMRSA at the University hospital Innsbruck, smaller district hospitals and among patients of practitioners in the Tyrol (Austria).

Methods: 220 MRSA strains, isolated from patients of the University hospital Innsbruck (n = 174), district hospitals (n = 36) and practitioners in the Tyrol (n = 10) from 2003 to 2007, were investigated for the presence of lukS-lukF gene by PCR. For lukS-lukF-positive strains antibiotic resistance testing was performed and the presence of SCCmec element and accessory gene regulator (agr) was determined. Additionally all lukS-lukF-positive isolates were typed by automatic ribotyping.

Results: Among 220 MRSA isolates 16 lukS-lukF-positive caMRSA (7.3%) were identified: 10 from patients from the University hospital Innsbruck (5.7%), 3 from district hospitals (8.3%) and 3 from outpatients (30%). All caMRSA were resistant against the β-lactam antibiotics tested as expected and susceptible to cotrimoxazol, rifampicin, fosfomycin and vancomycin. Three strains showed resistance against fusidic acid. The majority of the strains carried SCCmec IV (n = 12), one strain each carried SCCmec I and SCCmec II. In two cases it was not possible to identify the SCCmec element. Agr-gene-typing revealed a majority of agr type 1 (n = 9), followed by agr type 3 (n = 4). One strain each carried agr type 2 and 4, the agr type of one strain could not be determined. Ribotpying revealed 8 different patterns, ribotpye 2 was the most prevalent (n = 5).

Conclusion: CaMRSA are rather common in the outpatient community in the Tyrol. Most caMRSA were similar in their genetic profile with SCCmec element IV and agr type 1 being the most prevalent. Determination of caMRSA should always be considered in the microbiological routine and especially practitioners should be alerted to pay more attention to the threat of caMRSA.

P1033 Typing and characterization of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* ST398 from pigs including detection of clindamycin resistance mediated by linA gene

C. Aspiroz, C. Lozano, F. Ruiz-Larrea, M. Zarazaga, C. Torres (Zaragoza, Logroño, ES)*

Introduction and Objective: Methicillin resistant *Staphylococcus aureus* (MRSA) isolates of sequence type ST398 have been identified to colonize and cause infections in animals and humans, especially in people in contact with pigs. The aim of this study was to characterize the resistance phenotypes and genotypes of MRSA isolated from pigs of a farm where one swine worker infected (skin lesion) and colonized by MRSA ST398 was previously detected (Aspiroz et al. SEIMC, 2009).

Methods: Nasal swabs were collected from 12 pigs of the farm and selective culture for MRSA recovery was performed on ORSAB agar plates (OXOID) and one isolate per positive sample was characterized. MRSA identification was confirmed by PCR of nuc and mecA genes. Antimicrobial susceptibility was determined by VITEK-2 system (bioMérieux) and by disc-diffusion method. MLST, SCCmec, agr and spa-typing was performed by PCR and sequencing. The presence of ermA, ermB, ermC, mrsA, linA, tet(K), tet(L), tet(M), tet(O), ant(4′), aph(3′), aac(6′)-aph(2′) and dfrK genes as well as PVL (lukF/lukS) genes were tested by PCR, and in some cases also by sequencing.

Results: MRSA ST398 were obtained from 11 of the 12 nasal swabs (91.6%). All 11 MRSA isolates showed resistance to tetracycline and were negative for PVL, and ten of them showed resistance to cotrimoxazole. Seven isolates were typed as spa-t108, SCCmecV and presented an unusual macrolide/lincosamides resistance phenotype: resistance to clindamycin and susceptibility to erythromycin and harboured linA in addition to tet(L), and dfrK genes. The four remaining MRSA-ST398 isolates were typed as spa-t011 and SCCmecIVa, and presented different resistance phenotypes, harbouring the following genes (number of isolates): ermA (2), ermB (2), ermC (2), tet(L) (11), tet(M) (5), dfrK (10), ant(4′) (4), aph(3′) (4), and aac(6′)-aph(2′) (3).

Conclusions:

1. A high rate of colonization by MRSA ST398 is detected in pigs, being t011 and t108 the spa-types detected.
2. MRSA ST398 presents a wide diversity of antimicrobial resistance phenotypes harbouring a high variety of resistance genes.
3. The unusual pattern of erythromycin susceptibility and clindamycin resistance detected in 7 of 11 MRSA ST398 was mediated by linA, being this gene the first time found in a ST398 isolate.
4. Clindamycin resistance can compromise treatment of skin lesions caused by MRSA ST398 of animal origin.

P1034 Methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* belonging to clonal lineage ST398: animal and human prevalence, human clinical impact in France

F. Laurent, E. Jouy, S. Granier, J. Vaucel, R. Ruimy, M. Bes, J-P. Rasigade, B. Felix, A. Le Roux, C. Chauvin, J. Etienne, A. Brisabois (Lyon, Ploufragan, Maisons Alfort, Saint Brieuc, Paris, FR)*

Objective: The aim of this study was to evaluate the prevalence and highlight the clinical impact of MRSA and MSSA belonging to clonal lineage ST398 among pigs and human in France.

Methods and Results: To assess the prevalence of MRSA carriage by French fattening pigs, we sampled 165 batches of 10 pigs by nasal swabbing from January and September 2007 in 21 slaughterhouses. Sampling was representative of national production. The prevalence study demonstrated that 29.0% of batches and 13.3% of pigs were MRSA carriers at slaughterhouse. ST398 was predominant (81%) and was retrieved for 8 spa-types (t011, t034, t108, t588, t899, t1184, t1456, t2370). ST398 MRSA isolates appear less prevalent but more diverse than in other European countries.

To evaluate the prevalence of ST398 MRSA and MSSA in French patients, we retrospectively screened all MRSA (n = 69) and MSSA (n = 139) collected at St Brieuc Hospital (in an area with high density of pig farming) from March to September 2009. No MRSA and only 4 MSSA belonged to clonal lineage ST398. These data indicate the low prevalence of ST398 isolates in French patients that need to be hospitalized, i.e, with severe infections without excluding possible carriage and uncomplicated infections in the community.

Clinical impact of some ST398 isolates:

- a first case of lethal necrotizing pneumonia due to a PVL+ ST398 MSSA strain in a healthy 14-year-old girl was reported in Orleans (France). Clinical features and medical history were highly consistent with those of previously published necrotizing pneumonia and ST398 *S. aureus* was isolated from various specimens.
- eight ST398 MSSA (7%) were identified among 114 MSSA isolates collected during the French national survey on infective endocarditis

(IE) conducted in 2008 while ST398 isolates are not classically detected in nasal carriers.
– two related noscomial pulmonary infections were detected in a ICU due to ST398 MSSA

Conclusion: The data collected indicate that the worldwide emerging threat of livestock-associated ST398 *S. aureus* in France is so far limited and especially focused on MSSA. The isolates are able to induce severe infections and nosocomial dissemination. They illustrate the ability of ST398 genetic background to acquire resistance and virulence factor genes. Therefore, further studies are required to monitor i) the temporal and geographical incidence in hospital and in the community; ii) the potential genetic drift of ST398 in France and throughout Europe in the future.

P1036 *Staphylococcus aureus*, methicillin-resistant *S. aureus* and the mecA gene in a municipal wastewater treatment plant

S. Börjesson, S. Melin, A. Matussek, S. Löfgren, P-E. Lindgren (Linköping, Jönköping, Gothenburg, SE)*

Objectives: Methicillin-resistant *S. aureus* (MRSA) and the mecA (the gene encoding methicillin resistance) were recently detected and quantified in wastewater. Knowledge is limited about their occurrence in non-clinical environments, e.g. wastewater, and their role as a potential human health hazard.

The project aims at

1. Investigating the occurrence of total *S. aureus*, MRSA and mecA in a wastewater treatment plant (WWTP) and the effects on mecA concentration, the prevalence of *S. aureus* and MRSA during the process. Characterising the composition of the MRSA populations and how the process affects it, in the WWTP.

Methods: The concentration of mecA and the occurrence of *S. aureus* were determined by a recently developed real-time PCR assay. The detection and quantification of MRSA was carried out by real-time PCR and cultivation by enrichment broth. MRSA were characterised by spa typing, SCCmec typing and antibiograms.

Results: *S. aureus* and mecA could be detected over the year and during the whole treatment process. No clear trend of the variation of the mecA concentration could be noticed. However, a reduction in the mecA concentration was found during the process.

MRSA could be detected by real-time PCR over a year, but mainly in the inlet and early treatment steps. The results were confirmed and examined in more detail by selective cultivation of MRSA over a two-month-period. Totally 189 MRSA colonies, belonging to 29 spa types, were detected. 10 of these spa types were isolated for the first time in Sweden, including 6 novel spa types. 65% of the isolates were resistant only to β-lactams, but 21% were resistant to more than two classes of antibiotics (defined as multi-resistant). 19 different spa types and 66% of the isolates were cultivated from inlet, compared to 15 spa types from the activated sludge step. All spa types carried either the SCCmec I or IV. In activated sludge 40% of spa types were multi-resistant, bu only 21% in the inlet. Most spa types showed close genetic relationship to clinical MRSA isolates, previously described in Sweden.

Conclusions:

1. *S. aureus* and mecA occur over the year and during the entire treatment process, but the mecA concentration is reduced during the treatment.
2. MRSA was for the first time detected and isolated from municipal wastewater, and showed a stable occurrence over time. However, the WWTP process reduced the amount and diversity.
3. Wastewater isolates of MRSA might reflect the carriage in the community.

P1037 Dynamic pattern and genotypic diversity of *Staphylococcus aureus* nasal carriage in healthy kindergarten children

S. Blumental, A. Vergison, S. Jourdain, A. Deplano, R. De Mendonça, S. Rottiers, E. Vandaele, P. Smeesters, M.J. Struelens, O. Denis (Brussels, BE; Stockholm, SE)*

Background: It is common wisdom that persistent carriage of *S. aureus* is more frequent in young children than in adults.

Objectives: To assess temporal carriage pattern of *S. aureus* among a healthy community of kindergarten children, with concomitant description of genotype diversity, toxin-encoding gene and antibiotic resistance profiles.

Materials and Methods: Children aged from 3 to 6 year attending 11 kindergartens in Belgium were followed over the school year. *S. aureus* nasal carriage was assessed longitudinally by culture of sequential nasopharyngeal aspirates collected during autumn, winter and spring. *S. aureus* identification was confirmed by PCR for detection of 16S/ nuc/ mecA genes. Antimicrobial susceptibility was tested by disk diffusion method. Toxin production profile was determined by PCR for PVL/ TSST-1/ETa/b toxin genes. Typing was performed by spa sequencing using Ridom StaphType 1.4 (Ridom GMbH).

Results: 898 samples were collected from 333 children, 286 (32%) yielded *S. aureus* from 185 children (55%) being carriers at least once over the school year. Among 271 children who underwent at least two consecutive samplings, 90 had a single positive culture and 68 had two or more. Based on consecutive genotype analysis, only 41/271 (15%) were classified as persistent carriers and the remaining 117 (44%) as intermittent carriers. Spa typing showed 89 types clustered into 13 spa clonal complexes (CC) using the BURP algorithm. Fourteen strains isolated from 11 (3%) children of five different schools were resistant to methicillin. Seven (50%) MRSA strains belonged to the unusual spa CC304 (corresponding to CC8 by MLST). MSSA isolates were more diverse with 54% distributed into five spa CC. Rate of antimicrobials resistance among MSSA strains reached 24% for erythromycin, 2% for clindamycin, 8% for tetracycline and 1.5% for ciprofloxacin. TSST-1 gene was present in 70 *S. aureus* isolates (24%). In contrast, PVL gene was carried only by four MSSA strains. Exfoliatin toxin genes were detected in 10 (3.5%) MSSA strains of which 5 were related to the impetigo clone CC121.

Conclusions: Although *S. aureus* nasal carriage was high among healthy kindergarten children, persistent carriage seems to be less frequent than previously reported. Prevalence of MRSA carriage was 3% but was not associated with PVL. As expected, MSSA isolates were genotypically diverse and included toxic shock and exfoliatin producing strains.

P1038 Diabetes is a risk factor for recurrent cellulitis

M. Karppelin, T. Siljander, A. Aromaa, H. Huhtala, J. Kere, J. Vuopio-Varkila, J. Syrjänen (Tampere, Helsinki, FI; Huddinge, SE)*

Objectives: In previous case–control studies obesity, but not diabetes, has been associated with acute and recurrent bacterial non-necrotizing cellulitis. We evaluated the role of diabetes as a risk factor for recurrent cellulitis in a retrospective case–control study.

Methods: A questionnaire and a consent form were sent to all those who, according to National Health Insurance Institution, had in year 2000 received reimbursement for benzathine penicillin, indicated for prevention of cellulitis. Medical records were obtained from the respective care providers to confirm the diagnosis of cellulitis. Based on the questionnaire the following variables were recorded: age, sex, height, weight and diabetic status (all types). Body mass index (BMI) was calculated as weight (kg) divided by square of height (m).

Control population consisted of 8005 Finnish subjects, aged 30 years or over, participating in Health 2000 Health Examination Survey, a national population based health survey, carried out in years 2000–2001 by the former Finnish National Public Health Institute (KTL). This survey consists of a randomly drawn, nationally representative sample of the Finnish population. Data regarding the corresponding variables

mentioned above were derived from the database. A logistic regression analysis (method enter) was performed using STATA software.
Results: Of 960 subjects contacted, 487 (50%) returned a consent and filled in a questionnaire. Recurrent cellulitis could be ascertained from the medical records of 398 patients of which 235 (59%) were female. The median age of the patients was 65 years (range 22–92). The mean BMI was 31.5 and 32.5 for male and female patients, respectively, and 32.1 for the whole patient population, compared to 26.9 for controls. Of the patients and controls 20.6% and 6.1% were diabetic, respectively. The multivariate analysis of risk factors is presented in table.
Conclusion: In the present study, comprising large patient and control populations, diabetes proved to be a risk factor for recurrent cellulitis, independently of obesity and age. The incidence of bacterial cellulitis has been suggested to be growing. Increasing prevalence of obesity and adult type diabetes along with increasing longevity may contribute to this.

Table. Multivariate analysis of risk factors for recurrent cellulitis in 398 patients and 8005 control subjects

Risk factor	OR	95% CI
Sex	1.19	0.95–1.49
Age	1.05	1.04–1.06
Body mass index	1.17	1.14–1.19
Diabetes	1.69	1.26–2.27

P1039 Complex nature of locally invasive odontogenic infections in patients requiring intensive care

L. Seppänen, A. Lauhio, C. Lindqvist, R. Rautemaa-Richardson (Helsinki, FI)*

Objectives: An increase in the number of locally invasive odontogenic infections requiring hospital care has been recently observed in many countries. Our aim was to analyse the microbiological findings of odontogenic abscesses in relation to the clinical characteristics of the patients and the need for intensive care.
Methods: Microbial findings and medical records of patients admitted to the Helsinki University Central Hospital due to locally invasive odontogenic infection in 2004 were analysed retrospectively. Microbiological reports were available from 69 patients and these patient cases were included in the study.
Results: Twenty-eight (41%) patients were in need for intensive care. Staphylococci, including *Staphylococcus aureus*, were a significantly more common finding in patients requiring intensive care compared to those patients who did not ($p < 0.0001$ and $p = 0.0484$). Staphylococci had been reported in 57% of the cases requiring intensive care in contrast to 10% of cases who did not. In addition, microaerophilic streptococci were more prevalent in patients requiring intensive care ($p = 0.0152$). There was no difference in the isolation rate of anaerobes between the two groups. Propionibacterium and *Lactobacillus* spp. were more commonly reported in patients requiring intensive care ($p = 0.0154$ and $p = 0.0368$). Many reported isolates demonstrated reduced susceptibility to penicillin and macrolides. Resistance to clindamycin was more common than to doxycyclin. The clinical course of these infections was more severe as reflected by significantly more elevated inflammatory markers. White blood cell count on admission (median 15.4 x 103/μL and 11.3 x 103/μL, $p = 0.0038$), and at maximum (median 16.2 x 103/μL and 11. x 103/μL, $p = 0.0002$), as well as C-reactive protein level on admission (median 161 mg/L and 76 mg/L, $p = 0.0002$) and at maximum (median 176 mg/L and 85 mg/L, $p < 0.0001$) were significantly higher in patients with the need for intensive care compared to patients without the need for intensive care. Length of stay was 8.1 days for patients with intensive care and 2.8 day in mean for patients without intensive care ($p < 0.0001$).
Conclusion: The microbial findings of this study suggest that staphylococci may have a greater role in severe odontogenic infections than generally appreciated. The possibility of their involvement should be considered in the empiric antibiotic therapy of infections requiring intensive care.

P1040 Evolution of methicillin-resistant *Staphylococcus aureus* isolated at a community healthcare centre in Spain

C. Potel, M. Álvarez, L. Constenla, F. Lago, C. Varela (Vigo, ES)*

Objective: To determine the distribution of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolated from outpatients at a community health care center serving a large population of 300.000 from the Northwest of Spain.
Methods: In total 1.913 *S. aureus* were isolated between 2003 and 2007. From these ones, 135 strains were MRSA.
The presence of mecA gene and pvl genes was identified in all MRSA strains by PCR. The isolates were studied by analysis of restriction fragment length polymorphism of the coagulase gene and digestion with cfoI (RFLP) and pulsed-field gel electrophoresis (PFGE). The staphylococcal chromosome cassette (SCC)mec and the accessory gene regulator (agr) types were determined by multiplex and duplex PCR respectively. Representative strains were analyzed by spa typing and multilocus sequence typing (MLST).
Results: The MRSA percentage ranged from 9% in 2003 to 12% in 2007. According to the RFLP patterns, the 135 MRSA isolates were classified in seven clonal groups; 33.4% were identified as t018-ST36-II-agr1 (British clone); 30% t002-ST5-IV-agr2 (New York/Japan clone); 25% t067-ST125-IV-agr2 or IVa-agr2; 4.5% t148-ST72-IVa-agr1; 4.5% t037-ST239-II-agr3 (Brazilian clone); 1.5% t008-ST8-IV-agr1 (USA300 clone); 0.8% t002-ST5-IV-agr1 (Paediatric clone).
The pvl genes were detected in four isolates (3%); two strains belonged to the USA300 clone (one was isolated in 2006 and the other in 2007), one strain to the Paediatric clone, and another one to the ST125 clone.
Conclusion: In other surveillance studies, we described the local epidemic lineages isolated in the hospital setting (1, 2). They were typically health-care associated clones; ST36 (actually it is endemic in our hospitals), ST5, ST125, and ST239 (it was endemic until 2002). In the present study these clones were isolated in outpatients, therefore they were also circulating in the community setting. The clones denominated as community-acquired MRSA were scarcely isolated, only two USA300 isolates. We can state that the community setting highlights the relationship between community and hospital MRSA strains whichever might have been their acquisition, so we think that it is useful to continue surveillance in this scenario.
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P1041 Community and healthcare associated methicillin-resistant *Staphylococcus aureus*: observational study in a large tertiary-care Italian hospital

A. Bartoloni, E. Mantengoli, F. Bartalesi, A. Cavallo, D. Cecconi, P. Pecile, E. Graglia, P. Nicoletti, G.M. Rossolini (Florence, Siena, IT)*

Objective: To investigate the role of methicillin resistant *Staphylococcus aureus* (MRSA) in community-associated (CA) infections in a large tertiary-care university hospital in Italy, and to compare it with the role of health-care associated MRSA (HCA-MRSA) infections in the same setting.
Methods: MRSA isolated from patients within 48 hours of hospitalization and without risk factors for HA-MRSA were defined CA-MRSA. HA-MRSA infections constituted the control group. A data questionnaire was used to collect patients demographics, clinical history, HA- and CA-MRSA risk factors.
Results: 30 patients were enrolled from April until September 2009: 6 infected by CA-MRSA and 24 by HA-MRSA. The mean ages were, respectively, 64 (45–84 years) and 74 years (29–100 years). All CA-MRSA were from skin and soft tissue infections (SSTI). HA-MRSA were from SSTI (46%), sputum or BAL (42%), blood (12%). 1 of 6 CA-MRSA infected patients had HIV as risk factor. Most of HA-MRSA infected patients (23/24) had at least one risk factor. Complications (septic shock, sepsis, pneumonia) were observed

in none of the patients infected by CA-MRSA but in 9 of 24 (63%) of those infected by HA-MRSA [p = 0.02]. 5 death (21%) were observed in the HA-MRSA infected patients and none in those with CA-MRSA [p = 0.5]). The susceptibility rates for HA- and CA-MRSA were, respectively: clindamycin 21% vs 50%; erythromycin 21% vs 33%; levofloxacin 8% vs 0%; gentamicin 29% vs 67%; tobramycin 17% vs 50%; rifampicin 87% and 100%; tetracycline 92% vs 83%; trimethoprim sulfamethoxazole 96% vs 100%; all isolates were susceptible to vancomycin, teicoplanin, linezolid and tigecycline. Molecular analysis showed heterogeneity of SCCmec cassettes (type I and IV in CA-MRSA and type I, II, and IV in HA-MRSA) and the presence of PVL genes in only one CA-MRSA.
Conclusions: This is the first report comparing molecular epidemiology, clinical impact, treatment outcome and risk factors for HA- and CA-MRSA infections in Italy. HA-MRSA infections had more complications and seem to have a worse prognosis. CA-MRSA showed in general an higher antibiotic susceptibility, even if we observed a remarkable reduced susceptibility to aminoglycosides and a total resistance to quinolones. HA-MRSA and CA-MRSA were found to share some molecular features, as also recently reported.

P1042 *Staphylococcus aureus* carriage in healthy humans in Spain: antibiotic resistance mechanisms, virulence traits and genetic lineages

C. Lozano, E. Gómez-Sanz, F. Ruiz-Larrea, M. Zarazaga, C. Torres (Logroño, ES)*

Objective: To study the prevalence, resistance mechanisms, virulence traits and genetic lineages of nasal *S. aureus* of healthy humans.
Methods: 135 nasal swabs were obtained from healthy people (aged from 1 to 74) from April to October 2009 in La Rioja (Spain). Samples were inoculated into BHI broth with 6.5% NaCl, incubated at 35ºC/24 h., and then seeded on ORSAB plates (OXOID) with 2 mg/L of oxacillin and Mannitol Salt Agar (BD) plates (for methicillin-resistant-*S. aureus* (MRSA) and *S. aureus* recovery, respectively), and incubated at 35ºC/36 h.. One isolate per positive sample was characterized. Identification was confirmed by PCR of nuc gene. Profile susceptibility to 16 antibiotics was determined by disk diffusion method according CLSI guidelines. Presence of mecA, ermA, ermB, ermC and msrA genes was studied by PCR. Mutations in quinolone targets were determined by sequence analysis of grlA and gyrA genes. spa and agr typing was implemented on all the *S. aureus* strains. Presence of toxin genes lukF/lukS, tsst1, eta, etb, hla, hlb, hld, hlg, and hlg-2 was investigated by PCR.
Results: 23 of 135 samples contained *S. aureus* (17%). A high diversity of spa-types were identified (t002, t008, t012, t018, t021, t084, t190, t209, t216, t270, t342, t571, t985, t1008, t1641), and four new spa-types were detected. The most frequent spa-type was t002 (3 strains, 13%). Isolates presented agr-type I (30.5%), II (39.1%), III (26.1%) and IV (4.3%). No MRSA were detected. Eight strains were resistant to erythromycin and the following genes were detected (no strains): ermA (5), ermB (1), ermC (1), and msrA (5). Two strains showed resistance to ciprofloxacin; one of them presented Ser80Phe in GrlA and Ser84Leu in GyrA amino acid changes, and Pro144Ser in GrlA and no mutation in GyrA the other one. All strains were susceptible to other antibiotics tested. The following toxin genes were detected (no strains): lukF/lukS (0), tsst1 (9), eta (2), etb (0), hla (23), hlb (14), hld (23) hlg (14), and hlg-2 (13).
Conclusions: A moderate prevalence of *S. aureus* nasal colonization, and a high genetic diversity (19 different spa-types) have been detected in healthy people in Spain. Four new spa-types were detected. No MRSA was found. Different resistance and toxin genes were detected. 39% of the strains were positive for tsst-1 gene and the majority of them belonging to agr-typeIII.

P1043 Emergence and characterization of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* infections in Canary Islands, Spain, 2003–2008

A. Cañas-Pedrosa, I. Horcajada, F. Artiles, A. Vindel, B. Lafarga (Las Palmas, Madrid, ES)*

Objective: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged rapidly in the United States and is now emerging in Europe. In this study we assessed the prevalence, microbiological characteristics, molecular epidemiology and outcomes of Panton-Valentine leukocidin-positive (PVL-positive) CA-MRSA infections in Gran Canaria over the last 6 years.
Methods: Patients with PVL-positive CA-MRSA infections between January 2003 and December 2008, were eligible for inclusion in this study. The PVL genes (lukS-PV and lukF-PV), mecA gene and arcA region of ACME gene were detected by PCR. SCCmec types, SCCmec IV subtypes and agr type were determined by using multiplex PCRs. Strains were genotyped by pulse field gel electrophoresis (PFGE) after SmaI digested, spa typing and assigned to clonal complexes (CCs).
Results: A total of 560 new MRSA cases (1 per patient) were found from 2003 to 2008. Of the MRSA cases, 2.7% (18) were PVL-positive CA-MRSA. The female-to-male ratio was 0.29 and the mean age was 20 years (range, 1–54). The proportions of PVL-positive CA-MRSA cases were less than 2% from 2003 to 2006, 3.6% in 2007 and 15% in 2008. In total, 22% (4/18) of the patients were of non-Spanish origin (Ecuador, Colombia and Filipinas). Skin and soft tissue infections (SSTIs) were predominant (16 cases [88%]), followed by invasive infections (arthritis and osteomyelitis) (2 cases [12%]). In 13 (82%) cases, SSTI was associated with an abscess. In total, 82% (13/16) of the isolates genotyped were identified as spa-CC008-SCCmec IV, agr I allele. Of these 13, three (23%) were found to be USA-300, ACME+, SCCmec IVa, spa type t008, and 10 (70%) had a USA300-like PFGE pulsotype, SCCmec IVc, spa type t008 (8) and t024 (2). Two isolates (12%) were t019/ST30-SCCmec IVc, agr III allele (South Pacific clone) and one isolate (6%) was t311/ST5-SCCmec IVa, agr II allele (paediatric clone).
Conclusions: Our results show an increase in the incidence of PVL-positive CA-MRSA in 2008. PVL-positive CA-MRSA predominantly caused SSTI in children, and abscesses were the most frequently encountered indication. Well-known CA-MRSA strains, such as, CC8:ST8-IV (USA300), CC30:ST30-IV (South Pacific CA-MRSA) and CC5:ST5-IV (paediatric clone) were found. However, CC80: ST80-IV (European CA-MRSA) was not present. Clinical and microbiological evidence suggests import and subsequent transmission as the most likely sources of many of the new CA-MRSA cases.

P1044 Skin and soft tissue infections due to *Staphylococcus aureus* producing Panton-Valentine leukocidin toxin in the Nord-Pas-de-Calais region, northern France, 2004–2008

C. Gros, Y. Yazdanpanah, M. Roussel-Delvallez, A. Vachet, E. Senneville, X. Lemaire (Tourcoing, Lille, Roubaix, FR)*

Background: The aim of this study was to describe epidemiological characteristics, clinical characteristics, and treatment of patients with skin and soft tissue infections due to *Staphylococcus aureus* (*S. aureus*) producing Panton-Valentine leukocidin (PVL) toxin in Nord-Pas-de-Calais region, Northern France (4 millions of inhabitants).
Methods: All patients with *S. aureus* specimens producing PVL seen in one of the 7 hospitals in the Nord-Pas-de-Calais region between 02/2004 and 04/2008 were first identified. Epidemiological and clinical characteristics for and antibiotics initiated in these patients were extracted from their medical records or through telephonic interviews using a standardized questionnaire.
Results: During the study period, *S. aureus* PVL was isolated in 64 cases from February 2004 to April 2008. 54 pts had skin and soft tissue infections (35 abscesses), 3 had pneumonia, and 7 had only

nasal carriage. Skin and soft tissue infections were abscesses with inflammatory signs in 64.8% of the cases. Only 20% of these abscesses were necrotic. Patients with skin and soft tissue infections were men in 63% and had a mean age of 24.7 years. Sixteen patients (29.6%) were from the city of Roubaix (100,000 of habitants). The median number of household members in a patient with *S. aureus* PVL was 4.5 (vs. 2.5 in Nord-Pas-de-Calais). 44.4% of patients had domestic animals. 70.3% of patients had a methicillin resistant strain. An antibiotic therapy was administered in 83.3% of patients and these antibiotics were considered to be inadequate in 53.3% of the cases. Surgery was performed in 83.3% of the patients. An exposition to antibiotic therapy in the previous 3 months was noted for 14 of the 38 patients with available data. A methicillin resistant strain was isolated in 10 of these 14 patients.
Conclusion: A high number of household members and a previous recent exposition to antibiotic therapy could lead to suspect a PVL in patients with skin abscess. In these patients, efforts should be done to obtain skin swab samples and cultures to adapt antibiotic use. Given the increasing number of *S. aureus* PVL infections in the Nord-Pas-de-Calais region, a prospective study should be initiated to better identify risk factors for developing skin lesions related to PVL.

P1045 Prevalence and clinical significance of infections caused by toxin-producing *Staphylococcus aureus* isolates

T. Nhan, R. Leclercq, V. Cattoir (Caen, FR)*

Objectives: Among virulence factors described in *Staphylococcus aureus*, several are of major concern such as PVL, TSST-1/SEB/SEC and ETA/ETB potentially implicated in complicated skin and skin structure infections and necrotizing pneumonia, toxic shock syndrome and staphylococcal scalded skin syndrome, respectively. The aim of this study was to screen consecutive *S. aureus* clinical isolates (resistant or not to methicillin) for the presence of six major toxin genes and to assess the clinical significance of these toxin-producing strains.
Methods: We screened all *S. aureus* clinical isolates recovered from May to June 2009 from patients hospitalized at the university hospital of Caen, France. Clinical data were obtained for each patient regarding age, gender, predisposing conditions, site of isolation and clinical presentation. PCR detection of pvl, tst, seb, sec, eta and etb genes was performed using two multiplex real-time PCR assays. We compared groups of patients presenting uncomplicated infections versus severe infections (severe sepsis, toxic or septic shock) according to the presence or not of toxin genes by using the Fischer's test.
Results: During the two-month study period, 186 *S. aureus* strains were isolated from 186 patients suffering from superficial and invasive infections (48.4% and 51.6%, respectively). Prevalence of nosocomial infections was 53.2%. The mean age was 54 years (range 0–97 years), sex ratio M/F was 1.6 and predisposing conditions were present in 94 patients (50.5%). At least one toxin gene was detected in 55 (29.6%) isolates as follows: pvl (n = 1), tst and sec (n = 5), seb (n = 19), seb and sec (n = 1), sec (n = 28), eta (n = 1) and etb (n = 0). The proportion of toxin-producing strains (40/151, 26.5%) in the group of patients presenting uncomplicated infection and in that of patients with severe infection (15/35, 42.9%) was not statistically different (P = 0.066) although severity of infection tended to be associated with the presence of a toxin gene.
Conclusion: This study contributed to estimate the prevalence of toxin gene among consecutive clinical isolates of methicillin-susceptible and -resistant *S. aureus* from France. Although the prevalence of toxin genes is relatively high (about 30%), no statistically significant relationship between severity of infection and presence of toxin gene has been found.

P1046 Pathogenic mechanisms of methicillin-resistant *Staphylococcus aureus*

A. Haggar, A. Nguyen Hoang, M. Svensson, G. Arakere, A. Norrby-Teglund (Stockholm, SE; Bangalor, IN)*

Objective: Community-associated (CA)-MRSA has been reported in association with highly aggressive infections, including necrotizing soft tissue or lung infections as well as septic shock. Here we have studied pathogenic responses elicited by MRSA isolates in human *in vitro* cell and tissue model systems.
Methods: CA-MRSA isolates (n = 17) collected in Bangalore were characterized with respect to agr type, superantigen gene profile and presence of the Panton-Valentine Leukocidin gene (PVL). Seven isolates with matched agr type, but varying toxin profiles, were selected for functional analyses and toxin-containing supernatants (SUPs) were prepared from stationary phase cultures. These SUPs were tested for ability to induce proliferation of peripheral blood mononuclear cells, and for cytotoxic (epithelial damage, HMGB-1 release) and/or inflammatory effects (IL8 expression) in a 3D organotypic lung tissue model. The latter analyses were done by immunostaining and in situ image analyses (ACIA) of SUP-exposed tissue.
Results: The proliferation assay showed that isolates clustered according to their agr type. Type I and II strains induced significantly stronger proliferation than did agr type III and IV isolates (ANOVA, p = 0.02). In fact, agr III and IV isolates induced a strong cytoxic response over a broad concentration range. The cytotoxicity could not be related to PVL-positivity, as the most cytoxic strain was PVL-negative. Western blot analyses of SUPs revealed higher amounts of α-hemolysin in cytotoxic, as compared to mitogenic, SUPs.
Two agr type II mitogenic isolates and three cytotoxic isolates (agr III and IV) were further tested in the lung tissue model. SUPs were added on the apical side of the epithelial layer in the tissue model and after 24 h of exposure the tissue was analyzed for IL-8 and HMGB-1. HMGB-1 was markedly increased in tissues exposed to the cytotoxic SUPs (ACIA=38–40) whereas tissue exposed to mitogenic SUPs was equal to control tissue (ACIA=19–23). IL-8 was upregulated in all tissues exposed to the SUPs (ACIA=20–45) as compared to unstimulated control tissue (ACIA=11).
Conclusion: Our findings reveal striking differences in mitogenic versus cytotoxic functions of MRSA isolates of varying agr types. These functional differences were also reflected in the responses elicited in the human lung tissue model. Furthermore, the data implicates α-hemolysin, rather than PVL, in these responses.

Streptococcal infections (non-*Enterococcus*)

P1047 Characteristics of longitudinal oropharyngeal asymptomatic colonization and of multicolonizaton by different strains of group A streptococci from Portugal

R. Pires, C. Alves, F. Esteves, D. Rolo, J.M.F. Almeida, T. Ramos, C. Gameiro, F. Andrade, A. Lopes, L. Romeiro, F. Vaz, A. Morais, A. Brito-Avô, J. Gonçalo-Marques, I. Santos-Sanches (Caparica, Oeiras, Lisbon, PT)*

Objective: To examine the long-term asymptomatic oropharyngeal colonization status by Group A streptococci (GAS) and if co-colonization of the oropharynx by multiple GAS strains occurs among asymptomatic persons.
Methods: Between 2000 and 2004, 5,494 oropharyngeal samples were taken during 12 sampling periods in October, February and May of each year from 1,729 healthy individuals. Bacterial identification was carried out by standard methods. The total number of GAS isolates was 626 (carriage rate 11.4%) out of which 243 (38.8%) were from 113 (6.5%) recurrent carriers – defined as colonized by GAS more than once during 2 to 12 sampling periods. These isolates were considered as associated with long-term or longitudinal colonization. All β-hemolytic colonies identified as GAS that were picked from each of the primary plates containing the swabs of 23 participants, out of 865 (2.7%) persons sampled in one sampling period, were considered as causing co-colonization or multicolonization of the same niche. Isolates of both groups – 243 causing longitudinal colonization and 74 isolates causing multicolonizaton – were typed by pulsed-field gel electrophoresis (PFGE) for strain definition and by sequencing part of the emm gene encoding the M surface protein (emm typing).

Results: Of the 113 recurrent carriers, 111 were colonized in 2 or 3 sampling periods and 2 were colonized in 4 sampling periods. In the majority of the recurrent carriers (n = 89, 78.8%), strain replacement was observed. Examples that occurred frequently were emm12 (PFGE.AB) replaced by emm1 (PFGE.X) or emm3 (PFGE.BG), and emm1 (PFGE.X) replaced by emm12 (PFGE.AP). The remaining 24 (21.2%) persons were carriers of a same strain. Also, out of the 113 persons, 54 (47.8%) were carriers at least once of emm12 strains. Of the 23 participants of the multicolonization study, 6 (26.1%) were colonized by multiple strains of different PFGE types and of a same emm type. An example was one individual that carried 3 emm75 strains (PFGE.CY, PFGE.FM and PFGE.FN types).
Conclusions: A replacement of GAS strains was frequently found among recurrent carriers and co-colonization of the oropharynx by multiple GAS strains was detected. emm12 isolates seem to be successful colonizers as they were identified as widely disseminated among epidemiological related and unrelated carriers and were persistent overtime.

P1048 Unique and changing epidemiology of group A streptococcal infection in western Australia

D. McLellan, L. O'Reilly, A. Levy, T.J. Inglis, J.R. Dyer (Fremantle, Perth, AU)*

Objective: The aim of this study was to examine the clinical and molecular epidemiology of group A streptococcal (GAS) disease over a 10-year period in Western Australia.
Methods: Isolates from patients with invasive GAS disease were collected in two time periods, 2000–03 and 2006–09. These isolates were subjected to emm typing and pulse field gel electrophoresis (PFGE). For comparison, 186 consecutive non-invasive isolates were also collected in 2007 and emm typed. Clinical information was collected from patients identified in 2000–03.
Results: 61 patients with invasive GAS disease were identified in the period 2000–03. 57% of cases were male and the mean age was 47. Nineteen cases (31.1%) occurred in indigenous Australians. Necrotizing fasciitis was evident in 6 patients (9.8%) and streptococcal toxic shock syndrome (STSS) in 7 (11.5%). Seven (11.5%) deaths could be directly attributed to GAS disease. In the 11 cases complicated by necrotizing fasciitis and/or STSS there were 5 deaths (45.5%).
Of the 61 isolates, 27 different emm types were identified. Emm type 8 was the most common type, representing 6 of 61 isolates (9.8%). PFGE analysis of the 61 isolates revealed a high concordance between emm type and PFGE pattern. The 6 emm type 8 isolates were indistinguishable based on their PFGE pattern.
For the period 2006–09, 51 patients with invasive GAS disease were identified. Of the 51 isolates, 17 different emm types were identified. The most common emm types were 1 (22%), 89 (18%), 28 (10%) and 12 (8%). Molecular analysis by PFGE of the emm type 1 isolates indicated they were clonally related.
Of the 186 non-invasive isolates collected in 2007, 41 different emm types were identified. The most common emm types were 4 (15.1%), 89 (10.2%), 49 (8.1%) and 87 (7%).
Conclusions: A remarkably heterogeneous population of emm types have been identified causing both invasive and non-invasive infection in Western Australia. This study also suggests that new emm types are entering this population at regular intervals and are responsible for severe disease. This epidemiology is different to what has been reported in the Northern Hemisphere and eastern Australia and likely reflects geographical isolation and other factors. This information has implications for M protein-based GAS vaccines currently under development.

P1049 Invasive group A streptococcal disease in Ireland, 2004–2008

J. Martin, S. Murchan, D. O'Flanagan, F. Fitzpatrick (Dublin, IE)*

Objectives: Diseases caused by Invasive Group A streptococcus (iGAS) are a major clinical and public health challenge. There has been an increase in prevalence of iGAS globally in recent decades. Many aspects of their occurrence and characteristics remain poorly understood. The aim of this study is to analyse the microbiological and epidemiological characteristics of iGAS in Ireland over the five year period 2004–2008.
Methods: Enhanced surveillance data and laboratory results were entered onto the national electronic reporting system. Data were entered on patient demographics, risk factors for iGAS, clinical presentation and outcomes associated with iGAS, in addition to source, timing and clusters of disease. Collection of antimicrobial susceptibility data commenced in 2008.
Results: Between Jan 1 2004 and Dec 31 2008, 270 cases of iGAS were notified. Males and females were affected equally. Cases occurred in all age groups but children up to 9 years and adults 55 year and over were most common affected (median age, 42 years; range 0–94 years). iGAS incidence increased from 2004 (0.9 per 100,000 population) to 2008 (1.6 per 100,000 in 2008). The most common risk factors associated with iGAS were age over 65 years and skin/wound lesions. Thirteen cases presented with streptococcal toxic shock syndrome (3 of whom died) and 11 with necrotising fasciitis (4 of who died). Over the five year period 21 people with iGAS died, eight of whom had iGAS identified as the main cause of death (case fatality rate of 3%). Antimicrobial susceptibility data was available on 38 of 68 isolates in 2008. All isolates tested were susceptible to penicillin (n = 37), clindamycin (n = 5) and vancomycin (n = 31). Resistance to erythromycin was reported in 3 of 30 (10%) isolates.
Conclusion: There appears to be an increase in iGAS infections over time. This could be a genuine increase in the incidence of iGAS or could represent better reporting of this disease, which only became notifiable in Ireland as of 2004. The number of cases notified in Ireland still remains low compared to other Northern European countries and the US. Certain serotypes of GAS are known to be more virulent than others, e.g. serotypes M1 and M3, but in the absence of a streptococcal reference laboratory in this country, no serological typing data are available to investigate such a link between Irish isolates and severity of disease.

P1050 Incidence and risk factors for chronic suppurative otitis media in a birth cohort of children in Greenland

A. Koch, P. Homøe, C. Pipper, T. Hjuler, M. Melbye (Copenhagen, DK)*

Objectives: Inuits of the Arctic experience very high rates of chronic suppurative otitis media (CSOM), yet world-wide very little is known about the epidemiology of CSOM. The study aims were to determine age-specific incidence, median age at debut, risk factors, and associated population-attributable risks for CSOM in children in Greenland in order to devise possible preventive measures.
Methods: A population-based cohort of 465 children aged 0–4 years was followed closely for a two-year period 1996–98 in Sisimiut, the second-biggest town of Greenland, and cases of CSOM and other respiratory tract infections were registered based on medical history and clinical examinations. Information on risk factors was obtained from questionnaires, and risk factor analyses were carried out using Cox regression models.
Results: The cumulative incidence rate of CSOM at 4 years of age was 14%, and median age at debut was 336 days. Significant risk factors were being of Greenlandic descent, attending childcare centers, having smokers in the household, having a mother who reported a history of purulent ear discharge, and having a high burden of upper respiratory tract infections. High population-attributable risks for CSOM were associated with use of childcare centers (51%) and living with smokers in the house (74%).
Conclusion: Greenlandic children have high rates of CSOM with debut early in life, but the identified risk factors and the associated population-attributable risks indicate that preventive measures regarding use of childcare centers and passive smoking may reduce the frequency of CSOM in this high risk population.

P1051 Outcomes of hospitalized patients with bacteraemic and non-bacteraemic community-acquired pneumonia caused by *Streptococcus pneumoniae* with high rates of resistance to β-lactam antibiotics

M.S. Hsu, Y.T. Huang, P.R. Hsueh (Panchiao, Taipei City, TW)*

Objectives: The impact of *S. pneumoniae* bacteraemia on the outcome of pneumococcal pneumonia is not fully known, particularly in regions with high rates of resistance to β-lactam antibiotics. This retrospective study compared the outcome of hospitalized patients with bacteraemic and nonbacteraemic pneumococcal community-acquired pneumonia (CAP) treated at a medical center from 2000 to 2008.
Methods: Case of pneumococcal pneumonia was diagnosed by on a positive blood culture for *S. pneumoniae* and positive *S. pneumoniae* urinary antigen test from January 2000 to December 2008 were identified from databases of the microbiology laboratory. Antimicrobial susceptibility testing and MIC were according to guideline of CLSI. Medical data were also collected.
Results: A total of 309 patients with pneumococcal pneumonia (137 nonbacteraemic and 172 bacteraemic) were included. Patients with bacteraemic pneumococcal pneumonia were older than those with nonbacteraemic pneumococcal pneumonia (42.9±31.2 years vs. 21.9±31.1 years; $P < 0.001$) and were more likely to have underlying medical diseases (61.6% vs. 34.3%; $P < 0.001$). The overall mortality rates at 7, 14, and 30 days were significantly higher in patients with bacteraemic than nonbacteraemic pneumococcal pneumonia (10.5% vs. 2.2%; 14% vs. 3.6%; 17.4% vs. 5.1%; $P < 0.01$ for all comparisons). The in-hospital mortality rate was higher in bacteraemic than nonbacteraemic patients (22.1% vs. 5.8%; odds ratio [OR], 4.57, $P < 0.001$). The difference of mortality rates between bacteraemic patients caused by penicillin susceptible and non-susceptible pneumococcal isolates was not significant (26% vs. 20.7%, respectively, $P = 0.57$). Multivariate regression analysis showed that pneumococcal bacteraemia was correlated with mortality (OR, 2.70; $P = 0.003$) and extrapulmonary involvement (OR, 5.0, $P < 0.001$).
Conclusions: Despite the decline in the incidence after pneumococcal vaccination and the advances in antimicrobial agents, the presence of pneumococcal bacteraemia increased the risk of mortality and extrapulmonary involvement in patients with pneumococcal pneumonia. Further study is needed to develop optimal antibiotic and vaccination strategy to improve the outcome of bacteraemic pneumococcal pneumonia.

P1052 Pneumococcal infections as a sudden death cause: forensic microbiological diagnosis

R. Abad Moralejo, M.A. Fernandez-Rodriguez (Madrid, Las Rozas, Madrid, ES)*

Objectives: Pneumococcal infection (PI) is a main cause of community-acquired pneumonia (CAP) and meningitis in adults, sometimes leading to a fatal outcome. The incidence of PI as responsible of sudden unexpected death (SUD) has not been established to date. Since SUD usually occurs outside of the hospital or soon after arrival in the emergency department, in most occasions a microbiological analysis is not performed at the hospital and a medico-legal autopsy is performed. The aim of this study was to determine the incidence of PI as a cause of SUD and to assess the utility of different techniques to detect this pathogen in forensic postmortem samples.
Methods: Specimens were received from the different Forensic Pathology Services all along Spain. We designed a flexible protocol including: (i) detection of capsular pneumococcal antigen by latex agglutination and immunocromatography, (ii) bacteriological culture of forensic samples, and (iii) detection of pneumococcal DNA by PCR (ply, lytA and psa genes). Antigenic detection was always considered a presumptive result. A positive result for a sample was defined when a pneumococcus had been isolated by culture and/or when a positive real time PCR result was obtained from a sterile fluid or tissue.
Results: We analyzed 377 SUD (255 in adults and 122 in infants) (age range 19 days-90 years) and 1971 samples. In 46 SUD cases a pneumococcus was detected, 36 in adults (age range 19–74) and 7 in infants (4–16months) (16 females and 30 males). The cause of death was directly attributed to pneumococcus in 44 of them according to histopathology. Concomitant diseases were present at least in 32 of them. One infant SUD had co-infection with the H1N1 virus. Other pathogens were detected in 8 cases. In 38.3% of the positive cases the diagnosis of PI was made by culture. In a 25.5% of the cases the diagnosis was established by both culture and PCR. In a 36.2% only the PCR assay permitted the detection of pneumococcus and, in 35.3% out of them, the only specimen where a positive PCR result was obtained was a paraffin-embedded tissue.
Conclusion: Pneumococcus is a pathogen involved in sudden death, mainly due to meningitis (52.2%), CAP (28.3%) and Waterhouse-Friederichen syndrome (15.2%). Attention should be paid when chronic underlying diseases are present. A combined protocol including culture and molecular techniques such a real time PCR is required to detect this pathogen in forensic samples.

P1053 Surveillance of invasive pneumococcal disease in the Czech Republic

J. Motlova, C. Benes, P. Kriz (Prague, CZ)*

Objectives: Pneumococcal disease has been in the focus of interest worldwide since the pneumococcal conjugate vaccine (PCV) became available. The international organizations such as the World Health Organisation, European Centre for Disease Control and Pneumococcal Awareness Council of Experts motivate the countries to implement a nationwide surveillance programme for invasive pneumococcal disease (IPD) and to include PCV into the national immunization programme (NIP) for infants. In the Czech Republic, PCV has been available since 2001 when the 7-valent vaccine (PCV-7) was registered, followed by the 10-valent vaccine (PCV-10) and 13-valent vaccine (PCV-13) in 2009. PCV will be included into the Czech NIP for infants in 2010. The aim of this project was to obtain information about the pre-vaccination IPD incidence and serotype distribution.
Methods: A laboratory-based IPD surveillance programme was carried out in 2000–2006 and a pilot enhanced IPD surveillance programme followed in 2007. A nationwide IPD surveillance programme was implemented in January 2008. An IPD case was defined by the isolation of *S. pneumoniae* from blood, cerebrospinal fluid, or other normally sterile sites. Each isolate was identified by standard methods and *S. pneumoniae* isolates were serotyped by the Quellung reaction using serotype-specific antisera.
Results: Based on the laboratory surveillance data, the IPD incidence rates varied from 2.3 to 4.3 per 100 000 population between 2000 and 2006. Based on the enhanced surveillance data, the incidence rates were 2.9 in 2007 and 3.3 in 2008. The age-specific IPD incidence rate was the highest in the <1 year-olds, reaching 15.7 per 100 000 according to the laboratory-based surveillance data and 16.6 per 100 000 according to the enhanced surveillance data. In the period 2000–2008, the coverage by PCV-7 was 60% in the under-1-year-olds and 62% in the under-5-year-olds, the coverage by PCV-10 was 71% in both age categories and the coverage by PCV-13 was 79% and 82%, respectively.
Conclusions: The incidence of IPD in the Czech Republic is comparable to the pre-vaccination data reported in other European countries. It is recommended to include PCV in the routine vaccination scheme for infants in the Czech Republic.

P1054 Invasive *Streptococcus pneumoniae* isolates before introduction of PCV7 in Ankara, Turkey: antimicrobial susceptibilities and serotype distribution

Ö. Köseoglu Eser, H. Uludag Altun, D. Gur, G. Hascelik (Ankara, TR)*

Objectives: The present study evaluated the antimicrobial susceptibilities and serotype distribution of invasive *Streptococcus pneumoniae* (SP)

isolates identified from patients admitted to Hacettepe Hospitals before the introduction of heptavalent pneumococcal conjugate vaccine (PCV-7) in Turkey between 1996 and 2008.
Methods: Invasive SP clinical isolates were collected from children and adults in Hacettepe Hospitals, Ankara. Susceptibilities of all isolates were determined by CLSI broth microdilution testing for six antimicrobial agents; penicillin(PEN), ceftriaxone(CRO), levofloxacin(LEV), erythromycin(EM), clindamycin(CD) and vancomycin(VAN). Serotyping was performed by Quellung reaction with specific antisera for SP.
Results: Of the 182 non-duplicated invasive pneumococcal isolates, 59 were isolated from children and 123 from adults. Of these, 32 were cerebrospinal fluid and 150 were blood isolates. Fifty seven (31.3%) isolates showed reduced susceptibility to PEN (MICs >0.12 μg/ml). Intermediate resistance (MIC 0.12–1 μg/ml) to PEN were found in 52(28.6%) penicillin-nonsusceptible isolates and high-level resistance (MIC ⩾2 μg/ml) in five (2.7%). Twenty three (12.6%) isolates revealed resistance to EM (MICs ⩾0.5 μg/ml), of these 11 were resistant to both EM and CD. Nine (4.9%) of invasive SP isolates showed a multi-drug resistance pattern. Antimicrobial susceptibilities of invasive SP isolates are shown in Table 1. In the paediatric age group, 17 different serotypes were recognized, while eight strains were nontypeable (NT). In the adult group, 23 different serotypes were observed, with 7 NTs. The most frequent serogroups in both age groups were 6, 3, 23, 9 and 5. The potential coverage of 7- and 13-valent pneumococcal conjugate vaccine were 44.1%, 66.1% in children and 39.8%, 71.5% in adults, respectively.
Conclusion: SP is the leading cause of morbidity and mortality worldwide, especially in children presenting with meningitis and bacteraemia. PVC-7 was licensed for use in Turkey in November, 2008. As surveillance data before PCV-7 vaccination are insufficient, this study may provide an important baseline to assess the potential changes in the epidemiology of IPD after PCV-7 introduction in Turkey. In addition, widespread vaccination programme may contribute to decrease the high penicillin and macrolide resistance patterns which are caused by the selective pressure resulting from misuse of antibiotics against invasive pneumococci.

Table 1. Antimicrobial susceptibilities of invasive pneumococcal isolates

	MIC_{50}	MIC_{90}	Range	Resistance	
				I, n (%)	R, n (%)
Penicillin	<0.06	1	<0.06–2	52 (28.6)	5 (2.7)
Ceftriaxone	<0.25	0.5	<0.25–1	0	0
Levofloxacin	1	1	<0.25–>8	0	2 (1.1)
Erythromycin	<0.25	1	<0.25–>8	2 (1.1)	21 (11.5)
Clindamycin	<0.25	<0.25	<0.25–>8	0	11 (6.0)
Vancomycin	<0.25	<0.25	<0.25–<0.25	0	0

P1055 Phenotypical and genotypical differences of *Streptococcus pneumoniae* carried by healthy children attending day care centres in big cities versus small communities of Hungary

A. Tóthpál, S. Kardos, S.G. Amyes, K. Nagy, O. Dobay (Budapest, HU; Edinburgh, UK)*

Objectives: *Streptococcus pneumoniae* is a pathogen causing a wide spectrum of severe diseases, which can be carried in the nasopharynx of healthy individuals, especially children attending community centres. In the present study, we have compared the carriage observed in day-care centres of big cities versus small villages in Hungary.
Methods: Nasal swabs were collected from 187 children (age 3–6 years) from 6 day-care centres (4 big cities and 2 small villages) in different parts of Hungary. The pneumococci were selected from the normal flora, and their identity was confirmed by optochin sensitivity and the presence of the lytA gene. Serotyping was done by the combination of the conventional method (antisera) and a PCR-based method. The antibiotic sensitivity was determined by E-tests, and the genetic relatedness of the strains was examined by PFGE.
Results: The pneumococcus carriage rate was 39.5% (n = 74). On one occasion, we could detect the simultaneous carriage of 2 pneumococcal strains. We could determine the serotype of 64 strains. The serotype distribution showed that in the villages only 2 or 3 serotypes were represented, while in the cities the diversity was much wider. The serotypes in ranking order were: 14 (35.6%), 6 (24.1%, 6A > 6B), 19F (9.2%), 15, 23F, 18C, 3, 19A, 13, 7. The strains were generally very sensitive to antibiotics, except for macrolides. In the villages we detected slightly lower penicillin MICs, but none of the strains were resistant (R) to penicillin. The highest MICs (0.5–1 mg/L) were detected in sero 14, 23F, 19F, and 18C (all part of the Prevenar vaccine). 18 strains showed high-level R to erythromycin, these were of different serotypes, and 14 carried the ermB gene. We could identify the presence of very distinct PFGE clones at the individual nurseries, e.g. different clones of serotype 14 strains from the different places.
Conclusions: There was no difference in the carriage rate between cities and villages, and little in antibiotic sensitivity. The difference lay in the serotype distribution. Based on our data and taking the relevant cross-protections into account, Prevenar (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) would cover 77.0% of the total isolates, but 100% in the villages. On the other hand, there was only one single strain, protection to which is not provided by Pneumovax. We could clearly show the presence of discrete clones within the nurseries, indicating the intensive exchange of bacteria between children.

P1057 Prevalence of *Streptococcus agalactiae* in different age groups presenting with vulvovaginitis

S. Baka, A. Panopoulou, A. Lykas, I. Tsouma, I. Patikas, E. Kouskouni (Athens, GR)*

Objectives: Vaginal culture is one of the most difficult cultures to be evaluated in clinical microbiology practice. *Streptococcus agalactiae* has been associated with serious infections and represents an important cause of neonatal infection due to maternal-infant transmission. The aim of the present study was to evaluate the prevalence of *S. agalactiae* when isolated in the vaginal secretions of different groups of patients presenting with vulvovaginitis.
Methods: A total of 2,130 vaginal cultures from different patients presenting at the outpatient clinic for obstetrics and gynecology of our hospital during January 2008 to October 2009 were studied. Cases were divided into 4 groups: 245 samples from girls 2–14 years old, 1,294 samples from reproductive age (18–40 years old) non-pregnant women, 386 samples from pregnant women in their third trimester of pregnancy and 205 postmenopausal women (50–65 years old). All women enrolled presented with signs and symptoms of vulvovaginitis (vulvovaginal irritation, itching and/or vaginal discharge) on physical examination. Samples were inoculated onto blood agar, MacConkey, Mannitol Salt, Sabouraud Dextrose agar, *Gardnerella* agar and Wilkins-Chalgren agar as well as Thayer-Martin and chocolate agar followed by incubation in aerobic, anaerobic or CO2 atmosphere at 37º C for 24 or 48 hours, as appropriate. Wet mount and Gram stain preparations were examined to investigate the presence of leukocytes and the type of microorganisms present. The identification of isolated strains and their susceptibility test to antibiotics were carried out with the API System and the automated system VITEK 2 (bioMérieux, Marcy l'Etoile, France).
Results: *S. agalactiae* was isolated from 6/245 (2.4%) of girls, in 88/1,294 (6.8%) of non-pregnant, reproductive age women, from 46/386 (11.9%) of pregnant women and 14/205 (6.8%) of postmenopausal women.
Conclusion: Among the four groups studied, *S. agalactiae* was more frequently isolated from women in late pregnancy. Although neonatal group B streptococcal infection can and has been reduced by routine screening and use of antibiotics, strict adherence to a culture-based screening strategy is important to identify the mother-infant pairs at risk of vertical transmission.

P1058 **Disease associations amongst bloodstream infections caused by the new species included in the *Streptococcus bovis* taxonomic group**

A. Burillo, Y. Gil, C. Flecha, M. Palomo, J. Gómez-Garcés (Madrid, ES)*

Objectives: Modern taxonomy ascribes all clinical *Streptococcus bovis* bacteria to a group that includes *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG), *S. gallolyticus* subsp. *pasteurianus* (SGP), *Streptococcus infantarius* subsp. *coli* (SIC), and *S. infantarius* subsp. *infantarius* (SII). We sought to identify possible disease associations amongst bloodstream infections (BSI) caused by these species by studying patient records.
Methods: Forty-four consecutive blood culture isolates initially designated *S. bovis* were further characterized using phenotypic methods (API 20 Strep®, bioMérieux). Patient records were examined.
Results: We identified 15 SGG, 24 SGP, 2 SIC and 3 SII isolates in 44 BSI episodes. Twenty-five (57%) of the 44 patients were men and 19 (43%) women. Median ages were 74 (IQR 65–81.5) years for the men and similar for all taxa detected and 79 (IQR 68–87) years for the women. The mean number of blood cultures positive for *S. bovis* was 2.2 (SD 1.2). Median follow-up was 7 months (IQR 1–23.5). Comorbidities detected were: diabetes mellitus, 27%; intravenous drug addiction, 2%; alcohol abuse, 17%; immunosuppression, 19%; chronic renal failure, 20.5%; chronic respiratory disease, 19%; cardiovascular disease, 56%; urinary tract infection, 12%; solid organ neoplasm, 19%; hematological malignancy, 7%; colon disease, 53% and hepato-biliary disease, 37%. A colonoscopy was performed in 22 (50%) of the patients, and colon disease was detected in 20 patients (adenomatous/vellous polyp in 10, colonic carcinoma in 5 and other diseases in 5). Endocarditis was recorded in a third of the patients.
Among the patients with SGG BSI, 64% and 36% presented colon polyps or colon carcinoma, respectively; for SGP BSI, these figures were 33% and 17%, respectively ($p = 0.023$). Hepato-biliary disease was more common in patients with SGP BSI than SGG BSI (61% vs. 0%, $p < 0.001$). Endocarditis was more frequent in patients with BSI caused by SGG (67% vs. 9.5%, $p = 0.001$). Seven of the patients died during the hospital stay in which *S. bovis* bacteremia occurred, but only in 2 cases was *S. bovis* bacteremia the single cause of death.
Conclusions: Our findings indicate that the association between *S. bovis* bacteremia and endocarditis and/or colon carcinoma is highly dependent on the causative species. SGG BSI is a surrogate for endocarditis and/or colon disease, whereas SGP BSI is a surrogate for hepato-biliary disease.

Molecular epidemiology of healthcare-associated MRSA

P1059 **Local acquisition of methicillin-resistance in predominant *Staphylococcus aureus* clones of western Switzerland**

P. Basset, L. Senn, V. Vogel, G. Zanetti, D.S. Blanc (Lausanne, CH)*

Objectives: Recent population genetic studies suggest that the Staphylococcal Chromosome Cassettes mec (SCCmec) was acquired at a global scale much more frequently than previously thought. We hypothesized that such acquisitions can also be observed at a local level. In the present study, we aimed at investigating the diversity of SCCmec in a local MRSA population, where the dissemination of four MRSA clones has been observed (JCM 2007, 45: 3729).
Methods: All the MRSA isolates (one per patient) recovered in the Vaud canton of Switzerland from January 2005 to December 2008 were analyzed in this study. We used the Double Locus Sequence Typing (DLST) method, based on clfB and spa loci, and the e-BURST algorithm to group the types with one allele in common (i.e. clone). To increase the discriminatory power of the DLST method, a third polymorphic marker (clfA) was further analyzed on a sub-sample of isolates. The SCCmec type of each isolate was determined with the first two PCRs of the Kondo scheme.
Results: DLST analysis indicated that 1884/2036 isolates (92.5%) belong to the four predominant clones. A majority of isolates in each clone harboured an identical SCCmec type: 61/64 (95%) isolates to DLST clone 1–1 SCCmec IV, 1282/1323 (97%) to clone 2–2 SCCmec II, 237/288 (82%) to clone 3–3 SCCmec IV, and 192/209 (92%) to clone 4–4 SCCmec I.
Unexpectedly, different SCCmec types were present in a single predominant DLST clone: SCCmec V plus one unusual type in 3 isolates of clone 1–1; SCCmec I, IV, V, VI plus two unusual types in 41 isolates of clone 2–2; SCCmec I, II, VI plus three unusual types in 51 isolates of clone 3–3; and SCCmec II, IV, V plus one unusual type in 17 isolates of clone 4–4. Interestingly, adding a third locus generally did not change the classification of incongruent SCCmec types, suggesting that these SCCmec elements have been acquired locally during the dissemination of the clones.
Conclusion: Although the SCCmec diversity within clones was relatively low at a local level, a significant proportion of isolates with different SCCmec have been identified in the four major clones. This suggests that the local acquisition of SCCmec elements is not a rare event and illustrates the great capacity of *S. aureus* to quickly adapt to its environment by acquiring new genetic elements.

P1060 **Genetic fingerprinting of MRSA from Abu Dhabi**

S. Weber, R. Ehricht, P. Slickers, L. Abdel-Wareth, G. Donnelly, M. Pitout, S. Monecke (Saarbruecken, Jena, DE; Abu Dhabi, AE; Dresden, DE)*

Methicillin-resistant *Staphylococcus aureus* (MRSA) have become a global problem. However, for many regions of the world only few, if any, data on the actual distribution and abundance of various epidemic or pandemic strains are available. This includes many countries of the Middle East. In order to obtain such typing data, clinical isolates from Sheik Khalifa Medical City, Abu Dhabi, have been characterised using diagnostic DNA-microarrays.
Out of 62 MRSA isolates tested, two carried SCCmec elements of type III. The most common SCCmec element was type IV which was present in a total of 54 isolates. SCCmec elements of type V were found in six isolates. SCCmec elements of types I and II were not detected. Common resistance determinants included blaZ, ermC and genes encoding aminoglycoside-modifying enzymes. Mupirocin resistance was rare with mupR being detected in a single isolate. PVL genes were present in as much as 26 isolates.
Based on array hybridisation profiles, eighteen strains could be distinguished which belonged to eleven clonal complexes. The most common clonal complex was CC22 (21 isolates), followed by CC8 (nine isolates, including USA300, ST72 and ST239 strains) and CC80 (seven isolates). Several unusual MRSA strains (such as ST6-MRSA-IV, CC361-MRSA-V or ST573/772-MRSA-V) were also found.
Generally, a high diversity of different strains as well as a rather high rate of PVL carriage were noted among isolates from Abu Dhabi. These results warrant further investigations on the epidemiology of MRSA in this region.

P1061 **Characterization of *S. aureus* strains from two Turkish hospitals**

A. Alalem, M.D. Avsaroglu, F. Bozoglu (Ankara, Van, TR)*

Objectives: To characterize the *S. aureus* strains obtained from two hospitals in Turkey during June-August 2007 according to their antimicrobial susceptibilities, some genetic determinants and PVL-gene content.
Methods: 305 samples were collected from Ankara and Hacettepe hospitals. The strains were isolated from different clinical origin samples on blood agar. Cultures were maintained on glass beads by using Microbank at –70ºC. Confirmation was done by Gram's staining, coagulase-, and catalase-tests followed by Phoenix Automated Microbiology System (BD Diagnostic Systems) analyses. Antimicrobial susceptibilities of the strains were investigated by disk diffusion method

against 12 different antimicrobials, and only when oxacillin resistance observed, the MIC value of oxacillin was detected by E-test by CLSI standards for all. Genetic determinants were analyzed for randomly selected 30 strains. The studies were carried out with the StaphPlex system for 18 genetic determinants and monitored with the Liquichip200 Workstation.
Results: In this study out of 305 isolates, 95.7% was confirmed as *S. aureus* and 4.3% were CoNS. Except 30, all isolates were resistant to one or more antimicrobials. All oxacillin resistant isolates displayed the MIC value of at ⩾256ug/ml There were 7 OXA resistant CoNS out of 13 isolates. Among *S. aureus* isolates, 46% were MSSA and 54% were MRSA. The VRSA was only 2% and there were no intermediate susceptibility observed. The majority MRSA isolates were multi-resistant to more than five classes of antibiotics (84.2%) and the resistant patterns differed. The results of genetic investigations indicated that the mecA gene was present in the majority of randomly selected 30 isolates; the community acquired MRSA type (ccr-BIV) was present in three and there was no case found to carry the hospital acquired MRSA type (ccr-BI, -II, -III). Besides the methicillin resistance gene, only tetracycline resistance determinants were obtained astetM and tetK. The prevalence of PVL gene was low (only one isolate).
Conclusion: Although there was high prevalence of *S. aureus* among tested clinical samples, there is no clonal spread of a specific isolate according to phenotypical characterization. Multi-resistance of MRSA isolates would be considered as a potential threat in Turkish hospitals based on two hospitals results. Low incidence of VRSA isolates indicates that vancomycin could be the drug for the treatment of *S. aureus* infections in Turkey.

P1062 Clonal evolution and the changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Spanish hospital

F. Menegotto, S. González Cabrero, A. Cubero, C. García Loygorri, A. Dueñas, M.P. Gutiérrez Rodríguez, A. Orduña, M.A. Bratos (Valladolid, ES)*

Introduction: The epidemiology of methicillin resistant *Staphylococcus aureus* (MRSA) has undergone a series of changes over the years. The predominant MRSA clones are constantly replaced by the new ones, showing the dynamic genetics of this pathogen.
Objective: This study aims to assess the clonal evolution and the change in the molecular epidemiology of the MRSA in a Spanish hospital.
Material and Methods: A total of 185 MRSA isolated were collected from 2005 to 2008 from the Clinical University Hospital of Valladolid (Spain) (800 beds). The most relevant genotypes characterized by PFGE were selected for MLST analysis. The sequence type (STs) and their alelic profiles were compared using the goeBURST algorithm, a globally optimized implementation of the eBURST algorithm.
Results and Discussion: We have identified two main clones, ST228-MRSA-I and ST125-MRSA-IV between the strains isolated in the year 2005. In that year, the ST228-MRSA-I clone was the most frequent (68%), and the cassette SCCmec type I (71%) was the most prevalent. In contrast, in the years 2007 and 2008, the cassette SCCmec type IV was identified in the 83% of isolates. The ST125-MRSA-IV has been the predominant clone (43%), followed by ST146-MRSA-IV (20%), ST8-MRSA-IV (20%), ST228-MRSA-I (15%) and ST239-MRSA-III (2%), underscoring the replacement of the ST228-MRSA-I clone by ST125-MRSA-IV between 2005 and 2008. Using the algorithm goeBURST, all the main clones joined two clonal complexes: CC5 and CC8. We have been able to verify that the prevalent clone in 2005 (ST228-MRSA-I) and the emerging clone (ST125-MRSA-IV) belong to the same clone complex (CC5), since their alelic profiles are DLVs. The ST146-MRSA-IV clone is also related with these clones, and all of these were derived from epidemic MSSA lineages (ST5-MSSA) who acquired the cassette SCCmec. The ST8-MRSA-IV and ST239-MRSA-III clones were only identified in the year 2007. These clones are SLVs and belonging to the CC8, which ancestor is the ST8-MSSA, also ancestor of the first MRSA strain isolated (ST250-MRSA-I).
Conclusions: The predominant ST228-MRSA-I clone in 2005 was replaced by the ST-125-MRSA-IV clone in 2008. In this period an increase in the prevalence of SSCmec type IV cassette was observed.

P1063 Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates from Thailand by SCCmec type, variable number of tandem repeats, and virulence determinants

C. Wilailuckana, C. Tribuddharat, P. Tishyadhigama (Khon Kaen, Bangkok, Nontaburi, TH)*

Objective: To characterize genetic background of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Thailand by SCCmec typing, variable number of tandem repeat typing (VNTR) typing, and distribution of virulence genes.
Methods: A total of 106 MRSA isolates from 18 hospitals in Thailand under an Antibiotic Resistance Surveillance Program, the National Institute of Health, Ministry of Public Health, were characterized by SCCmec typing, VNTR typing in spa gene and hypervariable region downstream of mecA gene, and detection of virulence genes such as sak, sea, seb, sec, sed, see, seg, sei, seh, lukSF-PV, and tst-1 by polymerase chain reaction.
Results: Five SCCmec types were identified as type-III (41.5%), type-IIIA (40.6%), type-III DCS (14.1%), type-II (1.9%), and type-I with class C mec complex (1.9%), whereas spa and HVR typing could distinguish MRSA into 5 and 12 groups, respectively. Eighty (75%), and 61 (58%) isolates carried staphylokinase (sak) and staphylococcal enterotoxin A (sea) gene, respectively. The major type of MRSA isolates (20.8%) were type III SCCmec with estimated 15 direct repeat units (DRUs) in HVR and 7 DRUs in spa gene, virulence genes of sea and sak. In addition, high virulent leukocidin, lukSF-PV, was also detected in three MRSA carrying type III, type III DCS and type II SCCmec. Combination of all genetic markers could distinguish 106 MRSA isolates into 35 groups.
Conclusion: Genetic diversity of MRSA in Thailand was demonstrated by SCCmec, VNTR, and virulence determinants. The major clone might be identified by these typing techniques. The genetic information may be useful for development of appropriate typing methods for the tertiary hospitals in our country.

P1064 Molecular epidemiology of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* invasive isolates in Russia

O. Dmitrenko, N. Lavrova, I. Alexandrova, S. Ghilina, V. Karabak, S. Rosanova, A. Gintsburg (Moscow, RU)*

Objectives: During investigation conducted in 2002–2006 we discovered circulation of several epidemic MRSA strains in hospitals in Russia. Two of them REMRSA-1(spa-037, SCCmec type III) and REMRSA-2 (spa-008, composite SCCmec: comlex mecB+, ccr1+, ccr2+) were discovered in different parts of the country and REMRSA-3 (spa-030, SCCmecIII) was identified only in one hospital of the Central Region. In the last few years the MRSA proportion increased dramatically especially among ICU patients. The increase incidents of invasive staphylococcal infections were observed two. The aim of the present study was to identify the MRSA genotypes currently circulating in the healthcare settings and to compare genotype and superantigen profiles of invasive MRSA and MSSA isolates.
Methods: 115 *Staphylococcus aureus* isolates were collected in 2005–2009 in 9 hospitals of Central, Ural and North-West region of Russia from blood (50), spinal fluid (4), low respiratory tract (61). Antimicrobial susceptibility was determined by standard methods. Genetic background was investigated by coa and spa typing. Structural components of SSCmec, sea, seb, sec, seh, seg, tst, pvl were tested by PCR additionally.
Results: 74 (64.3%) isolates were methicillin-resistant (MRSA). Among 30 MRSA recovered from blood and spinal fluid 14 (46.6%) were REMRSA-2 and 16 (53.4%) were REMRSA-3. Among 44 low respiratory tract isolates 42 (95.4%) were REMRSA-3, and 2 (4.6%)

were REMRSA-2. REMRSA-3 was detected in 8 of the 9 participating hospitals. MRSA isolates recovered in different hospitals differed by toxin genes profiles. 96.6% isolates carried sea. One isolate of REMRSA-2 (6.3%) was sea+pvl+. Among REMRSA-3 17.2% isolates carried sea,sec; 10.3% isolates carried sea,sec,tst. Isolates of REMRSA-1 were not discovered. In methicillin-sensitive *S. aureus* (MSSA) isolates recovered from blood (24) and respiratory tract (9) spa-types 002, 015, 078, 156, 159, 164, 284, 359, 647, 737, 841, 3665 were discovered. 16.6% of MSSA isolates carried tst or pvl.
Conclusion: Progressive increase of the proportion of the MRSA isolates in hospitals in Russia is a result of dissemination REMRSA-3 (spa-type t030, SCCmec III). This strain was able to accumulate the toxin genes successfully and replaced REMRSA-1 (spa t037, SCCmecIII). It became the predominant strain as a causative agent of staphylococcal ventilator-associated pneumonia. MSSA invasive isolates differed from MRSA by genetic background and toxin genes profiles.

P1065 Trends in the occurrence of MRSA strains in Upper Austria in a 4-year time period

K. Krziwanek, S. Metz-Gercek, H. Mittermayer on behalf of the MRSA Registry Upper Austria*

Objectives: The project "MRSA-Registry Upper Austria" exists since 2006 and aims to systematically collect and analyse data of all human MRSA-isolates which are detected in Upper Austria. All 9 Upper Austrian laboratories and 22 Upper Austrian hospitals take part in this project. In this study we investigated the trends in the occurrence of MRSA types and subtypes in Upper Austria within a time period of 4 years.
Methods: From January 2006 until October 2009 we received all consecutively collected primary clinical MRSA-isolates. All isolates were cultured and investigated for the presence of mecA/femA as well as for the PVL genes lukS-lukF via PCR. All isolates were typed using PFGE, the bigger part of the isolates were additionally investigated by spa-typing and MLST (multi locus sequence typing).
Results: We received 1,436 human primary MRSA isolates. The isolate number was slightly decreasing over the years (2006 n = 461, 2007 n = 374, 2008 n = 385, Jan-Oct 2009 n = 216). 29 different MRSA types were detected using PFGE as well as MLST. Both techniques showed that the 4 most frequent strains comprised 83% of all isolates: ST8 34%, ST5 32%, ST22 11% and ST228 6%. Within these MRSA types we recognised predominant PFGE-subtypes. Interestingly, these PFGE- and MLST-types as well as the predominant subtypes remained similar frequent throughout the 4 years. Investigating about 17% of all isolates by spa typing, we detected 61 different spa-types. Also, the main spa-types – t008, t190 (mostly CC8/ST8), t002, t003 (mostly CC5/ST5), t001, t041 (mostly CC5/ST228) – remained the dominant types throughout the described time period. The proportion of PVL-positive isolates, especially those belonging to ST8, increased since 2007 (2006 8.0%, 2007 5.6%, 2008 6.2%, 2009 11.1%). Most of the PVL-positive isolates belonged to ST8 (n = 21, 15 isolates thereof were USA300), ST152 (n = 21), ST5 (n = 18), ST777 (n = 11) and ST88 (n = 10).
Conclusions: The MRSA population in Upper Austria is not as dynamic as probably expected. No matter which typing method was used, the predominant MRSA strains as well as subtypes remained to be the most frequent ones throughout the last 4 years and were not replaced by minor clones. The increase of PVL-positive isolates since 2007 is partly a consequence of the occurrence of USA300 in Austria. Our data suggest to continue MRSA surveillance with an additional focus on community associated MRSA.

P1066 MRSA in north-eastern Scotland 2003–2007: evolving strain distribution and resistance patterns

A. Hunt, B. Edwards, K. Girvan, B. Cosgrove, G. Edwards, I. Gould (Aberdeen, Glasgow, UK)*

Objectives: This study explored strain distribution and resistance patterns of MRSA over a 5 year period in North East Scotland (NES).
Methods: MRSA isolates used in this study were obtained from hospital and GP specimens submitted to the Medical Microbiology Department, Aberdeen Royal Infirmary (ARI) during the period 2003–2007. During this time, all new isolates were submitted to the Scottish MRSA Reference Laboratory (SMRL), for typing by PFGE, extended antibiotic resistance testing, and in '07 for toxin gene testing.
Results: 7558 isolates were analysed. Over the five years, the number of MRSA isolates sent to the SMRL increased from 985 isolates in '03 to 1860 isolates in '07. 85–90% of the isolates each year were of either the two UK epidemic strains; E15 and E16. Unlike the rest of the UK, in NES, E16 has previously been present at a higher rate in comparison to E15. Over the course of '03–'07 there has been a shift in the relative rates of E15 and E16, with an increase in E15 from 51% of isolates in '03 to 82% in '07, and a corresponding decrease in the percentage of E16 isolates, bringing the pattern in line with the rest of the UK. An increase in the community acquired (CA) strains SM106 (equivalent to ST5) and SM153 (ST8 (US300)) has also been seen. In '07 eta was detected in 25% of SM106 and lukF/lukS were detected in 95% of SM153.
During '03–'07, the percentage of isolates resistant to trimethoprim and fusidic acid increased from 7.8% and 4.4% in '03 to 71.6% and 10.6% in '07 respectively. During the same time, the percentage resistance to clindamycin, kanamycin, novobiocin and tobramycin (cl/ka/no/to) decreased from around 43% to around 10%. The decrease in cl/ka/no/to resistance seems to be linked to the decrease of E16. However, E16 composes a decreasing proportion of the isolates resistant to cl/ka/no/to (from >90% in '03 to 40% in '07), showing that resistance to these agents is increasing elsewhere. SM111 (ST45) appears to be the main other strain in which this resistance pattern occurs. Although some of these trends can be linked to changes in strain distribution, from 2003 ARI had a hospital policy of eradicating throat carriage of MRSA with the oral agents trimethoprim and fusidic acid. This may have contributed to resistance.
Conclusion: The incidence of MRSA continues to increase despite the implementation of various control measures, especially CA strains. Strain distribution and antibiotic resistance patterns are also evolving.

P1067 The changing epidemiology of MRSA in Scotland

J. Wilson, A. Eastaway, G. Edwards, B. Cosgrove, C. Peter (Glasgow, UK)*

Objectives: Scotland has a mandatory reporting scheme whereby all first MRSA bacteraemia isolates are referred to the Scottish MRSA Reference Laboratory for typing and antibiotic susceptibility testing. Bacteraemia specimens are used as a proxy indicator of the level of MRSA infection in Scottish hospitals. Little is known about the characteristics of non-bacteraemia isolates at a national level. This study was designed to look at MRSA isolated from all specimen types and will monitor trends in strains, emerging antimicrobial resistance and toxin genes. The changing epidemiology of MRSA is a matter of Public Health concern and this study aims to provide epidemiological information on both hospital and community associated strains for Scotland.
Methods: Scottish diagnostic laboratories were asked to send all "new" MRSA isolates collected over a period of one week, 4 times a year to the Scottish MRSA Reference Laboratory for typing and toxin testing. A "new" isolate is defined as: "one which the diagnostic laboratory has no record of being isolated from that patient in the last twelve months". Epidemiological information was provided with each isolate. All isolates were phenotypically typed using biotype and antibiogram. Genotyping was performed using PCR-ribotyping and PFGE. Isolates were tested for resistance to 22 antibiotics. Those with reduced susceptibility to

mupirocin were further tested by PCR for the presence of the mupA gene. Toxin testing was performed: toxic shock syndrome toxin-1 (tst), two exfoliative toxins (eta and etb) and the PVL gene was tested for. Sequenced based typing (spa typing) was performed on all isolates. Surveillance period: Oct 2008–Mar 2009.
Results: Two hundred and seventy seven isolates were identified as EMRSA-15, 54 were EMRSA-16 and 26 isolates were characterised as "other". A high proportion of the EMRSA-15 and EMRSA-16 were resistant to ciprofloxacin, with slightly fewer resistant to erythromycin as is typical for these clones. Eight isolates had the PVL gene and one of these appears to be a true community associated MRSA strain. The majority of isolates were spa type t032.
Conclusion: This "snapshot" programme has provided additional information which has been used to augment the intelligence we already have from our mandatory *S. aureus* bacteraemia reporting scheme. This has provided us with a very clear picture of the epidemiology of *S. aureus* in Scotland including information on community and hospital acquired strains.

S. aureus susceptibility and GISA

P1068 Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates collected in 2008 at a Detroit medical centre

H. Salimnia, C. Vidaillac, K. Rossi, K. Kaye, M. Rybak (Detroit, US)*

Background: With a rising rate of *Staphylococcus aureus* infections in the health-care settings, prevalence and spread of methicillin-resistance *S. aureus* (MRSA) is a major clinical concern. We performed a surveillance of 5,533 *S. aureus* isolates collected at the DMC between January and December 2008.
Methods: Minimum Inhibitory Concentration (MIC) values of vancomycin (VAN), daptomycin (DAP), oxacillin (OXA), linezolid (LNZ), clindamycin (CLI), trimethoprim-sulfamethoxazole (SXT) and tetracycline (TCY) were determined by MicroScan PC29 panels for 2,309 clinical isolates of MSSA and 3,224 clinical strains of MRSA, defned on the basis of OXA MIC. Typing of 13 VISA MRSA isolates identifed by MicroScan and 1 hVISA confirmed by modified population analysis profile was performed by Pulse Field Gel Electrophoresis. SCCmec type, presence of the PVL gene and agr group were assessed by multiplex PCR reactions.
Results: MIC50/MIC90 values and non-susceptibility (NS) rates are reported in Table 1. No difference was observed between the MIC distributions with regard to the specimen sources and the susceptibility patterns of MSSA and MRSA collections (except for CLI). Antimicrobial NS rates were less than 1% for all tested antibiotics, except for CLI (5.5 and 27.4% for MSSA and MRSA isolates, respectively). MIC50/MIC90 values of the 14 hVISA/VISA group of isolates were 4/8 and 2/2 mg/L for VAN and DAP, respectively. USA 300 typing was positive for 57% of the VISA isolates, and 50% were PVL positive. Of interest, 67% of MRSA isolates exhibited a VAN MIC at 2 mg/L.
Conclusion: Of interet, a high percentage of MRSA had a VAN MIC at 2 mg/L and more than 50% of VISA isolates were USA 300. This surveillance of *S. aureus* isolates performed at Detroit Medical Center demonstrated low NS rates for VAN, DAP, and SXT.

Table 1. *In vitro* activity and resistance frequency for 7 selected antimicrobial agents against clinical MSSA and MRSA isolates

Agent	MSSA (n = 2309)			MRSA (n = 3224)		
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Resistance frequency (% of isolates)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Resistance frequency (% of isolates)
VAN	2	2	0.13	2	2	0.43
DAP	⩽0.25	1	0.26	⩽0.25	1	0.37
LNZ	2	4	0.04	2	4	0.03
SXT	⩽0.25	⩽0.25	0	⩽ 0.25	⩽0.25	0.37
CLI	⩽025	⩽0.25	5.50	⩽ 0.25	>4	27.39
TCY	⩽2	⩽2	9.00	⩽2	⩽2	8.41

P1069 Increasing vancomycin and daptomycin minimum inhibitory concentrations in methicillin-resistant *Staphylococcus aureus* blood isolates from a Canadian inner-city hospital

T. Wong, S. Champagne, M. Romney (Vancouver, CA)*

Objectives: To describe vancomycin and daptomycin minimum inhibitory concentration (MIC) trends in methicillin-resistant *Staphylococcus aureus* (MRSA) blood isolates at a Canadian tertiary care hospital over a three year period, specifically to assess whether "MIC creep" is present at this institution.
Methods: From July 2006 to June 2009, vancomycin and daptomycin MICs were determined via Etest for 200 consecutive MRSA blood isolates at St. Paul's Hospital, a tertiary-care, inner-city hospital in Vancouver, Canada. MIC determinations were read by a single observer and categorised as susceptible, intermediate, or resistant according to Clinical Laboratory Standards Institute (CLSI) breakpoints. Isolates were tested according to manufacturer's instructions. Only the first isolate for each patient was included. For statistical analysis, actual Etest MIC values were used instead of using traditional 2-fold MIC values. MIC50, MIC90, geometric mean, percentage susceptible and percentage above baseline median MIC were calculated according to 12 month reporting periods.
Results: Over the entire study period, all MRSA isolates were found to be susceptible to both vancomycin (MIC50/MIC90: 1.00/1.50 ug/mL) and daptomycin (MIC50/MIC90: 0.19/0.38 ug/mL). No vancomycin-intermediate or resistant MRSA isolates were detected. No daptomycin resistant isolates were detected. Geometric mean MIC for vancomycin increased from 0.91 ug/mL to 1.17 ug/mL during the study period. Geometric mean MIC for daptomycin also increased from 0.14 ug/mL to 0.29 ug/mL. There was a statistically significant increase in the percentage of isolates that were above the median MIC for both vancomycin (10.3% to 46.7%, $p < 0.0001$) and daptomycin (42.1% to 95.6%, $p < 0.0001$) compared to the MIC for the baseline study period.
Conclusions: Vancomycin and daptomycin MICs for MRSA increased over a period of three years at this institution. Despite the increase in MIC, all isolates remained under the CLSI susceptibility cutoff. Further studies to determine the clinical impact of this phenomenon are currently underway.

Proportion of MRSA isolates above baseline median MIC (n = 200)

	Baseline MIC (μg/mL)	Isolates above baseline MIC (%)			
		Period 1[1]	Period 2[2]	Period 3[3]	P value
Vancomycin	1.000	10.3	39.6	46.7	<0.0001
Daptomycin	0.125	42.1	68.8	95.6	<0.0001

[1]Period 1 = June 2006 – July 2007; [2]Period 2 = June 2007 – July 2008; [3]Period 3 = June 2008 – July 2009.

P1070 Effect of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* bloodstream isolates from a single centre between 1998 and 2009 on risk of complications following catheter-related bloodstream infection

C.E. Miller, R. Batra, A.K. Patel, N. Dawood, G. French, J. Edgeworth (London, UK)*

Objectives: A gradual increase in vancomycin (VAN) MIC of susceptible methicillin-resistant *Staphylococcus aureus* (MRSA) isolates over the past 5 years has been noted in some studies. Over the same period VAN breakpoints have fallen (4 to 2 mg/L) and concerns have arisen that infections with isolates near the breakpoint (1–2 mg/L) may fail therapy. On a background of declining institutional MRSA bloodstream infection rates, we assessed changes in VAN MICs of MRSA bloodstream isolates and whether higher MICs were associated with increased risk of deep-seated infection following MRSA catheter-related bloodstream infection (CRBSI).

Methods: VAN MIC (mg/L) of 886 clinically significant single-patient MRSA bloodstream isolates (Jan 1998–May 2009) were assessed by Etest over 3 months. Clinical data were available for 2002–2009. Complicated MRSA CRBSI was defined as development of deep-seated infection (endocarditis or osteomyelitis/septic arthritis) within 6 weeks. Individual serum VAN levels and hospital glycopeptide defined daily doses (DDDs) were obtained from computerised records. Hospital guidelines advised VAN therapy for all MRSA CRBSIs. Non-parametric and linear regression methods were used to assess trends/clinical outcomes.
Results: MRSA bacteraemias decreased by >75% over the 10 year period. All isolates had a VAN MIC$\leqslant$2. The proportion of isolates with VAN MIC$\geqslant$1.5 increased from 5% (8/153) in 1998/1999 to 42% (10/24) in 2008/2009 ($p < 0.0001$). The modal MIC increased from 0.5 in 1998/1999 to 1.5 in 2008/2009. Hospital glycopeptide DDDs doubled over the same period (6380 to 14190, $p = 0.0009$). 234/425 episodes (2002–2009) were CRBSIs. The occurrence of complicated CRBSIs for isolates with MIC $\geqslant$ 1.5(4/60) vs MIC < 1.5 (4/174) approached statistical significance ($p = 0.060$). There was no difference in persistent positive blood cultures at 3–7 or 8–42 days ($p = 0.244$ and $p = 0.365$) between MIC groups. Serum VAN levels were available for 170 (73%) CRBSIs; no difference in percentage of patients with a level <10 mg/l at any time $\leqslant$14 days between $\geqslant$1.5 vs <1.5 MIC groups was observed ($p = 0.551$).
Conclusion: VAN MIC of MRSA bloodstream isolates has increased over 10 years during which time MRSA BSI rates have decreased and glycopeptide use has increased. MRSA CRBSIs with a VAN MIC $\geqslant$1.5 are associated with a trend towards increased risk of metastatic complications.

P1071 Susceptibility and molecular characteristics of *Staphylococcus aureus* isolates associated with glycopeptide treatment failures

E. Mantengoli, C. Tascini, E. Tagliaferri, A. Leonildi, F. Menichetti, G.M. Rossolini (Siena, Pisa, IT)*

Objective: Emergence of *S. aureus* with reduced susceptibility to glycopeptides (VISA and hVISA) is known to be associated with glycopeptide treatment failures.
Methods: All *S. aureus* isolates associated to teicoplanin treatment failures or showing decreased teicoplanin susceptibility, isolated in 2008 in Pisa Cisanello Hospital (Italy) were studied. MICs of vancomycin (VA), teicoplanin (TC), linezolid (LZ), daptomycin (DPT) and tigecycline (TGC) were determined by CLSI broth and agar dilution methods or Etest. Population analysis profiling (PAP), determination of staphylococcal cassette chromosome mec (SCCmec) type, accessory gene regulator (agr) group and presence of Panton-Valentine leukocidin (PVL) were investigated. Genetic relatedness was analysed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
Results: PAP revealed a behavior typical of hVISA and VISA for 2 and 11 isolates, respectively, from diabetic foot infections (n = 3), SSTI (n = 3), peritonitis (n = 2), osteomyelitis (n = 2), sepsis (n = 1), pneumonia and pleural infection (n = 1). Ten isolates are methicillin resistant and three are susceptible. Ten patients failed treatment with TC (mean daily dose of 466±100 mg/die for a median time of 42±36 days). Using Etest isolates showed VA MIC of 2–4 μg/ml (median 3 μg/ml) and TC MIC of 2–6 μg/ml (median 2 μg/ml). All isolates were susceptible to LZ, 12 to TGC and only two to DPT. TC and VA were bactericidal (MBC/MIC < 16) against only two isolates and DPT against 4. Molecular typing of VISA and hVISA revealed: SCCmec cassette diversity [type I (4), type II (1), type III (1), type IV (3) and untypable (4)]; 9 and 4 isolates belonged to agr group I and II, respectively; 3 isolates were PVL-positive; all isolates were clonally unrelated excepted for two PVL-positive isolates. MLST confirmed clonal diversity. Infections were successfully treated with oxacillin, linezolid or tigecycline.
Conclusion: Spreading of unrelated VISA and hVISA isolates were observed in Pisa Cisanello Hospital in 2008. This phenomenon was probably related to the use of teicoplanin for long time in this setting. Daptomycin, as described, has a reduced activity and alternative treatments should be considered.

P1072 Methicillin-resistant *Staphylococcus aureus* with intermediate susceptibility to vancomycin: are strict additional contact measures sufficient?

I. Nahimana Tessemo, C. Bellini, J.M. Entenza, G. Zanetti, D.S. Blanc (Lausanne, CH)*

Background: A hospitalised patient infected with MRSA was found to harbour a VISA strain after 6 weeks of treatment with vancomycin. Additional contact measures were reinforced according to CDCs recommendations. We decide to evaluate if these applied control measures were effective.
Objective: To evaluate the efficacy of strict additional contact measures to contain the dissemination of VISA from an infected patient.
Methods: All patients from the unit were screened weekly for MRSA during a 6-week period, whereas health care workers (HCW) were screened only once. Screening specimen included nose, throat, groin, and clinical specimens for patients, and only nose and throat for HCW. Broth enrichment and chromogenic agar (MRSA-select) were used for MRSA detection. All MRSA isolates were tested on Van screen plates, and growing colonies were tested for MIC of vancomycin. MIC was performed using Etest. Population analysis was done for VISA confirmation. One strain per person was typed by Double Locus Sequence Typing (based on clfB and spa sequencing).
Results: 66 patients hospitalized in the same service during the 6 weeks and 55 HCW were screened for MRSA and VISA. MRSA was found in 16/66 (24%) patients and 1/55 (2%) HCW. 16/17 MRSA from patients belonged to the same genotype that the VISA strain. The remaining patient had a MRSA identical to the HCW isolate.
Among the 16 MRSA isolates sharing the same genotype than the VISA strain, two showed Etests vancomycin MIC of only 4 mg/L. MIC results were confirmed by the population analysis. They were not considered as VISA, but as MRSA with increased vancomycin MICs. Both isolates were obtained from two roommates.
Conclusion: Strict additional contact measures were found to be effective to contain VISA dissemination. However, the identification of two isolates with increased vancomycin MIC (4 mg/L) in two roommates raised the question of the need to routinely test this susceptibility and of adequate control measures for patients harbouring such isolates.

P1073 Methicillin-resistance and vancomycin heteroresistance in *Staphylococcus aureus* in cystic fibrosis patients

V. Cafiso, T. Bertuccio, D. Spina, F. Campanile, D. Bongiorno, M. Santagati, A. Sciacca, C. Sciuto, S. Stefani (Catania, IT)*

Objectives: Methicilllin-resistant *Staphylococcus aureus* (MRSA) infections are increasingly reported in cystic fibrosis (CF) populations worldwide. We sought to look at the epidemiology, the molecular characterization and the antibiotic resistance of MRSA isolates in our cohort of CF patients. All MRSA were collected prospectively at the University Hospital of Catania (I) in a two-year study between mid 2005 and mid 2007 and underwent molecular, pathotype and susceptibility characterizations.
Methods: We investigated the epidemiology, the molecular characterization of the strains by MLST, SCCmec typing and PFGE, the pathotype determination the virulence gene content including lukS/F-PVL by Multiplex-PCR, the biofilm production by spectrophotometric assays and the antibiotic-resistance of LEV, TOB, VA, TEC, LNZ, DAP, TYG by MIC according to the CLSI guidelines, and heteroresistance was determined by macroEtest (AB-Biodisk SE) and confirmed by population analysis (PAP).
Results: Our study demonstrates persisting infections with both HA- and CA-MRSA including PVL-positive strains in our CF population with an overall prevalence of 7.8%. We demonstrated that in these patients,

persistence was sustained by either identical clones that underwent subtle changes in their toxin content or by different clones over time. The isolation of MRSA in our CF population aged 7–24, was associated with an increased severity of the disease even if, due to the small sample of patients included and the paucity of data on the clinical outcome, these results cannot be conclusive. Furthermore, 35.7% of strains were hVISA, questioning the use of glycopeptides in the treatment of MRSA infections also in these patients.

Conclusion: We report cases of persistent infections caused by HA- and CA-MRSA including PVL-positive and hVISA isolates in our CF population and their possible correlation with an increased severity of the disease.

P1074 Increased rifampicin resistance among blood isolates of methicillin-resistant *Staphylococcus aureus* among patients who received current anti-tuberculosis treatment

M.S. Hsu, Y.T. Huang, P.R. Hsueh (Panchiao, Taipei City, TW)*

Objectives: The purpose of this study is to compare the rifampin resistance rate of methicillin-resistant *Staphylococcus aureus* (MRSA) recovered from patients with MRSA bacteremia that have or have not been exposed to rifampicin-containing anti-tuberculosis (TB) treatment.
Methods: Patients with MRSA bacteremia were selected from a database from year 2000 to 2008 in a 2000-bed tertiary hospital, and compared with a TB database to identify patients with previously confirmed pulmonary tuberculosis. Patients matched with sex, age and time of culture of the study group but without previously TB were selected in a 2:1 ratio as control group.

Table 1. MRSA bacteremia with or without previous anti-TB exposure

	With previous Anti-TB exposure (n = 49)	Without previous Anti-TB exposure (n = 90)	p
Age	69.6±15.5	70.8±14.5	0.641
Sex, Male (%)	36 (72.5)	65 (72.2)	0.875
Mortality %	**59.2**	**41.1**	**0.041**
Source of patients			0.948
Intensive care unit	16 (32.7)	27 (30.0)	
Isolation ward	1 (2.0)	2 (2.2)	
Ordinary ward	32 (65.3)	61 (67.8)	
Appropriate Antibiotics	39/49 (79.6)	74/90 (82.2)	0.704
MIC_{50} for rifampin, median (range)	**128 (0.02–256)**	**0.015 (0.02–256)**	**<0.001**
Drug resistant			
Rifampin	**29/49 (59.2)**	**15/90 (16.7)**	**<0.001**
Clindamycin	41/49 (83.7)	82/89 (92.1)	0.126
Erythromycin	38/41 (92.7)	76/80 (95.0)	0.605
Gentamicin	33/41 (68.8)	66/89 (76.4)	0.331
Minocycline	37/48 (77.1)	74/90 (82.2)	0.469
Trimethoprim–sulfamethoxazole	17/26 (65.4)	37/44 (84.1)	0.072
Underlying disease			
End stage renal disease	14/49 (28.6)	27/90 (30.0)	0.860
Diabetes	14/49 (28.6)	33/90 (36.7)	0.335
Liver cirrhosis	6/49 (12.2)	9/90 (10.0)	0.684
Steroid	7/49 (14.3)	5/90 (5.6)	0.113
Transplantation	1/49 (2.0)	3/90 (3.3)	1.000
HIV infection	1/49 (2.0)	0/90 (0)	0.353
Solid cancer	18/49 (36.7)	21/90 (23.3)	0.093
Hematological cancer	4/49 (8.2)	2/90 (2.2)	0.185
Site of Infection			
Pneumonia	28/49 (57.1)	51/90 (56.7)	0.957
Catheter	19/49 (38.8)	23/90 (25.6)	0.105
Soft tissue	15/49 (30.9)	28/90 (31.1)	0.952
Urinary tract infection	5/49 (10.2)	12/90 (13.3)	0.591
Arthritis	2/49 (4.1)	0/90 (0)	0.123
Central nervous system	0/49 (0)	3/90 (3.3)	0.552
Intraabdominal infection	0/49 (0)	2/90 (2.2)	0.540
Pericardial	0/49 (0)	1/90 (1.1)	1.000
Primary	6/49 (12.2)	20/90 (22.2)	0.150

Results: The data of 139 patients with MRSA bacteremia (49 patients with previous rifampin exposure and 90 patients without previous rifampin exposure) were analyzed. The rifampin-resistant rate is significantly higher in patient with rifampin exposure (59.2% vs 16.7%, $p < 0.001$). The MIC50 of rifampin against MRSA isolates from the study group were also significantly higher (128 vs 0.015, $p < 0.001$). The mortality rate was significantly higher in the study group (59.2% vs 41.1%, $p = 0.041$) (Table 1). Further analysis showed that MRSA isolated from the patients with current usage of anti-TB were more likely to be resistant to rifampin (83.3% vs 36%, $p = 0.001$) with higher MIC50 (256 vs 1, $p = 0.002$) and cause higher mortality rate than patients with remote usage of anti-TB (79.2% vs 40%, $p = 0.005$) (Table 2). Multivariate analysis showed that current anti-TB usage is the only risk factor for rifampicin resistance (OR 7.457, 95% CI 1.581–35.167) and mortality (OR 6.841, 95% CI 1.515–30.901) (Table 3).
Conclusions: Given the high rifampin resistant rate in patient with prior anti-TB treatment, rifampin susceptibility test should be done before considering combination treatment of rifampin in MRSA infection. The higher mortality rate in patient with current rifampin usage in MRSA bacteremia needs further investigation.

Table 2. MRSA bacteremia with current or remote Anti-TB exposure

	Current Anti-TB exposure (n = 24)	Remote Anti-TB exposure (n = 25)	p
Age	69.6±16.4	69.7±14.8	0.981
Sex Male (%)	14 (58.3)	22 (88.0)	0.875
Drug resistant			
Rifampin	**20/24 (83.3)**	**9/25 (36.0)**	**0.001**
Clindamycin	20/24/ (83.3)	21/25/ (84.0)	0.950
Erythromycin	19/20/ (95.0)	19/21/ (90.5)	0.578
Gentamycin	17/23/ (73.9)	16/25/ (64.0)	0.459
Minocycline	19/23/ (82.6)	18/25/ (72.0)	0.382
Trimethoprim–sulfamethoxazole	11/14/ (78.6)	6/12/ (50.0)	0.127
MIC_{50} for Rifampin, median (range)	**256 (0.02–256)**	**1 (0.02–256)**	**0.002**
Appropriate Antibiotics	19/24 (79.2)0	20/25 (80.0)	1.000
Mortality %	**79.2**	**40.0**	**0.005**

Table 3. Risk factors for Rifampin resistant in patients with MRSA bacteremia

	p		Odds ratio
	Univariate analysis	Logistic regression	(95% Confidence Interval)
Current Anti-TB usage	**0.001**	**0.011**	**7.457 (1.581–35.167)**
Steroid usage	0.018	0.628	1.873 (0.148–23.780)
Catheter-related infection	0.023	0.243	2.410 (0.551–10.543)
End Stage renal disease	0.045	0.555	1.666 (0.313–8.858)

Diagnostics in virology

P1075 Performance of the new Vidas EBV assays versus immunofluorescence for the detection of EBV antibodies in routine diagnostics

C. Koidl, B. Schweighofer, S. Fett, M. Bozic, M. Hübner, E. Marth (Graz, AT)*

Objective: To investigate the performance of the new bioMérieux VIDAS® EBV VCA IgM, VCA/EA IgG and EBNA IgG assays on the VIDAS® instrument (all bioMérieux SA, Marcy-l'Etoile, France) that provide qualitative detection of EBV antibodies in a routine diagnostic laboratory.
Methods: A total of 165 clinical serum samples were enrolled in this study. All samples were tested with an IVD labelled immunofluorescence EBV assay referred to as standard method. In parallel all samples were tested with the new VIDAS® EBV enzyme linked fluorescent assays that enable routine diagnosis of infectious mononucleosis and establishing

of the stage of infection on the VIDAS® instrument. For all samples with discordant results in interpretation of the EBV serological status, the samples were retested with an IVD labelled recombinant EBV IgM and IgG immunoblot assay. For the finding of final interpretation (non infected, primary infection and past infection) of serological status, immunofluorescence and immunoblot results were harmonized. All assays were performed according to the manufacturer's package inserts in an International Standards Organization (ISO9001, 2000)-certified routine clinical laboratory.

Results: With the standard method and optional immunoblot testing 29 EBV non infected, 52 EBV primary infections and 84 EBV past infections were identified. With the new VIDAS EBV assays 29 EBV non infected, 45 EBV primary infections and 84 EBV past infections were identified. In 4 samples with primary infection the VCA IgM on the VIDAS® instrument was found to be equivocal.

Conclusion: In conclusion, the new VIDAS® EBV assay includes all features required for detection of EBV antibodies and interpretation of serological results. It is important to check equivocal results with testing of follow-up samples collected 1 to 2 weeks after first blood collection. The new Vidas® EBV assays proved to be suitable for the routine diagnostic laboratory allowing a rapid and safe diagnosis of the serological status.

P1076 Comparison of four commercial systems for the diagnosis of Epstein–Barr virus primary infections

F. De Ory, M.E. Guisasola, I.M. García-Bermejo (Majadahonda, Getafe, ES)*

Objectives: To compare the performance characteristics of four diagnostics systems to establish EBV seroprofiles for the diagnosis of EBV primary infections.

Material and Methods: A total of 125 samples from 125 infectious mononucleosis cases were studied. The cases were classified as EBV primary recent infection [presence of VCA-IgM and/or -IgG, in absence of EBNA antibodies] (82), EBV past infection [presence of VCA-IgG and EBNA antibodies, in absence of VCA-IgM] (26 cases), and not infected [negative for the three markers] (17 cases). These samples were used to compare the individual serological markers of each system, and to establish the EBV seroprofiles, as defined. The following systems were tested: Two ChemiLuminiscence ImmunoAssays (CLIA), (Liason, Diasorin, Italy [CLIA-L] and Immulite 2000, Siemens, Germany [CLIA-I]), Immunofiltration (IF) (All.Diag, France), and ELISA (Diasorin). Specific IgM-VCA synthetic peptide p18 (in both CLIA and ELISA) and against ZEBRA protein (in IF), specific IgG-VCA synthetic peptide p18 (in CLIA-L, IF and ELISA) or against gp125 (CLIA-I), and IgG-EBNA-1 synthetic peptide (IF, CLIA-L and ELISA) or against a recombinant p72 (CLIA-I) were tested. As reference methods were used indirect immunofluorescence (IIF) for VCA-IgM and -IgG and anticomplement immunofluorescence for EBNA antibodies (Meridian Bioscience, USA). To evaluate the crossreaction of EBV- and CMV-IgM, 17 CMV-IgM positive, low avidity IgG additional samples were assayed in all the EBV-IgM assays.

Results: In the IgM assays the sensitivity ranged from 74% (ELISA) to 96.1% (CLIA-L), and the specificity from 93.8% (CLIA-L) to 100% (IF). In VCA-IgG assays, the sensitivity varied from 86.9% (CLIA-L) to 96.3% (CLIA-I), and the specificity from 94.4% (IF and CLIA-L) to 100% (CLIA-I). In EBNA assays, the sensitivity ranged from 81.3% (IF) to 100% (CLIA-L), and the specificity from 32.3% (CLIA-L) to 91.4% (IF). In relation to EBV profiles, IF showed sensitivity of 92.7% and specificity of 90.7%. The corresponding figures for CLIA-L, CLIA-I and ELISA were 91.5% and 93%; 89% and 97.7%; and 89.6% and 95.2%. When CMV cases were assayed positive EBV IgM result was obtained in 9 (52.9%) (IIF), 13 (74.5%) (IF and CLIA-L), 7 (41.2%) (CLIA-I), and 3 (17.6%) (ELISA).

Conclusion: Although there were limitations in some individual markers, the systems evaluated seems to be useful for the diagnosis of EBV infections by the establishment of EBV seroprofiles.

	IIF	Immunofiltration				CLIA Liason				CLIA Immulite				ELISA			
	IgM-VCA	IgM-ZEBRA	IgG-VCA (p18)	EBNA-1	Classification of cases	IgM-VCA (p18)	IgG-VCA (p18)	EBNA-1	Classification of cases	IgM-VCA (p18)	IgG-gp125	EBNA (p72r)	Classification of cases	IgM-VCA (p18)	IgG-VCA (p18)	EBNA-1	Classification of cases
Agreement		93.6	93.6	88.8		95.2	88	49.6		88	96.8	84		82.6	92.5	90.1	
Sensitivity		89.6	93.5	81.3	92.7	96.1	86.9	100	91.5	81	96.3	96.9	89	74	92.1	90.6	89.6
Specificity		100	94.4	91.4	90.7	93.8	94.4	32.3	93	95.8	100	79.6	97.7	95.8	94.7	89.9	95.2
CMV cases (17); positives:	9	13				13				7				3			
% of positives	52.9	74.5				74.5				41.2				17.6			

P1077 Evaluation of VCA IgM and ZEBRA IgM assays in diagnosing acute status of EBV-infection

P. Singer, F. Vlaspolder (Alkmaar, NL)*

Objectives: For many years, the VCA IgM assay has been the most widely used, yet limited, tool to diagnose the acute status of EBV-infection. Recently, a ZEBRA IgM assay has been introduced as a commercially available tool for this purpose as well. A widespread need exists to come to a clear interpretation of the status of the patient using EBV-serology. The focus of this research is therefore to deliver a valuable contribution in the development of these conclusions.

Methods: To determine the performance of a VCA IgM assay (Siemens Immulite 2000®) and a ZEBRA IgM assay (Clindia Benelux), we made a selection from randomly selected sera (107 out of 629 sera) for diagnosing EBV-infection in June and July 2009, based on their serological screening results in Immulite 2000® EBNA-1 IgG, VCA IgG and VCA IgM assays (see table).

Mostly profiles representing a clear result of a non-active status were excluded.

The final conclusions were based also on:

a. EBV-IgG (avidity) blot results using Mikrogen recomLine® EBV blot
b. Bloodsmear findings for 68 sera
c. Clinical diagnosis was traced in some cases

Results: The final conclusions from this 107 sera were:

30 active infections
6 recent infections
48 past infections
23 cases a conclusion wasn't possible

From this 107 sera, 85 sera were positive and 5 intermediate in the VCA IgM assay and just 58 sera were positive and 18 intermediate in the ZEBRA IgM assay.

The VCA IgM assay was positive or intermediate in 33 cases of active or recent EBV infections, against 34 in the Zebra IgM assay. Sera showing a positive result (46) in all of the 3 screening assays were the most difficult group to interpret. They revealed a percentage of 7.6 in the whole sera population. The 3 sera in this group that had an active or recent infection, were positive in both assays. In past infections the VCA IgM was 43 times positive and 1 time intermediate. The ZEBRA IgM assay however just 11 resp. 12 times.

Conclusion: The study revealed no significant variance in sensitivity between the VCA IgM and the ZEBRA IgM assay in active or recent infections.

The VCA IgM assay, however delivered significantly more positive and intermediate results than the ZEBRA IgM assay in past infections, which make interpretation difficult. Based on these findings, it can be stated that the ZEBRA IgM assay delivers a much higher positive predictive value, resulting in more reliable conclusions.

EBNA IgG	VCA IgG	VCA IgM	ZEBRA IgM results			
			intermediate	negative	positive	Total
intermediate	positive	negative			1	1
intermediate	positive	positive			1	1
negative	intermediate	positive			1	1
negative	positive	intermediate	1	1	3	5
negative	positive	negative	5	6	5	16
negative	positive	positive	2	2	33	37
positive	positive	positive	10	22	14	46
		Total	18	31	58	107

P1078 Performance of the Access® CMV IgM assay

N. Groleau, B. Roussel, V. Potelle, M. Bullenger, J. Poropane, C. Chandelier, R. Falcou-Briatte, F. Bouniort, J. He, O. Flecheux (Marnes-la-Coquette, FR; Nyon, CH; Chaska, US)*

Objectives: An automated CMV IgM assay has been developed by Beckman Coulter. The purpose of this study was to evaluate the reproducibility, analytical specificity and the clinical sensitivity and specificity of the Access® CMV IgM assay.
Methods: Reproducibility including intra-assay, inter-assay and inter-lot precision were performed according to CLSI guidance EP5A using 2 negative, 2 low, a medium and a high positive sample for CMV IgM. Clinical sensitivity was tested with 2 commercial seroconversion panels and 222 samples from commercial panels, blood banks and hospitals including pregnant women. Analytical specificity was validated with 369 samples from patients infected with other viruses or concerned with abnormal autoimmune diseases. The clinical specificity was studied with 1729 samples from blood donors, hospitalized patients and pregnant women. The studies were performed on the Beckman Coulter Access system with the bioMérieux Vidas*CMV IgM used as the comparative method.
Results: The average intra-assay, inter-assay and inter-lot CV's were 4.9%, 7.2% and 5.0%, respectively. The Access CMV IgM assay detected the seroconversion one sample earlier than Vidas for one panel and simultaneously for the second panel. The clinical sensitivity was 96.0% (213 / 222). Among the 369 samples tested for cross-reaction, 361 were found negative yielding an analytical specificity of 97.8%. The clinical specificity for blood donors, hospitalized patients and pregnant women was 97. 7% (428/438), 95.6% (770/805) and 96.5% (447/463), respectively.
Conclusion: The Access CMV IgM assay demonstrated excellent performance and can be performed on the family of Access immunoassay systems (Access2, UniCel DxI 600/800) and including the integrated platforms with closed tube sampling (clinical chemistry / immunochemistry).
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P1079 Validation of the rapid fluorescent focus inhibition test for rabies antibodies performed on microplates

S. Stankov, G. Stojadinovic (Novi Sad, RS)*

Objective: Except one recent publication of the rapid fluorescent focus inhibition test for determination of potency of rabies immunoglobulin by de Moura and colleagues in 2008, no articles which specifically address validation of this test have so far been published. Therefore a thorough examination of characteristics of the test was undertaken for the purpose of establishing its utility for examination of sera from individuals that have been vaccinated against rabies.
Methods: The performance of the test was assessed with regard to its accuracy, specificity, analytical sensitivity (i.e. detection limit), intra-assay precision, inter-assay precision and linearity.
Results: Rapid fluorescent focus inhibition test for rabies antibodies showed satisfactory accuracy, 100% specificity, intra-test variability of 14%, inter-test variability of 21%, and linearity in the range up to about 60 international units per milliliter.
Conclusions: Results of the test were meaningful from the standpoint of adequate rabies prophylaxis, taking into account the minimal satisfactory antibody level of 0.5 international units per milliliter of the serum sample. In other words, this investigation showed that the test is suitable for assessment of the satisfactory antibody level upon pre-exposure or post-exposure rabies immunization.

P1080 Advantages of the state-of-the-art ELISA test at verification of an early HIV infection

*I. Sharipova, N. Denisova, E. Baranova, M. Susekina, V. Puzyrev, K. Sarkisyan, M. Vorob'eva, A. Obriadina, A. Burkov, T. Ulanova * (Nizhny Novgorod, Moskow, RU)*

Background: One of the problems of EIA diagnostic HIV infection associated with the reliable confirmation of the screening assays results. The basic difficulties are connected with confirmation screening result at early stage of seroconversion because immune blot assays (WB) are not intended for detection of presence p24 antigen – early serological a marker of an HIV infection. The other problem is "indeterminate" WB result. The aim of present study was to evaluate EIA test "DS–EIA–HIV–Ab/Ag–spectrum" as a supplemental assay for confirmation of the HIV positive results at early stage of seroconversion.
Materials: The "DS–EIA–HIV–Ab/Ag–spectrum" is an *in vitro* qualitative EIA for the detection of antibodies to individual proteins of HIV 1 (including HIV 1 group 0), HIV 2 and HIV-1 p24 antigen in human serum or plasma. Wells of microtiter plate are separately coated by recombinant proteins comprising diagnostic relevant epitopes of HIV 1 structural proteins gp41 and HIV-1 group 0 gp 41, gp120, p24, p31 and gp36 of HIV 2 and mouse monoclonal antibodies to HIV 1 p24. Sensitivity of the test was evaluated by 16 commercial available seroconversion panel (total n = 167) [ZeptoMetrix and BBI (USA)].
Results: EIA test "DS–EIA–HIV–Ab/Ag–spectrum" permits confirmation of earlier detection of HIV infection. This assay is able to confirm 100 out of 167 seroconversion specimens as HIV positive. 28 of them were positive for p24 antigen only. The delay of HIV infection detection by EIA test "DS–EIA–HIV–Ab/Ag–spectrum" in comparison with detection of HIV virus RNA is 1.2 days. WB can confirm only 13 samples out of 167 as HIV positive and 54 samples as indeterminate. The delay of HIV infection detection in comparison with detection of HIV virus RNA is 18–19 days.
Conclusion: The received results demonstrated high diagnostic efficiency of new supplemental assay. Opportunity of detection p24 antigen and high specificity allow to confirm screening results from early stages of HIV infection and reducing number indeterminate results received by WB.

P1081 Comparison of the Avidity Index method with two 4th generation HIV assays

V. Regine, M. Raimondo, A. Rodella, C. Galli, N. Manca, L. Camoni, M.C. Salfa, B. Suligoi (Rome, Brescia, IT)*

Objective: To compare the performance of the Avidity Index (AI) in indentifying recent HIV infections by using two 4th generation HIV assays which detect HIV antigen and antibody.
Methods: 102 serum samples were collected from 29 HIV-positive individuals for whom the date of seroconversion was estimated as the midpoint between last negative and first positive HIV antibody test. Specimens positive for the Ag and specimens collected when the individual was under ARV treatment were excluded from the analysis. All samples were assayed for AI with both the AxSYM HIV Ag/Ab Combo and the Architect HIV Ag/Ab Combo (Abbott).
The sensitivity and specificity of the two assays were evaluated using for both a cut-off of 0.80 ($AI \leqslant 0.80$ = recent infection, $\leqslant$6 months from seroconversion; $AI > 0.80$ = long-standing infection, >6 months from seroconversion). We calculated the correlation and the concordance rate between the two assays; for the latter, a grey zone around the 0.80 cut-off was included, thus classifying the specimens in three groups: recent infection $AI < 0.75$, grey zone $0.75 \leqslant AI \leqslant 0.84$, long-standing infection $AI > 0.84$.
Results: Based on estimated date of seroconversion, 68 specimens (66.7%) were collected in the 6 months after seroconversion and 34 (33.3%) more than 6 months after seroconversion.
Sensitivity and specificity for recent infection were: for AxSYM HIV Ag/Ab Combo 88.2% and 85.3% respectively, and for Architect HIV

Ag/Ab Combo 89.7% and 76.5% respectively. The overall accuracy for identifying or excluding recent infections was 87.2% for AxSYM and 85.3% for Architect. The regression coefficient between the two assays was 0.74 (95% CI 0.59–0.85).
Of the 102 serum samples, 84 gave concordant results with the two assays, with an overall concordance rate of 82.4%. When excluding 20 specimens, classified in the grey zone, the concordance rate was 95.1% (78/82) (Table 1).
Conclusions: The two assays showed a similar accuracy; Architect HIV Ag/Ab Combo was slightly more sensitive but less specific. About 10% of the specimens fell in the grey zone around the cut-off of 0.80: when these specimens were excluded, the two assays showed a very good concordance.

Table 1. Comparison of the AI results obtained with Architect HIV Ag/Ab Combo and AxSYM HIV Ag/Ab Combo

Architect Combo	AxSYM Combo			
	Recent inf.	Grey zone	Long-standing inf.	Total
Recent inf.	54	4	3	61
Grey zone	5	2	5	12
Long-standing inf.	1	4	24	29
Total	60	10	32	102

Note: recent infection: AI < 0.75; grey zone: AI 0.75–0.84; long-standing infection: AI > 0.84.

P1082 Study of sensitivity and specificity of ELISA test system MONOLISA HCV Ag-Ab ULTRA

S. Mukomolov, E. Barantsevich, A. Mukomolova, N. Barantsevich, E. Schlyakhto (St. Petersburg, RU)*

Testing the blood samples for hepatitis C antibodies (anti-HCV) is a compulsory procedure for donor's blood approbation. The third generation of test systems based on EIA and chemiluminescent analysis are currently used. It is known that anti-HCV are appearing in the blood in hepatitis C infection later than hepatitis C virus RNA. There by it is highly important to determine seroconversions and core viral antigen early. Test-system, that allows to determine anti-HCV and HCVcore Ag simultaneously, has recently appeared in the market.
Objectives: The present study was aimed to determine sensitivity and specificity of ELISA test-system MONOLISA HCV Ag-Ab ULTRA.
Methods: 33 samples, that showed positive or uncertain result in donor blood primary testing for anti-HCV, were tested repeatedly with:
– test systems MONOLISA HCV Ag-Ab ULTRA (Bio-Rad Laboratories, France)
– test-systems Best anti-HCV (Vector-Best, Russia)
– test-systems for determination of hepatitis C RNA by PCR (Interlabservice, Russia)
Results: After repeated testing of serum samples by test-system MONOLISA HCV Ag-Ab ULTRA 14 samples were positive (42.4%). In Best anti-HCV 11 samples showed to be positive (33.3%). 8 samples showed concordance. 6 samples proved to be positive only by evaluation with test, that determine both HCV core antigen and antibodies. That could indicate that MONOLISA HCV Ag-Ab ULTRA test is more sensitive. All 33 samples were tested by PCR for hepatitis C viral RNA presence, it was confirmed in 12 serums. In 6 samples, that were positive only in MONOLISA HCV Ag-Ab ULTRA testing, hepatitis C RNA was determined in 4 (67%).
Conclusion: In the selective sampling (only uncertain results for hepatitis C virus samples) more than 10% (4 out of 33) were proved to contain only viral antigen and were not determined by anti-HCV testing. Early detection of hepatitis C infection before seroconversion and anti-HCV appearance presents MONOLISA HCV Ag-Ab ULTRA as an invaluable instrument for blood banks protection.

P1083 The highly sensitive enzyme immunoassay for HBsAg detection as the alternative method to nucleic acid testing

N. Egorova, S. Igolkina, I. Pyrenkova, I. Sharipova, V. Puzyrev, A. Obriadina, A. Burkov, T. Ulanova (Nizhniy Novgorod, RU; Saronno, IT)*

Background: Enhancement of sensitivity of the currently available EIA assays is very important be-cause the early stage of HBV infection when HBsAg level is below detection limit of the best available EIA kits (0.05–0.1 IU/ml) is one of the main reasons of transfusion-associated hepatitis A. The aim of the study was to evaluate the advantage of highly sensitive assay DS-EIA-HBsAg-0.01 (0.01 IU/ml Second Interna-tional Standard for HBsAg subtype adw2, genotype A, NIBSC code number: 00/588) for detection of HBsAg during seroconversion period.
Methods: The correlation between HBsAg and HBV DNA presence in seronversion samples has been studied. Twenty eight commercial panels PHM – 911, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935A(M), 935B (Boston Biomedica Inc.) and HBV – 6271, 6273, 6275, 6277, 6278, 6279, 6281, 11001, 11002, 11003, 11006, 11008, 11011, 11012, 11017 (ZeptoMetrix Corp.) were used. The aggregate score was calculated by summing the total number of reactive specimens in the all panels detected by each test. The mean number of days delay in detection of HBsAg and HBV DNA were also calculated.
Results: DS-EIA-HBsAg-0.01 assay detected 203 samples as HBsAg positive out of 283 seroconversion samples. Only 181 seronversion samples out of 283 were detected as HBV DNA positive.
The detection of HBsAg and HBV DNA ranged 0–133 days with the means delay in detection of HBsAg of 21.85 days and detection of HBV DNA of 24.48 days. DS-EIA-HBsAg-0.01 detected HBsAg in the specimens of the PHM (926, 931, 933), HBV (6277, 6279, 11001, 11012, 11017) panels by one bleed earlier and in the specimens of the panels PHM925, HBV11002, PHM932, HBV6275 by two, two, four and five bleeds, accordingly, earlier than the initial detection of HBV DNA (100–400 copies/ml) occurred.
With the use of the DS-EIA-HBsAg-0.01 for evaluation the specimens of the PHM (927, 928, 929, 930, 934, 935A(M)), HBV (6271, 6273, 6281, 11003, 11006, 11008, 11011) panels, the moment of detection of HBsAg coincided in the time of initial detection of HBV DNA. Hence using more highly sensitive assay allows determining HBsAg simultaneously to HBV DNA or even earlier.
Conclusion: Increasing sensitivity EIA for HBsAg detection up to a level comparable to sensitivity of nucleic acid testing (NAT) allows to consider it as the possible alternative to other methods which will raise quality of screening of donor blood, will allow to reduce the risk of posttransfusional hepatitis B infection.

P1084 The evaluation of the ELISA kit "EIA-anti-HCV" with new recombinant antigens

G.B. Bochkova, V. Puzyrev, A. Obriadina, A.N. Burkov, T. Ulanova (Nizhniy Novgorod, RU)*

Introduction: High heterogeneity is typical for hepatitis C virus. The new generation enzyme immunoassay "EIA-ANTI-HCV" intended for the detection of antibodies to viral hepatitis C in human serum or plasma was developed. The recombinant antigens comprising only diagnostically relevant regions of different variants of native HCV proteins were specially selected.
Aim: The evaluation of the ELISA kit "EIA-anti-HCV".
Objectives and Methods: The various sequences of recombinant antigens comprising HCV Core, NS3, NS4, NS5 were adsorbed on the microtiter plate. Diagnostic value of the assay was studied by testing 1004 anti-HCV positive samples of patients with confirmed hepatitis C diagnosis, including 338 samples with determined genotype 1–6 (76 samples of genotype 1, 51 sample of genotype 2, 38 samples of genotype 3, 25 samples of genotype 4, 5 samples of genotype 5, 8 samples of genotype 6); samples of 31 commercial seroconversion panels (BBI Inc., ZeptoMetrix), samples of the "Anti-HCV Mixed Titer

Performance Panel BBI PHV 206" (BBI Inc.) Sera samples of patients with acute (n = 30) and chronic (n = 439) hepatitis C were studied for the clinical efficiency assessment.
Diagnostic specificity was studied by testing samples of healthy donor blood (n = 8107), clinical patients (n = 1225), pregnant women (n = 735), patients with hepatitis B (n = 600).
Results: Out of 259 samples from 31 tested seroconversion panels the kit "EIA-ANTI-HCV" detected 101 samples (40%) as positive. The kit detected 23 positive results and indicated 2 negative results according to data available from insert package of the panel "Anti-HCV Mixed Titer Performance Panel BBI PHV 206". It should be mentioned that the value OD/Cut-off of the most of positive results were higher than the passport date. The diagnostic sensitivity of the kit "EIA-ANTI-HCV" at testing anti-HCV positive samples with clinical diagnosis of acute and chronic hepatitis C was 100%. Sensitivity of the kit during testing the samples with different genotypes was 100%.
The study showed high specificity of the kit "EIA-ANTI-HCV".
Conclusion: The received results demonstrated high diagnostic efficiency of ELISA kit in the combination with high specificity.

P1085 Performance evaluation of the Access® HCV Ab Plus and the Access HIV-1/2. New assays performed on the UniCel® DxI 800 in a virology laboratory

C. Roussel, P. Zawadzki, E. Le Moal, N. Groleau, R. Falcou-Briatte, G. Duwerlie (Amiens, Marnes-la-Coquette, FR)*

Objective: Access HCV Ab Plus and Access HIV-1/2 New are *in vitro* diagnostic assays for the qualitative detection of anti-HCV and anti-HIV antibodies respectively in human serum or plasma. The aim of this study was to evaluate the performance of these two assays in terms of sensitivity, specificity and precision in our routine laboratory.
Methods: Access HCV Ab Plus and Access HIV-1/2 New assays were evaluated on UniCel DxI 800 immunoassay system and compared to Abbott AxSYM® anti-HCV and AxSYM® HIV 1/2 gO assays respectively. For the performance studies, we used unselected serum samples, positive samples, seroconversion panels and different quality controls.
Results:
- Access HCV Ab Plus assay performance: On 500 unselected routine serum samples, the global agreement between Access and AxSYM was equal to 97.8% (96.1–98.9%). The clinical specificity for the Access HCV Ab Plus assay was 99.0% (97.6–99.7%) versus 99.2% (97.9–99.8%) for the AxSYM anti-HCV assay (2 equivocal samples were excluded from the calculation). The clinical sensitivity was 100% and the seroconversions were detected earlier with the Access HCV Ab Plus assay.
- Access HIV-1/2 New assay performance: From unselected serum samples tested on both Access and AxSYM assays, 6 were positive and 540 negative. The agreement between Access and AxSYM assays was 100%. The relative specificity was 100% and the seroconversion sensitivity was equivalent.

Precision for both HCV and HIV assays was better on UniCel DxI 800 than on AxSYM.
Conclusion: Both Access HCV Ab Plus and Access HIV-1/2 New assays showed good performance and good concordance with the AxSYM assays.
The Access assays can be easily used on UniCel DxI 800 in a virology laboratory with high volume of HCV and HIV screening tests.

P1086 Utility of rapid influenza antigen detection test for novel influenza virus (H1N1)

J. Acosta, J.R. Otero, L. Folgueira (Madrid, ES)*

Objectives: RT-PCR is the method of choice for diagnosis of pandemic H1N1 virus in respiratory samples; however such test cannot be readily performed in primary health care settings. It makes necessary to establish if currently commercially available rapid influenza diagnostic tests (RIDTs) can detect novel influenza A (H1N1) virus. The aim of this study was to compare the Directigen EZ Flu A+B® immunoassay with the real-time polymerase chain reaction (RT-PCR) assay developed by the Centers for Disease Control and Prevention (CDC) to detect pandemic influenza A/H1N1 2009 virus.
Methods: 140 samples (54 nasal aspirates and 86 nasopharyngeal swabs) collected from paediatric patients were submitted to the clinical virology lab for rapid influenza viruses testing during June, July and August. All tests were performed according to the manufacturer's instructions. Testing for pandemic influenza H1N1 2009 virus was performed using the CDC developed RT-PCR assay.
Results: Of 140 specimens, 71 (50.7%) were positive and 69 (49.3%) were negative by RT-PCR. Of the 71 specimens that tested positive by RT-PCR, 50 tested positive by RIDT for a test sensitivity of 70.4% and a positive predictive value of 100%. Of the 69 specimens that tested negative by RT-PCR, 69 tested negative by RIDT for a test specificity of 100% and a negative predictive value of 76.6%. The overall agreement between the two assays was 119/140 (85%).
Conclusions: RIDTs have lower sensitivity than RT-PCR or viral culture in the detection of seasonal influenza viruses. Several reports have shown that RIDTs have low sensitivity in detection pandemic influenza A/H1N1 2009 virus. In contrast, our data indicate that the sensitivity of BD Directigen EZ Flu A+B® influenza antigen detection was comparable to that of seasonal influenza previously reported for this assay, in samples collected from paediatric population.

P1087 The usefulness of the direct immunofluorescence assay for influenza A (H1N1) virus

J.H. Lee, H. Shim, J.H. Jo (Iksan, KR)*

Objectives: The aim of this study is to evaluate the diagnostic accuracy of direct immunofluorescence assay(DFA) of the laboratory-confirmed cases of influenza A (H1N1).
Methods: From August 2009 to October 2009, of 2302 patients with symptoms influenza-like illness, 1390 patients were performed DFA. The specimens were obtained by the nasopharyngeal flocked swabs and DFA were conducted with D3 DFA Screening and the ID kit. (Diagnostic Hybrid Inc., Athens, OH, USA) A positive result was indicated by the presence of two or more intact cells exhibiting the specific fluorescence pattern. We evaluated the clinical usefulness of the DFA in detecting influenza A(H1N1).
Results: The sensitivity of DFA were 62.9%. The sensitivity were different in age groups, showing the highest(71.1%) in patients below age of 10 years. When we stratified the results according to the patient's body temperature and the elapsed time from the onset of influenza like illness, the sensitivities of DFA were higher in subjects with body temperature above 38ºC (69.5% vs 54.6%), and in subjects who were done the DFA within 48 hours after onset of the symptoms(60.2% vs 52.6%). During the study period, the overall incidence of influenza A(H1N1) was 39.3%(905/2302) by RT PCR method. The incidences of novel influenza(based on the laboratory confirmed cases) were increased as follows; 10.7%(13/121) in August, 12.3%(77/624) in September, 30.1%(58/193) in the first half of October, and 55.6%(758/1364) in the second half of October. However, the DFA positive rates were not influenced by the increase of the incidence.
Conclusion: DFA for influenza A (H1N1) virus had a positive rate of 62.9%. DFA would be more useful to patients who have a body temperature above 38ºC or young children below age of 10 years.

P1088 Quantitative HHV-6B antigenemia test for the monitoring of transplant patients

R. Loginov, T. Karlsson, K. Höckerstedt, D. Ablashi, I. Lautenschlager (Helsinki, FI; Santa Barbara, US)*

Objectives: Human herpesvirus-6 (HHV-6) infection, mostly caused by the variant B, is common after solid organ transplantation, as previously demonstrated by antigenemia test. Here we report a new modified method

using a HHV-6B glycoprotein IgG-antibody, OHV-3, and attempt to quantify the HHV-6 antigenemia after liver transplantation. Twenty-four adult liver transplant recipients were frequently monitored by the qualitative HHV-6 antigenemia test, which detects the HHV-6B virion protein in peripheral blood mononuclear cells (PBMC).
Methods: HHV-6B antigens were now demonstrated using a glycoprotein OHV-3 IgG-antibody in the immunoperoxidase staining from the same specimens and quantified as positive cells/10,000 PBMC. The results were confirmed and quantified by DNA-hybridization in situ using biotinylated oligoprobe. Altogether 206 blood specimens were analyzed.
Results: During the first six months, HHV-6 antigenemia was detected in 17/24 (71%) recipients by using HHV-6B virion antibody. All, except one patient with a faint positivity, demonstrated positive findings also with the OHV-3 antibody. Altogether, 37% (77/206) of specimens were positive with the virion antibody and 39% (78/201) by the OHV-3 antibody. The peak number of OHV-3 positive cells in the PBMC's varied from 5 to 750/10,000 (mean 140/10,000).
Conclusions: The OHV-3 antibody was useful to quantify the HHV-6B antigenemia. The findings of the HHV-6B quantitative antigenemia using the OHV-3 antibody correlated well with the previous qualitative HHV-6 antigenemia assay, and can be used as a quantitative method in the monitoring of HHV-6 in transplant patients.

P1089 Comparison of nasopharyngeal aspirates and nasopharyngeal flocked swab specimens for the detection of respiratory viruses by indirect immunofluorescent method, culture, and multiplex PCR

J.O. Kang, H. Pai, H. Sung, N.Y. Lee, C.K. Lee, H. Kim, E.C. Kim, K. Lee, C. Kang (Guri, Seoul, KR)*

Objectives: Nasopharyngeal aspirates (NPA) have been considered the gold standard for the respiratory virus samples, but the procedure is uncomfortable for the patients and unpleasant for the medical staffs. Copan Diagnostics Inc. (Brescia, Italy) recently marketed flocked swab kits and they have been reported to get respiratory specimens more comfortably, and to improve the sensitivity of various diagnostic tests. We compared the Copan nasopharyngeal flocked swab (NPFS) kits with the NPA, whether the kits could get better respiratory specimens or increase sensitivity by the three most commonly employed diagnostic methods for the detection of the respiratory viruses.
Methods: A total of 111 infants and young children were enrolled who were admitted in Pediatric Department due to lower respiratory tract infection in a university hospital. For NPFS, a flocked swab was introduced into the right nostril against the pharyngeal tonsil, and rotated 3 times. For NPA, the catheter was inserted into the left nostril to the same depth, washed with saline, and aspirated out. All specimens were sent to laboratory for the detection of the seven respiratory viruses by indirect immunofluorescent (IF) method (Chemicon, Temecula, CA, USA), culture (R-Mix Too, Diagnostic Hybrids, USA, and multiplex PCR (Seeplex® RV12 ACE Detection kit, Seegene, Korea).
Results: The percentage of inadequate specimens for indirect IF methods due to lack of respiratory epithelial cells were 23.4% in NPA but 5.4% in NPFS. Positive rates of respiratory viruses detected by indirect IF method were 23.4% in NPA and 43.2% in NPFS ($P < 0.05$). By culture with R-Mix cells, positive rates were 27.9% for NPA and 38.7% for NPFS ($P < 0.05$). With multiplex PCR method, positive rates were 56.8% for NPA and 71.2% for NPFS ($P < 0.05$). PNFS sampling method was easier for the medical staff and more comfortable for the patients. The specimen quality variation by different medical staffs was much less in NPFS specimens.
Conclusion: The Copan NPFS was superior to NPA for sampling respiratory epithelial cells for the indirect IF method, obtaining virus for culture, or getting viral nucleic acids for multiplex PCR. It would be possible to request seven respiratory viral tests in emergency room or in outpatient clinic if PNFS were available. We confirmed that sampling method was very critical in detecting respiratory viral pathogens.

Emerging influenza virus: H1N1v and H5N1

P1090 Simplexa™ influenza A H1N1 (2009) assay for detection of 2009 pandemic influenza H1N1 with Microfluidic real-time PCR system

M. Aye, J. Chen, S. Dempsey, E. Eleazar, H. Mai, L. Nair, Y. Xie, Y.P. Chen, M. Exner (Cypress, US)*

Objective: In 2009, a new strain of Influenza A (H1N1) virus emerged and rapidly spread into a global influenza pandemic. Because of its unique sequence divergence by genetic shift, this virus was not readily subtyped by pre-existing tests. In this study, we report development of a real-time PCR-based Simplexa™ Influenza A H1N1 (2009) assay for rapid detection and differentiation of pandemic influenza virus from seasonal influenza A viruses.
Methods: Limit of detection studies and specificity studies were performed to determine analytical performance characteristics of the Simplexa™ Influenza A H1N1 (2009) assay. Clinical performance of the was determined by testing blind panels of clinical specimens and comparing results to those obtained using the CDC real-time RT-PCR assay for Detection and Characterization of Swine influenza (version 2009).
Results: Limit of detection studies showed that the Simplexa™ assay detected influenza A H1N1 (2009) and seasonal influenza H1 and H3 subtypes at less than 101 TCID50/mL. Sensitivity and specificity of the Simplexa assay for influenza A H1N1 (2009) and, seasonal influenza were 98.3% (59/60) and 99.1% (119/120), and 100% (118/118) and 96.8% (60/62), respectively, compared to CDC real-time RT-PCR assay.
Conclusions: The Simplexa assay (CE IVD) was demonstrated to be a sensitive and specific method for detecting and discriminating influenza A H1N1 (2009) from other seasonal influenza viruses. The assay is compatible with automated and manual sample preparation methods, and can produce the results in less than 90 min. Thus, Simplexa assay provides a compact high throughput system for detection and identification of 2009 pandemic influenza H1N1 virus.

P1091 Development of recombinant adeno-associated virus vector delivering short-hairpin RNAs to inhibit the replication of influenza A viruses

G. Zhang, M. Chen, B. Zheng (Hong Kong, HK)*

Objectives: RNA interference is a powerful tool to inhibit viral infection. Recombinant Adeno-associated virus (rAAV) vectors are promising and widely used for gene delivery. This study is to select nucleocapsid protein (NP) and matrix protein (M2) of influenza A virus as targets and to develop rAAV vector to deliver antiviral short hairpin RNAs (shRNAs) to combat influenza A.
Methods: rAAV plasmid (pAAV-H1-GFP) was constructed, which contained H1 promoter and GFP marker gene. shRNAs targeting M2(shM2) and NP(shNP) genes were inserted into the pAAV-H1-GFP. rAAV-shM2 and rAAV-shNP were packaged using a modified AAV Helper-Free system. The physical and biological titers of purified rAAV were quantified by real time PCR and flow cytometry and the shRNAs expression were checked by real-time RT-PCR. rAAV and rAAV-shM2 and rAAV-shNP infected MDCK cells respectively. At 48 hours (h) post-infection, MDCK cells were infected by H1N1 (MOI=1) and then incubated at 37ºC for10 h. After aspirating medium, the MDCK cells were washed with PBS and added fresh medium and then incubated at 37ºC for 1 h again to collect the supernatants to check the titers of H1N1 by plaque assay.
Results: The pAAV-H1-GFP was transfected into Hela, 293FT and MDCK cells and rAAV pseudo-virus infected MDCK cells. At 24 h post-transfection and 48 h post-infection, strong green flourenscence can be observed under fluorensence microscope and at 48 h post-infection, shRNA can be checked. The physical and biological titers of purified rAAV are more than 10^{13} and 10^{10}/ml respectively. The H1N1 virus titer of the collected supernatant for rAAV and rAAV-shNP and rAAV-shM2 is

2.9 ± 10^4, 1.1 ± 10^4 and 6.6 ± 10^3 PFU/ml, respectively. Therefore, 62.1% and 77.2% H1N1 virus were inhibited by rAAV-shNP and rAAV-shM2 respectively.
Conclusions: pAAV-H1-GFP which can express shRNAs and GFP has been constructed and rAAV pseudo-virus with high titer and infectivity has been packaged and purified successfully. rAAV-shM2 and rAAV-shNP can infect MDCK cells and efficiently knock down M2 and NP specific mRNA of H1N1 to inhibit the replication of the H1N1 in MDCK cells. These preliminary results show that rAAV vector can be developed to deliver antiviral-shRNAs to combat influenza A and encourage us to further evaluate anti-H5N1 effects of rAAV-shM2 and rAAV-shNP *in vitro* and *in vivo*.

P1092 *In vitro* selection and characterization of DNA aptamers against H5N1 virus nucleoprotein

Y.P. Lin, J.A. Tanner, B. Zheng (Hongkong, CN)*

Objective: To develop DNA aptamers against H5N1 virus nucleoprotein and study the ability to inhibit H5N1 viruses production.
Methods: Nucleoprotein of influenza A virus (A/vietnam1194/2004 (H5N1)) was cloned and expressed in *E. coli*. The single strand DNA library is composed of 30 random nucleotides flanked by constant sequences for PCR amplification. Magnetic beads-based approach was used to screen DNA aptamers binding purified His-tagged nucleoprotein. After 20 rounds of selection, the enriched pool was cloned and 40 colonies were sequenced. The structure of the G-quadruplex aptamers was characterized by circular dichroism spectroscopy. MDCK cells were transfected with representative aptamers by Lipofectamin 2000. Four hours after transfection, cells were challenged with 100TCID50 influenza A virus (A/Vietnam/1194/2004(H5N1)). The viral titer was determined by HA assay at 48 hours post-infection.
Results: The sequences from *in vitro* selection revealed fairly conserved and could be classified into two groups, G-quadruplex and T-rich DNA aptamers. G-quadruplex aptamers were found dominant in the population and comfirmed by circular dichroism spectroscopy. Circular dichroism spectroscopy gave ellipticity minima and maxima at 240 nm and 264 nm, indicating a parallel arrangement of the quadruplex strands. In the early stage of post-transfection, DNA aptamers were mainly localized in the nuclei where the replication and transcription of influenza virus genome take place exclusively, which may facilitate successful antiviral efficacy. The T-rich aptamer had only mild effect on the inhibition of H5N1 virus, whereas G-quadruplex forming aptamers exhibited potent antiviral effect.
Conclusions: This study has demonstrated that nucleoprotein could be a potential antiviral target for influenza H5N1 virus. Further investigation will aim to elucidate the interaction between the G-quadruplex aptamer and nucleoprotein, as well as the antiviral mechanism.

P1093 H1N1 influenza virus infection in north-Western Spain, summer 2009

J. Fernandez, S. Perez, J. Torres, O. Touza, E. Martinez, I. Lopez (Vigo, ES)*

Objective: To study the epidemiological characteristics of the Influenza A H1N1v infected patients during the Summer of 2009 in Galicia (North West Spain).
Methods: From June to September 1466 samples (upper respiratory tract) were received (MTV, 4ºC) in our laboratory from hospitals throughout the Region. They were from the same number of patients with flu symptoms. RNA extraction was carried out using QIAamp Viral Mini Kit (QIACube-Qiagen). The CDC rRT-PCR Flu Panel (Ag Path-ID One-Step RT-PCR Kit) was used in order to detect the H1N1v virus. Neuraminidase sequence analysis was performed in 20 positive cases (BigDye 1.1 Applied Biosystems) to detect mutations causing resistance to oseltamivir.
Results: 359 out of 1466 samples proved positive (24.5%). The characteristics of the infected patients were: in-patients 79 out of 438 (18.0%); outpatients 177 out of 582 (30.4%, $p < 0.0001$), unknown origin 103 out of 446 (23.1%). Gender: male 192 out of 797 (24.1%), female 167 out of 669 (25%). Age: <5 yrs: 10 out of 98 (10.2%); 5–15yrs: 78 out of 136 (57.4%, $p < 0.0001$); 16–39yrs: 194 out of 608 (31.9%), 40–64yrs: 61 out of 397 (15.4%); >65yrs: 9 out of 207 (4.3%, $p < 0.0001$). The Crossing point (Cp) of the positive samples: <20: 25; 20–24: 99; 25–29: 124; 30–34: 104; ⩾35: 7. No neuraminidase mutations were detected in the residues previously associated with oseltamivir resistance (275, 295).
Conclusions: (1) The higher incidence of H1N1v infection was found in outpatients and children between 5–15 yrs. (2) Patients more than 65 years old were the less affected. (3) Only a third of the positive samples had a $Cp < 25$. (4) No oseltamivir resistance was detected.

P1094 Presence of several respiratory viruses in samples from patients with suspected flu A/H1N1 infection

A. Álvarez-Buylla, E. Culebras, C. Betriu, J. Picazo (Madrid, ES)*

Objectives: Infections caused by the influenza virus have a significant repercussion on modern society and causes particular concern. Since many of the signs and symptoms of respiratory virus infections are similar, other respiratory virus pathogens can be overlooked when focusing only on FluA. The aim of this study was to determine the presence of different respiratory viruses in both positive and negative pandemic influenza A samples.
Materials and Methods: A total of 60 respiratory specimens collected from patients with influenza-like illness had been selected for the study and previously tested by real time PCR. Specimens included 30 positive and 30 negative for pandemic FluA (15+15 samples from June and 15+15 samples from October).
Samples were tested using commercial kit (CLART PneumoVir. Genomica®) that employ a combination of two multiplex RT-PCR followed by primer extension and microarray hybridization. This assay can detect adenovirus; bocavirus, coronavirus; enterovirus (echovirus); rhinoviruses (HRV); influenza viruses A (seasonal), B and C; human metapneumoviruses (HMPV) A and B, human respiratory syncytial viruses (HRSV) A and B; and parainfluenza viruses (PIV) of types 1, 2, 3 and 4.
Results: No other respiratory viruses were detected in pandemic FluA positive samples from June, whereas six positive specimens from October were identified as dual infections. These dual infections included: one type 4 PIV, one HRV, one type 1 PIV, one RSV and two seasonal FluA. In pandemic FluA negative samples 7 out of 15 specimens from June reveal the presence of another respiratory virus (two samples with adenovirus, 2 samples coronavirus, one type 4 PIV, one enterovirus and one FluC). Only three of the negative pandemic FluA samples analyzed from October were positive in our study (HRV the three specimens).
Conclusions: Different respiratory viruses are present in samples obtained from patients with influenza-like illness. Detection of these other respiratory viral etiologic agents can be difficult in cases in which the causal agent was not suspected and a specific test was not demanded by the clinicians. The circulation of different respiratory viruses during the same period of the year makes very complex to elucidate their individual contributions to the global respiratory disease.

P1095 Interim report on pandemic H1N1 influenza virus infections in Korea, August to October, 2009

S.H. Lee, B.H. Park, H.Y. Kang, K.O. Lee, G.Y. Kim (Seoul, KR)*

Objectives: In Early April 2009, the earliest confirmed case of H1N1 influenza A virus was reported in Mexico. Currently, H1N1 is rapidly transmitted from person to person worldwide. Rapid detection is essential to instigate appropriate public health management and for disease surveillance. We estimate the epidemiology and positive rates of novel influenza H1N1 virus during the fall season in Korea.

Methods: 52,899 nasopharyngeal samples in patients with influenza like illness were collected over the country during mid August to October in 2009. H1N1 positive case was defined by real time reverse transcription PCR (rRT-PCR). H1N1 virus test using RNA was extracted using RNA extraction kit (Zymo Research, CA, USA). For the detection of influenza A and novel H1N1 virus, Genekam kit (Genekam, Duisburg, Germany) were used according to the CDC protocols verified by WHO. We analyzed the prevalence of virus according to age range, sex, regional distribution, and time period.
Results: Of the 52,899 patient, H1N1 virus was detected in 29,554 samples (55.9%). The highest frequency was found in the 10–19 year-olds' group (M = 68.3% versus F = 64.8%) and followed by the 0–9 year-olds' group (M = 56.6% versus F = 54.7%). The positive rate of H1N1 virus decreased as age range increased in over the 30 years-olds' group. The mean positive rate was significantly higher in men than in women (M = 58.2% versus F = 53.2%). The prevalence of novel H1N1 virus was high in some areas such as Gangwon, Daejeon, Chungcheong and Busan among 13 provinces of Korea. The H1N1 positive rate increased over time and showed significantly a high peak at mid-September in 2009.
Conclusion: The positive rate of H1N1 virus is especially high in adolescences and children groups in Korea. A peak in prevalence is found at mid-September in 2009. Therefore, it is necessary to pay attention to the younger patients with influenza like illness as well as the high risk groups in epidemic season of H1N1 virus.

P1096 Predictive factors for mortality among in-hospital Mexican patients infected with influenza A/H1N1

J.A. Mata-Marin, I. Ascencio-Montiel, J.E. Gaytan-Martinez, R. Arias-Flores, C. Martinez-Chacon (Mexico City, MX)*

Objective: To determine predictive factors for mortality among in-hospital Mexican patients infected with influenza A/H1N1.
Methods: We performed a case–control study of the factors associated with unfavorable prognosis (mortality) among inpatients with documented influenza A/H1N1 infection. We enrolled patients seen from April to October 2009 at the Hospital de Infectologia, La Raza National Medical Center, in Mexico City. Cases were those patients who die during the hospitalization and controls were those who were discharged due to improvement. Patients were included if they had over 18 years old and a positive influenza A/H1N1 RT-PCR test. Data collected included, general attributes, medical history, presenting symptoms, records about respiratory evolution, and routine laboratory tests.
We compared median differences using t test or Mann–Whitney U test when data were skewed. Odds ratio and 95% confidence interval were calculated to assess the relationship between each predictive factor and the risk of mortality. To adjust for the effects of potential confounders, we used a logistic regression models. P-value was considering statistically significant if <0.05.
Results: We included 31 patients (11 cases and 20 controls), of the patients enrolled 12 (38.7%) were male: 7 (63.6%) in cases group and 5 (25%) in controls group. The median ($\pm$SD) age of our subjects was 36.2 ± 13.8 and 34.25 ± 11.47 respectively ($p = 0.685$).
The risk factors associated to mortality were: male sex OR 5.25 (CI 95% 1.07–25.79), delayed in medical attention (>3 days) OR 6.41 (95% CI 1.18–34.61), delayed to start anti-flu therapy (>3 days) OR 10.0 (95% CI 1.07–93.43), admission to the intensive care unit OR 9.9, (95% CI 1.51–64.52), required mechanical ventilation OR 9.3 (CI 95% 1.43–61.14), presence of chronic diseases OR 10.86 (CI 95% 1.02–114.57), and high creatinine levels (>1.0 mg/dl) at admission to hospital OR 11.2 (95% CI 1.05–120.32). After adjust for the effects of potential confounders in a logistic regression model, we found only delayed in medical attention aOR 22.68 ($p = 0.02$) and presence of chronic diseases aOR 47.62 ($p = 0.037$) as predictors of mortality.
Conclusion: An early medical attention is necessary to decrease mortality in patients with influenza A/H1N1. Presence of chronic diseases increased the possibility of mortality in in-patients with influenza A/H1N1 in Mexican population.

P1097 Epidemiologic characteristics of deaths associated with 2009 pandemic influenza A(H1N1) virus infection in Korea

H.S. Kim, J.H. Kim, S.Y. Shin, Y.A. Kang, H.G. Lee, J.S. Kim, J.K. Lee (Seoul, KR)*

Objectives: To describe clinical and epidemiologic features of fatal cases associated with 2009 influenza A(H1N1) infection in Korea.
Methods: Nationwide enhanced public health surveillance of Korea residents who were died with laboratory evidence of pandemic 2009 influenza A(H1N1) infection reported to the Korea Centers for Disease Control and Prevention between August 15 and November 05, 2009.
Results: During the study period there were 51 cases of death due to pandemic 2009 influenza A(H1N1) infection reported in Korea. Among the 51 cases, the median age was 55 years (range, 2 months-83 years); 27(52.9%) were male and 24(47.1%) were female. The median time form symptom onset to diagnosis was 3days(range: 1–16days) and from symptom onset to death was 6days. Antiviral drugs were administerd to 42patients(82.4%). 24patients(57.1%) of them start antiviral medication before diagnosis and others start antiviral medications as soon as the influenza confirmed. 42 patients(82.4%) had risk factors for seasonal influenza complications such as 1) age less than 59months were 4 patients, 2) age more than 65years were 22 patients(19 cases of these had chronic medical conditions), 3) chronic medical conditions were 16 patients(malignant tumor, DM, chronic lung disease and chronic renal disease).
46 patients (90.2%) of those had pulmonary complication like pneumonia or acute respiratory distress syndrome. Evidence of coinfection was found in specimens from 19.6%(10/51) patients, including 6 caused by bacteria and 2 caused by funfus.
Conclusions: Fatal cases from 2009 influenza A(H1N1) in Korea occurred in high-risk patients and was associated with viral pneumonia and severe acute respiratory distress syndrome. Considering current situation, we need to manage high-risk patients actively.

P1098 Dynamics of clinical symptoms in a case with pandemic influenza A (H1N1)

C.S. Lee, J.H. Lee (Jeonju, KR)*

Background: The clinical spectrum of 2009 pandemic influenza A virus infection is still being defined, but both self-limited illness and severe outcomes, including respiratory failure and death, have been observed.
Method: The 2009 H1N1 influenza was confirmed by means of a polymerase chain reaction assay (PCR). Upper respiratory symptoms were defined as rhinorrhea, sore throat, and nasal congestion. Lower respiratory symptoms were defined as cough, and dyspnea. Systemic symptoms were defined as subjective fever, headache, and myalgia. Gastrointestinal symptoms were defined as nausea, vomiting, and diarrhea. The symptom score was average, and the range was from 0 to 3. All subjects were asked to complete a daily log at about the same time every morning to document the severity of their influenza-related symptoms on a 4-point scale sheet.
Result: During the evaluation period, 239 patients were diagnosed with pandemic influenza A infection. Among them, 118 patients were admitted to the hospital, and the other 121 were given oseltamivir and sent home to rest. Among 118 patients, 85 patients (>9 years old), who filled out the symptoms score completely, were analyzed for the study. Of the 85 patients studied, 57 (67.1%) were male. The mean age, hospital stay, and the interval from initial symptoms to oseltamivir administration were 23.4 ± 14.3 years, 5.7 ± 1.5 days, and 2.3 ± 1.4 days, respectively. Among the clinical manifestations, fever (91.9%) and cough (91.9%) were the most frequent, followed by sore throat (69.8%), rhinorrhea (64.0%), headache (60.5%), nasal congestion (55.8%), myalgia (43.2%), diarrhea (31.4%), nausea (24.4%), dyspnea (22.1%), and vomiting (15.1%). The duration of cough was the longest, followed by myalgia, and sore throat. Regarding the severity of symptoms, systemic symptoms peaked the earliest (by day 2) and resolved faster than lower respiratory symptoms (LRS), and upper respiratory symptoms (Fig 1). The LRS

resolved slowly over many days. The gastrointestinal symptoms were mild and short lived.

Conclusion: The pandemic H1N1 virus involve primarily lower respiratory tract as in H5N1 virus. The risk groups with any chronic lung disease should be advised to do their best to protect themselves from this pandemic influenza virus.

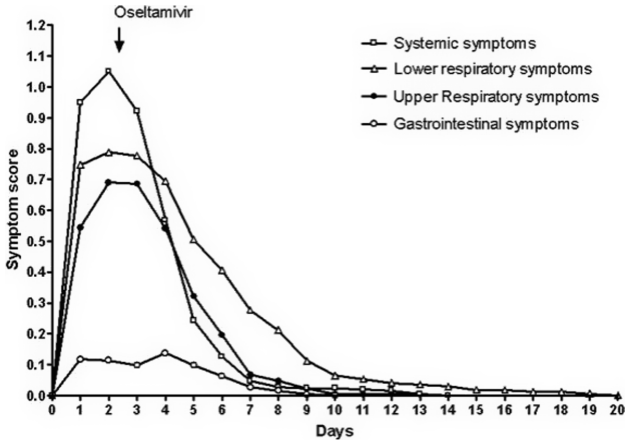


Figure 1. The dynamics of clinical symptoms of the patients infected with 2009 pandemic influenza (H1N1).

P1099 Investigation of the frequency of pandemic influenza A 2009 H1N1 virus and other respiratory viruses in patients with acute respiratory tract infections

C. Cicek, H. Pullukcu, E.U. Saz, G. Cok, B. Arda, F. Bacakoglu (Izmir, TR)*

Objectives: The aim of this study is to establish the frequency of respiratory viruses (RSV, influenza viruses type A and B, parainfluenza viruses type 1, 2, and 3, and adenovirus) and pandemic influenza A 2009 H1N1 virus in patients with acute respiratory tract infections in Izmir since January 2009.

Methods: Nasopharyngeal swab specimens were collected from 480 [251 (52.3%) male, 229 (47.7%) female] patients with acute respiratory tract infections between January 1st and November 17th 2009. The age range of patients is between 6 days to 82 years (median: 15 years). Direct immunofluorescent antibody (DFA) test and shell vial cell culture method were performed when all the specimens arrived at the laboratory. Cytocentrifuged specimens were stained with Respiratory DFA Viral Screening & Identification Kit (Light Diagnostics, Millipore, USA) according to the manufacturer's instructions. For the recovery of respiratory viruses were used HEp-2 (RSV), A-549 (adenovirus), Vero (parainfluenza viruses type 1, 2, and 3), and MDCK (influenza viruses type A and B) cells line and shell vial cell culture method were used. Coverslips were stained with a fluorescein isothiocynate labelled monoclonal antibody specific for each virus (Light Diagnostics, Millipore, USA) according to the manufacturer's instructions. Pandemic influenza A 2009 H1N1 virus was detected by real time RT PCR assay (Influenza A H1N1 primer and probe set, SuperScript™ III Platinum® One-Step qRT-PCR System, Invitrogen, USA) by using ABI 7500 system.

Results: Of the 480 patients, 141 (29.4%) were positive for respiratory viruses by using the combination of DFA and shell vial cell culture method and five of these patients were infected by two different viruses [18 (12.3%) RSV, 99 (67.8%) influenza type A, 5 (3.4%) influenza type B, 9 (6.2%) parainfluenza viruses, and 15 (10.3%) adenovirus]. Of 99 influenza A viruses, 94 (94.9%) were typed as pandemic influenza A 2009 H1N1 virus.

Conclusion: Pandemic influenza A 2009 H1N1 virus was not very common until the second week of November in Izmir. This may be due to the temperate climatic conditions during this period (20–240C) in Izmir. Two-thirds of pandemic influenza A 2009 H1N1 virus was detected between November 9th and 17th due to cold weather. The frequency of the other respiratory viruses decreased after September. Except for influenza A virus, no other respiratory viruses have been detected in patients since the first of November.

P1100 Epidemiological and antiviral susceptibility data of A(H1N1)v in northern Greece

A. Melidou, G. Gioula, M. Exindari, A. Papoutsi, D. Chatzidimitriou, E. Diza, N. Malissiovas (Thessaloniki, GR)*

This study presents up-to-date information on the epidemiological characteristics of influenza A(H1N1)v virus infections, as well as antiviral susceptibility data, obtained from the northern part of Greece until the 16th of November. 2149 pharyngeal swabs and/or washes were examined at the National Influenza Centre for northern Greece. RNA was extracted with the Qiagen Viral RNA mini kit and A(H1N1)v infections were confirmed using the CDC swine influenza real-time RT-PCR kit following WHO recommendations. Results were reported to HCIDC, WHO and ECDC. One step RT-PCR and sequencing of the neuraminidase gene of 15 representative isolates was also done in order to test the susceptibility of circulating strains to oseltamivir. 688 were confirmed A(H1N1)v infections (32%). Confirmed cases were presented with ILI, with fever temperature ranging from 38ºC to 40.5ºC, acute onset of symptoms, cough, catarrh, sore throat, fatigue, headache, muscle pain and in some cases conjunctivitis, diarrhoea and/or vomitting. There were 172 patients with pneumonia symptoms, of which 23 were found positive for A(H1N1)v infection (13.4%). The mortality rate is 0.14, with a total of 3 deaths. There were also a small number of asymptomatic infections, mostly detected during the summer period. The age distribution showed a trend towards young adults. Sex distribution showed similar infection rates at both sexes. During the first wave of the pandemic, until mid July, the majority (70%) of infected individuals were travellers. Regarding the antiviral treatment, at the onset of the pandemic all patients received oseltamivir (Tamiflu) after the confirmation of A(H1N1)v infection, but only 21% of the cases were treated from the onset of symptoms. After widespread transmission within the country, patients no longer received antiviral treatment unless necessary. 15 isolates were tested for antiviral resistance and they were all found sensitive to oseltamivir. As already known, all isolates are resistant to amantadine. A(H1N1)v infection rates in northern Greece showed an initial peak during weeks 29–34 and the ongoing second peak initiated at week 42 and is still growing. Epidemiology data of the ongoing pandemic A(H1N1)v infections, as well the susceptibility of circulating strains to antiviral drugs is considered essential at this point of the pandemic.

P1101 Evaluation of three commercial Rapid Antigen assays for the detection of influenza An/H1N1

J. Perez, E. Culebras, A. Alvarez-Buylla, C. Betriu, J. Picazo (Madrid, ES)*

Objectives: Pandemic influenza An/H1N1 emerged in mid-April 2009 leaving laboratories around the world scrambling to establish a diagnostic test to detect this novel influenza virus. Reverse-transcriptase polymerase chain reaction (RT-PCR) is considered the reference test for diagnosis of influenza because of its high sensitivity and specificity as well as its rapid turnaround time. However, RT-PCR is expensive and requires trained expertise and batch testing, which delays reporting. Rapid influenza antigen test might prove useful, because they have a fast turnaround time (10–15 min) and require minimal training to perform. We evaluated three rapid antigen detection test for their ability to detect influenza An/H1N1. We calculated the sensitivity for each test.

Materials and Methods: We tested 3 different rapid influenza antigen tests for pandemic infuenza An/H1N1 virus. We checked BinaxNow® Influenza A&B (CV. Inverness Medical), Influ A&B Uni-Strip (IAB. Materlab) and QuickVue® Influeza A+B test (QV. Biomerieux). Twenty two nasopharyngeal specimens that were positive

for pandemic influenza A virus by RT-PCR (Roche) were included. We included samples with viral load between 1×10^2 and 1×10^8 copies/ml. Only 5 samples were tested for the IAB test.
Results: Sensitivities of test were low to moderate: BinaxNow® Influenza A&B (Inverness Medical) 3 of 22 specimens and QuickVue® Influeza A+B test (Biomerieux) 10 of 22 specimens. The detection limit of both systems was 1×10^6 copies/ml and 1×10^4 copies/ml respectively. IAB test was positive only in samples with >1times;10^6 copies/ml. All samples with viral load below 9×10^3 copies/ml were negative with all tests.
Conclusions:
1. QV test showed the highest sensitivity of the evaluated tests.
2. Positive results for rapid antigen tests correlates with higher viral load.
3. Negative rapid influenza diagnostic test does not rule out infection with novel influenza A (H1N1) virus.
4. Rapid influenza antigen tests can be used to avoid the RT-PCR assay in samples positive for the rapid tests.

P1102 A comparative study between 2009 H1N1 influenza A virus and seasonal influenza virus infections at a Bangkok hospital, Thailand

E. Thiansukhon, L. Paitoonpong, G. Suwanpimolkul, P. Bhattarakosol, C. Suankratay for the Division of Infectious Diseases & Virology, King Chulalongkorn Memorial Hospital, Bangkok*

Objective: To compare the epidemiology and clinical features between the patients with laboratory-confirmed 2009 H1N1 influenza A and seasonal influenza virus infections.
Methods: A retrospective study of laboratory-confirmed 2009 H1N1 influenza A and seasonal influenza virus infected patients at King Chulalongkorn Memorial hospital was carried out from June 3 to August 20, 2009. Reverse transcriptase polymerase chain reaction tests were used to confirmed diagnosis.
Results: There were 167 and 57 patients with 2009 H1N1 influenza A and seasonal influenza infections. The 2009 H1N1 influenza A group had less mean age (29.1+19.5 years) than the seasonal influenza virus group (36.5+21.6 years) because there are less patients that are more than 50 years old in 2009 H1N1 influenza A group. Of all 224 patients with influenza infection, the most common symptom was fever (89.7%), followed by cough (83.4%), rhinorhea (61.9%), sore throat (54.3%), myalgia (45.3%), and dyspnea (22.9%). There was no significant difference in most clinical manifestations between the two groups except a higher frequency of diarrhea in the 2009 H1N1 influenza A group than the seasonal influenza virus group (16.3 vs 5.3%, p = 0.042). Pneumonia was noted in 41 (24.7%) patients in the 2009 H1N1 influenza A group, in comparison with 5 (8.8%) patients in the seasonal influenza virus group (p = 0.013). The mortality rate was 1.8% in the 2009 H1N1 influenza A group, but no patient in the seasonal influenza virus group died.

Clinical data of H1N1 2009 influenza A and seasonal influenza infections in King Chulalongkorn Memorial Hospital

	Total, n (%)	H1N1 2009 (n = 167)	Seasonal (n = 75)	P-value
Fever	200 (89.7)	147 (88.6)	53 (93)	0.453
Cough	186 (83.4)	144 (86.7)	42 (73.7)	0.037
Myalgia	101 (45.3)	72 (43.4)	29 (50.9)	0.357
Rhinorrhea	138 (61.9)	99 (59.6)	39 (68.4)	0.271
Sore throat	121 (54.3)	89 (53.6)	32 (56.1)	0.76
Dyspnea	51 (22.9)	42 (25.3)	9 (15.8)	0.2
Diarrhea	30 (13.5)	27 (16.3)	3 (5.3)	0.042
Vomit	29 (13)	26 (15.7)	3 (5.3)	0.065
Pneumonia	46 (20.6)	41 (24.7)	5 (8.8)	0.013
Dead	3 (1.3)	3 (1.8)	0	0.572

Conclusions: The patients with 2009 H1N1 influenza A infection are younger than seasonal influenza virus infected patients. Diarrhea is only symptom that is more common in 2009 H1N1 influenza A infected patients. 2009 H1N1 influenza A infection seems to be more severe because there are more pneumonia and death.

P1103 Multiplex assay for simultaneous typing of influenza A viruses and subtyping into H1N1 and H3N2, including novel 2009 swine origin influenza A H1N1 using a microarray-based platform

A. Alemán, O. Salazar, M.L. Villahermosa, R. Cospedal (Coslada-Madrid, ES)*

Objectives: Recent outbreak of novel influenza A (H1N1) virus (swine origin influenza virus [S-OIV]) makes necessary the design of well-validated diagnostic tools for the rapid, sensitive and specific identification of the 2009 pandemic H1N1 influenza virus. We have developed and validated a multiplex PCR assay followed for a hybridization in a low-density microarray (CLART® FluAVir) for simultaneously typing and subtyping of Influenza A viruses including novel H1N1 swine origin influenza virus [S-OIV], human H1N1 and H3N2 influenza A and generic Influenza A viruses.
Methods: The assay targets the genes NP (nucleoprotein) to type the influenza A viruses and subtype human influenza A H1N1 and N3N2, and the gene HA (haemagglutinin) to subtype and S-OIV H1N1. 446 clinical isolates were typed and subtyped using CLART® FluAVir assay.
Results: The assay achieved an analytical sensitivity of 10 to 10^2 copies of transcripts per reaction for each of the genes. The assay correctly typed and subtyped 446 clinical isolates, including 228 S-OIV H1N1, 15 A/H1N1 human, 60 A/H3N2 human, and 62 from other viruses and 113 negative samples. Testing of the analytical specificities revealed no-cross reactivity and demonstrated reproducibility of results. Clinical tests showed 98.3 of sensitivity for Influenza A H3N2 and 100% sensitivity and specificity for the rest of the types, including S-OIV H1N1. Comparisons of the CLART® FluAVir results with multiple validated in-house molecular assays, and CDC-validates FDA-approved assay demonstrates a 100% positive agreement for all the viruses.
Conclusions: CLART® FluAVir is a rapid, sensitive, specific and multiparametric assay for the typing and subtyping of influenza A viruses, which allow to process up to 96 samples simultaneously in a working day, being useful for de diagnosis and surveillance of 2009 pandemic (H1N1) influenza virus and necessary for clinical and public health decisions.

P1104 Emergence of influenza A H1N1v oseltamivir-resistant strain in a 2 year-old child with acute lymphatic leukaemia

G. Campanini, A. Piralla, F. Rovida, E. Percivalle, F. Locatelli, F. Baldanti (Pavia, IT)*

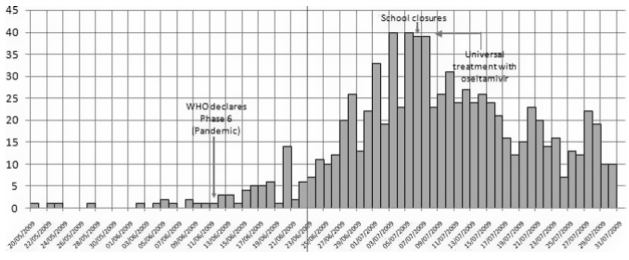
Our Center is part of a National network of 22 laboratoires monitoring the evolution of the current influenza A pandemic sustained by the H1N1v strain.
Methods: Emergence of oseltamivir resistance-associated mutations was monitored in about 1/10 of untreated influenza A H1N1v strains recovered at our center. In addition, all patients still positive after 10 days of antiviral treatment were analyzed for the emergence of drug-resistant virus strains.
Results: Thus far, influenza A H1N1 neuraminidase gene was sequenced in 35 untreated patients and oseltamivir- or zanamivir-associated mutations were not detected. H1N1v neuraminidase gene was also sequenced in virus strains from the nasal swab of 5 immunocompromised patients undergoing oseltamivir administration and still positive after more than 10 days of treatment. In 4 patients no drug-resistence mutations were detected, while in a 2 years-old girl with acute lymphatic leukemia the H274Y mutation was shown. Influenza A H1N1v was detected by realtime RT-PCR in this patient on Oct. 27, 2009 in the presence of a mild influenza like illness (ILI). Due the underlying disease, the patient was administered oseltamivir (30 mg Bid) starting from Oct. 29, 2009 and presence of virus RNA was monitored every

other day. On Nov. 14 the patient was still positive for influenza RNA in nasal swab and the virus was sequenced showing the presence of a resistant variant. Despite the emergence of a drug-resistant variant and leading to failure of oseltamivir treatment, ILI is slowly improving in this patient. This is the first reported drug resistant influenza A H1N1v strain in Italy since the beginning of the pandemic, while 43 resistant strains have been reported worldwide.
Conclusions: (i) Emergence of drug resistant strains is a rare event in this pandemic, (ii) monitoring of drug resistance is warranted in patients undergoing prolonged antiviral administration.

P1105 Clinical and epidemiological characteristics of outpatients with influenza-like illness during influenza A H1N1 pandemic in Buenos Aires, Argentina

C. Rodriguez, E. Cordova, G. Oviedo, W. Cornistein, A. Noel, L. Vazquez (Buenos Aires, AR)*

Objectives: Describe the clinical and epidemiological characteristics of a series of cases of influenza-like illness (ILI) attended at Infectious Diseases Outpatient Department, 'Cosme Argerich' Acute General Hospital, Buenos Aires, Argentina.
Methods: Descriptive and retrospective study of clinical and epidemiological findings of patients with ILI attended at Infectious Diseases Outpatient Department from 20/05/2009 to 31/07/2009 in the context of influenza A H1N1 pandemic. ILI was defined as documented fever $\geqslant$38ºC and cough or sore throat in the absence of another cause. According to National Health Ministry recommendations no virological diagnostic tests were performed to these patients.
Results: N = 864. Male sex: 352 (41%). Median age (range): 34 years (3 months-91 years). The age group of 20–29 years old was the most affected by ILI: 210 cases (24%). Domicile: Buenos Aires city (75%). The major incidence was seen between epidemiological week 26–28, accounting for 63% of the cases.
Main clinical presentation: headache (98%), fever $\geqslant$38ºC (91%), myalgia (86%), mild cough (63%). Median time between onset of symptoms to medical assistance: 24 hours (range 0–17 days). History of contact with a suspect case: 169/310 (55%). Previous immunization with seasonal influenza vaccine 2009: 164/812 (20%). Among health care workers with ILI this antecedent was only present in 44/91 (48%). Risk factors for ILI: 199/836 (24%), corresponding to: asthma/COPD (11%), diabetes (3%) and cardiopathy (3%). Chest X ray: interstitial infiltrate (56%), interstitial-alveolar infiltrate (6%), alveolar infiltrate (3%), normal (36%).
Treatment with oseltamivir: 783/816 (96%). Complications: 301/457 (66%), being more frequent: pulmonary compromise of probably viral etiology, follow by acute sinusitis, acute otitis media and bacterial pneumonia.
Conclusions: Different to what is commonly described for seasonal influenza, young adults with no comorbidities were the age group more frequently affected by ILI. The main complication observed during this period was pulmonary compromise of probably viral etiology. A low rate of vaccination for seasonal influenza was seen among health care workers.



Outpatients with influenza-like illness during influenza A H1N1 pandemic attended at Infectious Diseases Outpatient Department 'Cosme Argerich' Acute General Hospital, Buenos Aires, Argentina.

P1106 Antibodies to interferon-gamma in ultra-low doses: a new option for pandemic influenza

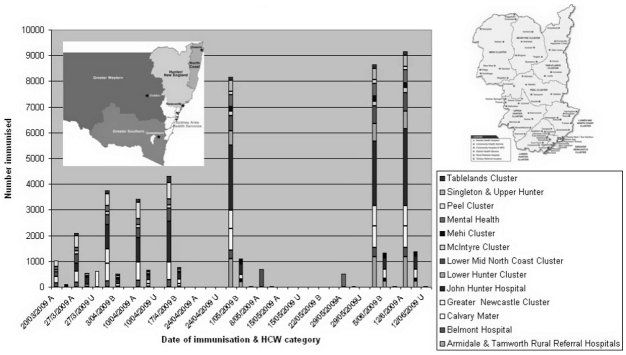
S. Tarasov, V. Zarubaev, J. Tafani, J. Dugina, S. Sergeeva, O. Epstein (Moscow, St. Petersburg, RU; Maisons Alfort, FR)*

Objectives: To assess antiviral activity of antibodies to interferon-gamma in ultralow doses (ULDabIFNg) against influenza A/H1N1 viruses.
Methods: Antiviral activity of ULDabIFNg (Anaferon for children®) was studied in experimental models of lethal infection of mice infected by different influenza A/H1N1 virus strains. Studies were conducted in Influenza Research Institute (Russia) and in APcis (France) in 2009. In Influenza Research Institute 100 female outbred mice (16–18 g., 20 mice/group) were infected intranasally with 10LD50 of influenza virus A/California/07/2009swl. In APcis 60 female Balb/c mice (10–12 g., 20 mice/group) were infected intranasally with 3LD50 of influenza virus A/NewCaledonia/20/99. In both studies ULDabIFNg given as water solution were administered according to prophylactic/treatment regimen (5 days before and 12 days after inoculation) via oral gavage (0.2 ml/mice 2 times/day). Besides ULDabIFNg were given instead of drinking water. Control mice were given distilled water according to the same schedule. In negative control group mice were neither infected nor treated. In Influenza Research Institute efficacy of combination of ULDabIFNg with oseltamivir against monotherapy with oseltamivir was also studied. Oseltamivir was administered according to prophylactic/treatment regimen (25 hours and 1 hour before and 3 days after inoculation) via oral gavage (0.2 ml/mice 2 times/day at dose 20 mg/kg/day) or (Apcis) from 1 hour to 5 days after inoculation (10 mg/kg/day). Combination of ULDabIFNg with oseltamivir was prepared by dissolving oseltamivir in water solution of ULDabIFNg. Mortality rate and body weight change were evaluated.
Results: In Influenza Research Institute ULDabIFNg significantly reduced mortality. In control group survival was 12.5%, in oseltamivir group – 10%, in ULDabIFNg group – 50%, in oseltamivir+ULDabIFNg group – 35%. In the second study (APcis) in the control group inoculation caused deaths 60% of mice on day 7, treatment with ULDabIFNg resulted in a later disease onset (on day 5 in ULDabIFNg group mortality was 15% vs 40% in control group). In both studies mean body weight of alive mice was constantly a positive function of time in group treated with ULDabIFNg.
Conclusion: Antiviral activity of ULDabIFNg against two strains of influenza A/H1N1 viruses including the pandemic one is comparable to oseltamivir. Combination of ULDabIFNg with oseltamivir increases the efficacy of monotherapy with oseltamivir.

P1107 Influenza vaccination without compulsion for healthcare workers

R. Givney on behalf of the Hunter New England Health V-Day Program*

Immunisation of healthcare workers against influenza has been promoted as a method of preventing disease in patients. Healthcare worker immunisation rates against influenza are historically poor but recently high rates of immunisation have been reported from mandatory vaccination programs despite ethical concerns & the uncertainty about their value in protecting patients.
We present data from a series of annual programs of voluntary staff influenza seasonal influenza immunisation programs (2007–9) and from the initial pandemic H1N1(2009) vaccination program in the Hunter New England Area Health Service (16700 staff, 130000 square km) in New South Wales in eastern Australia.
We demonstrate the effectiveness of voluntary program (>60%) when there are committed immunisers, an effective communication strategy about the benefit to the workers, motivated leadership & funding to provide vaccination services at the places where people are working at the times they are working.



2009 HNEH Healthcare Worker (HCW) seasonal influenza immunisation by category [A, B or Unclassified (U)] and date of immunisation.

Viral hepatitis – seroepidemiology

P1108 Phylogenetic analysis of hepatitis E virus isolates originated from Czech patients

P. Vasickova, M. Slany, P. Chalupa, M. Holub, R. Svoboda, I. Pavlik (Brno, Prague, CZ)*

Objective: Hepatitis E virus (HEV) is the leading cause of hepatitis non-A, non-B worldwide. Outbreaks of hepatitis E (HE) are associated with faecal contamination of drinking water, while sporadic cases are also linked with zoonotic potential of HEV. The virus is non-enveloped and its genome contains linear positive-strand RNA. According to phylogenetic analysis HEV isolates segregate into four genotypes. Relative conservation of genotypes I and II corresponds with primary circulation within humans, whilst diversity of genotypes III and IV is related to zoonotic and foodborne origin. The objective of this study was to perform phylogenetic analysis of HEV isolates from faeces of HE patients and thus determine the origin of virus.

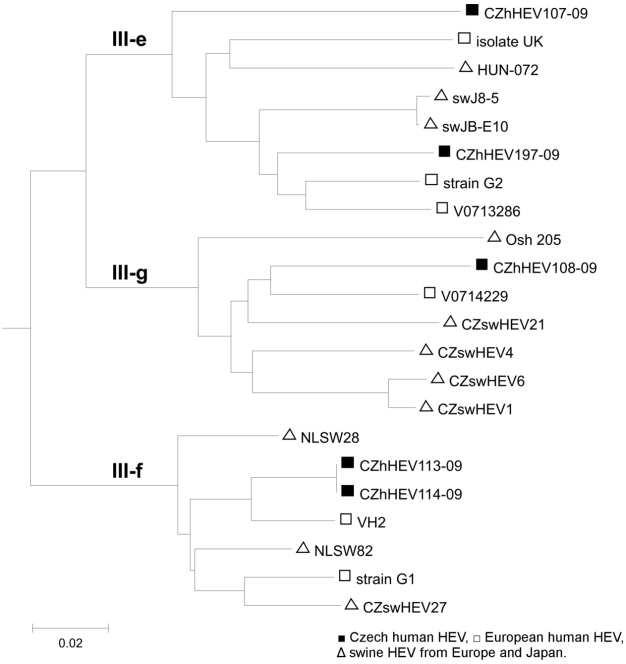


Figure 1. Part of the phylogenetic tree showing clustering of Czech hepatitis E virus isolates within genotype III of genus Hepevirus.

Methods: Faeces from eight serologically confirmed HE patients were tested. RNA was extracted using QIAamp Viral RNA kit (Qiagen) according to the manufacturer's instructions. The detection of HEV RNA was performed by nested RT-PCR targeting 287 bp long part of ORF 1. The sequences were analysed by MEGA 3.1 software and compared with the sequences in GenBank using the BLAST utility. Neighbor-joining method was used for phylogenetic analysis.

Results: HEV RNA was detected in faeces of five patients. Comparison of the obtained sequences with sequences in GenBank failed to find any 100% homologous HEV isolate. Sequence analyses showed that these isolates share 82.2 to 100% nucleotide identities with each other. Phylogenetic analysis revealed that all five Czech HEV isolates belong to genotype III subgroups e, f and g (Fig. 1).

Conclusion: We detected HEV excretion in faeces of five patients from a total number of eight serologically confirmed HE cases. Phylogenetic analysis suggests that Czech patients were infected in Europe. This hypothesis is supported by patients anamnestic data: e.g. patient 108–09 reported visit of Sushi bar in Germany, which is in concordance to 93.8% homology with German human isolate. 100% homology within isolates CZhHEV113–09 and CZhHEV114–09 hints possible identical source of infection. Due to the high genetic diversity of HEV isolates, phylogenetic analysis even of such small part of its genome can help to determine sources of HEV infection.

Acknowledgment: This work was supported by national Grants No. MZE0002716202, OC08045 and international Cost Action 929 ENVIRONET.

P1109 Isolated anti-HBc profile should be investigated in a country of intermediate endemicity for hepatitis B

N. Hannachi, O. Bahri, B. Boukatef, H. Triki, J. Boukadida (Sousse, Tunis, TN)*

Objectives: Isolated antibody to hepatitis B core antigen (anti-HBc) is a common serologic finding in diagnostic settings. However, it is not clear how this serological profile should be interpreted and whether further serological or molecular tests should be conducted routinely on these samples. The aim of this study was to assess the frequency and clinical significance of anti-HBc alone in central Tunisia.

Methods: This study included 9294 subjects divided into two groups: healthy group (857 blood donors and 2709 pregnant women) and a group of hospitalized patients especially in hematology, pediatric and infectious diseases services (5728 patients). HBs antigen (HBs Ag) and anti-HBc were performed for all sera. Negative HBs Ag sera were systematically tested for anti-HBs antibody (anti-HBs). Levels of anti-HBs below 10mUI/ml were considered as negative. Isolated anti-HBc sera were investigated for other hepatitis B markers (IgM anti-HBc, HBe antigen and anti-HBe antibody), for hepatitis C (anti-HCV), for hepatitis D (anti-HDV) and for human immunodeficiency virus infection (anti-HIV). Presence of hepatitis B virus DNA (HBV DNA) was studied by nested PCR.

Results: Prevalence of isolated anti HBc profile was 4.7% (436 cases). There was no statistical difference in this prevalence between healthy and hospitalized subjects. However, the frequency of this profile was higher in patients with elevated transaminase levels ($p < 0.0001$). Analysis of hepatitis B markers in isolated anti-HBc sera showed that a low reactivity of anti-HBs (between 1 and 9.9mUI/ml) was present in 163 cases (37%). In the remaining 273 isolated anti-HBc samples presence of anti-HBe, anti-HCV, anti-HDV and anti-HIV was detected in 23.4%, 3.8%, 5% and 1% of cases, respectively. Acute hepatitis B was established in one case. HBV DNA was detected in 4.1% of isolated anti-HBc tested samples.

Conclusion: In Tunisia, country of intermediate hepatitis B endemicity, the finding of isolated anti HBc profile is frequently indicative of past infection. Nevertheless, acute or occult hepatitis B can occur and must be researched especially in patients with abnormal liver function tests. Coinfection with hepatitis C and D or HIV are not rare and should be considered.

P1110 Anti-HCV negative viral hepatitis C in HIV-infected patients from AIDS centre, Prague

V. Aster, J. Konig, L. Machala, H. Rozsypal, O. Shivairova, M. Stankova (Prague, CZ)*

Objectives: Negative anti-HCV test is rare in common population. In contrast, in HIV infected persons with hepatitis C coinfection negative anti-HCV test is more frequent, especially in intravenous drug abusers. At the highest risk of seronegative HCV infection are HIV infected intravenous drug abusers with transaminase elevation or low CD4+ count. The aim of the study was to determine patients at the highest risk of seronegative HCV infection and to provide targeted HCV RNA testing using PCR method.
Methods: 666 HIV infected patients were followed-up at AIDS-Center Prague till August 2008. Data were collected through a retrospective search through the patients' clinical records. CD4+ count, transaminase value, and anti-HCV testing were done at up to a maximum 8 week interval. HCV RNA testing was started in HIV infected intravenous drug abusers with aminotransferase elevations or in those with CD4+ levels under 200/mcl.
Results: 49 (7.4%) of the 666 patients were intravenous drug abusers. In 14 of them transaminase elevation was observed; in 13, a CD4+ count of under 200/mcl was observed. 16 patients out of these 17 (14+13) were anti-HCV negative. To date to 7 of these anti-HCV negative patients HCV RNA test has been provided. 6 patients were HCV RNA positive and 1 patient HCV RNA negative.
Conclusion: Negative anti-HCV test in HIV/HCV coinfected patients, more so in intravenous drug abusers with transaminase elevations or with low CD4+ count, is more frequent than in patients with HCV infection alone. HCV RNA testing in HIV infected patients should be performed generally in anti-HCV positive patients, but also in selected anti-HCV negative patients. The study is funded by the grant Nr/9288–3 from the Internal Grant Agency of the Ministry of Health of the Czech Republic.

P1111 Hepatitis C virus genotyping and possible routes of transmission in a Greek population

D. Kouniaki, K. Tarassi, V. Kapsimali, T. Athanassiades, V. Kitsiou, K. Gizori, C. Papasteriades (Athens, GR)*

Hepatitis C virus (HCV) is classified into 6 genotypes (1 to 6) and more than 70 subtypes (termed a, b, c, d, . . .), according to the International standardization of the Nomenclature. On the other hand, the distribution of HCV genotypes is linked to geographical location and mode of transmission.
Objectives: The aim of the present study was to define the distribution of HCV genotypes/subtypes in a cohort of Greek patients and the possible routes of transmission.
Subjects and Methods: in 258 serum samples (from 154 males and 104 females), HCV viral load was determined by Cobas Amplicor kits and subsequent HCV genotyping was performed using the Versant (Lipa) assay, detecting the 1a, 1b, 1a/1b, 2a/2c, 2b, 3a, 3b, 3c, 3k, 4a/4c/4d, 4c/4d, 4b, 4e, 4f, 4 h, 5a, 6a/6b subtypes.
Results: the frequencies of HCV genotypes/subtypes found in serum samples tested have as follows: genotype 1: 40.7% (males 30.5%, females 56.7%), genotype 2: 8.9% (males 6.5%, females 12.5%), genotype 3: 38.3% (males 48%, females 24%), genotype 4: 12% (males 15.6%, females 6.7%) and particularly subtype 1a: 11.6% (males 9.1%, females 15.4%), subtype 1b: 25.6% (males 17.5%, females 38.5%), subtype 1a/1b: 1.5% (males 1.9%, females 0.9%), subtype 2b: 0.4% (males 0%, females 0.9%), subtype 2a/2c: 8.5% (males 6.5%, females 11.5%), subtype 3a: 38.3% (males 48%, females 24%), subtype 4a/4c/4d: 0.7% (males 0.6%, females 0.9%), subtype 4c/4d: 2.3% (males 2.6%, females 1.9%), subtype 4 h: 4.2% (males 6.5%, females 0.9%).
Conclusions: (1) The two major HCV genotypes among Greek patients are 1 and 3 (particularly subtypes 1b and 3a), like in other populations in Europe and North America. (2) The high frequency of subtype 3a (48%) in males may be attributed to needle-sharing in intravenous drug users, whereas iatrogenic procedures (blood-transfusions, operations etc) may be responsible for the high incidence of subtype 1b (38.5%) among females. (3) It is also pointed-out the relatively high frequency of genotype 4 (12%) in Greek patients, which mainly characterizes patients from Middle East and North Africa.
Besides epidemiological considerations, HCV genotyping in Greek patients has also a clinical impact, as particular HCV genotypes are associated with severity of liver disease and response to antiviral therapy (e.g. subtype 1b is correlated with lower rates of response to interferon-α).

P1113 Patients PCR status and hepatitis C avidity assay

S. Klimashevskaya, T. Ulanova, A. Burkov, H. Fields, A. Obriadina (Nizhny Novgorod, RU; Atlanta, US; Saronno, IT)*

Objectives: Antibody Avidity is serological marker of primary infection and avidity test is a reliable method to distinguish acute primary HCV infections and chronic HCV infections for certain. But during this study some limitations for the avidity assay were found.
Methods: 220 samples from 21 commercially available seroconversion panels, 480 samples from anti-HCV and HCV RNA-positive blood donors and samples from 21 anti-HCV positive, but HCV RNA negative blood donors with resolved infection were tested. The detection of antibody avidity was based on an indirect ELISA method using a mixture of antigens, containing epitopes to core-1b, NS3−1a, 1b and NS4 (the artificial mosaic protein contains the HCV NS4 immunodominant regions from 1, 2, 3, 5 genotypes).
Results: The mean AI value for seroconversion samples, obtained <65 days after the last anti-HCV negative result was 18.6% (95% CL, 3.5% to 33.7%). Samples from anti-HCV and HCV RNA positive patients with chronic HCV infection showed the mean AI value of 100% (95% CL, 83.1% to 116.9%). Samples from patients with resolved infection showed a mean AI of 54% (95% CL, 32.8% to 75%). The observed differences were significant ($P < 0.001$).
Patients with high PCR level had an AI increase during a shortest time than patients with low PCR Level. The best correlation between AI value and time after infection onset was observed from patients with PCR status more than 1,000,000 copies: $y = 0.83x + 1.5$ against $y = 0.3x + 31$ for patients with PCR status 10,000–100,000 copies or $p = 0.08x + 49$ for patients with PCR status <1,000 copies.
Conclusions: The AI may depends on patient PCR status. Persons with low PCR level or PCR negative (resolved infection) may have the low AI for a long time and the avidity assay is more reliable for PCR positive specimens with high PCR level.

P1114 Switching of two genotypes of hepatitis A virus and clinical relevance in the Republic of Korea over the past 10 years

Y.K. Yoon, H.S. Sim, J.Y. Kim, D.W. Park, J.W. Sohn, J.E. Yeon, J.H. Kim, M.J. Kim (Seoul, KR)*

Objectives: As a result of declining seroprevalence of hepatitis A virus (HAV) in the age of 10–20 years adolescents and young adults, an outbreak of HAV infection with severe presentation among adults has sustained in the Republic of Korea (ROK) since 2008. This study aimed to investigate clinical features of HAV infection and the predominant HAV genotypes in relation to influencing factors on disease severity over the past ten years.
Methods: Study subjects included acute hepatitis patients with anti-HAV IgM in 1997–2000 years (group 1, $n = 49$) and in 2009 year (group 2, $n = 66$). Groups stratified by year, HAV genotype or disease severity (the level of ALT) were compared for clinical features. To determine HAV genotypes, nested RT-PCR targeting the VP1/2A and VP1 regions and sequencing were carried out from serum or stool samples collected. The VP1 region (~900 bp) was used for phylogenetic analysis.
Results: The patients in group 2 were older (21.7 $\pm$6.0 vs 33.6 $\pm$6.7, $p < 0.0001$) and had a longer hospitalization (5.6 $\pm$4.8 vs 18.8 $\pm$9.4, $p < 0.0001$), a higher level of ALT (915.8 $\pm$2341.4 vs 3134.8

±1817.5, $p < 0.0001$) and INR of prothrombin time (1.1 ±0.3 vs 1.3 ±0.4, $p = 0.003$), and lower levels of platelets (219.1 ±81.4 vs 161.8 ±68.0, $p < 0.0001$) and albumin (4.14±0.47 vs 3.60±0.40, $p < 0.0001$). Genotyping of 82 HAV isolates revealed that the predominantly HAV strains circulating during the period of 1997–2000 and year 2009 was genotype IA (96.4%) and genotype IIIA (92.6%) respectively. In multivariate analyses, there was significant differences between two groups stratified by year in age [odds ratios= 1.47, 95% confidence interval= 1.08–2.00, $p = 0.015$] and genotype [OR = 134.46, CI= 10.31–1752.78, $p < 0.0001$]. All 27 isolates in group 1 and 4 isolates in group 2 belonged to genotype IA showed high identities at the nucleotide level with each other with homology 97–100% and 98–100% respectively. Between group 1 and group 2, homology of genotype IA isolates was 97–99%. Comparison by HAV genotype (IA or IIIA) in multivariate analyses presented no difference in clinical features. Comparison by disease severity in multivariate analyses revealed the significant factors of patient's age [OR = 57.50, CI= 14.12–234.22, $p < 0.0001$] and IIIA genotype [OR = 22.49, CI= 6.74–75.08, $p < 0.0001$].

Conclusion: Our study indicates the changing molecular epidemiology as well as host factors might be associated with severe clinical features of HAV infection in the ROK.

P1115 Hepatitis C genotyping in haemodialysis patients and the development of genotyping method based on Taqman probe real-time PCR

H.A. Hamzah, M.I. Mustafa, M.S. Abdul-M, A. Nasarudin, M.H. Bin Hasmani (Kuantan, MY)*

Objectives: This is a seroprevalence study of HCV genotypes amongst infected haemodialysis patients in Pahang, Malaysia and the development of genotyping method using real-time PCR.

Methods: Patients were screened using ELISA by detecting the anti-HCV in the sera. HCV RNA was detected by RT-PCR technique targeting 5′UTR region. All negative first-round PCR products were re-tested by nested PCR. The base sequence of the PCR products was determined using the same primers as for the RT-PCR. By comparing the obtained nucleotide sequence data with sequences of known genotypes from the NCBI homepage, we deduced that our local isolates could be assigned to genotypes 1, 3, 4 and 6. Based on the data, TaqMan® probes were designed for the simultaneous identification and quantitation of these genotypes. A new batch of blood samples was recollected from patients and one step real-time RT-PCR (Applied Biosystem) assay was conducted for genotyping and viral load estimation.

Results: Out of 472 patients, 43 (26 males) were diagnosed positive for anti-HCV by ELISA. Two seropositive patients were excluded as they refused to give consent. Excluding another two patients who were seroconverted to HCV negative, 66.6% (26/39) were of genotype 3, 23.1% (9/39) of genotype 1, 5.1% (2/39) of genotype 6, followed by 2.6% (1/39) of genotype 4 and one patient gave a discordant result with the sequencing analysis.

Conclusion: Genotype 3 was the most prevalent genotype followed by genotype 1, 6 and 4. TaqMan real-time PCR has the potential as a method for rapid HCV genotyping.

P1116 Hepatitis D virus replication – clinical impact and viral replicative interference with hepatitis B virus

G. Popescu, D. Otelea, C. Popescu, M. Tinischi, S. Paraschiv, E. Neaga, L. Gavriliu, M. Popa, E. Benea, A. Streinu-Cercel on behalf of the HED-DM Group*

A viral replicative interference between hepatitis B virus (HBV) and hepatitis delta virus (HDV) with HDV dominance seems to exist; we studied the extent of this interference. The clinical outcome of chronic HBV hepatitis is worse with a dual HBV-HDV infection; we would to evaluate if a replicative HDV infection is a supplemental risk factor for these patients.

Methods: The study took place at Matei Bals Infectious Diseases National Institute (Bucharest, Romania), between June 2006 and December 2006. We included 223 chronic HBV infected patients positive for anti-HDV assay. Controls consisted of 112 chronic HBV monoinfected patients. The interactions between HDV and HBV were studied by measuring HBV-DNA and HDV-RNA levels. Clinical data were collected for both groups: ALT level, platelets count, prothrombin level, and severe liver disease.

Results: From study group, 179 patients (80.27%) were viremic for at least one virus, and 61 patients had dual HBV/HDV viremia (27.35%). HBV viremia was reported in 64.57% patients in study group and in 81.11% patients in control group ($p = 0.006$); difference is conserved for naïve treatment patients from both groups: 67.33% versus 86.21% (pYates=0.001). HBV-DNA significant level (>104 copies/ml) was less frequent in anti-HDV positive group: 18.39% versus 33.33% patients. The detection rate of HDV RNA among anti-HDV-positive patients was 42.60% versus only 2.68% in control group ($p = 0.0001$). HBV replication was not different in the HDV viremic and non-viremic patients from study group: 22.1% versus 24.2%. All analyzed clinical parameters were more altered in anti-HDV positive group; clinical parameters were more altered for HDV viremic versus HDV non-viremic patients: elevated ALT values: 58.95% versus 28.91% (OR 3.40; $p = 0.00001$), thrombocytopenia (OR 2.22; $p = 0.003$), decreased prothrombin index (OR 2.01; $p = 0.03$), but not for end-stage liver disease: 18.95% versus 10.93% (OR 1.90; $p = 0.09$).

Conclusion: HDV is acting as a "dominant" virus in HBV-HDV dual infection; HBV replication is less frequent and at lower levels as in HBV monoinfected patients. Clinical parameters are worse in anti-HDV patients, and worst in those who are HDV viremic. Less than half anti-HDV positive patients are HDV viremic, raising the question of optimal treatment for those with only HBV replicative infection.

P1117 Epidemiology of hepatitis D virus infection in Bucharest area (HED-DM study)

G. Popescu, C. Popescu, M. Ivan, L. Gavriliu, D. Antonica, A. Paise, G. Ducu, M. Popa, E. Benea, A. Streinu-Cercel on behalf of the HED-DM Group*

Several studies indicated a decreasing trend in the hepatitis D virus (HDV) prevalence in different regions. However, in the past twelve years no studies were conducted to evaluate if there is a similar epidemiological change in the Bucharest area.

Objective: This study was undertaken to obtain regional epidemiological data on hepatitis delta virus (HDV) in symptomatic or asymptomatic patients infected with hepatitis B virus (HBV).

Methods: We conducted a cross-sectional study at "Matei Bals" National Institute of Infectious Diseases (Bucharest, Romania), between June 2006 and November 2006. A total of 1094 patients with HBsAg positivity were evaluated for HDV antibodies with SmarTest Diagnostics (Orgenics Ltd, USA). We explored the association between anti-HDV positivity and several epidemiological and clinical parameters; HDV genotypes were evaluated in PCR positive patients.

Results: Anti-HDV was positive in 223 of 1094 patients – 20.38%, CI95% (18.10%; 22.87%), significantly lower than 30.09% in a previous study, $p < 10^{-7}$. A significant association between the age over 40 years and anti-HDV positivity was found RR = 1.58 (1.19; 2.09), $p = 0.0009$. Gender, HBeAg positivity, liver disease stage and HIV or HCV co-infection were not significantly associated with anti-HDV presence ($p > 0.05$). Delta antigen was present in only one patient with anti-HDV antibodies (0.45%). Dual HDV and HBV infection was associated more frequently with elevated ALAT ($p = 0.0004$), with thrombocytopenia (RR = 8.46, $p < 10^{-7}$) and with end-stage liver disease RR=6.05 (4.52; 10.42), $p < 10^{-7}$. All the 26 HDV srains belonged to genotype I.

Conclusion: The results suggest that delta infection remains common in our patients, but a transition towards a decreasing prevalence is possible in Bucharest area. We found a significant relationship between the age of the patients (possible longer HBV infected status) and anti-HDV positivity; delta antigen can not be considered as a marker of HDV replication.

P1118 Prevailing HCV genotypes and subtypes among HIV-infected patients in Georgia

M. Karchava, L. Sharvadze, L. Gatserelia, N. Badridze, T. Tsertsvadze (Tbilisi, GE)*

Introduction: Recent analysis of ART program data in Georgia showed that end-stage liver disease is leading cause of death among HIV/HCV co infected patients on ART after initiation of ART program in 2005. Therefore, treatment of hepatitis C among HIV infected individuals is essential for sustaining success of ART in Georgia and to improve survival rates of HIV infected patients. HCV treatment response rate and sustained viral suppression after antiviral treatment largely depends on the HCV genotypes along with the influence of HIV co-infection itself. The objective of this retrospective study was to study the prevailing genotypes and subtypes of HCV virus in a cohort of HIV infected patients in order to obtain the preliminary data.
Material and Methods: We analyzed 1490 HIV infected patients co infected with HCV virus. Among them 59% were HCV coinfected. Measurement of HCV RNA viral load was done by COBAS TaqMan HCV-2.0 Test and HCV genotyping by reverse hybridization line probe assay using VERSANT HCV Genotype kit 2.0 respectively.
Results: Detectable HCV RNA was found among 680 patients (91.%). Less than 10 Iu/ml was found among among 67 (9%). This numbers indicate low rate of self clearance of the virus among HIV persons. Greater HCV RNA levels were associated with a greater chance to be infected with HCV genotypes 1. Most prevalent genotypes were genotype 1 accounting for 41.6%, followed by genotype 3 (34.7%) and 2 (17.6%), inter genotype recombinants were found among 5.8%.
Conclusion: Study demonstrated high prevalence of HCV infection among HIV-infected patients and reveals 1 as predominant genotype. The differences of prevailing HCV genotypes among general population and HIV co infected group was probably attributed to the different methods for sample selection used. Another option can be possible influence of diverse transmission networks among HIV infected group. The high number of intergenotype recombinants might be results of continues parenteral exposure to different HCV genotypes during drug injection paraphernalia. Study highlights and strengthens the need for careful follow-up of HCV/HIV co infected patients, effective management and therapies against HCV in order to reduce liver related death rates in patients on ART.

P1119 Hepatitis D in Switzerland: a silent epidemic

I. Rossi, D. Genné (La Chaux-de-Fonds, CH)*

Objectives: The seroprevalence of co-infection with the hepatitis B virus (HBV) and the hepatitis D virus (HDV) is well known in many European countries, starting from 6.8% in Germany to more than 27% in some Turkish areas. To better describe this infection in Switzerland, and to characterize affected people, a questionnaire was sent to all Swiss gastroenterologists, hepatologists and infectologists.
Methods: 369 physicians received a questionnaire concerning their HBV- and HDV-infected patients.
Results: A cohort of 101 HDV-positive patients followed by 78 specialists were analyzed. The physicians were in charge of 1699 patients with HBV (HBsAg-positive), giving a 5.9% prevalence of HDV infection in HBV-positive patients. A predominance of males (75%) from Switzerland (39%), and African origin (21%) was recorded. Co-infection with HBV was reported at 16% and superinfection for 3% of them; the relationship of time between the two infections was unknown for 73% of the patients. Most were contaminated by intravenous drug use (62%), followed by vertical transmission (15%), sexual contact (13%), or transfusion (2%). The majority (74%) had a very low ($<10^3$ UI/ml) HBV viral load and 75% were HBeAg-negative. 76% of those who had a liver biopsy had an advanced disease ($\geqslant$ F2, 77%), and only 21% had received a standard therapy (interferon or pegylated interferon α). Meanwhile, 10% had healed spontaneously (anti-HBs-positive).
Conclusion: With a prevalence of 5.9% of hepatitis D in HBsAg-positive patients, Switzerland seems less affected than most other European countries; it is, however, possible that this infection is underdiagnosed. Intravenous drug use is the main risk factor. Associated advanced liver disease is also very common.

P1120 Epidemiological analysis of potential risk factors contributing to infection of HBV, HCV and HIV among the population in Tripoli Area, Libya

A. Shabash, M. Habas, A. Fara, M. Daw (Tripoli, LY)*

Hepatitis B, Hepatitis C and Human Immune Deficiency viruses are a major cause of concern among health institution with great social and economic impacts on each country. Their epidemiology and associated factors varied geographically. Tripoli is the largest populated city in Libya.
The objectives of this study were to: (1) determine the prevalence of HBV.HCV and HIV among Tripoli population. (2) Study the potential risk factors associated with the infections of these viruses. (3) Study social clustering of these viruses among the infected individuals. (4) Alarm the public health authorities about the risky population categories.
Methods: A total of 878 persons screened to be positive of one or more of the Viruses(HBV, HCV, & HIV) using ELISA test were studied. Their age varies from 14 to 72 years. The risk factors including, IV drug abuse, blood transfusion, surgical and dentistry involvement etc were analyzed. The social back ground and the social clustering were studied and the data were analyzed using Microsoft word excel 2007.
Results: Of the total individuals studied, 654 (74.4%) were HBV positive, 204 (23.2%) HCV positive, and 47 (5.3%) HIV positive. 13 (1.4%) persons were seropositive for both HIV and HCV antibodies, eight (0.9%) persons were seropositive for both HBV and HCV, while only three (0.3%) persons were seropositive for HBV, HCV and HIV. 726 (82%) males were infected with one of the viruses, though its only 152 (17%) females were infected. Male to female ratio were (6.8:1) for HIV infected individuals followed by HCV (4.6:1) and then HBV (2.4:1). The age peak of the infected individuals with HBV were range between 18–29 years, and with HCV were range between 33–40 years, HIV ranged between 20–36 years with highest peak were 20 years. The most infected areas were in a form of clusters. The potential risk factors were surgical intervention, dentist visit, blood transfusion, and drug abuse respectively.
Conclusion: HBV, HCV, and HIV infections were a major problem among Tripoli population with young people were more borne to be infected, this were associated with certain clustering among the society. This however is an alarming problem to the Libyan society and hence then specific programs have to be implemented to tackle the problem.

P1121 Six-year study of hepatitis A, B and C infection prevalence in a Greek major hospital

V. Karabassi, M. Pouyiouka, K. Achilleos-Geladaris, C. Petrocheilou, A. Kiriakaki, M. Toutouza (Athens, GR)*

Objectives: The purpose of this study was to perform a serological analysis of Hepatitis A, B and C virus (HAV, HBV and HCV) infection prevalence in the patients of a Greek major hospital within a six-year period.
Methods: During the six last years 1/7/2003–30/6/2009, sera from 31580 patients (26180 in-patients and 5400 out-patients), were tested for the detection of HAV, HBV and HCV markers. There were 4 groups of patients, Group A: 10130 patients (7490 in-patients, 2640 out-patients) who were controlled for HBV surface antigen (HBsAg), Group B: 21450 patients (18690 in-patients and 2760 out-patients) who were controlled for the HBV markers: HBsAg, HBeAg, antiHBe, antiHBcore, antiHBs, Group C: 22320 patients (19200 in-patients, 3120 out-patients) who were controlled at the same time for HCV-antibodies and Group D: 3220 patients (2460 in-patients, 760 out-patients) who were controlled

for HAV- antibodies (total and IgM). All tests were performed during 2003–2004 by MEIA methodology (AXSYM – Abbott) and during 2005–2009 by immunoluminometric assay method (ARCHITECT – Abbott).
Results: In Group A of the 7490 in-patients and 2640 out-patients were found to be positive for HBsAg 150 (2%) and 82 (3.1%) respectively. In Group B of the 18690 in-patients and 2760 out-patients were respectively: 8728 (46.7%), 1220 (44.2%) seronegative for HBV markers, 1214 (6.5%), 345 (12.5%) positive for HBsAg, 2952 (15.8%), 174 (6.3%) positive for antiHBs due to HBV vaccination, 5794 (31%), 1020 (37%) negative for HBsAg and positive for the rest HBV markers. In Group C of the 19200 in-patients and 3120 out-patients, HCV-antibodies positive was found in 442 (2.3%) and 188 (6%) respectively. 2% of them were at the same time positive for HBsAg. In Group D of the 2460 in-patients and 760 out-patients, HAV- antibodies total positive was found in 1304 (53%) and 388 (51%) and HAV- antibodies IgM positive was found in 50 (2%) and 10 (1.3%) respectively.
Conclusions: This study showed that there is a significant prevalence of HAV, HBV and HCV infection in the patients of our hospital (53%, 37.5%, 2.3% for in-patients, 51%, 49.5%, 6% for out-patients respectively). The effort for the prevention must be continual and the control measures with the systematic Hepatitis A and Hepatitis B vaccination should be strictly enforced.

P1122 HBV infection in immigrant population in Barcelona, Spain. Immune tolerant phase and description of an atypical serological pattern

E. Dopico, E. Grenzner, J. Ros, L. Guerrero, E. Castro, L. Hernández, T. Vinuesa (Barcelona, ES)*

Introduction: In low-prevalence areas, like Spain, HBV infection is acquired mainly among young adults by sexual or parenteral transmission and people with high replicative viral activity present hepatic injury.
In some world regions, like Eastern Asia, the most common transmission occurs in the perinatal or early childhood period. On those cases, the immune tolerant phase characterized by absence of hepatic damage can span of more than 20 years.
Objective: The aim of the present study was to describe the cases of chronic hepatitis B in patients showing a high replicative activity and a normal hepatic function.
Methods: We included in the study 37 patients with chronic HBV infection and HBeAg reactivity, detected from January 2008 to October 2009, in the Clinical Laboratory of l'Hospitalet-Cornellà of the Public Health System that attends around 750000 inhabitants.
HBeAg and anti HBc were analyzed by chemiluminescent immunoassay (Vitros® Johnson & Johnson), and HBV-DNA was quantified by Abbott RealTime HBV DNA Assay®. The immune tolerant phase was defined by HBeAg presence, viral DNA $>10^6$ UI/mL and normal serum ALT levels.
Results: Of the 37 patients, there were 15 men and 22 women, 7 of them were pregnant.
Fifteen (40.5%) had normal ALT levels and 6 of them were in the immune tolerant phase as they had viral loads >106 copies/ml.
The origin of those 15 patients was: 9 Eastern Asia, 3 Eastern Europe, 2 Northern Africa and 1 Western Africa. The immune tolerant patients came in 6 cases from Eastern Asia and one from Western Africa. In five of the 37 patients, anti HBc was negative and their viral loads were $>10^6$ UI/mL.
Conclusions: In our environment is not uncommon to find patients infected by HBV with a high replicative activity and normal hepatic function. They come from high-prevalence areas, mostly Eastern Asia. Some of them are in the immune tolerant phase. The infections detected in pregnant women were coming from the screening procedure. A widely used diagnostic algorithm for screening hepatitis B virus infection is based in anti HBc detection. We have to consider the atypical serological pattern of anti HBc absence and high levels of viral DNA in serum, especially in patients from Eastern Asia. Those patients have a high risk of sexual and perinatal transmission.

P1123 Forecast modelling for prediction of hepatitis B and hepatitis C seropositivity among Libyan population

A. Shabash, M. Habas, A. Alhajrasi, A. Furarah, A. Bouzedi, M. Daw (Tripoli, LY)*

Blood born hepatitis (HBV, HCV) is a major health problem particularly among developing countries. Hence then specific strategies have to be implemented to overcome such problem. In Libya national strategies has been developed to overcome such problem.
Objectives of this study were to: 1) determine the prevalence of hepatitis B and C seropositivity among healthy Libyan population; 2) implement a new model for short and long term forecasting of HBV and HCV seropositivity; 3) outline the major steps to reduce the risk of such viruses.
Methods: Data of hepatitis B surface antigen and HCV antibody screening for 100,8214 person were reviewed between 2003–2008 at three main laboratories in Tripoli Libya using ELISA test. The data subjected to forecasting function of excel 2007 to predict the number of seropositive patient after 2, 5 and 10 years.
Results: Of the total patients studied: 19878 (1.97%) were HBV positive, and 17087 (1.77%) were HCV positive during the study period. The prevalence of HBV seropositivity were decreased during the study period from 3.5% on 2003 to 1.8% on 2008, while HCV prevalence decreased from 2.6% on 2003 to 1.5% on 2005 though it was 1.8% on 2006, and 1.7% during the last two years. The prevalence of HBV were more than that of HCV throughout the study. Using the forecasting function test of excel the predicted number of HBV seropositive person after two, five and ten years in comparison to 2008 (2010:2008, 2013:2008, 2018:2008) the ratio were 1.5:1, 2:1, 2.5:1, while the expected HCV positive person ratio were 1.5:1, 2:1, 2.8:1.
Conclusion: Our study reveal that, HBV and HCV infection was an alarming problem among Libyan. Further evaluation is needed to determine possible new strategies to control the spread of infection, forecast model was valuable in highlighting such problem. In addition assessment of effectiveness and continuity of the previous preventive measures like vaccination and education programs is mandatory.

P1124 Seroepidemiological analysis of hepatitis C genotypes among Libyan population and its association with risk factors

H. Elasifer, B. Elagy, M. Eltaghdy, M. Daw (Tripoli, LY)*

Introduction: Hepatitis C virus is major public health problem and it is one of the most important causes of chronic liver disease. It has high rate of spontaneous mutation which lead to a marked degree of heterogeneity with a high molecular diversity world wide.
Objectives: To determine (1) epidemiology of HCV genotypes among different Libyan patients, (2) its association with the risk factors involved among the patient studied.
Methods: 479 patients with hepatitis C virus were studied. They were recruited from the Department of Infectious Diseases at Tripoli Medical Centre, from January 2003 to October 2008. The data collected was designed to extract information from patients. The patients were positive for HCV antibody. HCV genotyping was performed. The data were analyzed using SPSS version 11.5 to identify the distribution of different genotypes and its association with gender, age, and risk factors.
Results: 479 patients were studied. Their ages ranged from 16 to 84 years. Genotypes 5 and 6 were not detected in any patient. The prevalence of genotypes were (G1) 32.6%, G2; (15%), G3; (16.7%) and G4; (35.7%), according to subtypes 28% were unclassified G4, 14.6% were G1b and some patients infected with more than one subtype (2.3% G4c/d, 1% G2a/c), according to gender in male G4 the commonest while in female G4 is the commonest the relationship between HCV genotype and gender was significant (P value=0.00). All genotypes were common in patients less than 44 years except genotype 2 (G1 were 71.8%, G2 were 31.9%, G3 were 88.8%, G4 70.2% their age less than 44 years). Genotype1 and genotype 4 were common in patients with history of dental procedures, history of surgery was common in

patients with G4 and G1, blood transfusion history was common in patients with G4, IVDA history was common in patients with G1 and G3, according to subtypes G1b was commonest in blood transfusion and surgical procedure. The relationship was insignificant (P = 0.180). The relationship between HCV risk factor for infection and gender was significant 'P = 0.000'.
Conclusion: the epidemiology of HCV among Libyan patients was variable and all genotypes were detected except genotypes 5 and 6, further more specific genotypes were associated with certain risk factors, hence then HCV genotypes could be used to monitor and follow HCV infection in Libya.

P1125 Prevalence of hepatitis C virus among patients attending a hospital in Sirte, Libya

S. Abddulsalam Bagar, B. Abdallah, F. Neferro (Sirte, LY)*

Background: Hepatitis C virus is a major global health care problem. The WHO estimates that up to 3% of the world's population has been infected with the virus, equating to more than 170 million carriers of HCV worldwide.
Objective: The present study was aimed to estimate the prevalence of hepatitis C virus in this area and compare that with other regions worldwide and other regions in Libya.
Patients and Methods: A retrospective study included 17419 patient's serum attended the medical center at Ibn Sina Hospital in Sirt region during the years 2007 and 2008 for the presence of anti HCV antibodies among them by ELISA method.
Results: Out of 17419 patients, 640 cases were HCV positive (3.67%). Among then 482 males (75.31%) and 158 females (24.69%). The majority of the positive cases belong to the age group 31–40 years.
Conclusions: The percentage of the HCV positive result study was 3.67% and that correlate with the results in other regions of the world. Another study should be carried out and the necessary measures to be taken to know the carriers and the infected individuals.

P1126 Prevalence of hepatitis B surface antigen among patients attending a hospital in Sirte, Libya

S. Abddulsalam Bagar, B. Abdallah (Sirte, LY)*

Background: Hepatitis B virus (HBV) infection is a global health problem, with an estimated 400 million being chronic carriers of the virus that equating up to 5–10% of the world's population, most of them in south of the desert in Africa and in Far East countries.
Objective: The present study was aimed to estimate the prevalence of hepatitis B virus in the Sirt region and compare that with other regions in Libya, and also with other regions in the world.
Patients and Methods: A retrospective study included 17419 patient's sera attended the medical centre at Ibn Sina Hospital in Sirt region during the years 2007 and 2008 for the detection of the presence of HBs antigen by ELISA method.
Results: Out of 17419 patients, 1325 cases were HBV positive (7.66%). Among them 925 males (69.25%) and 410 females (30.71%). The majority of the positive individuals belong to the age group 31–40 years.
Conclusions: The percentage of the HBV positive in the present study was (7.66%) and that correlate with other results in other regions of the world which carried out in the same measures as in the present study.

P1127 High frequency of SEN virus viraemia in patients with hepatitis B virus and hepatitis C virus infection in Iran

A. Karimi-Rastehkenari, M. Bouzari (Isfahan, IR)*

Objectives: After nomination of two viruses of HGV/GBV-C and TT virus as possible causes of inaugurating hepatitis with unknown etiology, there has been a continuing search for detection of additional hepatitis viruses. On July 20, 1999, SEN virus (SENV) was discovered in the serum of a human immunodeficiency virus type 1 (HIV-1)-infected patient possessing hepatitis with unknown etiology in Italy, and was named on the basis of initials of the patient. SEN virus is a blood-borne, circular single-stranded DNA virus. Among nine genotypes of SENV (A to I), SENV-D and SENV-H genotypes have strong association with non-A-E hepatitis.
Methods: Nested-PCR was used for detection of partial ORF1 of the SENV-D and SENV-H genotypes in 120, 50 and 50 sera from healthy blood donors and patients with hepatitis B virus and hepatitis C virus infection, respectively in Guilan Province, North of Iran. Also, to explicate a possible role of SEN virus associated with HBV and HCV on progression the liver inflammation, alanine and aspartate aminotransferases were measured.
Results: SENV was identified in 109 out of 120 healthy blood donors (90.8%), in 47 out of 50 patients with HBV infection (94%) and in 48 out of 50 patients with HCV infection (96%). Frequency of SENV-D and co-infection (both SENV-D and SENV-H) viraemia was significantly higher among patients with hepatitis B and C infection than healthy blood donors ($P < 0.05$). Frequency of SENV-H viraemia was significantly higher than SENV-D among healthy individuals. Alanine and aspartate aminotransferases levels were significantly correlated with HBV and HCV viraemia but no with SENV viraemia. High genomic homology observed between our sequences and some of the TT virus isolates.
Conclusion: The high rate of co-infection shows that different genotypes of the virus have no negative effects on each other. The high frequency of SENV infection in patients with hepatitis B virus and hepatitis C virus indicate that there is the common route of blood transfusion among these viruses. High frequency of SENV infection in healthy blood donors indicates that other routes rather than blood transfusion also are important. High genomic homology observed between our sequences and some of the TT virus isolates may be the outlook to the evolutionary history of SENV in relation to TT virus. SEN virus with high frequency in patients with hepatitis B and C infection and healthy individuals is capable of infecting all age groups that can be nominated as an endemic virus in Guilan Province, North of Iran.

P1128 Prevalence of hepatitis E (HEV) infection in pregnant women and clinical features associated to the detection of antibodies to HEV in Spain

G. Gabilondo Alvarez, B. Romero, M. Rodriguez, O. Martin, M. Mateos (Madrid, ES)*

Objective: hepatitis E virus (HEV) is the main cause of acute viral hepatitis in developing countries. Acute infection by HEV generally causes mild and self-limited hepatitis but it can also produce fulminant hepatic failure in pregnant women and is associated with very high mortality particularly in the third trimester of pregnancy.
The goal of this study was to investigate the seroprevalence of HEV infection in pregnant women and the clinical features related to the detection of antibodies to HEV in blood.
Methods: 769 pregnant women attended in the Obstetric Department for routine screening were included in this study. 181 patients were studied in 2007 and 588 in 2009. The range of age was 14 to 45 years (media: 30.6 years). None of the patients presented clinical symptoms associated with hepatitis when the blood sample was withdrawn. ALT/AST levels and clinical symptoms were assessed in all patients with positive results. IgG anti-HEV antibodies were detected in serum by a commercial immunoenzymatic method and all positive samples were further studied for the presence of IgM anti-HEV antibodies (HEV Ab and HEV IgM, Dia.Pro Diagnostic Bioprobes, Milan, Italia).
A result was considered positive when the sample's optical density/cut off optical density was superior to 2. Positive results by the immunoenzymatic method were confirmed by Western Blot analysis (RecomBlot HEV IgG/IgM, Mikrogen, Martinsried, Germany).
Results: IgG anti-HEV antibodies were found in 2.1% (5/181) in the 2007 group, and 4.08% (24/588) in the 2009 group, and the overall prevalence was 3.7% (29/769).
IgM anti-HEV antibodies were detected in 0.52% (4/769) suggesting acute or recent infection. None of them presented clinical symptoms

related to acute viral hepatitis. ALT and AST had normal values in all the patients who presented IgG and IgM anti-HEV in serum.
Conclusions: asymptomatic HEV infection may be detected in pregnant women in non-endemic countries. Seroprevalence of IgG HEV antibodies was 3.7% and it has not increased in our country in these recent years in spite of the increase in immigration and travels to endemic areas.

P1129 Outcomes of infants born from chronic hepatitis B virus infected mothers

M. Hasanjani Roushan, M. Soleimani Amiri, M. Baiany, M. Javanian, M. Sadeghi (Babol, IR)*

Objectives: Infants born from chronic hepatitis B virus infected mothers are predisposed for developing of HBV infection. The purpose of this study was to assess the outcomes of infants born from chronic HBV infected mothers.
Methods: From April 2004 to September 2009, infants born from chronic HBV infected mothers were received Hepatitis B Immune Globulin 0.5 cc and the first dose of hepatitis B vaccine at birth. The second and third doses of HBV vaccine were administered at 1 and six months of age. Hepatitis B surface antigen (HBsAg) and anti-HBs were performed at 12 months of age. Those who had anti-HBs titers <10 mIU/ml received the second series of HBV vaccine.
Results: Thirty-four and 201 infants born from chronic HBeAg or anti-HBe+ mothers, respectively. HBsAg was detected in 6 (17.6%) cases born from HBeAg positive mothers and in 3 (1.5%) cases born from anti-HBe+ mothers ($p = 0.0001$). Anti-HBs >10 mIU/ml was developed in 204 (86.8%) cases. The mean anti-HBs levels in infants born from HBeAg+ mothers was 284.23 ± 257.2 and for infants born from anti HBe+ mothers was 367.2 ± 322.8 mIU/ml ($p = 0.213$). Twenty-two (9.4%) cases were not responder and among them 11 cases responded to the second series of HBV vaccine.
Conclusion: The results of this study show that infants of HBeAg positive mothers have higher risk of developing of HBV infection due to higher viral load. Reduction of the viral load in this group of mothers is recommended.

P1130 Pregnancy and chronic hepatitis B infection: a 3-year evaluation

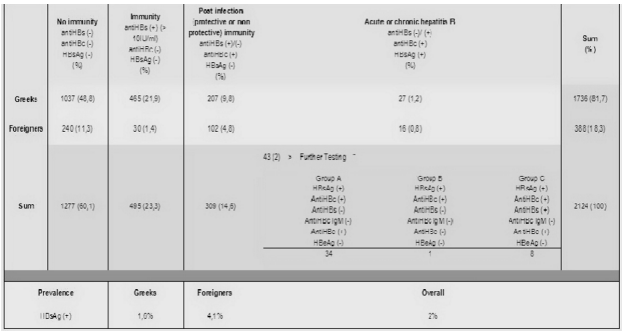
B. Kurtaran, H.S. Aksu, A. Candevir, A.S. Inal, S. Komur, Y. Tasova (Adana, TR)*

Objectives: Vertical transmission of HBV is an important risk factor for chronic infection in newborns. Pregnant women with positive hepatitis B surface antigen should be screened in the early period of pregnancy. Babies who born to mothers with hepatitis B infection should be immunized after delivery as soon as possible, especially in 24 hours. This study evaluates the risk factors, epidemiology, the progression of disease and the assessment of antiviral treatment for chronic HBV infection during pregnancy.
Methods: Pregnant patients with chronic hepatitis B infection followed in the Department of Infectious Diseases in the Medical School of Çukurova University. The study comprised between January 2007 and March 2009.
Results: Totally 46 pregnant patients were followed with a mean age of 29 ± 6 (number of the patients were 19;19; 8 according to years). Median age of pregnancy was 16 weeks at the time of first visit (minimum 4, maximum 36 weeks). Chronic hepatitis B infection diagnosis was made in 54.4% of the patients before and 45.6% patients during the pregnancy. Eighty three percent of the patients were HBe Ag negative. Family history was positive in 48% of patients and no additional risk factors were found. Delta hepatitis co/super infection was not detected. Median ALT and HBV DNA values in first visit were 22.5 u/L (8–508 u/L) and 857 iu/ml ($0–11 \times 10^7$ iu/ml) respectively. Patients of 39% were treated. Patients with or higher than 2000 iu/mL HBV DNA level were treated with lamivudine in the last trimester of their pregnancy. Seven patients had a significant increase in liver disease activity after pregnancy, defined as a three times increase in alanine aminotransferase (ALT) within 6 months after delivery. These seven patients (of those under treatment) used lamivudine during last trimester of their pregnancy. Three patients continued lamivudine for six months after delivery. All newborns vaccinated and applied HBV vaccine and HBIG. Only 30% of those patients and their babies could follow after delivery.
Conclusion: Chronic hepatitis B diagnosed in second trimester of majority pregnant women in spite of family history. Small percent of mothers came to follow up after delivery. This means need more attention and education for family practice.

P1131 Hepatitis B serological profile and HBsAg(+) prevalence among women inpatients of an obstetrics-gynaecological unit in Greece

A. Papadimitriou, D. Theocharidou, V. Koulourida, E. Siskou, E. Martziou, S. Charalampidou, E. Sidiropoulos (Thessaloniki, GR)*

Objectives: Vertical transmission from mother to infant is a very important factor for HBV infection transmission. In Greece, HBsAg(+) prevalence is 2%. Also, it must be evaluated women's immigration from countries with endemic HBV infection. The purpose of this study is to evaluate the serological profile of Greek and foreigner women admitted to the obstetrics-gynecological unit of a Greek general hospital.
Methods: 2124 women, admitted to the Obstetrics-Gynecological Unit of Papageorgiou General Hospital during 1/9/2008–31/8/2009, underwent a screening serological testing process, which included HBsAg, AntiHBs, AntiHBc, and further tests such as AntiHBcIgM, HBeAg, AntiHBe, whether required (ABBOTT® kits applied on the ARCHITECT® analyzer). SPSS 16.0 was used for data statistical analysis.
Results: The 388 (18.3%) foreigner's and 1736 (81.7%) Greek's serological profile is shown in the table included. 1277 (60.1%) had negative serological profile and no sign of either past or present infection, or vaccination. 495 (23.3%) were found with antiHBs >10IU/ml. Among 43 (2%) patients with HBsAg(+), who had a median age of 34 years, 27 were Greeks and 16 foreigners and none had signs of acute HBV infection [AntiHBcIgM(−)]. 34 women (Group A) suffered from chronic HBV infection. 1 patient (Group B) was a HBsAg carrier with no signs of HBV replication. Among 8 women (Group C) we observed the presence of both HBsAg and low titles of AntiHBs. Although, the possibility of blood or plasma transfusion before serological testing could not be eliminated, we couldn't ignore the possibility of co-infection with another HBV serotype. As far as, 27 (63.8%) of 43 HBsAg(+) were women of reproductive age (range 17–44), their HBeAg(−)/AntiHBe(+) status showed low risk of vertical transmission during a present or future pregnancy.
Conclusion: Overall HBsAg(+) prevalence reported here is 2%. It is significantly higher ($p = 0.002 < 0.05$, OR: 2.722, 95% CI: 1.452–5.104) among foreign patients (4.1%) than among Greeks (1.6%). Immunization rate (antiHBs >10IU/ml) reported here is 23.3%, significantly higher ($p < 0.05$ OR: 4.366 95% CI: 2.964–6.432) among Greeks (26.8%) than among foreigners (7.7%). Our study shows that immigration can change the epidemiology of HBV in our country, and the necessity of an intensive vaccination program.



P1132 Current vaccination coverage against hepatitis B in pregnant women in Greece: far from the ideal target

I. Elefsiniotis, H. Brokalaki, I. Glynou, I. Zorou, E. Vezali, E. Apostolopoulou, H. Kada, G. Saroglou (Athens, GR)*

Objectives: Vertical transmission of Hepatitis B virus (HBV) infection occurs usually in perinatal period and is responsible for the majority of the disease burden in endemic areas, so screening of pregnant women for HBV serological markers is mandatory. Data concerning the current serological status as well as the vaccination coverage against HBV in women at reproductive age in Greece are limited.
Methods: Between September 2008 and September 2009 a total of 1826 pregnant women (mean age 29.9 years) who gave birth at the Departments of Obstetrics and Gynaecology of 'Elena Venizelou' Hospital of Athens were prospectively evaluated for serological markers of HBV infection.
All women in the study population were screened during delivery process for HBsAg, anti-HBc and anti-HBs, whereas HBeAg and anti-HBe were evaluated only in those who tested positive for HBsAg.
Results: More than half of the study population was originally from Greece (56%), 30.9% was from Albania and 13.1% from Eastern European countries. Overall, 3.83% (70/1826) of pregnant women were HBsAg(+) and the vast majority of them (50/70, 71.42%) were Albanian. Two of 70 (2.85%) HBsAg(+) women were HBeAg(+) whereas the vast majority of them (97.15%) were HBeAg(−). About half (48.6%, 274/564) of the Albanian women exhibited anti-HBc seropositivity followed by Eastern European women (21.3%, 51/239) whereas only 6% (61/1023) of Greek women presented serological markers of previous HBV exposure. Moreover, serological markers of past HBV infection with spontaneous recovery [antiHBc(+) and antiHBs(+)] were observed in 13.8% (252/1826) of the whole study population whereas only 19.76% (361/1826) exhibited vaccination-induced protection [characterised by the presence of isolated antiHBs(+)]. Importantly, vaccination-induced protection rates were significantly higher in Greek women [89.6% of antiHBs(+) Greek women were isolated antiHBs(+)] compared to Albanian [16.2% of antiHBs(+) Albanian women were isolated antiHBs(+)] and Eastern European women [30% of antiHBs(+) Eastern European women were isolated antiHBs(+)] ($p < 0.001$, in all comparisons).
Conclusions: Current vaccination-induced protection rates against HBV are very low among pregnant women in Greece, so surveillance and immunisation programmes targeted at pregnant women are necessary in order to avoid vertical transmission of the infection.
Acknowledgements: The study was partially supported by the Hellenic Foundation of Gastroenterology.

P1133 Prevalence of serum HBV markers among pregnant women in a secondary care hospital, Greece

I. Passalidou, P. Karapavlidou (Kastoria, GR)*

Infection with hepatitis B virus is a special problem for pregnant women. Not only does a pregnant woman face the risks of hepatitis herself, she also can pass the virus to her baby. Hepatitis B virus (HBV) has a high rate of vertical transmission, causing fetal and neonatal hepatitis. Additionally, mother-to-child transmission of HBV infection predisposes to carriage, liver cirrhosis, and hepatocellular carcinoma in young adults. Testing for the HBV is generally a standard, routine test performed in all pregnant women at or before their first antenatal visit, (usually before 12 to 14 weeks of pregnancy).
Objectives: To evaluate the seroprevalence of hepatitis B surface antigen (HBsAg) in a group of pregnant women and to examine the presence of hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe) as well as anti-HBs, anti-HBc-IgG, anti-HBc-IgM antibodies.
Methods: During a period of 3 years, a total of 974 pregnant women were included in the study. Blood was collected during the first trimester of pregnancy. All samples were analyzed for HBsAg, HBeAg, anti-HBs, anti-HBc-IgG, anti-HBc-IgM and anti-HBe. Blood analyses were performed in the Roche Elecsys 2010 immunoenzymic analyzer.
Results: The majority of the study population were from Greece (87.58%) whereas 121 (12.42%) of them were from Albania. Overall, 19 of 974 females (1.9%) were HBsAg(+). The seroprevalence of HBsAg among Greek women was 1.63% (14/853) and among Albanian were 4.13% (5/121). All of HBsAg(+) women were HBeAg(−)/anti-HBe(+). Also, among Greek women: 290 (34%) have been vaccinated, 139 (16.30%) were anti-HBs(+)/anti-HBc-IgG(+)/IgM(−)/anti-HBe(+), and 410 (16.30%) were serum negative.
Among Albanian women: 1 (0.83%) has been vaccinated, 31 (25.62%) were anti-HBs(+)/anti-HBc-IgG(+)/IgM(−)/anti-HBe(+), and 63 (52.07%) were serum negative.
Conclusions: Our results suggest that: (1) The overall seroprevalence rate of HBsAg is 1.95% among pregnant women in Greece. (2) The percentage of serum negative Greek women is relatively low but is higher enough among Albanian, suggesting possibly that the vast majority of the Albanian females have not been vaccinated.

P1135 Prevalence of viral hepatitis among inmates of Bulgarian prisons

G. Popov, K. Plochev, K. Ramshev, Z. Ramsheva (Sofia, BG)*

Objective: To determine the prevalence of viral hepatitis and examine risk factors for this infection among inmates of Bulgarian prisons.
Methods: This study was carried out in four Bulgarian prisons (for men and women) and a juvenile correctional facility. Anonymous cross-sectional data were collected for prisoners who agreed to participate in the study and who were interviewed using a standard questionnaire including demographic, imprisonment history and viral hepatitis related risk behaviors items. Thereafter, the blood drawn from the participants was tested for anti-HAV, anti-HBc, anti-HCV and anti-HDV antibodies and HBsAg and HBeAg by ELISA tests.
Results: A total number of 498 inmates participated in our study. Three hundred and sixty seven (74%) were men and one hundred thirty one (26%) were women. The overall rate of antibody positivity for anti-HAV was 368 (74%), anti-HBc – 292 (59%), anti-HCV – 123 (25%) and anti-HDV – 46 (9%). The presence of huge number of prisoners with viral hepatitis B and C are due to use of i.v. drugs, unprotected sexual contacts, tattoo and other manipulations with skin and mucosa lesions.
Conclusion: The seroprevalence of viral hepatitis among prisoners in comparison with the general population in Bulgaria is very high. Our results indicate the importance of policies to prevent transmission of viral hepatitis during and following incarceration. We recommend providing of free of charge condoms, needles and syringes as well as possibility of voluntary testing for HBV, HCV and HIV.

P1136 Anti-HAV seroprevalence in Turkish military personnel and its relation to demographic properties

H.C. Gul, I.Y. Avci, Ö. Coskun, R. Ogur, Y.H. Basaran, Ç. Güney, B. Besirbellioglu, C.H. Acikel, C.P. Eyigün, A. Pahsa (Ankara, TR)*

Aim: Turkey is a middle endemic area in terms of Hepatitis A Virus (HAV) infection. Previous studies in Turkey showed that most residents had been infected with HAV by the second decade of life. In this study we aimed to detect the anti-HAV seroprevalence rate in Turkish military personnel and its relation with demographic properties.
Material and Methods: Randomly selected 1049 military personnel were enrolled in the study. All were male and their average age was 24.97 ± 6.48 (Range: 20 to 50) years. A written questionnaire about his demographic properties and an informed consent was obtained from each subject. Statistical analysis was performed with SSPS 10.0 software (SSPS, Inc, Chicago, III). Differences were considered significant when $P < 0.05$ for the two tails.

Results: Anti-HAV-IgG test yielded positive result in 889 (84.7%) subjects. The result was positive for 89.9% of the subjects older than 31 years old and 83.3% of the younger than 31 years old ($P > 0.05$).
Of these 1049 subjects, 67.2% were married. Of the anti-HAV-IgG positive subjects, 91.6% were married whereas 81.8% of the anti-HAV-IgG negative subjects were married ($P < 0.01$). Being a villager was observed as a risk factor for HAV infection and HAV endemicity in Marmara and Karadeniz regions was significantly lower than that in the other regions. The educational level of the subjects and also their parents have seen affect Anti-HAV seropositivity. As the educational level of the subjects and their parents increased, the Anti-HAV IgG seropositivity decreased ($P < 0.01$).
Conclusion: The results of our study reveal that HAV exposure may increase due to risk factors such as socioeconomic and infrastructural problems, personal hygiene, sources of water and food. Improvement in these factors will lead to fewer exposures to HAV.

P1137 High-risk of hepatitis B and C infection in Tunisian multi-transfused patients

N. Hannachi, L. Boughammoura, N. Ladhari, A. Khlif, S. Soussi, H. Skouri, J. Boukadida (Sousse, TN)*

Objective: Transfusion-transmitted infections continue to be a major challenge for blood transfusion organizations across the world. Risk depends on the epidemiology of infections in each country. We aimed to evaluate the rate of seropositivity to hepatitis B and C infections among adults and children receiving multiple transfusions in central Tunisia compared with healthy controls.
Methods: This study includes 107 multitransfused patients (59 adults and 48 children) and 317 controls (257 blood donors and 60 healthy children). Blood was collected over a period of 3 months (December 2008 to February 2009) and serum samples were screened for HBs antigen (HBs Ag) and anti hepatitis C antibody (anti-HCV) by the enzyme linked immunosorbent assay (ELISA) method. The χ^2 test was used for comparisons and a p value less than 0.05 was considered as significant.
Results: Mean age of multitransfused adults was 34.8 years old and 31.3 years old for blood donors. Mean age of children was 7.3 years old for multitransfused group and 7.9 years old for healthy group. The prevalence of HBs Ag was higher in polytransfused group (8.4%) than control group (3.1%) ($p = 0.03$). Prevalence of anti-HCV was 9.5% in the multitransfused patients and 0.4% in controls, the difference between the two groups was significant ($p < 0.0001$). Age-stratified analysis showed that risk of Hepatitis B or C was significantly higher in adults (older than 15 years). Risk of hepatitis C was less important after the date of instauration of systemic HCV serological screening in Tunisian transfusion centers. Significant high frequency of hepatitis B infection was found in patients transfused after 1973 (date of instauration of systemic HBs Ag screening in Tunisian transfusion centers).
Conclusion: Tunisian multitransfused adult patients must be considered at high risk of hepatitis C infection even if hepatitis C endemicity is low. Hepatitis B infection still occurs in multitransfused Tunisian patients in spite of systemic HBs Ag screening. Deferring all reactive anti-HBc antibody units of blood strategy is not defendable in country of important prevalence of hepatitis B. Therefore, implementation of hepatitis B virus DNA screening in transfusion centers should be considered especially for multitransfused patients.

P1138 Seroepidemiology of hepatitis C in cases with history of intravenous drug use in Isfahan province, Iran

B. Ataei, P. Adibi, M. Yaran, N. Kassaian, Z. Nokhodian, M. Meshkati, R. Fadaei Nobari (Isfahan, IR)*

Objectives: Hepatitis C virus (HCV) which is prevalent among estimated 3% of the world population continues to be a major disease burden on the world.
Several studies have documented that injecting drug use is a primary mode of transmission for HCV. But, there is limited information of HCV detection among intravenous drug users (IVDU) in Iran and in Isfahan there is not any documentation about it. Hence, the present study was conducted to understand the status of HCV infection among IVDUs in Isfahan province, Iran.
Methods: In a cross sectional study, during November 2008-March 2009, the cases with history of intravenous drug using in Isfahan, Iran from drug treatment centers, prisons and community, came into study. HCV-Ab was tested by ELISA, Diapro kit (Italy) and confirmed by RIBA test.
Results: There were 3284 cases (3251 male and 33 women): 1006 in prisons, 531 in drop in centers (DIC) and 1747 in community. The totally prevalence of HCV was 38% which was 40% in prisons, 47% in DICs and 34% from community announcement.
Conclusion: It is important that high prevalence of HCV infection be taken into consideration. Comprehensive harm reduction programs and routine testing are needed for IVDUs in Iran in order to best control further transmission of this infection.

Viral hepatitis – pathogenesis and treatment aspects

P1139 The relation between the cytokine levels and histopathological activity and fibrosis in chronic hepatitis B and chronic hepatitis C infections

A. Tigli, F.Z. Akcam, O. Kaya, M. Ciris (Isparta, TR)*

Objectives: We ivestigated that possible correlation between the levels of TNF-α, IL-18, IL-10, TGF-β, and INF-γ and the Histological Activity Index (HAI) and fibrosis levels in patients with Chronic hepatitis B and C.
Methods: Fifty patients were included in the study. Serum samples were obtained and liver needle biopsy were performed on the same day. Cyctokine levels were investigated by ELISA. Histopathological examinations were carried out by the same pathologist.
Results: There was a statistically significant positive mid-level correlation between TNF-α with HAI and fibrosis ($p = 0.004$ $r = 0.401$, $p = 0.003$ $r = 0.407$). No significant correlation was found between HAI and fibrosis with IFN-γ, IL-10, IL-18, ve TGF-β.
Conclusion: HBV and HCV infections have elaborated pathogenetic mechanisms. The cytokin activity has important impacts on clinical severity and progression. The results obtained in this study shows that further investigations on possible effects of cytokins on hepatocellular damage and fibrosis should be done. Thus, new immunotherapeutic approaches in viral hepatitis may be arranged if this issue is further studied.

P1140 Association of candidate susceptible loci with chronic hepatitis B virus infection in a Chinese population

D. Chen, B. Zheng (Hong Kong, CN)*

Objectives: A number of genetic loci have been proposed to be associated with persistent hepatitis B virus (HBV) infection. In this study, we aimed to evaluate the association and interaction of susceptible genes with HBV persistence in Chinese population.
Methods: Seventeen polymorphisms in 9 candidate genes were studied in 361 Chinese chronic hepatitis B patients (CHP group) and 304

spontaneously recovered individuals (SRI). Distribution frequencies of polymorphisms associated with disease were analyzed in healthy Chinese and Caucasian populations. Gene-gene interactions were tested by the multifactor dimensionality reduction (MDR) method.
Results: The TNF −308 G/G genotype and G allele, IL-10RB codon 47 A allele, and MCP-1 −2518 G/G genotype and G allele were more frequent in patients than controls ($P < 0.01$, after multiple corrections $Pc < 0.05$), while TNF −308 A/G genotype and IL-10 −592 A/A genotype were significantly higher in the SRI group than in the CHP group ($Pc < 0.05$). The distribution frequency of risk allele MCP-1 −2518 G and CTLA4 6230 G were much higher in Chinese than Caucasian groups ($P < 0.001$). An interaction between CCR5 −2459, TNFA −863, IL-10RB codon 47 and MCP-1 −2518 was detected by MDR ($P = 0.001$).
Conclusion: Our results suggest that genetic determinants may affect the outcome of HBV infection in both independent and synergic manners.

P1141 Establishment of a non-transgenic mouse model for chronic hepatitis B virus infection

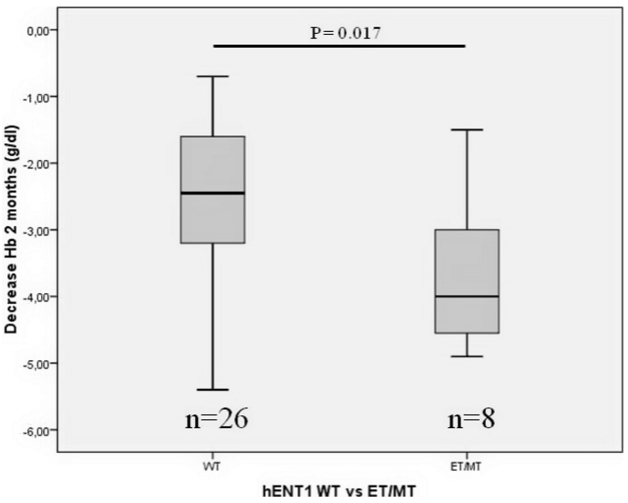
*L. Yang *, B. Zheng (Hong Kong, HK)*

Objectives: Chronic hepatitis B virus (HBV) infection is a severe worldwide public health problem. Although HBV transgenic mice have been developed to mimic the immune tolerant state of chronic HBV infection, lack of a non-transgenic mouse model make it difficult, to address what happen in the early phase of infection and how the outcome of viral infection (to be eliminated or persistent) is determined. This study aimed to develop a chronic HBV infection mouse model using a humanized mouse strain with HLA-transgenic H-2 knockout background (MHC-TgKO mouse).
Methods: 20 ug of pAAV/HBV1.2, a plasmid encoding 1.2-fold of HBV genome, was delivered into mouse tail rein by hydrodynamic injection. HBsAg, HBeAg and HBV DNA were monitored in one-week intervals during a period of over 6 months. Early immune responses to HBV antigens were also determined.
Results: The results showed that, over 60% of injected MHC-TgKO mice persistently produced HBsAg and HBV DNA for over 6 months, which met the criteria of chronic HBV infection in humans. HBV replicative intermediates and transcripts were exclusively detected in the liver carrier mice, but not in other organs. Similar to that in human, injected female mice had lower HBsAg persistent rate, HBsAg concentration and HBV DNA levels than the male. A significant lower immune response to HBcAg was detected in MHC-TgKO mice than that in C57BL/6 by ELISPOT assay, which may explain the high persistent rate in MHC-TgKO mice.
Conclusion: This study has established a mouse model for chronic HBV infection, and further investigations are on going to characterize the virological and immunological properties in carrier mice.

P1142 SLC29A1 −706G>C predict haemoglobin decrease in patients with hepatitis C treated with interferon and ribavirin

A. D'Avolio, M. Siccardi, A. Ciancio, L. Baietto, M. Simiele, S. Patanella, D. Aguilar Marucco, G. Cariti, A. Calcagno, M. Sciandra, A. Smedile, F.G. De Rosa, S. Bonora, M. Rizzetto, G. Di Perri (Turin, IT)*

Objective: Ribavirin (RBV) and pegylated interferon are the standard of treatment in chornic hepatitis C. RBV plasma exposure correlates with the achievement of sustained virological response and hematological toxicity. The role of the equilibrative nucleoside transporter 1 (ENT1), encoded by SLC29A1 gene, in the absorption, transport, metabolism and erythrocyte disposition of RBV has been recently reported. The objective of our investigation was to illustrate whether SNP −706G>C in this gene has an effect on the hematological toxicity and its possible role as a predictor of haemoglobin decrease.
Methods: Patients were recruited in Amedeo di Savoia Hospital and Molinette Hospital in Turin, Italy. Sampling was performed after written informed consent was obtained in accordance with local ethics committee indications. Patients receiving RBV (1000 mg/die) and pegylated interferon therapy were included in this study. Main inclusion criteria were: no concomitant interacting drugs, no renal function impairment, self-reported adherence >95%. Genotyping was conducted by real time PCR based allelic discrimination using standard methodology. Statistical analysis was conducted by SPSS software.
Results: 34 patients were included in the study meeting the inclusion criteria. No associations between patient demographics with ENT1 polymorphism were found. After 2 and 3 months of treatment with RBV the median haemoglobin decrease, expressed as g/dL, in patients with the mutant allele (GC or CC, n = 8) for SNP −706G>C was higher compared to patients with wild-type genotype (GG, n = 26); after 2 months: −4.00 (−4.57 to −2.90) Vs. −2.45 (−3.22 to −1.60), $p = 0.017$; and after 3 months: −3.90 (−4.82 to −2.95) Vs. −2.7 (−3.50 to −1.66), $p = 0.031$; In multivariate linear regressions analyses the presence of the mutant allele was the only independent predictor of higher decrease of haemoglobin after 2 (b = −1.13, 95% CI −2.04 to −0.23, $p = 0.016$) and 3 months (b = −1.05, 95% CI −2.00 to −0.10, $p = 0.032$).
Conclusions: The mutant allele C correlates with an higher decrease of haemoglobin after 2 and 3 months of treatment. This result suggests that hENT1–706G>C may have an important influence on RBV-induced anemia. Further studies are required to confirm this association and to clarify its clinical value in predicting RBV-associated hematological toxicity.



hENT1 and Hb after 2 months of ribavirin treatment.

P1143 Factors related to the effectiveness of the chronic hepatitis C virus infection therapy in patients with or without HIV infection

A. Puerta García, A. Salinas Botrán, E. Martínez Alfaro, J. Blanch Sancho, F. Mateos Rodríguez (Albacete, ES)*

Objective: To describe factors related to the effectiveness of the hepatitis C treatment in coinfected and monoinfected patients.
Methods: Descriptive, retrospective study of 101 patients with chronic hepatitis C with and without HIV infection treated with interferon and ribavirin (guideline A) or with pegylated interferon and ribavirin (guideline B) in our hospital during 9 years.
Results: 37 patients presented HIV co-infection and 64 were monoinfected. Within the group of patients with HIV infection: 7 patients presented sustained virologic response (SVR) (18.9%), 1 out of 6 with guideline A (16%) and 6 out of 31 patients treated with guideline B (19%). 3 patients responded during the first month and among them there was SVR in 100%, 1patient responded during the third month and got SVR as well, 4 responded during the sixth month and none of them got SVR and 4 patients responded during the ninth month and 1 got SVR ($p = 0.03$). Among the patients who got undetectable viral load during the treatment, patients with SVR had an average of weeks for the treatment

with undetectable viral load of the HCV of 30.6 whereas there were 18.3 weeks in patients without SVR (p 0.03). Regarding genotypes, from 26 patients with genotype 1, 11.5% presented SVR, from 6 patients with genotype 3, 50% presented SVR and from 5 patients with genotype 4, 25% presented SVR (p = 0.14).
Within the group of monoinfected patients, 41 patients presented SVR (64%). Patients treated with guideline A responded 5/15 (33%) and patients treated with guideline B responded 36/49 (73%), with statistically significant differences (p = 0.026). From 19 patients that responded in the first month 17 presented SVR, from 21 patients that responded in the third month, 18 presented SVR. In patients who got undetectable viral load during the treatment, patients with SVR had an average of weeks for the treatment with undetectable viral load of the HCV of 32.7 whereas there were 19.1 weeks in patients without SVR (p 0.03). Regarding genotypes, 22 out of 40 patients with genotype 1 (55%) presented SVR, 18 out of 22 patients with genotype 3 (81%) and 1 out of 2 with genotype 4 (50%).
Conclusions: In patients with chronic hepatitis C virus infection with or without HIV coinfection the longer with undetectable viral load during the treatment is related to SVR in a significant way, therefore, it could be possible to individualize treatment according to the viral response to make SVR better.

P1144 The influence of HCV genotypes and other predicting factors in virological response in patients treated with INF α or PEG INF α 2a in combination with ribavirin

H. Elasifer, B. Elagy, M. Eltaghdy, A. Draah, M. Daw (Tripoli, LY)*

Introduction: Recombinant interferon α or pegylated interferon alone or in combination with ribavirin are known treatment of hepatitis C virus infection, HCV genotypes commonly used to outline such treatment.
Objectives: were (1) to determine the efficacy of two different regimens of therapies, (2) to asses the impact of virus genotype, gender, ALT level and viral load on such treatments.
Methods: 479 of chronic HCV infected patients registered at Tripoli Medical Centre were followed from January 2003 to October 2008. The information were reviewed the data were collected. These include age, gender, risk factors, ALT level, viral load at diagnosis, viral genotype, the regimen of therapy 'INF α or pegylated INF α 2a in combination with ribavirin', ETR, SVR. Only 229 were given therapy. The statistical analyzed by using SPSS version 11.5.
Results: 86 patients were given INF in based therapy and 54% of them had End Treatment Response (ETR) and 28% had Sustained Virological Response (SVR). 143 patients were given PEG INF α 2a in based therapy, 69% had ETR and 36% had SVR. The relationship between ETR, SVR and gender was significant in patients who were given INF based therapy (ETR 'P = 0.009', SVR 'P = 0.016'), and was insignificant in patients who were given PEG INF based therapy. The relationship between ETR, SVR and age, ALT at diagnosis, basal viral load, was insignificant in both regimens. The ETR in patients who were given INF based therapy was G1 33.3%, G2 69.2%, G3 52.4% and G4 64.3%, and SVR were G1 16.7%, G2 30.8, G3 23.8% and G4 39.3%. ETR in patients who were given Peg INF based therapy was G1 66.1%, G2 82.4%, G3 68.8% and G4 66.7% responded and SVR was G1 32.1%, G2 35.3%, in G3 43.7% and G4 37%. The relationship between the genotype and ETR to INF α regimen was statistically significant (P = 0.037) and was insignificant in PEG INF regimes (P = 0.345). The relationship between the genotypes and SVR to INF α regimen was insignificant (P = 0.271), in PEG INF the relation was insignificant (P value=0.792).
Conclusion: HCV genotypes was found to be important in predicting ETR in patients who were given INF α based therapy. The gender was also important in predicting ETR and SVR in patients who were given INF α based therapy. Gender and genotype were not significant in patients who were given PEG INF based therapy. Moreover, the patient's age, basal viral load and basal ALT were found insignificant.

P1145 Prevalence of HBV Lamivudine-resistance mutations in a cohort of Iranian HBV-infected patients with and without HIV co-infection

M.R. Aghasadeghi, A. Farahani Far, G. Bahramali, S.M. Sadat, A. Memarnejadian, S.D. Siadat, M. Mohraz, M. Foroughi, F. Javadi, F. Roohvand, R. Vahabpour, M.S. Pouriayevali (Tehran, IR)*

Objectives: Individuals co-infected with human immunodeficiency virus 1 (HIV-1) and hepatitis B virus (HBV) often receive treatment with an antiretroviral regimen including lamivudine (LAM) that is a nucleoside analogue and inhibits HIV/HBV reverse transcriptase (RT). However, its efficacy is limited by the development of LAM-resistant strains within the YMDD motif of the HBV polymerase gene. Prevalence of mutations between HBV/HIV co-infected (HIVpos) and HBV mono-infected (HIVneg) individuals on LAM therapy have not been extensively documented in the Middle-East. The aim of this study was to determine the prevalence of HBV LAM-resistance mutations in Iranian HBV infected patients with and without HIV infection.
Methods: Fifty eight chronic hepatitis B patients including 30 HBV mono-infected and 28 HIV/HBV co-infected patients were studied, while receiving LAM as a part of antiretroviral therapy. The YMDD motif was PCR-amplified and directly sequenced in HBV isolations. HBeAg, HBV genotype and polymerase gene mutations were additionally assessed.
Results: The average LAM exposure was 24.35 ±21.91 and 25.3 ±13.94 months in HIVpos and HIVneg groups, respectively. All the patients were infected with HBV genotype-D and the study revealed a significant correlation between the HBV/HIV co-infection and both HBV subtypes of ayw3 and ayw4 ($P < 0.001$). Seventeen out of 28 HIVpos patients and 19 out of 30 HIVneg infected ones were shown to be HBeAg negative. No association was observed between the type of infection and HBeAg status ($P > 0.05$). M204V, M204I and L180M mutations were observed in 2 (6.66%), 7 (23.33%) and 5 (16.66%) HIVneg patients, respectively. The dual mutations (M204V/I+L180M) were detected in 5 (16.66%) HIVneg patients. Interestingly, LAM-resistance mutations were not detected in HBV/HIV co-infected individuals.
Conclusion: In accordance to previous studies in Iran, HBV-D was the only detected genotype in both HIVpos and HIVneg patients. This study suggested that individuals With HBV infection that carry ayw4 and ayw3 subtypes are more likely to be infected with HIV. Moreover, in contrast to several European investigations, reporting the LAM-resistance mutations in mono- and co-infections, our study for the first time in Iran indicated these mutations just in HBV mono-infected patients. However, further studies to clarify the role of HIV in HBV LAM-resistance mutations are needed.

P1146 High rate of non-response and relapse associated with peginterferon-alfa monotherapy for the treatment of acute hepatitis C in HIV-infected patients

J.E. Arends, S. van Assen, A.J. Wensing, C. Stek, T. Mudrikova, D. van Baarle, H.G. Sprenger, A. Hoepelman (Utrecht, Groningen, NL)*

Background: The incidence of acute hepatitis C virus infection (HCV) in patients infected with human immunodeficiency virus (HIV) is rising. Because of low patient numbers and a wide variety of inclusion criteria between studies, the optimal treatment regimen is under debate. We advocated peginterferon-alfa monotherapy (pegIFN-α) for acute HCV in HIV-coinfected patients (Arends-JE et al. AIDS 2008; 22(11):1381–138).
Methods: In HIV-infected patients acute HCV was diagnosed by anti-HCV serology and quantitative PCR (HCV-RNA), in combination with clinical signs or elevated alanine aminotransferase (ALAT), and a negative serology within 1 year prior to diagnosis. All patients started treatment with peginterferon alfa-2a (180 μgr /weekly) and continued if a rapid viral response at week 4 (RVR, i.e. HCV-RNA <50 IU/ml) was reached. If no RVR was reached, weight based ribavirin was added based on the physician's preference. Early viral response (EVR) was defined as $>2 \log_{10}$ decrease or undetectable HCV-RNA at week 12 of therapy.

Results: Until July 2009 a total of 23 HIV-patients were diagnosed in both centers (UMCU and UMCG) with acute HCV-infection (17 genotype 1 and 6 genotype 4) of whom 19 started peginterferon alfa-2a monotherapy. A RVR was reached by 7 patients (37%) while 10 patients (53%) achieved an EVR. 2 patients reached an EVR with addition of ribavirin from week 4 onwards. Nine patients (47%) were non-responders with a less than 2log10 drop in HCV-RNA at week 12 with 1 patient receiving additional ribavirin from week 4 onwards. All non-responders discontinued treatment at week 12 of therapy. With respect to time between seroconversion and start of therapy, baseline HCV-RNA viral load, maximum ALAT reached, baseline CD4 count and HIV-RNA viral load, no statistical differences were observed between responders (RVR and EVR) and non-responders. Interestingly, 5 out of 7 patients achieving a RVR also completed their 24-weeks of therapy. Unexpectedly, 3 patients in this group experienced a relapse of their HCV-infection. This relapse was confirmed by sequence analysis of the NS5B-region comparing the baseline quasi-species pool with the relapse strains.
Conclusion: Peginterferon-alfa monotherapy resulted in a high percentage of non-responders. Furthermore, relapse of HCV-infection in patients achieving a RVR, was common after completion of treatment. Combination with ribavirin seems to be essential in HIV-infected patients with acute HCV infection.

P1147 Correlates and prognostic value of hepatitis C virus core antigen serum levels during chronic infection and treatment

E. Durante-Mangoni, L. Vallefuoco, E. Perna, D. Iossa, R. Andini, C. Caianiello, E. Ragone, R. Utili, G. Portella (Naples, IT)*

Objectives: The current assessment of chronic hepatitis C patients includes determination of aminotransferases, viral load and HCV genotype. Recently, a novel assay has been developed that allows quantification of HCV core antigen (HCVcoAg) with a sensitivity of 3 fmol/L. In this study, we analysed HCVcoAg levels in a cohort of chronic hepatitis C patients to assess their correlation with clinical and virological parameters and their changes during antiviral treatment.
Methods: We studied 26 chronic hepatitis C patients treated with standard doses of pegylated interferon α and ribavirin. Baseline data incuded quantitative HCV-RNA, HCV genotype, ALT, GGT as well as histology parameters including necroinflammation, fibrosis and steatosis. HCV-RNA and HCVcoAg levels were also measured in samples obtained 2, 14 and 28 days after antiviral treatment start.
Results: HCVcoAg baseline levels showed a good correlation with HCV-RNA ($r = 0.26$) and ALT ($r = 0.32$) levels and with liver necroinflammatory activity ($r = 0.24$) but not fibrosis or steatosis. Genotype 3 patients showed significantly lower baseline levels of HCVcoAg than both genotype 1 and 2 patients (1024 vs 8062 and 5538, respectively; $p < 0.05$). Pre-treatment HCVcoAg levels did not show any predictive value for final treatment outcome. However, the early decline of HCVcoAg from baseline to day 2 of treatment was significantly greater in patients who subsequently reached a sustained virological response compared to those nonresponders or relapsers to treatment.
Conclusion: Our data suggest that the evaluation of HCVcoAg serum levels may provide relevant data in the baseline clinical evaluation of patients with chronic hepatitis C. In addition, early changes of HCVcoAg serum levels during treatment mirror those observed with HCV-RNA measurement. Further studies are underway to determine whether a cheaper and faster assay such as HCVcoAg measurement may replace HCV-RNA in the early assessment of virological response to peg-interferon/ribavirin regimens.

P1148 A tertiary care facility experience on pegylated IFN efficacy in chronic hepatitis B patients

Y. Kurtoglu Gül, C. Bulut, S. Kinikli, Z. Koçak Tufan, C. Ataman Hatipoglu, F.S. Erdinc, A.P. Demiroz (Ankara, TR)*

Introduction: Pegylated interferons (Peg-IFN) have been used for the treatment of chronic hepatitis B (CHB). The main properties of these drugs with certain therapy durations and no resistance improvement made them even more preferable. In this study we aimed to evaluate the efficacy of Peg-IFNs in our CHB patients.
Methods: This prospective study was conducted in our clinic between 2006 and 2008. The CHB patients admitted and treated with Peg-IFN are included.
Results: Totally 40 patients (17 female, 23 male) were included. Mean age was 37 (min 16, max 65). 13 of total were HBeAg(+), 24 were Anti-HBeAg(+) while two were both HBeAg(+) and AntiHbe(+) and one was negative for both.
HBeAg (+) patients: They treated for 24 weeks. Virological response rate was 10%, biochemical response rate was 40%, sustained response rate was 10% and primary nonresponse rate was 60% at the end of treatment. Mean alanine aminotransferase (ALT) levels at the beginning and end of treatment were 93.2 ± 52.4 and 73.8 ± 59.7 respectively ($p = 0.413$). Mean decrease in the HBV DNA level during this perios was $2.4 \log_{10}$ ($p < 0.05$). HBeAg seroconversion was seen in two (20%) patients. HBsAg seroconversion was not seen in any patients.
HBeAg(–) patients: They were treated for 48 weeks. Virological and biochemical response rates at the end of treatment were 76% and 67% respectively. Mean ALT levels at the beginning and end of treatment were 116.7 ± 92.4 and 41.5 ± 27.3 respectively ($p = 0.003$). Mean decrease in the HBV DNA level during this period was $4.3 \log_{10}$ ($p = 0.000$). Sustained response rate was 14%. HBsAg seroconversion was seen only in one patient.
Conclusion: Peg-IFN therapy was found to be more effective in HBeAg(–) patients than HBeAg(+) ones. The treatment duration of HbeAg(+) patiens are still not clear and prolonging the treatment time to one year like the negative ones could be detected as well in further studies.

P1149 Evolution of hepatitis B virus mutation during nucleoside/tide analogues long-term therapy in chronic hepatitis B patients

E. Arrese, M. Basaras, S. Blanco, P. Ruiz, R. Cisterna (Vitoia-Gasteiz, Bilbao, ES)*

Objective: The aim of the present study was to evaluate the therapy with nucleos(t)ide analogues (adefovir dipivoxil (ADV), lamivudine (LAM), entecavir (ETV)) in chronic hepatitis B virus (HBV) infected patients with frequent measurements of DNA levels, to characterize HBV genotypes, and to determine the emergence of nucleos(t)ide analogues mutants before and during the two years of therapy by direct sequencing.
Patients and Methods: A total of twenty patients with chronic HBV infection were treated with nucleos(t)ide analogues for at least two years. We studied nucleos(t)ide analogue-naïve patients as well as patients previously treated with another nucleos(t)ide analogue. A virologic breakthrough was defined as an increase in serum HBV DNA level and resistance to therapy was defined as the emergence of resistance mutations by direct sequencing of the polymerase gene, which was looked for in all HBV DNA positive serum samples.
Results: Viral genotype was determined showing the presence of genotype D (55%) in eleven patients, genotype A (40%) in eight patients and genotype C (5%) in one patient. In the viral response to long-term treatment, three patients developed LAM resistance mutations (rtM204V/I+rtL180M; M204I; M204I+rtQ215S), one patient developed ETV resistance (rtM204V+rtL180M+rtT184A+rtS202G), one patient developed ADV resistance mutations (rtA181V+rtQ215S) and another patient developed ADV+LAM resistant mutations (rtL180M+rtA181V+rtN236T). In another two patients, the viral load did

not respond sufficiently to therapy but they did no developed nucleotide changes in the polymerase gene associated with viral resistance. On the other hand, in twelve patients the viral load was undetectable after two years of treatment.

Conclusions:

1. Frequent assessment of quantitative serum HBV DNA remains the best approach to early detection of resistance.
2. Direct sequence analysis is an essential tool to detect mutations and to optimize therapeutic management of HBV chronic infection in clinical practice in order to choose the appropriate nucleos(t)ide analogues.

P1150 Pregnancy outcome in women with chronic hepatitis B virus infection – the value of serum HBsAg quantification

I. Elefsiniotis, I. Glynou, I. Zorou, E. Vezali, H. Kada, G. Saroglou (Athens, GR)*

Objectives: Preterm birth is the leading cause of perinatal morbidity and mortality. The impact of maternal chronic HBV infection on pregnancy outcome has been retrospectively evaluated in some studies mainly from South-Eastern Asia but their results are controversial. The aim of this study was to prospectively evaluate the spontaneous preterm birth (SPB) rates in association with chronic HBV infection in a multinational population of pregnant women (n = 1826) without several known risk factors for preterm delivery. Moreover we examined the role of serum HBsAg as well as serum HBV-DNA levels during perinatal period in respect to SPB, in a subgroup (n = 44) of chronic HBV infected women of our study population.

Methods: All women were screened for HBsAg, anti-HBc and anti-HBs, whereas HBeAg and anti-HBe were evaluated only in those who tested positive for HBsAg. Serum HBV-DNA was determined by using the Cobas TaqMan HBV Test. HBsAg quantification was done using the Abbott ARCHITECT HBsAg assay.

Results: More than half of the study population was originally from Greece (56%), 30.9% was from Albania and 13.1% from Eastern European countries.

Overall, 3.83% (70/1826) of pregnant women were HBsAg(+) and the vast majority of them (71.42%) were Albanian. Among Albanian women the prevalence of HBsAg was 8.9% followed by 3.3% among women from Eastern European countries. Thirty-three women (1.83%) presented SPB (Greek women 1.5%, Albanian women 1.6%, Eastern European women 3.8%, p = 0.051). A strong relationship between SPB and HBsAg status was observed [7.25% (5/64) of HBsAg(+) mothers presented SBP compared to 1.62% (28/1703) of HBsAg(−) ones (p = 0.007)]. The relative risk of preterm delivery in HBsAg(+) mothers was 4.5 times higher than in HBsAg(−) mothers.

SPB was not related either to the presence or absence of viremia (p = 0.576) or to the level of viremia (p = 0.761), among HBsAg(+) women. A trend for relationship between SPB and the HBsAg levels [2520.87 IU/ml for HBsAg(+) women who had SBP vs 2120.51 IU/ml for HBsAg(+) women without SPB, p = 0.079] was observed.

Conclusions: HBsAg(+) pregnant women exhibit a higher risk for spontaneous preterm delivery compared to HBsAg(−) women, especially those with relatively higher HBsAg levels during perinatal period.

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P1151 HBV-DNA presence in cord blood does not reduce the efficacy of the immunoprophylaxis schedule in neonates of HBeAg-negative chronic HBV-infected women

I. Elefsiniotis, M. Papadakis, G. Vlachos, E. Vezali, C. Mihas, G. Saroglou, A. Antsaklis (Athens, GR)*

Background/Aim: Intrauterine/transplacental transmission of HBV infection is observed in a significant proportion of pregnancies, resulting in passive-active immunoprophylaxis failure. In our study we evaluated the efficacy of the currently used passive-active immunoprophylaxis schedule in neonates of HBeAg-negative chronic HBV infected pregnant women, in respect to maternal laboratory data during perinatal period as well as to the presence or absence of HBV-DNA in cord blood samples.

Methods: 76 chronic HBV infected pregnant women were clinically, haematologically, serologically and virologically evaluated during perinatal period. Cord blood was obtained at the time of delivery and the samples were evaluated for the presence of HBV-DNA (Cobas Amplicor HBV Test).

Results: HBV-DNA was detectable in 13 (17.8%) cord blood samples evaluated. Cord blood HBV-DNA positivity was significantly correlated with maternal viral load during perinatal period (p = 0.002). Chronic HBV infected pregnant women with serum HBV-DNA levels higher than 10.000 copies/ml during perinatal period exhibited 5.8 times higher risk of HBV-DNA presence in their cord blood compared to women with serum HBV-DNA lower than 10.000 copies/ml (50% vs 8.6% respectively, p = 0.003). Median cord blood HBV-DNA levels were significantly lower than median maternal serum HBV-DNA levels (316 copies/ml vs 2.667 copies/ml respectively, p < 0.001). None of 44 infants evaluated exhibit immunoprophylaxis failure.

Conclusions: The presence of HBV-DNA in cord blood does not reduce the efficacy of the currently used immunoprophylaxis schedule in neonates of HBeAg-negative chronic HBV infected women.

P1152 Factors associated with severe liver fibrosis in HIV/HCV co-infected patients, ANRS CO13 HEPAVIH cohort

M.A. Loko, F. Bani-Sadr, P. Carrieri, M. Winnock, K. Lacombe, D. Neau, P. Morlat, P. Sogni, F. Dabis, D. Salmon on behalf of the ANRS CO 13 HEPAVIH*

Background: HCV-induced liver disease is a leading cause of death among HIV/HCV coinfected patients. Liver stiffness measurement is known to be well correlated to liver fibrosis.

Aim: To assess factors associated with liver fibrosis in HIV–HCV co-infected patients.

Methods: The study focused on 671 HIV/HCV-coinfected patients enrolled in the ANRS CO13 HEPAVIH cohort since January 2006 and for whom successful results of liver stiffness measurements evaluated by elastometry were available. The study outcome was severe liver fibrosis, defined as a liver stiffness >9.5kPa. Logistic regression models were used to identify correlates of severe liver fibrosis among patients' characteristics (input variables). Patients with anti-HCV treatment at the time of liver stiffness measurements, as well as those with end-stage liver disease were not included. Variables with P-values <0.10 in univariate analysis were included in multivariate models, carried out using a backwards stepwise procedure.

Results: Among the 671 evaluated patients, 67% patients were male, with a mean age of 45 (SD: 6.1) years. 67% were IVDU. The mean CD4 cell count was 506±295 /mm^3 at the time of liver stiffness measurement, 186 (27.7%) presented a severe liver fibrosis. In multivariate analyses, factors associated with severe liver fibrosis were male gender (OR: 2.4, [CI: 1.4–4.1], p = 0.002), receiving opioid substitution treatments (methadone or buprenorphine), both proxies of longer history of HCV infection (OR: 2.3, [CI:1.3–4.0], p = 0.004), presence of lipodystrophy (OR: 2.0, [CI:1.2–3.3], p = 0.005), increased HOMA-insulin resistance (OR: 1.1, [CI:1.0–1.2], p = 0.002) and a long duration under ART (OR: 1.1, [CI:1.0–1.1], p = 0.03).

Conclusion: Our study shows that a long exposure to ART and the presence of metabolic disorders are associated with a more severe liver fibrosis in HIV–HCV co-infected patients. This finding may have implications for the management of HIV–HCV co-infected individuals.

P1153 Peripheral blood and intrahepatic natural killer cells in chronic hepatitis C: relation to disease activity and hepatic fibrosis

H. El Aggan, F.S. Mohamed, N.M. El Deeb, M.M. El Sawy, M.S. Shater (Alexandria, EG)*

Objectives: Cellular immune responses are thought to play a key role in the pathogenesis of hepatitis C virus (HCV)-related liver damage. Recently, increasing attention has been drawn towards components of the innate immune system to HCV, including natural killer (NK) cells. The present work was designed to study peripheral blood and intrahepatic NK cells in patients with chronic hepatitis C in relation to disease activity and severity of hepatic fibrosis.
Methods: Fifteen patients with untreated CHC and 12 healthy subjects were included in the current study. The NK and NKT cells in fresh whole blood samples were identified using two-color flow cytometry as CD3-CD56+ and CD3+CD56+ cells respectively and the results were expressed as percentages of the total lymphocyte count. Liver biopsies were taken from all patients and the specimens were evaluated as regards the histological activity grade and fibrosis stage according to METAVIR scoring system and for the presence and grade of steatosis. Immunohistochemical staining was done using antibodies against CD56 and smooth muscle actin (SMA) for detection of intrahepatic NK cells and activated hepatic stellate cells (HSCs) respectively. A semi-quantitative method was used to score the intensity of immunostaining.
Results: The percentages of CD3-CD56+ NK cells and CD3+CD56+ NKT cells in peripheral blood showed significant decreases in patients with CHC compared with healthy subjects ($P < 0.01$) and was positively correlated with the intensity of intrahepatic NK cells ($P = 0.001$). The CD56+ NK cell infiltrate was found to be absent or minimal in about 70% of the liver biopsies of patients with CHC. Patients presented with chronic fatigue showed significantly lower percentages of circulating NK and NKT cells and intensity of intrahepatic NK cells than patients who were asymptomatic ($P < 0.05$). The percentages of peripheral blood NK cells and NKT cells and the intensity of intrahepatic NK cells showed significant inverse correlations with serum HCV RNA levels, steatosis grade, METAVIR fibrosis stage and intensity of activated HSCs ($P < 0.05$).
Conclusions: The deficiency of peripheral blood and intrahepatic NK cells in patients with CHC may provide a mechanism for immunosuppression resulting in viral persistence, disease chronicity and progression of hepatic fibrosis.

P1154 Recombinant mammalian cell derived hepatitis C virus-like particles induce neutralizing antibody responses to hepatitis C virus

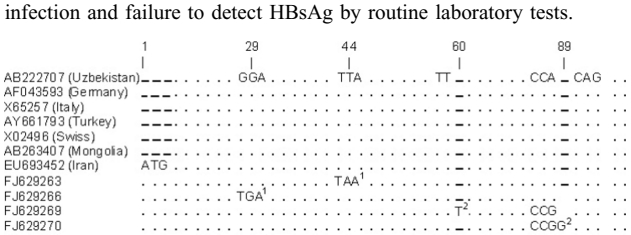
D.F. Johnson, R. Chin, L. Earnest-Silveira, H. Zentgraf, T. Bock, B. Chua, D.C. Jackson, J. Torresi (Melbourne, AU; Heidelberg, Tuebingen, DE)*

Objective: Clearance of Hepatitis C virus (HCV) requires a strong and broadly cross-reactive CD4+, CD8+ T cell and neutralising antibody (Ab) responses. Virus like particles (VLPs) resemble mature parent virus inducing protective humoral and cellular immune responses against HCV and provide a viable prophylactic vaccine candidate.
Methods: Recombinant adenoviruses expressing HCVcore-E1-E2 were used to infect Huh7 (hepatoma) cells and produce HCV VLPs. These were isolated from cell lysates and purified by Iodixanol density gradient ultracentrifugation. E1 and E2 glycoproteins of the correct size in HCV VLPs were confirmed by Western immunoblot. HCV VLPs were analysed by testing for maturation of Dendritic cells (DC) and mice were immunised with HCV VLPs alone and with alum and Freunds adjuvants. The mice were assessed for (1) humoral responses against both VLPs and a recombinant E2 protein of HCV, (2) production of mouse antibody secreting cells (memory B cells) in splenocytes using B cell Elispot assays and (3) neutralizing Ab in a Huh 7 cell entry assay. A second group of mice were immunised with VLPs and 2 novel adjuvants.
Results: We have produced, purified and confirmed the presence of HCV VLPs of genotype 1a by western immunoblot, electron microscopy (EM) and immunogold EM. HCV VLPs efficiently stimulate the maturation of dendritic cells to level that are comparable to lipopolysaccharide (LPS). Mice immunised induced strong humoral responses to E2 and VLPs and mouse anti-HCV VLP serum neutralized VLP entry into Huh7 cells. B cell elispot assays, using mouse splenocytes, demonstrated production of mouse antibody secreting cells / memory B cells. Mice immunised with VLPs and 2 novel adjuvants demonstrated a greater humoral response and increased levels of mouse antibody secreting cells compared with mice immunised with HCV VLPs alone or in Alum.
Conclusion: Mammalian cell derived HCV VLPs exhibit similar morphological, biophysical and immunological properties as putative HCV virions and are a viable vaccine strategy for HCV. HCV VLPs of genotypes 1b, 3a and 4 are currently being produced. Studies of CD8 responses and methods to improve VLP immunogenicity and alternative adjuvanting are ongoing.

P1155 Mutations in the S gene region of hepatitis B virus in high-risk patients with occult HBV infection

A. Ramezani, M. Mohraz, R. Hamkar, A. Aghakhani, S. Soufian, A. Eslamifar, M. Banifazl (Tehran, Arak, IR)*

Objective: Occult hepatitis B virus (HBV) infection is characterized by presence of HBV infection with undetectable hepatitis B surface antigen (HBsAg). Surface gene mutants (S mutants) have been reported in a variety of patient groups, with variable rates of occurrence. Due to the apparent increase in prevalence of S mutants and limited data regarding these mutations in patients with occult HBV infections; we aimed to determine the surface gene mutations of HBV among high risk patients with occult HBV infection.
Patients and Methods: In this study, 395 patients including 289 patients on chronic hemodialysis (HD) and 106 HIV infected subjects were enrolled. The presence of HBV-DNA was determined in plasma samples of patients with isolated anti-HBc (HBsAg negative, anti-HBs negative and anti-HBc positive) by real-time PCR. In HBV-DNA positive patients, surface gene region was amplified by nested PCR and surface gene mutations were analyzed after direct sequencing.
Results: HBV-DNA was detectable in 12 out of 40 patients (30%, 95% CI, 15.8%-44.2%) who had isolated anti-HBc. Of these 12 patients, 9 of them were HD patients and 3 of them were HIV infected subjects. Plasma HBV-DNA load was less than 50 IU/ml in all of these patients. In 8 patients (5 HD and 3 HIV infected patients) the amount of DNA was enough for analysis. In these patients, genotype and surface gene mutations were analyzed after direct sequencing. Phylogenetic analysis revealed that all of the HBV isolates were clustered in the Genotype D. Insertion of a T residue at position 60 and a G residue at position 89 were detected in 2 isolates. Premature stop codons were created in 2 other isolates via replacing of T by A at position 44 and G by T at position 28. The TTA to TAA stop mutation led to a premature stop codon at position Leu15 and the GGA to TGA led to a premature stop codon at position Gly10 in the S gene. Serine to asparagine substitution at residue 207 (S207N), due to a G to A transition at nucleotide position 620 was found in the other 4 sequences.
Conclusion: HBV genotype D was the only detectable genotype in Iranian patients with occult HBV infection. No "a" determinant mutations were detected in our isolates. This study suggested that the "a" region mutations did not play a major role in HBsAg detection and other mutations may be responsible for the existence of occult HBV infection and failure to detect HBsAg by routine laboratory tests.



Community-acquired gastroenteritis

P1156 Outbreak of *Listeria monocytogenes* caused by beef meat from a meals-on-wheel's delivery, Denmark, 2009

B. Smith (Copenhagen, DK)*

Objectives: To describe the first outbreak of listeriosis in Denmark since 1986, this occurred in May 2009. Furthermore to describe the value of rapid molecular typing of isolates in identifying cases being part of the outbreak as a prerequisite for subsequent epidemiological investigation and identification of a possible single source of infection.
Methods: Two primary diagnostic laboratories each isolated *L. monocytogenes* from the blood or cere-brospinal fluid of three patients within a few days. The isolated bacteria were referred to the national reference laboratory and typed by MLVA and PFGE together with other isolates from the last month. Patients identified from MLVA typing or their close relatives were interviewed by telephone or in their homes about eating habits and food delivery.
Results: The typing of the isolates showed that eight patients had identical MLVA patterns distinguish-able from other isolates from Danish patients. The patients were all adults; the median age was 78 years (range 44–94 years). All patients had predisposing risk factors. All patients developed septicaemia and one patient presented with meningitis. Two of the patients died. All patients except one had had ready-made food delivered from the same catering company. From the records of the catering company it was found that each of the seven patients had individual diets, and the only meal they had in common was sliced beef served with different sauces and vegetables, prepared three weeks prior to onset of disease. Five samples obtained from remains of the meat were sent for bacteriological examination without positive culture of *L. monocytogenes* in any of them.
Conclusion: This outbreak incurred substantial health costs and caused considerable public concern be-cause recipients of meals-on-wheels constitute a large group of people with impaired health who are especially susceptible to *Listeria* infection. The meal was intended for heating in the homes before consumption this didn't happened. Food that may be mistaken for ready-to-eat food should be free of *L. monocytogenes.*

P1157 Viral gastrointestinal infections in a paediatric hospital

E. Nika, T. Guajardo, K. Papavasileiou, A. Makri, I. Mammas, E. Papavasileiou, A. Voyatzi (Athens, GR)*

Objectives: To investigate the burden of etiological agents of childhood viral gastroenteritis (V.G.E) with regard to age and to estimate the prevalence and seasonal distribution of Rotavirus (R.v), Adenovirus (A.v), Norovirus (N.v) and Astrovirus (As.v).
Material and Methods: During a two year period (2008–2009) a total of 1352 faeces samples were examined for enteric viruses. The study population were children <5 years which visited the outpatient clinics with symptoms of acute gastroenteritis for medical advice or hospitalized. Clinical features and possible transmission mode of patients was recorded. R.v group A, A.v and N.v genogroups I and II were screened by rapid chromatographic immunoassays, while As.v was tested using enzyme immunoassay.
Results: V.G.E was diagnosed in 394 cases (an overall rate 29%). Dehydration. diarrhoea, vomiting and fever were the predominant clinical manifestations. The prevalence for R.v, A.v, N.v, and As.v were 16%, 7%, 5% and 1% respectively. Co-infections were found in 32 cases (8%) and were associated with R.v group A and A.v. N.v was identified as single leading cause of the illness in prematurity cases. V.G.E occurred in 53% boys versus 47% girls. In a high rate of V.G.E (90.5%) suffered children aged less than 3 years while 47.6% belonged in the age group of 6–24 months. Two nosocomial outbreaks of R.v occurred in born preterm neonates. A seasonal fluctuation of the R.v disease was observed with the highest rate of the illness (68%) documented between Winter and early Spring, while N.v infections peaked in Winter (78%). In contract A.v infections occurred all year-round.
Conclusions: R.v remain still the main causative viral agent for the majority of V.G.E cases in the paediatric population in Greece. Most serious symptoms were presented in hospitalized children with R.v infections compare to others viral agents. Our data showed the need to develope a critical management for V.G.E in order to avoid the spread of viruses (especially for R.v and N.v) in paediatric population.

P1158 Typhoid fever in Jordan

T.M. Al-Sanouri, B. Paglietti, A. Haddadin, M. Murgia, D. Bacciu, M. Youssef, S. Rubino (Amman, JO; Sassari, IT; Lyon, FR)*

Background: Enteric fever caused by *Salmonella enterica* serovar Typhi has not been adequately explored in Jordan.
Methodology: In this study we investigated antibiotic resistance patterns and resistance determinants coupled with fingerprint methods of forty-eight isolates of S. Typhi obtained from 113 patients with suspected enteric fever admitted at six governmental hospitals in different directorates in Jordan. Twenty-four isolates were from an outbreak of typhoid fever that occurred between October 2004 and January 2005, and another twenty-four were from sporadic cases from 2005.
Results: All isolates of S. Typhi were resistant to streptomycin. A multidrug resistant (MDR) pattern of ampicillin, chloramphenicol, co-trimoxazole with tetracycline and streptomycin (R-type ACCoTS) was found in 58% of the epidemic strains causing the outbreak and in 98% of the strains from sporadic cases. MDR isolates harbored a single IncHI1 plasmid containing a class 1 integron (dfrA7). Plasmid conjugation studies demonstrated a genetic transfer of resistance (ACCoT). S. Typhi isolates were all sensitive to fluoroquinolones and cefotaxime, the alternative drugs recommended for treatment of typhoid fever. The genomic analysis using PFGE showed: a) the outbreak was caused by an introduced circulating clone with/without an MDR plasmid, and b) isolates from the sporadic cases from 2005 are the same MDR clone that persisted and spread in the country.
Conclusion: The emergence of MDR S. Typhi strains is a major important public health issue in Jordan. This study should guide selection of effective antibiotic therapy for the treatment of typhoid and monitoring of the spread of MDR of S. Typhi.

P1159 Playground sand as an environmental source of a localized community salmonella outbreak

M. Staff, J. Musto, G. Hogg (Sydney, Melbourne, AU)*

Objectives: To identify the source of a protracted *Salmonella* Paratyphi B biovar Java (S. Java) outbreak localized to a single local government area within metropolitan Sydney, Australia.
Methods: From 2007 to 2009 S. Java cases notified under public health legislation to the Northern Sydney Central Coast Area Health Service were interviewed to collect clinical information and to identify likely sources of infection. Human isolates were classified by phage typing and Multi-locus Variable number tandem repeat Analysis (MLVA). Environmental samples were collected from various sources and isolates classified using both phage typing and MLVA. Contaminated sand from one playground was tested second monthly to assess environmental persistence of the bacterium. An age-matched case control study was conducted for outbreak cases identified in the first half of 2008 to test the hypothesis that close contact with playground sand was the source of outbreak.
Results: A total of 69 cases of S. Java were notified among residents in a single local government area (population 57,000) between 2007 and 2009 with 41% aged less than 2yrs. Most cases had significant gastrointestinal symptoms (including bloody diarrhoea) lasting a median of 7 days. Of the 65 human isolates for which phage typing was available 72% were reported as Dundee with remainder typed as reacts does not conform (RDNC). MLVA typing identified a specific outbreak strain with no variation at the first and fifth loci and only minor variation at the second locus. Case interviews implicated playground sand as a possible source and subsequent environmental testing isolated S. Java with the

outbreak MLVA pattern from 5 of 13 playgrounds tested. Second monthly testing of sand from one playground left undisturbed and closed to public access demonstrated that the organism could survive for at least 6 months in sand. The case control study confirmed playground sand exposure as the source of the outbreak with cases being significantly more likely to have played with sand in known contaminated playgrounds (odds ratio 3.7, 95% ci 1.1 to 12.1).
Conclusion: This research demonstrates that S. Java can survive in sand for a prolonged period and lead to a sustained community outbreak of human disease. The use of sand in playgrounds can pose a microbiological hazard especially to young children.

P1160 Two consecutive outbreaks of gastroenteritis due to infections with *Salmonella Typhimurium* DT193 in the Austrian Armed Forces, 2009

J. Pichler, R. Fretz, P. Much, C. Kornschober, G. Mayr, A. Leodolter, F. Allerberger (Vienna, Graz, Ried im Innkreis, AT)*

In 2008, the Austrian National Reference Centre for *Salmonella*, AGES, reported 21 isolates of *Salmonella Typhimurium* definite type 193 (ST DT193) in humans. In June 2009, 99 isolates, and in September 2009, 28 isolates from members of two military caserns in the province of Upper Austria were diagnosed with ST DT193. Consequently, AGES was assigned to investigate the outbreaks. The objective was to determine the magnitude of the outbreak and to identify the chain of transmission.
Methods: A descriptive-epidemiological investigation, a cohort study of the first outbreak, and a broad microbiological investigation were carried out. An outbreak case was defined as a person who (i) fell ill with diarrhoea between May 25 and June 7, respectively between Aug 31 and Oct 2, (ii) was on military duty in the relevant time period, or (iii) had a microbiologically confirmed infection with ST DT193.
Results: The attack rates were 27% in the 1st outbreak (122/450 persons from camp A), respectively 31% in the 2nd outbreak (61/200 members of a battalion during a field exercise, formed by persons of camps A+B). A continuous common-source outbreak was assumed in the 1st outbreak, based on the findings of descriptive and analytical epidemiology. Being on military duty between May 25–31 (RR 9.3; $p < 0.001$) and consuming several different meals in the dining hall of casern A (RRs 2.0–9.09; $p < 0.017$) in the same time period were associated with illness. However, the further conducted microbiological investigations (such as eggs from the local egg producer) were all *Salmonella*-negative; and the vehicle of infection was not identified. The most probable link between the two outbreaks were the use of mobile cooking units and heating boxes from camp A, which underwent inadequate cleaning and thermal disinfection due to a failure of the steamer used. Furthermore, the washing facility in camp A was in a very poor hygienic condition and a consequent resoiling of cooking utensils from the floor was very likely.
Conclusion: In spite of extensive investigations in a clearly closed setting, the vehicle of infection was not identified. However, failure in the cleaning procedure of cooking utensils and poor kitchen hygiene in the camp A may account for the 2nd outbreak.

P1161 Phenotypic and molecular characterization of a shigellosis outbreak in Antofagasta, Chile, during the year 2008

J. Silva, J. Gaona, N. Herrera, C. Chambel, B. Alcaide, K. Guerrero, D. Gonzalez, N. Valenzuela, A. Marcoleta, R. del Campo on behalf of the CIBERESP*

Objectives: Shigellosis is a major cause of diarrhea with a high morbidity and mortality, especially in the developing countries, being *Shigella flexneri* the more frequent specie detected in South America. During the year 2008, we observed a dramatically increase in the *Shigella sonnei* incidence from patients attended in different Health Centers of Antofagasta (North of Chile). The aim of this work was to analyze the phenotypic and molecular characteristics of these strains.
Methods: We recovered prospectively (January-June 2008) information about identification, serology and antibiotic susceptibility of 265 *Shigella* isolates obtained in three different Health Centers: a Public Hospital, a Private Clinic, and a Primary Health Center. Biochemical and serological tests were applied for specie identification, which was confirmed by specific PCR amplifications. Antimicrobial susceptibility to ampicillin (AM), ceftriaxone (CTR), ciprofloxacin (CIP), cloramphenicol (CH), cotrimoxazole (SXT), and nitrofurantoin (NF) was determined by the agar dilution method. Plasmid presence was analyzed in all strains and their genetic diversity was explored by PFGE.
Results: Specie identification of the isolates corresponded to *S. sonnei* (79%), *S. flexneri* (13%), *S. boydii* (3%), *S. dysenteriae* (2%) and *Shigella* spp (3%). About 50% of the diarrhea process in children with $\leqslant$10 years was caused by *S. sonnei,* and we focused our study in forty randomized isolates (23 from children and 17 from adults) of this specie. Most of patients were living in the same geographic zone; the north area of Antofagasta, supporting that contaminated water was the most probably the cause of the disease. High resistance to ampicillin (94%), chloramphenicol (89%) and cotrimoxazole (89%) was detected in these strains, being the 84% of the strains co-resistant to the three antibiotics. A particular plasmid was observed in almost all forty isolated. Genetic PFGE studies revealed that 95% of *S. sonnei* isolates grouped in the same clone.
Conclusion: We detected a Sighella sonnei outbreak in our region, affecting different patients from different Health Centers, being contaminated water the most probable route of transmission.

P1162 Non-pathogenic *Vibrio* environmental strains carrying virulence, fitness and antibiotic resistance genes

M. Gennari, V. Ghidini, M. Tafi, M. Lleò (Verona, IT)*

Objective: Vibrios are important members of the autochthonous flora of aquatic environments and include non pathogenic and pathogenic species. Although it has been usually report that environmental bacteria lack virulence and antibiotic resistance genes generally found in clinical strains, recent studies indicate that acquisition of such genes might have place in the aquatic environment. A collection of non-pathogenic vibrios has been screened for genes generally found in pathogenic species.
Methods: *Vibrio* strains were isolated from water, plankton and sediment samples obtained in the northern Adriatic Sea. The DNA extracted from 114 environmental isolates was subjected to PCR to screen a battery of virulence, fitness and antibiotic resistance genes usually found in *V. cholerae* and *V. parahaemolyticus.* Two of the genes, yopP and trh, are considered virulence determinants in *V. parahaemolyticus* while the nanH gene from *V. cholerae* encodes a neuraminidase. flrA is a gene involved in the regulation of *V. cholerae* flagella synthesis and response to environmental changes. *V. cholerae* vpsR is involved in biofilm formation and environmental persistence, while *V. cholerae* luxA gene is involved in bioluminescent expression. int15′cs is a gene included in a cassette of antibiotic resistance determinants in *V. cholerae.*
Results: 40 marine strains resulted positive to PCR using primers selected on the described genes: 11 strains carried the gene nanH and 3 the yopP gene while no strains carried the trh gene. 13, 6 and 7 strains presented respectively the expected amplicons for flrA, vpsR and luxA while 7 strains carried int15′cs, a sequence from an integron involved in antibiotic resistance. Further analysis of the strains resulting positive to the presence of the gene nanH, which in *V. cholerae* is located in the pathogenicity island VPI-2, showed that 5 out of 9 strains carried this mobile genetic element.
Conclusions: The data obtained provide further support to the fact that gene exchange occurs in the aquatic environment between pathogenic and non pathogenic bacterial species and that in this exchanging may be included large genetic elements such as integrons and entire or fragments of pathogenicity islands. For this reason, environmental vibrios strains could constitute a marine reservoir of virulence and antibiotic resistance genes and might represent a risk for human health.

P1163 Role of toxR on survival strategies of *Vibrio cholerae* O1 El Tor

S. Valeru, H. Abd, G. Sandström (Solna, SE)*

Objectives: *Vibrio cholerae*, the causative agent of diarrhoeal disease cholera, utilises different survival strategies in aquatic environments. The bacterium can survive as free-living or in association with zooplankton and under starvation it can build biofilm and rugose colonies. *V. cholerae* expresses cholera toxin and toxin-coregulated pilus as the main virulence factors, which are co-regulated by a transcriptional regulator toxR.
Aim of the current study was to investigate role of toxR gene on survival of *V. cholerae* O1 El Tor under starvation or in association with the free-living amoeba *Acanthamoeba castellanii*.
Methods: Starvation survival test, rugose switching test, biofilm analysis, analysis of protein expression, analysis of toxR expression and bacteria-amoeba association assay.
Results: The results showed that toxR mutant can shift to rugose colony morphology in response to nutrient starvation characterised by wrinkled colony morphology, which shows increased biofilm formation and enhanced survival under specific conditions such different media and different temperatures. The toxR mutant strain shows high frequency of rugose switching at stationary phase when compared with wild-type *V. cholerae* strains. Immunoblot analysis showed that expression of Omp T was increased in rugose strains and Omp U seemed to be reduced.
The association assay of the bacteria with amoebae showed that presence of *A. castellanii* enhanced growth and survival of *V. cholerae* wild-type and toxR mutant to 100 fold at day 10. In comparison, growth of bacteria decreased 10000 fold from day 4 in absence of the amoebae. Despite *A. castellanii* grew well in absence of wild-type and toxR mutant bacterial strains, presence of the each bacterial strain did not inhibit growth of the amoebae.
Conclusions: The variation in expression of outer membrane proteins seem to play an important role in *V. cholerae* O1 El Tor (strain 1552) rugose and smooth switching. Rugose to smooth switching mechanism in *V. cholerae* may be toxR dependent, and the toxR gene is also important for the survival of *V. cholerae* in starvation conditions. *A. castellanii* enhanced growth of both wild-type and toxR mutant *V. cholerae* strains indicating that toxR has no affect on the interaction of *V. cholerae* with *A. castellanii* and that the bacteria are adapted to survive with the amoebae possibly in the aquatic environments.

P1164 A *Campylobacter jejuni* outbreak investigation in Crete, Greece: indications for waterborne spread

I. Karagiannis, T. Sideroglou, K. Gkolfinopoulou, E.N. Velonakis, E. Scoulica, T. Panagiotopoulos, K. Mellou, S. Bonovas (Athens, Heraklion, GR)*

Objectives: To present the results of the investigation of a *C. jejuni* outbreak, which took place around Chania, a town in Crete, Greece, in late May to early June 2009.
Methods: Two analytical studies were conducted in parallel: a case–control and a case-crossover study. Fifty cases were included in the two studies and 124 controls, with respiratory track symptoms, frequency matched for age, were recruited in the case–control study. The case definition was the same for both studies. The questionnaires were completed via telephone interviews with the children's parents in July 2009. STATA v11.0 was used for data analysis. Stool cultures, PFGE and MLST sub-typing in human samples and PFGE in chicken samples were conducted. Water quality tests were run in the outbreak area.
Results: Thirty-seven cases and 79 controls responded. The median age of cases and controls was 2 years. Sex distribution did not differ between cases and controls. The most commonly reported symptoms were diarrhoea (100%), fever (58.3%) and bloody stool (58.3%). In the univariate analysis consumption of tap water from the town's supply system was protective against Campylobacteriosis (OR: 0.23, 95% C.I: 0.06–0.77). In the stratified analysis by tap water supplier, consumption of tap water at home could be computed for rural areas only (ORMH=3.26, 95% C.I: 0.98–10.82). For these areas statistically significant factors were: consumption of bottled water (OR: 0.15, 95% C.I: 0.04–0.54), use of a tap water filter (OR: 0.00, 95% C.I: 0.00–0.53), use of dishwashers for children's utensils (OR: 0.16, 95% C.I: 0.03–0.75), consumption of concentrated milk (OR: 2.68, 95% C.I: 0.98–7.37) and consumption of milk types that need to be diluted with water (OR: 3.05, 95% C.I: 0.99–10.49). In the multivariable analysis, the town water supplier (OR: 0.17, 95% C.I.: 0.05–0.57) and drinking tap water (OR: 4.39, 95% C.I: 1.30–14.8) were statistically significant. Case-crossover design revealed no risk factors. The strains from patients' samples were identical but different from that of the chicken sample. No *Campylobacter* was found in any water sample.
Conclusions: Although there are indications that tap water quality was poor during and exactly before the outbreak, no *Campylobacter* was found in any of the environmental samples. However, there is strong epidemiological proof that tap water was the vehicle of the outbreak.

P1165 An outbreak of *Campylobacter jejuni* gastroenteritis at an Australian boarding school: consistency between fla-A typing and multi-locus sequence typing

C.R. Moffatt, A.S. Cameron, L. Mickan, R. Givney (Canberra, Adelaide, Newcastle, AU)*

Campylobacter jejuni causes an estimated 2 million cases of gastroenteritis each year in the United States more than either *Shigella* or *Salmonella* infections. In Australia during 2007, there were approximately 17,000 cases notified, giving a national incidence rate of 120.2 per 100,000 population.
Despite the frequency and potential severity of *Campylobacter* infections, point source outbreaks are rarely reported.
One reason for this is the lack of a standardised *Campylobacter jejuni* sub-typing method to distinguish clusters of related cases from the background of sporadic cases/
In this study an outbreak of *Campylobacter jejuni* gastroenteritis occurring at a boarding school was investigated using a retrospective cohort study and environmental health investigation. Molecular typing of human and environmental isolates using Restriction Fragment Length Polymorphism (RFLP) flaA typing and multi-locus sequence typing (MLST) was undertaken.
Thirty-five cases of gastroenteritis were reported among 58 persons exposed, with 14 confirmed as being *C. jejuni* infections. Attendance at one evening meal was statistically associated with illness (Ratio of proportions of 3.094, (95% CI, 1.206–11.11, $p < 0.0085$). There was no statistically significant association between any single food provided at the implicated evening meal and illness, suggesting a potential cause of the outbreak was a cross-contamination event.
Among human isolates two distinct RFLP fla-A subtypes were found. Results from subsequent MLST data were consistent with the fla-A typing results, indicating that RFLP fla-A may be a potential candidate method for *Campylobacter jejuni* sub-typing for public health epidemiology.

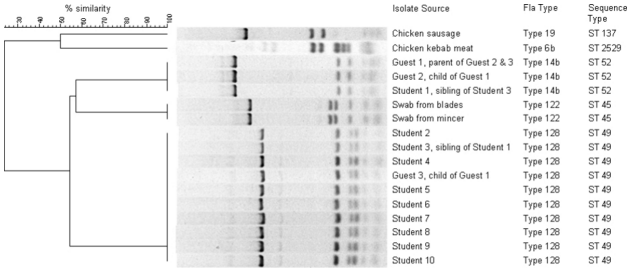
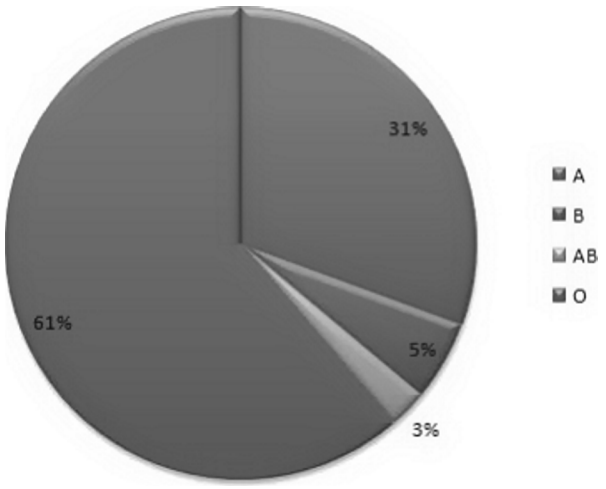


Figure. UPGAM dendrogram of the *fla*A RFLP patterns of *C. jejuni* isolates from human and environmental sources.

P1166 ***Helicobacter pylori*** **infection: association with blood group, demographics and life style in a Pakistani population**

A.K. Khuwaja, F. Wahab, A. Valliani, B. Ahmed (Karachi, PK)*

Background: It is well known that blood group antigens are related to the development of peptic ulcer and gastric carcinoma. *H. pylori* infection is one of the most widespread infection in the world. Its intensification in the individual populations is strongly related to economic conditions. Developing countries (Asia, Africa, Central and South America, Eastern Europe) are at high risk, due to people living in poor socioeconomic conditions. This study sought to determine the relationship between *H. pylori* and ABO blood groups, age, gender, smoking and life style.
Methods: Cross-sectional prospective study was conducted at endoscopy suit in Public Sector Hospitals of Karachi from Sep 2008 to Nov 2008. All the symptomatic patients coming for upper GIT endoscopy were included in this study.
Results: Biopsy for histopathology was taken from 558 patients out of them 222 (39.8%) were males with age range 18–65 years. Age group of 21–40 years was found to be related with *H. pylori* infection. Out of 558 patients 216 (38.7%) were turned out *H. pylori* positive with a significant male preponderance ($p \leqslant 0.05$). Distribution of ABO blood groups in H. Pylori group was A = 66/216 (30.5%), B = 12/216 (5.5%), AB= 6/216 (2.7%) and O = 132/216 (61.1%) which is statistically significant ($p \leqslant 0.05$). Rh factor was also related to *H. pylori* infection ($p = 0.514$). *H. pylori* could not be related with smoking ($p = 0.075$). Excessive tea consumption was related to *H. pylori* infection.
Conclusion: This study demonstrates that *H. pylori* infection can be related to ABO blood group, age, gender and even lifestyle. People of blood group O are more prone to develop infection related gastritis, ulcers, and even perforations, so they should be cautious against transmission of it. Although there was no correlation found with smoking in this study, other studies have shown otherwise. We suggest that there is a need for making aware people to promote healthy life style, especially after knowing their own health condition and demography.



H. pylori infection association with ABO blood group (n = 558).

Norovirus and rotavirus

P1167 **A foodborne norovirus outbreak in a healthcare facility, Austria, 2009**

H.W. Kuo, M. Hell, S. Kasper, I. Lederer, F. Allerberger, D. Schmid (Vienna, Salzburg, AT)*

Objectives: A foodborne norovirus outbreak occurred in a health care facility in Austria, from March 15 to March 27, including a total of 167 cases. Six out of ten cases tested were positive for norovirus. With a maximum of 91 cases on March 17, the pattern of the outbreak spread indicated foodborne origin, followed by a person-to-person spread. The kitchen was disinfected on March 17, and it can be assumed that 114 resident-cases with disease onset from March 16 to March 18 were suspected to be foodborne. A case-cohort study was performed among the residents of the affected premises to test the hypotheses that exposure to specific food items served on March 14, 15 and 16 was associated with increased risk of norovirus infection.
Methods: Out of the cohort-at-risk, including 510 residents who were exposed to food at least on one of the three risky days (March 14, 15 and 16), 274 persons were randomly selected regardless of the disease status for the reference-group. The study subjects were interviewed by self-administered questionnaire or face-to-face interviews on their exposure to food items served on the risky days. Out of the 388 study subjects, 350 responded to the questionnaire (response rate: 90%) and therefore remained for the case-cohort analyses.
Results: The analyses revealed that consumption of a particular cold cut (sausage) served on March 15 (OR = 3.98, 95% CI: 1.18–14, $p = 0.0166$), a soup served with sliced bread (OR = 1.88, 95% CI: 1.11–3.18, $p = 0.0176$), a meat dish with rice and salad (OR = 2.03, 95% CI: 1.16–3.55, $p = 0.0112$), and a rolled pancake filled with spinach served on March 16 (OR = 1.72, 95% CI: 1.03–2.88, $p = 0.037$) were associated with increased risk of a norovirus infection (Table 1).
Conclusion: Each of these epidemiologically identified risk-associated food items could have been easily contaminated with norovirus during preparation. Out of the 26 kitchen staff-members tested, 11 were positive for norovirus. Out of these, 5 kitchen staff members – who stated to have been asymptomatic – were on duty during the weekend prior to the outbreak. Repeated training of food handlers working in health care facilities in hand hygiene and environmental disinfection, and the implementation of the concept of hazard analysis and critical control points are indispensable to prevent foodborne norovirus outbreaks in health care facilities.

Table 1. Food items associated with increased risk of norovirus infection, odds ratio (OR) and 95% confidence interval (95% CI)

Food items	Univariable analyses		
	OR	95% CI	p
March 15 breakfast			
Particular cold cut (sausage)	3.98	1.18–14	0.0166
March 16 lunch			
Soup served with sliced bread	1.88	1.11–3.18	0.0176
Meat dish with rice and salad	2.03	1.16–3.55	0.0112
March 16 dinner			
Rolled pancake filled with spinach	1.72	1.03–2.88	0.037

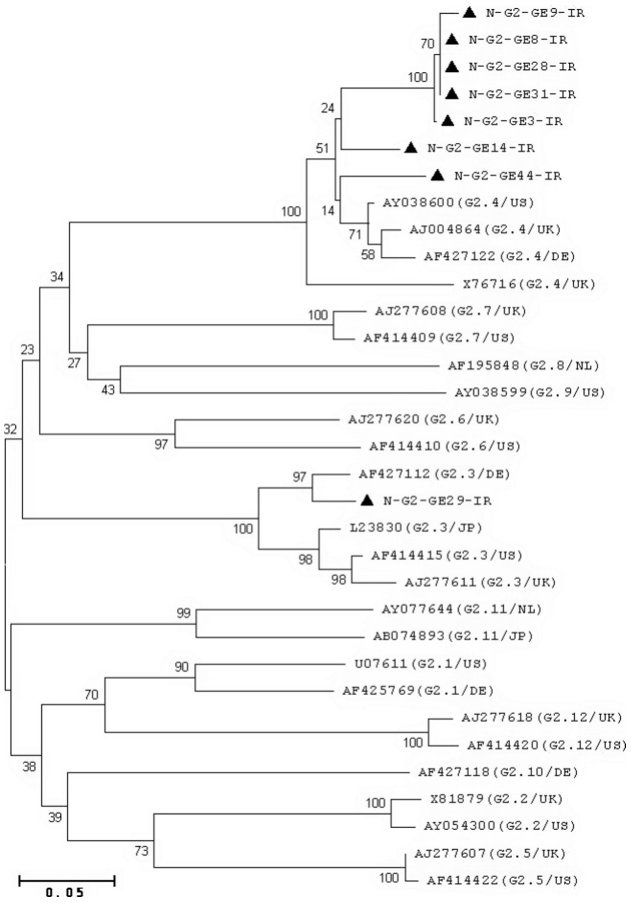
P1168 **High mortality following norovirus enteritis in hospitalized elderly patients**

L. Gustavsson, L.M. Andersson, M. Lindh, J. Westin (Gothenburg, SE)*

Objectives: Norovirus (NoV), particularly genogroup II (GII), is the leading cause of epidemic gastroenteritis in high-income countries. NoV may contribute to excess mortality in the elderly and chronically ill. The objective of this study was to retrospectively describe the all-cause mortality following NoV infection in hospitalised patients.

Methods: All hospitalised adult patients with a stool sample positive for NoV GII by PCR treated at Sahlgrenska University Hospital, Gothenburg, Sweden, from August 2008 through June 2009, were included. Patient files were reviewed and subjects characterised by age, sex and concurrent medical conditions (immune suppression, renal failure, diabetes, heart failure, malignancy, systemic inflammatory disease or other significant chronic illness). Deaths up to 90 days following the time of positive sampling were noted. In-hospital as well as 30- and 90-day mortality was calculated. χ^2 test was used to compare proportions. A p-value <0.05 was considered significant.
Results: 606 patients were initially included in the study. There were no deaths among patients less than 60 years old during the study period and these were not included in further analysis. The remaining 539 patients were aged 60 to 101 years, with 58% females. Sixty-two percent were diagnosed with at least one underlying condition. Total mortality rates in-hospital, within 30 and 90 days were 5.9%, 8.5% and 16.7%, respectively. Mortality rates increased with increasing age (see table). Total mortality rates were higher in patients with underlying conditions listed above, as compared to patients without these conditions (all ages combined: 10.5% vs. 5.3% at 30 days, $p < 0.05$; 21.6% vs. 8.7% at 90 days, $p < 0.001$). However, when looking at the age group >90 years isolated ($n = 106$), this difference was not seen.
Conclusion: We found considerable all-cause mortality, in chronically ill as well as in otherwise healthy elderly patients, within the 90 days following a NoV infection, suggesting that NoV may cause excess mortality in these patients.

Age (y)	Patients (n)	30-day mortality (%)	90-day mortality (%)
60–69	63	1.6	11.1
70–79	108	5.6	13.1
80–89	262	9.2	16.8
90+	106	14.2	23.6



P1169 Hospital-based phylogenetic analysis of noroviruses isolated from children with acute gastroenteritis in Iran

Z. Fazeli Bavandpour, A. Moradi, N. Baghaie, S.A. Nadji (Tehran, IR)*

Objectives: Noroviruses are the most important cause of acute gastroenteritis worldwide. Phylogenetic analysis revealed that the noroviruses could be divided into five genogroups (GI, GII, GIII, GIV and GV). The genogroups are further classified according to amino acid sequences from the capsid gene. The aim of the study was to determine the prevalence and analysis of norovirus phylogeny in children with acute gastroenteritis admitted to NRITLD, Iran.
Methods: A total of 47 fecal samples were collected from children up to 17 years of age, with acute gastroenteritis from 2006 to 2008 in Masih Daneshvari hospital in Tehran, Iran. For screening, nested PCR were performed to amplify sequences of the RNA polymerase region. The positive samples were re-amplified by using the region D primer sets (genogroup specific for GI and GII). 8 positive samples were subjected to sequencing for VP1 (D region) gene region. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1.
Results: The mean age of the study subjects was 30.26±28.61 (SD) months (range 3–120). The gender ratio (male: female) was 1.47. The Norovirus was detected in 21.3% of the subjects. Statistical differences were not observed in gender, age group and seasonal distribution of noroviruses. All isolates were identified as genogroup GII. The phylogenetic analysis revealed that 7 samples belong to genotype GII.4 and only 1 sample belongs to genotype GII.3 (Figure 1). There were 95–100% sequence identity at the amino acid level and 89.5–99.5% identity at nucleotide level among GII.4 isolates.
Conclusions: Our results show that noroviruses, after rotaviruses, are indeed a major cause of acute gastroenteritis in children and genogroup GII, mainly GII.4 genotype, is the most common type.

P1170 Aetiology of diarrhoea in patients at a university hospital in Sarajevo, Bosnia and Herzegovina

A. Dedeic-Ljubovic, M. Hukic, I. Salimovic-Besic, D. Bekic, M. Babic, S. Arapcic, S. Ramadani (Sarajevo, BA)*

Objectives: A prospective study was carried out to investigate the commonest etiologic agents of diarrhea for all ages from hospitalized patients.
Methods: Stool samples from 890 patients (adults and pediatric) with diarrhea admitted to the clinical center university of Sarajevo, Bosnia and Herzegovina from May 01 to October 31, 2009 were collected and processed for viral, bacteral, parasitic and yeast examination using standard methods.
Results: Overall, in 51% (458/890) of patients a potential pathogen is identified. A viral cause was found in 26% (233/890), bacterial in 17% (151/890), parasitic in 6.8% (24/890) and yeast in 5.6% (50/890) of cases. The occurrence of enteropathogens identified was as follows: rotavirus (151/458) 33%, *Salmonella* (122/458) 26.6%, with *Salmonella enteritidis* as the most common, astrovirus (54/458) 11.8%, diarrhoeagenic *Escherichia coli* (20/458) 4.3%, with enteropathogenic *E. coli* (EPEC) 044:K74, and enterohaemorrhagic *E. coli* (EHEC) O103:K- as the most common; *Entamoeba histolytica* (15/458) 3.2%, adenoviruses (9/458) 2%, *Giardia lamblia* (7/458) 1.5%, *Campylobacter* spp., *Clostridium difficile* toxin A+B and *Shigella sonnei* (3/458) 0.65%. No *Yersinia enterocolitica* was isolated. 9.4% of patients had mixed infection with two viral agent. Mixed viral and bacterial infection occured in 4% of patients. Most diarrheal episodes of viral and diarrhoeagenic *E. coli* etiology occured during the first three years of life. 10% (50/458) of patients with primarily non-gastrointestinal infection had elevated *Candida* counts in the stool. The majority of these patients were on antibiotic treatment. The most common species were *Candida albicans* and *Candida glabrata*. *Salmonella* and *Shigella* isolates were

tested for their susceptibility to common antimicrobial agents and most of the isolates were sensitive to all tested antimicrobials.
Conclusion: This study demonstrated that rotavirus and *Salmonella* were significant enteropathogens. This study also, confirms that diarrhoeagenic *E. coli* is important causal agent of diarrheal diseases in pediatric patients in Bosnia and Herzegovina. Our results also suggest that antibiotics make an important contribution to the incidence of the elevated *Candida* counts in the stool from patients with diarrhea.

CMV

P1171 Brain damage in foetuses congenitally infected by cytomegalovirus

L. Gabrielli, M.P. Bonasoni, S. Lega, D. Santini, F. Baccolini, G. Piccirilli, A. Chiereghin, E. Petrisli, B. Guerra, G. Gardini, M.P. Landini, T. Lazzarotto (Bologna, Reggio-Emilia, IT)*

Objectives: Cytomegalovirus (CMV) is the major infectious cause of congenital abnormalities in the central nervous system due to intrauterine infection in humans, with an average incidence of approximately 1.0% live births. The aim of this study was to understand the histopathogenesis of cerebral damage in 27 foetuses with congenital CMV infection documented at 20–21 weeks gestation by invasive prenatal diagnosis (amniotic fluid CMV culture and PCR positive with a viral load $>10^5$ GE/ml). All pregnancies were electively terminated at 22 weeks gestation.
Methods: Foetal sections were studied with haematoxylin and eosin staining and immunohistochemistry for CMV early antigen (ppUL44) and leukocyte common antigen CD45. Inflammatory infiltrate in the brain was characterized by immunohistochemistry for CD68PGM1, CD20, CD3, CD4, CD8 and granzyme B.
Results: All of the foetuses had a disseminated CMV infection. Inflammatory infiltrate was almost always present in CMV-infected foetal organs and the severity of the inflammatory response was correlated with the organ damage. Six out of 15 CMV-positive brains showed mild inflammatory response without necrosis and only mild telencephalic leukencephalopathy. Four CMV-positive brains showed a moderate CD45-positive inflammatory response associated with focal microglial nodules and telencephalic leukencephalopathy. The inflammatory response in the remaining five CMV-positive brains was severe and associated with diffuse cerebral damage such as cortical necrosis, polymicrogyria and periventricular leukomalacia. Characterization of inflammatory infiltration revealed the presence of activated CD8 T lymphocytes around cytomegalic cells often associated with apoptotic bodies. Diffuse chronic villitis with necrosis was found in all of the placentas of foetuses with severe brain damage.
Conclusion: Brain damage seems to be the results of a combined effect of viral infection and inflammatory infiltration with activated CD8 T-lymphocytes. The hypoxia due to severe placentitis may be a further way in which CMV could damage the foetal brain.

P1172 Cytomegalovirus infection in neonatal intensive care units

A. Senok, S. Al-Khawaja, A. Ismaeel, G. Botta (Sharjah, AE; Manama, BH; Udine, IT)*

Background: Human cytomegalovirus (HCMV) infections are among the most prevalent viral infections worldwide with rates of congenital infections varying in different populations. This study was to determine the prevalence of congenital and perinatal HCMV infections among newborns in two major neonatal intensive care units (NICU) in Bahrain.
Methods: Preterm and term babies admitted to the NICU during the study period (February-June 2006) as well as their mothers were included in the study. During the first six weeks of life, urine and saliva was obtained from the babies weekly and serial breast milk samples were obtained from the mothers. Maternal serum HCMV IgG was measured. Virus isolation and detection was by shell vial culture and nested PCR. HCMV infection was diagnosed in infants with virus excretion detected by both methods on at least two occasions or in two different specimen types.
Results: A total of 100 newborns (84 preterm; 16 term) and their mothers were included in the study. Maternal HCMV IgG-seropositivity was 100%. Congenital HCMV infection was confirmed in 3 newborns (preterm 2/84; 1.9%; term 1/16; 6.3%). HCMV DNA was detected in the breast milk samples obtained during the first 10days postpartum from mothers whose babies had congenital HCMV. Among breast milk samples collected 4–6weeks post-partum 22.5% were HCMV DNA positive. It was determined that five pre-term babies acquired perinatal infections via HCMV positive breast milk at 4–6weeks postpartum. There were no significant difference in the detection of HCMV using shell vial culture versus nested PCR method.
Conclusion: Our findings suggest that neonatal ICU care should include neonatal HCMV screening to detect the infection during early postnatal period. Shell vial culture and PCR amplification represent rapid and sensitive methods can be used for diagnosis of suspected congenital HCMV infections.

P1173 Prevention of cytomegalovirus disease in transplant recipients. Evaluation of real-time PCR CMV, clinic correlation and cutoff determination of pre-emptive therapy

C. Martín-Gandul, E. Cordero, M. Sánchez, V. Cabello, J. Fijo, C. Bernal, E. Lage, F. De la Cruz, M. Aguilar, M.J. Rodriguez, E. García for the Spanish Network for Research in Infectious Disease*

Objectives: Preemtive therapy guided by threshold viral replication allows optimizing cytomegalovirus (CMV) infection therapy. There is not a standarised and validated cut off point to guide preemptive CMV therapy in organ transplant patients. The aims of this sudy are: 1. To study the correlationship between CMV viral load and the spectrum of CMV infection in transplant recipients. 2. To set up a cutoff point of CMV viral load to start preemtive therapy.
Methods: A prospective cohort study was performed from october 2008 to june 2009. Every trasplant recipients with viral replication detected by PCR CMV were included. SPSS V15.0 was used for descriptive analysis and G-Stat 2.0 for analysis by ROC (Receiver Operating Characteristics) curves of sensitivity and specificity in the full spectrum of cutoff points in the over range of observed results.
Results: One hundred and twenty patients were included: 104 solid organ transplant recipients (28 liver, 63 renal, 1 liver-renal, 11 heart) and 16 allogeneic hematopoietic stem cell transplant recipients. Median age was 53 years (range 9–73), 64.7% were male and 16.6% were at high risk for CMV infection. There were 7 episodes (5.83%) of CMV organ disease (four gastritis, one colitis, one duodenitis, one pneumonitis and retinitis), 6 episodes (5%) of viral syndrome and 107 episodes (89.17%) of asyntomatic infection. Fifty-two patients (43.3%) received preemptive therapy. The median CMV copies at the start of therapy was 4325 copies /ml, interquartile range (1662.50 to 9265). After assessing the different cutoff points by ROC curves, 2130 copies/ml was defined as the best cutoff point for early therapy initiation, with specificity 94.67%, sensitivy 84.62%, negative predective value 99.37% and positive predictive value 9.09%. The area under the curve (AUC) ROC of 87.9% represents a high accuracy in the diagnostic test.
Conclusions: 1. Real time PCR CMV is an effective predictive dignostic test to guide preemptive therapy. 2. 2130 copies /ml is defined as the best cutoff point for starting preemptive therapy. 3. A cutoff point higher than 5000 and 10000 copies/ml as suggested in the literature is not acceptable in our experience because there were lower levels of CMV replication in a significant proportion of patients with CMV organ disease. 4. New studies are needed to validate this cutoff point in a external cohort.

P1174 Diagnosis and screening for congenital CMV infection in pregnant women in Cuba as prognostic markers of congenital cytomegalovirus in newborns

C. Correa, V. Kouri, D. Verdasquera, P.A. Martinez, A. Alvarez, Y. Aleman, L. Perez, J. Viera, R. Gonzalez, E. Perez, I. Moro, M. Navarro, P. Melin (Havana, CU; Liège, BE)*

Objective: Human cytomegalovirus (HCMV) has established itself as the most significant cause of congenital infection in the developed world, leading to mental and developmental retardation. The objective of this research was to identify pregnant women and newborns at risk of developing congenital infection due to HCMV.
Methods: A diagnostic algorithm utilizing Immunoglobulin G (IgG), IgM, and, IgG avidity was used to prospectively screen serum from 1131 pregnant women enrolled from three municipalities from Havana City, Cuba during 2007–2008. Qualitative nested PCR and quantitative Real Time-based PCR testing of DNA from urine and saliva were performed on women detected with active infection and their newborn. The identification of different clinical, demographical and epidemiological factors was associated with the virological results, aiming to identify prognostic markers of congenital infection.
Results: The majority of women were seropositive to HCMV (92.7%), with 2.38% (27 women), having active infection (IgM positive or IgG seroconversion). Primary infection was detected in 20 pregnant women (1.77%) while 7 patients (0.62%) had active non primary infection. HCMV DNA was found to be positive in 9 of the 27 pregnant women. CMV congenital infection was diagnosed in 12 (1.06%) of the 26 children born from mothers with active infection, for a vertical transmission rate of 46.2%. Two newborns were symptomatic at birth and two showed sequels during the follow up until 6 month age. It was found that mothers with primary or non-primary active infection had significant risk, RR: 1.11 and RR: 1.16, respectively, to have congenital infected children, furthermore, women with active infection showed significant risk (RR: 1.35) to have congenitally infected children.
Conclusions: It constitutes the first study of diagnosis and screening for congenital HCMV infection in pregnant women and their newborn in Cuba, demonstrating high prevalence rates, although there is an important group of women at risk to acquire infection during pregnancy and produce congenital infection on their offspring. Prenatal diagnosis, and early identification and follow up of congenital CMV infected infants are important for the confirmation and/or definition of the medical conduct with the positive infant, trying to avoid or reducing some possible sequels.

P1175 Prenatal diagnosis of congenital cytomegalovirus infection and outcome in 598 pregnant women undergoing a primary cytomegalovirus infection

T. Lazzarotto, L. Gabrielli, F. Baccolini, B. Guerra, C. Puccetti, M. Lanari, A. Chiereghin, E. Petrisli, G. Piccirilli, M.P. Landini (Bologna, IT)*

Objectives: PCR detection of cytomegalovirus (CMV) in amniotic fluid (AF) will differentiate uninfected from infected fetuses in mothers with primary CMV infection. Ultrasound has the advantage of not being invasive and will disclose any structural and/or growth abnormalities caused by CMV infection, but its sensitivity is poor.
Methods: This study describes a cohort of 598 pregnancies at risk of CMV transmission in utero of which 598 were tested by AF sampling followed by Real Time assay (target region: UL123) (Q-CMV Real Time, Nanogen Advanced Diagnostics, Italy). PCR results were reported as number of DNA copies per mL of AF.
603 AF samples were collected at 20–21 weeks gestation and at least 6–8 weeks after the onset of maternal CMV infection. Outcome was fully documented in 545 newborns and 59 fetuses.
Results: Fewer than 500 copies were detected in 497 AF samples. No congenital CMV infection was identified in 468 cases, while 29 newborns were infected without symptoms at birth or during subsequent monitoring. 59 infected fetuses and 48 newborns were found in 106 AF samples with >500 copies.
A total of 77 newborns were CMV infected, 20 were symptomatic and 57 asymptomatic. Among symptomatic and asymptomatic newborns, the mean viral load was significantly higher in symptomatic newborns ($P = 0.023$) (mean values 2.1×10^6 vs 8.7×10^4).
The 59 infected fetuses were classified as having histological damage in the brain and in 2 or more organs (group 1) or having histological damage only in 2 or more organs (group 2).
When qPCR disclosed more than 10^6 copies/mL of AF, 60.7% of fetuses had disseminated infection and brain damage (group 1) against 39.3% of fetuses with disseminated infection alone (group 2). When viral load in AF was between 10^5 and 10^6 copies only 40% of fetuses were classified in group 1 that includes certain cases of more severe infection.
Conclusion: Negative PCR in AF at 20–21 weeks gestation and at least 6–8 weeks after maternal infection excludes congenital CMV infection. Positive PCR in AF at 20–21 weeks gestation and at least 6–8 weeks after maternal infection identifies CMV infected fetuses.
Low viral loads (<500 copies/mL) in the AF, sampled at the appropriate times, may be a good indicator for ruling out fetal damage. High viral loads may be associated with symptomatic or asymptomatic congenital infections, but the highest values tend to indicate more severe infection.

P1176 Multicentre evaluation of the CMV IgM assay on the family of Access® immunoassay systems

L. Grangeot-Keros, C. Vauloup-Fellous, N. Gaidot, M. Rawlins, N. Groleau, J. Ritchie, C. Darte, F. Bouniort, M. Olson (Clamart, FR; Salt Lake City, US; Marnes-la-Coquette, FR; Atlanta, US; Marseille, FR; Nyon, CH; Chaska, US)*

Objectives: The human cytomegalovirus (CMV) is a member of the Herpesviridae family. CMV is transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation. CMV infection is a serious concern for women of child-bearing age because it is a leading cause of hearing and vision loss, as well as mental retardation among congenitally-infected children. Detection of CMV-IgM antibodies is only the first step in the differential diagnosis and confirmation of active CMV infection. The Access® CMV IgM assay is a two-step enyzyme immunoassay using paramagnetic particles coated with inactivated CMV antigen, alkaline phosphatase-conjugated sheep polyclonal anti-human IgM antibody and chemiluminescent signal detection.
Methods: Access CMV IgM assay reproducibility was evaluated at three centers using a panel of eight samples with varying degrees of reactivity and two quality control materials (negative and positive). Five replicates of each sample were analyzed each day for seven days to obtain at least five days of valid data. Concordance (percent agreement) with the bioMérieux VIDAS* and the DiaSorin Liaison* CMV IgM assays was evaluated at one site. De-identified residual non-selected samples from adult males, as well as adult pregnant and non-pregnant females ($n = 1{,}485$) who had CMV IgM serology testing ordered were analyzed using Access2 Immunoassay Systems.
Results: At the cut-off (1.0 S/CO), within site total %CV ranged from 4.9% to 8.8%. The initial overall agreement (excluding equivocal samples) of the Access CMV IgM assay with the VIDAS CMV IgM assay was 99% (95% CI, 98% to 99%). The initial overall agreement (excluding equivocal samples) of the Access CMV IgM assay with the Liaison CMV IgM assay was 98% (95% CI, 97% to 99%).
Conclusion: The Access CMV IgM assay provides excellent concordance with the comparison methods. The assay can aid in the diagnosis of CMV infection and may be used to assess the serological status of pregnant women with the advantage of a rapid, automated random-access immunoassay system.
*All trademarks are property of their respective owners.
† Pending submission to and clearance by the United States FDA, not yet available for diagnostic use in the U.S.

P1177 Measurement of CMV specific IgG avidity by Architect2000SR

D. Malotova, J. Trbusek (Sternberk, Prague, CZ)*

Objectives: Human cytomegalovirus (HCMV) is a member of the Betaherpesviridae subfamily. HCMV is the common cause of intrauterine infections and congenital abnormities. CMV also represents a serious threat to immunodeficient or immunosuppressed patients. Measurement of CMV specific IgG avidity has proven to be a powerful tool for distinguishing primary from non primary CMV infection.
The aim of our pilot study was to evaluate the new diagnostic test and get into everyday praxis.
Methods:40 patients with positive IgG antibodies were examined for IgG avidity. The samples were measured by instrument Architect 2000SR from company Abbott Laboratories. The Architect CMV IgG Avidity assay is a modified chemiluminescent microparticle immunoassay for determination in serum or plasma and is called Architect AVIcomp assay technology.
Results: From our group had 5 low IgG avidity and 35 high IgG avidity. We compared it with diagnoses and clinical symptoms. After this pilot study we have started this test to make in routine praxis.
Conclusion: Presence of high avidity IgG excludes the possibility that infection occurred within the previous 4 months. Low IgG avidity is a reliable indicator of infection within the previous 6 to 8 months. Architect 2000SR is only one fully automated instrument for CMV avidity testing.

Human papillomavirus

P1178 Epidemiology of high-risk human papillomavirus infections and associated cervical lesions in women over 30 years of age in St. Petersburg, Russia

E. Zolotoverkhaya, E. Shipitsyna, D. Kuevda, O. Shipulina, I. Kostyuchek, A. Savicheva (St. Petersburg, Moscow, RU)*

Objectives: The study aimed to determine the prevalence of high-risk human papillomavirus (HR HPV) types and associated cervical diseases in Russian women over 30 years of age, as well as to identify socio-demographic, behavioral and clinical predictors of HR HPV infection, with a view to consider implementation of HPV-based strategies in cervical cancer prevention in Russia.
Methods: Consecutive women aged 30 to 65 years (n = 823), not pregnant and with no history of treatment for high-grade cervical intraepithelial neoplasia (CIN), receiving routine gynaecological care in the outpatient department of the D.O. Ott Research Institute of Obstetrics and Gynaecology, St. Petersburg, Russia from June 2008 to April 2009 were enrolled. Cervical swabs were evaluated for cytological abnormalities and HR HPV types using Hybrid Capture 2 (HC2) assay with subsequent genotyping of HC2 positive samples with real-time PCR. Women with cytological abnormalities higher than atypical squamous cells of undetermined significance (ASCUS) and those who were positive for HR HPV were referred to colposcopy and biopsy confirmation.
Results: Infection with HR HPV was present in 108 (13%) women. Cytological abnormalities were found in 81 (9.8%) patients, including 59 (7.2%) women with ASCUS, 21 (2.5%) with low-grade squamous intraepithelial lesions (LSIL), and one (0.1%) with high-grade SIL. In one patient with normal cytology and positive HR HPV test biopsy showed high-grade CIN and in one patient with LSIL and positive HR HPV test biopsy showed invasive cancer. Of the 59 patients with ASCUS, 48 (81.4%) were HR HPV negative. The prevalence of HR HPV infection was highest (18.6%) among women aged 30–34 years and lowest (7.8%) among those aged 55 years or older. HPV DNA was less likely to be found in women who have been pregnant more than once and those who have been given live birth at least once. The only highly significant predictor of HR HPV infection was having symptoms of a urogenital infection. The most common HPV types were HPV 16 (31%), HPV 31 (21%), HPV 52 (13%), and HPV 33 (11%).
Conclusion: The present study emphasizes the importance of implementation of new cervical screening protocols in Russia that would include testing for HR HPV. Moreover, this study provides data on baseline distribution of different HPV types before the introduction of vaccination against HR HPV.
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P1179 Prevalence of human papillomavirus in anal samples of homosexual men

G. Gabilondo Alvarez, B. Romero, T. Hellin, J. Chacon, I. Sanz, M. Mateos (Madrid, ES)*

Objective: Persistent infection of human papillomavirus (HPV) is related to a higher risk of developing anal intraepithelial lesions and anal cancer. The aim of this study was to determine the prevalence of high risk genotypes of HPV (HR-HPV) and its association with human immunodeficiency virus.
Methods: 54 anal samples from 51 homosexual men were included in this study. The range of age was 20–65 years (media 38.5 years) and 23 of them were from HIV positive. All patients were attended in the Sexual Transmitted Diseases (STD) Unit of the Ramon y Cajal Hospital from June to November 2009. Anal samples were collected with cervical brushes (Cervix-Brush ®) and stored in PreservCyt ® medium. An anal cytology using the Bethesda classification and HPV genotyping (Linear Array®, Roche Diagnostics, Mannheim, Germany) were performed in all samples.
Results: Cytological results were obtained from 45 of the 54 samples. In the remaining 9, cytological results could not be evaluated due to an insufficient cellularity. The cytology result was normal in 31.5% (17/54), ASCUS in 7.4% (4/54), LSIL in 31.5% (17/54), HSIL in 7.4% (4/54) and ISIL in 5.5% (3/54). HPV was not detected in 16.6% (9/54), being 5 normal, 1 LSIL and 3 not valuable. HPV-51 and HPV-73 were the most frequent genotypes found (22.2%, 12/54); HPV-31 was found in 18.5% (10/54) and genotypes 16 and 18 were found in only 12.9% and 9.2% respectively. Mixed co-infections were seen in 64.8% (35/54), ranging from 1 to 9 different HR genotypes in the same sample. The overall prevalence of HR-HPV was 74% (40/54); 78.3% (18/23) in the samples from HIV+ patients, and 70.9% (22/31) in the negative ones.
Conclusions: The results suggest that HR-HPV 51 and 73 are the most prevalent in anal samples of homosexual men in our series. Genotypes 16 and 18 were not so frequently found as expected. This data must be considered for vaccine administration. Mixed co-infections were frequently observed. We could not find any differences in prevalence of HR-HPV genotypes between HIV positive and negative patients. The follow up of these patients is very important to prevent anal cancer.

P1180 Relation between E6/E7 transcripts expression and clinical and viro-immunolgical parameters in HIV/HPV co-infected women

G. Orlando, M. Fasolo, E. Casolati, M.R. Gismondo, C. Antonacci, F. Mazza, S. Rimoldi, E. Omodeo Zorini, R. Beretta, P. Pileri, F. Pagano, A. Agarossi, N. Zanchetta on behalf of the GISPAP (Gruppo Interdisciplinare per lo Studio delle Patologie Associate al Papillomavirus)*

Introduction: HIV infected women have a higher oncogenic risk when infected from HPV. Cancer screening and follow up with conventional cytology or HPV typing need to be revisited due to the high prevalence of mild conditions, the high sensitivity of DNA assays and the different pattern of regression/progression in immunocompromized if compared to immunocompetent people. Factors involved in progression to pre-invasive and invasive lesions are virus and host dependant but, once the HPV genome has integrated in host cell, an over-expression of the HPV E6 and E7 genes can be detected.

Here we want to evaluate the correlation between HPV E6 and E7 expression with clinical and immuno-virological parameters to verify clinical performance of this test in the triage of HIV infected women.
Methods: HIV/HPV co-infected women included in the GISPAP cohort (L Sacco University Hospital, Milan – Italy) have been included in this analysis. Pts have been evaluated with cytology, HPV genotyping, HPV E6/E7 mRNA analysis, colposcopy and biopsy of suspected lesions; CDC stage of HIV infection, HIV RNA, CD4 and antiretroviral treatments were collected from clinical records of the patients.
Results: Among 150 HIV/HPV co-infected women included in this analysis, 209 high and low risk HPV genotypes were identified, HPV-52, 16 and 66 being the most represented. Pap smears were neg for cytological lesions in 81 patients, ASC in 3 pts, LSIL in 48 pts and HSIL in 12 pts, unavailable in 6 pts. Colposcopic evaluation in 61 pts revealed 21 abnormal patterns. Biopsies performed in 18 pts revealed 3 CIN-1, 5 CIN-2, 2 CIN-3 and 8 were neg for intraepithelial lesions.
E6/E7 test was positive in 68 (45.33%) of the cases. In a univariate logistic regression model, HR genotypes, LSIL or HSIL, ANTZ1–2, CD4 <200 and multi-experienced for antiretroviral treatment patients, are strongly related to HPV E6/E7 mRNA expression (tab 1).
Discussion: E6/E7 mRNA expression is highly predictive of HPV related lesions in HIV infected women. However, in severely immunosuppressed patients and antiretroviral multiexperienced patients the odds ratio of a positive result for mRNA transcripts is 8 and 4 timer higher respectively, thus suggesting an important role of host interaction in promoting HPV integrated onchogenic evolution.

Table 1. Clinical and immuno-virological parameters and odds ratio of HPV E6/E7 positive results

Variable	E6/E7 mRNA (N pos/N neg)	Fisher's exact test	OR	95% CI
CDC stage (78 pts)				
A	6/9		1	
B	17/20	0.76	1.27	0.38 to 4.31
C	16/10	0.21	2.40	0.65 to 8.81
CD4 (138 pts)				
>500	14/28		1	
200–500	37/44	0.2471	1.682	0.7736 to 3.656
<200	12/3	**0.0025**	**8.000**	**1.936 to 33.06**
HIV RNA (138 pts)				
<50	44/58			
50–500	7/4	0.2186	2.307	0.6351 to 8.378
>500	12/13	0.4810	1.896	0.4412 to 8.147
TARV (141 pts)				
No	6/8		1	
Yes	59/68	1.0000	1.157	0.3795 to 3.527
ARV regimen experienced (81 pts)				
0	6/8		1	
1–2	10/6	0.4642	2.222	0.5136 to 9.616
3–5	6/10	1.0000	0.8000	0.1849 to 3.462
>5	22/6	**0.0362**	**4.889**	**1.216 to 19.66**
HPV genotypes (204 genotypes)				
LR	30/41		1	
HR	81/54	**0.0186**	**2.050**	**1.144 to 3.674**
Cytology (144 pts)				
NEG/inadequate	25/56		1	
ASC	1/2	1.0000	1.120	0.09695 to 12.94
LSIL	31/17	**0.0002**	**4.085**	**1.917 to 8.704**
HSIL	9/3	**0.0074**	**6.720**	**1.675 to 26.96**
Colposcopy (61 pts)				
NTZ	11/29		1	
ANTZ1	10/4	**0.0091**	**6.591**	**1.706 to 25.47**
ANTZ2	7/0	**0.0005**	**38.48**	**2.027 to 730.3**
Biopsy (18 pts)				
Neg	4/4		1	
CIN 1–2–3	8/0	0.0769	17.00	0.7373 to 392.0
Other STI* (81 pts)				
Yes	38/36		1	
No	2/5	0.4321	0.3789	0.06906 to 2.079

*STI: 1 HSV, 4 candida, 2 bacterial vaginosis. Boldface type indicates statistically significant results.

P1181 E6/E7 expression in the triage of HIV-HPV co-infected patients

G. Orlando, R. Beretta, A. Agarossi, S. Rimoldi, C. Antonacci, F. Mazza, N. Zanchetta, F. Pagano, M. Fasolo, P. Pileri, E. Omodeo Zorini, E. Casolati, M.R. Gismondo on behalf of the GISPAP (Gruppo Interdisciplinare per lo Studio delle Patologie Associate al Papillomavirus)*

Objective: Anal and cervical HPV related infections occur frequently in HIV infected pts and progression to invasive lesions depends both on HPV types and on host immune response. HPV E6/E7 genes are over-expressed in high-grade lesions and cancers as a consequence of HPV integrated cycle. Detection of E6/E7 mRNA could be a specific marker for high-grade disease and cancer.
We want to evaluate the rate of HPV E6/E7 mRNA expression according to cytology, CD4 cells and HIV-RNA in anal and cervical samples in HIV positive persons to define the potential effectiveness of this molecular approach in the primary screening of such pts.
Methods: E6/E7 mRNA expression was assessed by APTIMA® HPV assay (Gen-Probe Inc, San Diego, CA, USA) in 235 (85 anal and 150 cervical) samples of HIV pos Males and Females included in the GISPAP cohort – L Sacco Univ Hosp (Milan-Italy). Cervical and anal cytology was evaluated according to Bethesda system 2001. HIV related immuno-virological data were collected from clinical records.
Results: Diagnoses on cytology were: 85 neg, 5 inadequate, 3 ASC, 109 LSIL, 27 HSIL.
Proportion of E6/E7 expression was 81.48%, 73.39% and 30.95% in HSIL, LSIL, and neg respectively (χ^2 test $p < 0.0001$). Decreasing rates of mRNA expression in HSIL, LSIL and neg samples were confirmed when analysed according to site of infection (anal or cervical), HIV RNA and CD4 levels. Severely immunosuppressed pts (CD4 <200/microL) expressed E6/E7 in higher proportions when compared to pts with higher CD4 levels with 75% of E6/E7 expression also in neg samples.
High sensitivity/NPV and low specificity/PPV in identifying HSIL was assessed for any different group analysed (tab 1).
Conclusion: The molecular approach for HPV screening is aimed to reduce over-treatment of low-grade and, often, transient abnormalities by increasing specificity and PPV. Expression of E6/E7 mRNA is generally strongly associated with the severity of histological diagnosis while negative results, when 14 HPV genotypes are targeted, could have a high NPV.
In HIV infected pts we found a very low specificity and PPV of E6/E7 mRNA test in identifying HSIL lesions thus preventing its use as primary screening test to reduce the second level assessment need. The meaning of E6/E7 expression in LSIL or neg samples, could be the sign that the shift toward the integrated cycle has already begun and, added to other screening tools, could identify pts with the highest risk of progressive disease.

Table 1. Sensitivity, specificity, positive and negative predictive values of HPV-mRNA detection in predicting HSIL

	p	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Likelihood ratio
All the patients	**0.0031**	0.81 (0.62–0.94)	0.50 (0.43–0.58)	0.20 (0.13–0.29)	0.94 (0.88–0.98)	**1.64**
Anal samples	0.72	0.87 (0.59–0.98)	0.22 (0.12–0.34)	0.21 (0.11–0.33)	0.87 (0.62–0.98)	1.11
Cervical samples	0.0655	0.75 (0.43–0.94)	0.57 (0.48–0.65)	0.14 (0.065–0.25)	0.96 (0.89–0.99)	1.73
Patients with						
CD4 <200/μL	1.0000	1.00 (0.16–1.00)	0.13 (0.02–0.40)	0.13 (0.02–0.40)	1.00 (0.16–1.00)	1.15
CD4 200–500/μL	**0.0371**	0.85 (0.54–0.98)	0.47 (0.37–0.58)	0.18 (0.09–0.30)	0.96 (0.85–0.99)	**1.606**
CD4 > 500/μL	**0.0385**	0.89 (0.52–0.99)	0.49 (0.37–0.61)	0.17 (0.08–0.31)	0.97 (0.86–0.99)	**1.73**
Patients with						
HIV viral load <50 copies/μL	**0.0127**	0.87 (0.59–0.98)	0.48 (0.39–0.57)	0.16 (0.09–0.26)	0.97 (0.89–0.99)	**1.66**
HIV viral load 50–500 copies/μL	0.5286	1.00 (0.29–1.00)	0.31 (0.09–0.61)	0.25 (0.05–0.57)	1.00 (0.4–1.00)	1.44
HIV viral load >500 copies/μL	0.3783	0.83 (0.36–0.99)	0.44 (0.28–0.60)	0.18 (0.06–0.37)	0.95 (0.74–0.99)	1.49

Boldface type indicates statistically significant results. Likelihood ratio defined as sensitivity/(1.0 – specificity).

P1182 Detection and typing of human papillomaviruses in malignant, dysplastic, non-dysplastic and normal oral epithelium by nested polymerase chain reaction

E. Blioumi, D. Chatzidimitriou, C. Pazartzi, T. Katopodi, G. Tzimagiorgis, A. Melidou, E. Emmanouil-Nikoloussi, A. Markopoulos, N. Lazaridis, Z. Skouras, E. Diza, D. Antoniades (Thessaloniki, GR)*

Introduction: Studies on human papillomaviruses (HPV) participation in initiation and progression of oral neoplasia have generated conflicting results. Specifically, HPV infection in oral carcinomas ranges from 0%-100% and in the normal oral cavity from 0–60%.
Aim: This study aims at the detection of the HPV DNA in oral carcinomas, dysplastic leukoplakias, non dysplastic leukoplakias, fibromas and normal mucosa by nested polymerase chain reaction (nested-PCR) and the correlation of HPV presence with other factors obtained from the patient's records, such as histologic diagnosis, gender, age, anatomic location, smoking and alcohol drinking.

Material and Methods: 258 formalin-fixed paraffin-embedded tissues (63 carcinomas, 57 leukoplakias with dysplasia, 53 leukoplakia without dysplasia, 50 fibromas and 35 normal tissues) were deparaffinised, After DNA extraction, a nPCR was performed using primers targeting the HPV L1 gene; in the first step MY09/11 and in the second step GP5+/GP6+. Positive PCR products were sequenced for further HPV typing.
Results: HPV was detected in 22.2% of carcinomas, 31.5% of dyspalsias, 35.8% of non-dysplastic leukoplakias, 40% of fibromas and 40% of normal mucosas. HPV 16 was detected in 90% of positive samples. Statistical analysis with X2 and pearson correlation test shows significant negative correlation between HPV prevalence and age. No significant correlation was detected between HPV prevalence and gender, histological diagnosis, histological grading, location, smoking or alcohol drinking.
Conclusions: HPV was detected in all study groups, though in normal tissues more often than dysplastic or malignant. This fact does not exclude nor confirm the participation of HPV with the early stages of oral carcinogenesis.

P1183 Immunohistochemical expression of Ki67, C-erbB-2 in infiltrating duct carcinoma of the breast. Does C-erbB-2 cooperate with human papillomavirus in breast carcinoma pathogenesis?

R. Harfoush, N. Kamel (Alexandria, EG)*

Objectives: Breast cancer encompasses a heterogeneous group of neoplasms. Recently, studies have been suggesting that some types of viruses might be involved in the pathogenesis of breast cancer especially the human papilloma virus (HPV). Several factors can predict the biological behavior and clinical outcome of breast cancer: proliferation index, over expression of oncoprotein C-erb B2, tumor size and grade of malignancy. Our aim was to investigate the presence of low/intermediate/high risk types of HPV (DNA and/or antigen) in association with the immunohistochemical expression of C-erbB2, and Ki-67 as prognostic markers and to correlate the intensity of expression with tumor size, grade and lymph node status.
Material and Methods: The present study included 20 retrospective cases of infiltrating duct carcinoma of the breast. All samples were analyzed for the presence of HPV DNA of 32 HPV types using HPV LCD-Array Kit (HPV type 3.5), subjected to H&E staining and immunohistochemical staining using HER2/neu, ki67 and HPV antibodies.
Results: The gene sequences specific for HPV types were not detected. Positive immunohistochemical expression of HER2/neu (3+) was detected in 7 cases (35%), 6 cases (30%) were equivocal (2+), and 7 cases (35%) were considered negative (1+, 0). Positive immunohistochemical expression of Ki 67 was detected in 15 out of 20 studied cases (75%). A statistical significant relation was detected between immunohistochemical expression of HER2/neu (2+, 3+) on one hand. and increasing age ($P = 0.0012$), increasing size of tumor ($P = 0.041$), and high grade ($P = 0.02$) on the other hand. A statistical significant relation was detected between moderate to strong Ki-67 immunohistochemical expression on one hand and increasing age ($P = 0.012$), and increasing grade ($P = 0.01$) on the other hand. A statistically significant relation was detected between positive and equivocal immunohistochemical expression of HER2/neu and increasing positivity of Ki 67 ($P = 0.026$).
Conclusion: Our analysis was not able to support a role of HPV infection in breast cancer. It was therefore not possible to establish a correlation between the oncogene expression of c-erbB-2 and the HPV-DNA types. However, HER2 status might be incorporated into a clinical decision along with other prognostic factors, regarding whether to give any adjuvant systemic therapy. The usage of ki67 immunohistochemical stain as a prognostic marker is not recommended.

HIV genotypes

P1184 The HIV-1 protease L10I minor mutation decreases replication capacity and confers resistance to saquinavir

F. Flor-Parra, A. Perez-Pulido, J. Pachon, P. Perez-Romero (Seville, ES)*

Objectives: The objective of this study was to characterize the HIV protease minor mutations of low prevalence, L10I and L10V, the highly prevalent minor mutations, M36I and L63P, and the major mutations D30N and I84V and their impact on viral fitness, cytophatic effect and resistance to protease inhibitors.
Methods: We studied the prevalence of the protease mutations L10I, D30N, M36I, L63P and I84V, in treatment-naïve patients and patients receiving ART. To study their effect on RC, cell-killing and resistance to PIs the mutations were introduced by directed mutagenesis into the provirus pNL4.3ren. A structure prediction of the protease carrying the L10I mutation was determined by molecular modeling.
Results: The prevalence of the major mutations D30N and I84V was low in naïve patients with 0% and 0.6% respectively, and it was significantly higher in treated patients with prevalences of 2.6% to 5.8%, respectively. The prevalence of the minor mutations M36I and L63P in naïve patients was significantly higher compared to major mutations, 28.1% and 48% respectively and decreased in treated patients to 16.7% and 43.9%, respectively. Interestingly, the prevalence of the minor mutation L10I had a pattern similar to that found for major mutations, with a low prevalence in naïve patients and a significantly higher in treated patients, 4.9% and 12.9% respectively. Furthermore, viruses carrying the major mutations D30N or I84V or the minor mutation L10I showed a 90% decrease in RC, while viruses carrying the minor mutations M36I or L63P had RC similar to wild type (wt) virus. Cells infected with the virus carrying the L10I mutation showed 50% less cytophatic effect than wt virus infected cells at 4 days post-infection. In addition, the L10I mutation confered complete resistance to saquinavir and partial resistance to lopinavir. Molecular modeling of the protease carrying the L10I mutation suggests that L10I affects the conformation of Leu23, a critical residue in the substrate binding site.
Conclusions: The L10I mutation impairs RC and confers resistance to saquinavir and lopinavir, similarly to other major mutations. These effects may be related with changes in the binding site of the protease, since the L10I mutation appear to affect the conformation of Leu23.

P1185 Genotyping analysis of the protease and reverse transcriptase of newly diagnostic (naïve) HIV-1 non-B patients

E. Papadimitriou, A. Papoutsi, L. Skoura, B. Haidich, S. Metallidis, P. Kollaras, P. Nikolaides, N. Malissiovas (Thessaloniki, GR)*

Objective: The aim of this study was the identification of treatment-related mutations in non-B strains which might be relevant for resistance to current antiretrovirals.
Methods: 215 naïve subjects, reported to the National AIDS Reference Laboratory of Northern Greece between 2000–2008, were included in the study. The naïve non-B patients had never been exposed to antiretroviral drugs. Genotyping resistance testing was performed at the time of diagnosis with a sequence-based assay (TruGene Genotyping Kit – Siemens). 235 sequences from naïve subjects infected with subtype B HIV-1 virus were used as controls for comparison with those from naïve non-B individuals.
Results: The distribution of subtypes among the 215 non-B patients was as follows: 151 A (70.23%), 34 CRF01/AE (15.81%), 12 other CRFs (02/AE, 02/B, 04) (5.58%), 9 G (4.18%) and 9 C (4.18%).
34 of 215 patients harboured a virus with at least one mutation associated with a phenotypic resistance. 5/215 with mutations to protease inhibitors, especially D30N, M46IL and N88D. 7/215 with mutations associated with resistance to nucleoside reverse-transcriptase inhibitors(NRTIs) especially M184V and K219QE. 22/215 with mutations to non-nucleoside reverse-transcriptase inhibitors (NNRTIs), especially K103N,

Y181C and V179D. Most of the mutations were revealed from sequences from subtype A.
Conclusion: Our results suggest that NNRTI-resistance mutations were more prevalent than either NRTI mutations or PI mutations, underlying the importance of genotyping resistance testing in HIV-1 non-B patients before starting treatment.

P1186 Analysis of the mutations associated to integrase inhibitor resistance in subtype B and non-B human immunodeficiency virus type 1

O. Turriziani, C. Montagna, F. Falasca, G. Russo, M. Bucci, F. Graziano, P. Maida, K. Monteleone, L. Antonelli, G. Antonelli (Rome, IT)*

Objective: The integrase inhibitors (e.g. Raltegravir) are a new class of antiretroviral drugs that have recently become available for the treatment of HIV patients. The emergence of mutations that confer resistance to the integrase inhibitors has been observed and characterized but viral polymorphisms at the integrase gene caused by immune selection and/or associated with non-subtype B is not completely understood. Here we plan to study the genotypic resistance pattern associated to integrase inhibitor resistance in plasma-HIV RNA derived from B and non B HIV-1 infected individuals.
Methods: Thirty integrase inhibitors naïve HIV-1 infected patients were studied. Twenty non-B HIV-1 samples were collected from patients from Cameroon and 10 subtype-B HIV-1 samples derived from Italian patients. RNA was extracted from 140 μL of plasma using a QIAmp Viral RNA kit (Qiagen, Milan, Italy) following the manufacturer's instructions. Sequencing was undertaken using CLIP, a DNA sequencing technique for direct sequencing of small quantities of amplified template (Open Kit-TruGene HIV-1, Siemens Healthcare Diagnostics).
Results: Among the 20 non B strains the subtype, the distribution was as follows: 10 subtypes CRF02_AG, 3 subtypes CRF02_AE, 3 subtypes D, 1 subtype CRF11_cpx, 1 subtype F2 and 2 subtypes G. No major mutations associated with resistance to integrase inhibitor were detected in HIV-1 subtype B samples. On the contrary major mutations were found in non-B subtype samples. Specifically, 25% of samples showed Y143 H/R/C mutation (4 CRF02_AG and 2 CRF02_AE) and at the same position the Y143S polymorphism was detected in all non-B strain except in subtype F and G. Interestingly in 1 sample belong to subtype G the Q148H pathway (Q148H+E138A+G140S) was found. Other mutations detected at the resistance sites were E92D and E157G/K. E92D was found in 10% of subtype B samples and in 5% of non-B strains (1 CRF11_cpx). E157G/K was detected in 10% of B subtype and in 10% of non-B subtype (1CRF01_AE and 1 CRF02_AG).
Conclusions: These findings indicate that integrase inhibitor resistance mutations can be detected in non-B subtype patients in the absence of drug exposure. This underlies the need for studies to further evaluate the role of integrase inhibitors mutations in the management of HAART in HIV patients.

P1187 Genotypic and phenotypic resistance to reverse transcriptase inhibitors among 102 Cambodian children with AIDS treated with HAART

*I. Beldjebel *, V. Krcmery, J. Benca, M. Utesena, V. Noskova, J. Sokolova, J. Chomroun, A. Shahum, L. Rabarova, P. Slavikova (Phnom Penh, KH; Bratislava, Trnava, SK)*

Objectives: The aim o this study is to describe few failures on 1st line highly active antiretroviral therapy (HAART) in children treated since 2003 in Phnom Penh, St. Maximillian Kolbe Tropical Pediatric Clinic and asses the efficacy of second line treatment among the first line failures.
Methods: We analyzed 102 children infected by HIV in age 4–16 years in our orphanages in Phnom Penh, Cambodia in 2003–2009 on genotypic resistance as cause of 1st line failure and for adherence to HAART, virologic, clinical and immunologic response to HAART. CD4 cells were investigated once per 3 months and viral load in 6 months interval. Genotypic/phenotypic resistance tests were performed in case of clinical or immunological failure by National Pediatric Hospital laboratory affiliated to Institute Pasteur Cambodge, Phnom Penh.
Results: Mortality on AIDS and AIDS related opportunistic infections in our group of 102 children was less than 3%, only tree children died in 2003–2009. Two died even before the onset of therapy, one at the beginning of HAART. The rest 98 children were treated with HAART from 18 to 72 months: 7 of 99 (7%) failed between 15 to 36 months. As a failure we considered, when CD4 count decreased (2 measurements within 6 months) and viral load (VL) showed increasing tendency (>1000 copies/ml). Only VL increase (without immunologic correlate 1 child) or only CD4 decrease (discordant failure 2 children) was not considered as indicator for replacement of the 1st line. Most of the children failed on stavudine (d4T) + lamivudine (3TC) + nevirapine (NVP), which was replaced by abacavir (ABC) or didanosine (DD1) or zidovudine (ZDV) or tenofovir (TDF) with protease inhibitors (PI) – lopinavir/ritonavir (LOP/RIT) or efavirenz. Commonest mutations of the reverse transcriptase among NRTI's were M184V (5 children), V75M (3 children), and T215Y, among NNRTI commonest mutations were K103N (2 childeren) and K101E (2 children). Those who failed on 1st line were treated by TDF, ABC or ZDV and DDI. DDI was still left in the NRTI backbone with TDF or ZDV or ABC plus PI was added.
Conclusion: Prevalence of deaths (3%) and virological/immunological failures (7%) among our group of children in Cambodia within 6 years of follow up were low. Probably high proportion of those receiving efavirenz (90%) versus nevirapin (8%) due to concomitant anti-TB therapy for tuberculosis may explain our 7% failures rate (after 6 years follow up).

P1188 Characterization of vpr gene of HIV-1 subtype A variant prevailing in Russia

I. Lapovok, E. Kazennova, V. Laga, M. Bobkova (Moscow, RU)*

Objectives: The HIV-1 viral protein R (Vpr) has a great number of activities associated with reverse transcription, nuclear import of pre-integration complex, cell cycle progression, regulation of apoptosis, HIV 5′-LTR transactivation. It makes Vpr an attractive target for antiretroviral therapy.
There are numerous reported mutations in vpr gene associated with secondary structure changes and altered protein functions. For example, Vpr carrying W54R has high mutation rate in reverse transcription process that may reduce the viral reproduction. Mutations E25K, I63K, Q65E and L67S impair host cell nuclear localization and accumulation of Vpr. Mutations L64P/A/R dramatically enhance Vpr pro-apoptotic potential. Mutation I61A has the contrary effect.
The aim of work was to study vpr gene of HIV-1 subtype A variant prevailing in Russia for the presence of possible peculiarities in Vpr structure and activity.
Methods: 45 samples of peripheral blood mononuclear cells from HIV-infected individuals have been studied. The analysis of gag and env genes proved the belonging of all the specimens to HIV-1 A subtype. The sequence analysis of vpr gene derived from all the specimens was carried out followed by the comparison to sequences and consensuses of other group M HIV-1 subtypes. All sequences have then been used for the phylogenetic analysis.
Results: All sequences of vpr gene studied in this work clustered with the HIV-1 subtype A isolates from Russia and Ukraine. We revealed no mutations in vpr gene previously found to be associated with changes of Vpr activities. Additionally, we showed the high rate of polymorphism of C-terminal region of Vpr protein, for example in position 89.
Conclusion: The results of phylogenetic analysis confirmed the statement about the link between HIV pandemic in Russia and Ukraine. HIV-1 Vpr protein of the variant prevailing in Russia has no mutations potentially influencing the functions of this protein.

P1189 Trends of transmitted antiretroviral resistance among patients newly diagnosed with HIV infection in Taiwan

Y. Tseng, C.L. Lu, C.C. Hung, M. Chen, S. Hsieh, W.H. Sheng, S.Y. Chang (Taipei, TW)*

Objectives: We aimed to determine the prevalence and trends of antiretroviral drug resistance among newly diagnosed HIV-1-infected patients who were antiretroviral-naive from 1999 to 2009.
Methods: Blood samples collected from 934 HIV-1-infected patients were subjected to genotypic assays using nested reverse transcription-PCR amplification of a 1.2-kb fragment encompassing the protease gene and the first 240 amino acids of the RT gene. Drug resistance mutations were identified in accordance with the drug-resistance mutation list of the International AIDS Society-USA Consensus Guidelines. Prevalence of antiretroviral resistance was compared among three study periods: period 1, 1999 to 2003 (n = 424); period 2, 2004 to 2006 (n = 362); and period 3, 2007–2009 (n = 208).
Results: Of 934 patients with a median age of 32 years, 65.5% of the patients were men who have sex with men (MSM), while heterosexuals and injecting drug users accounted for 15.8% and 11.9%, respectively. The median CD4 was 240 cells per cubic millimeter and plasma HIV RNA load was 5.02 log10 copies per milliliter. 77.2% of the isolates were subtype B and 9.6% CRF07_BC. From period 1 to period 3, the median CD4 count of the enrolled patients and the proportions of patients who were MSM and infected with subtype B were increasing significantly. The prevalence of HIV-1 isolates that harbored one or more primary mutations associated with antiretroviral resistance to either reverse-transcriptase inhibitors or protease inhibitors was 6.6%, 12.7%, and 10.1% in periods 1, 2 and 3, respectively. In periods 1, 2, and 3, the prevalence of resistance to protease inhibitors was 1.2%, 4.4%, and 2.0%, respectively, while that to non-nucleoside reverse-transcriptase inhibitors was 2.8%, 5.5%, and 4.7%, respectively; and to nucleoside reverse-transcriptase inhibitors, 4.5%, 6.1%, 4.1%, respectively. Prevalence of isolates with multi-drug resistance (resistance to 2 classes or greater) decreased from 1.7% in period 1 and 2.5% in period 2 to 0.7% in period 3.
Conclusions: Our findings suggest that the overall prevalence of antiretroviral resistance to any of the three classes of antiretroviral agents did not continue to increase in Taiwan over the past 10 years and the trends of resistance varied with each class of antiretroviral agents. Compared with period 2, prevalence of isolates with multi-drug resistance was decreasing in period 3.

MDS and HIV infection

P1190 Antiretroviral treatment and expression of the mRNA levels for Pgp, MRP1, MRP4 and MRP5 in HIV antiretroviral naïve patients. Follow-up at 48 weeks

F. Falasca, F. Graziano, L. Antonelli, P. Maida, C. Montagna, B. Rizzo, L. Chiavozzo, V. Renda, G. Antonelli, O. Turriziani (Rome, IT)*

Aim: The ATP-binding cassette genes represent the largest family of transmembrane proteins [including multidrug resistant proteins (MRPs) and P-glycoprotein (Pgp)] able to drive the transport of various molecules across cell membranes. Several studies have demonstrated that most of the above transporter are also able to transport antiretrovirals. The aim of this study was to evaluate whether the antiretroviral treatment might affect the mRNA expression of Pgp and some MRPs.
Material and Methods: Blood samples were collected from 13 HIV-positive patients treatment naïve. After the beginning of the treatment, samples were collected at 12, 24, 36 and 48 weeks. Eight patients were treated with Kaletra and Truvada (group I) and five patients with Efavirenz and NRTIs (truvada or combivir) (Gropu II). Expression of mRNA of the Pgp, MRP1, MRP4, and MRP5 was evaluated by real-time-PCR using the TaqMan technology (ABI Prism 7000; Applied Biosystems).
Results: MDR1 and MRP4 expression was not affected by treatment with PI and NRTI. In fact, at all time analyzed the mRNA levels of these transporters did not significantly differed from the mRNA levels detected before the beginning of treatment. As far as MRP1 and MRP5 are concerned, a modest, but not significant, reduction in the mRNA expression levels was observed after beginning of treatment. In patients belonging to the group II basically the same results were obtained. Looking at the individual trend of the mRNA expression of the above transporters in each patient it can be seen that the expression levels of these transporters seems to change during follow up but it is independent of type and time of treatment. The expression of the mRNA levels of these transporters appears to increase in some patients and decrease in other individuals suggesting that a high interindividual variability in the modulation of these mRNA does exist.
Conclusions: Antiretroviral treatment does not significantly affect the expression levels of mRNA of transported analyzed. However an interindividual variability in the expression of these mRNA has been documented during the follow up and further studies are needed to evaluate whether the over-expression of these mRNA may affect the success of therapy.

P1191 Clinical experience in Serbia concerning the effect of antiretroviral therapy on immunological and virological outcome in AIDS patents with terminal immunodeficiency

I. Pesic Pavlovic, J. Nikolic, D. Salemovic, J. Ranin, S. Zerjav, D. Jevtovic (Belgrade, RS)*

Purpose of this study was to evaluate efficacy of antiretroviral therapy (ART) through examination of immunological and virological outcome in patients with terminal immunodeficiency (CD4 $<50/mm^3$). This retrospective study included subpopulation of ART-naive 171 patients (29.3% of total population of 582 HIV positive patients) with terminal immunodeficiency, initiated with ART during 1997–2007 at the Clinic for Infectious and tropical Diseases, Belgrade. Optimal success of ART was defined as an undetectable HIV RNA (HIV RNA <50 copies/ml ($1.7 \log_{10}$)), and elevation of CD4 $>400/mm^3$. Therapeutic failure was defined as HIV RNA $>2 \log_{10}$, despite of possible immunological recovery. Third possible outcome was dissociation of immunological and virological response (achievement of optimal suppression of viral replication without immunological recovery).
Study group included 171 patients (36.8% female, 63.2% male), 36±9 years old, (67.8% were older than 40 years), treated for the duration 3.6±2.1 years (range 1–10 years). Optimal therapeutic outcome was achieved in 23.2%, dissociative response in 50.4%, and therapeutic failure was noted in 26.4% ($p < 0.05$). The outcome was significantly affected by choice of therapeutic protocol, including combinations of all three classes of medications ($p < 0.01$).
This analysis suggests that ART can be provided successfully within the limited choice of medications and when genotype resistance testing is unavailable.

P1192 ICU mortality, in-hospital mortality as well as long-term follow-up outcomes are significantly improved in HIV-infected patients admitted to ICU in the HAART era

C.M. Wind, S.F. van Lelyveld, T. Mudrikova, H.J. van Leeuwen, D.W. de Lange, A. Hoepelman (Utrecht, NL)*

Objectives: Mortality data of patients infected with human immunodeficiency virus (HIV) admitted to an intensive care unit (ICU) in the highly active antiretroviral therapy (HAART) era are inconsistent and often a debate arises about the necessity for ICU care of HIV infected patients. We investigated whether mortality after admission to ICU has changed since the introduction of HAART.
Methods: In this retrospective cohort study, auto-intoxications and post surgery patients were excluded (n = 36). Patient characteristics, laboratory parameters, information of HIV infection and ICU admission data were collected from medical records. We compared patients admitted in the pre-HAART era ('91–'96) to those admitted in the HAART era ('96–'08). The latter group was divided into two cohorts

(early HAART '96–'03 and late HAART '03–'08). Primary outcomes were in-ICU, in-hospital, 1-year and 5-year all cause mortality.
Results: 127 ICU admissions of 116 HIV infected patients were included: 47 ICU admissions before and 80 after the introduction of HAART ('96–'03; 42 and '03–'08; 38). In the HAART era 36% used HAART at admission, median CD4+ count was not different (83 versus 60 pre-HAART) and 20% had an undetectable viral load. Baseline characteristics (pre-HAART vs. HAART) did not show statistically significant differences, except for mechanical ventilation (57 vs. 85%) and ICU admission indication (neurologic 2 vs. 16%; hemodynamic 30 vs. 8%). Primary outcomes showed a significant decrease of in-ICU (47 vs. 31%), in-hospital (60 vs. 45%) and 1-year (74 vs. 50%) mortality. Moreover, 5-year mortality significantly improved in the early HAART, compared to the pre-HAART era (62 vs. 91%).
Conclusion: This study shows a statistically significant reduction in mortality of HIV infected patients admitted to ICU in the HAART era. Therefore, there is no reason for excluding HIV infected patients from ICU care.

P1193 Risk factors of atherosclerotic plaque measured by carotid Doppler ultrasonography in HIV-infected Koreans receiving antiretroviral treatment

J. Baek, S. Han, B. Chin, H.K. Choi, S.Y. Shin, Y. Chae, S. Jin, Y.K. Kim, C.O. Kim, J.Y. Choi, Y.G. Song, H.C. Lee, J. Kim (Seoul, Wonju, KR)*

Objective: Antiretroviral therapy (ART) in HIV infection produces a spectrum of metabolic complications, including dyslipidemia, insulin resistance, lipodystrophy. It has been reported that ART is independently associated with the increase in the rate of myocardial infarction. We examined the risk factors for the existence of plaque in carotid artery, which are predisposing conditions of cardiovascular disease, in HIV-infected Koreans receiving ART.
Methods: Total 137 HIV-infected Koreans who have been treated with ART during more than 6 months were examined the carotid intima media thickness (IMT) and plaque existence by carotid doppler ultrasonography from August 2007 to March 2008 at Severance Hospital, a 2,000-bed tertiary care university hospital and referral center in Seoul, Korea. Anthropometric parameters were examined by bioelectrical impedance analysis and physical examination. The mean of the bilateral maximal common carotid artery (CCA) IMT was used as the carotid IMT values in our analyses, because it has been shown to have the strongest association with cardiovascular risk factors. The atherosclerotic plaques were defined as localized echo structures protruding from the vessel lumen. Only lesions for which the thickness was >1 mm at either the right or left CCA were considered as carotid plaques.
Results: Total duration of ART in total participants was 26.9 months. Carotid plaque was observed in 33 (24.1%) patients. Carotid IMT values in total patients had significantly positive correlations with waist-to-hip ratio ($r = 0.190$, $p = 0.027$), body mass index ($r = 0.235$, $p = 0.006$), total cholesterol ($r = 0.201$, $p = 0.019$), LDL cholesterol ($r = 0.269$, $p = 0.005$), total exposed duration of zidovudine ($r = 0.247$, $p = 0.017$) after the adjusting for age. In multivariate logistic regression model, age and history of hypertension were significantly associated with the existence of plaque (OR 1.09, 95% CI 1.02–1.17, $p = 0.018$, and OR 10.75, 95% CI 2.06–56.14, respectively). The total duration of HAART or total exposed duration of each antiretroviral drug did not show any association with the existence of plaque.
Conclusion: The increased carotid IMT and plaque existence in HIV-infected Koreans receiving ART were associated with the traditional risk factors of atherosclerosis known in non-HIV-infected individuals. The carotid IMT and plaque measurement should be regularly examined especially in the HIV-infected patients receiving ART with older age to prevent cardiovascular disease.

P1194 Long-term safety and efficacy of nevirapine-based HAART

C. Hidalgo, B. Alvarez, F. Bécares, R. García Delgado, M. Fernández Guerrero, M. Górgolas (Madrid, ES)*

Objectives: To evaluate the safety and efficacy of long term NVP based HAART in HIV infected patients.
Methods: Retrospective study of 107 HIV infected patients who started NVP based HAART during 1998, 1999 or 2000, either as a first line therapy or as a simplification strategy.
Results: 107p with a mean age of 34.8 years, (81% men and 19% women), 82.2% and 18.8% acquired the infection by sexual and parenteral route respectively. The reasons to start NVP were: (a) simplification with undetectable viral load from PI based regimen 81p (75.7%), (b) previous PI failing regimen 13p (12.1%) and (c) naïve 13p (12.1%).
Overall, the mean time on NVP based HAART was 63m, median 48m (range 9–132m). 43p (40.1%) were still on NVP with undetectable viral load after 104 m (range 12–130m) of follow up. 64p (59.9%) discontinued NVP due to the following reasons: 1) Structured treatment discontinuation 27p (25%); 2) Virologic failure 14p (13%); 3) Toxicity 11p (10.2%); 4) Change in treatment strategy 6p (5.6%); 5) Other reasons 6p (5.6%). From baseline to end of follow up the mean increment of CD4 was 139 ± 241 cells, the mean decrement in triglycerides was 48 ± 178 mg/dL, and the mean total cholesterol did not change.
Conclusion: Nevirapine based HAART is a long term (more than 8 years) safe and effective therapy for HIV infected patients. Virologic failures ocurred in 13% of cases and drug related toxicity in 10.2%.

P1195 Experience of antiretroviral adverse effects in a Malawian clinic

D. Morrison, C. McGoldrick, L. Munthali (Aberdeen, UK; Ekwendeni, MW)*

Objectives: A high HIV prevalence exists in Malawi, the world's third poorest country, and only a fraction of those needing antiretrovirals (ARV) receive them. For those who are treated, 1st line therapy consists of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP). As there is a perceived risk of adverse effects (e.g. peripheral neuropathy) with this older-fashioned regimen, our objective was to assess morbidity from such effects, as well as to assess adherence in one Malawian antiretroviral clinic.
Methods: An observational study was conducted retrospectively in November 2008 at Ekwendeni Hospital, Malawi. Using a proforma, data was collected from ARV clinic notes for individuals with HIV infection, whose first clinic attendance was between January 1st and June 30th 2006. Individuals whose care was subsequently transferred to another facility were excluded.
Results: 160 individuals were identified and included (mean age 35.95 years, male to female ratio 0.62:1). 75.6% (121/160) had been taking their most recent regimen in excess of 730 days. d4T, 3TC and NVP was the last prescribed regimen in 97.5% (156/160). 1.875% (3/160) had changed from this to zidovudine (ZDV), 3TC and NVP because of peripheral neuropathy, and 0.625% (1/160) had changed to ZDV, 3TC and efavirenz (EFV) for unrecorded reasons. Adverse effects of 1st line regimens were reported in 30.6% (49/160); 34.7% (17/49) of whom developed skin rash and 65.3% (32/49) of whom developed peripheral neuropathy. 95.6% (153/160) continued to work or attend school. Pill counting showed 90–100% adherence in 64.4% (103/160).
Conclusion: Most individuals were able to continue on first line antiretroviral regimens without change, and to continue to attend school or work, despite almost one-third experiencing adverse effects (skin rash or peripheral neuropathy). However, adherence was lower than expected and this may have resulted from their experience of such adverse drug side-effects.

P1196 Differences between healthy volunteers and HIV-infected patients in the activities of CYP3A, CYP2D6 and P-glycoprotein

A. Jetter, G. Fätkenheuer, D. Frank, T. Klaassen, A. Lazar, A. Seeringer, O. Doroshyenko, J. Kirchheiner, W. Hein, E. Schömig, U. Fuhr, C. Wyen (Zurich, CH; Koeln, Ulm, DE)*

Introduction: In inflammation and infection, cytochrome P450 enzyme activities are down-regulated. However, information on possible discrepancies in activities of cytochrome P450 enzymes and drug-transporters between HIV-infected patients and healthy people is scanty. We compared the activities of CYP3A, CYP2D6 and P-glycoprotein between 12 healthy Caucasians and 30 therapy-naïve HIV-infected patients using midazolam, dextromethorphan and digoxin.
Material and Methods: A "phenotyping cocktail" consisting of 1.5 mg midazolam (intestinal and hepatic CYP3A), 30 mg dextromethorphan (CYP2D6), and 0.5 mg digoxin (P-glycoprotein) administered orally, and of 1.0 mg midazolam intravenously four hours later (hepatic CYP3A activity), was administered to 30 therapy-naïve HIV-infected patients and to 12 healthy male controls. Plasma samples were analysed using immunoassays for digoxin and LC-MS/MS for all other analytes. Pharmacokinetics were calculated noncompartimentally. A parallel-group average bioequivalence approach was chosen for the comparison between patients and volunteers. The studies were endorsed by the Ethics Committee of the University of Cologne, Germany, and conducted in accordance with the laws of Germany. Written informed consent of each participant.
Results: Overall CYP3A activity (apparent oral midazolam clearance) was 0.490-fold lower in patients than in healthy volunteers (90% confidence interval CI, 0.377–0.638). The CYP2D6 activity (plasma ratio AUCdextromethorphan / AUCdextrorphan) was essentially unchanged (point estimate 1.289, 90% CI 0.778–2.136). P-glycoprotein activity was lower in patients (digoxin Cmax point estimate 1.304, 90% CI 1.034–1.644). Additionally, activity changes after a single dose of ritonavir-boosted lopinavir in healthy Caucasians were not useful to predict changes in patients during chronic therapy.
Conclusion: The activities of CYP3A and P-glycoprotein were lower in HIV-infected patients than in healthy volunteers, but, besides overall CYP3A activity (roughly 50% lower overall CYP3A activity), differences were small. The variability within the groups was much higher than the discrepancy between them It may be prudent to use lower doses of CYP3A substrates with narrow therapeutic indices in this population. Single-dose inhibition studies in healthy volunteers are not useful to accurately predict the interaction potential in HIV infected patients under chronic therapy.

P1197 Comparative evaluation of two commercial viral load assays for monitoring human immunodeficiency virus type 1 RNA in plasma: automated NucliSens EasyMAG/EasyQ v.1.2 vs. High Pure/Cobas Taqman HIV-1

A. Papoutsi, A. Karava, P. Kollaras, P. Nikolaides, N. Malissiovas (Thessaloniki, Athens, GR)*

Objective: Quantification of HIV viral load is used to evaluate response to antiretroviral therapy and its monitoring. The aim of this study was to evaluate the NucliSens EasyMAG/EasyQ HIV v1.2 (bioMérieux) in terms of relevance in determination of viral load, reproducibility, linearity and dynamic range of detection in comparison to the High Pure Viral RNA kit/COBAS Taqman (Roche Diagnostics) used routinely in the Northern Greece AIDS Reference lab for the last three years.
Methods: 251 plasma samples were collected randomly from 224 patients attending the HIV Treatment Center at First Pathology Clinic of the AHEPA University Hospital of Thessaloniki. Fifty four of these samples were consecutive follow up specimens from 27 individuals. The majority of the patients included in this study were under antiretroviral therapy. All viral load values reported according to the limits set by each assay and the viral detection protocols applied by the manufacturer's instructions.
Results: The two techniques showed overall agreement 95.2% (239/251). The relevance coefficient of samples found positive with both techniques was found to be $r = 0.91$ while the reproducibility of both methods was excellent $r = 0.96$. No significant differences were shown in the dynamic range of detection for both NucliSens EasyMAG/EasyQ and COBAS Taqman (2–7 logs). In samples from 27 patients with consecutive follow up specimens, the mean log difference estimated for each patient with the two techniques in their consecutive samples was found to be 0.15.
Conclusions: The NucliSens system requested less handling time than COBAS Taqman (3.6 h vs. 6.1 h for 48 samples). Both quantitative techniques are found to be equally reliable to be used for accurate determination of HIV-1 viral load. So, it depends on the discrete consideration of each laboratory to determine which one should be used, in terms of clinician and patient convenience, workload and personnel availability as well as financial resources.

P1198 The first knowledge, attitude, behaviour and practices surveillance on HIV/AIDS amongst troops of Indian armed forces stationed in Jaipur, India

M. Singh, A. Guleri (Jaipur, IN; Blackpool, UK)*

Background: 2.31 million people are estimated to be living with HIV in India. While HIV/AIDS is an important medical problem, it has a considerable socio-economic impact. Indian Armed Forces[IAF] have a successful containment programme. This first knowledge, attitude, behaviour and practices [KABP] surveillance was undertaken in Sept–Oct 2008 amongst troops of IAF stationed in the pink city (Jaipur, India). The objective was to inform strategic planning & consolidation of the revised local containment programme.
Method: 250 soldiers [randomly selected] responded to the KABP questionnaire. The entire procedure was explained in native language, confidentiality assured and verbal consent obtained. The questionnaire included various aspects of basic knowledge, myths/misconceptions, perception about high-risk behaviour, attitude towards HIV positive patients, knowledge regarding condom usage, knowledge about HIV/AIDS related services, etc.
Results: There were 250 male respondents (between 21–55yrs of age; 82.8% married). The mode of awareness was media (TV/radio/newspapers) 43.5%; Awareness sessions 17.1% & peer education sessions 16.9%. Regarding risk of transmission during sex – 17% were unaware of transmission risk associated with unprotected sex; 27% were unaware and 10% disagreed potential risk of transmission associated with unprotected sex with asymptomatic HIV carrier. Regarding condom usage and safer sex, 57% agree to condom reducing risk during sex with wife while 43% married women should use condoms with their husbands; 87% agree to need for single persons to use it with their partners; 12% disagreed with reduction in transmission risk by correct and consistent use of condoms. Between 12.8% and 18.4% respondents were unwilling to co-exist with a HIV positive person (at work, dine with, domestic help, share house/family member, send child to school with).
Conclusions: The results of this KABP surveillance were extremely useful in informing the strategic planning of the local HIV/AIDS containment programme. We believe that there is no room for complacency despite commendable programme run by the IAF. Action plan for stepping up efforts towards environment building and investing in human resource through aggressive IEC campaigns and training programs for peer educators. The results reveal negative bias and lack of empathy for co-existence with HIV positive persons. Human resource development, enhanced engagement, awareness campaign, greater transparency and mentoring for more trained peereducators were included.

P1199 Designing and biological evaluation of single-cycle replicable HIV-1 system as a potential vaccine strategy

S.M. Sadat, R. Zabihollahi, R. Vahabpour, K. Azadmanesh, F. Javadi, S.D. Siadat, A. Memarnejadian, K. Parivar, H. Khanahmad Shahreza, R. Arabi Mianroodi, S. Hekmat, M.R. Aghasadeghi (Tehran, Isfahan, IR)*

Objectives: Infection with human immunodeficiency virus (HIV) is a spreading world health problem, so a vaccine is desperately needed to control the AIDS pandemic. Inactive HIV particles may represent suitable vaccine candidates as they have all the immunogenic structural and surface antigens in their native forms. Accordingly, several preclinical studies have already shown that inactive and attenuated HIV virions can elicit protective humoral and cellular immune responses. Herein, we designed and constructed a novel HIV-1 virion, capable of replicating in a single cycle that provides a more immunogenicity, while prevents any pathologic effects and further evaluated its biological properties.
Methods: The pmzNL4–3 plasmid, containing the complete HIV genome of NL4–3 strain with a 2-kb MlsI-digested fragment deletion in reverse transcriptase (RT) and integrase (IN) genes was constructed, confirmed by sequencing reactions and transfected into HEK 293T cell line. By further co-transfection of psPAX2 and pMD2.G plasmids, which encoded HIV Gag-pro-pol and vesicular stomatitis virus surface glycoprotein, into the same pmzNL4–3-harboring cells, pseudotyped virions were produced, evaluated by electron microscopy and quantified using P24 end-point ELISA assay. Infectivity of mzNL4–3 virions and their efficiency towards the syncytium formation was evaluated on HIV-sensitive MT-2 cells.
Results: Despite the occurrence of a designed long deletion within the RT and IN genes the production of HIV virions was indicated by the level of P24 protein in culture supernatant of transfected cells and was further confirmed by electron microscopy. Formation of syncytia in MT-2 cells also evidenced for the functionality of the surface glycoproteins in produced pseudotyped virions. Interestingly, infectivity analysis verified that the second generation virions were completely non-replicative.
Conclusion: While the introduced single cycle replicable (SCR) HIV system completely maintained the antigenic structures of HIV-1, by its one cycle replicating properties represented a good implication as a potential vaccine candidate and this guarantees the further investigations towards the assessment of its immunogenicity, which are currently under process. Also, replacement of the deleted fragment in pmzNL4–3 with various immunostimulatory sequences may present another interesting approach towards the improvement of its application in HIV vaccine researches.

P1200 Frequency of CCR5 delta 32 polymorphism and its relation to disease progression in Iranian HIV-1 positive individuals

B. Moradmand Badie, H. Najmabadi, G. Esmaeeli Djavid, P. Kheirandish, F. Payvar, M. Akhlaghkhah, S. Jam, M. Rasoolinejad (Tehran, IR)*

Objectives: The (CC) β-chemokine receptor 5(CCR5) is an important co-receptor for entry of human immunodeficiency virus type 1 (HIV-1) to CD4+ T cells. Homozygosity or heterozygosity for 32-nucleotide deletion (delta 32) within CCR5 gene may influence HIV acquisition and progression in HIV-1 exposed or positive individuals respectively. In this study, frequency of heterozygosity for CCR5 mutation in Iranian HIV positive population was determined and its relation with their disease progression was evaluated.
Methods: A total of 194 HIV positives patients from a referral health care center of HIV/AIDS in Tehran, were enrolled in this study in 2008. The CCR5 delta32 in peripheral blood mononuclear cells was detected by the polymerase chain reaction (PCR) and gel electrophoresis. The disease progression was determined based on changes in CD4 cell counts or clinical presentation of recruited patients.
Results: Eight (4.1%) of all 194 enrolled patients were heterozygous for (CCR5 delta 32) deletion 32 genotype (wt/del 32). We found no homozygosity for the mutated (del 32/del 32). One hundred three patients (42.9%) were in AIDS that through of them, 3 patients were heterozygote. Although frequency of rapid progression to AIDS was less in heterozygous individuals (37.5%) than in wild type patients (53.2%), this difference was not statistically significant ($p = 0.4$).
Conclusion: It seems that prevalence of CCR5 mutation in HIV-1 infected individuals is varying in Iranian normal population and it could be related to extensive ethnicity variation. Studies on larger population based on ethnicity may help to clarify the role of this mutation in progression pattern in Iran.

P1201 Correlation between HIV viral load, increased fibrogenesis and HCV genotypes among HIV positive patients in Georgia

M. Karchava, L. Sharvadze, E. Dolmazashvili, A. Abutidze, L. Gatserelia, N. Dvali, L. Dzigua, T. Tsertsvadze (Tbilisi, GE)*

Background: The progression of hepatitis C virus (HCV)-related liver disease is accelerated in patients infected with HIV. The aim of this study was to evaluate risk factors for the development of fibrosis in co-infected patients at baseline visit.
Methods: In a prospective analysis of 249 HCV co-infected patients, associations between liver fibrosis score, and liver enzime levels, HCV viral loads, HIV viral loads, HCV genotypes, CD4 counts were assessed. All analises were done on patients at baseine visit before ART initiation. Measurement of HIV and HCV RNA viral load was done by COBAS TaqMan 48 analiser. HCV genotyping was done by reverse hybridization line probe assay using VERSANT HCV Genotype kit 2.0 respectively. Transient elastography was performed by Fibroscan (Echosens, Paris, France). The median value of 10 successful acquisitions, expressed in kilopascal (kpa) with a success rate of at least 60% is used for liver stiffness measurement for BMI < 28 and 35% for the liver stiffness measurement in case of BMI > 28. LS < 5.5 kPa was considered as fibrosis stage F0-F1 by Metavir, 5.5–8.0 kPa – fibrosis stage F2, 8.0–10.0 kPa – fibrosis stage F2–F3, 10.0–12.5 kPa – fibrosis stage F3, 12.5–14 kPa – fibrosis stage F3–F4 and LS > 14.0 kPa – fibrosis stage F4 by Metavir.
Results: Statistical analysis demonstrated a significant correlation between both ALT and AST levels and HIV viral loads with at least mild fibrosis. Higher number of HIV viral load, length of HIV infection was assosiated with the high number of LS. Study revealed no correlations between CD4 cell counts, HCV viral loads or different HCV genotypes with fibrosis.
Conclusion: Our study revealed coreletion of HIV viral loads ALT and AST levels with liver fibrosis. These findings suggest a direct role for HIV in development of liver fibrosis as well as high liver enzime levels in HCV co-infected individual. This findings support the need to maintain low HIV viral loads and along the need for initation of HCV treatment in order to minimize HCV disease progression among HIV infected patients.

P1202 Prevalence and correlates of co-infection with human immunodeficiency virus and hepatitis C virus in male injection-drug users in Iran

S. Seyed Alinaghi, P. Kheirandish, G. Esmaeili Djavid, H. Shirzad, N. Karami, M. Hosseini, M. Jahani, S. Azimi, F. Payvarmehr, M. Mohraz, W. McFarland (Tehran, IR; San Francisco, US)*

Objective: Aim of the study was to evaluate the HIV and hepatitis C virus (HCV) coinfection and associated risk behaviours among Injection Drug Users (IDU) in Detention, Tehran, Iran.
Methods: A cross-sectional survey included 499 male IDU arrested by police during a predetermined police sweep in Tehran (February, 2006). At the temporary detention centre, they were screened using a urine test and a physical examination for injection marks. Those who were identified as injectors were sent to the rehabilitation centre for 3 months. A questionnaire was filled out for each individual by interview. Blood specimens were collected for HIV and HCV testing.

The variables associated with HIV/ HCV coinfection at a significance level of $p < 0.10$ were considered in multivariate analysis.
Results: Of the 417 participants, 100 (24.0%) had HIV/ HCV coinfection (95% CI 19.9–28.4). Factors independently associated with HIV / HCV coinfection included history of using opioid in jail, and age ($P < 0.05$). There were not any association between other demographic characteristics (marital status, birthplace, residence and education), type and years of drug abuse, age of first injection, years of injection, sharing needles inside and outside of jail, injection in jail, history of tattooing, any sexual behaviour, and history of sexually transmitted diseases (STDs) with HIV / HCV coinfection ($P > 0.05$).
Conclusions: This study supports that incarceration is contributing to the increased spread of HIV/HCV coinfection. So, there is urgent need for effective harm reduction programs, particularly among incarcerated IDU.

P1203 HIV and viral hepatitis (HBV and HCV) among four vulnerable groups in Lebanon

S. Ramia, Z. Mahfoud, R. Afifi, J. Dejong, K. Kreidieh, S. Shamra, K. Kassak for the HIV/AIDS Group*

Objectives: AIDS-related policies and programs in the Middle East and North Africa (MENA) region are highly constrained by the lack of accurate information about the full scale of the HIV epidemic. Recently we conducted an integrated bio-behavioral surveillance study among four major vulnerable groups in Lebanon, namely prisoners, men who have sex with men (MSM), female sex workers (FSWs) and injecting drug users (IDUs). One of our aims in this study was to provide an estimate of HIV prevalence as well as an estimate of HBV and HCV prevalence and their co-infection with HIV in these populations. Furthermore, the distribution of HCV genotypes was conducted among the anti-HCV positive IDUs and the immune status of HBV infection was evaluated among MSM and FSWs.
Methods: Two types of sampling methods were utilized: simple random sampling for recruiting the prisoners in Roumyeh Prison and respondent driven sampling to target MSM, FSWs and IDUs. Blood samples were collected as dried blood spots and then eluted to be tested for HIV, HBV and HCV by ELISA. Anti-HCV positive IDUs' samples were subjected to RNA extraction followed by qualitative detection and genotyping.
Results: A total of 580 prisoners, 101 MSM, 103 FSWs and 106 IDUs participated in study. Prisoners showed a significantly higher seroprevalence of HBV (1.7%) and HCV (3.4%) than reported in the general population. Only 1 prisoner (0.17%) was confirmed as anti-HIV-positive. Among the MSM, only 1 (0.99%) was HBsAg carrier and 1 (0.99%) was confirmed anti-HIV positive. No HCV cases were detected in this population and only 10% of the MSM were immune to HBV. Regarding FSWs, none were infected with HIV, HBV or HCV and 30% were immune to HBV. Among IDUs, 56 (52.8%) were anti-HCV-positive with Genotype 3 the predominant one (57.1%) followed by genotype 1 (21%). 3 (2.8%) IDUs were HBsAg carriers and 1 (0.9%) was confirmed as anti-HIV-positive. None of the 3 HIV-positive patients was co-infected with HBV or HCV.
Conclusion: Although HIV does not seem to be a major problem among the four high-risk groups studied in Lebanon however, HBV and HCV seem to pose a serious health concern. Our results highlight the urgent need to raise awareness among prisoners, MSM, FSWs and IDUs and their health care providers of the availability and benefits of HBV vaccination in Lebanon. In addition, and due to the absence of vaccines against HCV and HIV, education programs aiming at behavioral changes should be intensified.

P1204 High diversity of HHV-8 molecular subtypes in AIDS-KS Cuban individuals and its relation with HHV-8 load: evidence of subtype B expansion

P. Martínez, V. Kouri, Y. Aleman, O. Blanco, V. Capó, M.E. Rodríguez, M. Dovigny, L. Cardellá, A. Gala, N. Jiménez, C. Luzardo, C. Correa, L. Pérez, A. Alvarez, U. Hengge (Havana, Dusseldorf, CU)*

Objectives: With the aim of investigate the distribution of KSHV genotypes and its association with diseases pathogenesis, 30 Cuban AIDS-KS patients diagnosed between 2005 and 2007 were studied.
Methods: Samples of plasma, saliva, peripheral blood mononuclear cells (PBMC) and affected tissue were obtained for KSHV quantification. KSHV genotyping was performed by the nucleotide sequencing of the variable ORFK1 gene from tissue samples. Amino acid sequences of this gene were used to construct a neighbour-joining phylogenetic tree. The median KSHV viral load (copies/100ng DNA) were 39, 151, 830603 and 143 in plasma, saliva, tissue and PBMC, respectively; being the viral load from affected tissue and saliva significantly higher in comparison to the other samples.
Results: Phylogenetic characterization of Cuban specimens showed a wide variety of subtypes [(A: $n = 6$ (20%), five being A5 (16.7%); C: $n = 6$ (20%); B: $n = 10$ (33.3%); and subtype E: $n = 3$ (10%)], showing an increasing tendency in the incidence of African genotypes (B and A5) among the new AIDS-KS cases in comparison with previous reported Cuban KSHV sequences. Interestingly, detection of type B KSHV strains was associated with higher viral load in tissue and saliva samples ($p < 0.01$).
Conclusion: The present result suggests that KSHV subtype B might have evolve to more efficient transmission over other subtypes among Cuban AIDS-KS patients, replacing KSHV A subtype that used to be more frequent before 2006.

P1205 HIV testing in tuberculosis patients in the United Kingdom

M.M. Raza, R. Mukherjee, M. Bhattacharya, D. Mital (Milton Keynes, UK)*

Objectives: Universal HIV testing is recommended for those diagnosed with tuberculosis (TB) under the UK National Guidelines for HIV testing 2008. Offering and uptake of such testing is an auditable standard which is reviewed annually by the Chest/Infectious Diseases team. The audit was done to ascertain the practice of universal HIV testing in this setting and to identify measures to improve things if necessary.
Methods: This audit was done in a UK district general hospital and included all cases of TB notified for the year 2008. The pathology IT system was used to identify which cases had an HIV test. Case notes were then reviewed to establish if the test was offered in cases where a result was not available and if the test was done as part of diagnostic work up or routinely after the diagnosis of TB.
Results: 40 cases were notified and included in the audit. 6 cases were children and among the adults 2 were never seen by the hospital services as one was diagnosed post-mortem and one had returned overseas at the time of diagnosis and 3 were treated in a private clinic and 1 in another specialist centre. As a result only 28 adult cases were seen in our hospital clinics and the analysis was limited to them. HIV testing was done and result documented in 16/28 cases (57.1%). HIV testing result or offer was documented in 20/28 cases (71.4%). In 50% of cases where HIV testing was done this was part of their diagnostic work up whereas in only 25% this was routine testing. Routine HIV testing was offered in only 8/28 (28.6%) of cases and uptake of routine testing was 62.5%.
Conclusions: HIV testing in adult patients seen in our hospital for TB is good (71.4% tested or offered) but the offer of routine HIV testing subsequent to the diagnosis of TB is poor (28.6%) although it's upatke is satisfactory (62.5%). To improve the offering and uptake of universal testing in TB patients (target 100%) we plan to introduce a proforma for all new TB diagnoses which will include offer of HIV testing and a patient information leaflet to explain HIV testing in this setting.

P1206 Human immune deficiency virus infection in patients diagnosed at age 50 years or later

F. Almasi (Courbevoie, FR)*

Objectives: Age related immune deficiency is a well known entity. Having considered this fact, immune deficiency due to HIV infection may be more severe in older patients.
Methods: A retrospective observational study in registered HIV infected patients in order to review epidemiologic, clinical, laboratory data and response to treatment in patients diagnosed at age of 50 or older.
Results: Among 1680 registered HIV infected patient there are 91 HIV infected patients who found their HIV positive situation at age of 50–74, aged 50–84 years. There are 22 women (24%) and 69 men (76%). HIV transmission risk factor Is homosexual-bisexual contact in 44% of cases, heterosexual contact in 44%, blood and blood product transfusion in 2% and unknown risk factor in 10% respectively.
49 patients (54%) were asymptomatic (stage A of CDC), 16 patients (17.5%) symptomatic non-C(Stage B) and 26 patients (27.5%)present AIDS difining condition(stage C). CD4 count before treatment ranged between 4 and 754, as a mean 226 cells/ml. 35 patients (38.5%) present low CD4 count (less than 200) and 15 patients (16.5%) with CD4 less than 100 cells /ml.
79 patients (87%) are treated and 12 patients (13%) untreated including 1 patient with indication of treatment.
After treatment CD4 count ranged between 10 and 1511, as a mean 511. CD4 count before and after antiretroviral treatment was increased (285). After treatment there were 14 patients with viral load more than 500 copies /ml including 8 untreated patients. It means 6 patients (7.6%) under treatment in virologic failure. There are 9 patients (11%) with detectable viral load but less than 500 copies/ml.
Conclusion: 76% of diagnosed patients at age of 50 or later are men. Male-female ratio is 3/1. Homosexual-bisexual contact is still an important risk factor in patients diagnosed at age 50 or more.
Age related immune deficiency in patients older than 50 is an important factor. Mean CD4 count was 226 in spite of 54% asymptomatic patients (stage A). It means that this group of patients is more immune deficient. After antiretroviral treatment there is pretty increase in CD4 count, it means a nice immunologic response.
Virologic response after treatment is as well as young even better may be because of better observance.

P1207 Patients with newly-diagnosed HIV in 2004 versus 2008: no apparent difference in progression

A. Brown, C. Ní Bhuachalla, C. de Gascun, R. Hagan, C. Bergin (Dublin, IE)*

Objectives: Trends have recently been observed suggesting more rapid progression in newly-diagnosed HIV-positive patients. This data is limited by an inability to specifically identify time of infection. Our HIV-positive cohort displays heterogenous acquisition risk and significant immigration from countries of high prevalence, and is ideal to study changing epidemiology.
Methods: A retrospective cohort study was undertaken comparing newly diagnosed HIV patients attending in the first half of 2004 with those in 2008. Baseline demographics, laboratory and virological parameters were gathered. Progression was followed for the first year after diagnosis. Patients with known seroconversion were of particular interest – including those who were certain of time of infection, who recalled significant seroconversion illness and/or a negative test within the preceding 6 months. Others with previous negative tests outside this window were not designated as known seroconverters. Rapid progressors (RPs) were defined as those with documented seroconversion window <2 years prior to presentation, who progressed to CD4 $\leqslant$350 cells/mm^3 within first year of follow-up. Results were analysed using Fisher's exact, χ^2, student t tests and two-tailed p values with GraphPad InStat.
Results: Of the 200 charts reviewed 96 met inclusion criteria. Forty-two from 2004 and 54 from 2008 were analysed. Baseline characteristics are summarized in Table 1. CD4 $\leqslant$350 cellls/mm^3 at first presentation in 2004 and 2008 respectively was seen in 20 (48%) and 31 (57%) p = 0.42. CD4 count $\leqslant$350 by end of year 1 was 25 (60%) vs 35 (65%) p = 0.67. Mean change in CD4% from diagnosis to end of year 1 or pre-ART was −2.9% (SD5.1) vs +7.2% (SD 11.0) p < 0.0001 (95% CI −13.7718 to −6.4882). AIDS-defining illness in first year was seen in 6 (14%) vs 7 (13%) p = 1.0. Of those with CD4 $\geqslant$350 cells/mm^3 at first presentation, 18 (82%) vs 18 (78%) maintained CD4 $\geqslant$350 cells/mm^3 at end of year 1 untreated. Of those with known seroconversion window <2 years, rapid progression was seen in 3/7 in 2004 and 9/20 in 2008 (p = 1.0).
Conclusions: In a diverse group of HIV patients, there was no difference in progression to CD4 <350 cells/mm^3 in the first year of attendance between 2004 and 2008 cohorts. Definite knowledge of time of infection eliminates the bias of late presentation in assessing true progression. Further large-scale prospective studies should focus on progression from seroconversion and minimising late presentation.

Baseline characteristics	2004	2008
Age (yrs)		
Mean	32.2	34.5
SD	7.41	12.7
% male	64	62.9
Country of origin (%)		
Sub-Saharan Africa	38	41
Europe	62	52
Other	0	7
Acquisition (%)		
Heterosexual	45	57
IVDA	36	6
MSM	19	26
Other	0	5
Subtype		
A	5	6
B	45	46
CRFs	2	15
Other/Unknown	48	33
CD4 at diagnosis (cells/mm^3)		
Mean	402	425
SD	291	433
Known seroconversion (%)	19	43
Coinfections (%)	62	61

P1208 Uninfected children born to HIV-infected mothers: no evidence of impaired immune function at 15 months of age

L. Kolte, V. Rosenfeldt, L. Vang, D. Jeppesen, I. Karlsson, L. Ryder, K. Skogstrand, S. Nielsen (Hvidovre, Copenhagen, DK)*

Objectives: With the increasing number worldwide of uninfected children born to HIV-infected mothers, any health problem that they may have, even of minor character, may be an enormous health challenge especially in developing countries. HIV-exposed uninfected (HIV-EU) infants present haematological and immunological abnormalities at birth including low CD4 counts and reduced thymic output, and it remains to be clarified if these abnormalities persist beyond infancy.
Methods: Twenty HIV-EU children at 15 months of age and 10 age and sex matched controls were enrolled. Total and naive CD4 and CD8 counts, immune activation and regulatory T-cells (Tregs) were measured by flow cytometry. Thymic output estimated as CD4+ cells containing T-cell receptor excision circles (TRECs) was determined by PCR. Cytokines (interleukin (IL)-1b, IL-2, IL-4, IL-6, IL-8, IL-10, interferon-gamma, transforming growth factor-β) were analyzed using Luminex Technology, and *Haemophilus* Influenzae Type B (Hib) vaccination responses were measured by ELISA.
Results: CD4 and CD8 counts did not differ between HIV-EU and control children, neither did thymic output estimated as CD4+ cells expressing naive (CD45RA+CD62L+CD27+, P = 0.31) or recent thymic naive

(CD45RA+CD27+CD31+, P = 0.13) phenotype, or CD4+ cells containing TREC's (P = 0.47). HIV-EU children and controls had similar levels of activated cells (CD4+CD38+HLA-DR+, P = 0.87; CD8+CD38+HLA-DR+, P = 0.22), Tregs (CD4+CD25+CD127lowFOXP3+, P = 0.53) and naive Tregs (CD4+CD25+CD127lowFOXP3+CD45RA+CD27+, P = 0.65). Finally, comparable levels of Hib protective antibodies in the two groups were found (P = 0.43).

Conclusion: The study indicates that immune deficits in HIV-EU children are not long-term, and compared to children born to HIV-negative mothers HIV-EU children have neither quantitative nor qualitative immunological abnormalities at 15 months of age.

P1209 Prevalence of hyperphosphaturia and of proximal renal tubular dysfunction and their associated factors in HIV-infected patients, ANRS CO3 Aquitaine Cohort, 2009

F.A. Dauchy, S. Lawson-Ayayi, C. Rigothier, F. Bonnet, G. Miremont-Salamé, S. Delveaux, N. Mehsen, C. Greib, F. Dabis, M. Dupon for the Groupe d'Etude Clinique du SIDA en Aquitaine (GECSA)*

Objectives: Tubular dysfunction is of particular concern in the course of HIV infection, as it may subsequently induce bone abnormalities. The objectives of this study were to assess the prevalence of hyperphosphaturia (HP) and of proximal renal tubular dysfunction (PRTD) and to investigate their potential determinants in an open and unselected cohort.

Methods: Consecutively enrolled HIV-infected subjects were prospectively evaluated for PRTD. HP of tubular origin was defined as a ratio of maximum rate of renal tubular reabsorption of phosphate to glomerular filtration rate (TmP/GFR) $\leqslant$0.77 mmol/L. PRTD (Fanconi syndrome) was defined as the presence of HP with hypophosphatemia $\leqslant$0.80 mmol/L or at least 2 out of 4 of the following criteria: non-diabetic glucosuria, renal acidosis, b2-microglobulinuria, or low serum uric acid with a fractional excretion of uric acid >10%. Multivariable analyses were conducted using two separate models relating to clinical and biological patients' characteristics and all antiretroviral treatment exposure.

Results: 349 patients (268 males) were included in this survey. Median age was 47 years (Interquartile Range [IQR]: 42–54), median CD4 count 472/mm^3 (IQR: 342–638), 89% were treated with $\geqslant$3 antiretroviral drugs and 81% had HIV-RNA <40 copies/mL.

HP was present in 52 patients (prevalence: 14.9%; 95% confidence interval [CI]: 11.1–19.5). In the final model, male gender (OR = 11.1, p = 0.001), age $\geqslant$55 (OR = 2.54, p = 0.047), lower estimated GFR calculated with MDRD equation (OR = 7.96, p < 0.001), tenofovir [TDF] (OR = 1.15, p = 0.046), zidovudine [ZDV] (OR = 1.07, p = 0.119) and nelfinavir/r [NFV/r] (OR = 1.21, p = 0.013) exposure remained independently associated with HP.

PRTD was diagnosed in 30 patients (prevalence: 8.6%; 95% CI: 5.8–12.3%). Using the same analytical approach, male gender (OR = 4.3, p = 0.035), MDRD (OR = 4.64, p = 0.009), ZDV (OR = 1.11, p = 0.069), lamivudine (OR = 0.85, p = 0.023), lopinavir/r [LPV/r] (OR = 1.23, p = 0.027) and atazanavir/r [ATZ/r] (OR = 1.28, p = 0.027) exposure remained associated with the detection of PRTD. Longer exposure to TDF was not retained (OR = 1.15, p = 0.116).

Conclusion: PRTD was a prevalent abnormality in this French unselected cohort. Our patients were broadly exposed to TDF (64.8% in the sample). The combined used of TDF, LPV/r, ATZ/r or NFV/r increased the risk of tubular dysfunction. Further studies are needed to assess the impact of the chronic exposure of TDF alone or combined with protease inhibitors on the kidney function.

P1210 Findings from fibre optic bronchoscopy in HIV-positive patients in eastern London

P. Papineni, Y. Ahmed, S. Limb, H. Noble, T. O'Shaughnessy, G. Packe, G. Thomas (London, UK)*

Objectives: Respiratory disease is a common complication of HIV infection. Definitive diagnosis can be elusive with non-invasive testing. We aimed to characterise the diagnoses made in HIV positive patients following fibre optic bronchoscopy (FOB) and assess the hypothesis that FOB remains useful in the diagnosis of respiratory disease in HIV.

Methods: A retrospective observational analysis of FOB in HIV positive patients at Newham University Hospital in East London between March 2005 and August 2008.

Results: 63 FOBs were performed on 58 HIV positive patients. There were 64 diagnoses of which FOB supported or proved 26 (41%). The most common diagnosis (30%) was *Pneumocystis jirovecii* pneumonia (PJP) with 19 cases of which 12 were proven, all on bronchial washing cytology. Of these 12 patients, 25% had CD4 counts $>200\times10^6$/L. The second most common diagnosis (27%) was tuberculosis with 17 cases of which 15 were primarily pulmonary in focus. Culture of bronchial washings was positive in 10 of these cases. Of the 15 cases of pulmonary tuberculosis, 13 were sputum smear negative. Of these, 3 (23%) were afforded early diagnosis by FOB with positive microscopy whilst 8 (62%) were culture positive, confirming the diagnosis and providing sensitivities. The rate of FOB proven PJP appears to have decreased over time whilst that of pulmonary tuberculosis appears to have grown. Average CD4 counts did not change consistently over this period.

Other diagnoses made at FOB include Cryptococcal pneumonitis, pulmonary strongyloidiasis, interstial lung disease and lymphoma. Other organisms isolated at FOB include *Staphylococcus aureus* (11%), *Haemophilus* influenza, *Pseudomonas* spp. and Coliforms (each 5%) and *Aspergillus* spp. (3%). The significance of these organisms in the patient's illness was uncertain. *Candida albicans* was isolated following 18% of FOBs, nearly 3 times the rate found in our HIV negative patients. No significant association was found between organism isolated and CD4 count (student's t test), perhaps due to limitations in sample size.

Conclusions: PJP and pulmonary tuberculosis are the most common diagnoses following FOB in our HIV+ patients. FOB is useful in the diagnosis of PJP and smear negative TB in HIV.

P1211 Is procalcitonin a useful biomarker of severe bacterial infections in HIV-1-infected patients?

T. Mikula, M. Dabrowska, J. Cianciara, A. Wiercinska-Drapalo (Warsaw, PL)*

Objectives: The role of procalcitonin (PCT) in immunocompromised individuals is still investigated. The aim of our study was to evaluate the usefulness of PCT in diagnosis of severe bacterial infections in HIV-1-infected patients and its correlation with C-reactive protein (CRP), white blood cells count (WBC), CD4 cell count, and plasma HIV-1 viral load.

Methods: A cross-sectional study was performed in 92 HIV-1(+) subjects and 37 HIV-1(–) individuals (9 males and 28 females, mean age 39.7 years). HIV(+) patients were divided into two groups: group 1(n = 40, 33 males and 7 females, mean age 35.4 years, CDC A/B, without cART) and group 2 (n = 52, 45 males and 7 females, mean age 37.9 years, CDC C, on cART). Clinical symptoms of bacterial infection, Systemic Inflammatory Response Syndrome (SIRS) or sepsis lasting no longer than 3 days before the hospitalization were the main inclusion criteria. WBC, PCT and CRP were analyzed in the first 12 hours of hospitalization. Blood and urine bacterial culture were performed. PCT serum concentration were evaluated with quantitative immunoluminometric method (LUMITEST PCT kit, BRAHMS AG, Berlin, Germany). Plasma HIV-1 RNA was calculated using the Amplicor system. All statistical methods (median, standard deviation and Pearsons correlation ratio) were performed in Statistica 8.0.

Results: Positive blood and/or urine bacterial culture were observed in 14 (27%) and 8 (21%) HIV(+) subjects, respectively. Moreover, fifty seven(62%) HIV(+) individuals had SIRS/sepsis confirmation. Twenty seven(47.3%) of them had normal PCT concentration (8 subjects in group 1 and 19 subjects in group 2). No PCT elevation was observed in control group. PCT sensitivity and specificity were estimated in all studied groups. The lowest PCT sensitivity (48%) was estimated in group 2; however, its specificity was still higher than CRP. In both HIV(+) groups positive predictive values were low (46.4 and 64.0, respectively) and negative predictive values rather high (91.0 and 93.0,

respectively). No correlation between PCT level and WBC count, CD4 cell count and plasma HIV-1 viral load was observed.
Conclusion: Serum PCT concentrations in HIV-1-infected patients, especially with AIDS, are mostly normal even during severe bacterial infection. Therefore, the influence of immune deficits in assessment of PCT results should be always considered in the early diagnosis of SIRS/sepsis in immunocompromised subjects.

P1212 New Advia Centaur® HIV Ag/Ab combo, 4th generation screening assay, shows high level of specificity in a mixed population of a routine diagnostic laboratory

D. Burki, S. Pfister (Basel, CH)*

In HIV-screening fourth generation assays detecting both HIV-1/2 antibodies and HIV-1 p24-antigen are the recommended standard. While sensitivity is crucial in any HIV screening assay, it should not be maximized at the cost of specificity as false-reactive reports result in anxiety in the patient as well as additional costs for secondary testing.
With the aim of establishing specificity of the new Siemens ADVIA Centaur® HIV Ag/Ab combo (CHIV) forth generation HIV-screening assay (Siemens Healthcare Diagnostics, Tarrytown, USA) in a mixed population of both in- and outpatients in screening situations or in the investigation of a clinical suspicion of HIV infection, 1738 unselected serum samples of a large diagnostic laboratory were compared with the current routine method ABBOTT Architect HIV Ag/Ab combo (ABBOTT Diagnostic Division, Wiesbaden, Germany).
Six reactive samples were confirmed by immunoblot and viremia. 1732 samples were considered HIV-negative. Of these, one was reactive in both assays, one in Architect only, and two in ADVIA Centaur® only. Specificities at the cut-off of 1.00 S/CO were 99.71% for ADVIA Centaur and 99.77% for Architect. When applying an extended grey zone from 0.60 to 1.00 S/CO specificities were 98.79% for ADVIA Centaur and 99.77% for Architect. Median S/CO was 0.05 (= limit of detection) for ADVIA Centaur® and 0.16 for Architect.
Sensitivity of the new HIV screening assay was not addressed as it can only be determined in very large populations or, more conveniently, using seroconversion panels. Still, all six confirmed samples were correctly identified by ADVIA Centaur®.
Specificity, conversely, is best assessed using real populations including individuals with clinical suspicion of HIV-infection as well as individuals in mere screening situations. At the cut-off of 1.00, specificities of the two assays were comparable. A low median of S/CO values in ADVIA Centaur® suggested better discriminatory power than Architect. However, a trailing of values in the high-negative range may significantly impair specificity if an extended grey zone is applied in the ADVIA Centaur®
We conclude that in a mixed population the new Siemens ADVIA Centaur® CHIV forth generation assay proved highly specific and may therefore be introduced into routine testing.

P1213 Human immunodeficiency virus-1 F1 subtype is associated with late presentation in newly diagnosed Romanian patients

D. Florea, S. Paraschiv, M. Fratila, D. Otelea (Bucharest, RO)*

Objective: Late presenters at the time of the HIV-1 diagnostic are frequent in Romania. The purpose of the present study was to find out if these late presentations are correlated with particular virological and/or epidemiological features.
Methods: Newly diagnosed patients from several representative HIV Romanian centres were clinically, epidemiologically and virologically evaluated. Late presentation was defined as AIDS-related conditions and/ or <200 CD4/mm^3. The isolated HIV-1 strains were sequenced using with the commercial Viroseq™ HIV-1 Genotyping System. For subtyping purposes the nucleotide sequences (entire protease gene and two thirds of the RT gene) were analysed with the publicly available algorithm REGA HIV-1&2 Automated subtyping tool version 2.0. In order to do phylogenetic analysis we used for aligning ClustalW software as implemented in BioEdit and Mega 4 software for tree building.
Results: We analysed 250 patients diagnosed between 2007–2009. More than half of them (54%) were late presenters: 35% had AIDS-related conditions and 45% had CD4 counts <200 cells/mm^3. Most of the late presenters were infected with the F1 HIV subtype whereas the asymptomatic patients were infected with more diverse subtypes (F, C, B, A, G, CRFs). A significant number of AIDS cases at the time of diagnoses were in patients aged 15 to 22 years of age. Late presentation was correlated to lack of acknowledged risk behaviour.
Conclusions: HIV-1 F1 subtype is associated with later presentation in newly diagnosed Romanian patients. Our data suggest that these findings are related to the epidemiological distribution of subtypes at the time of infection rather than host related factors.

Miscellaneous viral infections

P1214 Herpes simplex encephalitis in a national prospective encephalitis cohort, France, 2007

A. Mailles, P. Morand, H. Peigue-Lafeuille, J.P. Stahl (Saint-Maurice, Grenoble, Clermont-Ferrand, FR)*

Objectives: The prognosis of herpes simplex encephalitis (HSE) has been dramatically improved by the generalisation of acyclovir. However, despite international recommendations, the exact regimen is still discussed, as are the criteria for discontinuing the treatment. We present the clinical features of HSE patients included in the French encephalitis study in 2007 and the management of acyclovir prescription in encephalitis case-patients with or without HSE diagnosis.
Methods: HSE case-patients were patients aged 28 days or older, hospitalised in mainland France in 2007 with an acute onset of illness, and at least one abnormality of the CSF ($\geqslant$4 white blood cells/mm^3 or $\geqslant$40 mg/dl protein), and fever $\geqslant$38ºC and decreased consciousness or seizures or altered mental status or focal neurological signs, and a positive HSV PCR on CSF. Clinical, biological and management details were collected using standardized questionnaires.
Results: Among 253 encephalitis patients, 55 had HSE, 76 had another aetiological diagnosis, and 122 had no aetiological diagnosis identified. The age of HSE patients ranged from 1 month to 85 years (median 58), and 31 (56%) were men. Upon admission, 48 (87%) had memory loss, 15 (27%) had focal neurological signs and 14 (25%) had speech disorders. All received acyclovir, 9 (16%) of them for 2 weeks and 41 (75%) for 3 weeks. Their case-fatality ratio was 5.5%, and 48 (87%) still had remaining neurological signs on discharge, mainly speech disorders or memory impairment. Among non-HSE patients, acyclovir was started in 175 (88%) and discontinued after a median length of 8 days (range 1 to 25 days).
Conclusion: Our study showed a lower fatality ratio in HSE patients compared with other studies. Among HSE patients, acyclovir was not systematically prescribed following international recommendations. The duration of acyclovir treatment in non HSE patients is considered with regards to other aetiologies identified and the timeline of diagnosis. We concluded that the dissemination of international recommendations regarding the management of HSE should be reinforced in France.

P1215 Impact of varicella zoster virus exposure on frequency and age of onset of herpes zoster in France: Mona study

J. Gaillat, V. Gajdos, O. Launay, D. Malvy, S. Pinchinat, T. Derrough, C. Sana, E. Caulin, B. Soubeyrand (Annecy, FR)*

Context: Repeated contact with children suffering from varicella might play a significant role in maintaining immunity against varicella zoster virus (VZV) and reduce the risk of herpes zoster in adulthood.
Objectives: The principal objective was to evaluate the frequency of herpes zoster in a population with little or no exposure to children (monks/nuns) compared to that in the general population. The reported age of onset of herpes zoster in these two populations was a secondary objective.

Method: A national, comparative, observational, multicentre, epidemiological study in an "exposed/non-exposed" design conducted by means of self-administered questionnaires. The non-exposed population consisted of monks and nuns in 40 isolated monasteries, excluding postulants or novices and those without a command of French. Monks and nuns who regularly came into contact with groups of children under 10 years of age were excluded from the principal analysis population. The sample of the general population was constituted from a pre-established file, based on the quota method, which was representative of the French population in terms of age group, sex, socio-occupational groups and regions. Individuals who failed to return their questionnaire were contacted by telephone. Those without a command of French and/or living in an institution were excluded.
Results: The principal analysis population comprised 920 monks/nuns (41.5% nuns) and 1,533 individuals (51.9% women) from the general population. The reported frequency of herpes zoster was 16.2% in the monks/nuns and 15.1% in the general population (p adjusted for sex and age = 0.27). Herpes zoster had been confirmed by a healthcare professional in 92% of cases in both populations. The mean reported age of onset of herpes zoster was 54.8 and 48.6 years, respectively ($p = 0.06$). Women exhibited herpes zoster significantly more often than men in both populations [21% of nuns and 13% of monks ($p = 0.002$) – 18% of women and 12% of men ($p = 0.002$)].
Conclusion: In this study no difference in reported frequency and age of onset of herpes zoster was demonstrated in these two populations with a very different exposure to children. These results suggest that, if exposure to the varicella virus does have a protective effect against the onset of herpes zoster in adults, the effect is necessarily weak.

P1216 Aetiology and cerebrospinal fluid findings in aseptic meningitis

I. Vanaga, A. Broduza, B. Rozentale (Riga, LV)*

Objectives: Assessment of the etiology and cerebrospinal fluid (CSF) findings in patients with aseptic meningitis (AM) and correlation between etiology, papilledema, cerebral trauma and CSF findings.
Methods: 222 medical records of patients with AM, treated at the Infectology Center of Latvia during 2001 to 2008, were analyzed. Patients with HIV, tuberculous and tick-borne encephalitis virus meningitis were excluded. The etiology was confirmed if a microbe or microbe-specific nucleic acid was detected in the CSF or specific IgM antibodies were detected in the blood. We analyzed CSF pleocytosis, neutrophils/lymphocytes (Neu/Ly) ratio, and protein level.
Results: 222 cases of AM were reviewed. The patients ranged in age from 15 to 82. The male/female ratio was 135/87. Etiology was defined in 33%. The most common causative agents were enteroviruses – 44, herpesviruses – 17, *B. burgdorferi* – 6, mumps viruses – 5.
The patients with mumps meningitis had highest total pleocytosis in CSF (774.2 ± 598.0 cells/mm^3), but the lowest was in meningitis caused by *B. burgdorferi* (214.8 ± 201.9 cells/mm^3). 14% of all AM cases had Neu predominance. Ly count was higher in cases of mumps (754.6 ± 592.1 cells/mm^3) and herpesviruses (632.1 ± 406.1 cells/mm^3) than enterovirus (339.8 ± 416.2 cells/mm^3) meningitis, respectively, $p = 0.049$, $p = 0.012$. In 27% cases of enterovirus meningitis Neu predominance was observed. The highest CSF protein concentration were in patients with *B. burgdorferi* caused meningitis (1.13 ± 1.26 g/l), but the lowest were in patients with mumps meningitis (0.57 ± 006 g/l). In cases with herpesviruses meningitis CSF protein level (0.95 ± 0.39 g/l) was higher than enterovirus meningitis (0.63 ± 0.43 g/l), respectively, $p = 0.005$.
The greatest part of *B. burgdorferi* caused meningitis patients had papilledema. None patients with mumps meningitis had papilledema. Papilledema and anamnestic head trauma didn't affected CSF pleocytosis, Neu/Ly ratio, and protein level.
Conclusion: 67% of AM the etiology remains undefined. The leading cause was enterovirus. The highest pleocytosis in CSF had patients with mumps meningitis. AM patients CSF was not characterised only as lymphocytar, in some cases, especially enterovirus meningitis, Neu predominate. *B. burgdorferi* meningitis CSF protein level was highest. Papilledema was more often in AM caused by *B. burgdorferi.*

P1217 Clinical presentations of echovirus 6 and 9 infections among neonates, younger and older children in Taiwan

H. Lee, Y.C. Huang, C.J. Chen, C. Chiu, C. Wu, T.Y. Lin (Taoyuan, TW)*

Objectives: Echovirus 6 and 9, the predominant types of non-polio enterovirus infections, have not been comprehensively evaluated. To delineate the clinical features associated with echovirus 6 or 9 infections, clinical presentations of infected neonates, younger (1 month-old and <3 year-old) and older children (3 year-old) were compared.
Methods: Culture-proven echovirus 6 or 9 infections were identified by immunofluorescent staining with virus-specific monoclonal antibody in 199 children in a teaching hospital from 2000 to 2008. The medical records of all cases were reviewed and analyzed according to their ages. Clinical manifestations, laboratory findings, treatments, and outcomes were collected from data of 174 inpatients without any other concomitant or secondary bacterial or virus infection.
Results: The mean age was 4.7 years (range, 4 days to 15 years) and more than a half of patients (61.8%) were male. The disease spectrums were similar for echovirus 6 ($n = 100$) and 9 ($n = 74$) infections, with aseptic meningitis (49.0% and 51.3%) being the most common syndrome, followed by meningismus (17.0 and 14.9%), upper respiratory tract infection (7.0% and 6.8%), pneumonia (5.0% and 8.0%), and herpangina (5.0% and 4.1%) (Table 1). Aseptic meningitis and meningismus, were common (70%) in neonates or older children, but relatively uncommon (40%) in younger children ($p < 0.001$). The laboratory values were of no significant difference except for a higher proportion of serum aspartate aminotransferase level >40U/L in echovirus 6 infections (27.0% vs. 1.4%, $p < 0.001$). Fever, headache and vomiting were "triad" found in almost all children older than 3-year-old with echovirus meningitis (all >90%), and mannitol or glycerol with antipyretic was beneficial for these patients. Longer duration of fever and more frequency of high temperature (>39ºC) were significantly found in echovirus 9 than in echovirus 6 infections (both $p < 0.001$). Six (3.4%) children were severe, including fatal, rescued by extra-corporeal membrane oxygenation (ECMO) or with long-term sequel with initial presentations of seizure with blood sugar >150 mg/dL, liver function impairment, brain infarction or herniation and diagnosis of myocarditis.
Conclusion: Aseptic meningitis was the most common manifestation of echovirus 6 and 9 infections in children requiring hospitalization. Fatal outcome or long term sequel in survivors was rare but may be encountered in children with specific presentations.

Table 1. Diagnosis of 174 children with echovirus 6 ($n = 100$) or 9 ($n = 74$) infections among three age distributions in Chang Gung Memorial Hospital, 2000–2008

Age	<1 month		1 month – 3 years		>3 years		All	
Serotype	6	9	6	9	6	9	6	9
Number (%)	10 (10.0)	5 (6.8)	30 (30.0)	18 (24.3)	60 (60.0)	51 (68.9)	100	74
Aseptic meningitis	6 (60.0)	4 (80.0)	9 (30.0)	3 (16.7)	34 (56.7)	31 (60.8)	49 (49.0)	38 (51.3)
Meningismus	1 (10.0)	1 (20.0)	3 (10.0)	0 (0)	13 (21.7)	10 (19.6)	17 (17.0)	11 (14.9)
Upper respiratory infection	0 (0)	0 (0)	4 (13.3)	3 (16.7)	4 (6.7)	3 (5.9)	7 (7.0)	5 (6.8)
Non-specific febrile illness	2 (20.0)	0 (0)	5 (16.7)	0 (0)	2 (3.3)	2 (3.9)	9 (9.0)	2 (2.7)
Pneumonia	0 (0)	0 (0)	2 (6.7)	3 (16.7)	3 (5.0)	3 (5.9)	5 (5.0)	6 (8.1)
Herpangina	0 (0)	0 (0)	3 (10.0)	2 (11.1)	2 (3.3)	1 (2.0)	5 (5.0)	3 (4.1)
Exanthema	0 (0)	0 (0)	0 (0)	5 (27.8)	0 (0)	1 (2.0)	0 (0)	6 (8.1)
Hand–foot–mouth disease	0 (0)	0 (0)	1 (3.3)	2 (11.1)	0 (0)	0 (0)	1 (1.0)	2 (2.7)
Encephalitis	0 (0)	0 (0)	2 (6.7)	0 (0)	0 (0)	0 (0)	2 (2.0)	0 (0)
Myocarditis	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.7)	0 (0)	1 (1.0)	0 (0)
Polio-like syndrome	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.7)	0 (0)	1 (1.0)	0 (0)
Acute gastroenteritis	0 (0)	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)
Hepatic necrosis with coagulopathy	1 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)

P1218 Longitudinal study of BK virus infection in adult allogeneic haematopoietic stem cell transplant

J. Corneille, N. Dhédin, C. Deback, M. Uzunov, S. Nguyen, J. Vernant, H. Agut, D. Boutolleau (Paris, FR)*

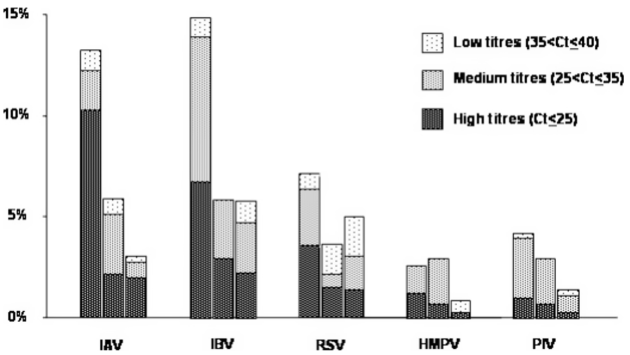
Objectives: In haematopoietic stem cell transplant (HSCT) recipients, BK virus (BKV) infection is associated with haemorrhagic cystitis (HC) occurring after engraftment. Nevertheless, no virological marker of late-onset HC has been clearly identified. The aim of this longitudinal study

was to determine the incidence and the risk factors of BKV infection in HSCT recipients, to analyse the association of BKV load in whole blood with HC, and to evaluate whether systematic follow-up of BKV infection is warranted.
Methods: BKV load was measured retrospectively using a real-time PCR assay in whole blood specimens from 61 consecutive allogeneic HSCT recipients (38 men, 23 women, median age: 45 years) collected at 0, 1, 2, 3, 6, 9, and 12 months post-transplantation.
Results: Fifteen patients (25%) developed BKV viraemia at a median of 2 months (1–3) after transplantation. Acute leukaemia as underlying disease was identified as a risk factor for BKV infection ($P = 0.03$). BKV infection was associated with acute graft-versus-host disease ($P = 0.003$) and haematuria ($P < 0.001$). The maximum BKV load was higher in patients with haematuria than in those without haematuria (median, 1685 vs. 160 copies/mL; $P = 0.05$). The occurrence of haematuria was statistically associated with a concomitant BKV load >500 copies/mL ($P = 0.04$) and with BKV detection in at least two consecutive blood specimens ($P = 0.04$). Lymphocyte recovery in patients with haematuria was delayed compared to those without haematuria. Ten patients (16%) experienced both BKV and cytomegalovirus (CMV) infections after transplantation. In all cases, CMV infection appeared either before ($n = 5$) or concomitantly ($n = 5$) with BKV infection. Overall, the frequency of BKV detection was statistically higher in CMV-positive samples than in CMV-negative samples (22% vs. 5%; $P < 0.001$). No adenovirus infection was evidenced in patients with haematuria.
Conclusion: BKV viraemia is highly associated with the occurrence of HC in HSCT recipients. Prolonged BKV viraemia and BKV load over 500 copies/mL of whole blood were identified as predictive markers for the development HC. The analysis of BKV load kinetics and lymphocyte recovery after transplantation underlines the potential role of cellular immunity in the pathogenesis of HC. Our results suggest a possible role of CMV in the process of BKV infection. The monitoring of BKV load appears useful to identify HSCT recipients at risk for developing post-engraftment HC.

P1219 Influenza and other respiratory viruses among febrile children with and without respiratory symptoms in Tanzania

V. D'Acremont, P. Cherpillod, M. Kilowoko, E. Kyungu, S. Philipina, W. Sangu, J. Kahama-Maro, P. Kibatala, L. Alamo, C. Lengeler, B. Genton, L. Kaiser (Basel, Geneva, CH; Dar es Salaam, Ifakara, TZ; Lausanne, CH)*

Objectives: Viral respiratory tract infections are the most frequent cause of febrile illnesses in infants and young children in industrialized countries but few studies have been conducted in Africa, especially in outpatients. To assess the contribution of viral infections to febrile episodes in children attending an outpatient clinic, we investigated the prevalence of common respiratory viruses among children with and without respiratory symptom/sign(s) in a rural and urban setting in Tanzania.
Methods: All consenting children aged 2 months to 10 years with temperature >38ºC were recruited between Apr-Dec 2008. Medical history and clinical examination were done and nasal/throat swabs taken for viral real-time PCR. Ct values were used to provide semi quantitative viral loads. X-ray was performed when IMCI criteria for clinical pneumonia were met. Other investigations were also undertaken to find a precise etiology of the fever episode with a probability level based on a pre-defined algorithm.
Results: 1005 children were recruited. 643 (64%) had at least one respiratory symptom/sign (RSS): 507 children had an acute respiratory infection (ARI) as primary diagnosis (high probability) (63% URTI, 20% clinical pneumonia with normal Xray, 8% bronchiolitis, 6% pneumonia confirmed by X-ray, 3% pneumonia with no Xray done) and 136 children had ARI as secondary diagnosis. 362 children had no RSS. Based on preliminary viral analysis and excluding picornaviruses, the proportion of children infected with at least one respiratory virus was 40% for children with ARI as primary diagnosis, 21% for children with ARI as secondary diagnosis and 15% for children without RSS. The proportion of children with picornavirus was the same in the 3 groups (36%, 35% and 33% respectively). The proportion of children with double infection was 15%, 5% and 6% respectively.
The proportion of children with presumably high viral titers ($Ct < 25$) were similar between the 3 groups, except for influenza A where more children with ARI as primary diagnosis than ARI as secondary diagnosis had high titres (see graph).
Conclusion: In these African children with febrile illnesses the prevalence of influenza A and B and of other respiratory viruses was higher than expected, especially in children without any respiratory symptom or sign. This latter observation suggests that these viruses may cause fever episodes without obvious respiratory manifestations.



Graph. Proportion of febrile children infected with each respiratory virus stratified by the absence/presence of ARI and/or RSS. Left column: ARI as primary diagnosis; Middle column: ARI as secondary diagnosis; Right column: no RSS.

P1220 Virological diagnosis of respiratory virus infection in patients attending an emergency department during the influenza season

A. Pierangeli, C. Scagnolari, S. Trombetti, M. Gentile, B. Rizzo, M.T. Spina, A. Iudicello, G. Bertazzoni, G. Antonelli (Rome, IT)*

Objectives: The aim was to investigate the burden of influenza and influenza-like respiratory illness in a hospital emergency department (ED) during the seasonal influenza months.
Methods: Consecutive patients attending an ED during February and March were enrolled within the framework of a respiratory infection surveillance project; 103 nasopharyngeal washes were tested for several common respiratory viruses using PCR-based methods.
Results: Influenza A viruses were detected in 25/103 samples (24%) whereas a rapid influenza test was positive in only 1/31 samples tested. Rhinovirus was detected in 15% of recruited patients, respiratory syncytial virus in only one, and influenza B was not detected. Only 16% of influenza A infections matched the influenza-like illness case definition at ED admission, whereas 19% of rhinovirus-infected individuals would have been diagnosed as having influenza-like illnesses.
Conclusion: Data suggest that triage of influenza cases based on syndromic occurrence of influenza would be insufficient and unsuitable for containing influenza virus spread during pandemic alerts, and could also lead to unnecessary isolation of patients infected with other respiratory viruses. The application of broader criteria at triage followed by timely molecular tests for the most common respiratory viruses could be effective in preventing transmission of new respiratory agents and in appropriate case management.

P1221 **Influenza like illness syndromes in an acute care geriatric unit in Edouard Herriot Hospital, Lyon, France during 5 seasons**

C. Régis, V. Escuret, B. Barret, C. Luxemburger, S. Pires-Cronenberger, C. Gorain, L. Pollissard, F. Morfin, F. Najioullah, B. Comte, B. Lina, P. Vanhems (Lyon, FR)*

Objective: A prospective study was performed during 5 influenza seasons (from November 2004 to April 2009) to report Influenza like illness syndromes (ILI) in a geriatric unit of a French university hospital and to describe cases of nosocomial influenza.
Method: Hospitalized patients (P) and healthcare workers (HCW) of an acute care geriatric unit of 18 beds with ILI syndrome were included in the study. An ILI syndrome was defined as fever >37.8ºC or cough or sore throat. Clinical diagnosis was confirmed by influenza detection using ELISA, viral culture and RT-PCR techniques performed on nasal samples. Medical data were collected. A nosocomial case was defined as a case occurring after at least 72 hours after hospitalization for P [Salgado; 2002] or exposure to an infected colleague or patient for HCW.
Results: During these 5 seasons, 135 P and 34 HCW were included. Among the ILI, 27 P (20%) and 2 HCW (5.9%) presented a confirmed influenza. Attack rate (AR) for 100 hospitalizations were calculated for the first 3 months of each year, we observed a median AR of 7.1% (min: 2.1; max: 24.7) for ILI and an AR of 1.1% (min: 0; max: 6.5) was observed for confirmed influenza.
Among patients, 95 (70%) presented the ILI before their hospitalization. According to the definition, 20 cases (15%) were nosocomial. No significant difference was observed for symptoms (cough and sore throat) between nococomial and community-acquired cases. 20 patients (15%) presented their ILI 24 to 48 hours after hospitalization; 9 of them were not hospitalized for a respiratory syndrome and 4 of them had a contact with an ILI case.
Among HCW, 24 (71%) ILI cases were nosocomial and 1 HCW presented nosocomial confirmed influenza. Nosocomial cases were observed from 28 potential transmission from P to HCW (n = 20) and from HCW to HCW (n = 8).
Conclusion: Despite the absence of virologicaly confirmed transmission of nosocomial cases from HCW to patient, many nosocomial cases of ILI were observed. These findings highlight the importance of the preventive measures completed by vaccination in this unit where high risk of contagiousness and high risk patients are present.

P1222 **Kaposi's sarcoma-associated herpesvirus selectively establishes latency and activates a subset of tonsillar B-cells**

L.M. Hassman, T.J. Ellison, D.H. Kedes (Charlottesville, US)*

To date, the identity of the subset(s) of B cells susceptible to KSHV infection has only been indirectly inferred from histological data (Chagas, et al., 2006) in which KSHV infection was observed in tonsillar regions with a high proportion of IgM+ B cells. Evidence supporting salivary transmission of KSHV, along with data showing that KSHV infected B cells in Multicentric Castleman's Disease (MCD) are also IgM+, are consistent with the hypothesis that KSHV may preferentially infect IgM+ tonsillar B cells *in vivo*.
Objectives: To establish which B cell subset(s) are susceptible to KSHV and to determine the effects of KSHV infection on tonsillar B cells.
Methods: We exposed cultures of purified tonsillar B cells to KSHV and analyzed the cells by multispectral imaging fluorescence cytometry (MIFC) as well as traditional flow cytometry and quantitative PCR (qPCR).
Results: Despite variation in susceptibility to latent infection (LANA expression) among individual donor tonsils, LANA+ B cells were almost exclusively IgM+ and expressed the lambda light chain. To assess whether this selectivity was due to differences in the level of viral entry or initiation of gene expression within the IgM+lambda population, we infected pre-sorted tonsillar B cell subsets and then measured intracellular viral genome copies and RNA expression by qPCR 24 h later. Despite differences in LANA expression, or the establishment of latent infection, approximately equivalent numbers of viral genomes as well as equivalent levels of initial viral transcripts were evident in all subsets. Latently infected B cells also displayed an activated phenotype (CD5 and high level surface immunoglobulin) and preliminary data suggest that infected B cells may show enhanced survival *in vitro*.
Conclusions: While multiple subsets of human tonsillar B cells were permissive to the initiation of KSHV infection, evidenced by viral entry and initial gene transcription, only IgM+lambda B cells displayed evidence of latent infection. Notably, the selectivity of KSHV in our *ex vivo* system corresponds to the phenotype of B cells found in MCD lesions, suggesting the possibility that IgM+lambda tonsillar B cells infected after exposure to salivary KSHV may serve as the cells of origin for this disease. Furthermore, KSHV infection activated B cells and we hypothesize that this activation may provide a survival advantage.

P1223 **Non-productive JC virus infection as a premise for cell transformation in colourectal malignancies**

I. Tsekov, D. Stoyanova, D. Dardanov, K. Kacarov, G. Kirov, Z. Kalvatchev (Sofia, BG)*

Objectives: Since the identification of the oncogenic JC virus (Polyomaviridae, Polyomavirus hominis 2) in samples from colorectal malignancies, the role of this infection has been a subject to controversial reports and further studies are needed. Our aim was to investigate a group of patients with colorectal cancers (CRC) in order to determine a possible role of JC virus for the development of the tumors. Furthermore, the functional state of the infection was evaluated in terms of productivity.
Methods: Biopsy materials from colorectal cancers or polyps, paired with matched normal, adjacent mucosa were collected from surgical resection specimens. A total of 35 fresh pairs were investigated. Following standard DNA extraction procedures, real time PCR assays were used for detection and characterization of JC genomic sequences. The amplified fragments were targeted: at the region encoding the viral regulatory protein termed large T antigen (LT); and the non-coding control region (NCCR). Melting curve analysis followed the cycling reactions in order that a characteristic profile of the amplicon was generated.
Results: Specific JC sequences from the LT genomic regions were found and characterized in 7 out of 18 polyps, and in 3 out of 17 cancer specimens. Additionally, 5 adjacent tissues were determined to harbor viral sequences. Only one specimen from a polyp was found positive for the NCCR target. All positive samples had a very low viral load in the range of 10–1000 starting copies. The results suggested either clonal integration of LT fragments or NCCR changes altering a productive course of the infection. Furthermore, the melting curve analysis depicted the melting temperature (Tm) profile of the amplified fragments. Seven more samples from tumors or polyps that were considered negative, had a similar pattern. This might be a result from changes in the targeted sequences, but further investigation is needed for clarification.
Conclusion: The results support a possible ethio-pathogenetic association of JC virus with colorectal malignancies. A non-productive course of the infection in colorectal cells is suggested as a premise for malignant transformation.

New antibacterials – clinical trials and experience

P1224 **Efficacy of novel formulation of josamycin for 7 and 10 days in paediatric patients with acute tonsillopharyngitis: results of multicentre study in Russia**

V. Krechikov, E. Bodyaeva, K. Shpynev, E. Danilova, S. Rachina, M. Galaktionova, S. Kozlov (Smolensk, Krasnoyarsk, RU)*

Objectives: To compare efficacy of 7- and 10-day courses of novel formulation of josamycin in children with acute tonsillopharyngitis (AT).

Methods: In this multicenter (4 sites) open-label study of josamycin (50 mg/kg/day in two doses) children aged 5 to 16 years with AT were randomized in two groups to receive either 7- or 10-day course of antibiotic. Novel tablet formulation (1000 mg) which can be either taken as a tablet or dissolved in water was used in the study. One of the inclusion criteria was assessment equal to 4–5 points on clinical score based on symptoms and signs of AT developed by Centor and modified by McIsaac, confirming high probability of streptococcal infection (>50%) and necessity of antibiotic prescription. Throat cultures were collected at the entry visit (V1, Day 1), at the end of treatment visit (V4, Day 11 for both groups) and at the follow-up visit (V5, Day 25). Clinical signs and symptoms were assessed at every visit. Information about episodes of AT and related antibacterial therapy in last three years was also collected.
Results: By the moment of data-cut a total of 117 children (5–16 years, mean 11.5 years) were enrolled in the study and randomized in two groups: 57 received josamycin for 7 days and 60 – for 10 days. Demographic characteristics and severity of disease was comparable in both groups. Twenty two patients (18.8%) received antibiotics in previous three years. Clinical efficacy at V4 was 94.9% in 7-day group and 96.1% in 10-day group, at V5–93.1% and 95.1% respectively. Forty seven isolates of group A streptococci (GAS) were isolated at the entry visit: 25 (in 43.9% of patients) in 7-day group and 22 (in 36.7% of patients) in 10-days group. Bacteriological efficacy at the end of therapy (V4) was comparable in both groups (97.4% – 7-day vs 95.2% – 10-day). The similar situation was observed at the follow-up visit: pathogen was eradicated in 95.2% and 91.5% of cases, respectively. Frequency of adverse events was low and comparable in both groups, the most frequent AE was nausea.
Conclusion: Novel formulation of josamycin showed high clinical and bacteriological efficacy for both 7- and 10-day regimens for treatment of AT in children.

P1225 A phase 2, randomized, double-blind, double-dummy study of IV sulopenem with switch to oral sulopenem etzadroxil compared to ceftriaxone with switch to amoxicillin/clavulanate in subjects with community-acquired pneumonia requiring hospitalization

K. Soma, A.M. Silvia, A. Hazra, S. Puttagunta, K. Durham, D. Kirby (New London, Groton, US)*

Objectives: Assess efficacy of 7–10 days (d) treatment for 3 regimens: single dose IV sulopenem (S) 600 mg with switch to oral sulopenem etzadroxil (SE) 1,000 mg BID (S-SIV); 2 d minimum IV S 600 mg BID with switch to SE 1,000 mg BID (S-MIV); and 2 d minimum IV ceftriaxone 2 gm QD with switch to amoxicillin/clavulanate suspension (800 mg/10 mL) BID (COMP).
Methods: Adult subjects were enrolled if presenting in Pneumonia Severity Index (PSI) risk class III-IV and with ⩾3 symptoms/signs of pneumonia. IV treatment could continue >2 days if needed and oral switch was allowed after: a minimum 2 d on IV treatment (active or placebo); improved cough and dyspnoea; afebrile or improved fever; improved WBC. The primary endpoint was the proportion of clinically evaluable (CE) subjects cured at the test of cure (TOC) visit, 7–14 days after end of treatment. Safety was assessed in the intent to treat group.
Results: Although a greater number of subjects was initially planned, 33 subjects were enrolled: 10, 11, and 12, respectively, for S-SIV, S-MIV, and COMP. Baseline demographic characteristics were similar. Ten, 8, and 8 subjects, respectively, met CE criteria. PSI risk class in the CE population for S-SIV, S-MIV, and COMP was III for 9, 5, and 6 subjects, and IV for 1, 3, and 2 subjects, respectively. Twenty-three subjects completed and 10 discontinued, with median treatment duration of 8.5, 10.0, and 8.0 d for S-SIV, S-MIV, and COMP, respectively. Subjects not assessed due to early discontinuation were considered failures. Results of the primary efficacy analysis are presented in the table. Cure rates for the CE population at TOC were 90%, 88%, and 63%, for S-SIV, S-MIV, and COMP, respectively.
Adverse drug reactions (ARs) occurred in 4/10 (40%), 3/11 (27%), and 4/12 (25%) subjects for S-SIV, S-MIV, and COMP, respectively, with gastrointestinal (GI) ARs reported in 2 (20%), 2 (18%), and 3 (25%) subjects, respectively. Four subjects (1 S-SIV, 3 COMP) discontinued for adverse events (AEs), one of which was an AR (COMP). Four subjects (2 S-MIV, 2 COMP) reported serious AEs, one of which was an AR (COMP). No deaths were reported.
Conclusions: These efficacy results were not statistically significant due to the small numbers enrolled; however, both S/SE regimens appeared to be comparable to COMP. ARs were comparable in all groups, including GI ARs. S/SE regimens should be investigated further for subjects with moderate to severe community acquired pneumonia.

Clinical response at TOC, CE population

	S-SIV, n (%)	S-MIV, n (%)	COMP, n (%)	S-SIV–COMP (%)	S-MIV–COMP (%)
Cure	9 (90.0)	7 (87.5)	5 (62.5)		
80% CI	(66.3, 99.0)	(59.4, 98.7)	(34.5, 85.3)		
Difference				27.5%	25.0%
80% CI				(–4.0, 55.3)	(–13.1, 57.9)

P1226 Effect of ceftobiprole on the normal human intestinal microflora

G. Panagiotidis, T. Bäckström, C. Asker-Hagelberg, M. Rashid, A. Weintraub, C.E. Nord (Stockholm, SE)*

Objectives: Administration of antimicrobial agents to patients can cause modifications of the normal microflora. Consequent reduction in the colonization resistance leading to overgrowth of new or already present microorganisms has been described. Increased resistance in many Gram-positive bacteria has driven the need to develop new antibacterial agents with activity against these pathogens including methicillin resistant *Staphylococcus aureus* (MRSA). Ceftobiprole is a new broad spectrum pyrrolidinone cephem active against *S. aureus* (MRSA and MSSA), vancomycin-resistant *Enterococcus faecalis* and Gram-negative bacteria such as Enterobacteriaceae and *Pseudomonas* spp. The purpose of the present study was to investigate the effect of administration of ceftobiprole on the normal intestinal microflora of healthy subjects.
Methods: Twelve healthy subjects (6 males and 6 females) 20–41 years of age received ceftobiprole 500 mg by intravenous infusion q8 h during 7 days. Plasma and faeces were collected on days 1, 4, 7, 10, 14 and 21 for determination of concentration by biological and chemical methods. For analysis of the microflora, the faecal specimens were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture, and identified to genus level. All new colonizing aerobic and anaerobic bacteria were tested for susceptibility to ceftobiprole.
Results: The plasma concentrations of ceftobiprole were as follows: On day 1, 14.7–23.6 mg/l; on day 4, 15.9–24.5 mg/l; and on day 7, 15.9–23.9 mg/l. No ceftobiprole was detected in plasma on days 10, 14 and 21. No measurable concentrations were found in faeces on days 1, 4, 7, 10, 14 and 21. There were minor changes in the numbers of enterobacteria, enterococci and *Candida albicans*, and there were moderate changes on numbers of bifidobacteria, lactobacilli, clostridia and bacteroides during the same period. No *Clostridium difficile* strains or toxins were found. No new colonizing aerobic and anaerobic bacteria with ceftobiprole minimum inhibitory concentrations of >4 mg/l were found.
Conclusion: Ceftobiprole had no significant ecological impact on the human intestinal microflora of healthy volunteers.

P1227 Complicated skin and soft tissue infections treated with daptomycin in the European Cubicin® Outcomes Registry and experience (EU-CORE)

A. Cogo, Z. Dailiana, P. Gargalianos-Kakolyris, T. Quast, V. Prisco, A. Segado, C. Iacoboni, P. Dohmen, M. Heep, H.J. Thurston, R.L. Chaves (Vicenza, IT; Larissa, Athens, GR; Bad Oeynhausen, DE; Mercato San Severino, IT; Madrid, ES; Cefalù, IT; Berlin, DE; Basel, CH)*

Objectives: Daptomycin (DAP), a cyclic lipopeptide antibiotic, was approved in Europe in January 2006 for the treatment of complicated skin and soft tissue infections (cSSTIs) caused by a variety of Gram-positive organisms. The aim of this retrospective, non-interventional records review was to evaluate clinical outcomes of patients (pts) with cSSTI receiving DAP therapy in Europe.
Methods: Clinically evaluable pts with a diagnosis of cSSTI who were treated with at least 1 dose of DAP were included. Patient demographic data, infection types, bacterial isolates, DAP dosing, and clinical outcomes (cure, improved, failure, or non-evaluable) at end of DAP treatment were collected and assessed by the investigators.
Results: A total of 1454 pts were included in the registry from Sept 2008 to Aug 2009. 484 pts (male 66%; age ⩾65 years 53.3%) were diagnosed with cSSTI with the following frequencies: wound 97 (20%), major abscess 40 (8%), diabetic ulcer (excluding diabetic foot) 24 (5%), diabetic foot 97 (20%), ulcer 53 (11%); surgical site infection (15% superficial, 14% deep incisional, 7% organ/space). 63% of inpatients received concomitant antibiotics, most commonly carbapenems (20%) and fluoroquinolones (18%). For 320 pts (66%), primary pathogens have been reported. *Staphylococcus aureus* (167/320) was the most frequent species with MRSA (n = 104) as predominant subset. The most frequent initial dose of DAP was 4 mg/kg (44%), 43% of pts received ⩾6 mg/kg. The overall clinical success rate was 84% (cure or improved). Failure was reported in 8% of pts and the rate of non-evaluable pts was also 8%. Clinical success rates for cSSTI subtypes were: wound 81%, major abscess 88%, diabetic ulcer (excluding diabetic foot) 92%, diabetic foot 85%, ulcer 85% and surgical site infection 88%, respectively. Adverse events (AEs) regardless of the study drug relationship which led to study drug discontinuation were reported in 2.9% of pts. Serious AEs, regardless of relationship, were reported in 3.5% of pts.
Conclusions: Daptomycin was effective and well tolerated for the treatment of cSSTI with high clinical success rate, in good agreement with results from registration trials. DAP is a useful therapeutic option for cSSTI caused by Gram-positive pathogens.

P1228 Daptomycin in the treatment of bacteraemia: clinical experience in Europe

A. Galloway, B. Galván-Guijo, A. Gonzalez-Ruiz, A. Rodríguez, B. Almirante, T. Lejko-Zupanc, V.J. González-Ramallo, I. Lampreabe Gaztelu, M. Heep, H.J. Thurston, R.L. Chaves (Newcastle upon Tyne, UK; Madrid, ES; Kent, UK; Tarragona, Barcelona, ES; Ljubljana, SI; Bizcaia, ES; Basel, CH)*

Objective: Daptomycin (DAP) is approved in Europe for the treatment of *Staphylococcus aureus* bacteraemia at a dose of 6 mg/kg once daily. Limited data are available for the treatment of bacteraemia in clinical practice. The aim of this registry was to describe the clinical experience with DAP for the treatment of bacteraemia.
Methods: The European Cubicin® Outcomes Registry and Experience (EU-CORE) is a retrospective analysis of patients' clinical outcomes with DAP therapy. Only those patients (pts) with bacteraemia who were treated with at least one dose of DAP were evaluated. Outcomes were assessed by the investigator (cured, improved, failure, non-evaluable) at the end of DAP therapy.
Results: Of the total of 1454 pts in the EU-CORE registry from Sept 2008 to Aug 2009 bacteraemia was diagnosed in 308 pts (21%), of which 53% were catheter related. Patients with bacteraemia were identified with the following demographics: male 71%; age ⩾65 years 47%. In total 39% of pts (120/308) received DAP in an ICU. Concomitant antibiotics were received by 72% of inpatients, most commonly with carbapenems (29%) and penicillins (22%). For 259 pts (84%) primary pathogens have been reported. Coagulase-negative Staphylococci (109/259) ranked first. *S. aureus* (97/259) was the second most frequent species, with MRSA (n = 45) a significant subset. *Enterococcus* spp. were seen in 23 cases. Most frequently used initial dose of DAP was ⩾6 mg/kg (75%).
The overall clinical success rate with DAP therapy was 74% (cure or improved). Outcomes of the primary infection for catheter related bacteraemia were: clinical success 76%, failure 11%, and non-evaluable 13%. For non-catheter related bacteraemia: clinical success 72%, failure 14% and non-evaluable 14%. Adverse events (AEs) regardless of the study drug relationship which led to study drug discontinuation were reported in 4% of pts. Serious AEs, regardless of relationship, were reported in 12% of pts.
Conclusion: DAP was effective and well tolerated for the treatment of Gram-positive catheter related bacteraemia and non-catheter related bacteraemia.

P1229 Efficacy and safety of high-dose daptomycin in *Staphylococcus aureus* infections

M. Bassetti, E. Nicco, E. Delfino, C. Fanciulli, C. Viscoli (Genoa, IT)*

Objectives: Daptomycin is a cyclic lipopeptide antibiotic approved for the treatment of complicated skin and soft-tissue infection (4 mg/kg) and *Staphylococcus aureus* bloodstream infection including right-sided endocarditis (6 mg/kg). On the basis of the drug's pharmacokinetic profile and concentration-dependent killing, higher doses may be beneficial in treating severe infections. There are limited safety data for high-dose and long-term daptomycin treatment. We describe our efficacy and safety experience with clinical use of daptomycin at doses of ⩾8 mg/kg for *Staphylococcus aureus* infections.
Methods: A retrospective chart review for all patients treated with daptomycin at San Martino University hospital, Genoa, Italy from January 2008 through September 2009 was performed. Patients were included in the study group if they received daptomycin at doses of ⩾8 mg/kg for *Staphylococcus aureus* infections. Clinical data were collected from their medical records (demographic conditions, clinical diagnosis, microbiologic isolate identification, antibiotic susceptibility, treatment indication, dose, duration and adverse events). Clinical and bacteriological outcomes were based on clinical assessment at baseline, end of treatment and follow-up. Safety was defined as adverse events (graded 1–4) documented in the medical record.

Characteristics	N = 21	%
Male gender	14	67
Age (years)	63	
Mean duration of hospitalisation (median, range)	35.2 days (21, 6–148)	
Co-morbid conditions		
Diabetes mellitus	3	14
Peripheral vascular disease	3	14
Immunocompromised	2	9.5
Neutropenia	1	4.7
Type of *S. aureus* infections		
Uncomplicated bloodstream infection	8	38
Complicated bloodstream infection	3	14
Skin and soft tissue infections	4	19.5
Left-side endocarditis	2	9.5
Right-side endocarditis	2	9.5
Osteomyelitis	2	9.5
Pathogens		
MRSA	19	90
MSSA	2	10
Vancomycin MIC 's		
⩽0.5	5	24
1	11	52
⩾2	**5**	24

Results: Twenty-one patients met the inclusion criteria. See Table 1 for clinical patient characteristics. Patients received a mean dose of 8 mg/kg (range, 7–9 mg/kg), for a median duration of 28 days of treatment (range,

10–162 days). The overall clinical and microbiological success rate at end of treatment was 95% (20/21 treated patients). No recurrences in the follow-up period (mean 75 days, range 30–180) were reported. Of 21 patients, 3 experienced grade 1 adverse events, and 1 experienced grade 3 CPK level elevation, with complete resolution after treatment discontinuation.
Conclusion: Our experience suggests that daptomycin was effective and well tolerated at a mean dose of 8 mg/kg for a median duration of 28 days for *S. aureus* infections. Determining the optimal dosing regimen for an antibiotic requires simultaneously maximising efficacy, minimising toxicity and minimising the selection of resistant organisms. Further study of high-dose daptomycin in larger cohorts are warranted.

P1230 First description of successful treatment of multiresistant typhoid fever with tigecycline

P. Pongratz, H. Laferl, J. Leitner, C. Wenisch (Vienna, AT)*

In typhoid fever, resistance to chloramphenicol, ampicillin and trimethoprim–sulfamethoxazole, extended spectrum cephalosporins and chinolones is widely spread and resistance to azithromycin and carbapenems has emerged Herein we describe 4 patients with travel (Pakistan) associated typhoid fever treated with tigecycline 1×150 mg for 14–21 days. All patients had at least one of the following risk factors, indicating a severe case: persistent vomiting, severe diarrhoea, abdominal distension requiring admission.
The demographic and clinical characteristics of these 4 patients with enteric fever are shown in table 1.
In multiresistant *S. typhi* infection tigecycline 1×150 mg iv daily could be an save and effective therapeutic option.

P1231 Experience with tigecycline in the treatment of multidrug-resistant pathogens at a university medical centre in Beirut

T. Baban, M. Yasmin, Z. Kanafani, A. El Zakhem, S. Kanj (Beirut, LB)*

Objectives: The rise in infections due to multidrug-resistant organisms coupled with the decrease in the development of new antibiotics has created a challenge to clinicians caring for critically-ill patients. Tigecycline is a newly introduced broad-spectrum antibiotic. Analyzing the use of tigecycline would help better evaluate and understand the value of tigecycline alone or in combination with other antimicrobials in treating various infections.
Methods: The aim of this study is to evaluate clinical characteristics and outcomes of patients who have received at least one dose of tigecycline for various infections between December 2006 and July 2009. A retrospective review of the patients' medical records was performed. Information was collected regarding basic demographics, infection type and location, concomitant antibiotic therapy, as well as microbiologic and clinical outcomes.
Results: A total of 116 patients were included in the analysis. The most common types of infection were ventilator-associated pneumonia (28.4%), hospital-acquired pneumonia (26.7%), abdominal infections (23.3%), skin infections (15.5%), and bloodstream infections (6%). 53.4% of infections were acquired in the intensive care unit. Tigecycline was used for directed therapy in 44% of cases and empirically in 56% of cases. In 58.6% of cases, concomitant antibiotics were required. The most commonly isolated resistant organisms in this patient population were imipenem resistant *Acinetobacter* spp. (27.4%), followed by organisms producing extended-spectrum β-lactometers (5.3%). Other outcomes of interest included sepsis (29.3%), superimposed infection (19%), and recurrent infection (5.2%). Adverse events that were observed with tigecycline treatment included elevated creatinine (6%), elevated transaminases (2.6%), rash (1.7%), *C. difficile* colitis (5.2%), and nausea and vomiting (2.6%). The overall mortality rate was 44.8%, whereas clinical and microbiological cure rates were 29.3% and 16.4%, respectively.
Conclusion: Patients who received tigecycline had low clinical cure rates and a high overall mortality. This might be explained by the fact that tigecycline was mostly used as salvage therapy rather than a first-line agent. A large proportion of patients were critically ill with a high expected mortality rate due to serious underlying conditions. In addition, the majority of patients had infections with an MDR organism where the available options for therapy were seriously limited.

P1232 Use of daptomycin in complicated cases of endocarditis: experience in a tertiary referral centre in the United Kingdom

I. Das, T. Saluja (Birmingham, UK)*

Background: Infective endocarditis (IE) is a serious form of infection necessitating specific antibiotic therapy for a prolonged period with or without surgery. Medical management can be a challenge because of pre-existing/new development of organ dysfunction, lack of clinical response or presence of allergic reaction to the specific antimicrobial agents. Presence of resistance in the causative organism to the commonly recommended antibiotics can also be a major issue as options on the recommended antibiotic therapy for IE are limited.
Aim and Objectives: An observational study assessing the usefulness of daptomycin, in the treatment of IE cases which are unresponsive or intolerant to the recommended first line agents. Cases for which the recommended first line agents could not be administered due to presence of resistance in the causative organisms were also included in the study.
Method: From 1 October 08 to 30 September 09, all cases of IE were followed up prospectively by the Microbiologists during regular ward visits. IE cases were noted following positive blood culture reports and consultation by the Cardiac unit.
Results: Eight of 89 cases of endocarditis were treated with daptomycin. Median age was 65 yrs (range 25–76 yrs), 50% were male. Native and prosthetic valves were affected in equal proportions (4 in each group). Mitral valves were affected in 3 and aortic valve in 5 cases. The causative organisms were known for all of the cases: *Staphylococcus aureus* (2), Coagulase negative staphylococci (2), *Enterococcus* spp (2), and viridians streptococci (2). Daptomycin was used as the second line therapy for all of the cases. The reason for change from the first line antibiotic therapy was: therapeutic failure (4), high level aminoglycoside resistance in *Enterococcus* (1), renal insufficiency (3), toxicity or allergic reaction with the first line regimen (3).
Prescribed daily doses daptomycin was 6 mg/kg (in presence of normal renal function) and an additional agent was used in 7 of 8 cases. Median duration of daptomycin therapy was 4–6 weeks with a range of 4–32 weeks. No untoward effect was observed in any of the cases. All of the cases were successfully treated with no further complications and have been discharged home.
Conclusion: Daptomycin is well tolerated and is an effective agent in the treatment of IE especially in cases of therapeutic failure or intolerance to the first line of agents.

P1233 Clinical experience with daptomycin from a regional Lancashire cardiac centre in northwest England

A. Kumar, M. Przybylo, S. Summers, A. Guleri (Blackpool, UK)*

Background: Lancashire cardiac centre is a tertiary centre located in Blackpool Victoria Hospital(BVH), a large district hospital in northwest England. Two years of clinical experience of daptomycin usage is presented here.
Methods: Outcomes of daptomycin usage were evaluated through retrospective data collection. Patients' age, sex, diagnosis, culture results, treatment outcomes and adverse events were recorded along with the response to the treatment. The treatment outcomes were categorized as success (defined as sum of cured and improved), failed and non-evaluable.
Results: Between Dec 2007 and Nov 2009, daptomycin was used in 40 evaluable patients. Daptomycin was used as second-line therapy or as concomitant antibiotic therapy for a range of serious infections. All the patients received treatment in the hospital. The average age of patients was 61.5 years and 87.5% were males.

Majority (60%) of the cohort were treated for cardiac infections (50% endocarditis, 5% pacemaker related infections and 5% aortic root abscess following cardiac surgery). Of the patients treated for infective endocarditis, 75% had left sided endocarditis of which 27% were infection of prosthetic valves. Daptomycin was used as monotherapy as well as in combination with rifampicin or fusidic acid.

Other indications of daptomycin usage were, soft tissue infections (22.5%), bacteraemia (7.5%), osteomyelitis (2.5%), pyelonephritis (2.5%), others(5%).

Confirmed infections with positive cultures were available in 67.5% of patients. *Staphylococcus aureus* was the most common isolated pathogen with only one case of MRSA. The dose of daptomycin ranged between 3.6 to 6.5 mg/kg. The most frequently used dose of daptomycin was 6 mg/kg (50% of patients).

Serious adverse events were reported in 5 patients (12.5%) and 2 patients (5%) discontinued the study drug due to side-effects. Details of use in patients with continuous veno-venous haemofiltration and creatine kinase monitoring to be presented.

The success rate with Daptomycin therapy was 65% (cured 25%, improved 40%), 10% patients failed to show any improvement while outcomes were non-evaluable in 25% of the patients.

Conclusions: Daptomycin was well tolerated in our cohort with overall success rate of 65% in treating a range of serious infections. It was predominantly used for cardiac patients and/or bacteraemia. Its been included in the local endocarditis management pathway. 32.5% use has been in empiric setting. Details to be presented.

Table 1. Infection types and outcomes

Infection type	Patients, n (%)			
	Cured	Improved	Failed	Non-evaluable
All	10 (25)	16 (40)	4 (10)	10 (25)
Endocarditis	8 (80)	5 (31)	1 (25)	6 (60)
Pacemaker infection	0 (0)	1 (6.25)	0 (0)	1 (10)
Aortic root abscess	1 (10)	1 (6.25)	0 (0)	0 (0)
Soft tissue infections	1 (10)	7 (44)	0 (0)	1 (10)
Bacteraemia	0 (0)	0 (0)	2 (50)	1 (10)
Osteomyelitis	0 (0)	1 (6.25)	0 (0)	0 (0)
Pyelonephritis	0 (0)	0 (0)	1 (25)	0 (0)
Others	0 (0)	1 (6.25)	0 (0)	1 (10)

P1234 Lack of serotonin syndrome among Veteran Affairs patients receiving linezolid

L. Tristani, N. Patel, B. Woo, A. Dihmess, H. VanDeWall, H. Li, R. Smith, T. Lodise (Albany, US)*

Objectives: Linezolid is a weak monoamine oxidase inhibitor and has the potential to cause SS, especially when administered with other serotonergic agents (SA). Limited data are available on the incidence of SS among patients receiving L. The objective of this study was to assess the incidence of serotonin syndrome among patients receiving L.

Methods: A retrospective, observational cohort study was conducted among all hospitalized patients who received L for ⩾48 h between 1/2005 and 8/2008 at the New York VA Health Care Network (VISN 2). Demographics, co-morbid conditions, laboratory values, microbiology, treatment data, concurrent SA, and clinical outcomes were collected from the patients' medical records. Patients that received L were evaluated for clinical symptoms of SS using the Sternbach and Hunter Serotonin Toxicity criteria. For SS and concomitant SA, pts were followed 35 d prior to start of L to 14 d after discontinuation of L.

Results: During the study period, 298 pts received L therapy and were included in the analysis. The median (IQR) duration of L therapy was 10 (6–16) days. The most common pathogens were *Staphylococcus* spp (45.0%) and *Enterococcus* spp (34.9%). Of the 298 pts, 287 (96.6%) were male with a mean (SD) age of 69.2 (12.3) years. The median number of comorbidities was 2 (IQR 1–4), and the mean (SD) APACHE-II score was 12.7 (6.5). Concomitant SA were used in 160 (53.7%) patients. The mean (SD) number of concomitant SA was 1.4 (0.6). Of the 160 pts receiving concurrent SA, 57 (35.6%) received an SSRI and 74 (46.3%) received fentanyl. None of the 298 VA patients met either the Sternbach or the Hunter Serotonin Toxicity criteria for SS.

Conclusion: In this VA population with high concomitant use of serotonergic agents, no cases of SS were observed while receiving L therapy.

P1235 Safety and effectiveness of daptomycin in patients with renal insufficiency not requiring renal replacement therapy

A. Gonzalez-Ruiz, K.C. Lamp, M.Y. Lin, R.L. Chaves (Kent, UK; Lexington, US; Basel, CH)*

Objectives: Daptomycin (DAP) is approved in the US and EU and other regions for the treatment of skin and soft tissue infections (SSTI), right-sided infective endocarditis (IE) and bacteraemia (BAC). However, the current approvals differ to some extent regarding pts with reduced renal function. There is still limited published experience with DAP in the treatment of patients with renal insufficiency. The objective of this analysis was to describe DAP use in pts with renal insufficiency.

Methods: US-CORE (USA) and EU-CORE (Europe and Argentina) are both retrospective multicenter observational registries. Pts enrolled from Jan 2007 to Dec 2007 in US-CORE and from Jan 2006 to Sep 2008 in EU-CORE were included. All pts with SSTI, IE or BAC and an initial estimated creatinine clearance (CrCl) were grouped into those with values <30, 30–<50, and ⩾50 ml/min. Pts receiving renal replacement therapy were excluded. Safety assessment: all adverse events noted from the first dose of DAP up to 30 days after the last dose of DAP were collected. Investigators assessed outcome (cure, improved, failure, nonevaluable) at the end of DAP therapy. Success was defined as cure or improved.

Results: A total of 1181 pts were identified; CrCl <30, n = 85 (7%); CrCl 30–<50, n = 195 (17%); CrCl ⩾50, n = 901 (76%). There were factors (for example, ICU, outpatient, concomitant antibiotics) that were different within the infection and renal groups that may have influenced the rates of possibly-related AE and success; the overall results are shown in the table.

Conclusion: In this group of pts, renal function was not associated with increased rates of adverse events possibly related to DAP. Additionally, no impact on the efficacy of DAP was observed. Future studies should control for differences in pt characteristics.

N (%)	CrCl (ml/min)		
	<30	30–<50	>50
SSTI (n = 627)	**(n = 35)**	**(n = 84)**	**(n = 508)**
Possibly-related AE	1 (3)	3 (4)	29 (6)
SSTI Success	27 (77)	71 (84)	434 (85)
SSTI Failure	2 (6)	2 (2)	26 (5)
SSTI Non-evaluable	6 (17)	11 (13)	48 (9)
BAC (n = 395)	**(n = 35)**	**(n = 74)**	**(n = 286)**
Possibly-related AE	1 (3)	5 (7)	24 (8)
BAC Success	28 (80)	58 (78)	208 (73)
BAC Failure	2 (6)	6 (8)	33 (12)
BAC Non-evaluable	5 (14)	10 (14)	45 (16)
IE (n = 159)	**(n = 15)**	**(n = 37)**	**(n = 107)**
Possibly-related AE	0	4 (11)	6 (6)
IE Success	11 (73)	26 (70)	88 (82)
IE Failure	1 (7)	5 (14)	5 (5)
IE Non-evaluable	3 (20)	6 (16)	14 (13)

$P > 0.05$ for possibly related AE or success by renal group within infection types.

New antimicrobials

P1236 Spectrum of activity of ceftaroline/NXL104 and β-lactam comparator agents tested against methicillin-resistant *Staphylococcus aureus* carrying different SCCmec types and Gram-negative bacilli with well-characterized resistance mechanisms

D. Farrell, R. Mendes, G. Williams, I. Critchley, R. Jones, H. Sader (North Liberty, Oakland, US)*

Objective: To determine the spectrum of activity and potency of ceftaroline combined with a fixed 4 mg/L concentration of NXL104 (CPT104) and comparator antimicrobial agents tested against selected molecularly characterized MRSA carrying different SCCmec types and well-characterized Gram-negative strains harbouring different β-lactamase (BL)-encoding genes. Ceftaroline is a novel parenteral broad-spectrum cephalosporin with activity against Gram-positive (including MRSA and MDRSP) and -negative organisms, Ceftaroline has limited activity against extended-spectrum β-lactamase (ESBL)- and AmpC-producing strains. NXL104 is a novel non-β-lactam BL inhibitor that inhibits Ambler class A, C, and D enzymes (eg, ESBL, KPC, and AmpC).
Methods: Susceptibility testing for all antimicrobials was performed by CLSI broth microdilution method (M07-A8, 2009) on a total of 250 strains categorized as follows: MRSA (100 MRSA subcategorized by SCCmec type [types I-IV and type IV subtypes]); E-ESBL (50 Enterobacteriaceae [ENT] with ESBLs); E-AMPC (49 ENT with Amp-C enzymes [28 chromosomal, 21 plasmidic]); E-CARB (31 ENT with carbapenemases [25 KPC, 5 OXA-48, 1 SME]); ACB (10 *Acinetobacter baumannii* [3 wild-type, 7 OXA-23, -24, and -58]); and PSA (10 *Pseudomonas aeruginosa* [2 wild-type, others include AmpC, MexX, OMP, and metallo-BL producers]).
Results: All MRSA strains were inhibited at ⩽2/4 mg/L of CPT104 and ceftaroline MIC was not affected by addition of NXL104. CPT104 was also very active against a wide variety of ENT with ESBL, chromosomal/plasmidic AmpC, and carbapenemases (highest MIC, 4 mg/L). Ceftaroline without NXL104 was not active against the vast majority of these selected ENT. Although CPT104 demonstrated activity against wild-type ACB and PSA, activity was low against BL-producing strains. Ceftazidime and piperacillin/tazobactam (P/T) were inactive against all six organism categories. Imipenem was active against E-ESBL and E-AMPC but had very limited or no activity against other categories.
Conclusions: CPT104 was very active against all MRSA regardless of SCCmec type or subtype and all ENT regardless of BL type, but had limited intrinsic activity against ACB and PSA expressing a variety of BLs. CPT104 had a wider spectrum of activity against these resistant Gram-positive and -negative categories than the four comparators. CPT104 represents a potential therapeutic option for empiric therapy in settings where MRSA and BL-positive ENT predominate.

Antimicrobial	MIC_{50}/MIC_{90} (mg/L)					
	MRSA	E-ESBL	E-AMPC	E-CARB	ACB	PSA
Ceftaroline	1/2	>64/>64	32/>64	>64/>64	>64/>64	>64/>64
CPT104[a]	1/2	0.06/0.25	0.12/0.5	0.5/2	16/>32	16/>32
Ceftazidime	>16/>16	>16/>16	>16/>16	>16/>16	16/>16	>16/>16
P/T	64/>64	32/>64	32/>64	>64/>64	>64/>64	64/>64
Imipenem	>8/>8	0.12/0.5	0.5/1	8/>8	>8/>8	8/>8

[a]Concentrations reported in the table for CPT104 refer to the concentration of ceftaroline.

P1237 *In vitro* and *in vivo* activity of novel non-quinolone agents targeting DNA gyrase and topoisomerase IV

M. Mitton-Fry, L. Brennan, S. Brickner, J. Casavant, D. Fisher, D. Girard, J. Hamel, J. Hardink, T. Hoang, M. Huband, S. McCurdy, M. Plotkin, G. Stone, R. Zaniewski, C. Zook (Groton, US)*

Objectives: DNA gyrase and topoisomerase IV are proven targets of high value for antibacterial therapy. We sought to develop a novel series of non-quinolone agents which would inhibit these targets and thus prove useful in the treatment of infections caused by Gram-positive organisms, especially methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: Novel analogs were synthesized and assessed using a number of different techniques. Inhibitory activity against both DNA gyrase and topoisomerase IV from *S. aureus* was assessed *in vitro*. MIC90 studies using isolates of both *S. aureus* and *Streptococcus pyogenes* were also conducted. Analogs with appropriate *in vitro* potency and suitable pharmacokinetic properties were subsequently evaluated in both murine systemic and thigh infections caused by *S. aureus*. Finally, spontaneous resistance studies were also conducted in *S. aureus*. Target mutations and the resulting elevations in MICs for these mutants were evaluated.
Results: A novel series of analogs was prepared with excellent inhibition of DNA gyrase (typical IC50 = 1 uM) and a range of inhibition of the secondary target, topoisomerase IV (IC50 = 5–32 uM for selected analogs). MIC90 values as low as 0.125 ug/mL against *S. aureus* and 0.25 ug/mL against *S. pyogenes* were obtained. The compounds displayed promising *in vivo* activity, with PD50 values of ca. 10 mg/kg in a murine septicemia model. Doses required for achieving stasis in the murine thigh infection model were also favorable (ca. 100 mg/kg). Mutant *S. aureus* isolates selected under drug pressure typically expressed one of two key mutations in gyrA, either D83N or M121K. Improved MICs against these mutant organisms correlated well with superior inhibition of the secondary target, topoisomerase IV.
Conclusions: We have discovered a novel series of agents targeting DNA gyrase and topoisomerase IV. These compounds have been shown to display excellent activity both *in vitro* and *in vivo* against key causative pathogens for serious Gram-positive infections.

P1238 Activity of the investigational fluoroquinolone, finafloxacin and seven other antimicrobial agents against 114 obligately anaerobic bacteria

R. Schaumann, G.H. Genzel, W. Stubbings, C.S. Stingu, H. Labischinski, A.C. Rodloff (Leipzig, Berlin, DE)*

Objectives: Finafloxacin (FIN) is a novel fluoroquinolone antimicrobial belonging to a 8-cyano subclass and exhibits enhanced activity at slightly acidic pH. FIN is active against a wide range of Gram-positive and Gram-negative aerobes and anaerobes. With the present study, the activity of FIN was tested against 114 recently strains of obligately anaerobic bacteria including reference strains and compared with various other antimicrobials including investigations under acidic conditions.
Methods: The activity of FIN against 73 strains of the *Bacteroides fragilis* group, 10 other obligately Gram-negative rods and 31 *Clostridium difficile* strains was tested and compared with the activity of moxifloxacin (MOX), levofloxacin (LEV), ciprofloxacin (CIP), clindamycin (CLI), imipenem (IMP), piperacillin/tazobactam (PIT) and metronidazole (MET). MICs were determined employing the microdilution technique in Wilkens-Chalgren broth supplemented with vitamin K1 and haemin. Furthermore, the MICs of FIN against 11 selected *B. fragilis* strains, the 10 other Gram-negative rods and 10 selected *C. difficile* strains were tested under acidic conditions.
Results: The MIC50 and MIC90 values (μg/ml) of the 114 strains tested are listed in the Table.

Antimicrobial agent	*B. fragilis* group (n = 73)		Other obligately Gram-negative rods (n = 10)		*C. difficile* (n = 31)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
FIN	0.5	2	0.06	0.25	4	16
MOX	1	2	0.5	1	8	32
LEV	2	8	0.5	2	32	>64
CIP	8	16	1	16	16	⩾64
CLI	1	8	⩽0.03	⩾64	8	⩾64
IMP	0.25	0.5	⩽0.03	0.06	2	4
PIT	0.5	4	0.06	0.06	0.5	2
MET	0.5	1	0.25	1	0.125	1

Under acidic conditions FIN has an increased activity against the selected obligately anaerobes compared to the activity at a neutral pH (data not shown).

Conclusions: FIN has promising activity against several pathogenic species of the *B. fragilis* group and other Gram-negative rods and is slightly more active than MOX against the obligately anaerobic bacteria tested here. Under acidic conditions FIN is more active against obligately anaerobes compared to the activity under neutral conditions.

P1239 Assessment of oritavancin serum protein binding across species

F. Arhin, A. Belley, I. Sarmiento, G.A. McKay, T. Parr Jr., G. Moeck (Ville Saint Laurent, CA)*

Objective: Estimates of serum protein binding are essential to translate drug exposure from nonclinical studies to humans during assessments of toxicology, pharmacokinetics, and pharmacodynamics. Oritavancin is a late-stage investigational lipoglycopeptide under study for treatment of serious Gram-positive infections. Nonspecific binding of oritavancin to plastic, glass, and filtration/dialysis membrane surfaces confounds traditional biophysical methods to assess protein binding. We estimated binding of oritavancin to human, mouse, rat and beagle dog serum from serum-induced increases in minimal inhibitory concentrations (MICs) using arithmetic drug dilutions under conditions that minimize nonspecific binding to labware surfaces.

Methods: Serum ultrafiltrate from each species was prepared using Centricon Plus-50 ultrafilters, whose molecular weight cutoff (50 kDa) excludes serum albumin. Oritavancin, ceftriaxone and daptomycin MICs against *Staphylococcus aureus* ATCC 29213 were determined by broth microdilution using arithmetic drug dilutions in 95% serum and 95% serum ultrafiltrate (supplemented with 5% cation-adjusted Mueller-Hinton broth) in parallel to assess the impact of serum albumin on their antimicrobial activity. Serum protein binding for each drug was calculated as follows: % bound = (1 – [MIC in serum ultrafiltrate/ MIC in serum]) × 100%.

Results: Increases in arithmetic oritavancin MIC in serum vs. serum ultrafiltrate, by species, were similar across species (range, 5.5- to 7.8-fold). Such shifts yielded mean values of oritavancin serum binding that were similar for the four species tested (range, 81.9% to 87.1%). Daptomycin binding to serum protein was more variable across species, ranging from 65.6% to 82.9%. Ceftriaxone was highly bound to human serum (92.6%) but substantially less bound to serum from mouse, rat, and beagle dog (range, 20.9% to 37.5%).

Conclusions: The broth microdilution-based method described here allowed for estimation of oritavancin serum protein binding with minimal drug losses to labware vessels. This method would likely be suitable for other drugs exhibiting nonspecific binding to labware surfaces. Oritavancin binding to serum was consistent across the species tested, with serum protein binding values reported here approximating those reported previously for human serum. These values will support translation of oritavancin exposure from nonclinical studies to humans.

Agent	Human serum		Mouse serum		Rat serum		Beagle serum	
	fold MIC increase*	% bound	fold MIC increase*	% bound	fold MIC increase*	% bound	fold MIC increase*	% bound
Oritavancin	5.5	81.9	6.8	85.3	5.7	82.4	7.8	87.1
Ceftriaxone	13.5	92.6	1.6	37.5	1.5	34.0	1.3	20.9
Daptomycin	5.8	82.9	4.2	76.0	2.9	65.6	3.9	74.5

*The fold MIC increase is the ratio of the mean arithmetic MIC in 95% serum to the mean arithmetic MIC in 95% serum ultrafiltrate.

P1240 Efficacy of BAL30072, alone and combined with meropenem, against VIM-producing enterobacteria in a murine thigh infection model

V. Miriagou, C. Papagiannitsis, S. Kotsakis, A. Zioga, E. Siatravani, A. Loli, E. Tzelepi, L. Tzouvelekis, M. Donzelli, J. Spickermann, M. Page (Athens, GR; Basel, CH)*

Objectives: BAL30072 is a siderophore sulfactam active *in vitro* against Gram-negative bacteria. The *in vivo* efficacy of BAL30072 alone and combined with meropenem, against *Klebsiella pneumoniae* (K.pn.) and *Escherichia coli* (E.co.) strains producing VIM-1 metallo-β-lactamase was evaluated in a murine thigh infection model.

Methods: 3 K.pn. producing VIM-1 [V-KP], 3 K.pn. producing VIM-1+SHV-5 [V+S-KP]), 1 E.co. producing VIM-1+CMY-2 [V+C-EC] and 2 sensitive isolates were used. MIC and MBC of BAL30072 (BAL), meropenem (MEM) and a 1:1 combination (BAL:MEM) were determined by broth microdilution with inocula of 5×10^5 and 10^7 CFU/mL. Neutropenic female CD-1 mice were infected with 10^6 bacteria. BAL (30 and 60 mg/kg), BAL:MEM (30:30 and 60:60 mg/kg) or saline were administered i.p. every 2 h for 24 h. Mice were sacrificed at 0, 6 and 24 h and log10 [CFU/thigh] was determined. Pharmacokinetic parameters were determined in blood samples taken at intervals after i.p. injection of a single dose of BAL (30 or 60 mg/kg).

Results: BAL was active *in vitro* against V-KP (MIC⩽0.06, MBC⩽0.06) and V+C-EC (MIC⩽0.5, MBC⩽0.5) isolates, whereas the V+S-KP isolates were highly resistant to this compound (MIC⩽16–256, MBC⩽32–1024). All K.pn. isolates (MIC⩽16–128, MBC⩽16–128) and the V+C-EC isolate (MIC⩽8, MBC⩽8) were resistant to meropenem. Reduction of the MICs ⩾16 fold were observed when BAL:MEM was tested.

The Cmax values were 16.8 and 39.3 mg/L for BAL30 and BAL60 dosing schemes, respectively. Peaks were observed at 10min after i.p. injection, followed by a fast elimination (half-time of 12min). AUC were determined as 8.5 and 21.6 mg.h/L.

In the *in vivo* experiments, the mean log10 [CFU/thigh] reductions at 24 h for BAL30/BAL60 regimes were 0.93/1.6 for V-KP, 0/0 for V+S-KP and 1.03/2.76 for V+C-EC infected animals. BAL:MEM combinations exhibited higher efficacies resulting in substantial log10 [CFU/thigh] reductions at 24 h (means, respectively for the 30:30 mg/kg and 60:60 mg/kg regimes, of 4.1/4.2 for V-KP, 3.8/4.6 for V+S-KP and 6.7/7.8 for V+C-EC infected animals).

Conclusion: BAL was effective against enterobacterial isolates producing VIM-1 metallo-β-lactamase, but not SHV-5, in the murine thigh infection model. Co-administration with MEM enhanced the activity against all isolates, even those producing multiple enzymes which were resistant to BAL or MEM alone. Further *in vivo* studies are required to fully assess the therapeutic potential of BAL30072 and this combination.

P1241 The role of iron transport in the activity of the siderophore sulfactam BAL30072 against *Pseudomonas aeruginosa*

M. Page, C. Müller, B. Hofer, E. Desarbre, J. Dreier, F. Vidal (Basel, CH)*

Objective: BAL30072 (BAL) is a novel monocyclic β-lactam that is active against many aerobic Gram-negative bacteria, including multidrug-resistant *P. aeruginosa* (MDR-PA). BAL30072 has a siderophore side chain that could enhance its activity, especially against strains with restricted influx due to porin mutations. The role of iron and of siderophore receptors in the activity of BAL30072 has been studied in MDR-PA.

Methods: 35 clinical MDR-PA isolates, with a variety of resistance mechanisms, were investigated. MICs of BAL and comparators were determined by broth microdilution using cation-adjusted Müller-Hinton broth (MH) and Iso-Sensitest broth (IS)as provided by the manufacturer or supplemented with either 16 mg/L 2,2′ bipyridyl (BPL, to induce siderophore systems) or with 0.01 mM ammonium ferric citrate (AFC, to repress siderophore systems). Production of the siderophore pyoverdine was determined spectrophotometrically. Mutants were selected by serial passage in the presence of increasing concentrations of BAL30072 in IS broth. PCR primers specific for the major siderophore receptors (fpvA, fpvB, fptA, pfeA, pirA) were designed using the published sequences. Expression levels of the receptors were determined by quantitative RT-PCR.

Results: The BAL MICs for individual strains differed between MH and IS by 0–3 doubling dilution steps. Production of pyoverdine by individual strains differed between MH and IS 2–9 fold. There was little correlation between pyoverdine production and BAL MIC. Adding BPL

increased pyoverdine levels in most cultures (up to 10-fold increase) and adding AFC stopped its production in all cases. There was again no correlation between pyoverdine levels and BAL MIC. Individual strains showed increased MIC in the induced state while others had the expected decreased MIC, compared to the repressed state. There was no correlation between BAL MIC and the presence, or expression level, of any one of the major siderophore receptors. None of the mutants selected for resistance to BAL had a phenotype characteristic of a tonB defect or deficiency in iron-transport.
Conclusions: BAL does not require any one of the major siderophore receptors for its activity against MDR-PA, nor does it appear to depend on TonB-mediated uptake. It is possible that BAL is a weak substrate for several systems and can therefore exploit any one of these that is available. This contrasts with the behaviour of earlier siderophore β-lactams.

P1242 The antibacterial activity of MUT056399, a specific FabI inhibitor against staphylococci

S. Escaich, C. Soulama, F. Moreau, V. Gerusz, V. Vongsouthi (Paris, Romainville, FR)*

Background: MUT056399 was designed and optimized as a specific inhibitor of *S. aureus* FabI enzyme. Its spectrum of activity was investigated against a panel of 658 bacterial strains and fungi.
Methods: Strains used were methicillin-susceptible and non susceptible *S. aureus* (MSSA and MRSA), VISA and Linezolid-resistant isolates. Standard procedures were used for the MIC and time-kill experiments. Linezolid and Vancomycin were used as comparators.
Results: MUT056399 was highly active against 129 *S. aureus* isolates including MSSA, MRSA and CA-MRSA (MIC50/90:$\leqslant$0.03/0.12μg/ml). MUT056399 was also very potent against coagulase negative staphylococci (165 isolates Methicillin susceptible or resistant) with MIC50/90 =0.06/2μg/ml. MUT056399 had no activity against enterococci and streptococci. *A. baumannii* was susceptible, some isolates showed high MICs.
In Gram negative bacteria *H. influenzae* and Neisseria spp. were very susceptible to MUT056399 (MIC90 = 0.03 and 0.25μg/ml). The Enterobacteriaceae showed variable susceptibilities with high activity on *E. coli* and *P. mirabilis* (MIC50 = 0.5–1); *C. freundii; M. morganii* & *S. marcescens* being non-susceptible. *P. aeruginosa* was not inhibited by MUT056399. The intracellular pathogens *C. pneumoniae* was susceptible to MUT056399, but the atypical pathogen *M. pneumoniae* and *M. fortuitum* were not inhibited by MUT056399. *L. pneumophila* was susceptible with MIC90 = 0.5. Finally, MUT056399 was inactive against anaerobes and yeasts.
MUT056399 demonstrated an *in vitro* bacteriostatic activity at 4 times the MIC against MSSA, MRSA, Linezolid resistant, and VISA (mean reduction of 2 log10 cfu/ml at 24 hours). Spontaneous resistance frequency was low in 6 strains of *S. aureus* ($2.5–7\times10^{-9}$ at 4×MIC). *In vitro* resistant clones were isolated, and point mutations in the active site of the Fab I enzyme were linked to MUT056399 resistance.
Conclusions: The novel FabI inhibitor MUT056399 has a specific spectrum of activity for bacteria which are strictly dependant on the FabI enzyme for fatty acid synthesis. It is very potent against *S. aureus*, a range of resistant isolates and coagulase negative staphylococci. These properties support MUT056399 as a very promising new chemical entity to treat specifically the staphylococcal infections.

P1243 Action of novel copper compounds on the viability of *Helicobacter pylori*

F. Perna, T. Hall, S. Hickok, V. Gant, D. Vaira, J. Holton (Bologna, IT; London, UK)*

Objectives: Owing to the increasing prevalence of antibiotic resistance in *H. pylori* and the falling eradication rate, novel antimicrobial agents are being actively sought. One novel biocide is a series of highly reductive copper complexes that have broad antimicrobial activity. These compounds have no effect upon eukaryotic cell viability until an excess of 100ppm. Copper is also recognized as angiogenic and may have healing effects on ulcers. Therefore we have investigated the action of two of these compounds on the viability of *Helicobacter pylori*.
Methods: Cag A positive (NCTC11637); CagA negative (NCTC 12908 and ACTC J99) and 53 clinical isolates were tested. For 5 of the isolates a kill curve was performed with an inoculum of 107–8 cfu/ml in sterile water at differing concentrations (0.5, 1.0, 5.0, and 12ppm) of two copper compounds CuAL42 and CuPC33 for 15, 30, 60 and 120 minutes. At each time point samples were withdrawn, decimal diluted into $^1/_4$ strength Ringers lactate, plated and incubated. Subsequently all the isolates were tested at the same inoculum against 12 ppm for 60 and 120 mins. The plates were incubated for 5 days at 370 C in an atmosphere generated by CampyGen (Oxoid UK).
Results: CUAL42 was more active than CUPC33. At 5ppm the viable count was reduced by 5–6 logs at 2hrs whilst for CUAL42 at 1 hour and 2 hours respectively 20 and 40 isolates were completely killed compared to CUPC33, where 16 and 34 isolates were killed at 1 and 2 hours exposure. Neither Cag A status nor antibiotic sensitivity bore any relationship to the efficacy of the copper compounds.
Conclusions: These novel copper compounds deserve further study in relation to eradication of *Helicobacter pylori*

P1244 Global screening of therapeutic bacteriophage preparations against clinical isolates of *Pseudomonas aeruginosa*

S. Morales, G. Mearns, A. Smithyman (Sydney, AU)*

The ongoing rise in antibiotic resistance has prompted renewed interest in bacteriophage (phage) therapy as an alternative or complimentary means of treating bacterial infection. Phage therapy refers to the targeted use of lytic phages to destroy pathogenic bacteria. By their nature phages are generally highly specific, with a spectrum of reactivity from the genus to sub-isolate level.
This gives phage therapy a major advantage over antibiotics as normal bacterial flora are unaffected by treatment. However such specificity can also be a disadvantage in that a phage preparation developed in one country against the dominant isolates of a particular bacterial pathogen may prove too specific to that region and thus ineffective in a different geographical location.
Objectives: To investigate the reactivity of therapeutic bacteriophages developed in Australia against clinical strains of *P. aeruginosa* in four geographical locations, namely Australia, the United Kingdom, United States and Central America.
Methods: Lytic phages were isolated from environmental sources and screened against a representative panels of 100 *P. aeruginosa* clinical isolates collected in Australia. Combinations of these phages were then tested for their specific and broad activity against 550 isolates in four separate geographical regions.
Results: Therapeutic phages showed a very broad host range infecting over 85% of the *P. aeruginosa* isolates tested.
Conclusion: Lytic phages with broad specificity for *P. aeruginosa* were combined into phage cocktails and tested *in vitro* against a wide range of clinical targets from four geographical areas. The phage cocktails reacted with over 85% against the isolates tested, with only minor variations between the geographical areas screened. This study showed that the specificity of bacteriophages can be addressed during the development of therapeutic products to ensure a wide coverage of the targeted bacteria around the world.

P1245 *In vitro* cytotoxicity testing of bacteriophages against *Acinetobacter* spp.

A.E. Henein, G.W. Hanlon (Brighton, UK)*

Objectives: *Acinetobacter* spp. have emerged as a major clinical challenge, due to multiple and pan-drug resistance, particularly in high-risk patients, including those with severe burns. Bacteriophages have shown promise in applications as systemic and topical antibacterials, as well as

FDA-approved food decontaminants. No previous comprehensive studies have been published investigating cytotoxicity and cell proliferation of purified phage suspensions on (human) dermal cells *in vitro*.

Methods and Results: A 3T3 mouse fibroblast cell line, human dermal fibroblasts (HDF) and keratinocytes (HDK) with 3T3 feeder layers were exposed to dilutions of purified phage. The cytotoxic impact and effect on cell proliferation were measured using a range of assays. No statistically significant difference could be detected between controls and phage with the Trypan blue method (3T3 cells only). 3T3 cell Hoechst Propidium Iodide stain experiments remained inconclusive; There was no statistically significant difference between concentrated phage samples and controls at any time points. After 72 hour incubation, controls gave rise to significantly higher cell counts than dilute phage preparations ($p < 0.001$). Lactate dehydrogenase (LDH) and MTS tetrazolium compound reduction results showed no evidence of statistically significant cytotoxic effect of phage on 3T3 cells, HDF or HDK. Some data suggested phage may have a beneficial effect on cell survival of HDK ($p < 0.001$) and proliferation of HDF ($p < 0.001$) and 3T3 cells ($p < 0.05$) compared to controls. Colony formation assays utilising a V79 hamster cell line were used to indicate the cytotoxicity of purified phage in different diluents. There was no statistically significant difference in terms of colony numbers or colony size (where measured) between phage samples and controls.

Conclusion: Bacteriophages lytic against *Acinetobacter* spp. had no cytotoxic effects on a range of cultured skin cells but may even exert a positive effect on cell proliferation. With the FDA having granted GRAS status to *Listeria* bacteriophages in food decontamination, the next logical step would be to consider concentrated phage suspensions as wound decontaminants and antibacterials in multiple-resistant *Acinetobacter* spp. infections, particularly in burns patients.

P1246 Antibacterial activity of supercritical *Usnea barbata* extract against staphylococci, streptococci and enterococci originated from animals

D. Misic, J. Ivanovic, I. Zizovic (Belgrade, RS)*

Objectives: Studies of antimicrobial activity of extracts obtained by the process of supercritical fluid extraction (SFE) are mainly performed on foodborne pathogens because of potential use of supercritical extracts as food additives. Due to the lack of new antibiotics on the market, studies of antibacterial activities of supercritical extracts on pathogenic bacteria are more present nowadays, with the objective to treat humans and animals in cases of infections. It is well known that plant extracts obtained by different technologycal processes have good antimicrobial activity, but mostly, they contain traces of organic solvents so they are unsuitable for pharmaceutical and nutritional use. Plant extracts obtained by SFE do not contain any solvents.

Methods: In this investigation, lichen *Usnea barbata* was used for the SFE. Supercritical extraction with CO2 as a slovent was performed in the Autoclave Engineers Screening System at pressure of 30 MPa and temperature of 60ºC. For the determination of MIC values of supercritical usnea extract, broth microdillution method (CLSI, 2008) was used, and the extract was previously dissolved in 1–2 propanediol. For comparison of the results, erythromycin was included too. The investigation has been performed on 14 *Staphylococcus*, *Streptococcus*, and *Enterococcus* strains of animal origin including VRE, MRSA and ATCC strains.

Results: Determined MIC values of the supercritical usnea extract for all strains ranged from $\leqslant$1.25 to 80 mcg/mL and MIC values of erythromycin ranged from 0.06 to >32 mcg/mL. The strongest antibacterial activity with MIC value of $\leqslant$1.25 mcg/mL usnea extract showed on two *S. intermedius* strains isolated from dogs' skin swabs. The weakest antibacterial activity with MIC value of 80 mcg/mL supercritical extract showed on *S. aureus* ATCC 11632 and on 1 MRSA strain isolated from dog's wound swab. Investigated supercritical extract showed good antibacterial activity against streptococci and enterococci including VRE with obtained MIC values of 10 to 40 mcg/mL. One methicillin-resistant *S. haemolyticus* strain isolated from ear swab of cat and 1 *S. uberis* strain originated in guinea pig were resistant to erythromycin with MIC value >32 mcg/mL but MIC values of supercritical extract for these two strains were significantly lower, 10 to 20 mcg/mL.

Conclusion: According to obtained low MIC values, investigated supercritical usnea extract showed very strong antibacterial activity against investigated bacteria.

P1247 The potential of a naturally-produced inhibitory substance (E226), which is produced by *Staphylococcus epidermidis* strain E226 and shows activity against epidemic MRSA-15

M. Al-Mahrous, J. Tagg, M. Upton (Manchester, UK; Dunedin, NZ)*

Objectives: In recent years, there has been much focus on a promising class of bacteriocins known as lantibiotics. The most prominent representative of lantibiotics, nisin, has already a long history of use in the protection of foodstuffs. Lantibiotics have also been considered for application in humans; however, they have not yet been used in the setting of chemotherapy on the same scale as traditional antibiotics.

We here investigate the cationic peptide antibiotic (E226) as a future opportunity for treating staphylococcal infections.

Methods:

1. Investigation of *S. epidermidis* strain E226 was performed using simultaneous and deferred-antagonism against MRSA
2. Purification and/or concentration of free inhibitors in broth supernatants was carried out using ammonium sulphate precipitation, Sep-Pak® cartridge, Speed-Vac®, Cation-exchange; then C18 reverse-phase chromatography.
3. MALDI TOF/TOF was used for mass analysis
4. Electron microscopy was used for ultra-structure diagnosis of a range of MRSA indicators
5. Biological activities were tested using spot-on-loan assay

Results: The biological activity of E226 is heat-stable and displaying specificity for the closely-related *S. aureus*.

The high ammonium sulphate saturation ($\geqslant$80%) needed for precipitating E226 suggests its small mass. The cationic-exchange chromatography (pH 5.2) and the late elution from C18 column suggest the cationic nature of E226. MALDI TOF/TOF showed 3 species and sized the mass in a window between 2000 and 2800Da (Figure 1). This suggests further purification using high resolution HPLC to eliminate, if any, unrelated species.

The Electron microscopy diagnosis reveals a clear damage in the cell wall.

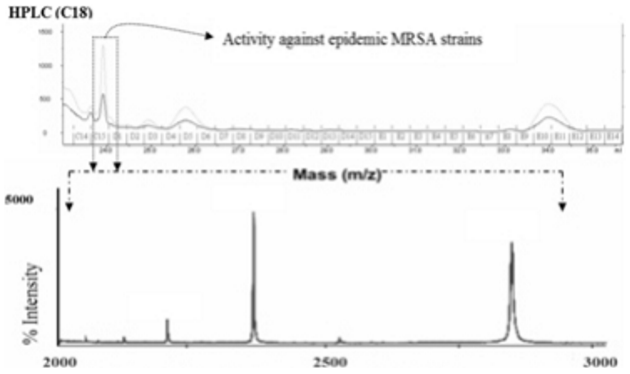


Figure 1. [A] Isolation of the active fraction using HPLC; [B] Detection of peptide species of E226 using mass spectrometry. Resulting amino acids were detected on a time-of-flight (TOF) mass spectrometer with matrix-assisted-laser-desorption ionization (MALDI).

Conclusions:

1. The biological activity of the highly-purified extract of the heat-stable small mass inhibitory agent E226, which shows specific inhibitory activity against Epidemic MRSA-15 and strains of MSSA, suggests its nature as a bacteriocin, possibly of Class-I.
2. The cationic-exchange separation and late elution of E226 from C18 reverse-phase column suggest that it is a hydrophobic in nature.

3. Based on the electron microscopy diagnosis, E226 shows obvious damage to the protective cell wall of the sensitive indicators. This postulates the binding of the hydrophobic E226 to the negatively-charged lipid-II in the cell-membrane resulting in its lysis.
4. E226 could have potential as topical therapeutic agents for treating highly drug-resistant staphylococcal infections.

P1248 Lethal photosensitization of *Enterococcus* using Fotolon®, a chlorine e6 derivative

E. Dworniczek, M. Trendel, M. Wawrzynska, A. Seniuk, W. Strek, B. Kowalska-Krochmal (Wroclaw, PL)*

Objective: The growing resistance of enterococci to conventional antimicrobial agents necessitates the development of alternative approaches to treating infections. Photodynamic inactivation (PDI) may be effective therapeutic option in which the non-toxic photosensitizers (PS) in combination with activating light, generate cytotoxic species. This investigations aimed to determine PDI efficiency using Fotolon® against *Enterococcus* strains.
Methods: PDI employed Fotolon®, (Belmedpreparaty, Belarus) and 662 nm diode laser light, against 9 clinical strains (4 *E. faecalis,* 4 *E. faecium,* 1 *E. casseliflavus)*, and 1 reference strain *(E. faecalis* ATCC 29212). The antibacterial spectrum of the photosensitizer was evaluated by zone of inhibiting testing. The minimal inhibitory concentration of PS was determined using the broth dilution method. For evaluation of phototoxic properties of Fotolon strains were incubated with different concentrations of the medication and exposed to fluencies of 662 nm laser light (5, 10, 20, 40 J/cm2). The viability of bacteria was assessed by a colony forming assay. Visualization of the bacterial structure pre- and post photodynamic inactivation was performed in transmission electron microscope (SEM).
Results: All strains of *Enterococcus* were susceptible to killing by PDI. The bactericidal effect depended on the concentration of Fotolon and light dose. Fotolon in concentration of 40 μg/mL activated by laser light 40 J/cm2 was the most effective and reduced >50% of bacterial populations tested. However, for some strains the best results in reduction of viable cells were obtained for lower concentration of Fotolon. The laser light alone did not affect the bacteria significantly. Maximal light dose (40 J/cm2) reduced <5% of enterococcal populations. Fotolon and light influenced the morphology of bacterial colonies. Different size colonies were observed after treatment with activated photosensitizer. SEM analysis revealed destructive changes in the cell structure. *E. faecalis* and *E. faecium* showed internal, electron lucent areas, not seen in control cells, which may indicate chromosomal alteration and condensation of DNA.
Conclusion: Photodynamic inactivation may be a useful approach in the treatment of enterococcal infections. These results showed that PDI with Fotolon acts lethally on different species of *Enterococcus*. However, the effectiveness of inactivation was dependent on the particular strain.

P1249 Ototoxic potential of ACHN-490 compared to gentamicin and amikacin in the guinea pig

C.F. Kostrub, D.F. Dolan, R.A. Altschuler, T.J. Baird, R.L. Tapp, J.A. Boggs, J.B. Bruss (San Francisco, Ann Arbor, Mattawan, US)*

Objectives: ACHN-490 is a "neoglycoside", a next-generation aminoglycoside (AG), currently being advanced through clinical development by Achaogen, Inc. ACHN-490 shows broad-spectrum bactericidal activity *in vitro*, and its potency is unaffected by most types of AG-modifying enzymes that confer resistance to AGs. In the present studies, the ototoxic potential of ACHN-490 relative to the AG comparators gentamicin and amikacin was investigated over 28 days in guinea pigs. The relative ototoxicity of shorter duration gentamicin treatment (1, 3, or 5 days) was also studied.
Methods: In the guinea pig model, compounds were administered once-daily for 14 days, and both structural and functional evaluations for ototoxicity were employed. Auditory function was quantified by comparing pre-dose and terminal auditory brainstem response (ABR) values at each of 3 stimulus frequencies (4, 10, and 20 kHz). In addition, cochleas were removed and examined histologically for evidence of hair cell damage. Short-term dosing was investigated by dosing separate cohorts of animals for 1, 3, or 5 days, and assessing ABRs both 1 day after the last dose for each cohort and again 28 days after the initial dose for all cohorts.
Results: Gentamicin at 80 or 100 mg/kg/day for 14 days produced substantial hearing loss. Amikacin, with less antibacterial potency but roughly an equivalent therapeutic index in comparison to gentamicin, was associated with profound hearing loss at a therapeutically comparable dose of 300 mg/kg/day. In both cases, histological analyses showed cochlear hair cell loss consistent with the functional ABR shift. By contrast, ACHN-490 demonstrated no significant effects on ABR thresholds up to a dose of 80 mg/kg/day, with no hair cell loss, as determined by cytocochleogram analyses. Gentamicin administration (80 mg/kg/day) for 1, 3, or 5 days did not result in significant functional ABR shift or histopathological evidence of ototoxicity.
Conclusions: The absence of ototoxicity observed for ACHN-490 in this 28-day guinea pig model suggests a lower ototoxic potential for ACHN-490 compared to gentamicin. The absence of hearing loss with shorter courses of gentamicin may support the hypothesis that the risk of AG-induced ototoxicity could be mitigated by avoiding extended treatment regimens. Taken together, these studies provide rationale for the continued clinical investigation of ACHN-490 in high-dose, short-course antimicrobial therapy.

P1250 Imidazolines as non-classical bioisosteres of N-acyl homoserine lactones and Quorum sensing inhibitors

A. Bucio-Cano, M. Montenegro-Sustaita, E. Curiel-Quesada, A. Reyes-Arellano, G. Aparicio-Ozores (Mexico City, MX)*

Objectives: Synthesis of six non classical N-acyl homoserin lactone bioisosters and evaluation of their inhibitory effects on quorum sensing in *Chromobacterium violaceum* and *Serratia marcescens*.
Methods and Results: Imidazolyl ethers were synthesized by alkylation of 4-hydroxibenzadehyde followed by cyclization with ethylenediamine. Imidazolyl amides were synthesized by reaction of 4-amino benzonitrile with organic acids followed by cyclization with ethylenediamine. Products were characterized by NMR and EM. Structures of synthesized bioisosters are shown in Figure 1.

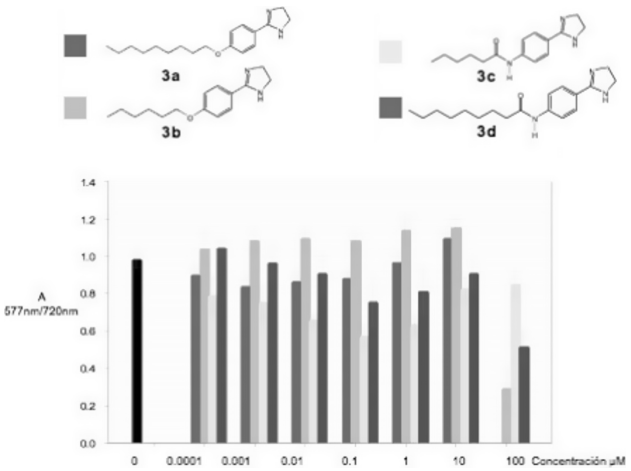


Figure 1. **3a**: R = O–CH_2–$(CH_2)_4$–CH_3; **3b**: R = O–CH_2–$(CH_2)_7$–CH_3; **3c**: R = NH–CO–CH_2–$(CH_2)_3$–CH_3; **3d**: NH–CO–CH_2–$(CH_2)_6$–CH_3.

In order to study their effect on QS, test bioisoster was added to bacterial cultures at final 100 pM to 1 mM concentrations and cultures were incubated for 15 h. Growth was monitored at OD720 while violacein production was measured at 577nm. For prodigiosin synthesis, *S. marcescens* was incubated for 48 h and pigment was recorded at 540 nm.

Figure 1 shows specific violacein production in the presence of four of the bioisosters assayed.

Molecular epidemiology of ESBL

P1251 Emergence of ESBL-producing *Klebsiella pneumoniae* in Danish hospitals: this is in part explained by spread of one CTX-M-15 clone in Zealand

C.H. Lester, S.S. Olsen, M. Arpi, K. Fuursted, D.S. Hansen, O. Heltberg, A. Holm, T. Højbjerg, K. Truberg Jensen, H. Krogh Johansen, U. Stenz Justesen, M. Kemp, J. Dahl Knudsen, B. Røder, N. Frimodt-Møller, A.M. Hammerum (Copenhagen, Herlev, Aarhus, Hillerød, Næstved, Odense, Aalborg, Esbjerg, Vejle, Hvidovre, Slagelse, DK)*

Objectives: The spread of resistance to third-generation cephalosporins in *Klebsiella pneumoniae* is a continuing cause of public health concern. Before 2007 the occurrence of third-generation cephalosporin resistance was low among *K. pneumoniae* isolated from bloodstream infections in Danish patients. However, the rate of resistance has increased from 4.4% in 2006 to 10.8% in 2008 (EARSS). The aim of the present study was to investigate the spread of third-generation cephalosporin resistant *K. pneumoniae* in Danish hospitals and to do a molecular characterisation of the collected isolates.
Methods: All Danish Departments of Clinical Microbiology were asked to send third-generation cephalosporin resistant *K. pneumoniae* isolated from blood in 2008 to Statens Serum Institut, Denmark. ESBL and/or AmpC phenotypes were tested using the NeoSensitabs double disk method (Rosco Diagnostica, Tåstrup, Denmark). Based on the obtained phenotypes, PCR amplification and sequencing was performed with primers specific to identify blaTEM, blaCTX_M, blaOXA, blaSHV and ampC β-lactamase genes. All ESBL and ampC positive isolates were PFGE typed. The PFGE patterns were compared using Bionumerics 6.01 (Applied Maths, Sint-Martens-Latem, Belgium).
Results: In total, 80 isolates were received: 77 isolates were ESBL positive, 2 isolates were ampC positive and 1 isolate was both ampC and ESBL positive. Among the ESBL positive isolates 47% (n = 37) belonged to the same PFGE cluster and had the genetic profile: CTX-M-15, SHV-28. The isolates in this cluster originated from eight hospitals all located in Zealand. Another cluster included nine isolates (12%) with the genetic profile CTX-M-15, SHV-1. The isolates in this cluster were all from hospitals in Copenhagen. The three ampC positive isolates were positive for the DHA-1 gene and PFGE showed that they were possibly related (>85% similarity). Among the 78 ESBL positive isolates, CTX-M-15 was detected in 64 isolates (82%); CTX-M-14 was detected in five isolates.
Conclusion: Emergence of third-generation cephalosporin resistance among *K. pneumoniae* in Denmark is mostly due to ESBL-producing isolates; 82% of the isolates contain CTX-M-15. Many of the isolates were clonally related (47%); these isolates were recovered from one region of the country (Zealand) indicating clonal spread within the hospitals in this region.

P1252 Molecular epidemiology and genetic background of extended-spectrum β-lactamase producing *Escherichia coli* isolated from human clinical samples in Hungary, 2006–2007

I. Damjanova, Á. Tóth, T. Mag, S. Tóth, G. Hajbel-Vékony, J. Pászti, M. Füzi (Budapest, HU)*

Objectives: There is limited information on dissemination of ESBL-producing *E. coli* in Hungary, thus study was conducted for characterizing representative collection of isolates from human clinical samples.
Materials and Methods: During 2006–2007 113 ESBL-producing *E. coli* clinical isolates were submitted to the National Center for Epidemiology for further investigation. Of these 45 isolates from 21 centres were selected for molecular typing and genetic characterization. Antibiotic susceptibility testing by agar dilution and serotyping were performed by standard methods. PCRs and sequencing of ESBL-genes (blaCTX-M, blaSHV, blaTEM), PCR for ST131 strains, PFGE and phylogenetic group (PG) determination were done.
Results: The majority of strains carried resistance to more than two drugs. Several β-lactamases were detected (SHV-2, -5, -12, TEM-1, CTX-M-1) with CTX-M-15 being the most common. Only 9 strains belonged to comensal PGs A and B1, 36 strains proved to be members of B2 and D PGs. 46% of strains (21/45) from 12 centres belonged to the international O25-ST131/B2 clone, while 9 isolates from 7 centers shared O15 serotype. Serotypes O8 and O9 were presented by two-two, and serotypes O1, O142 and O162 by one isolate each. 22 different PFGE profiles were distinguised, with 18 O25-ST131/B2 strains representing EC003 cluster, and 8 O15/D strains representing two closely related clusters (EC026 and EC027).
Conclusion: This is the first comprehensive study on molecular epidemiology of ESBL-producing *E. coli* clinical isolates in Hungary. The ciprofloxacin-resistant CTX-M-15-producing O25-ST131/B2 and O15/D strains proved to be widespread among Hungarian healthcare facilities.

P1253 Local epidemiology of ESBL and AmpC producing Enterobacteriaeceae seen in urine samples in Glasgow

P.M. Lansdell, J. Boyes, N. Khanna, A. Hamouda, A.M. Younes, S.G. Amyes (Glasgow, Edinburgh, UK)*

Aims:
1. Define the prevalence of AmpC/ESBL (extended spectrum β-lactamases) producing organisms in our urinary isolates.
2. Compare the distribution of isolates for age, location and organism type.
3. Identify common genotypes from community and hospital isolates.

Methods: Isolates were taken over a 12-week period from community and hospital urine samples received at our laboratory. The isolates underwent identification and antibiotic sensitivity testing as defined by the Health Protection Agency. Isolates resistant to amoxicillin and cephalexin had this confirmed by the Vitek2® system. Isolates sensitive to amoxicillin or cephalexin, or if they could not be identified by Vitek2®, were discarded. The final set then had their antibiograms confirmed using CLSI and Vitek2® methodologies. Phenotypic resistance mechanisms were identified using Vitek2®, HPA and MAST ID ESBL/AmpC methods. Of these ESBL and AmpC producing organisms a selection of underwent PCR genotyping.
Results: 9139 samples were processed of which 347 potential ESBL/AmpC producing isolates from 256 patients were identified. Of these isolates 219 were identified as *E. coli*, 56 as *Klebsiella* species, 30 *Enterobacter* species, 17 *Serratia* marcesans, 14 *Citrobacter* species, 8 *Morganella morganii* and 3 *Proteus* species. Amongst our *E. coli* 54.8% were phenotypically ESBLs, 16.9% were phenotypically AmpC producers and 3.2% showed duel mechanisms of resistance. We saw 116 isolates from the community and 231 isolates from inpatient locations. The predominant inpatient location was general medical wards. 53.3% of isolates were from the over 75 age group. A set of CTX-M ESBLs were genotyped; 90% were confirmed as CTX-M producers but only 60% were identified as being CTX-M-15, the commonest CTX-M type in the UK.

Organism	Number (%)	ESBL (%)	AmpC (%)	Duel (%)	Neg (%)
E. coli	**219 (63.1)**	***120 (54.8)***	***37 (16.9)***	7 (3.2)	***55 (25.1)***
Klebsiella species	**56 (16.1)**	***49 (87.4)***	3 (5.4)	1 (1.8)	3 (5.4)
Enterobacter species	**30 (8.6)**	0	***19 (63.3)***	9 (30)	2 (6.7)
S. marcescens	**17 (4.9)**	2 (11.8)	***14 (82.4)***	0	1 (5.8)
Citrobacter species	**14 (4)**	2 (14.3)	10 (71.4)	2 (14.3)	0
M. morganii	**8 (2.3)**	0	7 (87.5)	1 (12.5)	0
Proteus species	**3 (0.9)**	1 (33.3)	2 (66.7)	0	0
Total	***347***	***174 (50.1)***	***92 (26.5)***	***20 (5.8)***	***61 (17.6)***

Conclusions: Our study shows almost 14% of our Enterobacteriaeceae are potential AmpC/ESBL producers, with over 80% confirmed

phenotypically as producing either ESBL or AmpC or both. These organisms are seen most often in the over 75 age group and on general medical wards. In addition genotyping suggests common enzyme types in community and hospital isolates, raising concerns over treatment options for patients, with only parenteral antibiotics being available. In conclusion we feel current data on community and hospital prevalence, age distribution, resistance mechanism and organism type of ESBL/AmpC producers is lacking. We hope to raise awareness of these organisms and the impact they have on patient care.

P1254 Shifts in extended-spectrum β-lactamase types with increasing prevalence of *Escherichia coli* producing extended-spectrum β-lactamase in western Sweden

L. Helldal, N. Karami, S. Skovbjerg, K. Floren, C. Unosson, C. Welinder-Olsson, E. Moore, C. Ahren (Gothenburg, SE)*

Objectives: Contrary to other multidrug-resistant pathogens, the prevalence of bacteria producing extended-spectrum β-lactamase (ESBL) is increasing rapidly in Sweden. In Europe, ESBL of CTX-M-, TEM-, OXA- and SHV-types are generally associated with *E. coli* infections, CTX-M being the most predominant. We have investigated how the prevalence of these types has changed during the last five years in the low endemic setting of western Sweden.
Methods: Yearly resistance in urinary (approximately 10,000 isolates/year) and blood (approximately 250 isolates/year) *E. coli* during 2004–2008 were determined. Cephalosporin-resistant isolates were screened for ESBL, using a double-disk assay with clavulanic acid as the inhibitory agent. All ESBL-*E. coli* isolated in the region during the periods Sept 2003-April 2005 (n = 46) and April 2008-March 2009 (n = 256) were typed by multiplex-PCR, detecting CTX-M, TEM, OXA and SHV. CTX-M-positive isolates were sub-typed by real time Q-PCR for CTX-M-1, CTX-M-2 and CTXM-9 groups.
Results: During 2004–2008, ESBL-producing *E. coli* strains increased from 0.3–1.5% in urinary and 0–1.4% in blood isolates. Resistance to quinolones and trimethoprim was observed in 60–80% of strains, as compared to less than 8% in non-ESBL-producing *E. coli*. The majority of the ESBL-*E. coli* strains possessed the CTX-M gene-type, increasing from 78% (36/46) in 2003–2005 to 93% (238/256) in 2008–2009. Between these time-periods, a marked shift occurred in the distribution of CTX-M types, in that strains with the CTX-M-9 group decreased from 42% (15/36) of isolates to 21% (51/238, p = 0.01) and, simultaneously, strains with the CTX-M-1 group increased from 58% (21/36) to 78% (185/238, p = 0.02). Furthermore, strains of CTX-M-type exhibiting also TEM- and/or OXA increased to comprise 86% of cases, as compared to 75% previously. Similar trends were seen for community and hospital detected isolates and with no differences associated with age in affected patients.
Conclusion: A steady increase in multidrug-resistant ESBL-*E. coli*, possessing the genes for multiple ESBL-types, was observed in western Sweden, contrary to the patterns of other multidrug-resistant bacteria. As ESBL has increased during the five-year study period, we detected a shift in the prevalence of ESBL-types, currently dominated by the CTX-M-1 group. These observations suggest that a novel ESBL-producing *E. coli* clone may have emerged in the area, which will be further investigated and presented.

P1255 Continuous spread of *E. coli* producing extended-spectrum β-lactamases CTX-M-3 and CTX-M-15 in Bulgaria from 2002 to 2009

R. Markovska, I. Schneider, E. Keuleyan, M. Sredkova, D. Ivanova, B. Markova, T. Kostyanev, I. Mitov, A. Bauernfeind (Sofia, BG; Munich, DE; Pleven, BG)*

Objectives: Previous investigations have shown that CTX-M-type extended-spectrum β-lactamases (ESBLs) became the most widespread enzymes in Bulgaria since 2002, particularly in *E. coli*. The aim of this work was to characterize the epidemiology of CTX-M-producing *E. coli* in seven clinical centres from three towns in Bulgaria.
Methods: Antibiotic susceptibility was determined by the disc diffusion method (CLSI, 2005). The ESBL-production was confirmed by the CLSI double disc ESBL confirmatory method. Conjugation was performed on solid medium. Isoelectric focusing, followed by bioassay, ESBL-group specific PCR as well as sequencing of ESBL genes for representative isolates were carried out. Epidemiological analysis was performed by RAPD with ERIC-1A and ERIC-2 primers and PstI plasmid fingerprinting.
Results: During a survey on ESBLs in Bulgaria from 2002 to 2009, 196 ESBL-producing *E. coli* from seven centres in three different towns were detected. The most widespread enzymes were those of the CTX-M-type (86%). CTX-M producing *E. coli* could be divided into two main groups: 11 isolates (6%) from four centres harboured an enzyme with a pI of 8.6 which was identified as CTX-M-3. All other isolates (185; 94%; from seven centres) showed β-lactamases with a pI of 8.8 corresponding to CTX-M-15. 18 strains additionally produced a β-lactamases with a pI of 8.2 (SHV-12), while two *E. coli* co-produced TEM-139. Epidemiological analysis by RAPD revealed a broader diversity of RAPD-types among CTX-M-3 producing *E. coli*. In contrast, the CTX-M-3 encoding plasmids showed only one dominant type. Among 128 CTX-M-15 producers investigated, 95 isolates (74%) from four centres showed an identical ERIC type. The remaining isolates clustered in 14 ERIC types consisting of one to six members. However, plasmid fingerprinting showed a broader variety among CTX-M-15 encoding plasmids than blaCTX-M-3 harbouring plasmids. The dominant type of CTX-M-15 producing *E. coli* showed high resistance rates: Augmentin (88%), Cefoxitin (3%), Cefepime (89%), Tobramycin (95%), Gentamicin (85%), Ciprofloxacin (100%), Tetracycline (95%), Cotrimoxazole (48%), and Chloramphenicol (46%).
Conclusions: Our data demonstrate the dissemination of a multiple resistant CTX-M-15 producing *E. coli* clone in four centres in two towns in Bulgaria over eight years (2002 to 2009) and underline the necessity of more stringent measures to prevent further persistence and dissemination of resistant clones.

P1256 Molecular epidemiology of Gram-negative bacteria resistant to newer generation β-lactams in a rehabilitation centre

R. Izdebski, A. Baraniak, M. Herda, J. Fiett, A. Rossini, M.P. Balice, A. Salvia, S. Malhotra-Kumar, C. Lammens, H. Goossens, Y. Carmeli, M. Gniadkowski (Warsaw, PL; Rome, IT; Antwerp, BE; Tel-Aviv, IL)*

Objectives: To characterize Gram-negative strains resistant to newer generation β-lactams colonizing patients of a rehabilitation centre.
Methods: The Hospital Santa Lucia in Rome, Italy, is an institution specialized in functional recovery. Its two wards admitting patients with orthopedic, neurological and spinal disorders (106 beds) participate in the EU Project MOSAR. All patients are being screened for colonization with ESBL-producing enterobacteria at admission, then every two weeks, and at discharge. The material was collected in 02–03.2009 by plating rectal swabs on the Brilliance ESBL agar (Oxoid). Pure cultures were identified by API32 tests (bioMérieux) and checked for resistance to newer β-lactams by the ESBL double-disk test (with and w/o cloxacillin), and by carbapenem disks. Typing was performed by PFGE and MLST (*E. coli* and *K. pneumoniae*). β-Lactamases were profiled by isoelectric focusing and identified by PCR and sequencing.
Results: From 169 specimens, 208 enterobacterial or non-fermenting (n = 58) isolates were cultured, of which 134 isolates from 48 patients with relevant acquired resistance mechanisms were retained. Among 85 non-duplicates there were 39 *P. mirabilis* isolates, including one wide-spread PFGE type (21 isolates, 17 patients) with the CMY-16 AmpC β-lactamase. Other *P. mirabilis* isolates were of 11 types and produced TEM-92 or TEM-72 ESBLs. 24 *E. coli* isolates were classified into 18 PFGE types showing no significant clonal spread; however, 11 types (14 isolates) belonged to the international sequence type ST131. Other STs represented clonal complexes CC10, CC69 and CC405. The ESBL CTX-M-15 prevailed in *E. coli* (14 PFGE types), followed by other CTX-Ms and SHV-5. The ST131 isolates had CTX-M-15, CTX-M-1 or SHV-5. Clonal spread played also a little role among *K. pneumoniae*

with 12 isolates split into 7 PFGE types and STs. Only 4 patients had a single clone, ST101 with CTX-M-15, and this enzyme dominated in *K. pneumoniae* even more than in *E. coli* (5 PFGE types/STs). A single *P. aeruginosa* isolate produced MBL. More than 20 patients were colonized by several organisms, and in the majority of patients the colonization with a single strain persisted over the study period.
Conclusions: This is one of the first complex studies on resistance epidemiology in a specialized rehabilitation centre. It showed a remarkable variety of colonizing organisms with diverse β-lactamases and of epidemiologic phenomena in this type of environments.

P1257 pMLST typing of IncI1 plasmids in *E. coli* and *S. enterica* isolates derived from animals and humans

C.M. Dierikx, A. van Essen-Zandbergen, K. Veldman, D.J. Mevius (Lelystad, NL)*

Objective: A study on ESBL-producing *E. coli* and *S. enterica* isolates derived from Dutch poultry revealed that incI1 plasmids were the predominant replicon types associated with ESBL in both *E. coli* and *S. enterica* isolates. These incI1-plasmids carried the β-lactamase genes: CTX-M-1, TEM-1, TEM-20 and TEM-52. Also in human isolates incI1 has been associated with β-lactamase-genes (a.o. CTX-M-1, CMY-2, CTX-M-14, CTX-M-15). In the Netherlands CTX-M-1 has also been found on incI1 plasmids in enterobacteria derived from clinical infections in humans indicating a potential relation with an animal reservoir. The objective of this study was to determine the population structure of incI1 plasmids from different backgrounds and to compare subtypes of ESBL-carrying incI1 plasmids in *E. coli* and *S. enterica* of animal and human sources.
Methods: A collection of incI1 plasmids derived from animal or human *E. coli* or *Salmonella* isolates were typed by plasmid Multi Locus Sequence Typing (pMLST) as described in Garcia-Fernandez et al., 2008. These incI1 plasmids harboured CTX-M-1 (N = 19), TEM-52 (N = 6), TEM-20 (n = 4), TEM-1 (N = 8), intI1 (N = 6) and unknown resistance genes (N = 5).
Results: The population structure of incI1 was diverse with 10 different pMLST sequence types detected. Three major clusters were found: Clonal Complex 7 (CC-7) (N = 16), ST-3 (N = 11) and ST-10 (N = 6) for IncI1 plasmids harbouring ESBLs. CC-7 included ST-7 and ST-30 (single allele difference in the ardA-gene). All CC7 plasmids carried CTX-M-1, and all ST-10 plasmids carried TEM-52. These two plasmid clusters were present in both *E. coli* and *S. enterica* isolates from Dutch poultry. ST-3 plasmids associated with CTX-M-1 or TEM-20 were present in *E. coli* and *S. enterica* isolates. New ST's 24–30 associated with various genes were uploaded in the pMLST database.
Conclusion: pMLST typing of IncI1 plasmids is a valuable tool to understand the contribution of IncI1 plasmids in the transmission of β-lactamase genes. The results of this study illustrate that ESBL-genes are linked to specific IncI1-sequence types. Dissemination of sequence types occurs between bacterial species within the same host, but also between hosts. This knowledge can be used to study the attribution of the animal reservoir of β-lactamase genes to infections in humans.

P1258 Emergence and clonal spread of multidrug-resistant *Klebsiella pneumoniae* of sequence type 14 in a Spanish tertiary hospital, 2007–2009

M. Cubero, C. Ardanuy, L. Calatayud, F. Tubau, J. Ayats, M.A. Domínguez, C. Peña, R. Martín, J. Liñares (L'Hospitalet de Llobregat, ES)*

Objectives: To analyze the genotypes and the resistance mechanisms in a multidrug-resistant *K. pneumoniae* outbreak in our hospital.
Methods: Antimicrobial susceptibility was tested by microdilution and disk diffusion method. The β-lactamase family was screened by PCR. Sequencing of PCR products was performed in selected isolates. Integrons were analyzed by the Levesque method. The molecular characterization of isolates was performed by pulsed field gel electrophoresis (PFGE, XbaI) and by Multi Locus Sequence Typing (MLST). The quinolone-resistant determinant regions (QRDRs) of gyrA and parC were sequenced.
Results: From August 2007 until June 2009, 107 multidrug resistant *K. pneumoniae* isolates were detected from clinical samples of 44 patients, 73% of them admitted to the ICU. The source of isolates was: urine (n = 28), blood (n = 20), bronchial aspirate (n = 20), catheter (n = 13), abdominal samples (n = 12), wound swabs (n = 6), bronchoalveolar lavage (n = 5) and other (n = 3). The strains were resistant to amoxicillin/clavulanic acid, piperacillin/tazobactam, tobramycin, amikacin and ciprofloxacin and had diminished susceptibility to cefepime (MICs range 1–4μg/ml). This multidrug-resistant pattern was transferred by conjugation to *E. coli* J53–2. A common PFGE pattern was observed in all *K. pneumoniae* isolates related to ST14 (1–6-1–1-1–1-1) by MLST. *K. pneumoniae* isolates and their transconjugants had the blaOXA gene, confirmed as blaOXA-1 after sequencing. The blaOXA-1 gene was located in the variable region of a class I integron which also contains the aac(6′)Ib-cr gene. The sequencing of the QRDRs revealed a S83F change in GyrA and no changes in ParC.
Conclusion: The increase in multidrug-resistant *K. pneumoniae* isolates in our hospital was associated to the clonal spread of an epidemic strain belonging to ST14 and harboring blaOXA-1 and aac(6′)Ib-cr genes in a class I integron. Control measures and continuous surveillance are needed in order to prevent the spread of these epidemic strains.

P1259 Comparison of ESBL genes from extended-spectrum β-lactamase carrying *Escherichia coli* from sewage sludge and human urinary tract infection

G. Zarfel, G. Feierl, H. Galler, D. Haas, E. Leitner, F. Mascher, A. Melkes, J. Posch, I. Winter, L. Masoud, A.J. Grisold, E. Marth, F.F. Reinthaler (Graz, AT)*

Objectives: Extended-spectrum-β-lactamase carrying Enterobacteriaceae are a growing problem in hospital and community. Beside human reservoir, ESBL carrying Enterobacteriaceae can also be found in the environment. Different groups of β-lactamase genes are the genetic basis of the ESBL mediated resistance. In this study, ESBL carrying *Escherichia coli* from sewage sludge and human urinary tract infection were investigated for their phenotypes and the occurence of six different ESBL gene groups.
Methods: A sample of 20 ESBL *E. coli* from sewage sludge collected from five different sewage treatment plants in the area of Styria between January and September 009 and a sample of 20 ESBL *E. coli* strains from urinary tract infections, isolated at the Medical University Graz (Austria) in the same time period, were analysed. Strains were screened for 6 ESBL gene groups (CTX-M, TEM, SHV, OXA, VEB, GES) by PCR, and genes detected were subsequently sequenced. Further, strains were phenotypically characterized by the Phene-Plate™ System (DiaTeam, Austria) using PhP-RF microtiter plates 03 R PHP EC *E. coli*.
Results: In ESBL *E. coli* from sewage sludge CTX-M 1 was the most common ESBL gene and was present in nine of the 20 strains. CTX-M 15 was identified in six and CTX-M 3 in two strains. A total 17 (85%) sewage sludge strains contained a CTX-M gene. Further, one ESBL *E. coli* from sewage sludge harboured a SHV-15 gene, whereas two isolates had no positive PCR product for any of the tested ESBL gene groups. All 20 ESBL *E. coli* from urinary tract infection harboured a CTX-M gene; CTX-M 15 was detected in 15 strains and CTX-M 1 in five strains. 40% of all strains (16 strains from both sources) were carrying, in addition to CTX-M, the non ESBL β-lactamase TEM-1. Phenotyping of the strains resulted in four different phenotypical clusters (PHPtype clusters) with correlation coefficients higher than 0.95. 25 single strains could not be assigned to any of the clusters. Cluster one was formed by seven strains from urinary tract infection. The other clusters contained strains from both sources. The phenotypic data correlated with the PCR results, as within a cluster, all strains harboured the same CTX-M gene.
Conclusions: CTX-M genes were the dominant ESBL group in the analyzed samples. All ESBL gene variants present in human urinary

tract infection were also present in sewage sludge. Consistently, strains from both sources were assigned together into the same phenotypical clusters.

P1260 Paediatric faecal colonization with extended-spectrum β-lactamase producing Enterobacteriaceae in northern Portugal

D. Gonçalves, H. Rodrigues, H. Ferreira (Porto, PT)*

Objectives: Our previous work, in faecal colonization in the community, alerted us for the finding of extended-spectrum β-lactamase (ESBL) producers in particular niches, as nursing homes. In some Portuguese social solidarity institutions, we can find a day care centre (DCC) adjacent to a nursing home facility. In that way, the aim of our study was the detection and characterization of ESBL producing Enterobacteriaceae in the faecal flora of children attending DCC, in Northern Portugal.
Methods: Faecal samples of children from few months to 6 years old, attending two DCC, from the North of Portugal, were collected from April to July 2009. Samples were suspended in BHI. Isolates were selected in MacConkey agar with ceftazidime (2 mg/L), cefotaxime (2 mg/L), and aztreonam (2 mg/L). Lactose fermenters were randomly selected and susceptibility to antimicrobial agents was determined by agar diffusion methods. Screening of ESBL producers was performed by the double disk synergy test and confirmed according to the CLSI. Identification of the selected strains was achieved by API 20 E. β-lactamases were characterized by isoelectric focusing. Conjugation assays were performed with *Escherichia coli* HB101.
Results: Of 105 faecal samples of children attending two day care centres in the North of Portugal we screened 32 ESBL producing Enterobacteriaceae isolates: 18 *Escherichia coli*, 2 *Citrobacter freundii*, 3 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 2 *Enterobacter* sakazaki, 2 *Klebsiella* ornithinolytica, 2 *Hafnia alvei*, 1 Pantoea spp. and 1 *Klebsiella oxytoca*, predominantly showing an ESBL of pI > 8 alone or in association with β-lactamases of pIs 5.4, 7.4 and 7.8. Other ESBLs of pI approximately 8 and 7.6, were also present in some isolates. ESBL gene was successfully transferred coding a β-lactamase of pI > 8 and 5.4 plus approximately 8.
Conclusion: Our results showed that young children are colonized with ESBL producing Enterobacteriaceae. Isoelectric points of predominant β-lactamases, alert for the hypothesis of one successful track of community dispersion of a putative CTX-M-15, in this young population, in different combination with other β-lactamases, as in the CTX-M-15 producing ST131 *Escherichia coli* epidemic clone. The hypothesis of spread from the neighbour nursing homes to the young population needs to be assessed by strain relationship determination. This reality might create a cycle of dispersal of ESBL producers, to the healthy community.

β-lactam activity against Enterobacteriaceae

P1261 Comparative *in vitro* activity of cefditoren and other antimicrobials against Enterobacteriaceae causing community-acquired uncomplicated urinary tract infections in women: a Spanish nationwide multicentre study

O. Cuevas, E. Cercenado, M. Gimeno, M. Marin, P. Coronel, E. Bouza for the Spanish Urinary Tract Infection Study Group (SUTIS)*

Objectives: Cefditoren is a third generation orally administered cephalosporin with a broad spectrum of activity against Gram-positive and Gram-negative bacterial species. After an oral 400-mg single dose, the mean concentrations in urine are 186.5 mg/L at 2–4 h, and 12.7 mg/L at 8–12 h, and is a potential drug to be used in the treatment of urinary tract infection (UTI). We performed a multicenter nationwide study in Spain in order to determine the *in vitro* activity of cefditoren and other comparative agents against Enterobacteriaceae causing community-acquired uncomplicated UTI in women.
Methods: From June 2008 to March 2009, 89 institutions participated in the study. A total of 2152 Enterobacteriaceae were collected and sent to a reference laboratory where identification and antimicrobial susceptibility testing was performed against 20 antimicrobials using and automated microdilution method (MicroScan). Cefditoren MIC's were determined by the broth microdilution method (CLSI guidelines) using the same inoculum.
Results: Microorganisms isolated were *E. coli* (81.8%), *Klebsiella pneumoniae* (7.9%), *Proteus mirabilis* (5.2%), and others (5.1%). A total of 51 isolates (2.4%) were extended-spectrum β-lactamase (ESBL) producers, 3 (0.1%) produced plasmidic AmpC enzymes, and 64 (2.9%) chromosomal AmpC. The MIC50/MIC90 (mg/L) of cefditoren against all isolates was 0.12/0.5. Cefditoren inhibited 96.5% of isolates at 1 mg/L, and was uniformly active against all isolates with the exception of strains producing ESBLs or AmpC enzymes. The MIC50/MIC90 of other antimicrobials were: ampicillin (AMP) >16/>16; amoxicillin/clavulanic (A/C) ⩽8/4/16/8; cefuroxime (FUR) ⩽4/8; cefotaxime (CTX) ⩽1/⩽1; ciprofloxacin (CIP) ⩽0.12/>2; cotrimoxazole (SXT) ⩽2/38/>4/76; and fosfomycin (FOS) ⩽16/⩽16. The respective percentages of resistance were: 61%, 17.2%, 5.5%, 2.3%, 20.2%, 27.4%, and 4.8%.
Conclusions: The activity of cefditoren against Enterobacteriaceae producing community-acquired uncomplicated UTI in women was superior to that of AMP, A/C, FUR, CIP, SXT, and similar to that of FOS.

P1262 Susceptibility of Gram-negative pathogens isolated from intra-abdominal infections in Europe in 2008–2009 – The SMART Study

R. Badal, S. Bouchillon, D. Hoban, A. Johnson, M. Hackel (Schaumburg, US)*

Objectives: The Study for Monitoring Antimicrobial Resistance Trends (SMART) program has been monitoring activity of ertapenem (Etp), amikacin (Ak), cefepime (Cpe), cefoxitin (Cfx), ceftazidime (Caz), ceftriaxone (Cax), ciprofloxacin (Cp), imipenem (Imp), levofloxacin (Lvx), ampicillin-sulbactam (AS), and piperacillin/tazobactam (PT) vs. Gram-negative bacteria from intra-abdominal infections (IAI) since 2002. This report summarizes susceptibility levels for key IAI pathogens in Europe during 2008–2009.
Methods: 31 labs in Europe each collected up to 100 consecutive Gram-negative bacteria/year from IAI in 2008–2009. MICs were determined by broth microdilution, and interpreted using EUCAST guidelines if available. Susceptibility rates were determined for species with ⩾10 isolates.
Results: 3209 isolates were collected; however, only those with N⩾10 (3130, 97.5% of the total) were included in this analysis. The remaining 79 isolates represented 35 species. The table below shows % susceptible for each drug; values ⩾90% are shaded.
Conclusions: *E. coli* (~50% of all IAI pathogens) was ⩾90% susceptible vs. only 3 drugs: Imp, Etp, and Ak. *K. pneumoniae* (~11% of all IAI pathogens) was ⩾90% susceptible vs. only 2 drugs (Imp and Etp), and just 1 other (Ak) was >80%. No drug achieved even 80% susceptible vs. *P. aeruginosa*. Until definitive identification and susceptibility testing results are known, options for effective empirical therapy of IAI in Europe have diminished to include very few (e.g., carbapenems, amikacin) of the agents evaluated in this study.

Organism	N	Imp	Etp	Ak	Cpe	PT	Cax	Cfx	Caz	Lvx	Cp	AS
Escherichia coli	1563	100	99	92	87	87	85	85	85	76	75	44
Klebsiella pneumoniae	359	92	90	87	75	71	73	74	73	76	71	53
Pseudomonas aeruginosa	280	70	na	76	75	77	na	na	79	70	72	na
Enterobacter cloacae	211	99	85	96	77	67	56	56	58	91	87	22
Klebsiella oxytoca	136	99	100	100	94	89	91	94	99	99	96	71
Proteus mirabilis	131	83	100	97	100	99	82	61	63	88	81	1
Acinetobacter baumannii	93	47	na	54	na	na	na	na	na	16	16	na
Citrobacter freundii	79	100	100	97	87	78	69	69	68	99	97	50
Morganella morganii	72	83	100	97	100	99	82	61	63	88	81	1
Enterobacter aerogenes	56	93	88	96	84	61	52	54	48	93	93	25
Proteus vulgaris	37	89	100	97	92	97	51	78	84	97	97	54
Serratia marcescens	31	97	97	94	94	84	77	87	97	97	77	19
Stenotrophomonas maltophilia	29	10	7	14	10	10	10	10	38	86	14	na
Citrobacter koseri	21	100	100	100	95	100	95	95	95	95	95	90
Hafnia alvei	21	100	85	95	76	43	33	43	24	100	100	10
Citrobacter braakii	11	100	100	100	91	82	64	64	64	100	100	64

P1263 Comparison of antimicrobial susceptibility of Gram-negative pathogens from ICU vs. non-ICU – SMART, 2008–2009

R. Badal, S. Bouchillon, D. Hoban, A. Johnson, M. Hackel (Schaumburg, US)*

Objectives: Pathogens recovered from intensive care units (ICUs) tend to be more resistant than those from non-ICUs. The Study for Monitoring Antimicrobial Resistance Trends (SMART) program monitors the activity of ertapenem (Etp), amikacin (Ak), cefepime, cefoxitin, ceftazidime (Caz), cefotaxime (Cft), ceftriaxone (Cax), ciprofloxacin, imipenem, levofloxacin (Lvx), and piperacillin–tazobactam (PT) against Gram-negative bacteria (GNB) from intra-abdominal infections (IAI) globally. This report compares susceptibility levels for key IAI pathogens isolated from patients in ICUs vs. non-ICUs in 2008–2009.
Methods: 118 labs in 36 countries each collected up to 100 consecutive GNB per year from IAI in 2008–2009. MICs were determined by broth microdilution, and interpreted using CLSI guidelines. Susceptibility rates (%S) for isolates from ICU and non-ICU patients were determined for species with ⩾10 isolates in both locations. Differences were evaluated for significance using Fisher's Exact Test.
Results: 14 species (*A. baumannii*, *C. freundii, E. aerogenes, E. cloacae*, *Enterobacter* spp., *E. coli*, *K. oxytoca*, *K. pneumoniae*, *M. morganii, P. mirabilis, P. vulgaris, P. aeruginosa*, *S. marcescens*, and *S. maltophilia)* had ⩾10 isolates in both ICU and non-ICU locations. Of 168 organism/drug pairings, 15 could not be evaluated due to absence of CLSI S/I/R breakpoints; 10 were identical between ICU and non-ICU; in 30 cases the ICU %S was higher (none significant, $P > 0.05$); and in 113 cases the non-ICU %S was higher (40 significant, $P < 0.05$). Species with the highest number of significant differences were *E. coli*, *K. pneumoniae*, *P. mirabilis,* and *E. cloacae* (9, 8, 8, and 6 drugs, respectively). Drugs with the highest number of significant differences were PT (6), Caz (5), Cax (5), Cft (4), and Lvx (4). Etp was the only study drug to maintain ⩾89% susceptibility in ICU and non-ICU vs. all species for which it is indicated.
Conclusions: These data suggest that IAI pathogens from ICU are often more resistant to commonly-used drugs, but some species did not show significant differences. Etp and Ak showed the least number of significant differences between ICU and non-ICU. Etp had good *in vitro* activity vs. ICU and non-ICU isolates.

P1264 *In vitro* activity of tigecycline against multi-resistant *Klebsiella pneumoniae* isolates

A. Tsatsagias, O. Vasilaki, S. Spyroglou, E. Protonotariou, S. Mpalodimos, K. Karozis, S. Metallidis, F. Tsiapara, F. Frantzidou, E. Diza (Thessaloniki, GR)*

Objectives: Tigecycline (TGC), the first glycylcycline antibacterial agent, has been shown to be highly effective against a wide range of bacteria including multi-resistant Gram negative and positive pathogens. The purpose of our study was to evaluate the *in vitro* activity of tigecycline against multiresistant *K. pneumoniae* clinical isolates that phenotypically produce KPC.
Methods: From 11/11/08 to 12/11/09 we collected 37 non-duplicate multiresistant *Klebsiella pneumoniae* isolates recovered from blood, bronchoalveolar secretions, urine, trauma, pus and central venus catheter. The samples were isolated from patients hospitalized in ICU, surgical and internal departments of AHEPA University Hospital in Greece. Bacterial identification and initial antimicrobial susceptibility testing were performed by the VITEK 2-automated system (bioMérieux, France). All the isolates had reduced susceptibility or resistance to carbapenems. The isolates were phenotypically identified as possible KPC producers based on the positive synergy test using boronic acid and meropenem. The prescence of KPC genes was selectively performed using PCR in 10 isolates and all were positive for the blaKPC. MICs of tigecycline were determined by Etest following the manufacturer's instructions (AB Biodisk, Solna). All isolates with MIC < 2μg/ml were considered as susceptible according to FDA proposed breakpoints.
Results: All 37 *K. pneumoniae* isolates were resistant to almost all classes of antibiotics (including cephalosporines, carbapenems, fluoroquinolones, co-trimoxazole, aminoglycosides (except gentamicin). The only active agents were colistin and gentamicin. *In vitro* susceptibility testing with E-test showed that MICs for tigecycline ranged from 0.38 to 3 μg/ml and MIC50 and MIC90 values were 0.75 μg/ml and 3 μg/ml, respectively. Tigecycline was active against to most of the examined isolates (32/37). Five isolates (13.5%) were classidied as intermediate to tigecycline (MIC of 3 μg/ml). Overall, TGC resistance rates were 10.8% for *K. pneumoniae* isolates recovered from both ICU and non-ICU patients.
Conclusions: According to the current breakpoints established by FDA, tigecycline was active against most of the *K. pneumoniae* isolates tested inhibiting >90% in concentrations <2μg/ml. Nevertheless, it is of concern that higher MICs have begun to emerge in our hospital that might limit our therapeutic options in future.

P1265 Potent activity of ceftazidime/NXL104 tested against Enterobacteriaceae isolates carrying multiple β-lactamase enzymes

M. Castanheira, P. Rhomberg, R. Jones, D. Farrell (North Liberty, US)*

Objectives: Treatment of infections caused by pathogens carrying multiple β-lactamases (BLs) can be challenging due to the association of β-lactam-resistance mechanisms with other genetic determinants encoding resistance to non-β-lactam compounds. NXL104 is a broad spectrum BL-inhibitor and, combined with ceftazidime (CAZ), is undergoing clinical trials to treat hospital infections caused by Gram-negative pathogens, including those with multidrug resistance. The objective of this study was to assess the activity of CAZ/NXL104 against a panel of Enterobacteriaceae (ENT) carrying multiple BL enzymes.
Methods: CAZ activity combined with NXL104 at fixed 4 mg/L was evaluated by CLSI broth microdilution methods against 80 ENT strains (9 species) producing 2 to 4 BLs. BLs included: narrow-spectrum (3 SHV-, 2 OXA-types, TEM-1), ESBLs (4 SHV-, 4 CTX-M-, 2 OXY-variants, OXA-1/30), serine-carbapenemases (3 KPC-, 2 SME-types, NMC-A) and metallo-BLs (VIM-1, VIM-2) all identified by PCR and sequencing. ENT hyperproducing chromosomal AmpC were also tested.
Results: Strains producing 2 (59), 3 (19) and 4 (2) BLs were evaluated. 66 of 80 (82.5%) strains showed CAZ/NXL104 MIC results at ⩽2 mg/L. Inhibitory effect was observed despite the number of BLs (Table). Only 5 KPC-producers (1 *C. freundii* and 4 *E. cloacae*) and 5 SHV-12 (all *E. cloacae*) showed CAZ/NXL104 MIC results at 4 mg/L, but MIC reductions were 16- to 128-fold compared to CAZ alone. 2 KPC- and 2 VIM-producers (all *Enterobacter* spp.) had MICs at ⩾8 mg/L for CAZ/NXL104, though inhibitory effect (16- and 32-fold decrease) was noted for the KPC-producers. The great majority (85%) of ENT had MICs lowered 4- to 512-fold by NXL104. Four isolates showing no inhibitory effect carried MBL enzymes; one isolate had low/susceptible CAZ MIC value (⩽2 mg/L).

Organisms (no. tested)	MIC (mg/L) ⩽2	4	8	16	⩾32	MIC reduction with NXL104
2 β-lactamases (59)						
CAZ	12	2	1	3	41	
CAZ/NXL104	45	10	1	1	2	None[a] to >512-fold
3 β-lactamases (19)						
CAZ	1	3	1	3	11	
CAZ/NXL104	19	–	–	–	–	4- to 512-fold
4 β-lactamases (2)						
CAZ	–	–	–	–	2	
CAZ/NXL104	2	–	–	–	–	128- to 256-fold

[a]Four isolates showing no inhibitory effect carried MBL enzymes; one isolate had low/susceptible CAZ MIC value (⩽2 mg/L).

Conclusions: The addition of NXL104 to CAZ resulted in a 4- to 512-fold decrease in MIC for most isolates having multiple BLs.

CAZ/NXL104 MIC values were within the CLSI CAZ susceptibility range for the vast majority of ENT tested (>95% of strains), regardless of the number of BLs present or CAZ MIC. These data demonstrate that CAZ/NXL104 could be a significant therapeutic option for treatment of contemporary, most challenging BL-producing ENT species.

P1266 *In vitro* activity of cefixime combined with clavulanate vs. enterobacterial urinary isolates producing extended-spectrum β-lactamases

P. Khanna, D. Wareham (London, UK)*

Objectives: Treatment of UTI due to Enterobacteriaceae producing ESBLs is problematic. Conventional oral β-lactams and inhibitor combinations are inactive and many strains also exhibit co-resistance to quinolones, trimethoprim and nitrofurantoin. Cefixime combined with clavulanate is a potential oral treatment option. We therefore assessed its *in vitro* activity against a contemporary collection of ESBL producing urinary isolates.
Methods: Strains were isolated and identified from routine urine samples using CHROMagar orientation and the Microscan Walkaway system. Cefpodoxime resistant isolates were screened for ESBL production using commercial cefpodoxime / clavulanate combined discs and TEM, SHV and CTX-M genes were amplified by PCR. Susceptibility to cefixime in combination with clavulanate was determined by disc diffusion using 5 ug cefixime discs supplemented with 1.25, 2.5 and 4 ug of clavulanate. The MIC of cefixime with and without clavulanate was determined by Etest for 32 of the ESBL producers using isosensitest agar supplemented with clavulanate at 4 mg/L.
Results: 85 cefpodoxime resistant isolates were recovered over a 4 month period. All were resistant to amoxicillin, cefalexin and cefixime. 76.5% were also resistant to trimethoprim, 84.7% to ciprofloxacin and 16.5% to nitrofurantion. TEM, CTX-M and SHV genes were found in 100, 82.4 and 15.2% respectively. The mean zones of inhibition for cefixime discs supplemented with clavulanate at 1.25, 2.5 and 4 ug were 19.1 mm, 20.6 mm and 21.8 mm respectively. Using BSAC zone diameter interpretative breakpoints these concentrations restored susceptibility to cefixime in 41.2, 62.4 and 74.1% of isolates if breakpoints for systemic infection were used (sensitive >20 mm) and in 81.2, 89.4 and 94.1% isolates if urinary cephalosporins breakpoints were used (sensitive >16 mm). MICs in combination with clavaulante were all <1 mg/L in the 32 isolates tested by Etest, with an MIC50 of 0.38 and an MIC90 of 0.75 mg/L.
Conclusions: Due to the increasing involvement of ESBL producing bacteria in uncomplicated UTI, there is a rising need for effective oral treatments in mild cases. We found clavulanate restored the activity of cefixime against enterobacteria producing TEM, SHV and CTX-M ESBLs in up to 94% of isolates tested. There are anecdotal reports of the efficacy of this combination in the treatment of lower urinary tract infection but to our knowledge a clinical trial has not yet been undertaken.

P1267 Antimicrobial resistance of extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* strains from north-eastern Romania

C. Tuchilus, L.S. Iancu, B. Copacianu, A. Poiata, A. Badescu, A. Ambarus, C. Lapuste, D. Buiuc (Iassy, RO)*

Family Enterobacteriaceae have developed resistance to third- and fourth-generation cephalosporins via production of extended-spectrum β-lactamases (ESBL). The antimicrobial resistance patterns of ESBL-producing organisms and their prevalence may vary between geographic areas.
Objectives: to evaluate the *in vitro* antimicrobial resistance and the prevalence *Escherichia coli* and *Klebsiella pneumoniae* producing ESBL, isolated from hospital and community in 2009, January-October period.
Materials and Methods: We selected 112 strains (from which, 84 strains were identified as *E. coli* and 28 as *K. pneumoniae*) among 611 enterobacteria strains, by using disk diffusion method as initial screening for ESBL, according to Clinical and Laboratory Standard Institute (CLSI) guidelines. For selected strains, the ESBL phenotypic confirmation was performed with double disk test and the replacement disk method. The minimum inhibitory concentrations (MICs) for next antimicrobial agents tested: ceftazidime, cefotaxime, ceftriaxone, cefepime, cefpirome, imipenem, meropenem, ertapenem, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin were performed by agar dilution method. Resistance rates were reported using the CLSI breakpoints for the fully susceptible category; moderately susceptible isolates were classified as resistant. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used for quality control.
Results: All 112 tested strains (18.3%) were phenotypically confirmed as ESBL producer, when two phenotypic confirmatory tests were used. Resistance rates for ceftazidime, cefotaxime, ceftriaxone and aztreonam among tested strains were very high: 84.5%, 100%, 100% and 96.4% for *E. coli* and 98.8%, 98.8%, 97.6% and 89.2% for *K. pneumoniae*, with the MIC90 values >256 mg/l for either antimicrobial tested agent. The MICs for fourth generation cephalosporins ranges from 4 mg/l to 512 mg/l. The MICs for tested quinolones were >2 mg/l for 89.2% strains. Using breakpoint for susceptibility, all isolates were sensitive to ertapenem, imipenem and meropenem.
Conclusions: Results of this study have shown that ESBL producing *E. coli* and *K. pneumoniae* strains circulating in Romania are associated with multidrug resistance. In this study, carbapenems demonstrated the highest degree of activity and are the treatment of choice for ESBL producers.

P1268 *In vitro* interactions of colistin in combination with meropenem or ertapenem or piperacillin/tazobactam against metallo-β-lactamase-producing *Klebsiella pneumoniae* clinical isolates

M. Souli, P.D. Rekatsina, I. Galani, Z. Chryssouli, H. Giamarellou (Athens, GR)*

Objectives: The dissemination of metallo-β-lactamases (MBLs) in Enterobacteriaceae represents an emerging therapeutic challenge. These enzymes hydrolyse carbapenems although the MICs could be at the susceptible level. Treatment options against these strains are scarce and combination regimens are often used although the potential advantage of these combinations has not yet been studied. Our aim was to determine the interactions of colistin (COL) in combination with meropenem (MER) or ertapenem (ERT) or piperacillin/tazobactam (PIP/TAZ) against clinical isolates of VIM-producing *K. pneumoniae* (K. p.).
Methods: MICs were determined with standard methodology, according to CLSI. MBL-production was screened for using the imipenem-EDTA disc synergy test and confirmed by PCR with specific primers for blaVIM followed by RFLP analysis with SacI. Clonal relatedness among studied isolates was investigated using REP-PCR with specific primers. Interactions were investigated with time-kill methodology with an inoculum of 5×105 CFU/ml. Tubes containing each antibiotic alone and in combination at 1×MIC or 4×MIC for susceptible strains as well as at a concentration representing the steady state of each antibiotic (MER or ERT 10 mg/L, COL 5 mg/L, PIP/TAZ 35/7 mg/L) for non-susceptible strains were subcultured at 0, 3, 5 and 24 hours for colony counts.
Results: Thirty-three clonally distinct strains of K.p. were studied. All exhibited a positive synergy test and carried a blaVIM-1-type gene. The combination of COL-ERT showed synergy for 5 of 14 (35.7%) COL-S isolates and for 3 of 18 (16.7%) COL-R isolates. One of 14 (7.1%) COL-S and 2 of 18 (11.1%) COL-R isolates showed antagonism with ERT. The combination of COL-MER exhibited synergy for 3 of 10 (30%) COL-S isolates and for 3 of 18 (16.7%) COL-R isolates. Antagonism with MER was observed against 2 of 10 (20%) COL-S and 1 of 18 (5.6%) COL-R isolates. The combination COL-PIP/TAZ was synergistic against 5 of 12 (41.7%) COL-S and 1 of 17 (5.9%) COL-R isolates. Antagonism was observed against 4 of 12 (33.3%) COL-S isolates. All other isolates showed indifference.

Conclusion: The combinations of COL with either ERT, MER or PIP/TAZ although often used in clinical practice, result in strain-specific interactions. Indifference was most commonly observed in our study.

P1269 Susceptibility of Gram-negative pathogens to ceftobiprole, ceftazidime and cefepime isolated from centres in Austria, Germany and Switzerland

H. Seifert, S. Gatermann, W. Pfister, C. Poehlmann, S. Huhulescu, K. Muehlemann, J. Laeuffer, S. Decker-Burgard, M. Cassettari, I. Morrissey (Cologne, Bochum, Jena, Dresden, DE; Vienna, AT; Berne, Baar, CH; Neuss, DE; Fordham, UK)*

Objectives: Ceftobiprole (BPR) is a novel cephalosporin with bactericidal activity against both Gram-negative and Gram-positive bacteria. Here we report the comparative activity of BPR, ceftazidime (CAZ) and cefepime (CFP) against Gram-negative pathogens (GNP) isolated from hospitalized patients with serious infections collected by 18 centres across Austria, Germany and Switzerland.
Methods: 691 GNPs (including 200 *Escherichia coli* [EC], 172 *Pseudomonas aeruginosa* [PA], 127 *Klebsiella pneumoniae* [KP], 143 *Enterobacter* spp. [ES] and 49 *Haemophilus influenzae* [HI]) were collected from May to Oct 2008 from hospitalized patients with complicated skin and soft tissue infection (cSSTI), blood stream infections or nosocomial pneumonia including ventilator associated pneumonia (VAP). MICs were determined at each centre using Etest methodology.
Results: Against all GNPs included in the study, the three tested agents had an MIC90 of 4 mg/L and both BPR and CFP had an MIC50 of 0.06 mg/L compared with 0.25 mg/L CAZ. Enterobacteriaceae and HI were highly susceptible to all three cephalosporins with MIC90 of BPR, CAZ and CFP of 0.12/2/0.25 mg/L and 0.12/0.12/0.25 mg/L respectively. Against ES, BPR and CFP had an MIC90 of 1 and 2 mg/L compared with ⩾64 mg/L for CAZ. BPR, CAZ and CFP were comparable in activity to PA with an MIC50 of 2 mg/L and MIC90 of 16/8/8 mg/L respectively. BPR was at least 2-fold more active than CFP and CAZ against 33.4% and 73.7% of GNPs respectively and CFP was at least 2-fold more active than BPR and CAZ against 20.7% and 71.3% of GNPs respectively. BPR had identical MIC values to CFP and CAZ against 45.9% and 13.7% of GNPs respectively. CFP and CAZ had the same MIC values for 17.4% of GNPs.
Conclusion: Ceftobiprole was comparable to ceftazidime against *Pseudomonas aeruginosa* but generally more active against other GNPs and was comparable to or more active than cefepime against GNPs collected from centres in Austria, Germany and Switzerland in this study.

P1270 Antimicrobial activity of ceftaroline combined with NXL104 tested against a collection of organisms expressing multiple β-lactamases

H. Sader, G. Williams, G. Moet, M. Castanheira, I. Critchley, R. Jones (North Liberty, Oakland, US)*

Objective: To evaluate the activity of ceftaroline combined with NXL104 (fixed 4 mg/L; CPT104) against Enterobacteriaceae (ENT) with various types of β-lactamases (BL), with most strains carrying multiple BLs. Ceftaroline is a novel, parenteral cephalosporin with broad-spectrum activity against Gram-positive (including MRSA and MDRSP) and -negative organisms. Ceftaroline has limited activity against extended-spectrum β-lactamase (ESBL)- and AmpC-producing strains. NXL104 is a novel non-β-lactam BL inhibitor that inhibits Ambler class A, C, and D enzymes (eg, ESBL, KPC, and AmpC).
Methods: CPT104 and comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 148 clinical strains of ENT producing KPC + AmpC (26 strains), ESBL + AmpC (27), KPC + ESBL (7), multiple ESBLs (38 strains), SME or NMC-A carbapenemases (7), KPC (12), CTX-M (8), plasmidic AmpC (15), and metallo-BL (MBL; 8). Isolates were collected from 1999–2008 from global surveillance programs.
Results: CPT104 exhibited potent inhibitory effects against all BL types except MBLs. All isolates were inhibited at CPT104 MIC ⩽4 mg/L except the MBL-producing strains (Table). CPT104 was highly active against ENT producing KPC (MIC90, 0.5 mg/L), KPC + AmpC (MIC90, 2 mg/L), and KPC + ESBL (MIC100, 1 mg/L). CPT104 was more active than meropenem (MIC90, >8 mg/L) against these 3 groups. ENT-producing CTX-M (highest CPT104 MIC, 0.5 mg/L) and those producing more than one ESBL type (including CTX-M, SHV, TEM, OXA and OXY; MIC90, 1 mg/L) were also very S to CPT104. The highest CPT104 MIC observed among plasmidic AmpC was 0.5 mg/L. All strains producing SME or NMC-A were also inhibited at ⩽0.5 mg/L of CPT104. CPT104 (MIC, >32 mg/L) and all β-lactam compounds tested showed limited activity against MBL-producing ENT.
Conclusions: Results of this study clearly demonstrated that NXL104, when combined with ceftaroline, effectively lowers ceftaroline MIC for ENT that produce the most clinically significant BLs, except MBLs. CPT104 was highly active against ENT that produce KPC, various ESBL types, and AmpC (chromosomally derepressed or plasmid mediated), as well as those producing more than one of these BL types (Table). CPT104 represents a promising therapeutic option for treatment of infections caused by multidrug-resistant ENT.

BL type (no. tested)	Cumulative % inhibited at CPT104 MIC (mg/L):							
	⩽0.06	0.12	0.25	0.5	1	2	4	>4
KPC + AmpC (26)	3.8	3.8	8.6	30.8	73.1	96.2	100.0	–
ESBL + AmpC (27)	0.0	14.8	37.0	55.6	74.0	96.3	100.0	–
KPC + ESBL (7)	0.0	0.0	14.3	57.1	100.0	–	–	–
Multiple ESBLs (38)	2.6	60.5	86.8	89.5	97.4	100.0	–	–
SME/NMC-A (7)	0.0	14.3	42.9	100.0	–	–	–	–
KPC (12)	0.0	0.0	16.7	91.7	100.0	–	–	–
CTX-M (8)	12.5	75.0	87.5	100.0	–	–	–	–
Plasmidic AmpC (15)	13.3	60.0	86.7	100.0	–	–	–	–
MBL (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

P1271 Antibiotic resistance of *Escherichia coli* isolated from community-acquired urinary infections

A. Topan, D. Tatulescu, V. Zanc, A. Almas, M. Flonta, A. Slavcovici (Cluj-Napoca, RO)*

Objectives: Intensive use of fluoroquinolones in the treatment of urinary infections has led to the spread of resistant microorganisms. The aim of our study is to determine the antimicrobial resistance of uropathogenic *Escherichia coli* strains isolated from patients admitted in our hospital.
Methods: We retrospectively reviewed medical records of 86 patients aged 18 years or older, with urinary symptoms and cultured documented urinary tract infection (UTI) admitted to our hospital between 1st of January – 31st December 2008. Data abstract from each medical record included demographic aspects; the causative organism; the current use of antibiotics within 3 months; hospitalization within the prior year, previous UTI episodes, complicated or uncomplicated UTI.Hospital stay within prior month; indwelling urinary catheters and residence in long-term care facility were criteria of exclusion. The antibiotic sensitivity was determined by standard disc-diffusion method according to the NCCLS and CLSI standards. Categorical variables were compared using Fisher exact test. Significance was set at $p < 0.05$.
Results: Among the 86 positive cultures 57 (66.27%) were from female and 29 (33.72%) were from males. Median age of the patients was 61 (range 19–86). The most frequent agent as expected, was *Escherichia coli* 66.27% (n = 57), followed by *Klebsiella pneumoniae* 10.46% (n = 9). E. Coli presented a resistance rate to ampicillin of 66.66%, to TMP-SMX of 50.87% and to ciprofloxacin and norfloxacin to 26.33%. *E. coli* was sensitive to nitrofurantoin in 76.78% cases. *E. coli* resistant to fluroquinolone was significantly related to: age greater than 65 years ($p = 0.048$); current use of antibiotics within 3 months ($p = 0.0078$); prior hospitalization ($p = 0.007$). There was no statistical significance when we evaluated *E. coli* fluroquinolone resistant and sex ($p = 0.051$); previous episodes of UTIs ($p = 0.24$) or complicated UTIs ($p = 0.13$).

Conclusions: Resistance of *E. coli* to fluroquinolones in almost one third of cases shows that the broad use of these drugs needs to be revised. Current use of antibiotics within 3 months, prior hospitalization within 1 year and age over 65 years is a limitation to the use of fluoroquinolones as first choice for empirical therapy in community-acquired UTIs. Nitrofurantoin should be encouraged to use, especially for treatment of non-severe community acquired UTIs.

Carbapenem-resistant Gram-negatives

P1272 Outburst emergence of blaKPC-3-Tn4401a in Madrid (Spain) is associated with a pKPN3/4-like plasmid carried by a newly epidemic ST384-*Klebsiella pneumoniae* clone

T. Curiao, M.I. Morosini, P. Ruiz-Garbajosa, F. Baquero, T.M. Coque, R. Cantón (Madrid, ES)*

Objectives: KPC-2 and KPC-3 carbapenemase-producing *Klebsiella pneumoniae* isolates have been increasingly reported in different continents since first detection in USA in 1996. They have recently emerged in South European countries. We analyzed the first *K. pneumoniae* isolates expressing a KPC enzyme detected in our hospital.
Methods: Five *K. pneumoniae* isolates from 4 patients (Sep-Oct, 2009) hospitalized in different areas [internal medicine (n = 2), oncology (n = 1) and paediatrics (n = 1)] showing decreased susceptibility to carbapenems and a positive modified Hodge test were screened for the presence of carbapenemase encoding genes (PCR, sequencing, hybridization). Clonal relationship was established by PFGE (XbaI and SpeI) and MLST. Plasmid content, plasmid size determination (S1 nuclease), incompatibility group (PCR, hybridization and sequencing), and comparison of RFLP patterns (XhoI, HIndIII) were determined. The genetic environment of blaKPC was characterized by long PCR mapping based on known Tn4401 sequence, RFLP of amplicons and sequencing.
Results: Isolates were recovered from urine (n = 2), pharyngeal aspirate, wound and faeces (n = 1 each). They showed imipenem and meropenem MIC $\geqslant$8 mg/L and were susceptible to aminoglycosides and quinolones (both nalidixic acid and ciprofloxacin). All isolates showed related PFGE patterns and belonged to a newly characterized ST348 clone not previously detected among multi-resistant *K. pneumoniae* isolates from our institution. The blaKPC-3, blaOKP-5 and blaTEM-1 genes were detected in all isolates. They carried two plasmids, one of 70–90 kb (carrying blaKPC-3 and blaTEM-1) and a megaplasmid ranging from 180 to 320 kb. KPC-plasmids showed similar RFLP patterns and sequences highly homologous (97% identity) to pKPN3/pKPN4 from *K. pneumoniae* (GenBank CP000648, CP000649). The blaKPC-3 gene was located on a 10kb-Tn4401 containing a 100 pb-deletion between istB and blaKPC (isoform a, which has been mostly linked to blaKPC-2) in all isolates.
Conclusions: The emergence of a Tn4401-blaKPC-3 associated with a pKPN3/4-like plasmid and a newly defined ST384-*K. pneumoniae* clone was detected. Although KPC-3 has been scarcely reported in Europe when compared with USA, its location in both a widespread transposon and plasmid might fuel its future dissemination.

P1273 Characteristics and outcomes of intensive care unit patients with carbapenem-resistant *Klebsiella pneumoniae* bacteraemia

K. Vardakas, D. Matthaiou, E. Antupa, E. Pappas, G. Kechagioglou, A. Koteli, E. Antoniadou (Thessaloniki, GR)*

Background: Carbapenems are frequently used to treat infections due to extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. Thus, the emergence of infections due to carbapenem-resistant *K. pneumoniae* (CRKp) is a major public health concern.
Objective: To study the characteristics and outcomes of intensive care unit patients with carbapenem resistant *K. pneumoniae* (CRKp) bacteremia.
Methods: A retrospective cohort of patients requiring ICU treatment. The study was conducted in an 8-bed ICU between January 2006 and September 2009.
Results: During the study period 56 patients were diagnosed with CRKp bacteremia. The mean age of patients was 65.8±14.3 years. Thirty-two (57%) were males. The mean APACHE II score was 18.1±7.0. The median duration of hospital stay till bacteremia was 14.5 days. Twenty-nine patients (52%) had bacteremia of unknown origin, 17 (30%) had bacteremia secondary to other infections, and 10 (18%) had catheter-related bacteremia. Thirty-seven (66%) and 22 (39%) patients had previous and concurrent infections, respectively. Forty two (75%) patients died. The univariate analysis showed that APACHE II score ($p = 0.01$), cardiovascular disease ($p = 0.03$), red blood cell transfusion ($p = 0.02$), shock ($p = 0.01$) and acidosis ($p = 0.04$) immediately after the isolation of CRKp were the factors associated with death. None of these variables was an independent predictor of outcome in the multivariate analysis. Correct empirical treatment ($p = 0.02$) and successful outcome of the infection ($p < 0.001$) were the factors associated with survival.
Conclusions: Bacteremia due to CRKp in the ICU is associated with high mortality. Empirical treatment should include coverage against CRKp in ICUs with high prevalence of the infection.

P1274 Multiclonal dissemination of KPC-2-producing *Klebsiella pneumoniae* strains in a Greek hospital

A. Poulou, E. Voulgari, F. Markou, S. Pournaras, G. Vrioni, C. Katsiampeki, M. Lapatsi, A. Tsakris (Serres, Athens, Larissa, GR)*

Objectives: Class A KPC carbapenemases represent a new threat for the antimicrobial treatment of *K. pneumoniae* infections. The increase of KPC-producing *K. pneumoniae* isolates in different hospital settings has been associated with the dissemination of a single clonal type. This genotype conforms to a hyperepidemic clonal type, which has been spread in different countries, including the USA, Israel and Greece. We document the multiclonal dissemination of KPC-producing *K. pneumoniae* in a Greek health care facility.
Methods: During the study period (January 2008-September 2009) 63 *K. pneumoniae* isolates that exhibited reduced susceptibility to carbapenems (MIC >1 mg/L), were recovered from separate patients in our facility. Meropenem discs with and without boronic acid and EDTA were used for phenotypic detection of KPC and MBL genes, respectively and the modified CLSI ESBL confirmatory test was used for the detection of ESBLs among KPC producers. The production of KPC, VIM and ESBL enzymes was genotypically confirmed by PCR and sequencing assays. PFGE was performed to test the clonality of the isolates.
Results: The phenotypic assays showed that 20 isolates contained KPCs and 16 of them ESBLs. Molecular testing revealed that all 20 isolates carried blaKPC-2. Additionally, 7 isolates carried both blaKPC-2 and blaVIM-1 genes; two of them carried also ESBL genes. ESBL-positive isolates carried SHV-12 (n = 16), SHV-5 (n = 1) and CTX-M-15 (n = 1). PFGE revealed 5 clonal types among KPC producers; the major clonal type was detected in 15 isolates and contained two subtypes. The next more frequent type contained 7 isolates with two subtypes, while the remaining types contained from 3 to 1 isolate. The KPC-positive isolates were scattered among patients hospitalized in 8 hospital wards. The major clonal type was responsible for an outbreak among ICU patients. Medical history of the majority of the patients revealed previous hospitalization in different Greek hospitals.
Conclusions: In difference with the situation described in other geographic regions, in our hospital several distinct clonal types of *K. pneumoniae* have incorporated KPC genes. The transfer of patients between hospitals seems to contribute to the dissemination of clonally unrelated KPC-producing *K. pneumoniae* strains. Early laboratory detection and enforcement of infection control measures are needed to prevent the dissemination of KPC-producing clonal strains in our hospital environment.

P1275 Molecular characterization of blaKPC-containing plasmids from the four different clones of *Klebsiella pneumoniae* isolated in Greece

I. Galani, M. Souli, F. Panayea, Z. Chryssouli, H. Giamarellou (Athens, GR)*

Objectives: The aim of our study was the molecular characterization of KPC-producing *K. pneumoniae* isolates reported from our hospital during the 2008 outbreak.
Methods: One representative isolate of each of the four clones and one subclone (Kp 1–5) identified in the outbreak reported in our hospital were further examined. The presence of β-lactamases in these strains was detected by IEF and confirmed by PCR and sequencing. MICs were determined by Etest. Conjugation experiments were carried out by a filter method using a rifampin resistant *Escherichia coli* K12 laboratory strain as a recipient. Transformation experiments were carried out with *E. coli* DH5a laboratory strain. Molecular typing was performed by pulse-field gel electrophoresis of SpeI-restricted genomic DNA. Plasmid analysis was carried out by PFGE of S1 nuclease-digested total DNA as well as RFLP analysis. Aminoglycoside modifying enzymes were determined by a phenotypic method.
Results: The Kp1 belonged to clone A which was the predominant clone of the outbreak. The other four *K. pneumoniae* isolates (Kp2-Kp5) belonged to clones B1, B2, C and D. blaKPC-2 and blaTEM-1-like were identified in all isolates. Kp1 (clone A) and Kp5 (clone D) harboured also blaSHV-12 and blaSHV-11 while Kp3 (clone B2) and Kp4 (clone C) blaCTX-M-15 and blaSHV-11 or blaLEN-19. Kp2 (clone B1) coded also SHV-11. S1 nuclease method showed that all isolates harboured at least two plasmids. Conjugation experiments were repeatedly unsuccessful while transformation was successful for all cases. Plasmid RFLP analysis showed that Kp1 and Kp5 had the same plasmid coding for KPC-2 and TEM-1-like, conferring resistance only to β-lactams and inhibitor combinations. Kp3 and Kp4 harboured the same plasmid coding for KPC-2, CTX-M-15, TEM-1-like and aminoglycoside modifying enzymes (AAC(3)-V), conferring resistance to β-lactams, inhibitor combinations and aminoglycosides. Kp2 harboured a plasmid conferring resistance to β-lactams and inhibitor combinations (coding for KPC-2 and TEM-1-like) which had a different RFLP pattern than the previous plasmids.
Conclusions: KPC-producing Kp is an emerging threat in Greek hospitals. We documented a complex epidemiology of strains causing an outbreak in our hospital.

P1276 Emergence in Rome of an ertapenem-resistant *Klebsiella pneumoniae* clone ST37, carrying a novel OmpK36 porin variant

A. García-Fernández, A. Giordano, C. Venditti, V. Miriagou, C. Papagiannitsis, M. Venditti, C. Mancini, A. Carattoli (Rome, IT; Athens, GR)*

Objectives: In *Klebsiella pneumoniae* carbapenem resistance has been associated with the Extended-Spectrum β-Lactamase (ESBL) production and the loss of the major non-specific porins. Ertapenem resistant (ETP-R) *K. pneumoniae* clone ST37 caused a small outbreak in a hospital in Rome in 2008. This strain carried ESBLs and expressed a novel OmpK36 variant, showing an insertion of two codons in one of the most conserved site of the protein, the so called Loop 3. The objective of the study was to determine the diffusion of this clone within the hospital.
Methods: 60 multidrug resistant *K. pneumoniae* strains (one per patient) were collected in 2008–2009. 42/60 strains showed ETP-R. Representative strains from this collection were tested by PFGE, MLST, plasmid typing, β-lactamase identification, SDS-PAGE and analysis of the ompK36 gene. A specific PCR-based test was devised for a rapid screening of the ompK36V variant gene.
Results: All the ETP-R strains were ESBLs positive and exhibited ETP-MIC $\geqslant$8 mg/L. 32/42 showed, IPM-MIC $\leqslant$1.0 and MEM-MIC =1.0 mg/L, 9 exhibited IPM-MIC $\leqslant$1.0 mg/L and MEM-MIC $\geqslant$16 mg/L and one strain was resistant to the three carbapenems. In the latter the loss of the OmpK36 by an early termination of translation was detected. A PCR-based test positively amplified the ompK36V variant in strains showing the ETP-MIC$\geqslant$8.0, IPM-MIC$\leqslant$1.0 mg/L and MEM-MIC =1.0 phenotype. These strains belonged to ST37 and were recovered from patients in different wards, suggesting a wide circulation of the *K. pneumoniae* clone within the hospital. ompK36V was not amplified from strains showing MEM-MICs higher than the clonal strains, suggesting a different carbapenem resistance mechanism in these strains. All carbapenem susceptible strains tested were negative for the ompK36V.
Conclusion: This is the first report of a natural variant of the OmpK36 protein associated to the ETP-R in a *K. pneumoniae* clone disseminating within the hospital. The OmpK36 represents the main pathway for the penetration of ertapenem into the bacterial periplasmic space. In particular, the ertapenem has a net negative charge and two carboxylic groups are able to form hydrogen bonds with the basic residues of the channel. Amino acids mutations in the transmembrane β-strand loop 3 (L3) that constitutes the channel eyelet of enterobacterial porins are affecting the channel properties, reducing the uptake of carbapenems and cephalosporins.

P1277 Carbapenem-resistant *K. pneumoniae*: epidemiology and molecular analysis of a 6-year period (2004–2009)

H. Moraitou, M. Makarona, A. Mavrommati, I. Galani, N. Soufla, P. Tsarpali, A. Georgousi, C. Anthoulaki, V. Spyropoulos, S. Kanavaki (Athens, GR)*

Objectives: Carbapenem resistance in *K. pneumoniae* (CRKP) strains is emerging worldwide. The aim of this study was to retrospectively analyze the epidemiology and mechanisms of resistance of CRKP, in our Hospital throughout 2004–2009.
Materials and Methods: All CRKP isolates were collected since 2003, when the first resistant strain occurred in our hospital. From January 2004 to June 2009, 209 single patient/ CRKP strains were isolated both from ICU and non-ICU patients. Susceptibility testing was performed by Kirby Bauer, E-test and Vitek2 automated system, according to CLSI guidelines. All strains were further tested for the production of various β-lactamases, both phenotypically (VIM EDTA test, E-test imipenem, Hodge test, boronic acid/imipenem disc test) and by PCR for the detection of blaVIM and blaKPC gene. Rep-PCR was performed to investigate the clonal spread of isolates.
Results: The overall emergence of CRKP strains throughout the study period was 16.4% (187/1140 *K. pneumoniae*). In 2004, 18 CRKP were isolated out of 199 *K. pneumoniae* strains (9.0%). The following years (2005–08) the rate was 10.1%, 8.8%, 10.4%, 25.6% respectively, and up to 26.7% in the first semester of 2009. All CRKP isolates throughout 2004 to 2007 harbored the blaVIM gene (100%), while blaKPC emerged up to 23.3% (17/73) in 2008 and 73.7% (14/17) in 2009. Rep-PCR proved the clonal spread of resistant strains. The most of the strains originated from ICU patients (57.7%), although the rate decreased from 88.8% in 2004, to 45.2% in 2008 and 47.0% in the first semester of 2009.
Conclusions: There is an increasing rate of CRKP prevalence in our Hospital since 2004, when the first metallo-β lactamase producers emerged. It is of interest, that in 2004–07, when only blaVIM harbouring strains were detected, the rate of CRKP remained rather stable. When KPCs emerged in 2008, they dominated and were rapidly disseminated in several units, making KPC production the commonest mechanism of carbapenemase resistance in the Hospital the last years. The infection control committee should be alerted in any case and measures should be taken to avoid further dissemination. The microbiology laboratory could contribute to this purpose, by including phenotypic investigation of all multidrug resistant Enterobacteriaceae in routine practice.

P1278 The ongoing challenge of acquired carbapenemases: simultaneous production of VIM-1 and KPC-2 in *Klebsiella pneumoniae*

V. Miriagou, A. Zioga, E. Douzinas, M. Tsakiri, E. Tzelepi, L. Tzouvelekis, N. Legakis (Athens, GR)*

Objectives: VIM-1 and KPC-2 b-lactamases have been established among Enterobacteriaceae in Greek hospitals. We report here on the characteristics of 17 *K. pneumoniae* isolates of which five produced VIM-1, eight KPC-2 and the remaining four were positive for both carbapenemases.
Methods: Seventeen *Klebsiella pneumoniae* isolates exhibiting imipenem MICs >0.5 mg/L were derived from clinical material in Evgenidion hospital in Athens during March-August 2009. Carbapenemase activities were examined by conventional synergy techniques and biochemical methods (isoelectric focusing and photometric assays using imipenem as an indicator substrate). Entire blaVIM and blaKPC genes were amplified by PCR and sequenced. Isolates were typed by PFGE following digestion of genomic material with XbaI. Medical records of the respective patients were followed throughout the study period.
Results: PCR and biochemical assays documented that 13 isolates produced either KPC-2 (n = 8; group 1) or VIM-1 (n = 5, group 2) exhibiting the characteristic phenotypic traits conferred by the respective b-lactamases. The remaining four isolates (group 3) exhibited a resistance phenotype reminiscent of KPC production, yet they were positive for both KPC-2 and VIM-1. All group 3 isolates, while positive by the EDTA-based inhibition tests, appeared negative in the synergy tests combining carbapenems with boronates. This can be probably attributed to a masking effect of the VIM-1 b-lactamase. It could be hypothesized that the contribution of the latter enzyme in carbapenem resistance was more important than that of KPC-2. Nevertheless, total carbapenemase activities and carbapenem MICs did not significantly differ between the three groups.
PFGE typing classified the studied isolates into two distinct clusters, A (including group 1) and B (including both groups 2 and 3). Each cluster comprised isolates with highly similar macrorestriction patterns strongly suggesting common origins. It is therefore proposed that group 3 isolates evolved from an endemic VIM-1-producing strain through blaKPC-2 acquisition likely facilitated by a plasmid.
Conclusion: There have been indications in Athens hospitals for a partial "substitution" of KPC-for-VIM-producing enterobacteria. Nevertheless, this study documents establishment of at least one *K. pneumoniae* strain expressing both carbapenemases. Diffusion of such strains may be of serious consequences.

P1279 Interspecies spread of KPC carbapenemase in a single patient

I. Galani, M. Souli, F. Panayea, Z. Chryssouli, H. Giamarellou (Athens, GR)*

Objectives: We described an ICU patient who was sequentially colonized by two species of carbapenem-resistant Enterobacteriaceae, which produced KPC-2 β-lactamase and were further characterized.
Methods: A *K. pneumoniae* (Kp) and an *E. aerogenes* (Ea) carbapem-resistant isolates as well as a susceptible Ea isolated from the same patient were included in the study. The presence of β-lactamases in these strains was detected by IEF and confirmed by PCR and sequencing. MICs were determined by Phoenix automated system. Conjugation experiments were carried out by a filter method using a rifampin resistant *Escherichia coli* K12 laboratory strain as a recipient. Transformation experiments were carried out with *E. coli* DH5a laboratory strain. Molecular typing was performed by PFGE of SpeI-restricted genomic DNA. Plasmid analysis was carried out by PFGE of S1 nuclease-digested total DNA as well as RFLP analysis.
Results: The patient was admitted for pneumonia in Attikon University hospital ICU at 26 September 2008 and was colonized on day 10 by a KPC-positive Kp which was thought to be acquired by horizontal transmission. She developed bacteremia by this strain 17 days after her first colonization. Ea KPC-positive-strain was first isolated three months after the patients ICU admission, while earlier the patient was colonized by a susceptible Ea strain. The first Kp isolated from faeces and the Kp isolated from blood were identical and belonged to clone A which was the predominant clone of the outbreak that was reported in our hospital during 2008. These isolates were resistant to β-lactams, inhibitor combinations, amikacin and cotrimoxazole, with imipenem (IMP) MIC 8 mg/L. Ea KPC producing strain belonged to the same clone as the sensitive strain. It was resistant to β-lactams, inhibitor combinations, amikacin and colistin, with IMP MIC >8 mg/L. The Kp isolates harboured blaKPC-2, blaSHV-12, blaSHV-11 and blaTEM-1-like while the Ea harboured blaKPC-2, and blaTEM-1-like. S1 nuclease method showed that these isolates harboured three and two plasmids, respectively. Conjugation experiments were repeatedly unsuccessful while transformation was successful for both cases. Plasmid RFLP analysis showed that Kp and Ea had the same plasmid coding for KPC-2 and TEM-1-like, conferring resistance only to β-lactams and inhibitor combinations (IMP MIC 2 mg/L).
Conclusions: We documented the spread of a plasmid harbouring blaKPC-2 from a Kp to an Ea isolate colonizing the same patient.

P1280 Metallo-β-lactamases among Enterobacteriaceae from routine samples in an Italian tertiary care hospital and long-term care facilities during 2009

R. Aschbacher, L. Pagani, G. Spoladore, P. Innocenti, L. Moroder, R. Meyer, E. Pagani, D. Livermore, C. Larcher (Bolzano, IT; London, UK)*

Objectives: Metallo-β-lactamase (MBLs) producing Enterobacteriaceae have been isolated since 2005 in a 850-bed tertiary care hospital and associated long-term care facilities, serving an area of 200,000 inhabitants, in Bolzano, Italy. All routine isolates in 2009 were screened for MBLs and case details were reviewed for source patients.
Methods: Basic screening of Enterobacteriaceae with the Vitek 2 automatic system using cards that included an ESBL-confirmation test. Carbapenemase and MBL activity were sought with Hodge-cloverleaf and EDTA-synergy tests, respectively; VIM- genes were sought by PCR. Clinical data were collected retrospectively.
Results: The prevalence of MBL producing Enterobacteriaceae from in- and out-patient samples during the first 9 months in 2009 was 0.67% (28/4200), almost exactly the same as in 2008 (0.65%; 36/5,500). All the producer strains from both years had VIM enzymes. Prevalence rates in 2009 varied from 0.4% (13/3,000) for *E. coli*, 0.8% (1/126) for *E. cloacae*, 1.6% (6/372) for *K. pneumoniae*, 2.2% (1/46) for *C. freundii,* 2.3% (1/44) for *E. aerogenes,* 2.3% (3/129) for *K. oxytoca* up to 3.1% (2/64) for *M. morganii,* together with 1/1 for *C. amalonaticus.* These 28 MBL-producers were from 26 patients (one patient had MBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca*) in 8 hospital departments (14 isolates), 4 long-term care facilities (7 isolates) or outpatients (7 isolates); risk factors in the latter group remain unspecified. Seventeen were from urine samples (8 *E. coli*, 4 *K. pneumoniae*, 2 *M. morganii,* 1 *E. cloacae*, 1 *E. aerogenes* and 1 *C. amalonaticus)*; two were from blood (1 *K. pneumoniae* and 1 *C. freundii)*, 7 were from respiratory specimens (4 *E. coli*, 2 *K. pneumoniae* and 3 *K. oxytoca*); the remaining 2 were from peritoneal fluid (*E. coli*) and an ulcer (*K. pneumoniae*). Altogether 88 MBL-producing Enterobacteriaceae belonging to 10 species have been isolated since 2005.
Conclusion: Isolation of VIM MBL-producing Enterobacteriaceae from eight hospital departments and four long-term care facilities or nursing homes confirms the endemic persistance of this mode of resistance and its encoding elements in the Bolzano health care district.

P1281 Dissemination of a clone KPC-3-producing *Klebsiella pneumoniae* in hospitals, Bogota, Colombia

S.Y. Saavedra, C.A. Alvarez, S.I. Cuervo, N. Olarte, J.A. Escobar, A.L. Leal, J.S. Castillo Londoño (Bogotá, CO)*

Objective: Typing molecular from isolates carbapenem-resistant *Klebsiella* pneumonia and to detected microbiological and molecular ocurrence from carbapenemases.
Methods: Fifty-eight isolates carbapenem-resistant *K. pneumoniae* recovered from patients in six hospitals of Bogota were collected from 2008 to 2009. The identification and the susceptibility testing were performed with automated system; imipenem, meropenem and ertapenem susceptibilities were determined by disk diffusion and the carbapenemases production was confirmed with the modified Hodge test. The detection of genes encoding metallo and KPC-type carbapenemases was performed by PCR using specific primers for blaIMP, blaVIM and blaKPC genes. Amplification products were analyzed by restriction disgestion of the amplicom with the enzymes BstNI and RsaI and confirmed by sequentiation. The characterisation molecular by assessment the relatedness of the isolates was realised by ERIC-PCR, the cluster analysis was realised with Fingerprinting II software (Bio-Rad).
Results: All Isolates were resistant to ertapenem, ceftriaxone, ceftazidime, cefotaxime, eleven isolates were susceptible to imipenem and seven to meropenem. All isolates were phenotypically identified as possible carbapenemases producers and the presence of blaKPC gene was confirmed by PCR. Sequencing and restriction test confirmed the presence of KPC-3 in all isolates. Typing results showed presence of a clone predominant conformed by fifty-seven isolates from five hospitals and a fingerprint unique genomic.
Conclusions: Our results indicate the ocurrence of a clone KPC-3 producing *K. pneumoniae* in five hospitals from Bogota. This is the first report of dissemination of KPC-3 in hospitals from Colombia.

P1282 First two isolates of metallo-β-lactamase-producing *Serratia marcescens* in the Czech Republic

E. Chudackova, M. Fridrichová, E. Bebrova, P. Urbaskova, H. Zemlickova, J. Hrabák (Plzen, Prague, CZ)*

Objectives: Resistance to carbapenems in Enterobacteriaceae is mediated by the production of various carbapenemases and/or by the decreasing permeability of the outer cell membrane together with a hyperproduction of extended spectrum or AmpC β-lactamases. In the Czech Republic, Gram-negative bacteria with acquired metallo-β-lactamases (MBLs) are sporadic, with the first isolates of IMP-7 producing strains of *Pseudomonas aeruginosa* identified in 2008. The first two strains of *Serratia marcescens* resistant to carbapenems were sent to the National Reference Laboratory for Antibiotics at the end of 2008.
Methods: Production of β-lactamases was analyzed by the modified double disk synergy test. Carbapenemase activity was detected by imipenem hydrolysis assay. β-lactamases were visualized by isoelectric focusing followed by bioassay. Genes of β-lactamases were amplified and sequenced. The β-lactamase gene environments were determined as well as a replicon typing of plasmids was performed. Strains were compared by PFGE.
Results: The strains were isolated in different hospitals in the central region of the Czech Republic. Both strains were highly resistant to antibiotics; produced VIM-1 carbapenemase carried out on class 1 integron and intrinsic AmpC β-lactamase. The strains represented different PFGE profiles. One isolate also produced β-lactamases with pIs of 5.9 and 7.9 that were identified as TEM-6 (extended-spectrum β-lactamase) and DHA-1 respectively. blaDHA-1 was carried out on the complex class 1 integron with a different genetic organization than observed in *Klebsiella pneumoniae* strains endemic in some Czech hospitals. β-lactamases were localized on the plasmids with FIB, L/M and A/C replicons.
Conclusions: This is the first observation of MBL producing enterobacteria in the Czech Republic. To our knowledge, this is also the first identification of an inducible DHA-1 AmpC in *S. marcescens*.
This work was financed by a research project grant NS9717–4/2008.

P1283 First outbreak of carbapenemase-producing *Klebsiella pneumoniae* in a tuberculosis care facility in the Netherlands

N. Meessen, J. Wiersinga, K. van der Zwaluw, R. van Altena (Groningen, Bilthoven, NL)*

Background: Carbapenems are drugs of choice for serious infections caused by ESBL producers. *Klebsiella pneumoniae* carbapenemase (KPC) is a β-lactamase which confers resistance to all β-lactam antibiotics, including carbapenems. Clonal expansion of KPC producing *K. pneumoniae* (KPC-Kp) is emerging in Europe. KPC producing strains are particularly alarming due to the multidrug resistance which is carried on plasmids.
Methods: Identification and susceptibility testing of isolates were performed with Vitek2 and Etest. Carbapenemase and other β-lactamase genes were identified by PCR. PFGE was used for epidemiological comparisons. Medical records of patients that harboured KPC producers were retrospectively reviewed to ascertain factors that may have influenced the acquisition and persistence of the organism.
Results: After admission to the university hospital, a multidrug-resistant Kp was isolated from a wound of a leukaemic patient. The patient was isolated and no intrahospital transmission was observed. However, screening of all patients in the 20-bed extramural TB-care facility where the patient initially stayed, revealed another 6 patients with carbapenem-resistant Kp. Cohort isolation was applied and hygiene measures intensified. Due to poor compliance the KPC-Kp could spread further. In total 13 other patients proved to be colonized with KPC2-Kp, Environmental cultures also yielded KPC-Kp, despite extra cleaning.
Isolates expressed high-level resistance to all non-carbapenem β-lactam antibiotics, aminoglycosides (exept for gentamycin), cotrimoxazol, aztreonam, and quinolones. MICs of imipenem and meropenem ranged from 1.5 to 32 microg/ml. MIC90 values for colistine and tigecycline were 1 microg/ml and 0.75 microg/ml, respectively.
Prior to acquisition, almost all patients were on TB medication, in some cases including moxifloxacine and amikacin. Most patients were asylum-seeker and originated from non-Europe countries. Some of the African patients travelled to The Netherlands via Greece where KPC-Kp is highly-endemic.
Conclusions: Social and cultural aspects of behaviour contributed to the poor hygiene-compliance of the patients involved in this first outbreak of KPC-Kp described in The Netherlands. Since treatment options for infections with KPC-producers are extremely limited, spread should be avoided. Strict infection control measurements are effective. To achieve more awareness, local and national surveillance is needed.

P1284 The first carbapenemase-producing *Klebsiella pneumoniae* strains in the Netherlands are associated with international travel

J.W. Cohen Stuart, G. Voets, D. Versteeg, J. Scharringa, M. Tersmette, E. Roelofsen, A.C. Fluit, M. Leverstein-van Hall (Utrecht, Nieuwegein, Groningen, NL)*

Objectives: Carbapenems are often used as a last resort for treating serious infections caused by multidrug-resistant Gram-negatives (MDR-GN). Carbapenemase-producing Enterobacteriaceae are emerging worldwide, but, until now, none have been reported in The Netherlands. In this study, we determined whether the elevated carbapenem MICs of three *K. pneumoniae* isolates (KPN) isolated in 3 different Dutch hospitals were due to carbapenemase production. Isolate KPN-1 en KPN-2 were isolated from 2 patients that had recently returned from a holiday trip in India. During their trip they had not visited any medical care facility but had taken unspecified tablets against enteritis. The KPN-3 was from a patient transferred from a Greek hospital to which he had been admitted during his holiday.

Methods: MICs were determined using broth micro-dilution (MBD), VITEK and Etests (bioMérieux, Paris, France). For phenotypic confirmation a modified Hodge test, imipenem-EDTA and imipenem-boronic acid (BA) synergy tests were performed. Genotypic confirmation was done by PCR and sequencing.
Results: Table 2 shows the results of the susceptibility tests for 3 carbapenems and the confirmation tests. KPN-1 and 2 were only susceptible to colistin, tigecycline and chloramphenicol. KPN-1 contained a New-Delhi metallo-carbapenemase gene (NDM-1), a CTX-M-1 group ESBL gene, and two AmpC genes (CMY-6, DHA-1) while in KPN-2 only NDM-1was detected. These findings are in line with previous reports on NDM-1 positive isolates obtained from patients with prior medical treatment in India.
KPN-3 was only susceptible to gentamicin, tobramycin, amikacin, colistin, and tigecycline. In this strain a KPC-2 gene and a SHV-12 ESBL gene were identified, consistent with the hyperepidemic clone described in Greek hospitals.
Conclusion: The first carbapenemase-producing KPN in The Netherlands were travel-associated. Patients undergoing medical treatment in countries with high prevalence of MDR-GN have an increased risk of acquiring these micro-organisms. In low endemic countries, the in-hospital spread of MDR-GN may be prevented by identifying those patients treated in high risk countries, and to screen them at admission. However, when travelers may also acquire acquire carbapenemase producing isolates without medical treatment, this control strategy may fail, resulting in an uncontrollable introduction and spread of carbapenemases in low endemic countries such as The Netherlands.

				Meropenem			Imipenem		Ertapenem		
	Hodge test	Imi-EDTA	Imi-BA	MBD	Etest	Vitek	MBD	Etest	Vitek	MBD	Etest
KPN-1	+/–	+	–	>32	>32	4	2	>32	8	1	>32
KPN-2	+	+	–	>32	>32	nd	8	>32	nd	>8	>32
KPN-3	+	–	+	>32	>32	>16	8	>32	8	>8	>32

P1285 Molecular characterization of VIM-producing *Klebsiella pneumoniae* from Scandinavia

Ø. Samuelsen, M. Toleman, V. Hasseltvedt, K. Fuursted, T.M. Leegaard, T. Walsh, A. Sundsfjord, C.G. Giske (Tromsø, NO; Cardiff, UK; Lillehammer, NO; Aarhus, DK; Oslo, NO; Stockholm, SE)*

Objectives: The emergence and dissemination of MBL-producing *Klebsiella pneumoniae* has become a public health problem in certain countries. The aim of this project was to characterise MBL-producing *K. pneumoniae* isolates identified in Scandinavia.
Materials and Methods: The study included eight MBL-producing isolates of *K. pneumoniae* identified in Scandinavia (Norway, Sweden and Denmark) year 2005–08. The isolates were characterised with respect to resistance profile (Etest), clonal relatedness (multi-locus sequence typing) and by PCR assays for the presence of MBLs, ESBLs, plasmid-mediated quinolone resistance (qnr), 16S rRNA methylases and for plasmid-replicons. Plasmid transfer was assessed by conjugation and transformation experiments. Epidemiological data were collected retrospectively.
Results: Epidemiological data revealed that 7/8 isolates were associated with recent hospitalization outside Scandinavia (Greece, n = 6 and Turkey n = 1). All eight isolates harboured blaVIM and expressed a multi-drug resistant phenotype. MLST analysis showed that four isolates belonged to ST147 while the other isolates belonged to ST36, ST272, ST273 and ST383. All isolates supported *in vitro* transfer of blaVIM consistent with plasmid localisation. PCR-replicon typing of transconjugants/transformants (TC/TF) suggested that blaVIM was associated with IncN (n = 3) and A/C (n = 1) replicons. Four TC/TF were negative for the eighteen tested replicons. ESBL-genes were found in six isolates; CTX-M-3 (n = 1), CTX-M gr. 1 (n = 1), SHV-5 (n = 2), and SHV-12 (n = 2). The 16S rRNA methylase armA and the plasmid-mediated quinolone resistance gene qnrS was identified in one and two isolates, respectively. Co-transfer of blaVIM, blaCTX-M-3, armA, and qnrS was observed.
Conclusion: Emergence of MBL-producing *K. pneumoniae* in Scandinavia is still related to strain import. Characterisation of the isolates shows that blaVIM is co-localised on plasmids along with other major resistance determinants resulting in multi-drug resistant isolates.

P1286 Infections caused by KPC-2-producing *K. pneumoniae*: the impact of antimicrobial treatment on patients' outcome

O. Zarkotou, G. Chrysos, S. Pournaras, E. Voulgari, T. Pittaras, D. Voutsinas, A. Prekates, K. Digalaki, A. Tsakris (Athens, Larissa, GR)*

Objectives: KPC-producing *K. pneumoniae* (KPC-KP) infections pose a new therapeutic challenge. The results of a cohort study regarding antimicrobial treatment of KPC-KP infections and patients' outcome are presented.
Methods: All patients identified as infected by genotypically-confirmed KPC-KP between May 2008 and September 2009, were evaluated. Clinical and laboratory records were reviewed.
Results: 63 patients (37 males; median age, 72 years) were infected by KPC-2-producing KP. The mean time of hospital stay prior to isolation was 19.8 days, the majority of patients had >2 comorbidities (38/63), were mechanically ventilated at isolation (35/63) and had prior hospitalizations (42/63). Bloodstream infections were prevalent (38/63) followed by urinary tract (11/63), skin and soft tissue (7/63), hospital-acquired pneumonia (5/63), intra-abdominal (1/63) and CNS infections (1/63). The overall mortality was 46% and infection mortality was 28.6%. Appropriate antimicrobial treatment (defined by *in vitro* activity and treatment duration >3 days) was received by 40 patients; among those, infection mortality (IM) was 15% and microbiological response was observed in 23/25 patients having cultures available. IM was significantly higher (52.2%; p = 0.002) among patients who did not receive appropriate treatment. The most commonly used treatment regimen was tigecycline (22/40), either as monotherapy (10/40, IM 10%), or in combination with: colistin (6/40, IM 16.7%), gentamicin (4/40, IM 0%), meropenem (1/40, IM 0%), and colistin plus meropenem (1/40). Colistin was administered as monotherapy to 5/40 patients (IM 20%), while 5/40 and 2/40 patients received colistin in combination with gentamicin (IM 20%) and meropenem (IM 50%) respectively. No significant differences regarding IM were detected when administration of one active antimicrobial (IM 17.6%) was compared with combination schemes (IM 13%, p = 0.67).
Conclusion: KPC-KP has emerged as a common cause of infections associated with high infection mortality, among severely ill patients in our region. Appropriate antimicrobial treatment contributes significantly to a favorable patient's outcome.

P1287 Outbreak of multiresistant *Klebsiella pneumoniae* producing DHA-cephamycinase and resistant to carbapenems due to porin loss in an intensive care unit

M. Lorenzo, M.R. Gómez-Gil, Y. Moatassim, E. Salvarelli, B. Galvan, A. Gutiérrez, J. Mingorance (La Paz, ES)*

Objective: Description of an outbreak of *K. pneumoniae* producing DHA cephamycinase associated with an OXA extended spectrum β-lactamase (ESBL) and resistance to carbapenems due to loss of porins in the burn intensive care unit of Hospital Universitario La Paz (Madrid) between April 2008 and June 2009.
Materials and Methods: We studied 26 isolates of *K. pneumoniae* isolated from 21 patients of the burn intensive care unit of HU La Paz (Madrid, Spain) between April 2008 and June 2009 with resistance to cefotaxime, cefoxitin and cefepime. The isolates were obtained from blood (13), urine (7), burns (2), bronchoalveolar lavage fluids (2), catheter (1) and tracheal fluid (1). The identification and antibiotic susceptibility assays were done by MIC/ID Wider® (Soria Melguizo) or Vitek ® (Biomerieux) using CLSI breakpoints. ESBLs were confirmed in all isolates with CMI > 1 microg/mL to cefepime using E-test® with cefepime and cefepime with clavulanic acid. Hodge's test was done to rule out carbapenemases. Specific PCR reactions were done to detect

the genes coding for β-lactamases of the SHV, OXA, TEM, CTX-M groups and the plasmidic AmpC families (MOX, CIT, DHA, ACC, EBC, FOX). Clonality was assessed by Rep-PCR using the DiversiLab system. The presence of porins was analyzed by SDS-PAGE analysis of outer membrane preparations.
Results: All the isolates were resistant to cephalosporines and cephamycin and to β-lactams with β-lactamase inhibitors. Among the 26 isolates, 24 had an OXA ESBL gene, and 23 of these had a DHA-1 gene. These 23 isolates were clonally related. Ten isolates had intermediate susceptibility or were resistant to carbapenems according to CLSI's criteria. Loss of the porins OmpK 35 and 36 was observed in all ten. Hodge's test was negative in all the cases, as were KPC-specific PCR assays. All the isolates had resistance to quinolones, aminoglycosides and trimethopim-sulfamethoxazole.
Conclusions: We describe the first outbreak in Spain of *K. pneumoniae* producing OXA ESBL and DHA cephamycinase with resistance to all the β-lactams, including combinations with β-lactamase inhibitors, quinolones, gentamycin and tobramycin and trimethopim-sulfamethoxazole. Development of resistance to carbapenems by loss of OmpK35/36 porins was observed during the outbreak.

P1288 Imipenem-resistant *K. pneumoniae* and *E. coli* in 49 German ICUs, 2005–2008

E. Meyer, F. Schwab, B. Schroeren-Boersch, P. Gastmeier (Berlin, Freiburg, DE)*

Objectives: To analyse imipenem resistance (imiR) in *K. pneumoniae* and *E. coli* in German intensive care units (ICUs) participating in the SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units) and to look for temporal changes.
Methods: Prospective unit and laboratory based surveillance in 49 German ICUs from 2005–2008. The data were specified as imipenem resistant by the clinical laboratory using interpretive criteria recommended by CLSI or DIN. The proportion of non-duplicate resistant isolates (RP) and resistance densities (RD; i.e. the number of resistant isolates of a species per 1000 patient days) were calculated. Resistance data were tested by incidence density test.
Results: 49 ICUs reported data on 3128 *K. pneumoniae* and 8100 *E. coli* isolates tested to imipenem. In 2008, the pooled mean of imiR *K. pneumoniae* was 1.1% corresponding to 7 resistant isolates and was 0.1% (n = 2) of imiR *E. coli*. Two of the 7 isolates were tested and identified as KPC-producing bacteria by molecular confirmation tests.
From 2005–2007, the burden of resistance i.e. imiR *K. pneumoniae* per 1000 pd significantly increased from 0.011 to 0.047 in 2008 (p = 0.009). RD of imiR *E. coli* was 0.019 in 2005 and 0.013 in 2008.
Conclusion: There was a significant increase imiR *K. pneumoniae* in 2008 compared to the previous years indicating that KPC producing *K. pneumoniae* have arrived in German ICUs.

Miscellaneous – antibacterial susceptibility and drug interaction

P1289 Impact of a hypoxic environment on the efficacy of antimicrobials to intracellular pathogens

K. Shima, J. Gieffers, W. Solbach, J. Rupp (Lubeck, DE)*

Objectives: Chlamydiae are obligate intracellular pathogens that cause infections of the respiratory tract, the urogenital tract and the eyes. Currently available antimicrobials are effective in treating acute infections with *C. pneumoniae* (Cpn) or *C. trachomatis* (Ctr), but fail to eradicate persistent chlamydial infections in diseased organs. We found out recently that a microenvironment of low oxygen, that can be found even under physiological conditions in the urogenital tract and the lung, strongly induces chlamydial growth and progeny. The aim of this study was to investigate the activity of different antimicrobials on intracelllular chlamydial growth under hypoxic conditions.
Methods: We determined the MIC of doxycyclin, rifampin, erythromicin and moxifloxacin on chlamydial growth in HEp-2 cells under normoxic and hypoxic (2% O2) conditions. Uptake kinetics of C14-labeled moxifloxacin were monitored to assess changes in the intracellular accumulation under hypoxia. To analyze differences in the antimicrobial efficacy to different chlamydial developmental stages time-kill curves were performed.
Results: Culture in hypoxia strongly enhanced chlamydial growth and progeny to a more than 3-fold increase compared to normoxic conditions. Nevertheless, MIC for doxycyclin, rifampin, erythromicin and moxifloxacin on Cpn and Ctr did not differ between normoxia and hypoxia. Using C14-labeled moxifloxacin we could prove that intracellular uptake and accumulation was not affected by a low oxygen environment, showing high intracellular concentrations of the drug within 5 min after stimulation. Interestingly, drug-dependent differences to kill Ctr in the early phase of the developmental cycle (2 to 8 h p.i.) were observed between normoxia and hypoxia. Thus, reduction in chlamydial growth was less effective in doxycyline (2 μg/mL) treated cells when cultured under hypoxia than in normoxia. No differences were observed in cells treated with rifampin (8 μg/mL).
Conclusion: Low oxygen concentrations have a strong impact on the transcriptional activity and metabolism of host cells that have to be considered in testing antimicrobial efficacy to intracellular pathogens. We suggest to perform time-kill curves instead of standard MIC testing to calculate efficacy of antimicrobials under low oxygen concentrations. Further studies will show whether reduced antibiotic efficacy under hypoxia contributes to the emergence of persistent chlamydial infections in humans.

P1290 Antimicrobial effects of different wound dressings: an *in vitro* study

S. Baka, E. Kouskouni, D. Rizos, S. Demeridou, Y. Skalkidis, V. Arapoglou (Athens, GR)*

Objectives: Wound dressings have been developed for several decades to keep the wound clean by absorbing the exudate, thus contributing to the healing process. They help in the treatment of moderately to highly exuding leg ulcers mostly when healing is delayed due to the bacteria present. This study aimed to compare the effect of five commercially available wound dressings, *in vitro*, on the survival and proliferation of five different microorganisms frequently isolated from leg ulcers in our hospital.
Methods: Two protocols were used to test first, the ability of each dressing to inhibit bacterial growth on an already inoculated surface and second, to resist bacterial colonization after impregnation with the same concentration of each of the microorganisms tested. The antimicrobial activity of Contreet / Biatain Ag (a silver-containing foam dressing) and Biatain – Ibu (a foam dressing containing ibuprofen) was compared to Biatain (a plain foam dressing) (all foam dressings from Coloplast A/S, Humlebaek, Denmark) serving as control, while two gauze dressings (both from Dermagenics Europe BV, Kaatsheuvel, The Netherlands), MelMax® [impregnated with a mixture of metalloproteinase-regulating Poly Hydrated Ionogens (PHI-5) and buckwheat honey] and PHI – 5® (DerMax®) (containing zinc and rubidium ions) were compared to sterile gauze used in the operation rooms which served as a general control and as specific control for the 2 types of gauze dressings. Based on the prevalence in our hospital, five microorganisms were used to test the ability of the wound dressing to resist infection in both protocols: *Staphylococcus epidermidis*, ATCC 35984, *S. aureus*, ATCC 29213, Clinical isolate of methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, ATCC 35218, *Pseudomonas aeruginosa*, ATCC 27853.
Results: In both protocols Contreet/Biatain Ag foam dressing displayed antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* for 9 days, *Staphylococcus epidermidis* for 10 and 7 days, respectively, *Staphylococcus aureus* growth was inhibited for 10 and 6 days, respectively, while MRSA for 11 and 10 days, respectively. Two other dressings, MelMax® and PHI-5® inhibited MRSA and *S. epidermidis* growth, respectively, for 4 days.

Conclusion: Contreet/Biatain Ag foam dressing displayed a broader antibacterial activity in both protocols performed, suggesting that it can be left in place for a longer period of time.

P1291 *In vitro* activity of tigecycline against *Brucella* sp. isolated from 1997 to 2009

S. Al Johani, Z. Memish (Riyadh, SA)*

Background: Brucellosis is an endemic zoonotic infectious disease in Saudi Arabia. To date, there is no optimum antibiotic therapy for brucellosis due to relatively high rates of relapse and treatment failure. The use of new antibiotics, such as tigecycline, may hold future promise.
Objective: The aim of this study was to investigate *in vitro* activity of tigecycline (TIG) against *Brucella* spp isolated at Microbiology Laboratory, King AbdulAziz Medical City over the last 12 years.
Material and Methods: A total of 471 *Brucella* strains, isolated from blood and sterile body fluid and saved at −80 degrees from January 1, 1997 to November 2009, was sub cultured twice then tested against TIG using E-test (AB Biodisk, Sweden ®), and the minimal inhibitory concentration (MIC) was measured, along with MIC50 and MIC90.
Results: A total of 471 *Brucella* strain sub cultured, out of the 471, there were 8 isolates excluded because it fail to grow. Of the remaining 463 isolates were tested against TIG using E-test, the MIC were determined for each isolates. MIC range (0.064–0.125 μg/ml), MIC50 (0.064 μg/ml) and MIC90 (0.094 μg/ml). Out of 464 isolates, there were 286 isolates (61.77%) has an MIC below 0.125 μg/ml, and the rest 176 (38.01%) has an MIC between (0.250–1.50 μg/ml).
Of the 287 presumed susceptible strains, there were 136 isolates (47.39%) where their MIC below (0.064 μg/ml) and 151 (52.61%) has MIC between (0.064–0.125 μg/ml).
Conclusion: The results of this *in vitro* study suggest that tigecycline has a promising future to be used as a therapeutic alternative for brucellosis. These observations need to be supported with clinical trials. This is the first study to analyze a large number of brucella isolates at our region.

P1292 Antibiotics effect on *S. aureus* adhesion to human fibronectin

O. Dumitrescu, A. Moulay, A. Tristan, M. Bes, F. Vandenesch, J. Etienne, G. Lina (Lyon, FR)*

Background: *Staphylococcus aureus* is a human pathogen responsible of a large variety of severe infections. Its pathogenicity is strongly related to the production of many virulence factors. Among these, fibronectin binding molecules (FnBPA and FnBPB) are involved in the invasion and the intracellular passage of *S. aureus*, causing persistent infections non-responding to usual antibiotic treatment. Fibronectin binding molecules are major determinants of the pathogenesis of staphylococcal endocarditis and osteoarthritis. Unsuccessful outcome in these infections may be linked to the impaired diffusion of antibiotics at the infection site. In this work, we explored the effect of main anti-staphylococcal antibiotics, at subinhibitory concentrations, on *S. aureus* adhesion to human fibronectin.
Methods: We assayed for the adhesion to fibronectin of six different *S. aureus* strains by using a microplate adhesion test. Prior to the adhesion assay, the strains have been cultured in presence of subinhibitory concentrations of antibiotics (1/4 of the MIC). The antibiotics tested were: oxacillin, gentamicin, vancomycin, rifampicin, linezolid and moxifloxacin. By using relative quantitative RT-PCR, we also explored the effect of the antibiotics mentioned on the transcription of fnbA and fnbB genes coding for FnBPA and FnBPB.
Results: The results showed that the antibiotics differently modulate *S. aureus* adhesion to human fibronectin. Oxacilline, moxifloxacin and linezolid treatment led to the acquisition of a hyperadhesive phenotype consistent with the increased FnBPA and FnBPB mRNA levels after oxacilline, moxifloxacin and linezolid treatment. On the contrary, all the strains treated with rifampicin showed significantly decreased adhesion to fibronectin, while gentamicin and vancomycin had little impact on *S. aureus* adhesion to fibronectin.
Conclusion: Our results, showing an inhibitory effect of rifampicin on *S. aureus* adhesion to human fibronectin, support the use of rifampicin, in association with other anti-staphylococcal agents, for the treatment of severe *S. aureus* infections. Additionally, our observations may provide explanation for the poor outcome of staphylococcal infections in case of impaired antibiotics diffusion.

P1293 Gram-negative anaerobe susceptibility rates in six European countries, 2007–2009

S. Bouchillon, M. Renteria, M. Hackel, B. Johnson, M. Dowzicky (Schaumburg, Collegeville, US)*

Background: The Tigecycline European Surveillance Trial (TEST) monitors susceptibility of European aerobic and anaerobic bacteria to tigecycline (Tig), cefoxitin (Cfx), clindamycin (Cd), meropenem (Mer), metronidazole (Met), and piperacillin–tazobactam (P/T). This report summarizes susceptibility of Gram-negative anaerobes in 6 countries to these drugs.
Methods: Anaerobic isolates collected by 43 cumulative labs in 6 countries from 2007–2009 were sent to a central lab in the United States for agar dilution susceptibility testing, following CLSI guidelines. Results were interpreted by EUCAST breakpoints and guidelines; FDA breakpoints were used for tigecycline where none are defined by EUCAST.
Results: % Susceptible of each organism group in each country is summarized in the table below.
Conclusions: The most active drugs were tigecycline, meropenem, metronidazole, and piperacillin–tazobactam, each inhibiting >90% of all Gram-negative anaerobes at their respective breakpoints. Cefoxitin and clindamycin showed wide ranges in susceptibilities between countries and organism groups. Hungary demonstrated statistically lower susceptibility rates to cefoxitin and clindamycin against Gram-negative anaerobes than other countries in this study.

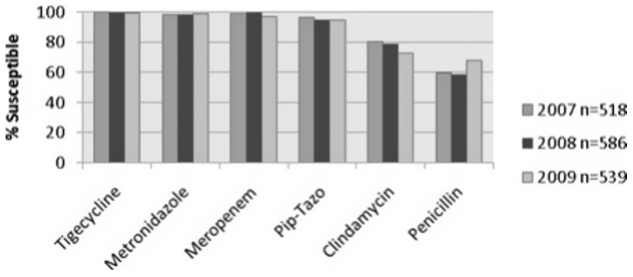
Organism	Demographics	N	Tig	Cfx	Cd	Mer	Met	P/T
Bacteroides spp.	Belgium	86	97.7	80.2	74.4	95.4	98.8	91.9
	Czech Republic	100	98.0	86.0	71.0	96.0	100.0	92.0
	France	316	96.5	81.7	70.3	97.5	100.0	91.5
	Germany	504	96.0	85.5	77.6	96.8	99.0	91.1
	Hungary	404	96.3	73.3	73.5	97.5	99.8	87.6
	United Kingdom	10	100.0	100.0	90.0	100.0	100.0	100.0
Prevotella spp.	Belgium	45	95.6	100.0	71.1	100.0	97.8	100.0
	Czech Republic	41	100.0	97.6	85.4	100.0	100.0	100.0
	France	93	98.9	98.9	79.6	98.9	98.9	98.9
	Germany	250	99.6	97.6	85.6	100.0	96.4	98.0
	Hungary	111	97.3	78.4	58.6	99.1	99.1	91.0
	United Kingdom	3	100.0	100.0	100.0	100.0	100.0	100.0
Total		**1,963**	**97.1**	**84.6**	**75.3**	**97.8**	**99.0**	**92.2**

P1294 Three-year susceptibility analysis of Gram-positive anaerobes in Europe – TEST, 2007–2009

R. Badal, S. Bouchillon, M. Hackel, B. Johnson, D. Hoban, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)*

Objectives: Tigecycline has a broad spectrum of activity that includes anaerobic bacteria. The Tigecycline European Surveillance Trial (TEST) has been monitoring susceptibility of anaerobes in Europe since 2007. This report compares the most recent data from 2009 to susceptibility levels observed in 2007 and 2008 of Gram-positive anaerobes in Europe.
Methods: 1643 Gram-positive anaerobic pathogens (518 in 2007, 586 in 2008, 539 in 2009) were collected and identified from 44 sites in 7 countries in Europe, and sent to a central laboratory in the USA where MICs of tigecycline and five comparators were determined using CLSI agar dilution. Tigecycline MICs were interpreted using US FDA breakpoints, while EUCAST guidelines were used for all other drugs.
Results: Overall % susceptibility for Gram-positive anaerobes is shown in the table.
Conclusions: Tigecycline retained excellent *in vitro* activity against Gram-positive anaerobic bacteria isolated from European hospitals in 2009, inhibiting nearly 100% of all isolates. Metronidazole and

meropenem were nearly as active as tigecycline, while clindamycin and penicillin inhibited less than 80% of Gram-positive isolates in 2009.



Susceptibility of Gram-positive anaerobes in Europe, 2007–2009.

P1295 Streptococcal bacteriocin-like inhibitory activity against potential upper respiratory tract pathogens

M. Santagati, M. Scillato, V. Metoldo, R. Burtone, F. Patanè, S. Stefani (Catania, IT)*

Objectives: The use of bacteria as a probiotic is in continuous development thanks to capacity to maintain or restore a host's natural microbiota by interference and inhibition with other microrganisms. This is due to the production of antimicrobial peptides such as bacteriocins. In the oral cavity, α-haemolytic streptococci such as *S. salivarius, S. mitis, S. mutans, S. sanguis* are the major producers of bacteriocin which are able to reduce the frequency of colonization of the main pathogens involved in upper respiratory tract infections. Bacteriae-therapy may offer a valid alternative in the prevention or treatment of bacterial infections. In our study, we analyzed 15 α-haemolytic streptococci from nasal and pharyngeal swabs of children to: i) evaluate the bacteriocin-like inhibitory activity against potential pathogens; ii) determine bacteriocin products and iii) to assay the capacity of adhesion on Hep-2 cell lines.
Methods: Thirty-one children were enrolled in this study (2–12 years). All samples were plated onto Mitis Salivarius agar at 37ºC in 5% CO2. All strains were identified by API Strep and sequencing of 16S rDNA and SodA gene. The bacteriocin production was evaluated by deferred antagonism test and the structural genes were investigated by PCR and sequencing. The adherence assays based on Benga L. et al. 2004.
Results: Among 81 α-haemolytic streptococci isolated from 31 samples, only 15 were bacteriocin producers. We found that three *S. mitis* and seven *S. salivarius* were active against *S. pneumoniae,* two strains – *S. sanguis* and *S. mitis* – against *M. catharralis* and two *S. mitis* showed a broad inhibitory activity against *S. pyogenes, S. pneumoniae, S. aureus* and *S. salivarius* and carried salivaricin B. Only one *S. mitis* with *S. pyogenes* inhibitory activity, contained the salA gene. The preliminary results of *S. salivarius* tests adhesion on Hep-2 cell lines demonstrated its excellent adhesion capacity.
Conclusion: This study focused on the streptococcal inhibitory activity demonstrating the capability of commensal flora of upper airways to interference and/or inhibit the growth of potential pathogens associated with their ability to produce bacteriocis. It is interesting to note that two *S. mitis* carrying salB act with a broad spectrum of activity.

P1296 Frequency and antibiotic susceptibility of *Ureaplasma urealyticum* isolated from the genital tract of sexually active individuals

E. Ostorházi, B. Farkas, I. Bencze-Györi, J. Gordos, K. Pónyai, S. Kárpáti, F. Rozgonyi (Budapest, HU)*

Objectives: The aim of this study was to examine the incidence and antibiotic sensitivity of *Ureaplasma urealyticum* strains cultured from urethra and vagina of sexually active individuals attended to our STD outpatient service.
Methods: Samples were taken from the urethra and cervix with universal swab (Biolab®) into Urea-Myco DUO kit (Bio-Rad®) and incubated in ambient air for 48 h at 37 Co. The determination of antibiotic sensitivity was performed in U9 broth using SIR *Mycoplasma* kit (Bio-Rad) under the same conditions. Only one isolate per patient was taken into consideration.
Results: Between May 2008 and October 2009 a total of 169 strains were obtained from urethra (38) and cervix (131). Most strains were sensitive to tetracycline (95%), doxycycline (97%), pristinamycin (96%), azithromycin (95%), josamycin (95%) and ofloxacin (93) but not to erythromycin (13%) and clindamycin (22%). 75% of the strains were resistant simultaneously to erythromycin-clindamycin combination also, suggesting that ex juvantibus administration any of them may select cross-resistant strains for both antibiotics.
Conclusions: Not only cultivation but also prior antibiotic sensitivity testing is needed for successful treatment of subclinical or manifest *U. urealyticum* infection since none of the analyzed strains was fully susceptible to all examined antimicrobials. In case of *U. urealyticum* infections or *Chlamydia trachomatis* and *U. urealyticum* coinfections, azithromycin is the drug of choice since it is effective against both agents. However, clarithromycin is also recommended since it inhibits biofilm formation of *U. urealyticum.*

Surveillance and molecular epidemiology of *Acinetobacter* spp.

P1297 Extended nosocomial outbreak with multidrug-resistant *Acinetobacter baumannii* in a French university hospital

C. Landelle, P. Lesprit, P. Legrand, F. Cizeau, S. Soing-Altrach, C. Brun-Buisson, E. Girou (Créteil, FR)*

Objective: Describe the course and measures taken to control an outbreak with multidrug-resistant *Acinetobacter baumannii* (MRAB) in a university hospital.
Methods: During the epidemic period, all intensive care units (ICUs) patients (pts) were screened weekly in rectal and throat swabs. The identity of isolates was confirmed by pulsed-field electrophoresis.
Results: Between 01/08 and 06/09, the same strain of MRAB (sensitive only to colistin) was identified in screening specimens for 28 pts and in clinical samples for 58 pts; 36 pts also had co-colonization with *Klebsiella pneumoniae* producing extended-spectrum β-lactamase. After the admission of a pt with toxic epidermal necrolysis (TEN) and MRAB carriage from Tahiti, 15 cross transmissions (CT) occurred in 2 ICUs between 01 and 04/08. Adherence to standard precautions was reinforced; contact precautions, auditing health-care workers (HCW) practices and environmental screening were implemented; rooms were cleaned with hydrogen peroxide mist disinfection after pt discharge or death, until the outbreak stopped. Admission of a second TEN pt with the same MRAB strain from the same Tahitian hospital led to 69 secondary cases in 4 ICUs and 3 wards during 4 periods between 06/08 and 05/09 (table). Previous measures were resumed. Because MRAB was detected in air, surfaces and from sink traps and pts' washing basins in one ICU, a weekly cleansing protocol with sodium hypochlorite solution for all sinks was initiated. Use of sink water for nasogastric tube rinsing or oral medication administration was forbidden. Care and cleaning procedures were revised or implemented. Chlorhexidine skin decontamination was initiated. ICU A, where most cases occurred was closed to new admissions and moved to another ICU four times (table). In 05/09, all MRAB pts in wards or discharged from ICU A were cohorted to an isolation unit, and cared for by trained and dedicated HCW. The outbreak of MRAB then stopped. Most likely modes of transmission were CT from colonized or infected pts via the hands of HCW, poor cleaning of equipment, splashing of contaminated water from sink traps, and reintroduction in the ICU of MRAB from pts previously colonized or personnel.
Conclusions: MRAB outbreaks are notoriously difficult to control. This extended outbreak involving several ICUs was controlled only after implementation of an extensive control program and eventual cohorting of all carriers of MRAB in a dedicated area with a dedicated staff.

Number of MRAB cross transmissions after transfer of Tahitian patient with MRAB carriage

	ICU A	ICU B	ICU C	ICU D	ICU E	Medical ward	Surgical ward A	Surgical ward B
	Admission of a patient with MRAB carriage							
Period 1: 01/08–06/09	10	5						
	Outbreak stopped – Admission of a second patient with MRAB carriage							
Period 2: 06–09/08	12	1				1		
	Admissions in ICU A were stopped and the activity was moved in ICU D while ICU A was cleaned							
Period 3: 10–11/08	11						2	
	Admissions in ICU A were stopped and the activity was moved in ICU D while ICU A was cleaned							
Period 4: 12/08–02/09	12	7		1				
	Admissions in ICU A were stopped and the activity was moved in a new ICU E							
Period 5: 03–05/09	–	3	4		11	2	1	1
	Admissions in ICU E were stopped until discharge of all MRAB carriers – all MRAB patients of wards or discharged from ICU E were cohorted into a isolation unit, with dedicated HCW							

P1298 Emergence of OXA-carbapenemase- and ArmA-producing *Acinetobacter baumannii* isolates of European clonal lineages I and II in Norway

N. Karah, B. Haldorsen, N.O. Hermansen, Y. Tveten, E. Ragnhildstveit, D.H. Skutlaberg, S. Tofteland, A. Sundsfjord, Ø. Samuelsen (Tromsø, Oslo, Tønsberg, Fredrikstad, Bergen, Kristiansand, NO)*

Objectives: *Acinetobacter baumannii* has emerged as an important cause of hospital-acquired infections due to its remarkable propensity to acquire resistance determinants and environmental resilience. The aim of this project was to characterise carbapenem-resistant *A. baumannii* isolates identified in Norway.
Materials and Methods: The study included 10 carbapenem-resistant isolates of *A. baumannii* submitted to the Reference Centre for Detection of Antimicrobial Resistance. The isolates were collected between 2004–09 from various clinical specimens in six different laboratories. Susceptibility testing was performed by Etest. PCR assays were performed for detection of OXA-carbapenemase genes, 16S rRNA methylase genes, ISAba1, ISAba3, intI1 and for determination of epidemic clonal lineages. Determination of the full-length sequences of blaOXA-51-like genes and the occurrence of mutations in the quinolone resistance determining regions (QRDRs) was done by sequencing.
Results: All ten isolates expressed multidrug resistance (MDR), but were consistently susceptible to colistin. Epidemiological data showed that all isolates were associated with hospitalization abroad; Greece, Thailand (n = 2), China, India (n = 3), Cyprus, Italy and Pakistan. Carbapenem resistance was explained by the presence of OXA-carbapenemases; blaOXA-23-like (n = 8), blaOXA-58-like (n = 1) and blaOXA-24-like (n = 1). ISAba1 was detected upstream of blaOXA-23-like genes in all isolates and downstream in five isolates. ISAba3 was detected downstream of the blaOXA-58-like gene. The 16S rRNA methylase gene armA was detected in the three isolates with high level aminoglycoside resistance to all tested aminoglycosides. All ten isolates were resistant to ciprofloxacin due to mutations in the QRDR region (Leu83Ser in GyrA and Leu80Ser in ParC). The intI1 gene was detected in 6/10 isolates. Seven isolates contained the blaOXA-66 variant and were all of European clone II. The remaining three isolates contained the blaOXA-69 variant with one of them belonging to European clone I.
Conclusion: MDR *A. baumannii* isolates belonging to European clones I and II have emerged in Norway, all associated with hospitalization abroad. Resistance mechanisms included OXA-carbapenemases, 16S rRNA methylases and mutations in the QRDRs. Three isolates of European clone II co-produced blaOXA-23-like and armA.

P1299 Molecular characterization of carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Portugal by Multilocus sequence typing

N. Mendonça, A. Duarte, G.J. da Silva (Coimbra, Lisbon, PT)*

Acinetobacter baumannii is an emergent nosocomial pathogen often resistant to multiple antibiotics. The emergence of carbapenem resistant strains has been increasingly reported worldwide.

Objective: The goal of the study was to genotype carbapenem resistant *A. baumannii* (CRAb) isolates recovered from different Portuguese hospitals. Methods. Representative isolates were selected from the collection recovered since 1998 until 2007 from hospitals of different geographic regions. Antimicrobial susceptibility was determined by disk diffusion method for β-lactams, quinolones (QNL) and aminoglycosides (AG). MICs for β-lactams were determined by E-test. Molecular typing was performed by random amplified polymorphic DNA (RAPD) and in representative strains by multilocus sequencing typing (MLST) to confirm the genotype and allow a global comparison of results with others ST types described worldwide.
Results: According to antimicrobial susceptibilities were formed three groups: R1, isolates resistant to all β-lactams but susceptible to QNL and a AG (only 3 collected in 1998/99); R2, those resistant to all antibiotics except tobramycin and/or amikacin and colistin (1999–2007); R3, isolates resistant to all antibiotics except AG and colistin (2004–2007). For all isolates the MICs for imipenem and ceftazidime were >32 mg/L and >256 mg/L, respectively. RAPD fingerprinting showed two distinct patterns: A (group R1) and B (groups R2 and R3). The isolates included in groups R2 and R3 were not identical but were genetically related. A new MSLT profile deposed as ST55 at *Acinetobacter* MLST website correspond to isolates of group R1. This strain produced the IMP-5 metalo-β-lactamase. Analysis of website data showed that it was only related with one strain, sharing the gdh-B, rec-A and rpoD genes. Groups R2 and R3 belong to MSLT profile ST33, and isolates from both groups produced an OXA-40 carbapenemase. The results showed that isolates with ST33 profile are endemic in Portugal for years and, according website data, worldwide.
Conclusion: The study showed that not all CRAb have capacity of dissemination. However, the need for surveillance of CRAb strains and the knowledge of well identified clones with defined characteristics is crucial to track potential epidemic strains and to improve infection control measures. We describe for the first time a new MLST profile corresponding to the IMP-5 producer strain apparently with no epidemic potential.

P1300 Multifocal spreading of an OXA-23-producing *Acinetobacter baumannii* clone in different Italian clinical settings

L. Pagani, R. Migliavacca, G. Fugazza, E. Nucleo, M. Spalla, M.M. D'Andrea, T. Giani, R. Daturi, M. Balzaretti, M. Tinelli, D. Marchetti, G.M. Rossolini (Pavia, Siena, Milan, Lodi, Bologna, IT)*

Objectives: *A. baumannii* is an oppotunistic pathogen responsible of nosocomial infections in intensive-care-units (ICUs) and in patients exposed to elevated risk factors. Infections caused by multidrug-resistant (MDR) strains of *A. baumannii* are difficult to treat, and reports of carbapenem resistance (CR) have accumulated worldwide. In our country CR in *A. baumannii* has been mainly attributed to acquisition of blaOXA-58 determinant, even though nowadays class D carbapenemase OXA-23 is an emerging problem. Objective of this study was to evaluate the spread of OXA-23 producing *A. baumannii* in different italian settings and to investigate clonal relationship of such isolates.
Methods: 43 non-replicate carbapenem resistant *A. baumannii*, negative by PCR for blaOXA-24/58 genes, were collected from different wards of 5 Acute Care Hospitals and 1 Long Term Care and Rehabilitation Facility between August 2008-September 2009. Isolates were identified and tested for antimicrobial susceptibility using the GNI card (Vitek System, bioMérieux) and panels NMIC/ID4 (Phoenix System, BD), respectively. Molecular identification of the species level was performed by PCR amplification of blaOXA-51-like allele. Analytical isoelectric focusing (IEF) of crude extracts was performed. The nature and presence of blaOXA-23 and blaOXA-51-like determinants were investigated by PCR and sequencing. Genotyping was performed by PFGE analysis and the assignment of sequence type group was performed by multiplex-PCR.
Results: IEF for all the 43 *A. baumannii* showed the presence of enzymes with pI 9.0 and 6.7, the latter consistent with the expression of the OXA-23 enzyme. PCR, followed by direct amplicon sequencing confirmed the

presence of blaOXA-23 gene in all isolates. PFGE analysis showed that all the isolates were clonally related (>75% similarity); multiplex-PCR showed that isolates belonged to the sequence type Group 1, related to European clone II. Interestingly, the number of isolates from different settings varied significantly (ranging from 1 to 35) on the basis of infection control measures adopted.
Conclusions: Results showed an intra- and inter-hospital spread in different italian settings of an OXA-23 producing clone of *A. baumannii* and emphasize the ability of such pathogen to become epidemic/endemic acquiring resistance genes in the hospital environment if the diffusion is not promptly limited.

P1301 Clonal diversity of OXA-23 producing *Acinetobacter baumannii* isolates from Rio de Janeiro, Brazil

F. Grosso, S. Quinteira, A. Ramos, A. Assef, K. Carvalho, M. Asensi, L. Peixe (Porto, Vila Nova de Famalicão, PT; Rio de Janeiro, BR)*

Objectives: Multidrug-resistant (MDR) *Acinetobacter baumannii* (Ab) are a leading cause of nosocomial infections in Brazilian hospitals, being OXA-23 producers disseminated in several hospitals.
The aim of this study was to assess the diversity of OXA-23-producing Ab isolates disseminated throughout hospitals in Rio de Janeiro (2006–2007) and to analyse blaOXA-23 carrying genetic structure.
Methods: From a collection of 96 OXA-23 producing Ab recovered in 8 hospitals (January 2006 to September 2007) from Rio de Janeiro, Brazil, isolates representative of different pulsotypes (n = 5) were selected for further characterization. Susceptibility tests and identification by rDNA sequencing were performed. Pulsotypes were determined by PFGE with ApaI. The genomic location of the bla OXA-23 gene was determined by I-CeuI technique and with PCR using specific primers for the composite transposon Tn2006. MLST scheme was conducted according to the Ab MLST database (http://pubmlst.org/abaumannii/). The blaOXA-51 type was identified by PCR and sequencing.
Results: Ab isolates were resistant to several b-lactams, presenting variable susceptibility to amikacin (clones A and E were susceptible), kanamycin and sulfamethoxazole/trimethoprim (clone E was susceptible). In all isolates, the blaOXA-23 gene was inserted in Tn2006 and located on the chromosome. The 5 pulsotypes clustered in 4 different STs that presented 4 new allelic combinations and 2 new gpi alleles. These new allelic profiles were related to other STs found in Latin America, but not related with ST22, already described in several European countries and Korea which is also associated to OXA-23 producers. Genotype A (69% of the OXA-23 producing isolates), the most prevalent and found in seven hospitals, was assigned in the same ST of clone D (1%) both presenting blaOXA-66. Genotypes B (25%) C (4%) and E (1%) presented blaOXA-132, blaOXA-95 and blaOXA-69, respectively.
Conclusions: This is the first study describing the population structure of MDR OXA-23-producing Ab clones in Brazil. Our findings indicate an ongoing spread of blaOXA-23 associate to new and diverse ST of *A. baumannii* in hospitals of Rio de Janeiro. The identification of the blaOXA-66 as the predominant lineage confirms its ability to disseminate and establish in the hospital setting.

P1302 Analysis of carbapenem resistance genes in *Acinetobacter baumannii* isolates from Kuwait hospitals

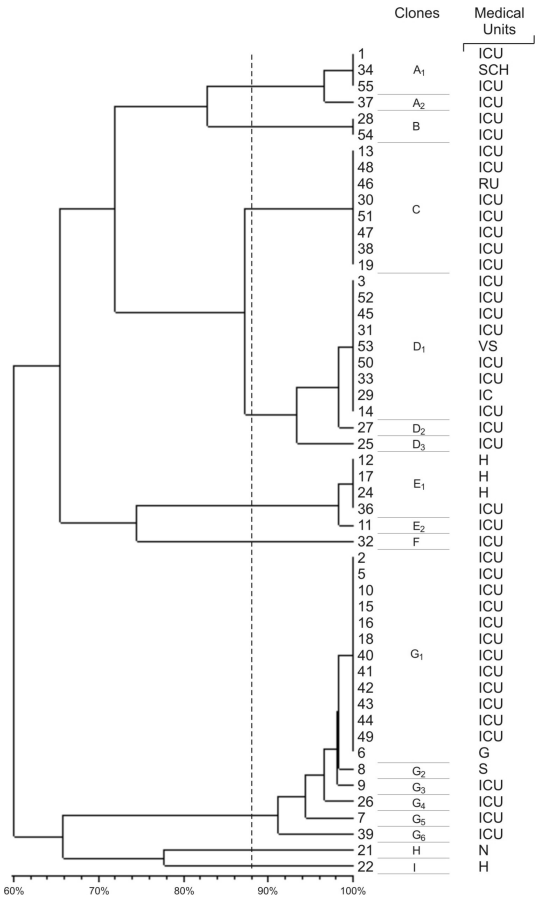
N. Al-Sweih, M. Al-Hubail, V.O. Rotimi (Kuwait, KW)*

Objectives: This study was designed to investigate the molecular epidemiology and genetic basis of the carbapenem resistance in clinical isolates of *Acinetobacter baumannii* obtained from all government hospitals in Kuwait.
Methods: A total of 250 clinical isolates were collected from 8 hospitals. Their susceptibility to 18 antibiotics was determined by E test method. Carbapenems resistant isolates were screened for phenotypic MBL production by disk approximation test (DAT) and MBL E test. Genetic characterization of the resistant mechanisms was performed by PCR. Their clonal relatedness was assessed by PFGE.
Results: All the isolates were multidrug resistant. Of the 250 isolates, 93 (37.2%) were resistant to carbapenems. The prevalence of MBL-producing isolates was 74.2% and 57% by DAT and MBL Etest, respectively. Sixty-five (69.9%) of the carbapenem-resistant isolates were positive for one or more resistance genes; 37 were positive for blaIMP-1, 17 blaVIM-1, 43 blaVIM2, 24 blaSPM-1, 32 blaOXA-23, 1 blaOXA-24. PFGE demonstrated widespread clones of similar strains in different hospitals and a cluster of 3 clones found only in one particular hospital.
Conclusion: Resistance clinical isolates to carbapenem has reached unacceptable levels in Kuwait and MBLs, as well as oxacilinases, are highly prevalent among *A. baumannii* in our hospitals.
Acknowledgment: This work was supported by a Kuwait University Research Grant no. YM 01/08 which is gratefully acknowledged.

P1303 Genetic relatedness of *Acinetobacter baumannii* strains isolated from patients in a university hospital in Bialystok, Poland

P. Wieczorek, P. Sacha, E. Tryniszewska (Bialystok, PL)*

Objectives: *Acinetobacter baumannii* is an opportunistic pathogen that gives rise to nosocomial infections and outbreaks, in particular, in the intensive care unit. The increasing resistance to antibiotics leads to appearing of multi-drug resistant strains. Examining the genetic diversity of randomly chosen *A. baumannii* strains isolated in University Hospital in Bialystok and the examination of sensitivity to antibiotics were a purposes of the study.



Analysis of genetic relatedness of *Acinetobacter baumannii* strains. ICU, intensive care unit; SCH, surgery of the chest; RU, rescue unit; VS, vascular surgery; IC, invasive cardiology; H, haematology; G, gastroenterology; S, surgery; N, neurology.

Methods: There were examined 51 strains of *A. baumannii* that were biochemically identificated by using VITEK2 apparatus according to the procedure of the producer. The identification to the species was confirmed genetically on the basis of the presence of carbapenemase blaOXA-51-like genes with the PCR technique.
Susceptibility to antibiotics was performed by using broth microdilution method. MIC value was determined and interpreted according to criteria proposed by Clinical and Laboratory Standards Institute for the following antibiotics: ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, ceftazidime, cefotaxime, cefepime, imipenem, meropenem, tetracycline, gentamicin, netilmicin, amikacin, ciprofloxacin.
The genetic relatedness of *A. baumannii* strains was examined by using pulsed-field gel electrophoresis (PFGE) with ApaI as the restriction enzyme. Cluster analysis was performed by the unweighted pair group method with mathematical averaging (UPGMA) and DNA relatedness was calculated by using the band-based Dice coefficient.
Results: Analysis of genetic diversity revealed nine different clones of which three clones C, D and G were dominated. The degree of the genetic similarity of strains was determined at $\geqslant$89% value. Majority of *A. baumannii* strains were isolated from patients treated in the intensive care unit.
Clone C was more resistant to the majority of antibiotics than two remaining clones. Strains belonging to the clone D and G were susceptible in the 100% to cefepime, meropenem, and piperacillin/tazobactam, meropenem, respectively. Three clones were resistant to piperacillin and ciprofloxacin.
Conclusion: Three clones of *A. baumannii* dominates in University Hospital in Bialystok, especially in intensive care unit, amongst which clone C demonstrates the multi-drug resistance phenotype, including resistance to carbapenems. All strains of the dominating clones were resistant to piperacillin and ciprofloxacin, and combination of ampicillin with sulbactam showed as the most active antibiotic.

P1304 Nosocomial spread of multidrug-resistant *Acinetobacter baumannii*: collateral damage from the war on terror

A. Troelstra, M. JagtZwetsloot, H. Blok, T.E. Hopmans, M. Bonten (Utrecht, NL)*

Background: Pre-emptive isolation and screening for multi-drug resistant bacteria in patients with increased risks for carriage is standard care in Dutch hospitals. This policy has been extremely successful in controlling nosocomial spread of MRSA and VRE. Repatriated soldiers from Afghanistan are considered at risk for carriage of multi-drug resistant *Acinetobacter* spp (MDR-AB), which can cause nosocomial outbreaks, colonisation and infections.
Our hospital (Dutch Central Military Hospital (CMH) as part of the UMC Utrecht) provides health services for all Dutch military patients.
Outbreaks: In april 2008, a wounded soldier repatriated form Uruzghan was admitted to our ICU and immediately (pre-emptively) treated in isolation. Screening- and clinical cultures on admission revealed MDR-AB (but not MRSA) in sputum and blood. The patient underwent several surgical procedures on several days. Subsequently, 3 patients that had received surgery in the same operation room (OR) as well as 2 patients treated in the same ICU acquired colonization with the same MDR-AB isolate (documented with DNA-fingerprinting) as the index-patient.
In august 2008, another wounded soldier was repatriated from Uruzghan and admitted to ICU. Again the patient was treated in isolation immediately, but in this case subsequent acquisition of MDR-AB was documented in 13 patients; 1 patient in the emergency room, 5 in ICU, 6 on a general ward and 1 patient in the revalidation department.
During both outbreaks, several interventions were implemented to control the situation. Screening of all contact-patients was performed, resulting in at least 2670 cultures taken from 178 patients for both outbreaks. Pre-empitve isolation was implemented until at least three sets of cultures taken from throat or sputum, axilla, wounds, groin and perineum revealed negative. Furthermore extended cleaning and disinfection procedures were performed on a daily basis.
Conclusion: Despite pre-emptive isolation and adherence to recommendations of our guidelines, MDR-AB introduced by 2 patients caused nosocomial spread to 18 patients. These outbreaks illustrate that a strategy, proven effective for controlling MRSA, can fail in controlling transmission of MDR-AB.

P1305 *Acinetobacter baumannii* infections in intensive care units patients: predictors of risk factors of multidrug resistance

A. Acar, O. Oncul, M. Ozyurt, S. Budak, L. Gorenek, T. Haznedaroglu (Istanbul, TR)*

Objective: This study was designed to evaluate the incidence, epidemiologic and clinical features and outcome of patients of *Acinetobacter* related nosocomial infections in our intensive carte units, and to identify predictors of multi drug resistance (MDR) for *Acinetobacter baumannii*.
Methods: Prospective study of all patients with nosocomial *A. baumannii* infections in ICU from January 1, 2007 to December 31, 2008. MDR in *A. baumannii* was defined as resistance to at least three classes antibiotics (antipseudomonal penicillins, antipseudomonal cephalosporins, antipseudomonal flouroquinolones, aminogllycosides, or trimethoprim-sulfamethoxazole) but susceptibility to carbapenems, and non-MDR was defined as suspectibility to carbapenems and other alternative antipseudomonal antimicrobial agents (eg, penicilins, cephalosporins, fluoroquinolones, or aminoglycoside).
Results: Among the 147 ICU patients, 197 *A. baumannii* infections were developed. Their mean age was 57.04$\pm$25.8 years. Infections site were: bloodstream 40.6%, respiratory tract 28.4%, and burn wounds 14.5%. Congestive heart failure (40.3%), cerebrovascular illness and diabetes mellitus (24%) were the most common underlying disease. Mortality was 56.7%. Of 197 episodes of *Acinetobacter* infections, 168 (85.3%) caused by MDR strains were identified. The most effective antibacterial agents for *A. baumannii* were netilmicin (61.4%), doxycycline (60.8%) and tobramycin (59.6%) respectively. Congestive cardiac failure ($p = 0.03$), chronic obstructive lung disease ($p = 0.02$), diabetes mellitus ($p = 0.04$), previous antibiotic usage in last three months ($p = 0.04$), steroid usage ($p = 0.03$), dopamine usage ($p < 0.001$), and previous emergence of *E. coli* infections ($p = 0.03$) were risk factors for multi drug resistance in univariate analysis. Multivariate analysis showed that steroid usage ($p = 0.91$; odds ratio [OR], 4.7; 95% confidence interval [CI], 1.4–16.4) and previous emergence of *E. coli* infections ($p = 0.004$; OR, 6.4; 95% CI, 1.8–21.2) as independent predictors of MDR.
Conclusion: *A. baumannii* infections in ICU patients are associated high mortality and MDR. Avoidance of unnecessary antibiotics and steroid is high priority and specific attention should be paid to patients with infections Gram-negative enteric pathogens especially *E. coli*.

P1306 Multidrug-resistant *Acinetobacter baumannii* in an intensive care unit: the never ending fight

E. Carretto, D. Barbarini, L. Carnevale, A. Amatu, A. Grosini, G. Iotti, P. Marone (Pavia, IT)*

Objective: *Acinetobacter baumannii* is an important cause of nosocomial infections in many hospitals, most often in critically ill patients admitted to intensive care units (ICUs). After the first isolation (April 2008) of a multi-drug resistant (MDR) *A. baumannii* strain (susceptible only to ampicillin-sulbactam and colistin) in an ICU of our Institution, we performed an active surveillance study up to July 2009. The results of the molecular typing of the strains collected and the molecular analysis of the mechanisms of carbapenems resistance are showed.
Methods: all the *Acinetobacter* strains showing a MDR phenotype were further characterized as belonging to the genomospecies 2/13 using the criteria of Gerner-Smidt et al. (JCM, 1991:277). We collected a single isolate for each patient, irrespective of the site of isolation. Susceptibility testing using the standardized disk-diffusion method (CLSI criteria) and E-test were performed. The molecular typing was performed using the rep-PCR Diversilab Microbial Typing System (bioMériéux, France). In the experiments the EU-AFLP type strains were

included as control (Dijkshoorn et al., 1996:1519). The mechanisms of carbapenems resistance were investigated initially using a phenotypic screening to detect metallo-β-lactamases (MBL). The MBL-negative strains were subsequently investigated using a multiplex PCR to detect genes encoding the OXA carbapenemases prevalent in *Acinetobacter* species, using the PCR protocol described in Woodford et al.(Int. J. Antimicrob. Agents 2006:351).
Results: 41 different MDR *Acinetobacter* strains belonging to genomospecies 2/13 were collected. The microorganism was recognized to be endemic, with 2–3 patients per month colonized or infected. During the study period we documented two large outbreaks (June 2008, 6 patients and March 2009, 11 patients involved). The rep-PCR allowed us to demonstrate two major clones. The first one comprised different isolates collected in the first 6 months of the study; this clone was then replaced by another one, strictly related to the EU clone II (from July 2008 to July 2009). None of our isolates was MBL positive; the wide majority of our strains carried the OXA51/OXA58 carbapenemases.
Conclusions: our study confirms the wide circulation of the *A. baumannii* EU clone II, that replaced another lineage not linked to other EU clones. As expected, the OXA51/OXA58 profile was most commonly involved in the high-level resistance to carbapenems of our strains.

P1307 Genospecies identification of *Acinetobacter baumannii* complex bacteraemia in critical patients

Y.C. Chuang, W.H. Sheng, S.C. Chang (YunLin, Taipei City, TW)*

Objectives: *Acinetobacter baumannii* (*A. baumannii*) is an emerging nosocomial pathogen. Due to rapid increase in the drug resistance of *A. baumannii*, the choices of antimicrobial agents were limited. However, it is difficult to differentiate *A. baumannii* (DNA group or genospecies 2), from genospecies 3 and 13TU by classic methods. We aimed to investigate the differences of characteristics, clinical presentations and outcomes between different genospecies within *A. baumannii* complex by genospecies identification.
Methods: 62 adult patients with *A. baumannii* complex bacteremia at the intensive care units of a teaching hospital in northern Taiwan between April 2008 and February 2009 were prospectively enrolled. *A. baumannii* complex was identified by classic methods and verified by the Phoneix system. 16S-23S rRNA intergenic-spacer sequences were used for genospecies identification.
Results: Among 62 patients of *A. baumannii* complex bacteremia, 43 (69.4%) had genospecies 2 bacteremia, 11 had genospecies 13TU, 2 had genospecies 3 and 6 had others. Genospecies 2 had significant higher MIC of Ciprofloxacin (MIC50 32 vs. 0.25), Amikacin (MIC50 >128 vs. 4), Imipenem (MIC50 16 vs. 0.5), Sulbactam(MIC50 16 vs. 1), and Tigecycline (MIC50 2 vs. 0.25) ($P < 0.001$, respectively). Genospecies 2 had less primary bacteremia (20.9% vs. 47.4%, $P = 0.03$). Genospecies 2 also caused more severe sepsis than the others ($P = 0.03$) (Pittsburgh bacteremia score: median 5, interquartile range (IQR) 4–7; median 4, IQR 2–5, respectively). Patients with genospecies 2 bacteremia were associated with a higher rate of mortality (81.4% vs. 52.6%, $P = 0.02$). Genospecies 2 bacteremia was the independent predictive factor (odds ratio 3.9, 95% CI: 1.11–13.62) for mortality after adjusting sex, age, severity of underlying disease, and empiric antibiotics use.
Conclusion: In patients with *A. baumannii* complex bacteremia, genospecies 2 was associated with higher resistance and mortality. Genospecies identification might be important in those high risk groups.

P1308 Carbapenem and multidrug-resistant *Acinetobacter baumannii* colonization/infection: epidemiology and factors associated to infection

A. Hernández-Torres, E. García-Vázquez, G. Yagüe, A. Fernández-Rufete, E. Moral-Escudero, J. Gómez, M. Canteras, H. Albendín-Iglesias, J.A. Herrero, J. Ruiz (Murcia, ES)*

Objective: To study an outbreak of nosocomial colonisation/infection due to multidrug and carbapenem resistant *A. baumannii* (ABMDR-C).
Patients and Methods: Prospective study of patients with ABMDR-C colonisation/infection (January 2007-June 2008). Epidemiological and clinical variables and predictors of infection versus colonization were analysed.
Results: 24 out of 101 cases were considered colonisations and 77 infections (27 bacteraemia); global mortality (colonisations and infections) was 42% (4 colonisations and 38 infections – 18 bacteraemia). All together incidence was 3.2/1000 admissions/day; 29% had been previously admitted and 79% had received previous antibiotic treatment (29% carbapenem; 34% piperacillin–tazobactam; 12.5% boths); 78% had an underlying condition; 81% were UCI patients; 90% had gone through invasive procedures; 65% had another microorganism isolated. In multivariate analysis, infection predictor factors were isolation of ABMDR-C in respiratory samples (OR 5.406; 95% CI 1.419–20.599); male patients (OR 8.842; 95% CI 1.988–39.325); previous hospitalization (OR 9.720; 95% CI 1.383–68.291) and initial clinical severity (OR 30.897; 95% CI 5.533–172.543).
Conclusions: Our cohort of patients with ABMDR-C colonisation/infection is characterised by its underlying comorbidity, the high rate of previous invasive procedures, previous hospitalisation and previous broad-spectrum β-lactam treatments (especially carbapenem); initial severity and respiratory samples with ABMDR-C isolates were predictors of infection versus colonisation.

Surveillance of MDR-Gram-negatives

P1309 Cross-transmission of *Klebsiella pneumoniae* in intensive care units: interhospital and intrahospital spread

A. Agodi, M. Barchitta, G. Valenti, R. Marzagalli, M.A. Romeo, L. Giaquinta, C. Santangelo, G. Castiglione, A. Tsakris (Catania, IT; Athens, GR)*

Objectives: In order to identify, assess and apply relevant evidence for better health care decision-making, our study was conducted to evaluate whether HCAIs due to "*Klebsiella pneumoniae*" in intensive care units (ICUs) originate mainly from patients' endogenous flora or from exogenous sources, by determining: i) the occurrence of "*K. pneumoniae*" carriage; ii) the ICU-acquired "*K. pneumoniae*" infection and colonization rates, by site; iii) the impact of cross-transmission using molecular typing data.
Methods: The study design integrated the patient-based and the laboratory-based surveillance approaches at the ICUs of two Italian Hospitals. Standard definitions of carriage and colonization were used. Infections were in accordance with the protocol of the Italian Nosocomial Infections Surveillance in Intensive Care Units (SPIN-UTI). Molecular typing of "*K. pneumoniae*" isolates was performed by macrorestriction analysis of the XbaI-digested genomic DNA. A cross-transmission episode was assumed when two patients had indistinguishable isolates.
Results: During a seven-months period, from October 2008 to April 2009, a total of 171 patients were enrolled in the study and a total of 107 isolates were collected from 51 patients. In particular, carriage was associated to 15.5% of isolates; colonization to 24.1%, infection to 23.1%, and colonization/infection accounted for the remaining 37.3% of isolates. Bloodstream infections were the most encountered (56%). Cumulative incidences of "*K. pneumoniae*"-sustained colonization and infections were respectively 15.2 per 100 patients and 14.6 per 100 patients. PFGE analysis of "*K. pneumoniae*" isolates led to the identification of a total of 27 unrelated pulse-types. Particularly, 4 clones were associated to cross-transmission and the remaining 23 were single patterns associated with sporadic strains. Two major clones were identified involving 38.3% and 22.4% of isolates, showing respectively interhospital as well as intrahospital spread. The impact of "*K. pneumoniae*" cross-transmission was estimated to be at least 56.5%, thus defining the exogenous preventable proportion of all cross-transmission episodes.
Conclusion: Our data underline the impact of "*K. pneumoniae*" in the ICU settings and highlight the need of appropriate epidemiologic

investigations to trace sources and transmission routes in order to provide evidences to address screening at admission and other control policies.

P1310 Epidemiology and clinical features of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in neonates and adults in Spain: a multicentre study (GEIH BLEE 2006)

J. Rodríguez-Baño, C. Ruiz de Alegría, M. Cano, M. Díaz, J. Calvo, J. Hernández-Bello, A. Pascual, L. Martínez-Martínez on behalf of GEIH and REIPI*

Objectives: To analyse the epidemiology and clinical features of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* (ESBLKP) in the era of CTX-M predominance.
Methods: A multicenter cohort study including all cases of ESBLKP colonization/infection in 33 Spanish hospitals in February-March 2006 was performed. Epidemiological and clinical data were prospectively collected. ESBLs were characterized by PCR and sequencing. Clonality was studied by REP-PCR and PFGE.
Results: ESBLKP was isolated from 162 different patients from 33 hospitals. Twenty-six were neonates; clusters of related isolates were found in 5 of 7 neonatal units with cases. Among neonates, the most frequent ESBL was CTX-M-15 (19 patients); 18 (69%) had a urinary catheter, 16 (62%) an umbilical catheter, 17 (65%) were intubated, and 15 (58%) had previously received antibiotics. Twenty-three neonates (88%) had an infection, and 8 were bacteraemic (35% of those with infection). Appropriate empirical therapy was administered to 14 (61% of patients with infection). Three patients died (crude mortality, 12%). There were 3 other paediatric patients, but data were available only for one. Finally, ESBLKP was isolated from 133 adult patients. Clusters of clonally related isolates were found in 16 of the 22 centers with >1 adult case. The most frequent ESBLs in adults were CTX-M-15 (47 isolates), SHV-12 (29), and CTX-M-14 (18). Clinical data were available from 102 adults (85/85 [100%] and 17/45 [38%] patients with nosocomial and non-nosocomial ESBLKP, respectively). Among the 85 nosocomial ESBLKP, the predisposing features of patients with clustered isolates (55) and sporadic isolates (30) were compared. Those with sporadic isolates more frequently had chronic pulmonary disease (39% vs 7%, $p = 0.01$) and recent antibiotic use (90% vs 53%, $p = 0.001$). Seventy patients had an infection (82%); of these, 7 (23%) were bacteraemic, 53 (76%) received appropriate empirical therapy, and 13 (19%) died. Among the 17 patients with non-nosocomial ESBLKP with available data, 12 patients had an infection (9 urinary tract, 2 pneumonia, 1 cellulitis), and 1 died.
Conclusion: Infections caused by ESBLKP mainly affected neonates and predisposed adults; bacteraemia was frequent. CTX-M enzymes, particularly CTX-M-15, were the most prevalent ESBLs. Clonally-related clusters of cases were apparent in most hospitals. Patients with sporadic isolates had more predisposing factors that those with epidemic isolates.

P1311 Relevance of mucosal surveillance cultures in predicting Gram-negative sepsis in neonatal intensive care units

Ü. Parm, E. Sepp, T. Metsvaht, M. Ilmoja, K. Pruunsild, L. Maipuu, M. Pauskar, I. Lutsar (Tartu, Tallinn, EE)*

Objective: To examine the spectrum and time course of bowel and nasopharyngeal (NP) colonisation with Gram-negative (GN) bacteriae and to define the value of surveillance cultures (SC) in predicting late onset sepsis (LOS) in neonates admitted to neonatal intensive care units (NICU) due to suspected or confirmed early onset sepsis (EOS).
Methods: From August 2006 until December 2007 NP and rectal swabs were collected on admission and thereafter twice weekly in all newborns admitted within the first 72 h of life with suspected or confirmed EOS. Blood cultures were obtained on admission and further as clinically indicated. The genetic relatedness between mucosal and bacteraemic strains of *K. pneumoniae*, *E. cloacae* and *A. baumannii* was defined by pulsed field gel electrophoresis (PFGE).
Results: A total of 278 patients (144 with BW <1500g) were recruited; 171 became colonised with GN bacteria. About half of the rectal (693/1250) and NP (558/1153) samples contained GN microorganisms. Among 877 sterile body fluid samples 31 (5.6%) were positive for GN organisms. The number of different species per patient ranged between 1 to 8 (mean 1.53; SD 0.11). The most common colonisers were *E. cloacae* (27.3% of patients), *K. pneumoniae* (18.3%), and *Acinetobacter* spp (16.9%). Culture proven GN LOS was diagnosed in 32 cases. Altogether 2108 invasive and mucosal culture pairs were analysed. The overall sensitivity, specificity, positive and negative predictive values were 27%, 66%, 4% and 94%, respectively. At all time points the sensitivity values for Enterobacteriaceae were greater than those for nonfermentative organisms (66% vs 22% on Day 2–5 prior LOS, 36% vs 14% on Day 7–10 and 50% vs 16% on Day 11–14). Compared with non-colonised patients those with prior colonisation of *K. pneumoniae* ($p = 0.011$), *K. oxytoca* ($p = 0.003$), *E. coli* ($p = 0.004$), *Stenotrophomonas* spp ($p = 0.032$), and *Pseudomonas* spp ($p = 0.005$) were more likely to have a LOS whereas no association was found for *A. baumannii*, *Serratia* spp. and *E. cloacae.* In the PFGE analysis the invasive and mucosal strains were identical and had similar antibiotic susceptibility pattern.
Conclusion: Routine mucosal cultures to all GN organisms are inefficient in predicting LOS in NICU. However, if targeted for specific organisms (e.g *Klebsiella* spp., *E. coli*, Stenotraphomonas and *Pseudomonas*) identification of a microbe in mucosal surfaces may offer the opportunity of implementation of infection control measures and timely initiation of appropriate antibiotic therapy.

P1312 Trends in multidrug resistance among *Escherichia coli* and klebsiellae in Germany, 1995–2007

M. Kresken, D. Hafner on behalf of the Antimicrobial Resistance Working Party*

Objectives: *Escherichia coli* (ECO) and klebsiellae are among the most common pathogens causing infections in in- and outpatients. Antimicrobial agents such as 3rd generation cephalosporins (3GC), carbapenems (carba) and fluoroquinolones (FQ) are essential drugs to treat serious illness. Treatment outcome, however, has increasingly been threatened by the emergence and dissemination of resistant (R) strains (multi-drug R [MDR] strains in particular). The objective of this study was to document changes in the R patterns of ECO, *Klebsiella pneumoniae* (KPN) and *Klebsiella oxytoca* (KOX) in Germany between 1995 and 2007.
Methods: Susceptibility data of clinical isolates collected in 21, 20, 21, 22 and 21 laboratories that participated in five surveillance studies conducted by the Paul-Ehrlich-Society in 1995, 1998, 2001, 2004 and 2007, respectively, were analysed. MICs of antimicrobial agents were determined by the broth microdilution procedure according to the standard DIN ISO and interpreted by EUCAST criteria. The CLSI MIC method using ceftazidime (CAZ)±clavulanic acid (CLA) and cefotaxime (CTX)±CLA was employed as screening test for ESBL-producing isolates. MDR was defined as antimicrobial R to more than two of the following four drug classes: 3-cef, carba, FQ, aminoglycosides.
Results: A total of 3,964 isolates primarily recovered from urine (33%), wounds (20%), respiratory specimens (19%), and blood (11%) were tested. Tere were 890 (24%) ICU isolates and 3,074 (76%) non-ICU isolates. Single R to ciprofloxacin (CIP) in ECO, KPN, and KOX increased from 6% (26/449), 4% (9/224) and 2% (2/83), respectively, in 1995 to 28% (115/418), 13% (25/190) and 17% (17/101) in 2007. R to CTX in all three species rose from 1–4% in 1995 to 11–15% in 2007. Concurrently, an increase in the percentage of strains showing the ESBL phenotype was observed, while R to carba (ertapenem [ERT], imipenem [IMP], meropenem [MEM]) remained uncommonly. MDR rates for each of the species and study years are shown in the table. Of the 36 MDR isolates recovered in 2007, none were susceptible (S) to CTX and CIP, 8% to gentamicin and 39% to piperacillin–tazobactam. In contrast, carba were active against MDR isolates, except one KOX (MICs of ERT, IMP, MEM 16–64 mg/l).

Conclusions: Single R to CIP and CTX, representing FQ and 3GC, respectively, as well as MDR in ECO and klebsiellae increased between 1995 and 2007. In contrast, activity of carba did not change over the last 10–15 years.

	Phenotype	1995	1998	2001	2005	2007
ECO	R to >1 drug class	3.8%	4.2%	6.6%	10.8%	15.6%
	MDR	0%	0.2%	1.2%	3.4%	5.3%
KPN	R to >1 drug class	1.8%	3.7%	5.8%	5.2%	10.5%
	MDR	0%	0%	2.6%	1.6%	6.3%
KOX	R to >1 drug class	1.2%	7.7%	0.9%	10.9%	8.9%
	MDR	0%	0%	0%	0.8%	2.0%

P1313 Transmission rate of ESBL-producing Enterobacteriaceae within the hospital and in households

B.Y. Betsch, S. Droz, K. Bögli-Stuber, K. Mühlemann (Berne, CH)*

Objective: We studied the transmission rate of *E. coli* and *Klebsiella* spp. with extended-spectrum β-lactamase (ESBL) production from index patients with ESBL carriage to hospital room mates and household members.
Methods: Patients with ESBL carriage newly detected during diagnostic work-up were recruited prospectively during the time period May 7 2008 to September 30 2009. Hospital contacts were defined as room-mates for ⩾48 hours. Screening was performed weekly for the duration of contact between index patient and roommate and continued for 1 week after separation from the index patient and included a screening at hospital discharge. Screening included always a fecal sample; additional samples were examined if the following risk factors were given: intubation/tracheostoma, skin lesions, catheters draining urin or body fluids. Fecal samples were collected from household contacts in a 3-monthly interval. Stool samples were analyzed with 3 different ESBL selective culture media: ChromID ESBL agar (Biomérieux) and ESBL agar (AES), a bi-plate with 2 selective media (MacConkey agar plus Ceftazidim and Drigalski agar plus Cefotaxim).
Results: 111 index patients, 67 inpatients (60.3%) and 44 outpatients (39.7%) were analyzed. Fecal carriage was more frequent in inpatients (76.1%) than in outpatients (52.3%) ($p = 0.039$). The proportion of ESBL *E. coli* was higher among outpatients (86.4%) than among inpatients (58.0%) ($p = 0.003$). ESBL carriage was detected in 27 of 64 (42.2%) of household (32 of 103 members) and in 20 of 153 (13.1%) hospital contact patients ($p = 0.00001$). Fecal ESBL carriage in index patients was slightly higher in households with transmission (76.9%) than among households with no transmission detected (62.2%) ($p = 0.33$). The index patient isolates were CTX-M-producers in 81.9% and SHV-producers in 13.3%. The percentages for the contact patients resp. household contacts were 76.5% and 17.6% resp. 93.1% and 3.4%. ESBL-specific TEM-production was detected in overall 2%.
Conclusions: Inhospital patient-to-patient transmission rates of ESBL producing *E. coli* and *Klebsiella* spp. are lower than transmission rates within households. The higher rate of positive household contacts might be explained by the closer and longer duration of contact compared to the hospital setting.

P1314 Investigation of gastrointestinal carriage of multi-resistant Gram-negative bacilli in a population served by a district general hospital

L. Jenkinson, J. Smullen, N. Weightman, K.G. Kerr (Harrogate, UK)*

Objectives: The purpose of this study was to determine the incidence of faecal carriage of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in hospital and community patients served by a small district hospital in the north of England and to investigate whether a change in incidence had occurred over a four year period.
Methods: One thousand and seventy six samples were screened at Harrogate District Hospital during May-August 2004 and from March-June 2009 (973 samples).
During both periods all faecal samples submitted to the laboratory for routine microbiological investigations were anonymised and screened for the presence of ESBL, AmpC and K1 enzyme production by inoculation onto modified CLED agar containing 6 mg/L vancomycin and 8 mg/L cefalexin. Colonies from this medium were assessed for cefpodoxime resistance (10 mcg disc) and resistant isolates were examined further using the double disc method in accordance with BSAC guidelines. In addition, all cefpodoxime-resistant isolates were identified to species level using the API system (Biomerieux, France).
Results: Of 1076 samples screened in 2005, 16 (1.48%), 52 (4.8%) and 7 (0.7%) were identified as ESBL, AmpC and K1 producers, respectively. Of in-patient samples 1.25% were positive for ESBL compared with 1.55% community samples.
In 2009, 973 samples were screened and 70 (7.2%), 100 (10.28%) and 1 (0.51%) yielded ESBL, AmpC and K1 producers, respectively. ESBL producers were identified in 6.67% of inpatients compared with 7.42% of specimens from the community.
Conclusion: We found a significant increase ($p < 0.05$; χ^2 test) in faecal carriage of multi-resistant Gram-negative bacilli (MRGNB) between 2004 and 2009 for both inpatient and non-inpatient groups with overall increases in AmpC and ESBL carriage of 5.48% and 5.7%, respectively. Over the same time periods bloodstream infections associated with MRGNB in our hospital increased from 8 to 23. We conclude that faecal carriage of MRGNB in our small semi-rural population is increasing in line with reports from centres which serve large urban communities. Given that colonisation likely precedes clinically overt infection these findings have important public health implications.

P1315 Population structure of multidrug-resistant non-typhoidal *Salmonella enterica* isolates from Portugal

P. Antunes, J. Mourão, A.R. Freitas, L. Peixe (Porto, PT)*

Objectives: Salmonellosis is one of the most common foodborne infections in Europe and multidrug-resistant (MDR) *Salmonella* is emerging worldwide with increasing involvement of particular clones. Our aim was to analyze the population structure of MDR nontyphoidal *Salmonella enterica* isolates in order to assess the relationship between antibiotic resistance genetic elements and MLST genotypes.
Methods: We selected 40 *Salmonella* isolates from a large Portuguese collection (2002–2008), representative of MDR clones from human infections (n = 21), food (n = 14), environment (n = 1) and piggeries (n = 4). Clonal relatedness was established by PFGE and MLST (http://mlst.ucc.ie/mlst). Analysis of antibiotic resistance genetic elements included characterization of plasmid and integron backbones (PCR, sequencing), transferability and genomic location (I-CeuI/S1 nuclease hybridization).
Results: The isolates studied (13 serotypes) corresponded to 24 PFGE types and 16 different sequence types (STs) including a new combination of known alleles. Most S. Typhimurium (n = 16/18; 4 PFGE types) belonged to the worldwide spread ST19 (n = 14) and to its SLV, ST313 (n = 1). They included the 3 most widespread clones in Portugal (DT104, OXA-30/CMY-2 and sul3) carrying different integron types and Inc plasmids (FIIA, A/C, I1, N). The globally disseminated ST11 was identified in all but one of the S. Enteritidis isolates (n = 4; 2 PFGE-types), including one carrying qnrS and belonging to a major clone. Isolates belonging to the emerging S. Rissen clone (n = 5) were assigned to the singleton ST469 or to its SLV, a new ST, only previously reported in European isolates of the same serotype. The remaining MDR isolates of different serotypes were identified as ST15, ST27, ST32, ST40, ST82, ST334 and the singletons ST48, ST64, ST102, ST306 and ST358. Mostly, a ST represented a single serotype. Distribution of different plasmids (IncFIIA, IncP, IncI1, IncN) and integron types was observed among different clonal lineage. Particular integrons and plasmids (IncA/C, IncHI1) were confined to specific STs.

Conclusions: This is the first study describing the population structure of *Salmonella* in Portugal. Our results confirm the prevalence of particular clusters enclosing major MDR clones that cause human infections. The selection and worldwide spread of particular STs might be influenced by the acquisition and horizontal transfer of specific resistance genes and genetic elements.

P1316 Extended-spectrum β-lactamase producing Enterobacteriaceae in retail meat

I.T.M.A. Overdevest, J. Kluytmans (Tilburg, Breda, NL)*

Objectives: Worldwide there is a rapid increase of resistance in Enterobacteriaceae causing infections in humans, especially of ESBL. Potential sources for ESBL in humans are food-products like meat. The aim of this study is to determine the prevalence of ESBL-producing Enterobacteriaceae in meat used for consumption.
Methods: A prospective observational study was performed between 17th august and 30th October, 2009. In this period parts of raw, non-spiced meat which was bought for consumption were collected. The meat was inoculated into a TSB broth which was incubated at 37ºC for at least 16 hours. Subsequently part of the initial broth was transferred to another TSB broth which contained vancomycin and cefotaxim. After at least 16 hours of incubation 10 ml of the broth was inoculated on a selective chromogenic agar (Biomerieux ESBL ChromID) and incubated again for at least 16 hours. Determination was done by Vitek2; and confirmation of the presence of ESBL was done by e-test.
Results: A total of 249 meat samples were included in this study (mean weight: 11.9 g). From 45.9% of the samples ESBL were recovered (95% CI: 39.5%-52.2%). The animal sources of the meat were in 92 (37.0%) chicken; 75 (30.1%) beef; 53 (21.9%) pork and 29 (11.6%) were from other animals. ESBL was most frequently recovered from chicken (88.0%, 95% CI: 79.6%-93.9%). Beef and pork had statistically significant lower recovery rates, 18.7% and 18.9% respectively. 83.9% of the meat included in the survey was not produced biologically. Non-biological produced meat had a higher rate of ESBL-carriage than biological produces meat (48.8% vs. 33.3%), but this was not statistical significant.
Conclusion: Almost half of the meat in retail stores contained ESBL producing bacteria. However, chicken contained significantly more often ESBL than pork or beef. This is a potential source of the current pandemic of ESBL and further investigations are warranted to elucidate the role of antimicrobial use in food production animals.

P1317 Epidemiology of multi-resistant Gram-negative bacteria isolated from 65 years and older residents of a nursing home in France

S. Thibaut, J. Caillon, N. Foucher, F. Ollivier, M.L. Meas, G. Grandjean, F. Ballereau on behalf of Medqual Network*

Background: The aim of this survey was to study the epidemiology of Multi-Resistant Bacteria (MRB) in French nursing home. The network MedQual has conducted this survey and has compared risk factors leading to an infection with MRB among elderly patients 65 old and more, living either at home or in nursing homes.
Methods: This prospective epidemiological survey has been carried out since January 1st 2009 with a network of 169 laboratories in five regions of western France: Pays de la Loire, Bretagne, Centre, Basse-Normandie and Poitou-Charentes. MedQual collected MRB antibiograms (MRSA and 3rd-generation cephalosporins (3GC)-resistant Enterobacteria) and socio-demographic patients' characteristics. 3 kinds of housing were considered: home (G1), nursing home without (G2) or with (G3) medical care.
Results: 875 patients MRB have been included, of which 539 (61.6%) were 3GC-resistant Enterobacteria: 381 in G1 (69.5%), 129 in G2 (47.8%), 29 in G3 (50.9%).
Within the last 12 months, 51.6% have experienced hospitalization (G1: 49.1%; G2: 55.0%; G3: 68.9%) and 70.5% have received an antibiotic treatment (G1: 70.6%; G2: 71.3%; G3: 65.5%). 94.9% of the 3GC-resistant Enterobacteria were isolated in urine and 70.3% were *E. coli* strain (G1: 73.9%; G2: 74.7%; G3: 68.9%). 58.8% of *E. coli* producing a ESBL enzyme (G1: 55.9%; G2: 67.4%; G3: 60.0%). Among the ESBL *E. coli*, 61.8% were resistant to ciprofloxacin, 55.6% to sulfamides. No difference was observed among the 3 kinds of housing for the resistance to these antibiotics. Only 4% of ESBL *E. coli* were resistant to furans.
Conclusion: MRB (MRSA and 3GC resistant Enterobacteria) distribution was significantly different between people living at home and living in nursing home (without or with medical care). Same risk factors were observed (in different proportions) among the 3 groups. Enterobacteria distribution was similar between the 3 kinds of housing, and the ration of ESBL *E. coli* didn't changed significantly according to the 3 groups of elderly patients. A better knowledge of the epidemiological behaviour of these MRB will contribute to better-adapted antibiotic strategies.

P1318 Predictor factors of ESBL versus non-ESBL *E. coli* bacteraemia at a university hospital, 2006–2007

A. García-Hernández, E. García-Vázquez, J. Gómez, A. Hernández-Torres, A. Fernández-Rufete, E. Moral-Escudero, J. Ruiz, J.A. Herrero, M. Cantero (Murcia, ES)*

Objectives: Incidence of ESBL *E. coli* bacteraemia (ESBLB) has progressively increased; the aim of this study was to evaluate predictor factors of ESB in a cohort of patients with *E. coli* bacteraemia (EB).
Patients and Methods: Observational and comparative study of a cohort of non-paediatric patients with EB admitted at a university affiliated hospital. Data collection from clinical records has been done according to a standard protocol. We analysed epidemiological, clinical, microbiological and laboratory data from January 2006 through May 2007. Patients with EB were identified by review of results of blood cultures from the hospital microbiology laboratory. *E. coli* isolation, identification and sensitivity test (VITEK-2; Biomerieux, France) were performed by standard criteria.
Results: 153 patients with EB were studied; prevalence of ESBLB was 22% (n = 34). Mean age was 59 years (range 14–94); in bivariate analysis, risk factors associated with ESBLB were: acquisition of the infection in Surgical Wards, nosocomial-acquired EB, previous antibiotic treatment, previous surgery, previous invasive techniques, structural renal diseases, comorbidity score of Charlson >3, severity of illness at onset according to Winston criteria "critical" or "poor" and shock on presentation. In the multivariate analysis, risk factors associated with ESBLB were previous antibiotic treatment (OR 2.6; 95% CI 1.1–6.2) and severity Winston score I-II (OR 9.8, 95% CI 3.4–28.3). In the multivariate analysis, risk factors associated with ESBLB were previous antibiotic treatment (OR 9.8, 95% CI 3.4–28.3) and severity Winston score I-II (OR 2.6; 95% CI 1.1–6.2).
Conclusions: Previous antibiotic treatment and severity Winston score I-II are the factors associated in our study to ESBL versus non-ESBL *E. coli* bacteraemia.

P1319 Bloodstream infections due to *Escherichia coli* and *Klebsiella* spp. producing extended-spectrum β-lactamase: factors influencing adequacy and mortality

G. Peralta, L. Anibarro, M. Lamelo, M. Velasco, A. Delgado-Iribarren, J.P. Horcajada, M. Montero, M.C. Fariñas, J. Alonso, M. Roiz, J.A. Capdevila for the SEMI-BLEE Study Group*

Objectives: Bloodstream infections due to *E. coli* and *Klebsiella* producing extended-spectrum β-lactamase (ESBL) are increasing. The precise role of factors influencing adequacy of empirical antibiotic treatment and outcome are controversial.
Methods: Retrospective study of cases *Escherichia coli* and *Klebsiella* spp ESBL bacteremia from 2003 to 2008, in 18 Hospitals of Spain. Cases were identified from Microbiology laboratory databases. Empirical antimicrobial therapy was judged to be either adequate or inadequate on the basis of the *in vitro* susceptibility of an isolated organism, and/or the

initiation of antibiotic treatment within 24 h of blood culture extraction. ESBL production was screened by the double disc synergy method as a complementary test for automated systems.
Results: In the period of study 372 cases of bacteremia (85% *E. coli*) with a mortality of 24.3% were identified. The origin of bacteremia were mostly urinary (50.7%), biliary (10.8%), and of unknown origin (10%). Of them 144 (38.8%) cases were nosocomial and 127 (34.4%) health related. Empirical antibiotic treatment was adequate in 45% of cases. In a logistic regression analysis the only clinical factor associated with the adequacy of empirical antibiotic therapy was the abdominal origin (ORa: 3.19; 95% CI: 1.26–8.11). Factors associated with mortality in the whole cohort were severe sepsis or shock (ORa: 8.84, 95% CI: 4.76–16.41), Charlson index score >2 (ORa: 2.92; 95% CI: 1.51–5.64), nosocomial acquisition (ORa: 2.26; 95% CI: 1.19–4.28) and adequate empirical treatment (ORa: 0.35; 95% CI: 0.21–0.94).
Conclusions: Mortality of *E. coli* and *Klebsiella* spp. ESBL bloodstream infections is high. Adequacy of empirical antibiotic treatment is an independent risk factor for mortality in these patients.

P1320 Impact of empiric cefepime therapy on mortality among patients with bloodstream infections due to extended-spectrum β-lactamase-producing pathogens

T. Chopra, J. Zhao, R. Tansek, D. Hatahet, J. Hothi, K. Chaudhry, H. Rahbar, T. Chen, T. Truong, V. Rodriguez, L. Bernabela, A. Bhargava, D. Marchaim, G. Alangaden, K. Kaye (Detroit, US)*

Objective: To study the impact of empiric cefepime for the treatment of blood stream infections (BSIs) due to extended spectrum β-lactamase-producing pathogens (ESBLs).
Methods: A cohort study was conducted at DMC from 1/05 to 12/07 in 5 hospitals. Retrospective chart review was conducted on patients with ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* (ESBLs). Patient variables collected included demographics, comorbid conditions, empiric treatment and in-hospital mortality. Empiric antibiotics were defined as antibiotics initiated during the time spanning from 2 days prior to culture to 3 days after culture. Logistic regression was used to create a prediction model for in-hospital mortality.
Results: 145 ESBL BSIs were identified; 83% were *K. pneumoniae* and 16.5% were *E. coli*. 35.2% of the BSI were catheter-related. The mean age of the patients was 66 years, 51% were female and 79.3% were African-American. The in-hospital mortality rate was 35% (n = 51). Cefepime was utilized as an empiric antibiotic in 68 patients (46.9%) and carbapenems in 40 (27.6%) patients. In logistic regression, predictors of in-hospital mortality included: admission to intensive care unit (OR = 2.14, 95% CI 0.96–4.76), central line present prior to positive culture (OR = 2.13, 95% CI 0.70–6.45), presence of a rapidly fatal condition at the time of admission (OR = 5.71, 95% CI 2.36–13.8) and prior hospitalization (OR = 1.75, 95% CI 0.76–4.02). Type of empiric antibiotic was not associated with mortality. In multivariate analysis, receipt of cefepime alone (n = 42) was associated with increased mortality, although this association did not reach statistical significance (OR = 1.58, 95% CI 0.67–3.70). There was a trend between empiric carbapenem therapy and decreased mortality (OR = 0.59, 95% 0.24–1.45). These associations remained unchanged after controlling for hospital and anatomic source of infection. A sub-analysis was performed on 42 patients who were treated with empiric cefepime alone, comparing survival and MIC to cefepime. No association was found between cefepime MIC and mortality in this sub-group.

MIC Cefepime (μg/ml)	In-hospital mortality [deaths/total (%)]
⩽2	4/12 (33.3%)
4	1/4 (25%)
8	1/2 (50%)
⩾16	10/24 (42%)

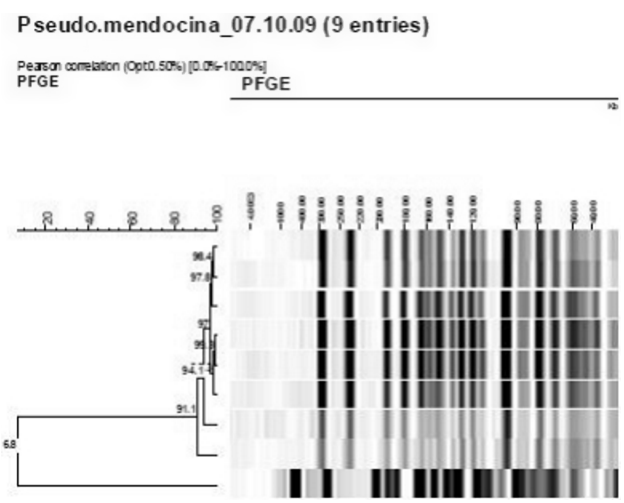
Conclusion: Empiric use of cefepime alone for BSI due to ESBLs was associated with a trend towards increased mortality. We were not able to demonstrate an association between MIC to cefepime and mortality, but were underpowered to do so. Additional study of the efficacy of cefepime therapy in treating ESBLs should be conducted.

P1321 Pseudo outbreak of *Pseudomonas mendocina* in stem cell-cultures

S. Tschudin, C. Arber, A. Tichelli, D. Nogarth, M. Dangel, R. Frei, A.F. Widmer (Basel, CH)*

Objectives: *P. mendocina* was first isolated in the 1970s from soil and water samples collected in the province of Mendoza, Argentina [1]. The first clinical case report was reported in 1992 with *P. mendocina* endocarditis, followed by two further case-reports of infections due to this organism. However, the association with human infections is largely unknown. We report a cluster of *P. mendocina* in diagnostic stem cell-cultures of the haematology unit.
Methods: We initiated an outbreak-investigation after the detection of Gram-negative bacteria by routine-microscopy of 21 diagnostic stem cell-cultures.
Identification of the Gram-negative bacteria was performed by conventional culture and additional confirmation by sequencing of 16s rDNA. Pulsed-field gel electrophoresis (PFGE) was performed to reveal the identity of the strains. For source-investigation environmental cultures of the involved surfaces in the affected laboratory, as well as water samples and the unopened fresh reagents used for the preparation of the diagnostic stem cell-cultures were examined.
Results: Culture and identification of the Gram-negative bacteria of three contaminated stem cell-cultures revealed *P. mendocina.* The environmental cultures and the water samples were negative for this pathogen. Culture of one of the reagents used however also revealed *P. mendocina.* PFGE revealed that the strains isolated were identical (Figure). Further outbreak investigation of the manufacturer confirmed contamination of their product. The computerized database of the clinical microbiology unit revealed that this pathogen has never been isolated before at our hospital in the last decade.
Conclusions: This is the first report of an outbreak caused by *P. mendocina.* We conclude that this environmental pathogen has the potential to cause contamination of reagents used in clinical settings. Our report demonstrates that commercial sterile product may be a source of outbreaks. During the entire outbreak no clinical isolate with *P. mendocina* was identified, nor was there evidence for an unusual increase of other *Pseudomonas* infections.
Reference(s)

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P1322 Molecular epidemiology and characterization of extensive drug-resistant *Pseudomonas aeruginosa* outbreak in a haematology unit

T.T. Tan, T.H. Koh, B.H. Tan (Singapore, SG)*

Objectives: Infections caused by extensive-drug-resistant *Pseudomonas aeruginosa* (XDR PAE) pose a serious problem in immunocompromised hosts. Metallo-β-lactamase (MBL) producing strains are now commonly seen around the world. We recently showed that clones of MBL-producing *P. aeruginosa* have not become established in our center. However, over a 24-mth period, XDR PAE was repeatedly isolated among our hematology patients. We describe the molecular epidemiology and characterization of these *P. aeruginosa* isolates as well as the measures taken to contain this outbreak.
Methods: All archived *P. aeruginosa* isolates kept during the study period were retrieved for analysis. These included antimicrobial susceptibility profile, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). The resistance mechanisms were elucidated by outermembrane analysis for porin expression and a real-time reverse transcription-PCR for the expression of various efflux pumps, β-lactamases as well as porins.
Results: The PFGE revealed a predominant clone that resurfaced many times over the course of the study. This clone was characterized by resistance to penicillins, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones. It remained susceptible only to colistin.
It was a non-MBL producing clone. Outermembrane analysis did not reveal the loss of any porins. The over expression of the MexXY-OprM efflux system appeared to be the predominant resistant mechanism. Furthermore, environmental survey found the presence of such XDR PAE clones in the drains of sinks in 2 rooms. Clinical isolates from previous occupants of the rooms with identical PFGE pattern suggested the sinks to possible contamination sites that help to propagate the outbreak. Strict contact and isolation protocols for known colonized patients were instituted. A change of sinks and drains that proved difficult to clean finally resulted in the termination of this outbreak. There was no further isolation of this clone of XDR PAE in the following 6 months.
Conclusion: This study illustrates the ability of non-MBL producing XDR PAE to persist in the environment and argues for the need to track the epidemiology MDR organisms to identify clonal outbreaks.

P1323 Risk factors for acquisition of multidrug-resistant *Pseudomonas aeruginosa* in patients with haematological malignancies

Y.X. Liew, W. Lee, T.T. Tan, J.L. Ng, A.L. Kwa (Singapore, SG)*

Objective: Multi-drug-resistant *Pseudomonas aeruginosa* (MDRPa) has been associated with increased mortality. Thus, the identification of risk factors for the acquisition of MDRPa will help to identify hospitalized patients who are at increased risk for harbouring MDRPa & implement interventions that can limit its spread & to identify potential causative factors leading to antimicrobial resistance.
Methods: A matched case (n = 28) – control (n = 61), study involving recruitment of patients from Singapore General Hospital (SGH), was conducted to examine the risk factors for MDRPa acquisition in Haematology ward retrospectively. Univariate & multivariate analysis (conditional logistic regression) via STATA MP10 were used. Pulsed-Field Gel Electrophoresis (PFGE) was also performed to ascertain the clonality of MDRPa strains.
Results: Forty-six males & 43 females, aged 46.57 (SD 1.50) were recruited from Dec 06 to Dec 08. Significant risk factors were length of hospitalization (Odds ratio [OR] = 1.06; Confidence interval [CI] = 1.02–1.10; P = 0.003), APACHE II score (OR = 1.31; CI = 1.08–1.58; P = 0.007), presence of central venous catheters (OR = 5.30; CI = 1.47–19.04; P = 0.010) & foley catheters (OR = 6.38; CI = 1.69–24.07; P = 0.006), antibiotic use before MDRPa isolation, namely aminoglycosides (OR = 5.03; CI = 1.79–14.10; P = 0.021), fluoroquinolones (OR = 4.60; CI = 1.29–16.90; P = 0.019), carbapenems (OR = 10.06; CI = 2.93–34.56, P = 0.001), vancomycin (OR = 6.15; CI = 2.00–18.90; P = 0.002) & metronidazole (OR = 8.13; CI = 1.72–38.42; P = 0.008) & prior high-dosed cytarabine used (OR = 5.13; CI = 1.24–31.34; P = 0.026). The risk of dying from a MDRPa infection is 41 times higher (P = 0.001; CI = 0.03–0.172) than from no Gram-negative bacteria infection. PFGE of the MDRPa revealed that half of all isolates were clonally related, where MexXY multi-drug efflux system overproduction may be the predominant mechanism of resistance.
Conclusion: We reported for the first time, the use of high dosed cytarabine was associated with acquisition of MDRPa. Prior aminoglycoside exposure may have induced the expression of MexXY multi-drug efflux system that renders cross-resistance to carbapenem & fluoroquinolones. Other significant risk factors include central venous & foley catheters, APACHE II score & length of hospitalization. Patients with these risk factors can be identified early for active surveillance & treated with appropriate antibiotics therapy empirically, if needed.

P1324 Genetic fingerprints of phenotypic different *Pseudomonas aeruginosa* strains from cystic fibrosis patients with DiversiLab® rep-PCR analysis

J. Posch, L. Masoud, G. Thalhammer, G. Zarfel, G. Feierl, E. Leitner, A.J. Grisold, U. Wagner-Eibel, D. Haas, E. Marth (Graz, AT)*

Objectives: *P. aeruginosa* is highly prevalent in cystic fibrosis (CF) patients, causing chronic airway infection. This study was conceived to investigate various aspects of colonization in order to answer the following questions:
1. Is one patient colonized by one specific genotype?
2. Are phenotypic different strains of one patient also genotypic dissimilar?
3. Are there epidemic strains colonizing two or more patients?
4. Are the results of rep-PCR comparable to pulsed-field gel electrophoresis (PFGE-Spe I)?

Methods: From July to October 2009 a total of 46 *P. aeruginosa* consecutive strains were collected from the sputum of 17 CF patients. Routine methods for identification and susceptibility testing were employed. Extraction of DNA, rep-PCR and generation of genetic fingerprints were done following the manufacturer's instructions. To determine the strain relatedness the DiversiLab® software was used. The strains were considered as "linked" with similarities >95% respectively a maximum of two peak changes. To evaluate the rep-PCR data 25 strains were also analyzed by PFGE-Spe I.
Results: In most cases patients were colonized by *P. aeruginosa* strains with only one specific genotype. A total of 30 strains from 12 patients showed at least one phenotypic difference concerning resistance pattern or morphology (mucus, colour). Nevertheless the major part of these strains was related to four main genotypic patterns. In regard to question three, we found that there are epidemic strains colonizing two up to six different patients. In comparison to PFGE the rep-PCR provided similar results in most cases. Four strains showed differences with a maximum of four band changes.
Conclusion: We conclude that there is an evidence for the presence of epidemic *P. aeruginosa* strains in CF patients. As a consequence of our findings further investigations will focus on sources and possible transmission routes to avoid cross-contamination. We are convinced of the superiority of the DiversiLab® system, because it provides results comparable to PFGE. Rapidity, easy handling and user friendly analysis are major advantages.

P1325 blaCTX-M-15 genetically linked to qnrB1 on conjugative plasmids from *Klebsiella pneumoniae*

Z. Zong, S.R. Partridge, J. Iredell (Chengdu, CN; Sydney, AU)*

Objective: blaCTX-M-15 appears to be the dominant extended-spectrum β-lactamase gene worldwide, including in Sydney. The qnrB1 quinolone resistance gene was first identified on a plasmid from *K. pneumoniae* that also carried blaCTX-M-15. These two important antibiotic resistance

genes have since been detected together in bacterial strains from different locations. Here, the multi-resistance regions (MRR) of conjugative plasmids carrying both genes were characterised to identify any direct link between them.

Methods: Two different *K. pneumoniae* clinical strains carrying blaCTX-M-15 and qnrB1, isolated at Westmead Hospital, Sydney in 2006, were mated with a rifampicin-resistant derivative of DH5α. Transconjugants identified as carrying both genes by PCR were analysed by S1 nuclease pulse-field gel electrophoresis and the plasmids subjected to PCR-based replicon typing and HpaI restriction digestion. A combination of PCR mapping with reference to known MRR (including that of pC15–1a, carrying blaCTX-M-15), inverse PCR and limited DNA sequencing was used to characterise MRR.

Results: Transconjugants from each strain contained a single plasmid carrying both blaCTX-M-15 and qnrB1. These plasmids, pJIE127 (~130 kb) and pJIE146 (~145 kb), appeared closely related and their replicons could not be typed. The large (>45 kb) MRR of pJIE146 consists of a region almost identical to the pC15–1a MRR (including blaTEM-1b, aac(3)-II, aac(6′)-Ib-cr, blaOXA-30, tetA(A), IS26, ISEcp1, Tn2, Tn1721 and Tn5403) flanked by other common MRR components and additional resistance genes (qnrB1, dfrA14, sul2, strB). The pJIE127 MRR contained all of these components in a different configuration plus additional copies of IS26.

Conclusions: qnrB1 is linked to blaCTX-M-15 within a single large MRR on each plasmid. The pJIE127 MRR could have arisen from the pJIE146 MRR by homologous recombination, illustrating the importance of this mechanism in the evolution of MRR. Mapping the pJIE127 and pJIE46 MRR on the basis of known MRR structures was feasible because of the mosaic nature of these regions, which appear to be composed of components from a limited set arranged in different ways. The relationship of these MRR to the MRR of pC15–1a, from Canada, demonstrates how large structures carrying multiple resistance genes may spread between geographic locations. The pJIE127 and pJIE146 MRR may provide the basis for examining other strains/plasmids carrying both blaCTX-M-15 and qnrB1.

P1326 First report of the plasmid-mediated quinolone-resistant gene qnrB5/19 on a 40 kb IncR plasmid in *Escherichia coli*, isolated from veal calves

J. Hordijk, J. Wagenaar, A. van Essen-Zandbergen, A.B. Bosman, D.J. Mevius (Utrecht, Lelystad, NL)*

Objective: The presence of plasmid mediated quinolone resistance (PMQR) is increasingly reported world wide. The majority of PMQR genes described are qnr genes, associated with various bacterial species on both chromosomal dna and plasmids. The qnr-B5/19 genes were detected previously on IncN, IncL/M, ColE1 plasmids in *Klebsiella* spp, Escherichia spp, *Salmonella enterica*. The aim of this study was to characterize a recently detected qnrB5/19-gene and its genetic environment on a 40 kb IncR plasmid in *E. coli*, isolated from veal calves in the Netherlands.

Methods: Plasmids were isolated from a qnr positive *E. coli* and transferred to DH10B(TM) cells by electroporation. Transformants were selected on MacConkey agar with 0.03 mg/L ciprofloxacin. Plasmids from the donor strain and the transformant DH10B(TM) cells were isolated and analyzed by Southern hybridization to locate the qnr-gene. PCR and sequencing was performed to detect mutations in the QRDR region of the gyrA/B and parC/E-genes and on the qnr flanking regions by primer walking. Incompatibility groups were determined by PCR-based replicon typing with additional PCR protocols for ColE, IncR and IncU. Furthermore, MIC values and the presence of resistance genes other than qnr were compared between the donor and recipient strain by broth microdilution and Identibac array analysis, respectively.

Results: No mutations were found in the gyrA/B and parC/E genes in the donor strain. The qnrB-gene was determined to be qnrB5/19. A final designation of the qnr-subtype is performed currently. The plasmid harbouring this gene was IncR, which is based on the detection of the repB gene in the donor and its transformant with 100% homology to that of the of reference plasmid pK245.

MIC-values and array analysis suggested that next to qnrB5/19, sul1, dfr12 and a class 1 integron are located on the same plasmid. Moreover, both donor and recipient show kanamycin and tetracycline-resistance (MICs > 128 and 64 mg/L, respectively).

The determination of the genetic environment of the qnr-gene in relation to the other resistance genes is in progress.

Conclusions: To our knowledge, this is the first report of a qnrB5/19 in *E. coli* of animal origin in the Netherlands and the first report of this gene on IncR.

P1327 High prevalence of qnr genes in *Escherichia coli* from healthy animals in Nigeria

D. Fortini, K. Fashae, A. García-Fernández, L. Villa, A. Carattoli (Rome, IT; Ibadan, NG)*

Objectives: The animal reservoir of plasmid-mediated quinolone resistance (PMQR) is still controversial and little information is available on the prevalence of these resistance determinants in developing countries. The aim of this study was to identify and characterize PMQR in a collection of *Escherichia coli* isolated from healthy chickens and pigs at slaughter, collected in November-December 2006 at Ibadan, Nigeria.

Methods: Faecal samples from 100 chickens and 100 pigs were plated on Eosin-methylene blue agar containing 100ug/mL ampicillin, recovering 96 *E. coli* from chickens and 66 from pigs. Strains were tested for antimicrobial susceptibility by disk diffusion assay. Breakpoints for ciprofloxacin (CIP) were determined by Etest (AB Biodisk, Solna Sweden). PMQR genes were screened by PCR and DNA sequencing. Clonal relatedness of the isolates was determined by ERIC-PCR. Plasmids were analysed for PMQR positive strains by conjugation or transformation, PCR-based replicon typing and restriction fragment length polymorphism (RFLP).

Results: 82 strains from chickens and 10 from pigs were resistant to nalidixid acid, 69 and 2, respectively, showed resistance to CIP (MIC > 4.0 μg/ml), 13 and 5, respectively showed reduced susceptibility to CIP (MICs 0.125–0.5μg/ml). Only one strain showed resistance to ceftazidime and cefotaxime. The ERIC-PCR showed that the isolates were distributed among 17 clonal groups. PMQR genes were detected in 18 strains (18/92, 19.6%) and 11 of them were in the group showing reduced susceptibility to CIP. 12 strains carried qnrS1, 3 qnrB19, 1 qnrB10 and 3 qepA; one strain carried both qepA and qnrB10. None of isolates carried qnrA, qnrC, qnrD and aac(6′)-1b-cr genes. qnrS1 was located on IncX, IncN, and IncI1 plasmids, qnrB19 was located on a small ColE-like plasmid and qepA was located on IncHI2. The strain showing CAZ and CTX resistance carried qnrS1 and the CTX-M-15 extended spectrum β-lactamase, but located on different plasmids.

Conclusion: To our best knowledge, this is the first study on the prevalence of qnr genes in bacteria from animal sources from Africa. Our findings suggest that food animals could represent an important reservoir of these resistance genes. Previous studies reported high prevalence of qnr genes in clinical isolates from humans in the Ivory Coast and Algeria, suggesting that the spread of these resistance determinants in Africa could be particularly relevant.

P1328 Analysis of plasmid-mediated quinolone resistance determinants among ESBL-producing Enterobacteriaceae in Belgium

H. Rodriguez-Villalobos, R. de Mendonça, P. Bogaerts, A. Cardentey-Reyes, I. Montesinos, C. Cardona, A. Deplano, M.J. Struelens, Y. Glupczynski (Brussels, BE; Stockholm, SE)*

Objectives: Plasmid-mediated quinolone resistance determinants are widespread among ESBL-producing organisms enhancing selection and transmission of multiresistance. qnr genes have not yet been reported

in clinical isolates from Belgium. We evaluated the prevalence of Qnr determinants in ESBL-producing isolates.
Methods: 117 ESBL-producing clinical isolates recovered from January 2000 to December 2007 at Erasme University Hospital (Brussels) with MIC of ceftazimime >8 mg/L and MIC of ciprofloxacin >0.25 mg/L were studied. Isolates included *E. coli* (n = 43), *K. pneumoniae* (n = 42), *E. cloacae* (n = 17), *E. aerogenes* (n = 9), *S. marcescens* (n = 2), *P. mirabilis* (n = 2) and *Providencia* spp.(n = 2). The presence of ESBL was confirmed by double combination disk test. ESBLs were characterized by PCR-sequencing assay. Qnr genes were analysed by multiplex PCR targeting blaqnrA, blaqnrB, blaqnrS and sequencing. The genetic environment of qnr genes was analysed by PCR mapping and sequencing. Clonality was assessed by PFGE. Conjugation assay was performed with *E. coli* J53 azide resistant as recipient strain.
Results: qnr genes were found in 37 isolates (32%). Only 1 CTX-M-15-producing *E. coli* isolate harboured qnrS1 (2%). Seventeen *E. cloacae* harboured qnrA1 (100%). These strains co-produced CTX-M-9 and SHV-12 (n = 15) or CTX-M-9 alone (n = 2). qnrA gene was present into a complex class 1 integron being flanked upstream by Orf 513 (ISCR1) and downstream by qacEdelta1 genetic elements (qac/sul 3′ end). No transconjugants were obtained neither from *E. coli* nor *E. cloacae.* 19/42 (45%) CTX-M-15 producing *K. pneumoniae* showed qnr genes (17 qnrS1 and 2 qnrB). These strains belong to a major epidemic clone in ICU. The analysis of transconjugants showed that blaqnrS1 was transferred with or without blaCTX-M-15, suggesting the location of qnr genes in different plasmids. blaqnrB was co-transferred with blaCTX-M-15. No qnr were found in the other species. Susceptibility to quinolones varied according to qnr type and host species.
Conclusion: This is the first description of qnr genes in ESBL-producing Enterobacteriaceae clinical isolates in Belgium. Our findings show a high prevalence of qnr genes among epidemic ESBL-producing *E. cloacae* and *K. pneumoniae*. They underline the need for caution in using quinolones for treatment of ESBL producers. Further study will examine the spread of qnr genes in other Belgian hospitals.

P1329 Identification of different CMY-2-producing strains of *Proteus mirabilis* in a Czech hospital

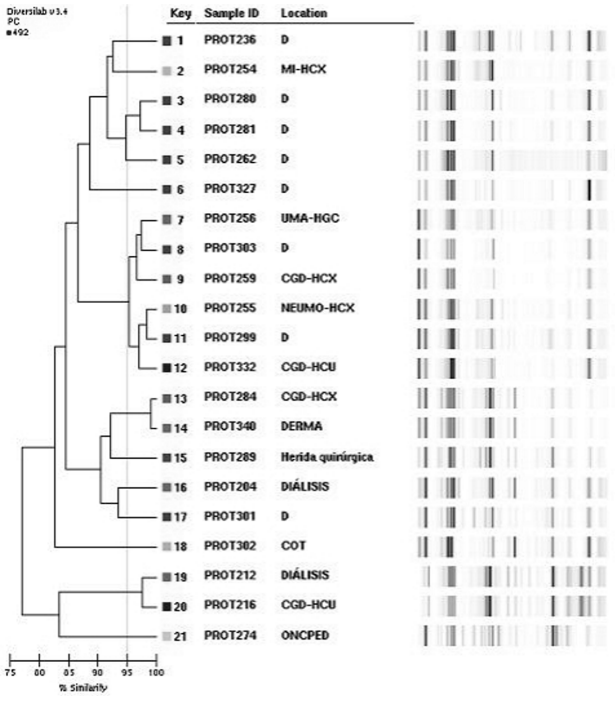
J. Hrabak, V. Jindrak, E. Chudackova, T. Bergerova, D. Cervena, P. Urbaskova (Plzen, Prague, CZ)*

Objectives: Acquired AmpC-type β-lactamases, especially DHA-1 producing strains of *Klebsiella pneumoniae* and *Serratia marcescens*, are endemic in some Czech hospitals. blaDHA-1 gene, mobilized by ISCR1 insertion sequence, has been localized as a part of class 1 integron in these strains. However, CMY type AmpC had only been described in veterinary isolates in this country. First two AmpC-producing isolates of *Proteus mirabilis* were sent to the Department of Microbiology of the Faculty of Medicine in Plzen for further analysis in spring of 2009.
Methods: Strains were identified by a standard microbiological method using ENTEROtest 12 (Pliva Lachema Diagnostika, Brno, Czech Republic). AmpC detection was performed by a modified double-disk synergy test on the plates with and without cloxacillin (128 mg/l). MICs to 24 antibiotics were determined as proposed by EUCAST. β-lactamase content was analyzed by isoelectric focusing followed by visualization with 0.5mM nitrocefin. Multiplex PCR was performed for preliminary identification of acquired AmpC-like genes. Entire gene was subsequently amplified and sequenced. Presence of ISEcp1 insertion sequence was determined by specific primers. Strains were compared by pulse-field gel electrophoresis after the digestion with XbaI endonuclease.
Results: The isolates showed different MIC and PFGE patterns. According to EUCAST criteria, both strains were intermediate susceptible to third generation cephalosporins and remained susceptible to aminoglycosides, fluoroquinolones and trimethoprime. In both isolates, CMY-2 AmpC-type β-lactamase was identified. Conjugative transfer of the blaCMY-2 gene was not successful as well as the presence of ISEcp1 element was not identified.
Conclusions: This is the first report of CMY type AmpC β-lactamase identified in clinical isolates as well as the first observation of acquired AmpC in *P. mirabilis* in the Czech Republic. Mobilization of blaCMY-2 by ISEcp1 seems to be common in other European countries, but this element was not found in described isolates that may indicate a different type of mobilization. Sequence analysis of blaCMY-2 gene environment is ongoing.
This work was financed by research project grants NS9717–4/2008, MSMT 2E08003 and MSM0021620819.

P1330 Occurrence of acquired AmpC β-lactamases and clonal relationship among *Proteus mirabilis* at a university hospital complex in Santiago de Compostela, Spain, 2006–2009

M. Treviño, L. Martínez-Lamas, A. García-Zabarte, C. García-Riestra, D. Peñalver, B.J. Regueiro (Santiago de Compostela, ES)*

Objectives: *Proteus mirabilis* is one of the most β-lactam-susceptible enterobacteria because it does not express AmpC cephalosporinase. Because of the increasing clinical relevance of this bacteria usually associated with complicated urinary tract infections an bacteriemia is of great importance to know the prevalence of multidrug-resistance by acquired AmpC β-lactamases and their epidemiology.
Methods: Isolates were obtained from routine cultures at the University Hospital Complex of Santiago de Compostela from 2007 to July 31th 2009. Only one isolate per patient was considered for analysis. Identification to the species level was achieved with Vitek 2 (bioMérieux). Antimicrobial susceptibility testing was assayed by Vitek 2. The isolates selected for this study were those showing intermediate or total resistance to amoxicillin–clavulanic, cefotaxime or ceftazidime according to CLSI breakpoints. AmpC screening were performed by double-disk synergy test with ceftazidime (30 mcg), cefotaxime (30 mcg) and cefoxitin (30 mcg) against cloxacillin (500 mcg) and boronic acid (300 mcg).
Molecular typing of the isolates was performed by automated rep-PCR using DiversiLab system (bioMérieux).



Results: Among the 2,217 isolates of *P. mirabilis* recovered, 35 were acquired AmpC β-lactamase producers (1.58%). This isolates were recovered from urine, blood, surgical wound, catheter tip, tracheal aspirate and skin ulcer. Forty percent of samples were from ambulatory patients. Resistance of the AmpC-producers to non-β-lactam antibiotic was not very high except for quinolones. Isolates showed resistance to

nalidixic acid (81%), ciprofloxacin (51%), trimethprim-sulfamethoxazole (19%), gentamicin (11%), tobramycin (5%). No resistance was seen against amikacin.
Fingerprinting analysis of the last 21 isolates recovered showed high genetic diversity. Hospital source strains tended to be arranged in clusters different from community source strains.
Conclusion: Knowledge about the prevalence and diffusion of this emergent resistance may be helpful to establish preventive measures that will curb their spread.

P1331 Effect of different environmental parameters upon the expression of certain virulence factors and resistance features in *Escherichia coli* aquatic strains

E. Panus, N. Rosoiu, C. Bleotu, L. Dascalu, L. Ditu, M.C. Chifiriuc Balotescu, V. Lazar, C. Iordache, C. Larion, O. Dracea, M. Bucur (Constantza, Bucharest, RO)*

Purpose: To investigate the expression of 8 virulence factors (VF) and bacterial resistance features of 20 *E. coli* aquatic strains isolated from Black Sea incubated at different temperatures and in variable culture media.
Material and Methods: The expression of cell-associated (i.e. adhesion to HeLa cells) and soluble VF (i.e. lecithinase, lipase, gelatinase, caseinase, amylase, aesculin hydrolysis, DN-ase) as well as the resistance to different bivalent metals (Cu, Co, Mn, Zn, Ni) were investigated at different incubation temperature, salinity and glucose concentration; pH in aerobic and respectively, anaerobic conditions.
Results: The tested strains have grown at 22ºC, 37ºC, 44ºC, irrespective to the salinity, pH and glucose concentration, in aerobic and anaerobic incubation conditions. The VF were better expressed at 37ºC, especially adherence ability, siderophores, amylase and caseinase production. At 0% NaCl only amylase and siderophores production was expressed. At 2% and 3% NaCl the amylase was better expressed. The best expression of siderophores and caseinase was at 6% NaCl. Thereafter, the expression of VF started to decrease till 10%. The adherence to HeLa cells was decreased by higher salinities. The tested strains proved high resistance to a broad range of pH from 4 to 9.6. The amylase and caseinase were better expressed at pH 9.6 and siderophores at pH 7. The higher glucose concentrations (3%) inhibited the expression of amylase and caseinase. The incubation conditions exhibited no significant differences on the VF expression. The metals resistance patterns varied with the tested parameter and the bivalent metal. The temperature growth induced an increase in susceptibility of the tested strains to Zn, Mn, Cu and Ni. The high salinity induced to occurrence of susceptibility to all tested metals. The higher glucose concentration of 3% and the alkaline pH induced higher rates of susceptibility to Mn, Zn and Ni.
Conclusion: Our results demonstrate the high adaptation ability of enterobacterial strains of water origin to different environmental stress conditions and the possibility of virulence potential expression even in limiting environmental conditions, demonstrating the role of these environmental parameters in the selection of resistance genes in the aquatic strains.

Factors influencing antibiotic consumption

P1332 A rapid aetiologic diagnosis using a multiplex RT-PCR panel can reduce antibiotic prescription rate in respiratory tract infections – a randomized prospective study

R. Brittain-Long, J. Westin, S. Olofsson, M. Lindh, L.M. Andersson (Gothenburg, SE)*

Objective: Etiologic diagnosis in acute respiratory infections (ARTI's) is difficult yet crucial for the decision to use antibiotics or not. Recently, multiplex real time PCR methods, that detect several common respiratory viruses, with high sensitivity allowing a rapid result, have been introduced. The objective of the study was to evaluate if the use of such a method in community-acquired ARTI would have an impact on the antibiotic prescription rate.
Methods and Study design: In an open randomized prospective study, nasopharyngeal and throat swabs were collected from adult patients, seeking primary health care with symptoms of respiratory tract infection during 3 consecutive winter seasons (Oct-Apr 2006–2009). Patients were randomized to receive either a quick result (the following day) or a delayed result (after 10 days ±2), which was given at a follow up consultation offered for all patients. Any treatment options were left at the discretion of the physician. The real-time multiplex PCR targeted the following 15 respiratory agents; parainfluensavirus 1–3 (PIV), influensavirus A (IfA) and B (IfB), human metapneumovirus (MPV), respiratory syncytial virus (RSV), rhinovirus (RV), enterovirus (EV), adenovirus (AdV), human coronaviruses 229E, OC43 and NL63, *M. pneumoniae* and *C. pneumoniae.*
Results: 426 patients were included in the study, of whom 206 (48%) were positive for one or more agents at initial visit. 13 patients (6.3%) had double infections. Influenza A virus was most commonly found (28%), followed by rhinovirus (22%) and coronaviruses (15%). Antibiotics were prescribed in 10.8% (n = 46) of the cases at initial visit. In the group randomized to a quick PCR result, 15 patients (7.2%) received antibiotic treatment, compared to 31 patients (14.2%) in the delayed result group ($p < 0.02$, χ^2 test).
Conclusion: The use of a multiplex real time PCR panel for etiologic diagnosis of viral ARTI's may be a useful tool in reducing overuse of antibiotics in an outpatient setting.

P1333 Factors associated with outcome and length of parenteral therapy in outpatient parenteral antibiotic therapy-treated patients with skin and soft tissue infections

R.A. Seaton, E. Bell, V. Bezlyak, C.J. Weir (Glasgow, UK)*

Objectives: The Glasgow outpatient parenteral antibiotic therapy (OPAT) service provides treatment for patients with skin and soft tissue infections (SSTIs) referred either via emergency departments, general practitioners or inpatient specialties. Factors associated with treatment failure or prolonged OPAT in SSTI were explored.
Methods: Treatment failure (readmission or switch to another parenteral agent following progression or adverse event) and duration of parenteral therapy were assessed at time of completion of therapy and recorded on a prospectively maintained data base (2001–2008). Baseline variables potentially influencing outcomes were identified from the database and explored via univariate and multivariate analysis. Logistic regression was used for factors related to outcome and linear regression analysis for factors associated with increase in (log-transformed) duration of OPAT.
Results: 963 patients were identified. 41.1% were male, median age 48 yrs. 4.9% were MRSA, 7.4% penicillin allergy, 8.8% diabetic, 3.1% vascular disease and 5.1% immune-compromised. 870 (90.3%) had cellulitis, 29 (3.0%) bursitis, 26 (2.7%) wound infection and 8 (0.8%) infected ulcer. 62.7% were referred from the community. 56.8% were managed through a nurse-led protocol (patient group direction). 811 (84.2%) received ceftriaxone and 144 (15%) teicoplanin as first-line treatment. Median duration of therapy was 3 days (inter-quartile range 2–5). There was a significant trend towards reduced duration of OPAT over time (χ^2 test for linear trend, $p < 0.0001$). 87.1% were treatment successes. In the multivariate models; treatment failure was associated with female gender, diabetes mellitus and teicoplanin therapy (table); prolongation of OPAT therapy was associated with increasing age, MRSA infection, vascular disease, teicoplanin therapy and a diagnosis other than cellulitis or infected ulcer. Reduced duration of therapy was observed in patients referred from the community and in those managed through patient group direction.
Conclusions: In patients with SSTI managed through OPAT we identified several factors independently associated with treatment failure or with prolongation of OPAT. Teicoplanin was the only factor associated with both prolongation of therapy and treatment failure. There may be other factors of importance that were not recorded in the study.

Table. Factors with significant association with treatment failure or duration of OPAT

		Lower 95% CI	Upper 95% CI	p
Parameter associated with treatment failure	**OR***			
Female	1.646	1.096	2.473	0.0163
Diabetes	2.023	1.116	3.668	0.0202
Teicoplanin	1.872	1.051	3.334	0.033
Parameter associated with duration of OPAT	**Effect size****			
Age (per additional 10 years)	1.030	1.007	1.053	0.0097
MRSA	1.468	1.170	1.843	0.0010
Vascular disease	1.288	1.011	1.641	0.0409
Teicoplanin	1.320	1.160	1.502	<0.0001
Other diagnosis (not cellulitis)	1.248	1.001	1.558	0.0494
Managed via PGD	0.708	0.652	0.770	<0.0001
Community referral	0.910	0.839	0.986	0.0211

*Multivariate logistic regression.
**Multivariate linear regression of log(OPAT duration); e.g. Effect size of 1.32 equates to a 32% increase in duration of OPAT compared to those without this risk factor.

P1334 Clinical and economic impact of pharmacy therapeutic substitution from piperacillin–tazobactam to cefepime and metronidazole

R.M. Chambers, S. Davis, E. Szandzik, M. Zervos (Detroit, US)*

Objectives: Therapeutic substitution (TS) is a strategy often employed by hospitals to maintain a closed formulary when agents with the same efficacy exist within a therapeutic class. To our knowledge, TS has not been previously applied between differing antibiotic classes. In February 2009, our institution implemented a pharmacist-driven criteria-based TS from piperacillin–tazobactam (PT) to cefepime with or without metronidazole (C/M). We sought to: 1. Compare clinical outcomes before and after implementation; 2. Evaluate the impact on pharmacy expenditures.
Methods: Quasi-experimental comparison of 50 patients who received PT prior to policy implementation and 47 patients who underwent TS to cefepime +/– metronidazole. Data collected: demographics, microbiology, antibiotic treatment, clinical and microbiologic success, length of stay, in-hospital mortality, *Clostridium difficile* rates, and cost of PT or C/M. Hospital wide antibacterial use was evaluated in the 4 months pre and post TS as defined daily doses per 1000 patient days (DDDs). Outcome analysis: χ^2 test to compare categorical variables and Student's t-test or Mann Whitney-U to compare continous variables. Economic analysis: Data log transformed and compared via Student's t-test.
Results: Infection types and outcomes were similar between groups (table 1). Median cost of therapy (US): $62.40 TS versus $330.72 PT ($p < 0.0001$). Hospitalwide cefepime use increased from 111 to 180 DDDs while PT use decreased from 80 to 16 DDDs. Total antibacterial use also decreased from 945 to 881 DDDs.
Conclusions: A pharmacist-driven TS from PT to C/M resulted in similar clinical outcomes before and after implementation of a hospital-wide policy. The TS policy was associated with reduction in antibacterial cost per patient and hospital wide antibacterial use.

Table 1

Variable	PT, n = 50 (%)	TS, n = 47 (%)	p-value
Site			NS
BSI	3 (6)	4 (9)	
Urine	5 (10)	7 (15)	
Respiratory	25 (50)	12 (26)	
Skin	12 (24)	16 (34)	
Intra-abdominal	6 (12)	9 (19)	
Other	7 (14)	6 (13)	
Confirmed Gram-negative infection	16 (32)	21 (45)	0.199
Appropriate initial dose	43 (86)	43 (92)	0.392
Clinical success	42 (84)	38 (81)	0.684
Microbiologic eradication	13/17 (77)	17/25 (68)	0.551
Mortality	5 (10)	6 (12.8)	0.668
C. difficile	6 (12)	4 (8.5)	0.572

P1335 Impact of National Hospital Evaluation Programme on surgical antibiotic prophylaxis in Korea

E.S. Kim, Y.G. Kwak, S.W. Park, C.S. Moon, C.S. Lee, B.N. Kim (Goyang, Busan, Seoul, Jeonju, KR)*

Objectives: Inappropriate antibiotic use in surgical prophylaxis is related to the development of surgical site infections (SSIs) and antibiotic resistance and an increase in cost. The quality assessment in surgical antimicrobial prophylaxis has been implemented as a part of national hospital evaluation program (NHEP) since 2007 in Korea. We investigated the use of prophylactic antibiotics in surgery before and after the assessment.
Methods: We retrospectively reviewed medical records of patients who underwent three types of operations (arthroplasty, gastrectomy, and hysterectomy), which were included for the assessment of NHEP, and two other types of operations (craniotomy and spine surgery), which were not, in six university-affiliated hospitals from 2006 to 2008. Data for the use of prophylactic antibiotics (regimen, timing, and duration) and SSI rates in each type of operations underwent from August to October in each year were collected for comparison.
Results: Among 2,207 operations, 1,949 were enrolled; 356 arthroplasties, 273 gastrectomies, 615 hysterectomies, 168 craniotomies, and 537 spine surgeries. There were no significant changes in gender distribution, mean ages, and National Nosocomial Infections Surveillance system (NNIS) risk index scores in each type of operations during the study period. The quality of surgical prophylaxis was significantly improved whether the operations were included for the assessment of NHEP or not. The changes of SSI rates were not significant (Table).
Conclusion: Implementation of the assessment of surgical antimicrobial prophylaxis as a part of NHEP resulted in dramatic improvement in the use of prophylactic antibiotics in major surgery in Korea. Further prospective studies are needed to investigate its effects on SSI rates and antibiotic cost.

Group	Quality indicators	2006	2007	2008	*P* Value
Arthroplasty	Adequate timing (%)	12.3	94.4	99.1	<**0.001***
	Adequate selection (%)	0.9	27.2	51.7	<**0.001***
	Duration (days)	21.6±9.6	10.3±7.2	8.5±5.5	<**0.001***
	SSI rates (%)	3.6	3.3	1.8	0.445
Gastrectomy	Adequate timing (%)	33.7	97.7	97.6	<**0.001***
	Adequate selection (%)	0	71.9	48.2	<**0.001***
	Duration (days)	11.6±7.9	6.7±3.1	8.8±5.3	<**0.001***
	SSI rates (%)	6.1	4.5	6.0	0.942
Hysterectomy	Adequate timing (%)	33.1	96.6	97.4	<**0.001***
	Adequate selection (%)	23.7	83.9	92.3	<**0.001***
	Duration (days)	12.8±2.8	6.9±4.8	7.0±4.8	<**0.001***
	SSI rates (%)	3.4	2.4	2.1	0.401
Craniotomy	Adequate timing (%)	25.5	52.8	50.9	0.01*
	Adequate selection (%)	0	15.1	23.3	<**0.001***
	Duration (days)	14.3±10.6	12.0±7.0	10.4±7.4	0.055
	SSI rates (%)	5.8	0	1.8	0.198
Spine surgery	Adequate timing (%)	13.1	73.0	79.9	<**0.001***
	Adequate selection (%)	0	2.6	5.9	0.001*
	Duration (days)	17.3±7.6	13.3±8.6	14.3±9.6	<**0.001***
	SSI rates (%)	4.5	5.3	2.5	0.289

P1336 Antibiotic consumption in 30 French nursing homes: a point prevalence study from the European Surveillance of Antimicrobial Consumption nursing home subproject

G. Gavazzi, P. GIbert, L. Fontaine, R. Stroobants, E. Hendrickx, A. Muller, V. Vankerckhoven, H. Goossens, B. Jans on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Project Group*

Objectives: Elderly individuals and particularly elderly living in Nursing home (NH) exhibit specific features for infections including diagnostic difficulties, increased multidrug resistant bacteria and high antibiotic (AB) use. Optimizing the use of ABs in the nursing home population is therefore an important priority of quality of care. However, considering AB consumption, no data are available in Europe. The objectives of the European Surveillance of Antimicrobial Consumption Nursing Home (ESAC-NH) subproject are to study AB use and prescriptions among European residents living in high skilled NH. The results presented here

are from the 30 French Nursing homes participating to the ESAC-NH sub-project.
Methods: In accordance with the methodology of the ESAC-NH subproject, a first point prevalence survey (PPS) was carried out in 30 NH in France within a one-month-period (one day in April 2009). Inclusion criteria for residents were to be resident in the NH (>24 hours) and to receive systemic AB(s) at the time of the PPS. Data were obtained from nursing notes, medication administration records and staff related to antimicrobial prescribing, facility and resident characteristics (e.g. prevalence of urinary and vascular catheter, wound, disorientation, bedridden). Indication, type and dosage for AB and, microorganisms were reported. Data were entered into customised web-based software and analysed descriptively.
Results: ABs were administered to 112 (4.8%) of 2318 eligible elderly residents. Characteristics of the residents were as follows: presence of urinary catheter (1.4%), vascular catheter (<0.01%), urinary incontinence (56.1%), disorientation (55.9%), wound (13.6%), bedridden (35.4%). Respiratory (RTI) and Urinary tract infections (UTI) were the most frequent infections (60.3% and 25.8%, respectively). Microbial identification was only present in 1.5% of RTI, and in 65.5% of UTI. The most frequent ABs were β-lactams for RTI (>70%, oral route 82%); quinolones(34.5%), cotrimoxazole (20.7%), and nitrofuranes (10.4%) were the most frequent ABs for UTI (intravenous route <10%).
Conclusion: Surprisingly in France, antibiotic use in the NH population was moderate. Although the PPS was organised during the Spring, which may explain the low rate of antibiotic use, the low prevalence of urinary catheter may have decreased the rate of AB use for UTI. This study indicates also that, to optimize antibiotic use in NH, guidelines should mainly focus on UTI and RTI.

Economic impact of infectious diseases and therapeutic regimens

P1337 MRSA complicated skin and skin structure infections in Glasgow; evaluation of hospital management and potential for earlier discharge

R.A. Seaton, J. Coia, R. Masterton, N. Reid, S. Cooper, B. Jones (Glasgow, Ayr, London, UK)*

Objectives: In the UK, MRSA skin and skin structure infections (cSSTI) are usually treated in hospital with glycopeptides. Opportunities for earlier hospital discharge include switch to an effective oral agent or outpatient parenteral antibiotic therapy (OPAT).
Methods: A retrospective review of MRSA SSTIs admitted to six acute hospitals in Glasgow was undertaken (September 2007 to December 2008) to identify patient treatment pathways and the potential for earlier discharge. Patients were identified through ICD-10 clinical coding and cross matched with patients known to have has a positive MRSA isolate.
Results: 605 (9.6%) patients had cSSTI and were MRSA positive. Of 476 case notes reviewed, 173 were confirmed with MRSA cSSTI. Median age was 68 years (54% female). cSSTI were surgical site (44.5%), cellulitis (23.7%), infected ulcer (18.5%) and abscess (5.8%). 61% were community-associated (isolate ⩽48 hours following admission). In 20.2% MRSA therapy was empiric, in 68.8% it followed a positive culture. 144 (83%) were managed entirely as inpatients, 19 (11%) had oral therapy post discharge and 10 (5.8%) received OPAT (following admission in one). 110 (63.6%) were treated with intravenous (IV) +/− oral therapy, 33 (19.1%) IV then oral switch, 8 (4.6%) oral therapy only and 22 (12.7%) did not receive anti-MRSA therapy. Vancomycin was the most commonly prescribed IV agent (68.8%) and linezolid the most commonly prescribed oral agent (9.2%). Median duration of stay following a positive MRSA result was 15 days (1–217 days) and 4352 days in total. 37 (34%) of those who received IV therapy in hospital were potentially suitable for earlier discharge (oral therapy or OPAT) as they received >72 hours IV therapy and were discharged within 72 hours of discontinuing therapy. 28 (16.2%) of patients died during their admission (cause not determined) and 15 (8.7%) were readmitted with cSSTI within 30 days of discharge.
Conclusions: MRSA infection is implicated in about 10% of cSSTI in Glasgow hospitals, the majority are community-associated, re-admission rate is 8.7% and mortality 16.2%. There is variability in management with approximately one third of patients who remain in hospital receiving IV therapy, potentially suitable for earlier hospital discharge either through IV to oral switch or OPAT. Earlier discharge should be considered in MRSA infected patients with SSTI in view of the potential economic, infection control and social benefits.

P1338 Outcomes and costs of previous vancomycin exposure in patients with complicated bacteraemia and infective endocarditis due to MRSA

J. Brown, K. Brown, A. Forrest, W. Wisniewski, R. Betts, F. Doloresco (Buffalo, Rochester, US)*

Objectives: MRSA is the primary cause of complicated bacteraemia (CB) and infective endocarditis (IE) in patients (pts). Standard treatment for these infections is vancomycin (V). Studies have questioned the costs/outcomes of V as compared to other therapies, yet to our knowledge no study has investigated the timing and degree of previous V on outcomes and associated hospital costs for pts with CB or IE due to MRSA.
Methods: We performed a retrospective review of pts treated for confirmed MRSA CB and IE from 7/1/06 to 6/31/08. Pts with CB or definite or possible IE as defined by the Modified Duke Criteria were included. CB was defined using standard accepted definitions. Data collection included clinical, microbiological, acuity, treatments, outcomes and hospital costs for pts exclusively treated at our institution. Failure was defined as clinical failure, microbiological failure, death or discontinuation of effective anti-MRSA therapy due to adverse events. Success and failure were evaluated using standard definitions. Hospital costs included: antibiotic costs, IV admixture/administration costs, antimicrobials for treatment failures, therapeutic drug monitoring, medications/procedures associated with the diagnosis/management of adverse drug events with possible/probable association and CB/IE associated length of hospitalization. Classification and regression tree analysis (CART) was used to identify prior V associated with failure. Students T test, Mann–Whitney test and Fishers Exact tests were used for univariate analysis and logistic regression was used for multivariate modeling. MICs were determined using E test.
Results: A total of 32 CB and 18 IE pts were included in the analysis. Overall, 34/50 had successful outcomes. The average total hospital costs for those with successful outcomes was $23,597 as compared to $37,505 for those experiencing failure ($p = 0.03$). Upon univariate analysis pts with any V in the prior 3 years (13/34 vs. 12/16, $p = 0.03$) and V MIC ⩾2 (7/34 vs. 9/16, $p = 0.02$) were associated with failure. The CART derived partition point for prior V was 18.75 grams or 14 days of V within the prior 3 years. In the final regression model only pts receiving ⩾14 days of V ($p = 0.002$) or ⩾18.75 grams ($p = 0.002$) in the prior 3 years were predictors of failure.
Conclusions: In our model of pts with MRSA CB or IE, predictors of failure included, pts receiving ⩾14 days of V or ⩾18.75 grams in the prior 3 years. Further analyses are warranted to explore/confirm these findings.

P1339 Prior vancomycin exposure and its impact on MRSA-complicated bacteraemia and infective endocarditis-related mortality

J. Brown, K. Brown, A. Forrest, W. Wisniewski, R. Betts, F. Doloresco (Buffalo, Rochester, US)*

Objectives: MRSA is a major cause of CB and IE. V traditionally has been the antibiotic of choice in these pts. Several analyses of CB/IE pts have found prior V an independent risk factor for poor outcomes and increasing V MICs. This exploratory study, quantitatively investigated the effects of prior V on MRSA CB and IE attributable mortality (AM).

Methods: We performed a retrospective review of pts treated for confirmed MRSA CB and IE from 7/1/06 to 6/31/08. Pts with CB or definite or possible infective endocarditis (IE) as defined by the Modified Duke Criteria were included. CB was defined using standard accepted definitions. Based on sample size calculations at = 0.05, 25 subjects with and without (50 total) prior V within the past 3 years were randomly selected. Data collection included clinical, microbiological, acuity and treatment information for pts exclusively treated at our institution. Classification and regression tree analysis (CART) was used to identify the amount of prior V exposure associated with an increased AM. Students T test, Mann–Whitney test and Fishers Exact tests were used for univariate analysis and logistic regression was used for multivariate modeling. MICs were determined using E test. AM was defined as deaths where CB or IE was documented as the main cause or where CB or IE was mentioned as the main diagnosis.
Results: A total of 32 CB and 18 IE pts were included in the analysis. The overall AM rate was 16% (8/50). Pts with any V in the prior 3 years (6/8 vs. 19/42, p = 0.25) and pts with IE (4/8 vs. 14/42, p = 0.436) were not associated with AM. The CART derived partition point for prior V was 45 grams or 31 days of V within the prior 3 years. Upon univariate analysis variables associated with AM included: APACHE II (12 vs. 10, p = 0.04), V MIC ⩾2 (6/8 vs. 10/42, p = 0.009), ⩾45 grams of V (5/8 vs. 2/42, p = 0.0001) or ⩾31 days (5/8 vs. 2/42, p = 0.0001) of V within the prior 3 years were. In the final regression model APACHE II (p = 0.04), pts receiving ⩾31 days of V (p = 0.002) or ⩾45 grams (p = 0.002) in the prior 3 years were predictors of AM.
Conclusions: In our model of pts with MRSA CB or IE, predictors of AM included higher APACHE II, pts receiving ⩾31 days of V or ⩾45 grams in the prior 3 years. Further analyses are warranted to explore and confirm these interesting findings.

P1340 Cost and outcome implications of the newly developed high-dose vancomycin consensus guidelines

R. Kullar, S. Davis, K. Kaye, T. Taylor, M. Rybak (Detroit, US)*

Objectives: Recent 2009 consensus guidelines on the therapeutic monitoring of vancomycin (VAN) has recommended using larger dosages of VAN for patients with complicated infections in order to achieve trough serum concentrations of 15–20 mg/L. We evaluated the costs and outcomes of patients with methicillin-resistant *Staphylococcus aureus* bacteraemia (MRSAB) before and after implementation in March 2007 of a policy to target high trough concentrations in complicated infections.
Methods: A quasi-experimental study conducted from 2005–2009 at Detroit Medical Centre. Consecutive adult patients treated with VAN for ⩾72 hours for confirmed MRSAB in two study phases: Pre (2005 – March 2007) and Post (March 2007–2009) policy implementation. We evaluated patient characteristics, clinical outcomes and variable direct costs, in U.S. dollars, in both phases. Costs were adjusted for inflation and non-parametric tests were used for skewed data.
Results: 214 eligible patients were identified (107 pre-implementation; 107 post-implementation). Baseline characteristics in patients were similar amongst pre- and post-implementation (Table 1). Median length of stay (LOS) was higher in patients pre- vs. post-implementation (18 days vs. 15 days, P = 0.22), although this was not statistically significant. Total variable hospital costs were higher in the pre-period compared to the post-period ($5,638.10 vs. $4,205.46, P = 0.017). However, VAN monitoring costs were significantly higher in patients post-policy implementation (Table 1). Likewise, the median VAN dose was statistically higher in patients during the post-period (2000 mg vs. 1450 mg, P = 0.006), which was associated with higher VAN drug cost ($166.40 vs. $116.48, P = 0.007). Rates of nephrotoxicity were numerically higher in patients following the policy implementation (18.7% vs. 16.8%, P = 0.60). Patients during the pre-period had significantly higher rates of VAN failure (57% vs. 44.9%, P = 0.048).
Conclusion: Implementation of a VAN dosing policy to achieve troughs of 15–20 mg/L resulted in improved clinical success and reduced LOS and hospital cost in a cohort of MRSAB. Treatment post implementation was associated with increased VAN drug and monitoring costs.

Table 1

	Pre (n = 107) Median (range) or n (%)	Post (n = 107) Median (range) or n (%)	P-value
Patients' Characteristics			
Age (years)	52 (27–89)	53 (20–94)	0.81
Apache II score	8 (0–22)	7 (0–26)	0.22
Weight (kg)	70.2 (35.4–178.0)	71.8 (36.1–186.0)	0.52
Creatinine clearance (ml/min)	68.2 (6.2–189.4)	64.8 (6.1–212.0)	0.41
Renal failure during hospitalization	35 (32.7%)	41 (38.3%)	0.39
Hemodialysis	15 (14.0%)	11 (10.3%)	0.39
Concomitant sites of infection			0.62
IV catheter	19 (17.8%)	24 (22.4%)	
Skin/wound	21 (19.6%)	13 (12.1%)	
Endocarditis	25 (23.4%)	19 (17.8%)	
Osteomyelitis	11 (10.3%)	11 (10.3%)	
Cost and Resource Utilization Data			
Total hospital variable cost ($)	5,638 (261–11,826)	4,205 (500–95,016)	0.017
VAN drug cost ($)	116 (8–648)	166 (12–1984)	0.007
Number of trough levels drawn	3 (0–14)	4 (0–27)	0.005
Cost of trough levels ($)	30.60 (0–142)	40 (0–275)	0.005
Monitoring labour cost (consult) ($)	56.56 (0–189)	68 (0–347)	0.006

P1341 The cost of a fictional β-lactam allergy

M. Ibeto, M. Przybylo, I. Maitland, M. Qulaghassi, R. Kato, A. Guleri (Blackpool, UK)*

Background: The British National Formulary [BNF]-standard UK drug manual, reports allergic reaction to penicillins occur in 1–10% of exposed individuals. It is an acceptable observation amongst clinicians that a large number of patients are labeled allergic to penicillin. Penicillin and other β-lactam allergies are conventional. Properly identifying patients who are erroneously labeled β-lactam allergic will lead to improved utilization of antibiotics while avoiding the use of less efficient/ more expensive alternatives.
Method: Cost identification of a 'penicillin allergy' was made by comparing the price of the alternative vs first line β-lactam antibiotic course for each patient prescribed over a month period at a general practice centre in Northwest England. Data was collected from the electronic medical record database. The history of each allergic reaction to penicillin was reviewed. The alternative antibiotics used were compared to their β-lactam equivalents. The price of each antibiotic course were obtained and compared from the BNF.
Results: Within one month, antibiotics were prescribed to 196 patients. 8% (16/196) of patients were labeled 'penicillin allergic'. 100% (16/16) had no detailed history of the allergic reaction to penicillin. The overall cost of all the non-β-lactam antibiotics used considering the penicillin allergy was £59.32. The cost of the first line β-lactam equivalents was £24.48. Non-β-lactam second line alternative antibiotics are two and a half times more expensive than their β-lactam equivalents.
Discussion: Although penicillin is still the most commonly reported allergy, less than 20% of all patients that believe that they have a penicillin allergy are truly allergic to penicillin. Penicillin allergy is an overused/ over prescribed diagnosis. Patients often report typical side-effects of β-lactams as a true allergy. This notion undermines the efforts to curb emerging resistance and has a big economic impact when considering how many people consider themselves β-lactam allergic. Investigations into true β-lactam allergies must be considered for future antibiotic consolidation and management. Clinical details of patients to be presented.

Community-acquired respiratory tract infections

P1342 Sensitivity of both PCR and serology for diagnosis of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* in a primary care setting

K. Loens, A. Vanderstraeten, C. Lammens, D. Ursi, S. Dettlaff, T. Verheij, P. Little, H. Goossens, M. Ieven and the GRACE study team*

Objectives: Serology and PCR are widely used for diagnosis of atypical bacteria such as *M. pneumoniae* (MP) and *C. pneumoniae* (CP) in respiratory tract infections. The aim of this study was to investigate the

role of these organisms in patients presenting with lower respiratory tract infections (LRTI) in 11 primary care networks (PCNs) in 8 European countries using sensitive real-time PCRs and serology.
Methods: From October 2007 through May 2008, a total of 861 adult patients with LRTI in the community were enrolled during the first winter period in a prospective study in 11 PCNs in 8 European countries. Among other samples, nasopharyngeal flocked swabs (COPAN), sputa if productive cough, and paired sera were collected and sent to the local laboratory to be frozen. Specimens were transported regularly to the central lab in Antwerp for in-house real-time PCR for MP, and CP detection. IgG serology was performed using *Mycoplasma* pneumonia-IgG-ELISA and *Chlamydia* pneumonia-IgG-ELISA plus kits (Medac GmbH, Wedel, Germany) for the detection of a IgG seroconversion or significant rise in anti MP/CP IgG in paired sera collected 3–4 weeks apart according to the instructions of the manufacturer. 43 and 59 paired sera were tested for the presence of MP and CP IgM, respectively.
Results: The results of the IgG serological study indicated that MP and CP were the causative agent in 1.6% (11/689) and 2.2% (15/690) of the studied adult patients with CA-LRTI. 7/43 patients were found positive for specific-IgM.
Only 1/8 patients showing a MP IgG seroconversion, tested positive for IgM. For the 4 MP positive patients identified by a significant rise in IgG, 2 tested IgM positive. Only 5 patients tested positive for the presence of CP IgM: 1 patient with a IgG seroconversion, 2 patients with a significant rise in IgG. For the other 2 patients, no significant change in IgG titer was observed.
In 0/689 and 3/689 (0.4%) nasopharyngeal flocked swabs MP and CP were detected by PCR, respectively. Three patients were found to be MP positive when PCR was applied to sputum. All PCR positive patients were also found positive by IgG serology.
Conclusion: Although nasopharyngeal sampling with flocked swabs is well tolerated and suitable to be used in an outpatient setting the rate of positive results for MP was higher when PCR applied to sputum or when examining paired sera for a significant rise/seroconversion in anti MP/CP IgG. IgM antibodies are of limited value in early diagnosis.

P1343 Is there a role for CRP or PCT as inflammatory parameters in the management of outpatients with community-acquired lower respiratory tract infections?

K. Loens, C. Lammens, A. Vanderstraeten, F. Coenjaerts, E. Claas, A. Van Loon, T. Verheij, P. Little, H. Goossens, M. Ieven on behalf of the GRACE study team*

Objectives: CRP and/or PCT measurement is often used for the management of patients with a Community-acquired Lower Respiratory Tract Infection (CA-LRTI). This study investigated the correlation between 2 CRP methods and between CRP and PCT as inflammatory markers for the management of adult outpatients presenting with a CA-LRTI in the GRACE network.
Materials and Methods: From 10/2007 to 05/2008, a total of 847 adult patients with CA-LRTI were enrolled during the first winter period in a prospective study in 11 primary care networks (PCNs) in 8 European countries. Among other samples, nasopharyngeal flocked swabs (COPAN), sputa and sera were collected and sent to the local lab to be frozen. Specimens were transported regularly to the central lab in Antwerp for in-house real-time PCR for detection of atypical bacteria and respiratory viruses. Sputa were cultured for bacterial etiology at the local labs. Nasopharyngeal flocked swabs were cultured for the presence of *S. pneumoniae* and *Haemophilus* spp at the central lab. CRP was measured by a lab-based test and a POCT (Orion), PCT was measured by the Kryptor (Brahms GmbH). Interpretation of PCT values was done according to Christ-Crain (Lancet 2004): <0.1μg/L: absence of bacterial infection; 0.1–0.25μg/L: bacterial infection unlikely; 0.25–0.5μg/L: possible and >0.5μg/L suggestive for a bacterial infection.
Results: For 430 patients of 6 PCNs, CRP, PCT, PCR and culture results are available.
A bacterial or viral etiology was found in 29.8% and 49.0% of patients, respectively: 13.3% of these were mixed infections. The concordance between the lab-based CRP and POCT CRP test varied between 98.9%, 97.8% and 90.6% for concentrations <20 mg/L, 20–50 mg/L and >50 mg/L respectively. Elevated CRP values of >20 mg/L were found in 125/430 (29.1%) of patients. PCT values of >0.25μg/L were found in only 10/430 (2.3%) of patients. CRP was >50 mg/L in 49/417 (11.8%) patients with PCT values <0.25μg/L. According to the PCT criteria used 97.0% of all infections belong to the groups "absence of bacterial infection" or "bacterial infection unlikely". For 96.1% of patients from whom bacteria were isolated, PCT values were not indicative for a bacterial infection.
Conclusions: Correlation between POCT CRP and lab based CRP is excellent. The measurement of PCT in primary care is of limited help for the GP to distinguish bacterial from viral infections. CRP seems more sensitive as inflammatory parameter than PCT in outpatients with CA-LRTI.

P1344 Epidemiology, clinical features, and outcomes of community-acquired pneumonia in patients with liver cirrhosis

D. Viasus, C. Garcia-Vidal, J. Castellote, J. Adamuz, R. Verdaguer, F. Gudiol, J. Carratalà (Barcelona, ES)*

Objective: Although liver cirrhosis is associated with an increased susceptibility to infection, little information is available regarding community-acquired pneumonia (CAP) in this setting. We aimed to ascertain epidemiology, clinical features, and outcomes of CAP in patients with liver cirrhosis. The prognostic value of several specific-disease scores was also analyzed.
Methods: Observational analysis of a prospective cohort of nonseverely immunosuppressed hospitalized adults with CAP (1995–2008). Liver cirrhosis was diagnosed by histology or by clinical, laboratory and imaging criteria.
Results: We documented 3421 CAP episodes, of which 90 cases occurred in patients with liver cirrhosis. The median value of model for end-stage liver disease (MELD) was 14 (range 6–36). According to the Child-Pugh (CP) score, 56% of patients were grade B and 21% grade C. Patients with liver cirrhosis were younger (61.4 vs 66.3 years; $p = 0.001$), more often male (80% vs 68%; $p = 0.021$), current smokers (41% vs 26%; $p = 0.002$) and heavy alcohol drinkers (35% vs 17%; $p < 0.001$) than the remaining patients. They had more frequently altered mental status at admission (33% vs 13%; $p < 0.001$), shock (13% vs 6%; $p = 0.011$), and were more commonly classified into high-risk pneumonia severity index (PSI) classes (group IV-V) (74% vs 57%; $p = 0.002$). *Streptococcus pneumoniae* (46% vs 33%; $p = 0.009$) and *Pseudomonas aeruginosa* (4.4% vs 0.9%: $p = 0.001$) were more frequently documented in patients with liver cirrhosis. Bacteraemia was also more common among these patients (21% vs 13%; $p = 0.023$). Specific-disease scores were comparable in predicting severe disease (30-day mortality and/or ICU admission); area under the ROC curve were 0.83 (MELD), 0.75 (CURB-65), 0.74 (CP), and 0.70 (PSI). Early (<48 h) (5.6% vs 2.1%; $p < 0.048$) and 30-day mortality rates (14.4% vs 7.4%; $p < 0.024$) were higher in patients with liver cirrhosis.
Conclusions: CAP is associated with a poor outcome in patients with liver cirrhosis. Specific-disease scores, especially MELD, may help identify patients at high risk of adverse outcomes. Strategies aimed to improve the management of CAP in liver cirrhosis patients are needed.

P1345 Causes and risk factors of poor outcome in patients with non-severe community-acquired pneumonia

M. Falguera, M. Velasco, O. Sacristán, I. Chica, P. Cabezas, A. Ruiz-González, J.M. Porcel (Lleida, ES)*

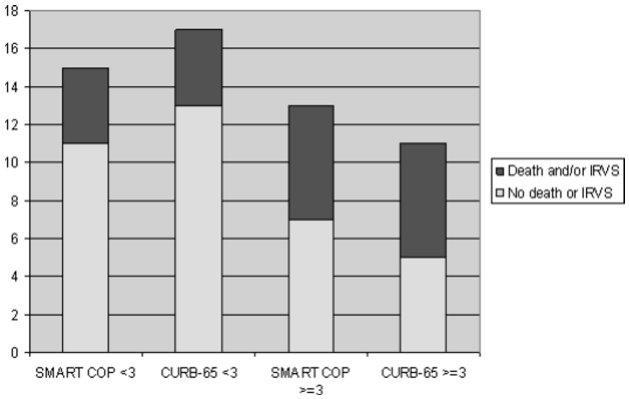
Objectives: To recognize causes and risk factors associated with poor outcome (mortality, long hospital stay and readmission) among patients with non-severe community-acquired pneumonia.
Methods: From a database of patients with community-acquired pneumonia, prospectively collected in a 10-year period, we selected those patients with non-severe disease according to the Pneumonia Severity

Index (PSI) (classes I to III). Mortality, long hospital stay (10 days or more) and readmission in 30 days were selected as poor outcome indicators. Causes were evaluated from clinical records and risk factors were determined by means of univariate and multivariate analyses.
Results: Eight of 706 (1%) patients died, directly related to pneumonia; risk factors associated with mortality in multivariate analyses were: oxygen saturation <88%, arterial systolic blood pressure <90 mmHg and plasmatic sodium level <133 mEq/L. Mortality rates were 0%, 2%, 10% and 100% for patients with 0, 1, 2, or 3 risk factors, respectively. A long hospital stay was recorded in 155 (22%) patients, directly attributable to pneumonia in 81, exacerbation of underlying diseases in 55, appearance of new events in 13, and others in 6; multivariate analyses selected the following predictive factors: presence of underlying diseases, pleural effusion, empyema, hematocrit level <32%, arterial systolic blood pressure <90 mmHg and arterial pO2 <60 mmHg. Finally, 52 (7%) patients were readmited in 30 days after discharge due to exacerbation of underlying diseases in 25, appearance of new events in 13 and pneumonia related causes in 14; risk factors determined by multivariate analyses were: presence of neoplasm, hematocrit level <30% and arterial pCO2 level >45 mm Hg.
Conclusions: In patients with non-severe community-acquired pneumonia, poor outcome seems predictable. Mortality is related to pneumonia; a long hospital stay can derive from pneumonia or from exacerbation of underlying diseases; and readmissions are frequently due to non-pneumonia related causes. Presence of risk factors can help to decide hospital admission.

P1346 SMART-COP in severe pneumonia – is it as smart as it seems?

J. Seddon, R. Vancheeswaran, D. Mack (London, Herts, UK)*

Objectives: Pneumonia severity is notoriously difficult to estimate with no single factor accurately predicting mortality or need for invasive ventilation. Composite scoring tools are vital to inform clinical decision-making and to predict risk of death. We audited management of pneumococcal pneumonia and compared the new SMART-COP tool with the traditionally used CURB-65 tool.
Method: We performed a retrospective case note review of all patients admitted to Barnet and Chase Farm Hospitals with pneumonia who had blood cultures positive for streptococcus pneumoniae between October 2008 and April 2009. We audited the use of CURB-65 by the admitting doctor and compared it with the SMART-COP score to determine the accuracy of each in predicting death and IRVS.
Results: We analysed data for 28 of the 32 patients with positive pneumococcal blood cultures. Due to small numbers, we pooled severity scores into high (⩾3) and low (<3) groups. Graph 1 shows patient numbers in each category. In addition, we found that 50% (3/6) of those that died had not received IRVS. CURB-65 score was documented in only 32% of patients. Antibiotic protocol compliance was only 50% when patients were CURB-65 scored appropriately.



Conclusion: Estimating pneumonia severity by means of a scoring tool has always been a dilemma between accuracy and simplicity of use. On opposite ends of this spectrum lie the precise, but time-consuming Pneumonia Severity Index (PSI) and the straightforward CURB-65. As CURB-65 is universally accessible it forms the backbone of treatment guidelines published by both the American and British Thoracic Societies. SMART-COP is appealing in forging a middle ground. We have shown that mortality and need for IRVS increases with higher CURB-65 and SMART-COP scores and that in our septicaemic patients with pneumonia there was no benefit in using a more complicated scoring tool. Moreover, we found poor use of CURB-65 with the score documented in only 32% of patients. Given that severity scoring guides antibiotic prescription, that only 50% of patients received antibiotics compliant with BTS guidelines was not a surprise. Clearly, if patients are not scored for severity, appropriate management becomes an even greater challenge. As has been shown in a number of studies, there are significant barriers to using even simple scoring tools. The lack of clear benefit, compounded by a more time-consuming and less memorable tool will limit widespread use of SMART-COP as a scoring system.

P1347 Invasive pneumococcal disease in a Spanish university hospital from 2007 to 2009: epidemiology and serotypes

I. Rebollo, J. Esperalba, M.P. Mendaza, R.M. Daza, P. Costa, A. Márquez, D. Dámaso (Majadahonda, ES)*

Objectives: A 3-year invasive pneumococcal disease (IPD) enhanced surveillance project in the "Hospital Puerta de Hierro de Majadahonda" (HPHM) between February 2007 and November 2009. It aims to describe the incidence of IPD and the clinical and epidemiological characteristics of cases in HPHM.
Methods: IPD cases were defined by isolation of *S. pneumoniae* from a normally sterile site. Data recorded for each isolate were age, gender, diagnosis and isolate type. Diagnosis was classified as bacteraemia, meningitis, pneumonia or empyema. *S. pneumoniae* cases were serotyped in a reference laboratory, using the Quellung reaction with serotype-specific antiserum and latex agglutination test (Pneumolatex). The HPHM is a 613-bed hospital and covers a population of approximately 5 hundred thousand people. The incidence of IPD was assessed using estimates of population from the Office for Local Statistics. Isolates from children <5 years were collected since October 2008.

Table 1. Summary of IPD isolates 2007–2009

	All	<5 years*	5–64 years	>65 years
Number of isolates	95	8	52	35
Number of males (%group)	55	6 (75%)	32 (62%)	17 (49%)
IPD incidence (per 100,000 persons)	6.2 [4.3–8.6]	23 [10.2–46.3]	5.9 [3.5–9.4]	36.3 [18.9–63.52]
Number of different serotypes identified	28	5	22	22
Rank serotype (number of isolates, more than 2)				
1	1 (18)	1 (2)	1 (15)	3 (8)
2	3 (13)	5 (2)	3 (4)	5 (3)
3	5 (8)	24F (2)	8 (4)	4 (2)
4	4 (5)	–	4 (3)	11A (2)
5	8 (5)	–	5 (3)	18C (2)
6	19A (5)		11A (2)	19A (2)
7	11A (4)		14 (2)	–
8	18C (4)		18C (2)	–
9	14 (3)		19A (2)	–
10	24F (3)		22F (2)	
Total isolates represented by top ten serotypes	71%	82%	75%	54%
PPV23 coverage	79 (83%)	9 (82%)	45 (87%)	28 (84%)
PCV7 coverage	15 (16%)	0	8 (15%)	7 (20%)

*Data from October 2008 to November 2009.
PPV23 serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. PCV7 serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F.

Results: A total of 95 strains were isolated between February 2007 and November 2009. 55 were men (58%). The overall IPD incidence in the HPHM population was 6.2 cases per 100,000 per annum [95% confidence interval (CI) 4.3–8.6]. The incidence in children under five was 23 [95% CI 10.2–46.3]. The incidence of serotypes included in the vaccine PPV23 (83%) was 5.1 [3.4–7.3]. A seasonal pattern of disease was observed. Twenty-eight serotypes were isolated in total, five different serotypes were found in the under fives (8 isolates), twenty-two in the over 65 age group (35 isolates) and twenty-two in the 5–65 age group (52 isolates). Most common serotypes in the general population were 1 (18 isolates), 3 (13 isolates), 5 (8 isolates), 4 (5 isolates),

8 (5 isolates), 19A (5 isolates), 11A (4 isolates) and 18C (4 isolates), compromising 62% of the total isolate collection. See Table 1. The diagnosis was known in 71% of patients. Of these, the most frequent being pneumonia 76%, followed by bacteraemia 11%, meningitis 7%, empyema 3% and other 3%.

Conclusions: The incidence of IPD varied with age, with the highest rates being among young children and the elderly, and is also coherent with data reported by other regions and developed countries. The proportion of cases by serotypes included in the PCV7 was lower than that observed in studies conducted by Madrid in prior years, primarily in children. Continuous surveillance of IPD is fundamental in order to assess the evolution of the overall incidence and serotypes.

P1348 Community-acquired pneumonia and pleural effusion. A comparative study between complicated and uncomplicated parapneumonic effusion

M. Falguera, J. Carratalà, I. Chica, C. García-Vidal, S. Bielsa, J. Dorca, F. Gudiol, J.M. Porcel (Lleida, Barcelona, ES)*

Objective: To determine the incidence, etiology and outcome of community-acquired pneumonia with parapneumonic effusion and to recognize predicitive factors for complicated fluid.

Methods: We analyzed databases from 2 university hospitals in Catalonia (Spain) that enrolled consecutive patients with community-acquired pneumonia for a 12-year period and selected subjects who had radiological evidence of pleural fluid. Complicated parapneumonic effusion was defined as empyema (presence of pus) or pleural fluid meeting one or more of the following criteria: pH < 7.20, glucose level <40 mg/dl or positive culture. Remaining cases were considered as having an uncomplicated effusion. Univariate and multivariate analyses were employed to compare patients with complicated and uncomplicated fluid.

Results: A total of 882 of 4715 (19%) patients with community-acquired pneumonia had radiologial evidence of pleural fluid, 261 (30%) of them met criteria for complicated parapneumonic effusion. In multivariate analyses, the development of complicated effusion was associated with 4 clinical factors: age <60 years ($p = 0.018$), alcoholism >60 g/day ($p = 0.002$), pleuritic pain ($p = 0.003$), and tachycardia >100 beats/minut ($p = 0.003$). Rates of complicated parapneumonic effusion were 12%, 21%, 32%, 50% and 63% in presence of 0, 1, 2, 3 or 4 risk factors, respectively. Patients with complicated effusion also showed substantial differences in percentages of causal agents with a greater incidence of conventional bacterial microorganisms, particularly non-pneumococcal Gram-positive cocci. Moreover, these patients had a significantly longer hospital stay, although the mortality was not increased.

Conclusions: In patients with community-acquired pneumonia and radiological evidence of pleural fluid (19% of cases), age <60 years, alcoholism, pleuritic pain and tachycardia increased the risk of complicated parapneumonic effusion (30% of cases). These patients also showed a greater incidence of infection caused by Gram-positive cocci and had a longer hospital stay, although the mortality was not increased.

Community-treated paediatric infections

P1349 Mono and mixed gastrointestinal paediatric infection: epidemiological and clinical features

S.G. Rimoldi, F. Stefani, C. Pagani, I. Di Bartolo, A. Lombardi, N. Zanchetta, D. Dilillo, F. Ruggeri, M.R. Gismondo (Milan, IT)*

Objective: Concerning the gastroenteritis surveillance in Italy the existing data about the viral agents are poorly known. This study was designed to assess the prevalence of viral agents among pediatric children admitted with acute gastroenteritis to L. Sacco University Hospital (Milan, Italy).

Methods: An eighteen months study (2008–2009) involving 118 patients, aged on average 30 months, was carried out. Stool specimens were tested for bacteria while Rotavirus was detected by enzymatic immunoassay card (Meridian).

Samples, stored at −80ºC until use, were processed for Norovirus (NoV), Bocavirus (HBoV), Adenovirus and Enterovirus by Real time PCR kit (Argene); Astrovirus with a multiplex PCR assay (Seegene).

Demographic and clinical data were collected in accordance with 20 point Ruska Vesikari Scoring System. Clinical information was obtained from the virology request forms and from case sheets. According to that, the severity of the gastroenteric disease was scored: minor (1–8), moderate (9–14) and serious (>15).

Results: 93 out of 118 children have shown diagnosis of gastroenteritis due to an infective cause (4 bacteria, 3 parasites, 86 viruses), 25 cases were without a cause. In the 75 mono-infection enteric viruses were: 35.6% for HRVes, 13.6% for NoVs, 11.0%for HBoV and 3.4% for Adenoviruses. Among the dual infection (11 cases) HRV/NoV were present in 4 cases (3.4%), HBoV/NoV in 1 case (0.8%), HBoV/HRV in 6 cases (5.1%). In a single case, a mixed infection supported by NoV/*C. difficile* was detected.

A moderate score of clinical severity(RVS)for HRV (1.5), Nov (2.15), HBoV (2.36)and Adenovirus (2.05) were reported. Moreover in mixed-infections synergic effects are likely to occur: NoV/HBoV infection showed the higher RVS (3) followed by HBoV/HRV (2.33) and NoV/HRV (2.00).

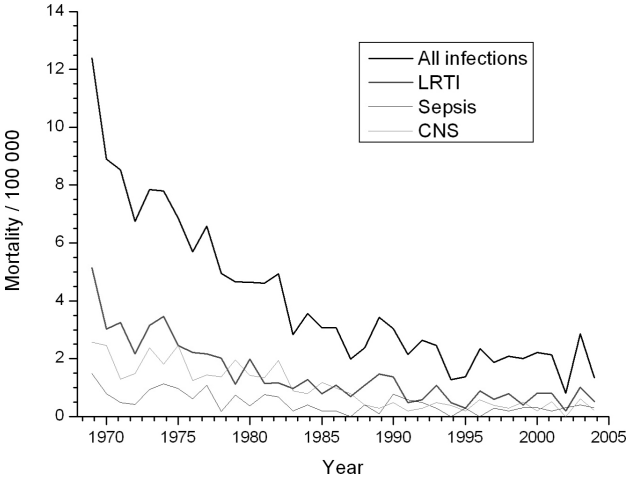
Conclusion: An high prevalence of HRV (53.9%) was detected following by NoV (22.5%), HBoVs (15.7%), Adenoviruses (4.5%). A severe score of seriousness was reached with HBoV in mixed infection with Nov and HRV. due to the synergic action of more pathogens that increases the clinical severity of illness or the different timing of viruses excretion and incubation.

P1350 Changes in infectious disease mortality in children, 1969–2004

M. Lantto, M. Renko, M. Uhari (Oulu, FI)*

Objectives: The aim was to evaluate the changes in infectious disease mortality in children in Finland from 1969 to 2004. Our main interest was in mortality of previously healthy children. We especially wanted to find out whether the infection mortality could be further reduced by the means of existent vaccines not included in current national vaccination programme.

Methods: We analysed infectious disease mortality data obtained from the official cause of death statistics in Finland for 1969–2004. Annual mortality rates were calculated in proportion to those at risk of dying. Infection mortality rates were conducted for neonatal group and children aged one month to 15 years.



Results: Mortality due to infectious diseases reduced by 89% in childhood group, from 0.12‰ in 1969 to 0.013‰ in 2004. In neonatal group relative decline was 69%, from 0.50‰ in 1969 to 0.16‰ in 2004. Pneumonia, CNS infections and septicaemia were the most common

fatal infections in childhood. Decline in mortality occurred evenly in all these groups, as well in all age groups in childhood.
Conclusions: Mortality from infectious diseases has decreased significantly during last decades in Finland. By the means of existent vaccines mortality could be still further reduced.

P1351 Characterization of *Haemophilus influenzae* strains isolated in Portuguese children with acute otitis media in the pre-vaccination period with PCV10-HiD

M. Bajanca-Lavado, C. Betencourt on behalf of ARSIP*

Objective: Non capsulated *Haemophilus influenzae* (HiNC) along with *Streptococcus pneumoniae* (Spn) are the most common cause of bacterial acute otitis media (AOM) in children. A vaccine with 10 Spn serotypes, each one conjugated to a recombinant non-lipidated form of protein D as carrier protein, a conserved cell surface protein of Hi, already showed to induce protection against non-capsulated (NC) Hi strains. Our aim is to characterize the serotype and to determine antibiotic susceptibility in 192 Hi strains isolated in children, with an otitis diagnose, to emphasise the importance of immunization against this disease.
Methods: From January 2001 to June 2009 we characterized 192 Hi strains isolated in 9 collaborating Hospital Laboratories, in Portugal. β-lactamase production was determined by a nitrocefin assay. Serotyping was performed by PCR, using primers specific to each capsule type (a to f). Minimum inhibitory concentrations (MIC; mg/L) were determined for 13 antibiotics by a micro dilution assay, according to CLSI.
Results: Serotype determination characterized all 192 Hi strains as NC (100%). β-lactamase production was detected in 8.3% of the strains. Thirteen β-lactamase negative strains (7.4%) had MIC values to ampicillin$\geqslant$1 mg/L, being considered ampicillin resistance-non-β-lactamase producers (BLNAR). High levels of resistance were obtained with cefaclor (32.3%) and SXT (22%).
Conclusions: Our results of 100% NC strains isolated from otitis are in accordance with the literature that refers HiNC as one of the principal causes of AOM episodes. This infection is one of the most common cause of antibiotic prescription, as well as one of the principal reasons to visit the paediatrician, which represents high economic costs. Several trials have indicated that the vaccine PCV10-HiD provide protection against HiNC disease, causing a decrease in more than 30% of the cases. In relation to resistance, we are worried about BLNAR strains, since this kind of strains are not detected in routine laboratory techniques. Molecular methods, that determine specific mutations in the resistance gene, are necessary to their characterization. The burden of the disease and the excessive antibiotic consumption justifies immune prophylaxis, preferentially by active immunization. The vaccine, reducing AOM episodes, also reduces antibiotic consumption, as well as resistance, which can be translated in clinical and public health impact.

P1352 Correlation between malnutrition and parasitic infection among children from rural areas in Constanta County, Romania

C.M. Mihai, R. Stoicescu, G. Taralunga, G. Lilios (Constanta, RO)*

Background: Malnutrition and intestinal parasitic infections are common public health problems of children from rural areas of Constanta County, Romania.
Objective: To establish the correlations between malnutrition and parasitosis in child from rural areas, Constanta County.
Methods: We examined these correlations in a cross-sectional program in Prodiagnostic Laboratory, Constanta, Romania. 339 children from 24 rural communities were screened for intestinal parasites. Examinations of stool specimens were done using fresh preparate and sedimentation and flotation techniques (ZnSO4 flotation). Children were assessed for the presence of malnutrition. Anthropometric measurements consisted of height and weight determination The body mass index (BMI) was determined by dividing the weight in kilogram by the squared value of the height in meters. Nutritional status was determined as weight-for-age (WFA), weight-for-height (WFH), and height-for-age (HFA). There were 159 girls and 180 boys, aged 6 months to 12 years.
Results: The relationship between the prevalence and the nutritional status of the children showed that 28.9% of examined children were infected with one parasite and 4.1% were infected with two or three parasites. Out of total number of the infected children, according to their BMI, 23.5% were malnourished and there was a significant difference ($P < 0.05$) of malnutrition between infected and non infected children. The prevalence of malnutrition was 19.2% (WFA), 10.1% (WFH), and 27.4% (HFA). Parasitosis were more prevalent in children less than 6 years of age with low HFA and WFA. In older children low WFA was more closely associated with parasitic infections. Ascaris and *Toxocara* were more prevalent in malnourished children.
Conclusions: Malnutrition associated with intestinal helminth infections is an important contributory factor for children's diseases from rural areas in our county. Therefore, it is recommended that the local health representatives should establish a guideline for regular examination, diagnosis and treatment for intestinal parasitic infections and associated malnutrition among children from rural areas.

P1353 Vulvovaginitis in pre-pubertal and pubertal girls

S. Baka, I. Patikas, A. Panopoulou, A. Lykas, A. Spathi, I. Tsouma, E. Kouskouni (Athens, GR)*

Objectives: Vulvovaginitis is the most common gynecological problem in prepubertal and pubertal girls. Nevertheless, the interpretation of the results of vaginal cultures performed in children is often difficult since clinical data and possible risk factors must be taken into consideration. This study aimed to evaluate the vaginal microflora in a group of prepubertal (PP) and pubertal (P) girls with vulvovaginitis.
Methods: A total of 245 girls aged 2 to 17 years, presenting at the outpatient clinic for pediatric and adolescent gynecology of our hospital during January 2008 to October 2009 with signs and symptoms of vulvovaginitis (vaginal redness and discharge and/or itching) were included. Cases were divided into 2 groups: 101 PP (age 2–11 years) and 144 P (age 12–17 years). Vaginal secretions were collected using a sterile newborn suction catheter carefully inserted into the vagina. Samples were inoculated onto blood agar, MacConkey, Mannitol Salt, Sabouraud Dextrose agar, *Gardnerella* agar and Wilkins-Chalgren agar as well as Thayer-Martin and chocolate agar followed by incubation in aerobic, anaerobic or CO2 atmosphere at 37º C for 24 or 48 hours, as appropriate. Wet mount and Gram stain preparations were examined to investigate the presence of leukocytes and the type of bacteria present. In order to rule out the presence of *Enterobius vermicularis* a Graham test was performed in all cases. The identification of isolated strains and their susceptibility test to antibiotics were carried out with the API System and the automated system VITEK 2 (bioMérieux, Marcy l'Etoile, France).
Results: Bacterial pathogens were isolated from 60/101 (59.4%) of PP girls and 99/144 (68.8%) of P girls. Interestingly, leukocytes were observed only in samples where pathogens were retrieved. Isolated bacteria in the PP and P groups included 12/101 (11.9%) and 72/144 (50.0%) anaerobes, 27/101 (26.7%) and 9/144 (6.3%) Gram-positive cocci, 21/101 (20.8%) and 10/144 (6.9%) Gram-negative rods, respectively. *Candida* species were isolated in 8/144 (5.6%) only in the P group. Finally, in 41/101 (40.6%) in PP group and 45/144 (31.2%) in P group no pathogen was isolated.
Conclusion: In the PP girls predominantly Gram-positive cocci and Gram-negative rods were isolated while in the P girls, anaerobes. *Candida* species were found only in the P group. The presence of leukocytes in vaginal samples increases the likelihood of finding pathogens which require specific treatment.

P1354 Pneumococcal carriage in the Warao Amerindians from Venezuela: high colonization rates and frequent serotype sharing among mothers and children

M.C. Sisco, I.A. Rivera-Olivero, M. Rodríguez, B. Del Nogal, P. Hermans, J. De Waard (Caracas, VE; Nijmegen, NL)*

Objectives: To obtain insight in the dynamics of pneumococcal transmission in the Warao people and to determine the role of the mother in the transmission of the pathogen we investigated pneumococcal colonization and serotype distribution among Warao children and their mothers in 157 families.
Methods: From July 2007 through August 2008, nasopharyngeal samples were obtained from 157 families, including 336 children between 2 months and 10 years old and 152 mothers. The mean number of children per family was 2.14. *S. pneumoniae* was isolated and identified according to standard microbiological procedures. Pneumococci were serotyped by multiplex PCR for the serotypes present in the 23 polysaccharide vaccine with exception of serotype 2.
Results: The overall pneumococcal nasopharyngeal carriage rate was 59.6% (n = 291) with 236 children (70.2%) and 55 mothers (36.2%) colonized. The most important capsular serotypes/serogroups found were 6 (25.3%), 19A (12.3%) and 23F (10.9%); 32.2% (n = 118) of the isolates were not typeable with the PCR method. We further observed that among children <2 years of age the carriage rate was 74.6%, among children between 3–5 years of age 75.2% and between 6 and 10 years of age 59.2%. In 42 (26.8%) families, only one individual was colonized representing 3 mothers and 39 children. In 91 families at least two members were colonized, and 48 families were colonized with the same serotype. In 28 of these 48 families, the serotype was shared between the siblings, and in 22 families the serotype was shared by the mother and at least one of the children.
Conclusion: This study shows high pneumococcal carriage rates in the Warao mothers (36.2%) and in their children (70.2%). Of the 55 mothers colonized with pneumococcus 22 mothers (40%) shared the serotype with their children, indication transmission between mother and child or vice versa. To determine the relationship between strains shared by mother and child molecular epidemiological studies are under way to determine the genotypes of the shared strains of these 22 families. Interestingly, 33 mothers were colonized with serotypes not found in their children, indicating a possible role of the mother bringing new serotypes into the family.

P1355 Macrolide and multidrug resistance in *Campylobacter* strains from acute gastroenteritis in childhood. Is there an emergency? A 5-year retrospective review

E. Staikou, A. Makri, K. Papavasileiou, F. Vagia, E. Papavasileiou, A. Voyatzi (Athens, GR)*

Objectives: 1. To investigate the prevalence of *Campylobacter* spp (Cspp) in our paediatric hospital during a five year period (2005–2009). 2. To compare the *in vitro* activities of novel and established antimicrobials as well as the emergence of macrolide and multidrug resistance.
Material and Methods: Out of 5200 feces specimen were tested for common enteropathogens (Cspp 44.8%, *Salmonella* 47.5%. *Shigella* 1.03%, *E. coli* EPEC 3.5%, *Y. enterocolitica* 2%, *A. hydrophila* 0.9% and Cl.difficile 0.27%). Identification and susceptibility patterns were performed according to CLSI guidelines. MIC values were determined by E-test and the examined agents were as follows: Erythromycin/EM, Nalidixic acid/NA, Ampicillin/AM, Amoxicillin–clavulanic/AMC, Tetracycline/TE, Ciprofloxacin/CIP, Gentamicin/GM, Chloramphenicol/CL, Levofloxacin/LE, Azithromycin/AZ, Roxithromycin/RO, Clarithromycin/CH. *C. jejuni* ATCC 33560 served as the control strain.
Results: Cspp were detected in 6.7% (348/5200) of the samples; 97.7% were identified as *C. jejuni* (C.j) and 2.3% as *C. coli.* Two strains of C.j were characterized as doylei subspecies. More than half of Cspp (52%) derived from paediatric clinics while 48% from outpatients ones. Males predominated over females (ratio 2:1). Isolates showed a significant resistance to NA 52%, CIP 48.9%, TE 33% followed by AM 12.35%, EM 4.31% and AMC 1%.All strains were susceptible to gentamicin. Only 7% of Cspp resistant to EM were susceptiple to CIP and LE. As a whole, 65.52% of Cspp strains presented resistance while multidrug resistance (⩾2 classes of agents) was found in 49%. The predominant resistance patterns (RP) in Cspp were determined as CIP/NA(RP) (52%), TE/CIP/NA(RP) (14.9%), E/CIP/NA(RP) (0.57%), TE/CIP/NA/AM(RP) (5.46%) and CIP/TE/AM/NA/E(RP) (1.43%) respectively. The examined Cspp strains showed the following MIC50/90 (mg/l): EM:0.5/2(mg/l), AZ:0.047/0.25(mg/l), CH:0.50/3(mg/l), RO:1/3(mg/l), AMC:0.75/1.5(mg/l), AM:4/48(mg/l), CIP:32/>32(mg/l), LE:8/32(mg/l), TE:16/256(mg/l), CL:2/4(mg/l), NA:256/256(mg/l). Remarkably, a gradual decrease in EM resistance was observed during the study period (2005:8.2%, 2009:3.0%).
Conclusions: 1. Cspp remain a leading cause of bacterial diarrhoea. 2. Among macrolides AZ was found to be the most active, followed by EM. Our data do not support emerging macrolide resistance. 3. Nevertheless, multidrug resistance poses a public health problem and global antimicrobial susceptibility surveillance is urgent.

P1356 Randomized trial of cranberry juice for the prevention of recurrences of urinary tract infections in children

J. Salo, T. Kontiokari, M. Helminen, M. Korppi, T. Nieminen, T. Pokka, M. Uhari (Oulu, Tampere, Kuopio, Helsinki, FI)*

Objectives: Urinary tract infection (UTI) has a great tendency to recur after the initial episode, and there is a great need for methods to prevent UTIs. Previously we have shown, that regular drinking of cranberry juice reduces the recurrence of UTIs in adult women. The objective of the study was to evaluate, whether cranberry juice is effective in preventing UTI recurrences in children who have had at least one episode of acute UTI.
Methods: Between 2001–2008 a double-blind randomized controlled trial was performed in seven pediatric departments. Altogether 263 children referred to pediatric departments because of UTI were randomized to receive either cranberry juice (n = 129) or placebo (n = 134) for six months. The patients were followed for 12 months, and all their recurrences of UTIs were recorded.
Results: Twenty (16%) patients in the cranberry group and 28 (22%) patients in the placebo group had at least one recurrence of UTI during the 12 months follow up (difference between the proportions 6%, 95% confidence interval 16% to 4%, P = 0.21). There were altogether 27 episodes of UTI during the follow up in the patients in the cranberry group and 47 in the patients in the placebo group. The cumulative incidence rate of the recurrences did not differ between the groups (Fig. 1).

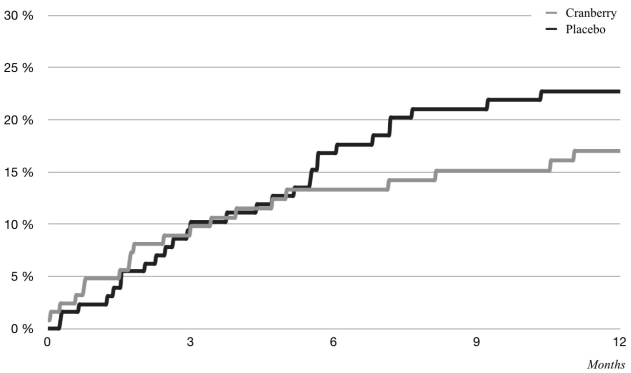


Figure 1. Time to the first UTI recurrence during 12 months follow up in patients receiving cranberry juice or placebo juice. The difference between the groups was not significant (P = 0.32).

Conclusion: Cranberry juice did not reduce clinically significantly the recurrence of UTI in children who had had at least one acute symptomatic UTI.

P1357 Norovirus outbreak in a hotel associated to a children's club

A. Domenech-Sanchez, C.I. Berrocal (Palma de Mallorca, ES)*

Objective: To determine the aetiological agent responsible for a gastroenteritis outbreak in a hotel located in northern Majorca.
Methods: We defined a case as any guest or employee of the hotel with acute onset of at least two of the following symptoms: diarrhoea, vomiting, abdominal clumps, or fever (>37ºC). Environmental samples, including food, drinking water, ice cubes and pool water were analyzed for bacterial pathogens. Stool samples were analyzed for intestinal pathogens, including members of *Salmonella*, *Shigella*, *Yersinia*, *Cryptosporidium* and norovirus. In children samples rotavirus and adenovirus were also analyzed.
Results: First case was described on June 18th. Ten of 685 guests were affected: 5 adults, 4 children and 1 baby (attack rates were 1.06%, 2.4% and 2.0%, respectively). Four of 90 employees were also affected (attack rate 4.4%). When only the entertainment team was analyzed, attack rate increased up to 66.7%, rising a 100% when working directly in the children club of the hotel. The most frequent symptoms were vomiting (93%), diarrhoea (50%), abdominal pain (29%) and fever (7%). The duration of symptoms ranged from 1 to 4 days. Two children and 1 woman were hospitalized for hydration support, with good outcome.
Four stool samples from 4 patients were taken during the acute phase of the gastroenteritis for culture. Genotype 2 norovirus was detected in 2 of the 4 samples, 1 from an adult and 1 from a child, by the ELISA test. All other pathogens tested negative. As for the environmental samples, microbiological levels were acceptable in all cases, ruling out their role as a source of infection. Once detected the problem related to gastroenteritis cases a specific control plan was rapidly implemented. Enhanced hand hygiene practices were established, and cleaning and disinfection of frequently contacted surfaces (doorknobs, phone keys . . .) with alcohol 70% were carried out. Common areas, especially toilets, were also frequently treated with bleach. Special care was taken at the children club and patients' rooms. Last case was detected on June 24th.
Conclusions: Genotype 2 norovirus was the responsible for the gastroenteritis outbreak. Immediate implementation of appropriate measures allowed a rapid control of the outbreak.

Miscellaneaous – community-onset infections

P1358 Infective endocarditis should be treated in experienced centres

N. Fernández-Hidalgo, B. Almirante, P. Tornos, M.T. González-Alujas, A.M. Planes, A. Pahissa (Barcelona, ES)*

Objectives: The aims of this study were: 1) to compare the characteristics of adult patients with left-sided infective endocarditis (IE) diagnosed and treated in a tertiary care hospital with those of patients referred from other centres because of the same diagnosis or suspicion; and 2) to establish the accuracy of diagnosis and treatment in referred patients and their influence in outcome.
Methods: A prospective, observational cohort study was conducted at Vall d'Hebron Hospital, Barcelona, Spain, a 1000-bed teaching hospital with all the medical and surgical services and a referral centre for cardiac surgery.
Results: Between January 2000 and September 2009, 337 episodes of left-sided IE in 334 patients were treated in our hospital. Of them, 114 (34%) were transferred from 35 hospitals. As compared with patients diagnosed in our hospital, transferred patients acquired IE less often in relation with health care system (16.7% vs 38.1%, $p < 0.0005$); were in better health before onset (Charlson index 1.44 ± 1.65 vs 2.36 ± 2.27, $p < 0.0005$); experienced more complications (94.7% vs 78.9%, $p < 0.0005$), including heart failure (68.4% vs 39%, $p < 0.0005$), myocardial abscess (38.6% vs 18.8%, $p < 0.0005$), and acute renal failure (69.3% vs 22.1%, $p < 0.0005$), underwent more surgeries (69.3% vs 22.1%, $p < 0.0005$) with a lower EuroSCORE (8.9 ± 3.3 vs 10.6 ± 3.7, $p < 0.0005$); and experienced a non-statistically different rate of in-hospital mortality (22.8% vs 31.4%, p=NS). Focused on the subgroup of 114 referred patients, only 52 (45.6%) initially received an antimicrobial regime included in the American, European or Spanish guidelines. In the remaining patients, the median days of incorrect treatment was 5 (IQR 3–10, range 1–42). Mortality was greater in patients without an appropriate antimicrobial regime than in the other group (32.3% vs 11.5%, OR 3.65, 95% CI 1.34–9.96, $p = 0.009$). Moreover, in 42 out of 114 patients (36.8%), an echocardiogram performed in our centre demonstrated a complication no diagnosed at origin. Although not statistically significant, this group of patients experienced greater in-hospital mortality than the other group (28% vs 18.8%, $p = 0.24$).
Conclusions: IE is a rare and severe condition that should be treated in experienced centres by multidisciplinary teams. Mistakes in the initial diagnosis and antimicrobial treatment are associated with greater mortality.

P1359 Risk factors for precocious mechanical ventilation in pneumococcal meningitis: a 12-year experience

P. Pagliano, V. Attanasio, M. Rossi, F. Fraganza, G. Esposito, M. Conte, F. Faella (Naples, IT)*

Despite continuing improving in the field of intensive cares and infectious diseases therapy, pneumococcal meningitis still represents a life-threatening disease. Aim of our study was to evaluate the characteristics of patients with pneumococcal meningitis receiving precocious mechanical ventilation in respect to those with less severe disease presentation.
Materials and Methods: In a prospective study we enrolled adult patients with pneumococcal meningitis admitted in our department during a 12-year period. Epidemiological, clinical and laboratory findings at admission were investigated. Statistical analysis was performed by two-tailed χ^2 test, by Fisher exact test, and by Mann–Whitney U-test. Data are expressed as median and Interquartile range(IQR).
Results: One-hundred-eighty-four cases of pneumococcal meningitis were enrolled [median age 58 years (IQR 39–66), males 55%]. Eighty-five (46%) patients received mechanical ventilation within 48 hours from admission (Group A), among the remaining 99 patients (Group B) only 2 had to receive mechanical ventilation during the course of disease. Condition leading to immunodepression were present in 40 (47%) group A patients and 25 (25%) group B patients ($p < 0.01$; RR= 1.86; 95% CI 1.24–2.8). No significant difference was observed in respect to the incidence of cases with sinus or middle ear infection and in respect to the time between first symptoms evidence and admission to the hospital. Penicillin susceptible strains of *Streptococcus pneumoniae* were cultured in 28% of Group A patients and 17% of Group B patients. Laboratory findings are reported in the table. Mortality rate was 48% for group A and 2% for group B patients.
Discussion: Immunodepressed patients with pneumococcal meningitis are at high risk for precocious respiratory insufficiency. Factors commonly associated to higher meningitis mortality, such as low CSF glucose, high CSF protein and low WBC counts are present more frequently in cases with rapidly progressive disease needing precocious mechanical ventilation. These evidences suggest a close relationship between some delay in the setting of precocious immune response and patients presentation and outcome.

Table 1. Laboratory findings in respect to the necessity of mechanical ventilation*

	CSF cells ($\times 10^3$/μL)	CSF glucose (mg/dl)	CSF protein (mg/dl)	WBC ($\times 10^3$/μL)	Platelets ($\times 10^3$/μL)
Group A	1.8 (0.6–7.8)	7 (2–17)	643 (360–950)	17.4 (12–26)	151 (121–239)
Group B	3.5 (0.8–9)	12 (5–31)	498 (314–685)	21.3 (16–28)	229 (165–299)
P	0.13	0.045	0.018	0.008	0.07

* Data are expressed as median (IQR).

P1360 Bacterial meningitis due to pericraneal fistula in adults: thirty years of experience

M. Bodro, C. Cabellos, R. Verdaguer, L. Lopez, F. Gudiol, J. Acebes, P. Fernandez Viladrich (Barcelona, ES)*

Objective: To know the etiology, clinical characteristics and outcome of bacterial meningitis due to pericranial fistula.
Methods: In our hospital all cases of bacterial meningitis are routinelly recorded in a 120 variables protocol. Pericranial fistula was diagnosed when pts referred patent rhino or otoliquorrhachia, when a bone defect was present in a CT scan or MRI, when isotopic cisternography revealed a CSF leak or with a history of neurosurgery trough nose or otologic surgery or cranial trauma. CSF and blood cultures were performed by standard methods. Early post surgical fistula was defined as that presenting in the first 20 days after surgery and late fistula as cases presenting later. Traumatic cases were classified in <1 yr, 1–10 yrs and >10 yrs according with the time of traumatic event.
Results: Between 1977 and 2008, 1256 episodes of bacterial meningitis in adults have been treated in our hospital. Among them 141 episodes in 129 pts were due to pericranial fistula. Pericranial fistula related episodes were present in 94 men (66%) and 47 women (34%). Mean age was 46.3 (16–93). Fistula was due to previous surgery in 60 episodes (42%), 31 (51%) due to early fistula and 29 (49%) to late fistula. Previous trauma was present in 67 (47%), 1 yr before in 31 (46.2%), 1 to 10 yrs in 20 (29.8%) and >10 yrs in 16 (24%). 38 episodes were recurrent meningitis. 2 pts presented 3 episodes. Etiology was *S. pneumoniae* in 63 (44%), *H. influenzae* in 17 (12%), other streptococcal in 11 (8%), Neisseria spp. in 2 (2%), anaerobical in 1 and unknown in 45 (32%). On admission fever was present in 115 (81%), headache in 113 (80%), nausea/vomiting in 86 (61%), GCS < 8 in 30 (21%), seizures in 19 (13%). Blood cultures were positive in 57 (40%) and CSF culture in 80 (56%). Sequelae were present in 7 (5%). Overall mortality was 8 (5.6%) (4 due to early neurological causes, 1 due to late neurological causes and 3 due to not related causes). Reparative surgery was performed in 52 pts. Among pneumococcal meningitis episodes a comparison was made in mortality among episodes due to fistula or other focus. Mortality was significantly lower in fistula related episodes 5/63 (8%) than in other pneumococcal episodes 56/200 (28%), $p < 0.05$.
Conclusion: Bacterial meningitis related to pericranial fistula is still due mainly to *S. pneumoniae* and *H. influenzae.* Trauma or neurosurgery may be a remote fact. Prognosis is good with a very low mortality and special efforts should be done to repair the fistula to avoid further episodes.

P1361 Epidemiological changes in community-acquired bacterial meningitis: 20 years of follow-up

A. Madurga, I. Sanfeliu, I. Pons, M. Lloret, P. Ferreros, M. Espasa, S. Capilla, D. Fontanals (Sabadell, ES)*

Objectives: The aim of this study is to know the aetiology and the epidemiological changes of community acquired bacterial meningitis in our centre over the past 20 years. To review the incidence of microorganisms included in the vaccination calendar and its variation in this period of time.
Methods: We reviewed 283 community acquired meningitis cases between 1990 and 2009. Hospital Parc Taulí has an adult as well as a paediatric reference area that varies from 380300 inhabitants in 1990 to 414080 inhabitants in 2009. Serotypes and serogroups of microorganisms were studied by The National Reference Centre of Microbiology in Majadahonda (Spain).
Results: The aetiology was: *N. meningitidis* 113 cases (39.9%), *S. pneumoniae* 74 (26.1%), *C. neoformans* 17 (6%), *L. monocytogenes* 16 (5.7%), *H. influenzae* 16 (5.7%), *S. agalactiae* 14 (4.9%), other microorganisms 33 (11.9%). Concerning *H. influenzae* serotype b, all cases were children <5 years old, of which 62.5% were male patients. It showed an incidence rate of $8.6/10^5$ inhabitants in 1994 that decreased successively since the introduction of the conjugated vaccine until its complete eradication. There were three cases of *H. influenzae* among adult patients non b serotype. As to *N. meningitidis,* it was present over the whole age spectrum and it was the leading cause of meningitis in young adults. It was slightly more common among women, 51.3%, and the incidence rate decreased from $6.2/10^5$ before the vaccination period, to $0.5/10^5$ in 2009. B serogroup was the most prevalent until 1997, >50% of the cases. During 1998 and 1999 C serogroup was present in 50% of the cases. After the introduction of the conjugated vaccine in 2000, serogroup C decreased until its complete disappearance. Finally, *S. pneumoniae* was more prevalent among adult patients of which 54.7% were men. Regarding children, 75% of them were younger than 5 years. The incidence rate showed a similar pattern, from $1.3/10^5$ to $1.1/10^5$. Serotypes 19A, 7F and 24 appeared after the vaccine introduction. Since 2002, 3 children had meningitis, which serotypes were 33, 7F and 14.
Conclusions: After the introduction of conjugated vaccines, we observed an important decrease of meningitis due to *H. influenzae* and *N. meningitidis.* Nevertheless, meningitis due to *S. pneumoniae* remained stable in time, with variations between serotypes depending on the period of time.

P1362 Clinical features and prognosis of *Escherichia coli* vertebral osteomyelitis. A descriptive study of 28 cases

M.E. Jiménez-Mejías, J.D. Ruíz-Mesa, E. García-Cabrera, A. Plata, J. Valencia, M. Delgado, J. Márquez-Rivas, J.D. Colmenero-Castillo (Seville, Málaga, ES)*

Objectives: To study the epidemiologic, clinical and diagnostic features, treatment and prognosis of vertebral osteomyelitis (VO) caused by *Escherichia coli*.
Patients and Methods: Multicentric, prospective, and descriptive study of 534 patients diagnosed of VO between January 1983 and October 2009. Inclusion criteria: 1) spinal inflammatory pain, or fever and spinal pain on physical examination; 2) compatible imaging findings with VO; and 3) aetologic diagnosis.
Results: Sixty-nine patients (13%) were diagnosed of VO caused by gramnegative bacili (GNB). *E. coli* was the responsible agent in 33 cases (48% of VO by GNB, and 6.2% of total VO). Five patients with polymicrobial isolates were excluded. Seventeen (61%) were females. Mean age: 63.9 (50% >65 years old). Ten (36%) patients had underlying diseases (5 diabetes, 3 inmunosuppression, 2 liver cirrhosis, 2 others). Primary infection foci was recorded in 23 (82%) cases: 17 genitourinary tract, 3 digestive, and 4 others. Previous bacteremia in 13 (46.4%). The vertebral levels involved were: 6 thoracic (21%), 2 dorso-lumbar, 15 lumbar (54%) and 5 (18%) lumbo-sacral. The mean duration of symptoms prior to diagnosis was 51.4 days (r:7–185). Clinical features: back pain 28 cases (100%), inflammatory pain 25 (89%), fever 24 (86%), constitutional symptoms 20 (71%), neurological deficits 19 (68%), sciatic pain 16 (57%). All cases had raised ESR, 85% high levels of C-reactive protein, and 50% elevated leucocyte count. Blood cultures were positive in 50%, vertebral biopsies in 10 of 12 cases (83%), adjacent infectious foci cultures in 75% (6 of 8)and urine cultures in 54% (12 of 22). Sixteen patients (73%) presented paravertebral masses, 12 (43%) epidural abscesses, and five (18%) psoas abscesses. All patients were treated with antimicrobial agents (mean:64 days, r:30–144 d.) and 11 (39%) required surgical treatment. Two patients (7%) showed therapeutic failures (one medical and one surgical failure). Twelve cases (42.8%) presented severe functional sequelae: 11 pain and one paraplegia. One patient died, but the death was not related with VO.
Conclusions: 1) *E. coli* is the microorganism more frequent isolated in VO by GNB. 2) Genitourinary tract infections and previous bacteremia by *E. coli* are the principal predisposing factors. 3) Blood, adjacent infectious foci and vertebral biopsy cultures showed high diagnostic yields. 4) Paravertebral masses and epidural abscesses and severe functional sequelae are frequents.

P1363 Clinical and microbiological features of pyogenic liver abscess. A multicentre study from Spain

F. Mateos Rodríguez, I. Terrancle, G. Peralta, L. Fuentes, J. Pérez-Arellano, P. Geijo, F. Marcos, M. García, J. Blanch Sancho, E. Martínez Alfaro for the Infectious Diseases Group of the Spanish Society for Internal Medicine (SEMI)*

Objectives: The aim of the present study was to examine our experience over pyogenic liver abscess (PLA).
Methods: Multicenter and retrospective review of the PLA diagnosed in six Spain's Hospitals during seven years were studied. Statistical analysis was performed with the SPSS software package.
Results: A total of 175 patients with PLA were managed. PLA was more common in males (61%). The median age was 65 (range 27–91). Concomitant medical problems included diabetes mellitus in 33 patients, neoplasm in 7 and transplantation in 2 (1 kidney and 1 liver). Most patients presented with non-specific clinical and biochemical features. Pre-admission, patients were symptomatic for a median 16 days, with the most common symptoms and signs being fever (85%) and abdominal pain/tenderness (70%). A raised ESR (median 81, range 2–159) was the most common laboratory found in about 86%. Ultrasonography was not as sensitive as computed tomographic scans in detecting abscesses. Single lesions were found in 119 patients, multiple lesions in 56. PLA occurring more frequently in the right hepatic lobe (70%). The microorganism responsible was identified in 126 (71%) of the cases, with enterobacteria being the greatest number isolated. 52% of the positive abscess cultures were polymicrobial. *Escherichia coli* was the most common etiological agent detected in cultures of blood and abscess aspirates. *E. coli* was cultured from the blood in 24 patients and 31 on abscess aspirate cultures. Abscesses were classified by the presumed route of hepatic invasion: (a) biliary tree (65, 37%), (b) portal vein (18, 9.5%), (c) hepatic artery (18, 9.5%), (d) direct extension from contiguous focus of infection (16, 8.6%) and (e) cryptogenic (58, 33%). All patients were treated with intravenous antibiotics. The most commonly used antibiotic combination was a cephalosporin 3^{d} G with metronidazole. 112 (63%) had both antibiotics and radiologically guided percutaneous catheter drainage. All these patients had abscesses with diameters measuring 2 or more cm. Only 24 (14%) need surgery; 16 because another illness who required surgery, 5 because of deterioration despite antibiotics and drainage and 3 because of failure of percutaneous drainage.
Conclusion: PLA require a high index of suspiction for early diagnosis. Imaging techniques provide the main support in both the diagnosis and treatment. When appropiate theraphy in the form of antibiotics in combination with percutaneous drainage is administered, morbility and mortality are low.

P1364 Prevalence of invasive meningococcal subtypes in Bilbao after immunization

M.J. Unzaga, C. Ezpeleta, J.A. Alava, I. Gerediaga, M. Sanchez, R. Cisterna (Bilbao, ES)*

Objectives: In 1990 an increasing number of serogroup C meningococcal strains were observed in Basque Country. These strains were characterized as C:2b: P1.2,5. As results a mass immunization campaign was conducted in 1997. The aim of our study was to determine the serotypes of invasive meningococcal disease nowadays, years after establishing vaccination.
Methods: The strains isolated from blood a cerebrospinal fluid in our hospital in 2008 and 2009 were sent to the Spanish Reference Laboratory for serotyping with monoclonal antibodies.
Results: The results of 18 meningococcal strains were analyzed. One strain was C serogroup C:2a:P1.5 and 17 strains were B serogroup. Completely serosubtypable were 10 strains: [seven: B:1:P1.14, one: B 4:P1.2,5 one B:2a:P1.5, one B:15:P1.15], Partially serosubtypable were five strains: [three, B 1:NT, and one respectively B:4:NT, and B:NT:P1.14] and B non-serosubtypable two strains. The outcome of invasive disease was not death whatever the serogroup involved. The most prevalent strain B:1:P1.14 was not involved in complex diseases in either case. The C serogroup was isolated from a male 39 y-old and B:2a: P1.5 from another male 33 y-old. The disease was severe in both cases with complications as pericardial and pleural fluid, internal malleolus abscess in C serogroup strain and arthritis in B serogroup strain. The only strain B: 15: P1.15 was involved in the development of a more severe disease with multiple organ failure. The two strains caused by B non-serosubtypable also developed complications such as arthritis and leg edema. The patients were an adult and a child respectively.
No strain was resistant to penicillin, while 11 of them showed intermediate sensitivity: 4 strains B:1:P1.14, the three strains B 1:NT, the two strains B non serosubtypable, B:15:P1.15 and the B 4:P1.2,5 strain. All of isolates were susceptible to cefotaxime, rifampin and ciprofloxacin.
Conclusions: The most prevalent meningococcal subtypes in our hospital is B:1:P1.14. The disease is not severe. The isolation of B:2a:P1.5 and C:2a:P1.5 strains from sporadic cases coincide with meningococcal C vaccination as in other regions and suggest that strong immunological pressure might be selecting these types of strains that evade the immune response produced by the vaccine. These strains behave more aggressive than other serotypes.

P1365 Factors influencing the length of hospitalization of elderly patients with cellulitis

D. Kofteridis, A. Valachis, D. Dimopoulou, M. Christofaki, S. Maraki, L. Sifaki, G. Samonis (Heraklion, GR)*

Objectives: Cellulitis in the elderly can be a serious disease with prolonged hospitalization and considerable cost. The aim of the study was to determine factors significantly associated with the length of hospitalization of elderly patients with cellulitis.
Patients and Methods: All records of patients aged >65 years, admitted to the department of Medicine of the University Hospital of Heraklion, Greece, diagnosed with cellulitis from January 2002 through July 2009 were retrospectively reviewed. Diagnosis was defined using established criteria. Patients with more than one episodes of cellulitis were considered to have recurrent disease. Patients were classified into 2 groups according to the length of hospital stay: those discharged in <11 days and those hospitalized for >11 days.
Results: A total of 107 episodes of cellulitis occurring in 102 patients have been evaluated. Fifty seven (56%) were women. Median age was 77 years (range: 65–102). The most common comorbidities were diabetes mellitus (45 patients; 44%), heart failure (29; 28%), chronic obstructive pulmonary disease (17; 17%), renal failure (12; 12%), obesity (9; 9%) and cancer (8; 8%). Portals of entry were known in 47 episodes (44%), with trauma being the most frequent (18 episodes; 38%), followed by chronic ulcer in (9; 19%). The predominant local predisposing factors were venous insufficiency (16; 15%), and presence of oedema (15; 14%). The most commonly infected site was the lower extremities (96 episodes; 90%), followed by the trunk (6; 6%), the face (4; 3%), and the upper extremities (1; 1%). Blood cultures were positive in 8 episodes (7.5%). The causative organisms were *Streptococcus viridans* in 2, *Streptococcus* group G in 2, *Streptococcus* group A in 1, *Staphylococcus epidermidis* in 2, and *Staphylococcus saprophyticus* in 1. The median length of hospitalization was 6 days, with a range from 2 to 60 days. Eighty-six patients (80.4%) were hospitalized for <11 days and 21 (19.6%) for more. Outcome was favourable in all cases. Logistic regression analysis showed that venous insufficiency ($P = 0.002$) and duration of symptoms for >3 days prior to admission ($P = 0.043$) were independently related to prolonged hospitalization.
Conclusions: The present study showed that venous insufficiency and duration of symptoms for >3 days prior to admission are independently associated with prolonged hospitalization in elderly patients with cellulitis.

P1366 Infectious bursitis: short-course adjuvant antibiotic therapy is not a risk factor for recurrence in adult hospitalized patients

A. Huttner, I. Uçkay, D. Lew (Geneva, CH)*

Objectives: No evidence-based recommendations exist for the management of infectious bursitis. We examined epidemiology and risk factors for recurrence of septic bursitis. Specifically, we compared outcome in patients receiving bursectomy plus short-course adjuvant antibiotic therapy (⩽7 days) to that of patients receiving bursectomy plus longer-course antibiotic therapy (>7 days).
Methods: Retrospective study of adult patients with infectious olecranon and patellar bursitis requiring hospitalization at Geneva University Hospital from January 1996 to March 2009.
Results: We identified 343 episodes of infectious bursitis (237 olecranon, 106 patellar). *Staphylococcus aureus* predominated among the 256 cases with an identifiable pathogen (85%). 312 cases (91%) were treated surgically, 142 (46%) with bursectomy and closure in one-stage, 146 in two-stage. All received antibiotics for a median duration of 13 days with a median intravenous component of 3 days. Cure was achieved in 293 (85%) episodes. Total duration of antibiotic therapy (OR 0.9, 0.8–1.1) showed no association with cure. In multivariate analysis, only immunosuppression was linked to recurrence (odds ratio 5.6, 95% CI 1.9–18.4). Eight to 14 days (OR 0.6, 0.1–2.9) or >14 days of antibiotic treatment (OR 0.9, 0.1–10.7) were equivalent in outcome to only ⩽7 days, while the duration of intravenous therapy also had no bearing on recurrence outcome (OR 1.1, 1.0–1.3).
Conclusions: In severe infectious bursitis requiring hospitalization, adjuvant antibiotic therapy may be limited to seven days in non-immunosuppressed patients.

P1367 Disease spectrum and diagnostic usefulness of needle aspiration biopsy in cervical lymphadenopathy

Y.J. Kim, K.W. Hong, S.I. Kim, S.H. Wie, Y.R. Kim, M.W. Kang (Seoul, KR)*

Objective: To evaluate the disease spectrum in patients with cervical lymphadenopathy and diagnostic utility of fine needle aspiration biopsy (FNAB) in various benign, tuberculosis (TB) and malignant lymphadenopathy.
Methods: From January 2003 up to December 2008, we reviewed retrospectively medical records of 447 adult patients with cervical lymphadenopathy in Seoul St. Mary's Hospital of Korea. When histologic diagnosis through FNAB was relevant to the histologic findings via excision biopsy and clinical course, it was regarded as "accurate".
Results: Three hundred five (82 male, 223 female) patients were performed FNAB among a total of 447 patients with cervical lymphadenopathy. The mean age was 36.7±14.5 years. Among the 305 FNABs, adequate material was obtained in 289 cases (96.4%). The histologic diagnoses were non-specific lymphadenitis in 161 (55.7%) patients, TB in 63 (21.8%) patients, Kikuchi's disease in 29 patients (10%) and malignancy in 11 cases (3.8%). It was accurate at 98% of non-specific lymphadenitis and Kikuchi's disease, 63% of TB lymphadenitis, and 90% of malignancy. Previous TB evidence (past history of TB or old TB scar on chest X-ray) was the significant predictors as an inaccurate diagnosis of FNAB in patients with TB lymphadenopathy ($p = 0.001$). There were no FNAB-related complications.
Conclusion: The disease spectrum of patients with cervical lymphadenopathy in Korean patients showed somewhat different from that of European, African, and American patients. Tuberculosis is still important cause of cervical lymphadeopathy in Korea. FNAB is a useful procedure for diagnosis of cervical lymphadenopathy. However in patients with previous TB evidence, diagnostic usefulness of FNAB has to be reassessed and early excisional procedure was considered to perform.

P1368 Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in children in a hospital, Kampala, Uganda

S. Samuel, L. Robert, N. Betty (Masaka, Kampala, Rakai, UG)*

Objectives: To evaluate the efficacy of fine needle aspiration biopsy (FNAB) in diagnosis of lymphadenopathy in children and determining the common causes of lymphadenopathy in children.
Methodology: A comparative cross sectional study was done with 94 children 12 years and bellow. FNAB and excision biopsy were done on one lymph mode from each patient and the histopathological and cytopathological specimen were examined by pathologists, sensitivity, specificity, PPV and NPV were calculated as well as the inter-rater agreement by the Kappa co-efficient.
Results: A high proportion of children were diagnosed with lymphadenopathic kaposi's sarcoma (14.6%), though majority revealed non-specific lymphadenitis (60%). Tuberculosis was also an important cause of lymphadenopathy (15.7%). Sensitivities were low (0 to 34.1%), but specificities were high (85.3 to 97.7%). The highest values being for diagnosis e.g "non-specific lymphadenitis". Similarly PPV was low (0–94.9%), where as NPV was high (86.0–97.6%). The kappa co-efficient was significant only for "non-specific lymphadenitis".
Conclusion: Kaposi's sarcoma is proving to be an increasingly important cause of lymphademopathy in children. FNAB proved to be only useful in diagnosing non-specific lymphadenitis.

P1369 RIFLE classification as predictive factor of 7-day mortality in severe sepsis patients

D.W. Park, B.C. Chun, J.W. Sohn, H.J. Cheong, J.Y. Choi, H.J. Choi, Y.H. Choi, H.Y. Kim, J.S. Eom, S.I. Kim, Y.G. Song, K.R. Peck, Y.S. Kim, J.M. Kim, M.J. Kim (Ansan, Seoul, Suwon, Wonju, KR)*

Objectives: Severe sepsis and septic shock, often complicated by acute kidney injury (AKI), are the most common causes of mortality in intensive care units (ICU). RIFLE, a newly developed international consensus classification for AKI, defines three grades of severity – risk (class R), injury (class I) and failure (class F). This study investigates the outcomes of patients with severe sepsis and elucidates the association between prognosis and RIFLE classification.
Methods: We performed retrospective analysis of prospectively collected data from the Korean Sepsis Registry System (KSRS). Community acquired severe sepsis patients were registered in KSRS from May 2005 to December 2008 by 12 teaching hospitals in Korea. Demographic, clinical, laboratory data were recorded for analysis as predictors of mortality and infectious characteristics of severe sepsis patients.
Results: A total of 1192 severe sepsis patients were enrolled in KSRS. 7-day mortality rate was 12.4% (147/1183). Acute kidney injury occurred in 62.5% (736/1178), with maximum RIFLE class R, class I and class F in 18.8%, 20.1% and 23.6%, respectively. 7-day mortality was significantly associated with male, underlying cancer, primary bloodstream infection, number of organ failure, Charlson's score, Acute Physiologic and Chronic Health Evaluation II (APACHE II) score, sequential organ failure assessment (SOFA) score and RIFLE classification ($p < 0.05$). The severity of RIFLE classification correlated with number of organ failure, Charlson's score, APACHE II score, SOFA score, and 7-day mortality. The area under receiver-operator characteristic curve (AUROC) analysis verified SOFA score, APACHE II score and RIFLE classification had significant discriminatory power in comparison of prognostic scoring systems, with AUROC of 0.75, 0.73 and 0.61, respectively.
Conclusion: RIFLE classification was significantly associated with 7-day mortality but it is less useful than other scores.

P1370 **Spectrum of pathogens causing acute rhinosinusitis in young adults and their antimicrobial susceptibility in prospective study using invasive technique**

R. Kozlov, I. Otwagin, A. Kolosov (Smolensk, RU)*

Objectives: Majority of published reports on etiology of acute rhinosinusitis use culture of nasal lavage samples and/or biopsy specimens of anterior ethmoidal mucosa. Historically, sinus aspirate was considered to be a 'gold standard' for microbiological diagnosis, though currently it is rarely used in many countries. The aim of our study was to determine the spectrum of pathogens causing rhinosinusitis and their susceptibility to the most commonly used antimicrobials.
Materials and Methods: Sinus aspirates were obtained from 112 patients with acute rhinosinusitis aged 19.4 + 1.4 years and transported within 2 hours to the single microbiological laboratory. Cultures were performed both in aerobic with increased CO2 concentration and anaerobic conditions, Susceptibility was determined used broth microdilution technique. Interpretation was performed using CLSI criteria (2009) were used for determination of susceptibility.
Results: Positive cultures were obtained in 62 (55.4%) of cases. Single pathogen was isolated in 90.3% of cases. The most common bacteria was *H. influenzae* (51.6%), followed by *S. pneumoniae* (21.0%) and *S. pyogenes* (11.3%). Susceptibility of *H. influenzae* to amoxicillin, amoxicillin/clavulanate and levofloxacin was 94%, 100% and 100%, respectively. *S. pneumoniae* was susceptible to penicillin, erythromycin and levofloxacin in 92%, 100% and 100% of cases.
Conclusion: In our study, *H. influenzae* was found to be the most prevalent pathogen, followed by *S. pneumoniae* and *S. pyogenes* with no *M. catarrhalis* isolated. In general, all predominant pathogens retain high percentage of susceptibility to the most commonly used antimicrobials in acute rhinosinusitis.

Current emerging infections in Europe

P1371 **The goat connection – a retrospective cohort study investigating the link between an identified veterinary source of Q fever and subsequent outbreak in humans**

V.H. Hackert, H.L. ter Waarbeek, N.H. Dukers-Muijrers, R. Boesten, E. Leclercq, C.J. Hoebe (Geleen, NL)*

Once a sporadic disease, Q fever has turned into an epidemic in the Netherlands since 2007, possibly linked to intensive dairy goat farming. Lack of firm evidence to support this assumption has impeded effective control measures. We are first to investigate a veterinary point source of Q fever in relation to subsequent human cases, in a region hitherto virtually Q-fever free, presenting the first out of several studies to deliver evidence for a causal link and assess routes of primary and secondary transmission and geographical spread of Q fever.
We received veterinary notification of a dairy-goat farm outbreak in March 2009. Based on contact tracing, we performed a retrospective cohort study. Primary cases were farm contacts seropositive for phase II IgM to *C. burnetii*. Secondary cases were household members of primary cases unexposed to the affected farm and seropositive with self-reported symptoms of Q fever. All contacts had a questionnaire on risk factors and transmission. We calculated attack rates (AR) and associations between outcome and risk determinants. Cases with no link to the farm, living in its wider surroundings, were notified to us by physicians in subsequent months.
Preliminary data show an AR of 100% in farm workers (25/25; 3 proved immune; response 68%(28/36)). AR in farm visitors, including two infected public health officials who had visited the farm on one short occasion, was 67% (20/30; response 94% (32/34)). High AR are congruent with high concentration of *Coxiella* found in environmental farm samples at 1 km. Primary contacts had mild symptoms, not requiring hospital admission. 8 of 17 secondary contacts were positive. An additional 206 cases were notified from neighbourhoods up to 12 km from the farm in the months following. Mean age in this group was significantly higher (49 years) compared to farm contacts (31 years), and so were admission rates and symptom severity. Most cases in this group occurred in the 3 months following veterinary notification, when removal of manure from the affected premises was banned.
Contact tracing and active case finding in farm workers and visitors at the moment of a veterinary outbreak of Q fever yielded an unexpectedly high attack rate. Even short-term visits seem to carry high risk of infection. Secondary transmission through contaminated fomites was frequent. Aerogenic spread seems the primary route of transmission in cases with no link to the farm. Disease seems more severe in non-farm cases.

P1372 **The role of *Clostridium difficile* culture in colo-rectal surgery**

S.N. Reddy, G. Fewster, B.J. Mander, R.G. Wilson, I.R. Poxton (Edinburgh, UK)*

Objectives: *Clostridium difficile* Infection (CDI) has become a growing concern world-wide with an increased reported incidence among patients admitted to surgery. Our aim was to review prospectively the number of colorectal surgical in-patients who had a delayed diagnosis or were undiagnosed using current testing practices from December 2007 to January 2009 inclusive.
Methods: All faecal samples, submitted to Lothian University Hospitals Division, were processed in a single enteric laboratory following national guidelines – all hospital diarrhoeal in-patient samples from those aged 1year and above were tested for *C. difficile* toxins A and B by enzyme immunoassay(EIA). The faecal samples submitted to the laboratory from colorectal surgical symptomatic in-patients were reclaimed for toxigenic culture (culture on selective media and EIA).
Results: 632 samples, from 483 patients (median age 71 years, ranging from 18–100 years) were reclaimed for toxigenic culture. Of these 105 samples (16.6%) were found by the laboratory to be positive by EIA. Following toxigenic culture a further 72 samples (11.4%) were identified as positive. 38 patients, who were symptomatic at the time of testing, were therefore not identified with CDI during their admission. The diagnosis of a further 17 patients was delayed by a median period of 6 days (range 1–19 days) prior to *C. difficile* toxin detection, from the time of their first negative sample being assessed to the ultimate positive sample, with a median of 3 (range 2–5) samples sent prior to detection. These patients were found to be toxigenic culture positive on their first sample. 32% of the patients whose diagnosis was delayed or not confirmed had undergone major colorectal surgery. Seven samples found to be toxin positive by the lab were culture negative.
Conclusion: CDI diagnosis or recognition at present may be delayed, as with current national guidelines CDI detection is based solely upon *C. difficile* toxins A+B EIA. Current resources cannot support toxigenic culture for all suspected faecal samples, however this therefore has implications in regards to delayed treatment, further patient management and infection control procedures. This cohort of patients is at high-risk for CDI and a provision should be available for toxigenic culture if a high index of clinical suspicion persists via multidisciplinary discussions between the clinical team and microbiology, whilst taking into account toxin positive-culture negative samples.

P1373 ***Staphylococcus simulans*** **as an authentic pathogenic agent of osteoarticular infections**

M. Mallet, X. Lemaire, C. Loiez, H. Melliez, Y. Yazdanpanah, E. Senneville (Tourcoing, Lille, FR)*

Objectives: To evaluate the role of *Staphylococcus simulans* (Ss) in osteoarticular infections (OAIs) and determine their main characteristics.
Methods: A search in the database of the microbiology laboratory of Lille's regional university hospital and Tourcoing's hospital was performed. Only results from blood, bone, orthopedic devices cultures were taken in account for hospitalized patients between January 2004 and January 2009. We considered cases in which Ss was the only bacteria isolated in all of the patient's biological samples with clinical and

biological signs of infection. For patients with complete medical records, we recorded their age, sex, and if they lived in a rural environment, the localization of the infection, the presence of orthopedic devices, antibiotic susceptibility, the antibiotic therapy and finally the clinical outcome.

Results: 7 cases of OAIs due to Ss were recorded, with 6 cases related to orthopedic devices infections. The median age was 48 years old and 3 patients lived in rural areas. All strains were susceptible to methicillin. Orthopedic device infections were localized in each case on the lower limbs and occurred less than one year after the initial procedure in 4 cases (3 and 20 years afterwards for the other cases). In 4 cases the initial pathology was traumatic (with an initially open wound for one patient in whom the infection occurred 3 years after). In 5 out of 6 patients, Ss was isolated in per operative samples exclusively, and in one patient only blood cultures were positive. For the last patient who was a farmer, Ss was isolated in blood cultures. This patient had a spondylodiscitis, and chronic foot ulcers due to gout disease were suspected to be the origin of the infection. All patients were healed after a mean follow-up of 12±3 months. Orthopedic devices were removes in 5 of the 6 patients concerned. The combination of rifampicin plus levofloxacin was used in 5 patients for duration of 3 months.

Conclusion: The present data suggests that even though Ss remains rarely observed in clinical pathology, its role in OAIs, especially in case of infected orthopedic device, is not exceptional. In our group of patient, almost half had a contact with a rural environment. As for the antibiotic treatment, the combination of rifampicin and levofloxacin seems to be an effective strategy, regarding our clinical results.

P1374 Surprising change in diagnosis and treatment of focal liver lesion

L. van Dommelen, J.H. Stoot, V.C. Cappendijk, M.A. Abdul Hamid, F.F. Stelma, L.M. Kortbeek, A.M. Oude Lashof (Maastricht, Bilthoven, NL)*

Case report: A 55-year-old female, with a history of multiple myxoid liposarcomas, presented at our hospital with cervical pain. Metastases in the cervical spinal column were suspected. MRI showed no abnormalities of the spinal column and FDG-PET-scan was negative. However, the diagnostic abdominal CT-scan showed seven hypodens lesions in the liver, suspect for metastases, not present 6 months earlier. Her liver enzymes were normal. After two chemotherapy cycles, partial hepatectomy was performed, leaving four lesions in situ.

Pathologic examination of the liver revealed three circumscribed nodules with central necrosis, a peripheral wall of histiocytes and containing foreign material, resembling *Echinococcus* structures. *Echinococcus* granulosis serology was borderline positive and therefore isolated DNA from unpreserved resected liver material was used for a nucleic acid amplification test and found positive for *E. multilocularis* (Em) using two targets (CO1 and NADH) and sequencing. Genotyping is in progress. After this diagnosis, our patient started with albendazole 400 mg twice daily.

Patient's travel history was only significant for three short holidays to Switzerland, Italy and Austria in 2006 and 2007. She was not extensively exposed to forest environment, did not consume forest fruits, had no contact with animals, did not work in the garden and bought her fruits and vegetables in regular supermarkets.

Discussion: This case displays two remarkable findings. Firstly, the PET-scan-negative, multiple, circumscript, hypodens lesions in the liver seen at regular CT in our patient are not classical for Em and represents a highly unlikely diagnosis in our region. Taking in account our patient's history, it is therefore reasonable to suspect metastases.

Secondly, our patient's history did not reveal behaviour associated with acquiring Em (like hunting and gardening) and only undertook short holidays to known endemic areas. Em however, is also prevalent in Dutch foxes and surveillance data have shown that Em is increasing its range by 2.7 kilometres per year in Northern direction. Considering duration of exposition, domestically acquired Em therefore seems most likely.

Conclusions: This patient probably represents the first case of *Echinococcus multilocularis* acquired in The Netherlands. Future research has to reveal whether Em is an emerging pathogen in Western Europe or whether we are facing isolated cases.

P1375 Emerging *Clostridium difficile* infections in neonatal piglets in the Netherlands

E.C. Keessen, L. Lipman, L.A. van Leengoed, D. Bakker, E.J. Kuijper (Utrecht, Leiden, NL)*

Objectives: *Clostridium difficile* infections (CDI) are emerging as a cause of neonatal diarrhoea in piglets. Isolates of piglet *C. difficile* type 078, which is considered the predominant ribotype in pigs, have a close genetic relatedness with human isolates of type 078. In humans with CD *C. difficile* type 078 is currently the third most common strain in the Netherlands and Europe. There are no surveillance on the prevalence of *C. difficile* in pigs in the Netherlands. The aim of this study was to investigate the presence and distribution of various *C. difficile* PCR ribotypes in piglets on farms with persistent problems with neonatal diarrhoea.

Methods: At eighteen pig breeding farms with persistent problems with neonatal diarrhoea, samples were taken from piglets from one to seven days old from at least three different litters. In total, 98 faecal samples were collected from piglets with diarrhoea and 87 faecal samples from piglets without diarrhoea.

All samples were cultured on *C. difficile* agar (bioMérieux) with ethanol shock pre-treatment and anaerobic incubated for 48 hours at 37ºC. Typical *C. difficile* colonies were inoculated on Schaedler agar (bioMérieux) and anaerobic incubated for 48 hours at 37ºC. Suspected colonies of *C. difficile* were transported to the Leiden University Medical Centre where additional identification tests and PCR ribotyping were performed The Pearson Chisquare test was used to asses the association between *C. difficile* isolation and presence of diarrhoea.

Results: *C. difficile* was isolated from 63 out of 98 samples of piglets with diarrhoea and from 31 of 87 samples of piglets without diarrhoea, leading to an odds ratio for diarrhoea when *C. difficile* is present of 3.3 (95% confidence interval: 1.8–5.9). Statistical analysis of the data showed a significant association of presence of *C. difficile* and diarrhoea ($P = 0.0001$). *C. difficile* was encountered at all farms investigated. At 16 farms all isolates belonged to type 078 whereas at 2 farms only type 045 was found. All isolates of type 078 and 045 contained genes for toxin A, toxin B and the binary toxins.

Conclusion: Of 18 pig farms with persistent problems of neonatal diarrhoea, *C. difficile* PCR ribotype 078 was present in 16 farms and type 045 was found in 2 farms. The results of this study indicate the importance of CDI in diarrhoeal piglets and emphasizes the need for further research to the association of *C. difficile* emerging disease in humans and piglets.

P1376 The use of a geographic information system to identify a goat dairy farm as the most likely source of an urban Q fever outbreak

B. Schimmer, T. Veenstra, R. ter Schegget, M. Wegdam-Blans, L. Züchner, A. de Bruin, P.M. Schneeberger, P. Vellema, W. van der Hoek (Bilthoven, Eindhoven, Helmond, The Hague, 's Hertogenbosch, Deventer, NL)*

Objectives: A Q fever outbreak occurred in an urban area in the south of the Netherlands in May 2008. Distribution of cases in time and place suggested a common source. We studied the spatial links between the residence locations of human cases and nearby small ruminant farms, of which one dairy goat farm had experienced abortions due to Q fever since mid April 2008. A generic geographic information system (GIS) was used to develop a method for source detection in the still evolving major epidemic of Q fever in the Netherlands.

Methods: All notified Q fever cases in the area were interviewed. Postal codes of cases and of small ruminant farms (size >40 animals)

located within 5 kilometres from the centre of the cluster area were geo-referenced as point locations in a GIS-model. For each farm, attack rates and relative risks were calculated for 5 concentric rings of 1 kilometre each, using the 5–10 kilometres zone as reference, to study the presence of a distance-response effect. These data were linked to the results of veterinary investigations.
Results: Persons living within 2 kilometres of an affected dairy goat farm (>400 animals) had a much higher risk for Q-fever than those living more than 5 kilometres away (Relative risk 31.1 [95% CI 16.4–59.1]). However, comparable attack rates and relative risks were found around two nearby sheep farms (around 50 animals each) that had experienced no clinical Q fever problems.
Conclusion: The study supported the assumption that a dairy goat farm was the source of the outbreak. GIS-based attack rate analysis is a promising tool for source detection in outbreaks of human Q fever.

P1377 Infection with *Clostridium difficile* in patients with ulcerative colitis

I. Chudzicka-Strugala, A. Szkaradkiewicz, R. Marciniak, P. Majewski, B. Zwozdziak, T.M. Karpinski (Poznañ, PL)*

Objectives: Numerical and qualitative disturbances in intestinal microflora are known to be associated with emergence of *Clostridium difficile* in the alimentary tract. Pathogenicity of the important enteropathogen is determined by two protein toxins: enterotoxin A (encoded by tcdA) and cytotoxin B (encoded by tcdB), which mediate diarrhoea and colitis (*C. difficile*-associated disease – CDAD). Taking the above into account present study aimed at analysis of manifestation of *Clostridium difficile* infection in active ulcerative colitis.
Methods: The investigated group included 20 patients (10 men and 10 women, age range 21–65 years) with active ulcerative colitis (UC), hospitalized at the Department of General, Gastroenterological and Endocrinological Surgery, University of Medical Sciences in Poznañ, Poland. The diagnosis was based on clinical, radiological, endoscopic and histological criteria. The standard surgical treatment was applied in all the patients. Due to the signs/symptoms of acute abdomen, three of the above mentioned patients were urgently subjected to colectomy. The diagnostic material included intra-operative sections of large intestines. DNA of *Clostridium difficile* was isolated from the intestinal samples using QIAamp DNA Mini Kits (Qiagen). For amplification of tcdB, hyplex ClosTox PCR Module was applied (BAG Healthcare). PCR product was detected using hybridization with the Multiplex-PCR-ELISA-System kits (hyplex ClosTox; BAG Healthcare) and the absorbance was read out at 450nm (Reader 250, bioMérieux). Values >0.3 were evaluated as positive.
Results: In 4 patients presence of tcdB *C. difficile* was detected in intestinal samples and, in parallel, histological examination demonstrated a vast inflammatory infiltrate which included muscularis layer. In the remaining 16 patients no tcdB *C. difficile* could be detected in intestinal samples and histologically the inflammatory infiltrate was restricted to mucosa of the large intestine.
Conclusion: The obtained results indicate that development of active ulcerative colitis may be accompanied by infection with *C. difficile*. Therefore, cases of UC should be monitored for presence of toxin-forming strains of *C. difficile* as well as novel diagnostic/therapeutic strategies should be worked out for the morbid unit.

P1378 MLVA patterns and antibiotic resistance of emerging *Clostridium difficile* PCR ribotype 078; an indication for zoonotic transmission?

M. Hensgens, E.C. Keessen, D. Bakker, L. Lipman, E.J. Kuijper (Leiden, Utrecht, NL)*

Objectives: In both veterinary and human medicine, *Clostridium difficile* infections (CDI) are emerging. The hypervirulent *C. difficile* strain 027 was identified as the major cause of nosocomially acquired outbreaks in humans. Type 078, however, is the most commonly detected strain in pigs. As type 078 is currently also the third most common strain in humans in the Netherlands, a common source for both human and animal CDI is suspected. Genetical relatedness and overlapping antibiotic susceptibility patterns of human and animal strains may indicate a high degree of similarity, and prompted us to study this for type 078 infections in the Netherlands.
Methods: Fifty human *C. difficile* type 078 isolates were selected at random from all samples submitted to the Dutch national reference laboratory between June 2006 and May 2009. From May 2009 fifty *C. difficile* type 078 isolates were collected from piglets with neonatal diarrhoea. To diminish selection bias due to one predominant epidemic strain, a maximum of two isolates per farm were included. In total, antibiotic susceptibility patterns of 100 isolates with PCR ribotype 078 were determined using the E-test method. A suspension of *C. difficile* was cultured on non selective blood agar plates in an anaerobic environment at 37ºC. Minimal inhibitory concentrations (MICs) for tetracycline, amoxicillin, co-trimoxazol, erythromycin, clindamycin, moxifloxacin, cefuroxim, imipemen and ciprofloxacin were determined after an incubation of 48 hours. For each antibiotic analysed, MICs were compared between the two groups. Genetic resemblance of type 078 isolates was determined by using multi-locus variable-number tandem repeat analysis (MLVA).
Results: As results are expected towards the end of December 2009, no preliminary results can be presented in this abstract.
Conclusion: Previous studies found similar antibiotic susceptibilities in type 078 from pigs and humans, however, very limited numbers of strains were investigated. This study will give an systematic, broad, view on antibiotic susceptibility patterns in CDI due to type 078. Furthermore, the degree of resemblance will provide insight in the (possible) common source of human and porcine CDI.

P1379 Epidemiological survey of hantavirus pulmonary syndrome in the north-eastern region of Rio Grande do Sul, Brazil

L. Michelim, J. Fracasso, P. Garrido, V. Buffon, A. Rocha, E. Kieling (Caxias do Sul, BR)*

Objectives: Hantavirus pulmonary syndrome (HPS), commonly referred to as hantavirus disease, is a febrile illness characterized by bilateral interstitial pulmonary infiltrates and respiratory compromise usually requiring supplemental oxygen and clinically resembling acute respiratory disease syndrome (ARDS). Humans can contract the disease when they come into contact with infected rodents or their urine and droppings. The aim of this study is to show an epidemiological survey of cases of HPS from 2002 to 2008 in the northeast region of Rio Grande do Sul – Brazil.
Methods: Retrospective review of all notified and confirmed cases of hantavirus pulmonary syndrome in the northeast region of Rio Grande do Sul/Brazil between January 2002 and December 2008. Cases were identified through the Local Health Epidemiology Service surveillance database and were supplemented by chart review.
Results: A total of 74 cases were identified during the study period of which 32 (43.3%) patients laboratory confirmed viral disease, 42 (56.7%) had clinical and epidemiological criteria, and 13 (17.5%) progressed to death. Incidence varied substantially by sex and race: 49 (66.2%) cases were male and 25 (33.8%) women; 53 (71.6%) were caucasian, 12 (16.2%) brown, 8 (10.8%) black and 1 (1.4%) yellow. The mean age of confirmed case patients is 27 years (range: 16 to 63 years). Potential occupational exposures have included grain farmers and agricultural, mill, construction workers. Many of these individuals had concurrent peridomestic exposures.
Conclusions: Based on these data, we can conclude that the hantavirus is an emerging disease of epidemiological importance in our region, requiring preventive measures to alert the population, control of rodents in rural areas and a higher degree of suspicion by physicians.

P1380 Colonization or infection? A case report of *Nocardia* isolation in a patient with cystic fibrosis

E. Fiscarelli, G. Ricciotti, C. Concato, L. Coltella, V. Lucidi, F. Callea (Rome, IT)*

Objectives: *Nocardia* spp. are Gram-positive, aerobic saprophytic bacteria with worldwide distribution. Infection by *Nocardia* spp. is relatively uncommon and usually manifests as primary pulmonary disease, acquired through inhalation of dust particles. Pulmonary diseases that compromise local airway defences, including chronic obstructive airway disease, bronchiectasis, pulmonary fibrosis, asthma, emphysema, and alveolar proteinosis are risk factors for developing nocardiosis. Surprisingly, the isolation of *Nocardia* spp. from the respiratory tract of cystic fibrosis (CF) patients is a very unusual finding. We describe clinical and microbiological features of our CF patient who presented *Nocardia* in her respiratory secretions with the aim of evaluating the clinical impact.
Methods: A 34 year old CF Caucasian woman was admitted to our Hospital with severe cough, increased sputum production and left thoracic pleuritic pain. WBC (11.7×10^9/L) and CRP (40.6 mg/L) were elevated. Chest CT showed nodular opacities in the left lower lobe and dense consolidation in the right one in addition to diffuse bronchiectasis. A sputum specimen was smear-and culture-positive for organism resembling *Nocardia* spp. *Staphylococcus aureus* was also isolated.
Results: The organism isolated from sputum was identified as *Nocardia asteroides*/cyriacigeorgica by 16S rDNA gene sequencing; a full gene sequencing of 16S rDNA was necessary to obtain an univocal identification. Antimicrobial susceptibility testing was performed by E-test against amikacin (2 mcg/mL), ceftazidime (⩾256 mcg/mL), ceftriaxone (0.5 mcg/mL), meropenem (4 mcg/mL), imipenem (2 mcg/mL), tobramycin (0.75 mcg/mL) and TMP/SMX (0.12 mcg/mL). The patient received amikacin and meropenem i.v. for a 4 week period; such treatment resulted in improvement of the clinical picture. Repeated sputum cultures performed in the following months (the last one 1 month ago) were again smear-and culture-positive for the same microorganism. No specific antinocardial therapy was started, due to the absence of broncho-pulmonary symptoms.
Conclusion: Colonization or infection? In the case described, we cannot attribute a specific pathogenetic role to a single microbial species. The presence of *Nocardia* from the respiratory tract of the CF patient does not necessarily imply the disease. We could suppose that *Staphylococcus aureus* was the cause of the symptoms and that it responded to the antimicrobial therapy.

P1381 Detection of a KPC-2 carbapenemase producing *Serratia marcescens* isolate in a pleural fluid sample: a case report

M. Kimouli, I. Petraki, I. Daniil, E. Voulgari, N. Rekliti, D. Petropoulou, A. Tsakris (Athens, GR)*

Objectives: KPC carbapenemases are among the most common β-lactamases mediating carbapenem resistance in Enterobacteriaceae. They have been widely spread among *Klebsiella pneumoniae* isolates in United States, South America, Israel and Greece. This is the first report of a KPC-2 carbapenemase producing *Serratia marcescens* in Europe.
Methods: A 62-year man was admitted to the cardiothoracic ward due to a significant right side pleural effusion. Over a two month period prior to his admittance he reported fatigue, fever and weight loss. A pleural needle biopsy was preformed and fluid was collected for cytological analysis and cultures. MICs of the clinical relevant isolate were determined by E-test. Phenotypic assays for carbapenemase production included the modified Hodge test, the combined EDTA test and the boronic acid potentiation disk tests. PCR and sequencing assays were used to identify β-lactamases (blaKPC, blaVIM, blaSME, blaSHV, blaCTX-M, blaTEM genes).
Results: On physical examination, the patient was afebrile, had a pulse of 75/min, blood oxygen saturation 95% and presented reduced right side respiratory sounds. Cytological examination of the turbid pleural fluid revealed the presence of numerous inflammatory cells, mainly lymphocytes. No malignant cells where detected. CBC showed a WBC count of 10,180/μl and a PLT count of 586,000/μl. Blood chemistry showed ALT 118 IU/L, ALP 484 IU/L, gGT 331 IU/L and CRP 121.2 mg/L. A Chest CT scan revealed the presence of a unilateral encysted hydropneumothorax and a notable increase in width of the pleura. Sputum samples were negative for acid-fast bacilli. A pleural fluid sample was processed with standard bacteriological methods and the BacTAlert 3D. Cultures yielded a Gram-negative rod, which was identified as *S. marcescens* by Vitek 2 automated system and API 20E. The isolate exhibited imipenem MIC ⩾16 mg/L, meropenem MIC 8 mg/L and ertapenem MIC ⩾8 mg/L and was phenotypically positive for KPC production. PCR assays and DNA sequencing identified the presence of blaKPC-2 and blaTEM-1 genes.
Conclusion: KPC-producing organisms have emerged as serious problem worldwide. Apart from *K. pneumoniae* isolates, which typically harbour blaKPC genes, other genera of Enterobacteriaceae have also sporadically detected as KPC producers. Effective measures for their early identification and control should be adopted in order to prevent their potential dissemination.

P1382 Seroprevalence of leptospirosis in Mazandaran Province, Iran using indirect immunofluorescence assay, 2006–2007

*S.P. Ziapour *, B. Esfandiari, M. Assmar, M. Alipour Espeh-Kolaie, M.R. Yousefi, H. Shokri, G. Amirbozorgi, B. Amini Navai, Z. Farzaneh-Nejad (Amol, Tehran, Babol, IR)*

Objectives: Leptospirosis is a worldwide zoonotic infection with a much greater in tropical and temperate regions and has now been identified as one of the emerging infectious diseases. Because of leptospirosis is significantly high in temperate regions, rice field workers and livestock animals; it will be necessary to evaluate condition of leptospirosis in Mazandaran province, Iran.
Methods: In this survey, 127 serum samples of suspected human were collected in different parts of Mazandaran province from November 2006 to July 2007. Serums were tested by Indirect Immunoflurescence Assay (IFA) in Pasteur Institute of Iran, Amol Research Center, Department Of Parasitology.
Results: The results showed that prevalence of Leptospirosis disease in Mazandaran province is 25.98% using IFA methods. In this survey, 33 samples were positive which 84.85% of them were belonged to male and 15.15% to female. Rice field workers showed the most frequent occurrence of infection and showed significant variance with other occupations ($P < 0.01$). Among 107 samples that were collected from males and 20 from females, 26.16% and 25% samples were positive, respectively. There was no significant variance between genus and leptospirosis ($P > 0.05$). Living place (urban or rural) and contact with animals did show no significant variance with leptospirosis. The most positive cases were found in age range of 40–50 years.
Conclusion: Because in Mazandaran Province, men and women work together at rice field, also urban peoples still relate to agriculture, it is acceptable that genus and living place have not significant variance with prevalence of leptospirosis. In order to as certain that the effect of contact with animals has no significant variance with the prevalence of leptospirosis, it needs more investigations, direct sampling from animals or their urine and comparison of them to the results of this study. The IFA test is a potentially valuable tool for the diagnosis of leptospirosis.

P1383 Continue active transmission foci of VEEV activity in coastal areas of Chiapas, Mexico

F.J. Ramirez Aguilar, S. Vázquez Corzo, R. Navarro Lopez, G. Leal, A. Travassos Da Rosa, S. Weaver, J.G. Estrada Franco for the International Group of Emerging Infectious Diseases*

Venezuelan equine encephalitis (VEE) is considered an emerging arboviral disease and a human health threat throughout the Americas.

The objective was to demonstrate through serosurveys the widespread presence of VEE in humans and dogs in coastal areas of the Mexican Pacific in the State of Chiapas and the identification of a putative hotspot of VEEV.
Methods: A cross-sectional study was conducted in 2008 and 2009 in a southern coastal village of the State of Chiapas, Mexico. Fasting venous blood samples (humans and dogs) were collected without anticoagulant to obtain serum. Oral informed consent was obtained from all individuals taking part in the study. All procedures were approved by the institutional review boards at the Chiapas State University and at the University of Texas Medical Branch. The serum samples from 142 individuals and 40 dogs (<6 months old) were analyzed using HI and PRNT assays.
Results: Human seroprevalence was 82% (116/142). Gender analysis showed 64% (91/142) males and 36% (51/142) females with VEEV antibodies. Odds ratio risk analysis using the stata program did not show any statistically significant association with several independent variables at the 95% CI including occupation, age, and clinical status of the sampled population. By occupation the highest percentage in a seropositive group were housewifes with 45% (n = 142, 74 positives), followed by students with 31% seropositives 44 seropositives (n = 142, 44 positives), agricultural workers with 18% (n = 26/142) and an undetermined group of 8 individuals that did not report any information about occupational status. By age most notably was the higher percentage of seropositive individuals in the group older than 60 years as seropositive (N = 26, 23 seropositives, 88% seropositivity). These rates suggest endemic transmission since VEEV PRNT values are known to last >25 years. Our dog seroprevalence was 37.5% 15/40 with high titers (7 dogs) HI and PRNT above ⩾1:620 suggesting recent infections. Our entomological studies and other vertebrate data reinforces our observations.
Conclusion: The results of our studies suggest a continuous active transmission foci of VEEV in coastal Chiapas. Sampling of the dog population suggests recent VEEV activity and even transmission within the household premises. We further discuss the complications arising from co-infections of other arboviral diseases circulating simultaneously in the same area (e.g. dengue and West Nile viruses) and the need to clearly conduct differential diagnosis in order to identify the disease burden of VEE and to distinguish this pathology from others.

P1384 Mosquito-borne diseases in Slovakia

N. Jalili, V. Krcmery, E. Kalavsky, F. Ondriska, P. Babál (Bratislava, SK)*

Objectives: Malaria was endemic in Slovakia up to 1959. After the elimination of malaria, the study of mosquitoes in Slovakia once again gained new impetus following the demonstration of their role as vectors of arboviruses in Central Europe. Bardoš & Danielová (1959) isolated an entirely new mosquito-borne arbovirus called Tahyna and later another arbovirus, Calovo was also discovered. West Nile virus was isolated from mosquitoes in 1974 and there is evidence for the activity of another mosquito-borne virus, Sindbis, isolated from birds, sentinel animals and frogs. Dirofilariosis of dogs, the causative agent of which is transmitted by mosquitoes, represents a serious problem for dogs and cats especially in several tropical and subtropical countries including Southern Europe. In connection to the global warming, recently this disease has been penetrated into Central Europe, Slovakia included.
Methods: Malaria was diagnosed in all suspected cases by microscopic examination of tick and thin blood smears, stained by Giemsa. Cases of cutaneous dirofilariasis were diagnosed clinically, histologically and determination of parasite genom by PCR.
Results: Since the eradication of malaria in the former Czechoslovakia, 150 malaria cases were reported into Slovakia, of which 50 cases imported by foreigners. From the identified species 45% of all malaria cases were due to *Plasmodium falciparum*; 40% due to *P. vivax;* 10% due to *P. malariae* and *P. ovale* species; the rest 4% were cased due to mixed infections, mainly by *P. falciparum* and *P. vivax* and 1% by *Plasmodium* sp. Dirofilariosis of dogs, the causative agent of which is transmitted by mosquitoes, has been spread to Slovakia recently. Two confirmed human cases are reported as well.
Conclusion: Although the number of imported malaria into Slovakia is not as high as in western European countries, but the possibility of its resurgence in the country still remains. Recently, due to global warming a new mosquito born disease became endemic. With respect to the fact, that mosquitoes are vectors not only of human malaria causative agents, but transmitting many other arboviruses and for this reasons their permanent monitoring, especially of their breeding places, is of constant importance even because of ever changing areas of pathogens transmitted by mosquitoes.
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P1385 Detection of *Leptospira* by PCR and comparison of different methods for early diagnosis of leptospirosis

P. Chandrasiri, P. Wahala, R. Ramesh, K. Kulathunge (Colombo, LK)*

Leptospirosis is a zoonotic disease of worldwide distribution. The infection is caused by *Leptospira interrogens*. The disease is maintained in nature by chronic renal infection of carrier animals which excrete bacteria in urine. Leptospirosis is endemic in Sri Lanka. The incidence is rising dramatically over the last few years & the number of deaths due to leptospirosis was 204 in 2008.
Objectives: To introduce a PCR technique for the early diagnosis of Leptospirosis. To compare the PCR results with existing methods such as culture & serology.
Methodology: Two teaching hospitals in Western province were selected for the study. Blood was taken from the patients suspected of having Leptospirosis as a part of the normal investigation process. The blood was collected for PCR, culture & serology prior to antibiotic therapy. The blood was inoculated into EMJH media at the bedside & incubated at room temperature. The genus specific microagglutination test (MAT) was performed using Leptospira Patoc strain. ELISA test was performed to detect IgM antibodies. PCR was done using G1/G2 primers.
Results: A total of 59 samples were tested. Up to 05 cells could be detected in the 50ul of PCR reaction mixture. Of the 59 samples 08 were posive by PCR test whereas only 06 were positive for culture. Seven samples gave diagnostic cut off titre of 1/800 with the MAT test & 17 gave positive by ELISA test. Out of the 08 PCR positive specimens only 1 gave positive with the MAT test whereas out of the 07 MAT positives only 01 was posive for PCR. Out of 08 PCR positives only 02 were positive for IgM whereas out of 17 ELISA positives only 2 were positive with PCR.
Conclusion: IgM Elisa is the most sensitive method to diagnose Leptospirosis in the early stage of the disease. MAT test is useful if the patient is presented later to the hospital. Specificity of the PCR method is 92.2% when compared to the gold standard culture method, PCR is more sensitive and rapid method than culture for the definitive diagnosis of Leptospirosis & is a valuable & reliable test to diagnose Leptospirosis in the initial stage of the disease.

P1386 Detection of human bocavirus in adenotonsillar tissue specimens by polymerase chain reaction

B. Gultekin, M. Eyigor, H. Eyigor, N. Aydin (Aydin, TR)*

Purpose: The human bocavirus (HBoV) is a recently identified parvovirus detected in respiratory secretions of children who had an infection of the lower respiratory tract. Several studies have identified HBoV in respiratory specimens from children with acute respiratory disease, but the full spectrum of clinical disease and the epidemiology of HBoV infection remain unclear. In this study, our objective was to detect whether adenoid and/or tonsillar tissue of patients diagnosed with chronic adenotonsilitis was a reservoir for HBoV.
Methods: This study was performed with 47 patients with the diagnosis of chronic tonsillitis and adenoid hypertrophy. The criteria for tonsillectomy was to have five or more tonsillitis attacks in a year, while the criteria for adenoidectomy was the 2/3 blockage of choana on endoscopy which causes obstruction symptoms. After the surgery

DNA extraction was performed with NucleoSpin Tissue (Macherey-Nagel, Germany) extraction kit from adenoid or tonsil tissues. Human bocavirus was searched by polymerase chain reaction (PCR). The primers were 188F (5′-GACCTCTGTAAGTACTATTAC-3′) and 542R (5′-CTCTGTGTTGACTGAATACAG-3′). The expected product size was 354 bp. All PCR products were sequenced to confirm that they were specific for HBoV.

Results: Eight (17%) patients underwent adenotonsillectomy, 12 (25.5%) patients underwent tonsillectomy, and 27 (57.5%) patients underwent adenoidectomy. Fifty-five (35 adenoid, 20 tonsil) tissue samples from 47 patients were included in the study. The average age of the patients varied from 4 to 42 (8±7.050). Bocavirus was detected in five (9%) specimens (one of them was tonsil and the others were adenoid specimens) from five children.

Conclusions: These findings indicate that the palatine tonsils can be colonized by HBoV. Further studies are needed to clarify the possible role of HBoV in upper aerodigestive tract diseases such adenotonsillitis.

P1387 Low prevalence of *Clostridium difficile* in retail meat in the Netherlands

J.T. Zwartkruis-Nahuis, C. Harmanus, E.J. Kuijper, E. de Boer (Zutphen, Leiden, NL)*

Objectives: Recent reports indicate that a large proportion of community-acquired *Clostridium difficile* infections (CDI)are not linked to recent antibiotic therapy, older age, significant comorbidity or previous hospitalization. The significance of contaminated food as source of *C. difficile* is unknown. Since data from US and Canada indicate that *C. difficile* contamination of food frequently occurs, we performed a surveillance study to the prevalence of *C. difficile* in meat.

Methods: A total of 500 samples of raw beef, pork, calf, lamb and chicken were collected from the retail trade in the period October 2008 to March 2009. The detection and isolation method used was based on the method described by Rodriguez-Palacios et al. (2007), using 5 g of meat added to 20 ml of selective *C. difficile* broth incubated for 10–15 days at 37ºC, under anaerobic conditions and shaking conditions. From the enriched broth, 2 ml was used for an alcohol shock treatment and streaked onto selective *C. difficile* moxolactam norfloxacin agar. Presumptive identification of suspect colonies was done by testing for agglutination using the *C. difficile* test kit (Oxoid DR1107A). Further identification was done by testing for the presence of tpi (topo isomerase), tcdA (toxin A) and tcdB (toxin B) genes using real-time PCR and by PCR ribotyping.

Results: The detection limit of the method applied for minced meat was in the range of 2 CFU per 5 g. In total, 500 raw meat samples were included which derived from beef (145), pork (63), calf (19), lamb (16), and chicken (257). Of 500 samples, only 8 (1.6%) were positive for the presence of *C. difficile*: 1 from lamb (6.3%) and 7 from chicken (2.7%). *C. difficile* from lamb was positive for TcdA, TcdB, binary toxin genes and belonged to PCR ribotype 060. Three isolates of chicken were negative for the presence of toxin genes and belonged to 3 different PCR ribotypes (071 and unknown types). The remaining four isolates belonged to PCR ribotype 003 (n = 2), 001 and 087; all contained TcdA and TcB, whereas binary toxin genes were absent.

Conclusion: In The Netherlands, retail meat is contaminated with *C. difficile* in a very low percentage. The strains which have been found in meat, are different from the types currently found in patients with hospital acquired CDI of community-acquired CDI.

P1388 Immune reconstitution syndrome associated with hip septic arthritis after successful treatment of visceral leishmaniasis: analysis of the immunomodulatory environment

F. Robert-Gangneux, Y. Poinsignon, V. Ciubotaru, C. Guiguen, J-P. Gangneux (Rennes, Vannes, FR)*

Objectives: Immune reconstitution syndrome (IRS) is well recognized during two main situations: AIDS and solid organ transplantation (SOT). After the advent of potent highly active antiretroviral therapy during AIDS or after reduction or withdrawal of iatrogenic immunosuppressive agents in SOT patients, concomitant infections were reported. Here, we observed that visceral leishmaniasis (VL) can also be an underlying condition for IRS and investigated the immunological environment during one episode.

Methods: A 75-year old patient living in Brittany, France, presented with febrile hepatosplenomegaly and pancytopenia. The only noteworthy background consisted in a right total hip arthroplasty for coxarthrosis 3 years before. However, the patient reported a pain of the right hip since his return of the South of France. The diagnosis of VL was established with a Giemsa stained bone marrow aspiration and a positive culture of promastigotes identified as *L. infantum.* After treatment with liposomal amphotericin B at a dose regimen of 3 mg/kg at days 1, 2, 3, 4, 5 and 10, the patient presented with septic shock in relation to an infection with *Staphylococcus aureus* of the right total hip arthroplasty. The echography revealed a 4×8 cm abcess of the cup. The treatment combined surgery (one-stage revision of the acetabular cup without femoral stem changing) and adapted antibiotherapy. A definitive cure of both VL and septic arthritis was assessed after a long term follow up of the patient, without relapse or sequela. In parallel, we performed blood dosages of IL-10 and IL-12 (by ELISA) and of soluble HLA-G, a type I MHC antigen previoulsly described as a powerful immunomodulator agent.

Results: The analysis of this case suggests that the septic hip infection was silent during the period of neutropenia in the active phase of VL. Dosages of cytokines showed that the patient had high serum levels of IL-10 and very low levels of IL-12 at the time of diagnosis, pointing to an inhibition of the Th1 immune pathway. Furthermore, particularly high levels of sHLA-G were detected, suggesting an immunomodulatory environment. The clinical expression was only observed after recovery of a normal count of polymorphonuclear neutrophils, and at a time point when the cytokinic balance was reversed, with a restoration of high IL-12 and low IL-10 levels.

Conclusion: We report for the first time an IRS-like syndrome following a successful VL treatment revealing a deep bacterial infection.

P1389 Importance of therapy compliance in patients with trichinellosis from Timis County, Romania

I. Marincu, L. Negrutiu, I. Iacobiciu, A. Neghina, R. Neghina (Timisoara, RO)*

Objectives: Numerous studies conducted on groups of patients with acute or chronic diseases highlight the role of therapy compliance in the evolution and prognosis of these diseases. Our study aimed to emphasize the importance of therapy compliance in a group of patients diagnosed with trichinellosis.

Methods: The authors have retroactively processed and analyzed data from the medical charts of 72 patients originating from an urban outbreak of trichinellosis broke out in 2006 in Timis County. Diagnosis was based on epidemiological aspects (the onset of infection following consumption of infected pork with *Trichinella spiralis*.), clinical features (fever, headache, chills, nausea, diarrhoea, facial/eyelid oedema, myalgia, urticaria) and laboratory tests (erythrocyte sedimentation rate, fibrinogen, anti-*Trichinella* IgM antibodies, leucocyte counts, eosinophil counts, serum protein electrophoresis, serum calcium, kalemia). Data of the epidemiological survey were collected from the Institute of Public Health. The statistical processing was done using Epi Info 3 software.

Results: All patients belonged to the gipsy ethnic minority with a low level of education and medical knowledge. Of the 72 subjects, 57 (79.2%) presented to the Hospital of Infectious Diseases in Timisoara for a medical evaluation, and 15 patients (20.8%) didn't present although they were advised by the doctors who have performed the epidemiological investigation of the outbreak. Ten patients were hospitalized with a moderately-severe clinical course of disease, and 22 individuals refused the admission to hospital. Although physicians prescribed ambulatory treatment for 40 patients (55.6%), only 8 followed the therapeutic cure with albendazole and the rest refused to purchase the drugs. None of the patients presented for the clinical

and laboratory post therapy re-evaluation. Patients were not informed about the danger of pork consumption from uncontrolled or unauthorized sources. Only 4 patients owned medical insurance and were following intermittent medical therapy for chronic disorders (ischemic heart disease, hypertension, chronic hepatitis).
Conclusions: Low therapy compliance associated with lack of alimentary and personal hygienic rules require implementation of specific projects. These must promote information and medical education in the gipsy community in order to prevent further trichinellosis or other infectious diseases outbreaks.

P1390 Acanthamoeba keratitis: clinical presentation, diagnosis, treatment and outcome

K. Tzanetou, D. Miltsakakis, K. Ziva, E. Tsianou, S. Strigari, E. Petrou, E. Marda, A. Goula, G. Ganteris, E. Malamou-Lada (Athens, GR)*

Acanthamoeba keratitis is a sight-threatening infection of the cornea caused by the ubiquitous free-living *Acanthamoeba* spp. The main risk factor for acquisition of this serious parasitic infection is the use of soft contact lenses.
Purpose: To review the clinical presentation, diagnosis, treatment, and outcome of patients with Acanthamoeba keratitis during a five year period.
Patients and Methods: All the patients, contact lens wearers, who presented with symptoms and signs of keratitis during the study period (from January, 2005 to October, 2009), underwent corneal scraping. The scrapings from the infected cornea were inoculated on 1.5% non-nutrient agar plates overlaid with *E. coli*, sheep blood agar, chocolate and sabouraud dextrose agar for isolation of *Acanthamoeba*, bacteria and fungi.
Results: Culture-proven Acanthamoeba keratitis was diagnosed in 25 patients. Seventeen of the 25 (68%) patients presented with symptoms and signs of early stage of the disease (pain, photophobia, epithelial and subepithelial opacities, dendritiform epithelial lesions, and radial keratoneuritis). Fourteen (82%) and six (35%) of early cases presented with pseudodendritic epitheliopathy (dendritiform epithelial lesions can mimic herpes simplex keratitis) and radial keratoneuritis (a nearly pathognomonic sign) respectively. Eight of the 25 (32%) patients presented with signs of late stage of the disease (annular and disciform stromal infiltration). The patients received topical treatment with polyhexamethylene biguanide (PHMB) 0.02% and hexamidine (desomedine or ophtamedine) 0.1% in combination or neosporine combined with desomedine or ophtamedine (duration of treatment was 6 to 12 months). The outcome of the early cases was clinical resolution and cure, while the advanced cases underwent therapeutic penetrating keratoplasty (one case complicated by scleritis, corneal ulceration and cataract).
Conclusions: The clinical presentation of Acanthamoeba keratitis may be pathognomonic (radial keratoneuritis), characteristic (ring-shaped stromal infiltrate) or non-specific. The early diagnosis and treatment with combined antiacanthamoeba agents are critical for a good outcome and vision maintenance.

P1391 An outbreak of *Plasmodium vivax* malaria in Lakonia, southern Greece, August-October 2009

P. Andriopoulos, A. Oikonomopoulou, K. Rigaki, N. Kaplanis, D. Rebelou, G. Assimakopoulos (Sparta, Athens, GR)*

Objectives: Malaria is considered to be eradicated in Europe. Only sporadic cases from travellers in endemic areas are occasionally reported. A cluster of Vivax malaria infected patients from Lakonia Greece is presented.
Methods: 8 patients were hospitalized from August to October 2009 in Sparta General Hospital with plasmodium vivax malaria. Epidemiological data, clinical symptoms, diagnosis and treatment are described.
Results: Two patients, 24 and 28 years old, immigrants from Pakistan and Afghanistan accordingly were admitted because of fever, jaundice and abdominal pain. The latter was treated for malaria with a 3-day regiment of chloroquine a year ago. Two weeks later and during a period of two months, six more patients, natives of Lakonia and living in different regions of the state from the first two, were admitted with similar symptoms. Blood smear tests were positive for all patients for plasmodium vivax which was verified with PCR testing. Sensitivity tests showed that all the strains were chloroquine-sensitive. All patients had irregular fever patterns, haemolytic anemia, thrombocytopenia, transient neutropenia, splenomegaly and elevated liver function tests. They were treated with combined regiment of chloroquine followed by primaquine and completed the treatment uneventfully.
Conclusion: A re-emergence of malaria may become a growing concern since populations migrate from Asia to Europe in poor sanitary conditions causing occasional local transmission. Surveillance and prevention are crucial in order to prevent further epidemics.

P1392 Risk factors for prolonged fever in patients with scrub typhus

Y.S. Kim, S. Park (Daejeon, KR)*

Objectives: Scrub typhus is an important rickettsial infection whose incidence has been rapidly increasing in Korea since 2004. With the increasing incidence of the disease, patients with delayed defervescence have become more common. We intended to compare the proportion of patients with prolonged fever in 2002 to those of 2008 and determine the risk factors.
Methods: We retrospectively analyzed data on the patients confirmed with scrub typhus by indirect immunofluorescence assay who were hospitalized in Chungnam National University Hospital. We used multiple logistic regression to determine risk factors for prolonged fever.
Results: The proportion of patients with fever $\geqslant$48 h increased from 14.9% (7 of 47 in 2002) to 41.3% (50 of 121 in 2008). Hypoxemia (OR, 3.58; 95% CI, 1.13–11.35) was more common in patients with prolonged fever. The choice of doxycycline or azithromycin for the treatment regimen did not affect the time to defervescence.
Conclusion: The proportion of patients with delayed response to antibiotic treatment has been increasing in Korea and hypoxemia was a risk factor. Disease severity may play a more important role in affecting the response to treatment than the choice of antibiotics.

P1393 Diversity and coexistence of *Borrelia* spp., *Anaplasma phagocytophilum*, *Rickettsia* spp. and *Babesia* spp. in *Ixodes ricinus* ticks in Middle Germany

A. Hildebrandt, K. Pauliks, A. Krämer, S. Sachse, E. Straube (Jena, DE)*

Objectives: So far, there are only few data about infection rates of *Anaplasma* spp., *Rickettsia* spp., *Babesia* spp. and their distribution in animal reservoirs in Germany and many other parts of Europe available. Possible coinfections with *Borrelia* spp. are of great importance since in case of human disease difficulties of diagnosis and treatment can occur. The aim of our study was to investigate the circulation and cocirculation of different tick-borne pathogens in a region of Middle Germany.
Methods: In 2006 and 2007 about 1000 Ixodes (I.) ricinus ticks were collected from a small recreational forest area in Middle Germany (Thuringia). Single ticks were investigated by PCR and RFLP or sequencing for *Borrelia* spp. (ospA gene), *Anaplasma* spp. (16S rRNA gene), *Rickettsia* spp. (gltA gene) and *Babesia* spp. (18S rRNA gene).
Results: Overall 27.0% (270/1000) of ticks were infected with *Borrelia* spp., 5.4% (54/1000) with *Anaplasma phagocytophilum*, 14.7% (147/1000) with *Rickettsia* spp. and in 5.0% (50/1000) of the ticks *Babesia* spp. specific DNA was present. *B. garinii* (133/270) was detected most frequently, followed by *B. burgdorferi* (70/270), *B. afzelii* (42/270), *B. valaisiana* subgroup I (28/270), not typable *Borrelia* spp. (5/270), *B. spielmanii* (3/270), *B. valaisiana* subgroup II (2/270) and *B. lusitaniae* (1/270). In 1.4% of investigated ticks several *Borrelia* species were found. At least two different *Rickettsia* spp. were identified: *Rickettsia helvetica* and *Rickettsia* monacensis. All *Babesia* spp. positive

amplicons were sequenced to distinguish between *Babesia microti* and *Babesia divergens*. In 7.0% (70/1000) of ticks two different pathogens were detected, 1.1% (11/1000) of ticks harboured more than 2 pathogens. Single, but also mixed infections occurred significantly more frequent in adult ticks compared to nymphs.
Conclusion: Systematic large-scale surveys of the coexistence of several tick associated pathogens are lacking. Our study is one of the limited numbers of smaller studies that have attempted to identify the prevalence of pathogens in individual ticks which is an important prerequisite for improved local risk assessment. Clinically, tick-borne mixed infections proceed often more severely than the corresponding diseases caused by a single pathogen. This implies the necessity of a comprehensive approach to the diagnosis, treatment and prophylaxis of these infections.

Table 1. Single and mixed infections of *Borrelia* spp., *Anaplasma phagocytophilum*, *Rickettsia* spp. and *Babesia* spp. in *Ixodes ricinus* ticks from a recreational forest area of Middle Germany (Thuringia)

	Nymphs 2006/2007 (%)	Females 2006/2007 (%)	Males 2006/2007 (%)	Total (%)
Number collected	430	293	277	1000
Single infections				
Borr	86 (20.0)	84 (28.7)	100 (36.1)	270 (27.0)
Ana	20 (4.7)	14 (4.8)	20 (7.2)	54 (5.4)
Rick	40 (9.3)	63 (21.5)	44 (15.9)	147 (14.7)
Bab	8 (1.9)	29 (9.9)	13 (4.7)	50 (5.0)
Total	154 (35.8)	190 (64.8)	177 (63.9)	521 (52.1)
Mixed infections				
Borr/Ana	4 (0.9)	4 (1.4)	4 (1.4)	12 (1.2)
Borr/Rick	4 (0.9)	15 (5.1)	11 (4.0)	30 (3.0)
Borr/Bab	0	5 (1.7)	5 (1.8)	10 (1.0)
Ana/Rick	1 (0.2)	1 (0.3)	3 (1.1)	5 (0.5)
Ana/Bab	0	1 (0.3)	0	1 (0.1)
Rick/Bab	3 (0.7)	7 (2.4)	2 (0.7)	12 (1.2)
Borr/Ana/Rick	1 (0.2)	1 (0.3)	2 (0.7)	4 (0.4)
Borr/Rick/Bab	0	2 (0.7)	4 (1.4)	6 (0.6)
Borr/Ana/Bab	0	0	0	0
Ana/Rick/Bab	0	1 (0.3)	0	1 (0.1)
Borr/Ana/Rick/Bab	0	0	0	0
Total	13 (3.0)	37 (12.6)	31 (11.2)	81 (8.1)

Borr – *Borrelia* spp.; Ana – *Anaplasma phagocytophilum*; Rick – *Rickettsia* species; Bab – *Babesia* spp.

P1394 Clinical course and microbiology of diphtheria in Latvia

R. Paberza, I. Vingre, M. Suvalova, F. Arsa, J. Storozenko, V. Kuse (Riga, LV)*

In recent years Latvia still remains the only country in the EU with regulary registered diphtheria cases. Sporadic cases continue to occur, but morbidity has decreased from 2006–2009.
Objectives and Methods: Using retrospective analysis of case – records summarize results of microbiology and imunological examinations, peculiarities of the course and outcomes of diphtheria in 56 patients treated in Infectology Center of Latvia (ICL) during period from 2006 till november 2009. The throat swabs for toxigenic corynebacteria were examined according to our standart protocols and the WHO guidelines. The level of antitoxic IgG antibodies were detected by ELISA.
Results: In period from 2006 till november 2009 at ICL were hospitalized 56 diphtheria patients: in 2006–24 patients, in 2007–12, in 2008–14 and in 2009–6 patients. Early hospitalisation before 5 days were in 34 cases. Only 13 patients were vaccinated according schemes, partially vaccinated and unvaccinated – 43. Toxigenic *C. diphtheriae* var. *gravis* were isolated in 54 cases, var.mitis – 1 case. The protective level of antitoxic IgG antibodies were insufficient in 35 cases. Course of diphtheria were mild in 26 cases, moderate – 7 cases, severe in 22 cases. 7 patients died. Severity criteria were: stridor, oedema of neck, skin petechia, circulatory collapse, acute renal insufficiency, myocarditis, motor paralysis. All patients with severe and those who died had not sufficient antitoxic IgG antibodies level. Because of severe course and clinical indications 12 patients were mehanically ventilated. From them 9 patients had pneumonia. In 3 cases from bronchial lavage and blood *Klebsiella pneumoniae* and multiresistant *Acinetobacter baumannii* was obtained.
Conclusions: Most of all diphtheria cases were idetected to unvaccinated, socially deprived adults. Pneumonia to mechanically ventilated patients was caused by gramnegative multiresistant microflora and treatment include broad spectrum antimicrobial. To provide further decrease of diphtheria level it is necessary to continue vaccination of adults.

P1395 The emergence of the major European clones of carbapenem-resistant *Acinetobacter baumannii* in Kuwait

A. Al-Hasan, A. Hamouda, S.G. Amyes (Edinburgh, UK)*

Objectives: *Acinetobacter baumannii* is an increasingly important nosocomial pathogen because of its ease of transmission and ability to become multidrug resistant. It has become a particular problem in Kuwaiti hospitals and this study aimed to examine the genotypic changes in the organism as it spread through one hospital.
Methods: A total of 88 *Acinetobacter baumannii* samples were collected from the Mubarak Al-Kabeer Hospital, over a three year period, 2006–2008, and they were identified phenotypically, by Vitek-2 systems, and then genotypically, by PCR amplification of blaOXA-51-like gene. The resistance to the carbapenems, Imipenem and Meropenem, was identified by use of the Minimal Inhibitory Concentration (MIC) test. Pulsed Field Gel Electrophoresis (PFGE) was used to type the strains and classify them into clonal groups. With this information, a dendogram showing the resistance profile and clonal relationship of all the isolates was constructed. Gene Sequencing was used to identify the blaOXA-51-like gene types of each of the isolates.
Results: All 88 isolates were identified as *Acinetobacter baumannii* by Vitek-2 system and were shown to carry a blaOXA-51-like gene. Resistance to Imipenem was found in 30% of the isolates, whereas resistance to Meropenem was found in 24% of the isolates. Overall carbapenem resistance was observed in 32% of the total isolates, with a slight increase in resistance of isolated over the 3 years of collection. In all, there were 10 different blaOXA-51-like genes identified. The sequences of these genes suggested there was some degree of real-time evolution of the blaOXA-51-like genes during the study period. There were four main clonal clusters. There were three main European clones (blaOXA-66, blaOXA-69, and blaOXA-71) plus a new clone-with blaOXA-51-like genes with sequences clustered around the blaOXA-98 gene.
Conclusion: This study has shown four major clones were found in the hospital during the study period, three closely associated with those found in Europe and elsewhere and one new clone, containing a blaOXA-98-like gene that appears to be more prevalent in this part of Asia.

P1396 Seroprevalence and genetic characterization of Dobrava and Saaremaa hantaviruses among rodents (*Apodemus agrarius*, *A. flavicollis)* in Hungary and Northern Croatia

V. Németh, M. Madai, B. Bérczi, A. Maráczi, G. Horváth, M. Oldal, T. Kovács, F. Jakab (Pécs, HU)*

Background: Dobrava and Saaremaa hantaviruses belongs to the genus Hantavirus, family Bunyaviridae and carried by yellow necked (*Apodemus flavicollis*) and striped field (*Apodemus agrarius*) mice. Dobrava hantavirus causes severe hemorrhagic fever with renal syndrome (HFRS) in many European countries, while Saaremma virus seems to causes milder form of HFRS.
Objectives: The goals of this study were (i) to determine the seroprevalence of Dobrava and Saaremaa hantaviruses in *A. flavicollis*

and *A. agrarius,* rodents, (ii) to genetically characterize those viruses detected by RT-PCR.
Methods: The rodents were trapped in seven different locations of the Transdanubian region of Hungary and Northern Croatia, during the summer and autumn seasons of 2005–2007. The rodents were dissected and lung tissues were used for hantavirus detection. The viral RNA was extracted from lung suspensions with TRIzol reagent according to the manufacturer's recommendation. Hantaviruses were detected by SYBR Green-based real-time PCR, using newly designed virus specific primers. Positive samples were selected for sequence and phylogenetic analysis. Representative Dobrava strain was selected for protein expression. Truncated nucleocapsid protein (rNP50) was expressed in BL21 *E. coli* cells using pET28a expression system. Recombinant protein contained 6×His tag; therefore it was purified using Ni-NTA column. The presence of IgG antibodies in the collected rodents were determined by ELISA reaction.
Results: During the study period a total of 130 *Apodemus* sp. (67 *A. agrarius,* 63 *A. flavicollis*) were tested for the presence of hantaviruses. Out of the 130 *Apodemus* rodents 10 (3 *A. agrarius* and 7 *A. flavicollis)* were infected with hantavirus. Based on the molecular sequence and phylogenetic analysis – as we expected – at least two hantavirus types, Dobrava and Saaremaa virus circulate parallel in the examined region. The seroprevalence of these hantaviruses were 15% in the collected rodents.
Conclusion: In this study we reported the occurrence and genetic characteristic of Dobrava and Saaremaa hantaviruses circulate in Hungary as well as in the northern part of Croatia. Based on the clinical experience and our new molecular and serologic data from this region we concluded that extended molecular and serological investigations might be important in the future.

P1397 Some data of leptospirosis in Albania

E. Puca, A. Ndreu, A. Pilaca, K. Shkurti, E. Puca, M. Josifi (Tirana, AL)*

Objective: To give same data of leptospirosis in Albania during January 2002–October 2009.
Methods: We study cases with leptospirosis during January 2002–October 2009 in our clinic UHC "Mother Teresa" which is the only tertiary center in Albania. All cases were confirmed serologically with ELISA (enzyme-linked immunoassay) positive for IgM antibodies.
Results: This study investigated retrospectively 38 consecutive patients with serologically confirmed leptospirosis admitted to our hospital during the period January 2002–October 2009. They were 89.4% males (34) and 10.6% females (4). Mean age at the time of diagnoses were 42.5 years old with 15.3±DS range 17–73. Distribution according to the season was: spring 15.8% (6 cases); summer 31.6% (12 cases); autumn 34.2%(13 cases); winter 18.4% (7 cases). Distribution according to the years were: 2002 2 cases; 2003 7 cases, 2004 1 case, 2005 2 cases, 2006 4 cases, 2007 2 cases, 2008 4 cases and 2009 14 cases. The time from the first symptoms until they presented to hospital were 7.5 days. Sign and symptoms were: myalgia 89.4%, temperature with average 38.8ºC in 86.8% of cases; fatigue 84.2%, headache 78.9%, jaundice in 68.4%, vomiting and abdominal pain 57.8%, respiratory signs in 44.7%, renal insufficiency in 23.9% of cases and 13.8% diagnosed during 2009. Laboratory results included: hyperleukocytosis in 55.2% of cases, thrombocytopenia in 28.9%, Average level of bilirubinaemia 4.82 mg/dl range and hyperbilirubinaemia were present in 73.7% of cases with average level 7.48 mg/dl, hepatic abnormalities(86.8%): AST (aspartate aminotrasferase) were elevated in 78.9% of cases with average level 467.6 UI/dl range 40–4000, ALT(alanine aminotransferase) were elevated in 82.4% of cases with average level 591.3UI/dl range 40–6000 and renal abnormalities: elevated serum creatinine 36.8%, proteinuria in 73.6% of cases, haematuria in 31.5% of cases, leukocyturia in 42.1% of cases. 38 (100%) patients received antibiotics. Mortality was 0%.
Conclusions: There was a predominance of the males in ratio 8.5:1. The disease was more frequent during summer-autumn. The study confirmed the variable clinical and biological symptoms of leptospirosis, and indicated that hepatic and renal abnormalities are common feature of leptospirosis.

P1398 The efficacy of ribavirin treatment in Crimean-Congo haemorrhagic fever in Turkey

G. Tuncer Ertem, F. Temocin, C. Ataman Hatipoglu, M. Sonmezer, B. Oral, N. Tulek (Ankara, TR)*

Objectives: Bunyaviridae family are known as generally sensitive to ribavirin. It was shown that ribavirin is effective against Crimean-Congo Haemorrhagic Fever (CCHF) *in vitro* and the positive effect of ribavirin was reported in some studies. However there are no randomized controlled clinical trials, ribavirin use in treatment of CCHF is suggested by World Health Organization. The aim of the study is to analyze the effect of ribavirin use on clinical course of the disease.
Methods: All patients with CCHF hospitalized at our clinic between 2007 and 2009 in Ankara Training and Research Hospital were evaluated retrospectively. All of the cases were confirmed at National Reference Laboratory by presence of IgM positivity by ELISA or positive RT-PCR tests. The patients were grouped on the basis of ribavirin use or not. Patients who did not use ribavirin served as control group. Then the patients who treated with ribavirin grouped according to receiving within 3 days of the onset of symptoms (Group 1) or later (Group 2). The levels of laboratory findings at the beginning, 5th day and end of treatment were compared for evaluation of the efficacy of ribavirin.
Results: Eighty-nine patients (48 female and 41 male) were included in our study. The mean of age the patients was 48 years (range: 16–80 years). Two patients were died (2.2%). Fourty-six (51.7%) of patients received oral ribavirin, 43 (48.3%) of not received. Died patients had entered to the hospital later (⩾3 days) but both of them received ribavirin. One of them died on next day of admission, other died four days later. The mean of platelet values was significantly higher in ribavirin receiving group on 5th day of treatment ($p = 0.04$). The mean of activated partial thromboplastin time (APTT) values on 5th day and prothrombin time (PT) values on 10th day were lower in ribavirin group (respectively $p = 0.04$, $p = 0.004$). Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) levels, platelet and APTT values rapidly normalized in ribavirin receiving group compared with nonreceiving group (respectively $p = 0.009$, $p = 0.018$, $p = 0.000$, $p = 0.015$). There were no statistically difference for mean of leukocyte, alanine aminotransferase (ALT), AST, LDH and creatine kinase (CK) levels in group 1 and 2. PLT values rapidly normalized in group 1 ($p = 0.033$), but for other parameters no difference was found.
Conclusion: We found a useful effect of ribavirin treatment especially on platelet, APTT, AST and LDH levels.

P1399 Public health importance of Crimean-Congo haemorrhagic fever in Iran as an emerging infectious disease

S. Chinikar, S.M. Ghiasi, M. Moradi, M. Bouloy, R. Flick, H. Feldmann (Tehran, IR; Paris, FR; Lowa, Bethesda, US)*

Background and Aims: Crimean-Congo Hemorrhagic Fever (CCHF) is a tick-born viral disease with high mortality rate which caused by CCHF virus, belongs to Nairovirus genus and Bunyaviridae family, and is transmitted to humans by infected tick bite, handling of infected blood or tissues or nosocomially. The disease contains four distinct phases including incubation, prehemorrhagic, hemorrhagic, and convalescence. In 1999 after re-emergence of CCHF in Iran, it was considered as a major public health problem and now most provinces of Iran became endemic. The CCHF is diagnosed by molecular and serological assays.
Methods: As national reference laboratory, human probable sera are collected from different parts of Iran. All sera were analyzed for CCHF detection by sandwich-IgM and sandwich-IgG specific ELISA and gel-based and real-time RT-PCR.
Results: As our data showed that the disease has infected 23 out of 30 provinces of Iran and has been continuously seen in some provinces such as Sistan and Baluchistan, Isfahan, Khorasan in the last 10 years, while

it was sporadically seen in some provinces such as Yazd in some years. Also, the epidemiological data implied that symptoms' severity and mortality of the CCHF differs in different provinces. By a phylogenetic survey, it was cleared that the majority of genome isolates from different part of Iran have close relationship to Matin (Pakistan) strain.
Conclusions: By respecting a major public health problem, CCHF is in the most important rank of viral hemorrhagic fevers in Iran. As the most infected province, Sistan and Baluchistan has faced to the disease annually, because it neighbors to Pakistan and Afghanistan with a large border where the CCHF is endemic. Moreover, the phylogenetic studies confirmed the origin of the CCHFV from Pakistan, but more pathogenesis and phylogenetic studies need to investigate in order to determine why the severity and mortality rate are different in different years and different provinces, it may perhaps be due to different strains or with different genotypes and or different pathogenicity.

P1400 Identification of virulent *L. monocytogenes* strains isolated from wastewater

L. Ballesteros, Y. Moreno, P. Santiago, G. Cuesta, M.A. Ferrús (Valencia, ES)*

Objectives: *Listeria monocytogenes* is the causal agent of a severe food-borne disease. It is also responsible for the highest hospitalisation rates (around 90%) amongst known food-borne pathogens *L. monocytogenes* is widely distributed and due to its capacity to resist adverse conditions it can survive in different environments as soil, animal and human faeces, superficial waters, sewage sludge and even chlorinated water.
Presence of *L. monocytogenes* in wastewater could represent a high health risk since a possible route of human infection could be the consumption of fresh vegetables irrigated with these contaminated treated waters.
The aim of this study was to determine the prevalence of *L. monocytogenes* virulent serotypes in wastewaters from a domestic and industrial treatment plant from Valencia, Spain to determine the possible infection risk through reutilization of these waters.
Methods: A total of 80 wastewater samples were analyzed. An amount of 100 ml was filtered through 0.45 μm membrane filters and membranes were incubated in modified Fraser selective broth and subsequently were platted on Aloa and Palcam agar. All colonies suspected to be *Listeria* were subcultured on blood agar plates to check the haemolytic activity. Oxidase, catalase and Gram-stained tests were also applied. Presumtive *L. monocytogenes* strains isolated were characterized by serology with *Listeria* antisera set (Denken Seiken. Co, Ltd., Tokio, Japan) according to manufacturers' instructions, biochemical profiles with API *Listeria* identification system (Biomerieux, Mercy L'Etoile, France), multiplex PCR and virulence characteristics determining the presence of lmo2821 gene were performed.
Results: Most of the 57 biochemicaly comfirmed *L. monocytogenes* isolates belonged to 4b and 1/2b serotypes, the main recovered from human listeriosis infection. A high percentage of *L. monocytogenes* isolated showed the presence of the region gene that encodes the fragment of the internalin protein lmo 2821 (a virulence gene).
Conclusions: This study show the high presence of virulent serotypes of *L. monocytogenes* in wastewater. Use of treated waters to irrigated crops could be an important route of the pathogen transmission due to the ingestion of uncooked contaminated vegetables, therefore a efficient treatment and control of these waters is a priority to prevent *Listeria* infection.

P1401 Interesting insights into the co-circulation of pathogens in *Ixodes ricinus* ticks from rodents, birds and in questing stages in Middle Germany

A. Hildebrandt, J. Fritzsch, J. Franke, S. Sachse, E. Straube (Jena, DE)*

Objectives: *Babesia* spp., *Anaplasma phagocytophilum* and *Rickettsia* spp. are potentially emerging tick-borne pathogens, whereas many issues about their ecology, e.g. reservoir host specificity, are still unclear. The aim of our study was to get an interesting insight into the circulation and co-circulation of different tick-borne pathogens in small mammals and birds as potential reservoirs, but also in questing ticks from the same area of investigation in Middle Germany.
Methods: From May until October 2007 a total of 658 *Ixodes ricinus* ticks were collected from rodents (273), birds (189) and questing stages (196) in Reifenstein in Norththuringia/Germany. They were investigated for *Babesia* spp. (18S rRNA gene), *Anaplasma* spp. (16S rRNA gene) and *Rickettsia* spp. (gltA gene) by PCR, RFLP and sequencing.
Results: Overall 13.1% (86/658) of the investigated ticks were infected with at least one pathogen. *Babesia* spp. specific DNA was detected in 9.7% (64/658) of ticks, 1.4% (9/658) were infected with *Anaplasma phagocytophilum* and 2.6% (17/658) harboured rickettsiae. *Babesia* spp. positive amplicons were sequenced to distinguish between *Babesia divergens* (33/64) and *Babesia microti* (29/64). Two amplicons were only characterized as *Babesia* spp. (2/64). At least two different *Rickettsia* spp. were detected: *Rickettsia helvetica* (7/17) and *Rickettsia* monacensis (2/17). The distribution among the different hosts showed the highest infection rate with *Babesia* spp. in birds (13.2%, 25/189), whereas in rodents (6.6%, 18/273) and questing ticks (10.7%, 21/196) lower infection rates were found. Rodents and birds were identified as reservoir or at least important co-vectors for the three pathogens, but in each host different tick developmental stages seem to be more susceptible for infections. Coinfections with two different pathogens occurred in 0.6% (4/658) of the ticks, only in subadult stages (*Babesia* spp./*Anaplasma* spp.: 2/658; *Babesia* spp./*Rickettsia* spp.: 2/658).
Conclusion: In Germany there are only few studies reporting about the prevalence of several tick-borne pathogens and their reservoirs. Our investigations contribute to the prevalence of *Babesia* spp., *Anaplasma* spp. and *Rickettsia* spp. in *Ixodes ricinus* ticks of small mammals, birds and questing ticks of the same investigation area in Middle Germany. Further systematic studies are necessary to detect interactions in transmission cycles and assess the risk for human diseases.

Coexistence of different pathogens in *Ixodes ricinus* ticks from rodents, birds and questing stages in the same investigation area of Middle Germany

	No. of ticks collected			No. (%) of positive specimens									Total
				Babesia spp.			*Anaplasma* spp.			*Rickettsia* spp.			
	larvae	nymphs	adults	larvae	nymphs	adults	larvae	nymphs	adults	larvae	nymphs	adults	
Rodents*	258	13	2	14/64 [1] (21.9)	4/64 (6.3)	0	3/9 [1] (33.3)	0	0	5/17 (29.4)	0	0	26/273 (9.5)
Birds**	62	127	0	12/64 [1] (18.8)	13/64 [1] (20.3)	0	2/9 [1] (22.2)	4/9 (44.4)	0	0	4/17 [1] (23.5)	0	35/189 (18.5)
Questing ticks	0	162	34	0	20/64 [1] (31.3)	1/64 (1.6)	0	0	0	0	7/17 [1] (41.2)	1/17 (5.9)	29/196 (14.8)
Total	320	302	36	26/64 (40.6)	37/64 (57.8)	1/64 (1.6)	5/9 (55.6)	4/9 (44.4)	0	5/17 (29.4)	11/17 (64.7)	1/17 (5.9)	90/658 (13.7)

No., number; [ ], number of coinfections.
*Yellow-necked mouse (*Apodemus flaicollis*), bank vole (*Myodes glareolus*).
**Blackbird (*Turdus merula*), European robin (*Erithacus rubecula*), marsh warbler (*Acrocephalus palustris*), Dunnock (*Prunella modularis*), Eurasian bullfinch (*Pyrrhula pyrrhula*).

P1402 Crimean-Congo haemorrhagic fever virus, south-eastern Bulgaria: a case report and review

N. Kalvatchev, I. Christova (Sofia, BG)*

Objectives: During the last decade Crimean-Congo hemorrhagic fever (CCHF) emerged or re-emerged in about 30 countries around the world including Bulgaria, Albania, former Yugoslavia, Ukraine, Russia, Turkey, and recently, in Greece. Our country is endemic region of CCHF and sporadic cases are observed every year. Rapid and accurate diagnostic algorithm in suspected patients is essential for a proper treatment of infection, favorable outcome and prevention of its spread.
Methods: A man residing in southeastern city of Bulgaria, Sliven, received a tick bite. Blood samples from the patient were drawn and transported to the laboratory. Molecular techniques like polymerase chain reaction (PCR) and its modifications real-time RT-PCR and nested RT-PCR were recently introduced and now used for diagnostics of CCHF.
Results: The present case report of this viral hemorrhagic fever was confirmed by the mentioned above molecular methods. New borne mice were infected with patient' blood and Crimean-Congo hemorrhagic fever virus (CCHFV) was isolated and determined by Complement

Fixation Assay (CFA). A sequence of the partial gene is performed and phylogenetic tree is designed.
Conclusion: We report here, the combined use of real-time RT-PCR, nested RT-PCR and CFA for detection of CCHFV in this patient. With this study we revealed and compared genetic heterogeneity of the virus in Southeastern Bulgaria and other close endemic area.

P1403 Recent emergence of tularaemia in the region of Burgundy (France): usefulness of real-time PCR for diagnosis of atypical presentations

S. Mahy, N. Roch, M. Duong, M. Grappin, M. Maurin (Dijon, Grenoble, FR)*

Background: Tularemia is a zoonotic disease caused by a Gram-negative bacterium, *Francisella tularensis*. A recent increase in cases of tularemia has been observed in France: in 2008, 105 cases were reported that is twice more than in 2007. Two endemic areas are known in France: the west and east (Alsace) areas. In the region of Burgundy, no cases were reported in 2007 whereas 8 cases were notified in 2008.
Objectives and Methods: To describe the clinical presentations of five cases of tularemia observed in the Department of Infectious diseases, CHU Dijon from 2008 to 2009 and to show the contribution of real-time PCR technology for early and accurate diagnosis of tularemia.
Results: Table 1: Clinical and biological characteristics of 5 patients with tularemia.
Discussion: The clinical presentations of tularemia are polymorphous and vary according to the routes of entry of bacteria (Table 1). As compared to serology, real-time PCR detection of *F. tularensis* DNA allows a more accurate diagnosis of tularemia, including in the early steps of the disease. It can be performed on various tissue samples such as skin or lymph node biopsies, conjunctival discharge or sputum.
Conclusion: Tularemia is an emerging infectious disease in the region of Burgundy, possibly by spreading from the tularemia-endemic Alsace region. Clinicians should be aware of the polymorphous clinical presentations of the disease. Real-time PCR detection of *F. tularensis* now supplants standard bacteriology, and allows earlier diagnosis than serology. It provides an accurate diagnosis and allows determination of the *F. tularensis* subspecies involved, which should be considered in the context of bioterrorism threat.

Table 1. Clinical and biological characteristics of 5 patients with tularemia

	Age	Suspected route of entry	Clinical form	Serology 1 (titre)	Serology 2 (titre)	PCR	Therapy
1	36	Inhalation	Pneumonia	1/1280	1/640	+ (lymph node)	Moxifloxacin
2	42	Contact with deadwood	Oculoglandular infection	<1/20	1/1280	+ (lymph node)	Doxycyclin
3	38	Contact with cold meat	Acute lymphadenitis	1/320	Not done	+ (lymph node)	Doxycyclin
4	48	Tick bites	Acute fever	<1/20	1/1280	+ (skin)	Levofloxacin
5	40	Tick bites	Chronic lymphadenitis	1/320	1/320	+ (lymph node)	Ciprofloxacin

P1404 Vibrio-associated gastroenteritis in Italy, 2006–2008

D. Ottaviani, F. Leoni, E. Rocchegiani, C. Canonico, S. Potenziani, S. Santarelli, L. Masini, L. D'annibale, A. Carraturo (Ancona, Perugia, Latina, IT)*

Objectives: Vibrio infections have been rarely reported in Italy in the past decades. For this reason, these microorganisms are not included in the Microbiological Surveillance System for Infectious Gastroenteritis. Results from 2006, described here show a different picture.
Methods: From January 2006 to December 2008, a passive surveillance study for Vibrio diseases was conducted at two Hospitals of Central Italy. A total of 100 stool specimens from hospitalized patients with acute diarrhoea, was examined. Samples were collected on the patients' admission to the hospital, before antibiotic treatment. Phenotypic and genotypic characterization of the isolates was performed by standardized procedures while susceptibility to antibiotics (amoxicillin–clavulanic acid, ampicillin, cephalotin, cephalexin, cephoperazone, ciprofloxacin, colistin, gentamicin, kanamycin, oxolinic acid, tetracycline, trimethoprim/sulphamethoxazole) was tested according to CLSI guidelines.
Results: Of 100 stool samples 2% were positive for *V. cholerae* non O1, non O139 and 3% for *V. parahaemolyticus,* in absence of other enteric pathogens. Their molecular, serological and antibiogram profiles are summarized in Table.
Conclusions: We report the isolation of *V. parahaemolyticus* O3:K6 pandemic clone in two sporadic cases occurred in Italy in two different years and the first case of a gastroenteritis with *V. parahaemolyticus* O1:KUT serotype in Europe. The recurrent report of Vibrio-associated gastroenteritis, with mussels as the most probable source of infection, emphasizes the need to include Vibrio spp in the epidemiological surveillance of gastrointestinal diseases and re-examine control programs for shellfish-harvesting areas in Italy.

Table 1. Results of serotyping, molecular and antibiogram patterns of Vibrios isolated from stools, Italy, 2006–2008

Isolates	Serotype	Origin	Source of infection	PCR*							Antimicrobial resistance†
				ctxA	*tcpA*	*stn/sto*	*hylA*	*tdh*	*trh*	*ToxRS*	
V. cholerae	O176	Diarrhoeal patient Central Italy 2006	prawn	–	–	–	+	n.p.	n.p.	n.p.	AMP; AMOX-CA
V. cholerae	O37	Diarrhoeal patient Central Italy 2006	mussels	–	–	–	+	n.p.	n.p.	n.p.	AMP
V. parahaemolyticus	O3:K6	Diarrhoeal patient Central Italy 2007	mussels	n.p.	n.p.	n.p.	n.p.	+	–	+	AMP; AMOX-CA; CEP; CEPH
V. parahaemolyticus	O3:K6	Diarrhoeal patient Central Italy 2008	mussels	n.p.	n.p.	n.p.	n.p.	+	–	+	AMP; AMOX-CA; CEP; CEPH
V. parahaemolyticus	O1:KUT	Diarrhoeal patient Central Italy 2008	mussels	n.p.	n.p.	n.p.	n.p.	–	+	–	AMP; AMOX-CA; CEP; CEPH; COL

**ctxA*, cholera-toxin gene; *tcpA*, toxin-coregulated pilus gene; *stn/sto*, heat stable enterotoxin gene; *hylA*, El Tor-like haemolysin gene; *tdh*, thermostable direct haemolysin gene; *trh*, TDH-related haemolysin gene; *toxRS*, pandemic marker of *V. parahaemolyticus*.
n.p., not performed; † AMP, ampicillin; AMOX-CA, amoxicillin–clavulanic acid; CEP, cephalotin; CEPH, cephalexin; COL, colistin.

P1405 A case of Crimean-Congo haemorrhagic fever with acalculous cholecystitis and intra-abdominal abscess

R. Caylan, I. Hasanoglu, D. Yapar, M.A. Tasyaran (Ankara, TR)*

Crimean Congo Haemorrhagic Fever (CCHF) is a fatal systemic viral infection and it is an important health problem in Turkey. Since it leads to diffuse endothelial damage, many complications can be seen during the course of the disease. We report here an atypical presentation of CCHF with acute acalculous cholecystitis (AAC) and intraabdominal abscess (IAA).
Case presentation: A 29-year old previously healthy female applied to a regional health-care facility with complaints of fever, headache, myalgia and fatigue. Despite no tick-bite history, her doctor recommended hospitalization based on elevated liver enzymes, leukopenia and thrombocytopenia with a prediagnosis of CCHF and referred to our hospital because of severe trombocytopenia.
Upon admission her body temperature was 39.7ºC and had a platelet count of 12000 K/mcL, aspartate aminotransferase (AST) 160 U/L, alanine aminotransferase (ALT) 68 U/mL and lactate dehydrogenase (LDH) 596 U/L. Her CCHF diagnosis was confirmed by RT-PCR. She had severe epistaxis and was treated with fresh frozen plasma, platelet transfusions and nasal packing. Meanwhile, she developed somnolence and had right hypocondrial and epigastric pain. Her liver enzymes elevated suddenly (AST 4045 U/L, ALT 1223 U/mL). Ultrasonography showed that her gallbladder wall thickness was 16 mm and there was no gallstone, so she was diagnosed as having CCHF with AAC. Her oral intake was ceased immediately and ciprofloxacin was initiated. Twenty days after admission, the patient's fever was still high despite antibiotheraphy. The abdominal tomography (CT) showed multiple IAA. Her clinical status improved with antibiotheraphy and percutaneous drainage. A control CT showed considerable reduction in the dimensions of the abcesses.
Since endothelial damage is a main contributor in the pathogenesis of CCHF, it can be seen in many different clinical manifestations and complications. Although it is known as a rare complication of viral haemorrhagic fevers, AAC hasn't been reported in the course of CCHF. Our case was also complicated with IAA which hasn't been reported as a complication of CCHF as well. It is difficult to explain IAA in this case while many aspects of the CCHF pathogenesis remain unclear. This may have been caused by bacterial translocation during the development

of multiple haemorrhage sites seen in cases with aggressive courses. In conclusion, CCHF is a systemic viral infection which can be seen with many complications such as AAC and IAA.

P1406 Sandfly surveillance within an emerging epidemic focus of cutaneous leishmaniasis in southern Iran

M.R. Yaghoobi-Ershadi, M. Hakimiparizi, A.A. Akhavan (Tehran, IR)*

Objectives: To determine the species richness and relative abundance of sandflies, their distribution, monthly prevalence, number of generations and leishmanial infection of sandflies in the study area.
Methods: The study was carried out from April to October of 2006 in Bahraman rural district of Kerman Province, south of Iran. Sandflies were collected biweekly from indoors and outdoors fixed places in the selected villages, using 30 sticky paper traps from the beginning to the end of the active season and they were mounted and identified. Some blood fed and gravid female sandflies of rodent burrows and indoors were dissected and examined microscopically for natural promastigote infection of leishmania parasite during August to September. The physiological age of each female was also determined by the presence or absence of granules in the accessory glands. All dissected sandflies were mounted and identified.
Results: In total, 2439 specimens comprising 8 species (3 Phlebotomus and 5 Segentomyia) were identified. The most common sandfly is *P. papatasi* and represents 87.1% of sandflies from indoors and 57.2% from outdoors. The activity of the species extended from April to end October. There are two peaks in the density curve of this species, one in June and the second in August. Natural promastigote infection was found in *P. papatasi* and it was calculated to be 12.7%. Examining the accessory glands of the female sandflies from rodent burrows showed that 85.45% of *P. papatasi* were parous and the rest were nuliparous and unknown.
Conclusions: Phlebotomus papatasi is considered as a probable vector among gerbils and to humans with a high percentage of promastigote infection in this new focus of cutaneous leishmaniasis. The Bahraman area which until recently was unknown as an endemic area seems now to represent a focus of zoonotic cutaneous leishmaniasis transmission in Iran.

P1407 Investigation for yellow fever virus exposure in humans from central/south-eastern Anatolia

K. Ergunay, M. Saygan, S. Aydogan, N. Litzba, M. Niedrig, N. Ozer, S. Bulut, D. Us (Ankara, TR; Berlin, DE)*

Objectives: Yellow fever is a viral hemorrhagic fever with high mortality that is transmitted by mosquitoes. The disease is present now in Africa and Central/South America, although historically, large outbreaks occurred in Europe and North America. Mosquitoes capable of transmitting yellow fever exist in regions where the disease does not presently occur. Data available on possible Yellow Fever virus (YFV) activity in Turkey is limited to a single study where total YFV antibodies were found in 9.7% in a survey of 1074 sera from Aegean region of Turkey but none could be confirmed. The aim of this study, partially supported by Hacettepe University Research Fund and Turkish Red Crescent Society, was to investigate YFV exposure in persons from Central and Southeast Anatolia. This is the first study to investigate YFV exposure in these regions.
Methods: A total of 1523 sera, that comprise 1454 sera from blood donors at 4 major branches (Ankara, Konya, Eskişehir and Zonguldak) of Turkish Red Crescent Middle Anatolia Regional Blood Center, along with 69 sera obtained from adults attending to outpatient clinics of State Medical Centers of Sanliurfa and Siverek (southeastern Turkey) for other laboratory analyses were included after informed consent. All individuals were asked to fill out a survey to reveal risk factors for vector-borne viral infections and persons with a history of Yellow Fever vaccination were excluded. All sera were previously evaluated with commercial ELISA assays for other flaviviruses (Anti-Dengue virus, Anti-TBE virus and Anti-West Nile virus ELISA, EUROIMMUN, Germany) and found to be negative. Evaluation for the presence of YFV IgG was performed by a commercial IgG indirect immunofluorescence test (IIFT) (Anti-Yellow Fever virus IgG IIFT, EUROIMMUN, Germany). All positive samples were retested twice and further evaluated by YFV plaque reduction neutralization assay to confirm antibody specificity.
Results: A total of 10 sera (10/1523, 0.65%) were positive in initial evaluation and repeat testing by the YFV IgG IIFT. All were negative in the plaque reduction neutralization assay.
Conclusions: No confirmed exposure to YFV could be demonstrated in this study. In order to rule out the presence of YFV activity in Anatolia, detailed vector surveillence data should be collected and thoroughly evaluted to identify mosquito species that may act as potential vectors.

P1408 Comparison of *Clostridium difficile* genotypes isolated from patients, environment and animals

V. Zidaric, S. Janezic, M. Ocepek, B. Kotnik Kevorkijan, N. Oresic, J. Rebersek Gorisek, M. Rupnik (Maribor, Ljubljana, SI)*

Objectives: *Clostridium difficile* is important human pathogen of health care-associated infections. Recent studies from North America have indicated large overlap between genotypes isolated from pigs and cattle with human isolates. Reports from the Netherland showed that strains of emerging ribotype 078 isolated from humans and pigs are indistingushable. The objective of this study was to compare genotypes present in humans in our region with animal and environmental strains isolated in the same geographic region in same time interval.
Methods: Strains were collected from January 2008 to June 2009. The majority of strains was isolated from humans and originated from the routine diagnostic laboratory. Animal strains were isolated from animal fecal samples collected at small and large farms (pig, poultry, and cattle farms) or from pets. Environmental samples included rivers and soil. Strains were identified as *C. difficile* accoding to cultural morphology and presence of sequences specific for toxigenic or nontoxigenic strains. All strains were PCR ribotyped and patterns were compared by Bionumerics software.
Results: Altogether, 327 strains were isolated from: patients in different health care settings (n = 116), different animal species (n = 48) and from environment (soil and water) (n = 163); 261 strains were toxinogenic and 67 nontoxinogenic. They were distributed into 58 PCR ribotypes.
Human strains were included into 42 PCR ribotypes, 5 of them were also found in animals and 19 in environmental samples.
Three PCR ribotypes were found only in animals and 13 only in environmental samples.
Three most prevalent PCR ribotypes were 014, 002, INT010 accounting for 22.3%, 12.0% and 10.1% strains, respectively. All of them were present in different hosts and environments.
Conclusion: Diversity of *C. difficile* genotypes in different hosts and environments is considerable, but almost 50% of all strains belonged to only three PCR ribotypes, all of them present in humans, most animal species and rivers.

P1409 Microbiological and clinical features of *Clostridium difficile* infection cases visiting emergency room in a tertiary care hospital

B. Shin, S. Mun, E. Kuak (Seoul, KR)*

Objectives: Clostidium difficile is one of the most important pathogens responsible for nosocomial diarrhea. Recently, community acquired *C. difficile* infection (CA-CDI) have been reported worldwide. We have experienced *C. difficile* positive cases among patients visiting emergency room in a tertiary hospital. The purpose of this study is to provide the basic features of the microbiological and clinical features of them to differentiate CA-CDI from hospital acquired CDI.
Methods: We performed 201 cases of toxin A/B EIA test, *C. difficile* culture and enteric pathogen (*Salmonella/Shigella*) culture from patients visiting ER from Feb, 2006 to Nov 2009. We amplified

toxin A and B genes in culture positive cases to differentiate toxin positive(tcdA+tcdB+), variant(tcdA−tcdB+) and negative(tcdA−tcdB−) strains. PCR-ribotyping was performed. Laboratory findings (albumin, creatinine, WBC), underlying diseases and histories about antibiotic usages were evaluated.
Results: *C. difficile* were isolated in 29 cases (14.4%). No *Salmonella* or *Shigella* spp. were isolated. Their mean age was 54 years (19–92) and no statistical difference was observed between male and female. Toxin gene PCR assay revealed that the proportion of positive, variant and negative strains were 76%, 17% and 7%, respectively. PCR ribotyping revealed diverse patterns among *C. difficile* strains except variant strains. Among the culture positive cases, 19 cases (65.5%) had no histories of antibiotics, but10 cases (34.5%) had the history of antibiotic usage and cephalosporin was the main antibiotic taken (80%). Mean value of albumin, creatinine and WBC of CDI cases were 3.42 g/dL, 1.16 mg/dL and 1,1240/uL, respectively. The main underlying diseases were malignancy, pneumonia and gastroenteritises.
Conclusion: Compared with HA-CDI cases, CDI cases visiting ER were relatively younger patients. Some of the CDI cases with antibiotic history were associated with cephalosporin more than quinolone. Toxin variant strain was also important strain among CDI cases visiting ER and diverse PCR ribotyping patterns were observed.

P1410 Detection and characterization of kobuviruses (in family Picornaviridae) in human and in new host species in Hungary

G. Reuter, Á. Boldizsár, Á. Boros, P. Pankovics (Pécs, HU)*

Objectives: Picornaviruses (family Picornaviridae) are small, non-enveloped viruses with single-stranded, positive-sense genomic RNA which is divided into eight genera. Kobuvirus genus consists of 2 officially recognised species, Aichi virus and Bovine kobuvirus. Aichi virus has been isolated in stool sample from human with gastroenteritis in Japan, in 1990. Bovine kobuvirus (strain U-1) has been detected in clinically healthy cattle in Japan, in 2003. Up to 80–95% of the human population at the age of 30–40 years has antibodies against Aichi virus.
Methods: Fecal samples collected from human (N = 65), cattle (N = 32), domestic pig (N = 60+60) and sheep (N = 8) were tested for kobuvirus by RT-PCR in Hungary. For detection and characterization of viral genome, newly designed screening, typing primers, "genome-walking" and sequencing methods were used.
Results: Aichi virus was found in 1 (1.5%) of the stool samples from children under age of 10 years with diarrhea with unknown origin. Bovine kobuvirus was detected in two cattle (6.25%) at the first time outside Asia. Serendipitously, new kobuvirus species were found from fecal samples collected from domestic pigs in Hungary. Porcine kobuvirus (S-1-HUN, EU787450) was found in healthy pigs and complete nucleotide (8,210nt), amino acid (2,488aa) sequences and genetic organization were determined. The 5′ untranslated region (UTR) forms an unique hepacivirus/pestivirus-like type IV IRES element. Two copies of 90 nucleotides long tandem repeat was detected in 2B and a unique sequence in 3′UTR. The genetic identity on coding region between Aichi, U-1 and S-1-HUN viruses are between 35% (L-protein) and 74% (3D region). By 21 months follow up study, high incidence (65% and 53%) and endemic circulation of porcine kobuvirus were found at the tested farm (*in vivo* evolution rate for VP1 is 6.75×10–3 substitutions/nucleotide/year). Porcine kobuvirus was also present in pig sera samples (32/60; 26.6%) indicating kobuvirus viraemia at the first time. Kobuvirus sequences related to bovine kobuvirus strains were also found in newborn sheep (N = 5, 62.5%).
Conclusion: Kobuviruses were identified in 4 host species including human. Our knowledge's (about diversity, pathogenesis, geographical distribution, transmission, epidemiology, virus organization etc.) are not complete related to enterically transmitted viruses especially kobuviruses.

Infections in the immunocompromised host and transplant patients

P1411 Epidemiology and outcome of Gram-positive bloodstream infection in solid organ transplant recipients

M. Fernández-Ruiz, L. Linares, C. Cervera, M. Almela, M. Tuset, F. Cofán, M.J. Ricart, M. Navasa, A. Escorsell, E. Roig, A. Moreno (Barcelona, ES)*

Aim: Bloodstream infection (BSI) is one of the most frequent complication in solid-organ transplantation (SOT) recipients. Our aim is to estimate the incidence, outcomes and risk factors for Gram-positive BSI in this population.
Methods: Prospective cohort study in the period between July, 2003 and December, 2007, including patients receiving renal, liver, heart and simultaneous double-organ transplantation. Demographics, underlying conditions, immunosuppressive schemes, and post-transplant complications were prospective collected. BSI was defined as significant according to the CDC criteria. BSI-related mortality was defined as a death occurring within the first 30 days. Multivariate logistic regression analysis was performed to identify variables associated with Gram-positive BSI after SOT.
Results: A total of 1,049 SOT were performed in the study period (528 kidney, 318 liver, 83 heart, and 120 double transplant). We diagnosed 186 recipients (17.7%) with at least one episode of Gram-positive infection. There were 72 episodes of Gram-positive BSI in 64 patients (6.1% of global cohort). The cumulative incidence according to the type of SOT was: kidney (2.6%), liver (12.5%), heart (2.4%) and double transplant (6.6%) ($p < 0.001$). The most frequent source of BSI was catheter-related (40.3%). Thirty-nine percent of episodes occurred during the first month after transplantation. Isolated species were: methicillin-resistant coagulase-negative staphylococci (CoNS) (27.8%), *Enterococcus faecalis* (23.6%), methicillin-susceptible CoNS (16.7%), methicillin-susceptible *Staphylococcus aureus* (12.5%), *Enterococcus faecium* (8.3%), methicillin-resistant *S. aureus* (MRSA) (1.2%), and others (9.9%). Vancomycin (29% of episodes) and teicoplanin (22%) figured as the most common first-line treatments. Multivariate analysis revealed as independent risk factors for Gram-positive BSI: liver transplantation (OR 3.74, CI 95% 2.18–6.39) and re-operation (OR 2.79, CI 95% 1.65–4.3). Twenty-three of 64 patients with Gram-positive BSI (35.9%) deceased, including 9 cases with related mortality.
Conclusion: The incidence of Gram-positive BSI after SOT was high, specifically in liver transplant recipients. Type of transplant and re-operation revealed as independent risk factors for the occurrence of such complication. Our results provide preliminary evidence of the decreasing prevalence of MRSA as a cause of BSI in SOT recipients, and the emergence of *Enterococcus* in this group of patients.

P1412 First isolation of catalase-negative *Staphylococcus aureus* from cerebrospinal fluid: case report and review

*M.R. Vicente *, E. Escribano, J. Lozano, M. Garcia-Sánchez, M.D. Crespo (Albacete, ES)*

Objectives: Isolation of catalase-negative *Staphylococcus aureus* (CNSA) from cases of human infection has occasionally been reported. We report a case of endocarditis and meningitis in which methicillin-resistant CNSA was isolated from cerebrospinal fluid (CSF), blood and, probably, from the prosthetic aortic valve.
Methods: Case report and review of the English and Spanish medical literature.
Results: On 7 September 2009, a 67-year-old woman was admitted to our hospital for fever, chills, vomiting, rigor and confusion. She had underwent mitral and aortic valves replacement two months before. For suspected central nervous system infection a lumbar puncture was performed. CSF showed 92 leucocites/mm^3 (74% PMN), proteins 0.85 g/L and glucose 98 mg/dL. She was started on

vancomycin and meropenem therapy. On hospital day 3, she began with signs of heart failure. Transesophageal echocardiogram (TEE) showed multiple vegetations on mitral valve, periprosthetic abscess and severe periprosthetic mitral regurgitation. At this time, methicillin-resistant *S. aureus* (MRSA) was isolated from blood and CSF cultures, so treatment was changed to vancomycin, gentamicin and rifampin. She underwent aortic and mitral prosthetic valves replacement. An external laboratory reported us the isolation of MRSA from the prosthetic aortic valve. She presented multiorganic failure with renal insufficiency and vancomycin was changed to linezolid. After six weeks with antibiotics, a new TEE showed new vegetations and periprosthetic mitral valve regurgitation, so new cardiac surgery was performed. *S. aureus* isolated from blood and CSF was repeatedly catalase negative. Reference laboratory reported that both strains belonged to the same mixed phage group. We have found other 25 documented cases of CNSA human infection or colonization. Most cases came from Europe (13/26). Patients were predominantly male (15/25), with a mean age of 53 years (range, 1–80) and frecuently had underlying conditions. Six out of 16 reported autcomes were fatal. The most frecuent culture sources were blood (12/25) and wound or soft tissue samples (10/25). Seven out of 20 strains were methicillin-resistant.

Conclusion: This is the first report of CNSA meningitis and the first report of methicillin-resistant CNSA human infection in Spain. Reports of infections caused by CNSA and caracterization of such strains must be encouraged to clarify the role of catalase in the pathogenicity of *Staphylococcus* sp.

P1413 Infectious endocarditis in patients with cirrhosis of the liver: unique causative agents and dreadful prognosis

M. Fernández Guerrero, M. Górgolas (Madrid, ES)*

Objectives: Infectious Endocarditis (IE) is, more than in the past, an infection of the chronically ill, fragile patient. We hypothesized that patients with cirrhosis of the liver (CL) may be at increased risk of developing IE in the community and the hospital settings, valve replacement is denied even when indicated and prognosis is gloomy.

Methods: Retrospective review of consecutive cases of IE in patients with CL during a period of 10-years in a single institution. Only cases of definite IE according to the modified Duke's criteria in patients with clinical or pathogical criteria for CL were included. Patients with chronic hepatitis were excluded. A control group composed by 2 patients without CL – the immediate preceding and the immediate following a case patient – was chosen for comparison.

Results: LC was found in 27 out of 286 cases of IE (9.4%). Alcoholism and hepatitis B and C virus were the main causes of CL. Patients with CL were younger than controls (58.6 vs 67.3 y). Differences on predisposing valve disorders or location of IE were not observed. Non-viridans streptococci (OR 4.9; CI95% 1.4–16.6; $p < 0.05$) and β-hemolytic streptococci (OR 12; CI95% 1.3–109; $p < 0.05$) were causative agents of IE more frequently in patients with CL than in controls. IE was hospital-acquired in 37% of patients with CL and in 12.9% of controls (OR 3.9; CI95% 1.3–12; $p < 0.05$). Venous catheters, Foley catheters, parenteral nutrition, colonoscopy, liver biopsy and Sengstaken-Blakemore baloon were the sources of nosocomial IE in patients with CL. Surgery was indicated in 16 cases (59.2%) with CL and in 23 of controls (42.5%); remarkably, while surgery was undertaken in 19 (82.6%) of controls, it was performed in only 4 (25%) of patients with CL (OR 0.07; CI95%: 0.01–0.33; $p < 0.05$). Mortality of patients with CL was significantly higher than in controls (48% vs 20%; OR 3.6; CI95%:1.3–9.9; $p < 0.05$).

Conclusions: Cirrhosis of the liver is nowadays a common underlying condition in patients with IE. Patients with CL have a serious hazard of acquiring IE during admission to hospital for invasive diagnostic or therapeutic procedures. "Virulent" microorganisms such as β-hemolytic streptococci and S aureus are the most frequent causative agents of IE in patients with CL. Sugery, even when indicated, is denied or refused and mortality is exceedingly high.

P1414 Incidence, aetiology and chronology of urinary tract infections after renal transplantation

A. Martín-Peña, E. Cordero, M.E. García-Prado, V. Cabello, G. Bernal, M.J. Rodríguez-Hernández for the Spanish Network for Research in Infectious Disease (REIPI)*

Objectives: Urinary tract infection (UTI) after renal transplantation remains a source of morbidity and graft faliure. The aim of this study was to evaluate the incidence, etiology and chronology of UTI in renal transplant recipients during the first year postransplantation.

Method: Retrospective and observational study of a cohort of adult recipients that consecutively received a renal transplant enrolled from January to December of 2008.

Results: During the study period, 89 patients (66.3% men) were included, with a median age of 52 (range: 17, 53) years and a median follow up of 262.5 (range: 65, 453) days. A total of 152 UTI were recorded in 61 patients of whom 124 (81.6%) were lower UTI and 28 (18.4%) pyelonephritis. Fourteen pyelonephritis (50%) were bacteremic. The global incidence of UTI was 70% (81.6% lower ITU, 18.4% pyelonephritis). Among patients with UTI, median number of episodes/patients was 1.7. The main etiology of UTI was *Escherichia coli* (91, 54.8%) being in nine cases extended βlactamase (ESBL) producers, followed by *Enterococcus faecalis* (22, 13.3%) and *Klebsiella* pneumonia (14, 9.6%). Etiology was polymicrobial in 14 cases. Lower UTI was caused in five episodes by *Candida glabrata*. ESBL producer *E. coli*, was more frequent in pyelonephritis 21.4% (3/14) than in lower UTI 4.8% (6/124), RR 4.4 CI95% 1.2–15.8, $P = 0.05$. Median time to UTI appearance after transplantation was 52 days (range: 1–436). Most of the UTI occurred during the first month post-transplantation (62, 40.8%) being lower UTI the most frequent clinical presentation (54, 87.1%). Between second to sixth month post transplantation, 72 UTI were diagnosed, 25% of them were pyelonephritis. After six months, 11.1% (2/18) of episodes were pyelonephritis. Most of UTI (95, 62.5%) had a good prognosis and 36.8% were recurrent infections. Seven patients (7.8%) death during the study period but only in one case (0.6%) death was considered infection related, a complicated pyelonephritis caused by ESBL producer *E. coli*.

Conclusions: Urinary tract infections are a common syndrome after renal trasplantation. *Escherichia coli* is the most common etiology of these infections with a high rate of ESBL producer strains mainly in pyelonephritis. Despite their frequency, urinary tract infections have a good prognosis.

P1415 A two-centre retrospective study on the microbiology of bisphosphonate associated osteonecrosis of the jaws

M. Jackson, J. Snäll, R. McFadzean, A. Smith, R. Rautemaa-Richardson (Glasgow, UK; Helsinki, FI)*

Objective: Bisphosphonate associated osteonecrosis (BON) of the jaws is a side effect of bisphosphonate therapy, which is commonly used in the management of osteoporosis, multiple myeloma and bone metastasis. The bisphosphonates act by inhibiting bone resorption resulting in an interference with the normal process of bone turnover. This creates the potential for aseptic necrosis of bone which may subsequently become infected. The mandible in particular appears at heightened risk for BON. There is very little information in the literature concerning the microbiology of infected BON of the jaws. The aim of this study was to determine the microbiology of specimens from BON lesions of the jaws.

Method: A review of cases with a clinical history of BON was determined by a data-base search over the period 2005–2007 (Helsinki) and by hand searching laboratory records over the period 2006–2009 (Glasgow), submitted to the respective regional diagnostic oral microbiology laboratories.

Results: In Helsinki (He), 18 cases of BON of the jaws were identified. Of these, specimens had been submitted for microbiological analyses in 11 cases. In Glasgow (Gl) 14 specimens had been analysed. All

specimens demonstrated mixed growth comprising *Actinomyces* spp. (8/11 specimens He, 2/14 Gl), *S. anginosus* group (2/11 He, 5/14 Gl), anaerobic cocci (4/11 He, 3/14 Gl) and anaerobic Gram negatives such as *Prevotella* and Fusobacterium spp (8/11 He, 1/14 Gl) in addition to Viridans group streptococci (10/11 He, 8/14 Gl). Many isolates demonstrated reduced susceptibility to penicillin and macrolides. Resistance to clindamycin was more common than to doxycyclin.
Conclusions: Our results from this small cohort of patients with infected BON of the jaws demonstrates that *Actinomyces* spp, *Streptococcus* spp. and Gram negative anaerobes are the predominant findings, many with reduced antimicrobial susceptibility. Clinicians should be encouraged to submit appropriate samples to aid in the management of this condition. This data should be considered when proposing guidelines for prophylaxis and therapy of infected BON of the jaws. It appears that the choice and duration of empiric antimicrobial therapy should be directed towards *Actinomyces* spp. Laboratory protocols should include prolonged culture for these slowly growing facultative and anaerobic pathogens.

P1416 Prostethic-joint infections in cancer patients

D. Nisichenko, N. Dmitrieva, I. Petukhova, Z. Grigoryevskaya, I. Shilnikova, E. Sokolova (Moscow, RU)*

Infections associated with prosthetic joints lead to significant morbidity, increase of financial costs and worsening of the quality of life.
Aim: to evaluate the postoperative rate of prosthetic joint infections (PJI) in cancer patients (pts) with various malignant tumors of bones.
Materials and Methods: 385 pts with primary or metastatic tumors of hip, tibia or shoulder after surgical bone resection and prosthesis implantation were included in the analysis. The time of onset symptoms due to infections was registered. Identification and susceptibility testing of microorganisms isolated from synovial fluid in cases of postoperative PJI was performed with VITEK-2 (bioMérieux, France).
Results: PJI were registered in 55/385 (14%) of pts. The rate of PJI varied due to tumor localization: 6% – after tumor resection with shoulder joint endoprosthesis, 16% – after resection with hip joint endoprosthesis, 8% and 20% ($p < 0.05$ compared to shoulder joint) after resection and knee joint endoprosthesis depending on the tumor localization in hip bone, and 15% after total prosthesis of the hip. Early infections (<3 month) were revealed in 27/55 (49%) of pts, delayed infections (from 3 month till 2 years) in 9/55 (16%) of pts and late infections (>2 years) in 19/55 (35%) of pts. Early and late infections were registered significantly more often ($p < 0.001$ and $p < 0.05$). Conservative treatment including antimicrobials was successful in 40% of patients. 5-year survival rate of prosthesis ranged between 26% (total hip prosthesis) and 74% (shoulder prosthesis). The main pathogens were *Staphylococcus aureus* and coagulase-negative staphylococci – 42–55% depending on prosthesis localization. *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, anaerobes (*Fusobacterium nucleatum* and *Peptostreptococcus* magnum) were rarely isolated.
Conclusion: PJI were registered more often in early and late periods after operation. Infection rate was maximal after resection with knee endoprosthesis. *Staphylococcus* spp. were the main cause of PJI in malignant bone tumors pts.

P1417 *Mycobacterium tuberculosis* in kidney transplant recipients: a single centre 19-year experience in Korea

S.I. Kim, K.W. Hong, Y.J. Kim, S.H. Wie, Y.R. Kim, M.W. Kang (Seoul, KR)*

Objectives: Transplant recipients are highly susceptible to various opportunistic infections. Epidemiologic data showed that *Mycobacterium tuberculosis* (MTB) is not common in western countries; however Korea has been one of endemic area for MTB. Special considerations are needed to prevent and to treat the MTB in transplant recipients. To make proper strategy, we reviewed medical records to find the incidence, manifestations and outcomes of MTB infection in renal transplant recipients of our center.
Methods: We retrospectively reviewed the medical records of 1,325 patients who had renal transplantation since January 1990 to September 2009.
Results: MTB was diagnosed in 5.05% (67/1,325) of renal transplant recipients. Mean age was 51 year-old, and 67.1% (45/67) were men.
Diabetes was seen in 34.3% (23/67) of patients, 40.2% (27/67) had episode of rejection before MTB manifestation, and 13.4% (9/67) had positive results for cytomegalovirus reactivation during MTB manifestation. Regarding past history, 25.3% (17/67) had previous history of MTB. Among them only 2 patients had received isoniazid prophylaxis before transplantation.
Pulmonary involvement [55.2% (37/67)] was more common than extrapulmonary involvement [44.7% (30/67)]. Disseminated MTB was 14.9% (10/67). The mean time between transplant and MTB was 43.5 months. Twenty-five patients (37.3%) were diagnosed with MTB within the first year after transplantation.
Mean duration of treatment was 12.4-month. Anti-TB therapy related renal toxicities were found in 26.8% (18/67) and hepatotoxicities were found in 5.9% (4/67). Drug interactions that need to modify the immunosuppressive agents were found in 43.2% (29/67).
Treatment success rate was 68.6% (46/67). Six patients (8.9%) died; among them one case was MTB related mortality. Nine patients (13.4%) experienced recurrence of MTB after cessation of anti-TB treatment and one patient experienced aggravation of MTB during treatment. Graft loss occurred 43.2% (29/67) of patients after MTB.
Conclusion: Our center showed 5.05% incidence of post-renal transplant MTB, which is much higher than general population (0.07%) and Western countries. MTB infection in renal transplant recipients showed lower rate of treatment success and was related with high rate of graft rejection. Considering that Korea is endemic area for MTB, patients with previous history of MTB, more aggressive preventive strategies are needed to reduce the risk of MTB reactivation and graft loss.

P1418 Prophylaxis vs. pre-emptive therapy for cytomegalovirus disease in (D+/R–) liver transplant recipients

N. Fernàndez-Sabé, L. Lladó, M. Bodro, J. Niubó, J. Castellote, E. Ramos, J. Fabregat, J. Carratalà (Hospitalet, ES)*

Objective: Cytomegalovirus (CMV)-seronegative recipients (R–) of CMV-seropositive donors (D+) have the highest risk of CMV disease. The most appropriate strategy to prevent CMV disease in this population is a matter of active debate. We aimed to compare prophylaxis and preemptive therapy for the prevention of CMV disease in D+/R– liver recipients.
Methods: Analysis of a prospective cohort of liver recipients (1992–2009). D+/R– patients were identified from the liver transplant program database.

	Prophylaxis n = 35	Preemptive therapy n = 39	p-value
Viremia (%)	3 (9)	15 (38)	0.003
Mean days after Tx	98	42	0.041
Viral syndrome (%)	2 (6)	6 (15)	0.267
Mean days after Tx	113	30	0.301
CMV disease (%)	3 (9)	13 (33)	0.010
Type of CMV disease			
Hepatitis	0	7	
Digestive disease	3	4	
Pneumonitis	0	2	
Mean days after Tx	104	45	0.143
Late-onset CMV disease (>90 days after Tx)	2	0	0.220
Allograft rejection (%)	6 (17)	6 (15)	0.838
Other opportunistic infections (%)	1 (3)	4 (10)	0.361
One-year case fatality rate	2 (6)	5 (13)	0.435

Results: Among 878 consecutive liver recipients, 80 (9%) were D+/R–. Six patients died within 30 days of transplantation (Tx) and were excluded. Of the remaining D+/R– patients, 35 (47%) received prophylaxis and 39 (53%) followed a preemptive strategy, based on CMV antigenemia surveillance. Fifty-four (73%) were men; mean age 49 yrs (15–68). Mean follow-up was 86 months (8–214). Baseline characteristics and initial immunosuppressive regimens were similar in the two groups. Either ganciclovir or valganciclovir were the antiviral drugs initially used in both strategy groups. The table details the main results.
Conclusions: In D+/R– liver patients, CMV disease significantly occurred more frequently among patients following preemptive therapy, whereas late onset CMV disease was more common in patients receiving prophylaxis. No significant differences in allograft rejection, other opportunistic infections and mortality were observed.

P1419 Herpesviridae viral infections following chemotherapy in patients with lymphoma: incidence, risk factors, and prevention

J.Y. Park, H. Kwon, H.S. Lee (Busan, Daegu, KR)*

Objectives: Herpesviridae viral infection(HVI) can cause serious complications such as dissemination and bacterial superinfection in lymphoma patients undergoing chemotherapy. But there was no consensus on the dose and the duration of antiviral prophylaxis during chemotherapy in lymphoma patients. We retrospectively analyzed the incidence and the risk factors for HVI.
Methods: 266 patients who newly diagnosed and received an chemotherapy without prophylaxis of acyclovir between June 1996 and August 2009 were enrolled retrospectively at a teaching hospital in Korea. HVI was confirmed based on clinical diagnosis, serologic test or pathologic diagnosis. The characteristics of the patients were as follows: the median age was 54 years (range 15–83) with a female-to-male ratio of 150:116. The included lymphomas were diffuse large B cell lymphoma (n = 151), Hodgkin's disease (n = 16), T cell lymphoma (n = 43) and other lymphoma (n = 56). The results were analyzed using a χ^2 test and independent samples T test. For the multivariate analysis, we used logistic regression test.
Results: Forty three patients (16.2%) developed HVI at a median of 5.43 months (range 0.43–51.33) after initial chemotherapy. In univariate analyses, risk factors for HVI were gender ($p = 0.002$, 10% in male vs 24.1% in female), cumulative dose of prednisone ($p < 0.001$, 4.0% in less than 4000 mg vs 31.6% in more than 4000 mg), duration of chemotherapy ($p = 0.009$, 11.8% in less than 6 months vs 24.0% in more than 6 months), presence of relapse ($p = 0.007$, 24.7% in relapse vs 11.9% in non-relapse), salvage chemotherapy ($p = 0.009$, 11.8% in no salvage chemotherapy vs 24.0% in salvage chemotherapy), and presence of neutropenic fever ($p = 0.019$, 26.9% in neutropenic fever vs 13.6% in no neutropenic fever). In multivariate analysis, the results confirmed 2 variables as independent predictive factors for the female ($p < 0.001$, hazard ratio (HR): 4.915, 95% confidence interval (CI) 2.200–10.981) and cumulative dose of prednisone ($p < 0.001$, HR: 14.269, 95% CI 5.241–38.848). There was no different mortality and survival rate between HVI and non-HVI group.
Conclusion: Female and high dose prednisone was seemed to be high risk for HVI in lymphoma patients undergoing chemotherapy without acyclovir prophylaxis. Antiviral prophylaxis for HVI may be needed in higher risk lymphoma patients undergoing chemotherapy.

P1420 Cytomegalovirus infection after liver transplantation – incidence and benefit of ganciclovir prophylaxis

P. Schnitzler, K. Weigand, J. Schmidt, F. Chahoud, D. Gotthardt, P. Schemmer, W. Stremmel, P. Sauer (Heidelberg, DE)*

Objectives: Cytomegalovirus (CMV) infection represents a complication after liver transplantation. Depending on CMV status of donor and recipient and individual risk profile antiviral prophylaxis is recommended in clinical practice guidelines.
Methods: Altogether 211 liver transplant recipients were retrospectively analyzed for CMV infection after transplantation, influence of donor and recipient CMV status and effect of antiviral prophylaxis. Underlying liver disease and immunosuppressive protocol was correlated to incidence of CMV infection. The mean follow-up time after transplantation was 24.5±13.5 month. Patients were divided in four groups according to CMV donor/recipient (D/R) profile: group A (D–/R–), Group B (D–/R+), Group C (D+/R+), Group D (D+/R–).
Results: The donor/recipient profile distributed as follows: Group A, 28 patients (pts), group B, 64 pts, group C, 79 pts and group D, 40 pts. CMV infection was observed in 17.9%, 29.7%, 24.1% and 22.5%, respectively and was not significantly different in groups A, B, C and D. In group A with a low risk profile a CMV infection occurred in 5 pts (17.9%), despite an antiviral prophylaxis in 4 pts. In contrast, in group D with a presumed high risk profile only in 9 of 40 pts (22.5%) CMV infection was observed in spite of prophylaxis in most patients. Most frequent infection rates were found in group B and C (R+ groups), with 29.7% and 24.1%, respectively. Underlying liver disease or immunosuppressive protocol had no influence on CMV infection.
Conclusion: Independently from donor/recipient CMV status, approximately one fourth of patients will acquire CMV infection after liver transplantation. Surprisingly, antiviral prophylaxis seems not sufficient to reduce this proportion of patients, neither in the high risk, nor in the low risk situation.

P1421 HHV-6-DNAemia after liver transplantation monitored by two quantitative real-time PCR methods

T. Karlsson, R. Loginov, K. Höckerstedt, I. Lautenschlager (Helsinki, FI)*

Objectives: HHV-6 is a ubiquitous virus with a high seroprevalence (>95%). Like other herpesviruses HHV-6 can reactivate under immunosuppression. In liver transplantation, most HHV-6 reactivations are asymptomatic, but also symptoms and graft dysfunction have been described. The aim of the study was to compare two quantitative HHV-6 PCR tests and correlate with HHV-6 antigenemia in liver transplant patients.
Methods: Thirty adult liver allograft recipients were frequently monitored for HHV-6 during the first 6 months after transplantation. HHV-6 reactivations were diagnosed by the antigenemia test in PBMC using indirect immunoperoxidase staining and monoclonal antibodies against HHV-6. Altogether, 170 whole blood specimens (mean 5 samples/patient) were analyzed. HHV-6-DNA was tested by using automated sample preparation system MagNa Pure LC and quantitative Taqman-based "in-house" real-time PCR and commercial quantitative Argene CMV, HHV6, 7, 8 R-gene™ kit. Argene's test amplifies a sequence of viral U57 gene and the "in-house" assay amplifies a sequence of viral U67 gene, detecting both HHV-6A and HHV-6B variants.
Results: HHV-6-antigenemia was recorded in 17/30 (56%) patients and HHV-6 DNAemia in 13/30 (43%). HHV-6-DNAemia usually occurred during the first weeks after transplantation. The viral loads by the "in-house" Taqman test varied between 520–19 700 copies/ml (median 1310) and by Argene's test 250–24 070 copies/ml (median 440). The correlation of viral loads between two quantitative tests was good (R = 0.93).
Conclusions: HHV-6 reactivations were common after liver transplantation. The results of the both PCR methods correlated mostly with HHV-6 antigenemia, and the correlations between the two quantitative PCR assays was good.

P1422 Incidence of invasive fungal infection in adult liver transplant recipients in the intensive care unit: a prospective validation of a risk stratification scheme

A. del Palacio, M.S. Cuétara, M.E. Alvarez, A. Alhambra, M. Catalán, J.C. Montejo, J. Pontón (Madrid, Leganés Madrid, Lejona-Vizcaya, ES)*

Objective: Hellinger et al (Liver Transpl 2005; 11:656) proposed a risk group stratification of invasive fungal disease (IFD) in adult liver transplant recipients (LTR). Our aim was to prospectively validate this proposal in LTR requiring mechanical ventilation in the intensive care unit (ICU) and the relationship between the incidence of IFD (per patient) and risk group.
Methods: Patients were stratified into 3 groups: (1) High risk LTR were patients on haemodialysis at the time of transplantation or with delay of hospital discharge beyond day 7 after transplantation due to allograft or renal insufficiency; (2) Intermediate risk – retransplantation or transplantation due to fulminant hepatic failure; (3) Low risk – absence of conditions in groups 1 and 2. Conventional diagnostic methods, biomarkers (Galactomannan, (1–3)-B-D-glucan), tissue biopsies and necropsies, with the assessment of risk factors, signs, symptoms and radiologic imaging were used for the diagnosis of IFD as defined by De Pauw et al (Clin Infect Dis 2008; 46: 1813).
Results: 43 adult LTR requiring ICU admission were prospectively studied. Evidence of IFD was documented in 8 patients (6 proven and 2 probable cases). The incidence of IFD in the high risk group was 29.6% (8/27) whilst the incidence of the intermediate and low risk groups was nil. The mortality in the IFD group was 62% (5/8) and in the nonIFD group was 42.85% (15/35). The rate of necropsies was 45% (9/20).
Conclusions: Our current data showed that the risk stratification scheme described and proposed by Hellinger et al is a valid method for identifying in the clinical setting those LTR patients at highest risk of developing IFD. In conclusion, readily identifiable patient characteristics can be used to stratify LTR for risk of developing IFD. Prospective studies with antifungal targeted prophylaxis given to high risk LTR may provide if this approach could lead to cost-effective prevention of IFD.
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P1423 Relevance, occurrence and significance of positive *Aspergillus* cultures: a prospective study in adult liver transplant recipients in an intensive care unit

M.S. Cuétara, M.E. Alvarez, A. Alhambra, M. Catalán, J.C. Montejo, J. Pontón, A. del Palacio (Madrid, Vizcaya, ES)*

Objective: Most *Aspergillus* culture isolates from respiratory samples do not represent invasive aspergillosis (IA). Our aim was to prospectively assess the significance of positive *Aspergillus* cultures of respiratory samples and their relationship with IA in a cohort of high risk liver transplant recipients (LTR) requiring mechanical ventilation in the intensive care unit (ICU).
Methods: Patients were stratified into 3 risk groups (high, intermediate and low) as proposed by Hellinger et al (Liver Transpl 2005: 11: 656). Conventional diagnostic methods, biomarkers (Galactomannan, (1 → 3)-B-D-glucan), tissue biopsies and necropsies, with the assessment of risk factors, signs, symptoms and radiologic imaging were used for the diagnosis of Invasive Fungal Disease (IFD) as defined by De Pauw et al (Clin Infect Dis 2008; 46: 1813).
Results: 43 adult LTR requiring ICU admission were prospectively studied. Evidence of IFD was documented in 8 patients (2 proven IA and 2 probable IA cases, as shown in the table). 14/43 (32.6%) patients had positive *Aspergillus* cultures: 3/14 (21.4%) were IA and 11/14 (78.6%) were colonizations (p = 0.0572). The respiratory samples were obtained one day after hospital admission for transplantation in 63.6% (7/11) patients. The overall *Aspergillus* colonization rate was 25.5% (11/43).
Conclusions: The mortality in the IFD group was 62% (5/8) and in the nonIFD group was 42.85% (15/35). The rate of necropsies was 45% (9/20). An early study of Kusne et al (J Infect Dis 1992; 166:1379), maintains that the isolation of *Aspergillus* has a high predictive value of IA in LTR, however in our study, the isolation of *Aspergillus* was not significant possibly due to its small size. It would appear that other factors are needed for the development of IA. The high rate of respiratory *Aspergillus* colonization one day after hospital admission suggests that fungal colonization of the lungs is present before entry in the hospital (Lass-Flörl C et al Br J Haematol 1999; 104: 745).
Acknowledgments: This investigation was supported by grants Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Proyecto Investigacion PI 070134 (to MSC), PI 070107 (to A d P) and PI 070376 (to JP), grant IT-26407 of Departamento de Educación, Universidades e Investigación del Gobierno Vasco (to JP) and Saiotek from Departamento de Industria, Comercio y Turismo del Gobierno Vasco (to JP) and an Educational grant from Pfizer (to A d P) and Gilead Spain (to A d P).

Table 1. Risk stratification in 43 LTR: significance of positive *Aspergillus* cultures in respiratory samples and relationship with invasive aspergillosis

Risk group	Non IFD patients			IFD patients	
	High (n = 19)	Intermediate (n = 3)	Low (n = 13)	High (n = 8)	
				Non IA[1] (n = 4)	IA (n = 4)
Patients with positive *Aspergillus* cultures, n (%)	5 (26.3)	1 (33.3)	5 (19.2)	0 (0)	3 (75)
A. fumigatus	1	1	2	0	0
A. niger	2	0	1	0	0
A. terreus	0	0	2	0	0
A. flavus	1	0	0	0	1
A. versicolor	0	0	0	0	2
A. fumigatus and *Rhizopus microsporus*	1	0	0	0	0
Dead patients with					
positive *Aspergillus* cultures	4	1	1	0	3
negative Aspergillus cultures	7	1	1	2	0
Patients with necropsy and					
positive *Aspergillus* cultures	2	1	0	0	3
negative *Aspergillus* cultures	2	0	0	1	0

[1] Non IA: non invasive aspergillosis (1 proven invasive candidiasis; 2 proven zygomycoses; 1 mixed proven invasive candidiasis and zygomycosis).

P1424 Two cases of atypical Guillain–Barré syndrome associated with *Chlamydia pneumoniae* infection

M. Pape, S. Prodromidou, C. Kanonidou, K. Chatzikosta-Mandraveli, S. Dionysopoulou, M. Paschalidou, E. Diza (Thessaloniki, GR)*

Objective: Guillain–Barré syndrome (GBS) is a disorder in which the body's immune system attacks part of the peripheral nervous system. Guillain–Barré has been associated with viral or bacterial infection.
We present two cases of atypical GBS associated with *Chlamydia pneumoniae* (CP) infection.
Methods: Case-1: A 45 year old female was admitted for acute weakness in the left arm that presented 2 weeks ago. The neurological examination revealed mild sensory disturbances in the left arm, absent reflexes and weakness. Case-2: A 22 year old female experienced mild left arm weakness 4 weeks before admission. The neurological examination revealed only left arm weakness, diminished reflexes and superficial hypoesthesia. Antecedent respiratory illness occurred before onset of neurological manifestations in both patients. Paired blood samples (time of admission-two weeks later) and Cerebrospinal fluid (CSF) were examined for DNA detection of CMV, EBV, HSV, VZV using Real-time PCR. Sera and CSF were also tested for the presence of antibodies against CMV, EBV, HSV, VZV *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Coxiella* burneti, *Bartonella henselae*/quintana, using the indirect immunofluorescent antibody (IFA) test and the enzyme-linked immunosorbent (ELISA) assay. CSF cytochemical examination and electrophoresis and computer tomographic scan were also conducted. No surgery or vaccination was reported.
Results: No viral genome was detected and there was no serological evidence of acute infection with CMV, EBV, HSV, VZV, *Mycoplasma pneumoniae*, *Bartonella henselae*/quintana and *Coxiella burnetii* in

blood and CSF specimens. CSF cytochemistry and electrophoresis were normal and computer tomographic scan showed no apparent abnormalities. Concerning CP the detection of IgM antibodies and seroconversion in paired blood samples strongly supported acute infection. They received doxicycline for 14 days. On follow up clinical improvement was noted in both patients.
Conclusions: Our cases suggest that CP should be added to the differential list of agents associated with the GBS syndrome.

P1425 Monoclonal subtyping of clinical and environmental-related *Legionella* strains in epidemiological investigations of legionellosis cases

L. Franzin, N. Bonfrate, V. Demarie (Turin, IT)*

Objective: Positive culture is the only method that allows the comparison of patient and environmental *Legionella* strains necessary to confirm or exclude a given environmental reservoir as the source of infection. Subtyping of *Legionella pneumophila* serogroup 1 strains by using monoclonal antibodies (MAb) is useful method in epidemiological investigations. The aim of the study is MAb subtyping of clinical and related environmental strains in epidemiological studies of culture positive legionellosis cases.
Methods: Culture was performed from 545 respiratory samples (sputum, bronchoalveolar fluid, bronchoaspirate, pulmonary biopsy) of patients with pneumonia. Aliquots of untreated, heat treated and acid-washed suspensions were plated on BCYE, BMPA and MWY. The strains of *Legionella* isolated were serologically typed. MAb subtyping of *L. pneumophila* 1 isolates was performed by immunofluorescence assay (Dresden panel). Culture of *Legionella* was performed from water of related environmental sites by quantitative methods. *L. pneumophila* 1 environmental strains (54) were typed by MAb and also by molecular techniques (PFGE, RAPD and SBT).
Results: *Legionella* culture was positive from respiratory samples of 48 patients. *L. pneumophila* 1 strains were isolated from samples of 45 patients. Clinical strains and related environmental strains were available in 9 epidemiological investigations. In all these studies, the strains isolated from the patient showed the same MAb subtype as the related environmental strains isolated from water. Five cases of legionellosis were acquired in different hospitals: four cases were caused by Pontiac group (2 Philadelphia and 2 Knoxville) strains and one by non-Pontiac group (OLDA) strains. Pontiac group strains were isolated in all the community acquired legionellosis cases. MAb subtype of clinical and related water strains isolated from two patients home was Philadelphia. Clinical and related environmental strains were France/Allentown subtype in the another case.
Conclusions: In these epidemiological investigations Pontiac group isolates (virulent MAb3/1 positive strains) were prevalent (8 out of 9). MAb subtyping is not specific enough to distinguish between closely related strains, but it very useful as first method to screen the isolates before molecular typing. MAb subtyping plays an important role in typing *L. pneumophila* 1 strains in source tracking and, when used with molecular techniques, can increase typing specificity.

P1426 Serotype-specific analysis of bacteraemic pneumococcal pneumonia in adults

Z. Blechova, O. Dzupova, M. Trojanek, T. Bergerova, A. Gabrielova, H. Zemlickova, J. Motlova, G. Krakorova, V. Maresova (Prague, Pilsen, CZ)*

Objectives: The objectives of this study were to describe the clinical course, laboratory features, risk factors and microbiological characteristics of adult cases of bacteraemic pneumococcal pneumonia (BPP), comparing patients hospitalized in two university hospitals.
Methods: Retrospective descriptive clinical study included adult patients (age over 18 years) with BPP hospitalized at Bulovka University Hospital and Pilsen University Hospital during 2000–2008. Clinical data were obtained from hospital records and microbiological characteristics of the isolates were determined at National Institute of Public Health in Prague.
Results: During the study period *S. pneumoniae* was isolated from blood in 135 patients hospitalized with community-acquired pneumonia (CAP). Age median was 57 years with male to female ratio 81:54. The most frequently identified risk factors were: smoking in 57 cases, chronic lung disease (40), chronic heart disease (34), diabetes mellitus in 24 cases. Disease severity was assessed using PORT/PSI with total score medians 93.5 (Bulovka UH) and 115.5 (UH in Pilsen). Parapneumonic effusion was observed in 58 patients and atelectasis in 24. Septic shock developed in 26 patients and the disease had lethal outcome in 21 patients (15.6%) with the highest case fatality ratio (27.3%) in the age cohort over 65 years. The most frequently isolated serotypes of *S. pneumoniae* were: 4 (22 cases), followed by 1 (18×), 3 (17×) and 8 (16×). Potential vaccine coverage with 23-valent polysaccharide vaccine was 91.6% and 71.7% with 13-valent conjugated vaccine. All isolates were susceptible to βlactam antibiotics (MIC's of penicillin $\leqslant 0.06$ mg/l) and resistance to other tested antibiotics was very rare.
Conclusion: Bacteraemic pneumococcal pneumonia is a serious disease with risk of complications and unfavourable outcome particularly in the elderly. The clinical and epidemiological characteristics of BPP are influenced by pneumococcal serotype. Study results imply to recommend the vaccination of the risk groups. The empirical antibiotic therapy in CAP should effectively cover *S. pneumoniae*. Susceptibility of the isolated strains to βlactams enables to recommend the use of penicillin in empirical treatment of pneumococcal infections in the Czech Republic.
Supported by a research grant A/CZ0046/2/0007 from Iceland, Liechtenstein and Norway through the EEA financial mechanism and by a research grant IGA-MZCR-9643–4.

Clinical characteristics of CAP cases caused by four most frequent serotypes

Serotype	4	1	3	8
Number of cases	22	18	17	16
Age (mean)	53.0	48.9	60.9	49.3
PORT/PSI (median)	106	68	132	71

P1427 Risk factors for microbiological contamination in allografts

V. Mirabet, M.D. Ocete, P. Solves, L. Villamayor, M. Calabuig, L. Larrea, N. Tormo, R. Roig, C. Gimeno (Valencia, ES)*

Objectives: Human cell and tissue banking involves several activities: donation, processing, storage, delivery, transplantation. Surgical procedures in the operation theatre and manipulation in the bank are the main factors related to microbiological contamination.
Methods: We have analyzed the results of microbiological cultures related to 8354 tissue pieces: 6613 musculoskeletal tissues, 533 heart valves, 1146 skin units (318 cm^2 each) and 62 vascular segments. We have considered the following variables: type of source (multiorgan donor, live donor), donor blood culture, number of pieces collected, sampling time (surgical procurement, processing, storage, delivery) and type of sample (tissue fragment, swabbing, liquid solution). In addition, we have assayed the efficacy of the disinfection protocol established in our laboratory with the use of an antibiotic solution (tobramycin, cotrimoxazole, vancomycin and amphotericin B, all of them 50μg/ml in Hanks' balanced salt solution).
Results: Samples at tissue collection are the most frequently contaminated. The bigger tissues and those requiring longer surgical procedures show higher contamination rates. Skin has especial consideration, because the presence of some microorganisms can be considered as normal. The use of a disinfection protocol reduces significantly the presence of microorganisms. The main species isolated correspond to air environmental microorganisms.
Conclusions: Algorithms for approval or rejection of tissues as allografts must be based in a quality assurance system, providing safe and clinically efficient products. Then, the process for taking decision

requires microbiological monitoring of all activities related to tissue banking and qualified personnel to analyze results.

Healthcare-associated infections: epidemiology and risk factors

P1428 National nosocomial infections surveillance network in intensive care units, France, 2004–2008

A. Savey, F. Nguyen, A. Lepape, F. L'Heriteau, P. Jarno, S. Boussat, A.G. Venier, B. Coignard on behalf of the REA-RAISIN working group*

Objectives: Within a nosocomial infections (NI) surveillance network, to assess and compare rates over time and amongst intensive care units (ICU), providing an evidence-based approach for improving infection control practices.
Methods: Since 2004, the national REA-RAISIN network conducts 6 months a year a patient-based NI surveillance in ICU. On a voluntary basis, participating ICUs collect data for each patient staying more than 2 days in the unit. Focusing on device-related infections, surveillance provides the units with incidence rates concerning ventilator-associated pneumonia (PNE), urinary tract infection (UTI) associated with indwelling urinary catheter (UC), central venous catheter (CVC) colonisation with or without catheter-associated bacteraemia (COL/CRB) and blood stream infection (BSI). Feedback of incidence data used rates per 1000 device-utilisation days, and standardised incidence ratio (SIR) for PNE.
Results: In 2008, 174 ICU included 25,225 patients. Patient characteristics were: mean age 61.0 yrs, sex-ratio 1.6, type of admission (medical 67%, emergency surgery 18%, scheduled surgery 15%), trauma 10%, impaired immunity 14%, patient origin (community 52%, acute care 41%, LTCF 4%, other ICU 3%), antibiotic treatment at admission 53%, mean SAPS II 41.9, mean length of stay 11.4 days. Device exposure was high: intubation 64%, CVC 61% and UC 85%, with device utilisation ratio being 58.9, 61.9 and 79.0 respectively.
Among 25,225 patients, 13.6% had at least one infection. Overall incidence rates were: 14.50 PNE/1000 intubation-days, 5.63 COL/1000 CVC-days (including 1.93 CRB), 3.46 BAC/1000 hosp.-days and 5.30 UTI/1000 UC-days. Unit distributions and SIR were used to compare units' performance.
From 2004 to 2008, changes in patient characteristics mainly concerned patient severity at admission (SAPSII from 39.4 to 41.9, antibiotic treatment +9.4% and impaired immunity +8.2%) and device exposure (intubation +9.0%, CVC +9.5%, UC +4.0%). Incidence rates decreased for UTI 36.3%, PNE – 10.8% and COL – 4.3%, but increased for BSI +4.5% and CRB +29.6%.
Conclusion: These data from a large sample of French hospitals represent a national reference to better document infectious risk in ICU. Feedback provides the participants with relevant information to improve care practices. Even if catheter-related infections are not frequent in ICU, their increase suggests that prevention efforts should focus on central venous catheter insertion and monitoring.

P1429 Epidemiology of nosocomial urinary tract infections in 165 French intensive care units

M. Giard, F. Nguyen, M. Leone, A. Lepape, B. Coignard, A. Savey on behalf of the REA-RAISIN working group*

Objectives: Urinary tract infections (UTI) are the most frequent nosocomial infections (NI) (30%) and the second after nosocomial pneumonia in Intensive Care Units (ICU). As they appear not very severe compared with other NI in ICU, they may not be considered as a priority in infection control. In the literature, UTI have been correlated to crude mortality ratio, but they are not considered as an independent risk factor for ICU and hospital mortality. The present epidemiological study aimed at determining the risk factors (RFs) and prognosis of UTI in ICU.
Methods: Data were collected from the national NI surveillance network (REA-RAISIN), covering 165 French ICU in 2007. A total of 22,927 patients hospitalised in ICU for at least two days were included. Both cumulative incidence of UTI per 100 admissions or 100 catheterised patients and incidence rate per 1000 urinary catheter (UC) days were assessed. Two multivariate logistic regression analyses were performed to identify the RFs for UTI and to determine if UTI is an independent RF for mortality. Confounders tested were: 1) age, gender, severity score (SAPS II), immunodeficiency, UC (percentage of exposure and duration), intubation, central venous catheter, diagnostic category, antibiotics at admission, ICU length of stay and patient origin for both analyses, and 2) additionally, nosocomial pneumonia and nosocomial bacteraemia for mortality RFs analysis. Results are expressed as percentages, medians and adjusted odds ratio (95% confidence interval).
Results: The UTI cumulative incidence was 5.7/100 admissions. The incidence rate was 6.5/1000 UC days. A total of 83.8% of the patients had a UC, 6.8% of whom developed a UTI. The duration of catheterisation was 6 [4–13] days. The most frequently isolated pathogens were *Escherichia coli* (30.5%), *Pseudomonas aeruginosa* (15.9%) and *Candida* sp. (14.8%). A bacteraemia followed UTI in 18.1% of UTI patients. Compared with patients without UTI, the UTI patients had longer lengths of stay in ICU (27 vs 6 days, $p < 0.001$) and higher crude mortality rate (23.5% vs 17.7%, $p < 0.001$). Independent RFs for UTI and mortality are described in the Table.
Conclusion: Prolonged duration (>5 days) of UC exposure increases UTI occurrence. Surprisingly, UTI are associated with a reduced risk of mortality. This may be due to the impact of antibiotic use in these patients. Nevertheless, the inconvenience for patients and the potential excess of cost should encourage specific preventive measures.

Table: Risk factors for urinary tract infection (UTI) and mortality (multivariate analysis)

	aOR[a]	95% CI[b]
UTI risk factors		
Urinary catheter duration (days)		
0	1	
1	3.38	0.78–14.67
2	0.93	0.32–2.71
3	0.73	0.37–1.43
4	1.69	0.95–2.98
5	2.68	1.55–4.63
6	3.81	2.21–6.56
⩾7	9.22	5.95–14.28
Female gender	1.94	1.71–2.21
Central venous catheter (CVC)	1.37	1.13–1.67
Intensive care unit (ICU) length of stay (1-day increment)	1.05	1.05–1.06
Antibiotics at admission	0.57	0.50–0.65
Mortality risk factors		
UTI	0.72	
Male gender	1.12	1.03–1.22
CVC	2.04	1.81–2.29
Nosocomial pneumonia	1.17	1.04–1.32
Nosocomial bacteraemia	1.90	1.61–2.24
Antibiotics at admission	1.14	1.05–1.25
Patient origin		
Community	1	
Long term care	1.11	0.93–1.34
Acute care	1.15	1.05–1.25
Other ICU	1.32	1.08–1.63
SAPS II[c]		
[0–10[	1	
[10–20[	0.28	0.11–0.70
[20–30[	0.76	0.35–1.70
[30–40[	1.31	0.59–2.91
[40–50[	2.23	1.01–4.94
[50–60[	3.89	1.76–8.62
[60–70[	5.52	2.49–12.26
[70 and over[	7.49	3.38–16.60

[a]Adjusted odds ratio, [b]95% confidence interval, [c]Simplified Acute Physiology Score II.

P1430 French multicentre survey of hospital mortality related to nosocomial infections: assessment of attributable and preventable part of deaths

A. Decoster, M.F. Demory, B. Grandbastien, V. Leclercq for the Regional Network "Nosocomial infection and mortality conference"*

Objectives: The aim of this study was 1: to evaluate the number of deaths associated with NI, 2: their relative contribution and 3: the preventable trait of NI and death through a mortality conference.
Methods: The study was performed in 14 French hospitals on 13,537 consecutive deaths (january 2007-december 2008). Patients with a Mac cabe score of 2 (short-term mortality) or who died within 2 days after admission were excluded. Medical records of the 2,355 eligible patients were reviewed for cause of death, NI and disease severity. The contribution of NI to death was assessed by an expert comity including hospital physicians and nurses in charge of the patient. Attributable and preventable trait of NI and of death were respectively assessed according to a 6-category scale and a 4-category scale of probability.
Results: Most of the 2,355 eligible patients came from medical wards (51%) or ICU (37%), and 23% (n = 552) presented at least one nosocomial infection: pneumonia (n = 280, 51%), bacteremia (n = 117, 21%), urinary tract infection (n = 55, 10%), surgical site infection (n = 33, 6%), GI tract infection (n = 33, 6%), skin infection (n = 9, 1.6%), catheter related infection (n = 8, 1.4%), bone infection (n = 7, 1.3%) and other infections (n = 7, 1.3%). Enterobacteriaceae (28%), *Staphylococcus aureus* (18%) and *Pseudomonas aeruginosa* (16%) were the most frequently identified microorganisms. The part of multidrug resistant bacteria was 57% for *S. aureus* (MRSA) and 16% for enterobacteria (ESBL). Mortality was attributable to NI for 182 patients (34% of NI) and estimated as preventable in 61 cases (11%). Death was considered as preventable in 35 cases (6%). Among them, 10 were attributable to a preventable NI in patients in which death was totally unexpected (1.8%).
Conclusion: Based on a consensual and thorough review of each patient's clinical story, this study confirms that NI is a leading cause of death and that a large part is preventable. If the same scale was used on a national level, the number of deaths attributable to NI in France would reach 3,500 (CI95%: 2,605–4,036), of which 1,300 NI (CI95%: 357–2,196) and 800 deaths (CI95%: 51–1,481) could be considered as preventable. To improve healthcare quality, mortality conferences are needed to identify circumstances that might be associated with severe NI contributing to death, and target as specifically as possible preventive measures. All healthcare workers must be associated to these mortality conferences.

P1431 Healthcare-associated infection surveillance in nursing homes in the Netherlands

N.A. Brunner, M-J. Veldman-Ariesen, A. Haenen, H. van de Sande, B.H. Benthem (Bilthoven, Leiden, NL)*

Objective: To determine risk factors of healthcare associated infections in nursing home residents.
Methods: Twenty-three nursing homes were recruited to participate in SNIV, the national sentinel surveillance network for infectious diseases in nursing homes, since 2009. Influenza like illness (ILI), gastro-enteritis (GE), and probable pneumonia (PP) cases from every nursing home were registered on a weekly basis. Infections were registered by nursing home physicians using standardized clinical definitions. Results were calculated over a 44 week period. From a baseline questionnaire, nursing home characteristics were derived, e.g. number of residents, living conditions, and influenza vaccination rate of residents and healthcare workers (HCWs). Poisson regression analysis was used to determine risk factors for the occurrence of ILI, GE, and PP.
Results: In total, 3643 nursing home residents were included. The average weekly incidence rate for GE and PP were in the same range; 3.8/1,000 cases per week and 3.7/1,000 cases per week. The average weekly incidence rate for ILI was much lower at a level of 1.9/1,000 cases per week. Table 1 shows odds ratios and confidence intervals of every significant determinant per infection. Nursing homes where residents share a toilet had a 2.6 higher risk of GE cases. Furthermore, nursing homes in which GE outbreaks occurred previously, GE incidence turned out to be higher in the current year ($p < 0.0001$). Nursing homes that didn't have single rooms only, had a 2.64 higher PP incidence ($p < 0.0001$). Furthermore, incidence was 42% higher when HCWs worked on multiple departments ($p = 0.0001$). Influenza vaccination degree of HCWs is a protecting determinant for ILI in residents, especially in the larger nursing homes and in nursing homes where HCWs are working on multiple departments.
Conclusion: Infections in nursing homes could be predicted by nursing home characteristics quite well, despite a relatively small sample size. Nevertheless, to achieve a real indication of incidence rates, infection rates should be registered for the whole year, taking seasonality of some infectious diseases into account.

Table 1. Determinants of gastro-enteritis, probable pneumonia, and influenza-like illness with odds ratios and 95% confidence intervals

	OR	95% CI
Gastro-enteritis		
– sharing of toilet	2.62	1.80–3.80
– Previous GE outbreak in 2008	4.14	2.82–6.07
Probable pneumonia		
– Sharing of rooms	2.64	1.83–3.82
– Interdepartmental labour	1.42	1.19–1.70
Influenza-like illness		
– Number of residents >158	1.41	0.98–2.03
– Interdepartmental labour	1.54	1.16–2.05
– Influenza vaccination degree of HCWs <16.3%	3.27	2.33–4.58

P1432 Incidence of and risk factors for nosocomial infection in a medical ward

E. Calbo, M. Riera, C. Nicolas, O. Monistrol, R. Font, N. Freixas, J. Garau (Terrassa, ES)*

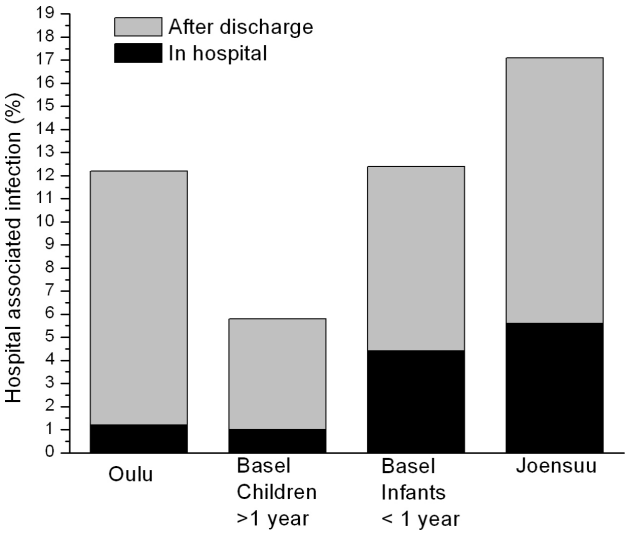
Objective: Prospective surveillance of nosocomial infection (NI) requires exhaustive resources and solid data are scarce. The aim of our study was to describe the incidence and risk factors for NI during a hand hygiene (HH) multimodal campaign in medical wards.
Methods: The study was conducted in all the medical wards of a 500-beds acute care University Hospital. All inpatients with >24 h of hospitalization were included. The surveillance was carried out in two 10 weeks periods, pre and post intervention. A multimodal campaign to improve HH compliance was conducted between the two periods. Data on potential intrinsic factors (demographics, co morbidities and functional status measured by Charlson and Barthel scores) and extrinsic risk factors (LOS, urinary and venous catheter days) for NI were collected. NI were prospectively identified by clinical findings and confirmed by laboratory and/or clinical data or physician diagnosis. NI were diagnosed and classified according to the standard definitions of the CDC 2008.
Results: 1963 patients were included. NI was diagnosed in 108 (5.5%)patients. Density of incidence was 6.8 NI/1000 admission days. Most frequently NIs identified were pneumonia 43 (39.7%), 15 of those attributed to aspiration (13.8%), and urinary tract infection 42 (38.8%). Patients with NI were more frequently female (59.6% vs. 40.4%, $p = 0.001$), were older (76 vs. 71 y-o, $p = 0.007$) and had a lower Barthel score (36 vs. 63, $p = 0.001$) as compared to non infected patients. Patients with NI had a longer LOS (18.6 vs. 8.1 days, $p < 0.001$), a higher use of venous catheters (11.7 vs. 4.8 mean days, $p < 0.001$) and urinary catheters (9.5 vs. 5.6 mean days, $p < 0.001$). No differences were found between both surveillance periods in the presence of intrinsic or extrinsic risk factors, or in the global NI rates. Independent risk factors for NI were female gender (OR 2.1, CI95% 1.3–3.5, $p = 0.003$); Barthel score (OR = 2, CI95%1.1–3.6, $p = 0.01$), LOS (OR = 1.1, CI95%=1.08–1.1; $p < 0.001$) and urinary catheter (OR = 1.1, CI95%1.05–1.1, $p < 0.001$).

Conclusions: In our hospital, the overall incidence of NI in medical wards was lower than expected according to the previous prevalence rates reported. This is important in order to accurately calculate sample size in studies designed to measure the impact of an intervention on the incidence of NI. Aspiration pneumonia was identified as an emerging problem in acute care hospitals, likely due to the increasing age of inpatients.

P1433 Hospital-associated infections in children – a prospective international multicentre post-discharge follow-up study

S. Kinnula, M. Büttcher, T. Tapiainen, M. Renko, K. Vepsäläinen, R. Lantto, U. Heininger, M. Uhari (Oulu, FI; Basel, CH; Joensuu, FI)*

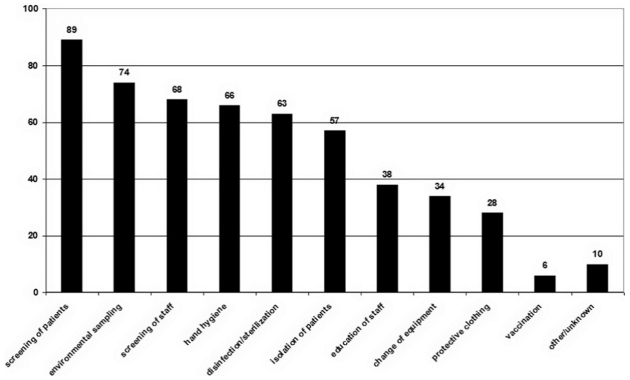
Objectives: Viruses, especially gastrointestinal and respiratory viruses, spread easily in paediatric wards causing hospital-associated infections (HAIs). Marked differences have been reported in HAI frequencies between different paediatric wards and hospitals. Reasons behind these differences are not well known. The study was done to evaluate the rate of viral HAIs and their risk factors in different paediatric settings in two hospitals in Finland (Oulu, Joensuu) and in one hospital in Switzerland (Basel).
Methods: One infectious diseases ward and three general paediatric wards were included in the survey. Data were collected prospectively for two years including a one-week follow-up after discharge with questionnaires. Post-discharge follow-up was done using a standardized questionnaire, where parents were asked for when the child was fully recovered, and if the child got symptoms of a new infection since hospital discharge and if so when the new symptoms had appeared. Altogether 5110 patients were hospitalized (mean age 3.7 years, standard deviation (SD) 4.2, mean time in hospital 4.0 days SD 6.9).
Results: Total HAI frequency was 13.5%. In hospital 3.7% of patients HAI, most often gastroenteritis. Of HAI, only 13% manifested during hospitalization whereas the great majority, 87%, became symptomatic within 72 hours after discharge. We found marked differences in HAI frequencies between the wards, the HAI frequency was the lowest in the general paediatric ward for older children in Basel and the highest in the general paediatric ward in Joensuu (Figure). The highest HAI rate was in a general paediatric ward where shared rooms were common. The lowest HAI rate was in a general paediatric ward where patients older than one year of age were hospitalized, mainly in single rooms. In logistic regression analysis shared room, young age, longer hospitalization time and antibiotic treatment were associated with the increased occurrence of HAI.
Conclusion: We suggest that single room bedding is effective in prevention of HAIs, especially preventing the spread of respiratory viruses. It also seems that treating patients with infectious diseases in their own unit is advantageous compared to a general ward.



P1434 Characteristics of polyphasic nosocomial outbreaks – a systematic review

S. Thon, R.P. Vonberg (Hannover, DE)*

Objectives: During the investigation of an outbreak, infection control efforts aim for terminating this incident at its most early stage. Some epidemic curves of nosocomial outbreaks show additional peaks of infection despite the introduction or enforcement of appropriate infection control measures. This systematic review shows the characteristics of such polyphasic nosocomial outbreaks.
Methods: A search of the medical literature was performed using the following sources: a) Worldwide Outbreak Database (www.outbreak-database.com), b) PubMed, and c) hand search of reference lists of relevant articles. Search terms applied were "Nosocomial", "Outbreak", "Epidemic", and "Polyphasic". Data got collected on setting (place, time, duration, ward, kind of unit), type of infection (number of cases, pathogen, type of infection), epidemiological features (source, mode of transmission, typing), and infection control measures. Findings from polyphasic outbreaks were then compared to 2.089 monophasic nosocomial outbreaks as filed in the Outbreak Database.
Results: 124 polyphasic nosocomial outbreaks got included, thereof 58 outbreaks in which intensive care units were involved. Surgical departments were more often affected by polyphasic outbreaks (33.9% vs. 21.9%; $p < 0.01$), and hepatitis B virus was determined as the predominanat causative agent (9.7% vs. 3.2%; $p < 0.01$). The following types of infection were more frequently observed in polyphasic outbreaks: blood stream infection ($p < 0.05$), respiratory tract infection ($p < 0.01$), wound infection ($p < 0.01$), urinary tract infection ($p < 0.01$), skin and soft tissue infection ($p < 0.05$), and meningitis ($p < 0.05$). The distribution of infection control measures is shown in FIGURE 1. These measures (except for change of equipment, protective clothing, and vaccination) were significantly more often applied in polyphasic outbreaks. There were 245 sources identified in 124 polyphasic outbreaks compared to only 2.169 sources in the 2.089 monophasic events.
Conclusion: The greater number of infections and infection control measures is most probably a consequence of the prolonged outbreak. Most likely the existence of an undiscovered additional source or the formation of such a secondary source during the first phase of the outbreak (rather than not identifying its primary source) was responsible for further outbreaks phases. Infection control staff should be aware of the possibility of a secondary source whenever investigating a nosocomial outbreak.



P1435 Five-year retrospective epidemiological survey of anaerobic bacteraemia in a university hospital

E. Urbán, G. Terhes, A. Lázár, E. Nagy (Szeged, HU)*

Objective: Bacteraemia due to anaerobic organisms occurs in 0.5–12% of blood cultures worldwide, however recent studies from EU and the US presented inconsistent data regarding the prevalence of anaerobic bacteraemias. We examined the occurrence of bacteraemias due to anaerobic bacteria during a 5-year period, in order to determine its

prevalence and evaluate the importance of anaerobic blood cultures in a university hospital in Szeged, Hungary.
Methods: Two bottles were routinely collected for blood culture: an aerobic bottle enabling preferential growth of aerobic microorganisms, and an anaerobic bottle enabling preferential growth of strict anaerobic bacteria. All blood culture bottles were placed in a BacTec system and monitored in accordance with the manufacturer's instructions. Clinical data was retrieved from patients' medical records.
Results: A total of 43,992 blood cultures were submitted to the laboratory in this period. From those, 305 (0.69%) anaerobic isolates were detected from blood cultures. The number of positive anaerobic blood culture results per 1,000 blood cultures performed decreased from 8.74 to 5.36 between 2005 and 2009. The *P. acnes* accounted for 57.7% of isolates (mainly contaminant), followed by *Clostridium* spp. (12.8%), *B. fragilis* group spp. (8.9%) and anaerobic Gram-positive cocci (5.9%) and Fusobacterium spp. (3%). During this period, the proportion of isolated anaerobic organisms compared to the number of all organisms isolated from blood cultures declined from 6.3% to 4.0%. Similar to the decrease in the number of anaerobic isolates, the number of patients with anaerobic bacteremia decreased from 69 patients to 44 patients. The most common risk factors were gastrointestinal surgery and active haematological malignancies with chemotherapy. The lower gastrointestinal tract and the oropharynx were the two most frequent presumed or proven sources for relevant bacteraemia. Fatal outcome correlated with the severity of underlying diseases and the immunosuppressed status of the patients rather than with the causative pathogen or the effectiveness of antimicrobial therapy.
Conclusions: In this report we retrospectively analyzed the low proportion of anaerobes out of total blood cultures and the distribution of different anaerobic bacteria isolated from positive anaerobic blood cultures in order to characterize their involvement in anaerobic blood cultures during a 5-year period.

P1436 Risk factors associated with inadequate initial empirical antibiotic therapy for nosocomial *Pseudomonas aeruginosa* bacteraemia in non-haematologic patients

H.H. Chang, S. Jung, S.W. Kim, K.H. Park, J.M. Lee, N. Kim (Daegu, Gwangju, KR)*

Background: There is a few report about risk factors associated with inadequate initial empirical antibiotic therapy in non-hematologic inpatients with nosocomial *Pseudomonas aeruginosa* bacteremia.
Methods: The medical records of adult patients (age >18 years) with *Pseudomonas aeruginosa* bacteremia in two tertiary teaching hospitals from January 2004 to June 2008 were evaluated. Among total 280 cases of *Pseudomonas* bacteremia occurred during study period, 88 cases of community acquired bacteremia and 69 cases that occurred in patients who had hematologic diseases or neutropenic fever were excluded. Demographic, clinical, laboratory and treatment data were collected, and statistical analysis was performed using SPSS® ver.17.0 program. Inadequate empirical antibiotic therapy was defined as no antibiotic or starting non-susceptible antibiotics within 48 hours after initial positive blood culture.
Results: 123 episodes of nosocomial *Pseudomonas aeruginosa* bacteremia were evaluated. The most common source of bacteremia was intra-abdominal infections(55, 44.7%), followed by pneumonia(24, 19.8%), urinary tract infections(23, 19.0%) and catheter-related bloodstream infection(17, 13.8%). 60 cases(48.8%) received appropriate initial empirical antibiotic therapy. In univariate analysis, the statistically significant risk factor for inadequate initial empirical antibiotic therapy was bacteremia occurred in general ward[odds ratio 1.786(95% C.I.1.041–3.063), p = 0.015], bacteremia originating from urinary tract infection [odds ratio 1.777 (95% C.I.0.933–3.384), p = 0.041], but catheter-related bacteremia was a major factor against inadequate initial empirical antibiotic therapy [odds ratio 0.527 (95% C.I. 0.387–0.718), p = 0.003]. In multivariate analysis, catheter-related bacteremia was a major factor against inadequate initial empirical antibiotic therapy[odds ratio 0.246 (95% C.I. 0.062–0.985), p = 0.047]. There was no significant difference in clinical course and outcomes between patients receiving inadequate and adequate antibiotic therapy.
Conclusions: Over a half of the patients with nosocomial *Pseudomonas aeruginosa* bacteremia received inadequate initial empirical antibiotic therapy. Bacteremia originating from urinary tract infections and occurring in general ward need more physician's attention about the probability of *Pseudomonas aeruginosa* bacteremia.

P1437 The resistible rise and fall of a burns and ICU-related hospital outbreak of multi-resistant *Acinetobacter baumannii*

T. Gottlieb, R. Bradbury, E. Cheong (Sydney, AU)*

Objectives: A report of a 9 year period of surveillance and management of *Acinetobacter baumannii* (MRAB) and its ultimate effective control.
Results: Concord Hospital provides the major state-wide Burns service for the state of NSW (Australia). In 2000, the first strains of carbapenem resistant OXA-23 producing *Acinetobacter baumannii* (MRAB) were identified in two Burns Unit patients. This resulted in a clonal outbreak affecting both Intensive Care and Burns unit patients. MRAB became endemic with peaks of prevalence related to difficulties in cleaning, crowding and infection control management. These related to events such as a change to a temporary intensive care unit, severe bushfires, mattress contamination and the Bali bombing in 2002. The latter event was due to polyclonal MRAB strains, but after a few months there was a reversion to the original MRAB clone. In the 12 months period from June-July 2000–01 there were 54 patients who became colonised with MRAB, a median of 13 days after admission. At its peak in 2001, 35% of burns inpatients became colonised by MRAB. Between 2000–2006, there were 59 bacteraemias with a 7 day mortality of 15% and 30 day mortality of 34%. Of clinical specimens collected in 2000–2006, 16% were from blood cultures, mostly related to iv cannula sepsis.
An intensified environmental screening and unit cleaning service was instituted in early 2005, and will be described. Since improvement in infection control management there has been a dramatic reduction in incidence of MRAB at Concord Hospital. In 2008 3 patients were colonised (an incidence of 3% of Burns patients), in 2009 no MRAB isolates have been identified on cultures to date. The rate of MRAB has fallen from 35% of all Burns patients colonised yearly in 2001 to 0% in 2009.
Conclusions: Hospital MRAB rates are reversible. Attention to scrupulous environmental cleaning and identification of patient and inanimate reservoirs are crucial for effective control but require considerable administrative and staff support.

P1438 Ranking the pathogens: a competing-risks model on the effect of ICU-acquired bacteraemia with different pathogens on ICU mortality

M.J. de Regt, A.M. de Smet, R. Willems, M. Bonten (Utrecht, NL)*

Objectives: In studies quantifying the effect of bacteremia on ICU-mortality, ICU discharge is a competing endpoint: after discharge patients can no longer die in ICU. Logistic regression addresses this issue, but cannot deal with the time-dependent nature of bacteremia, which might lead to time-dependent bias. Survival models can address time-dependency, but cannot account for competing endpoints. Competing risks models can incorporate competing endpoints and time-dependent covariates. We used this methodology to quantify prognosis of ICU-acquired (ICU-acq) bacteremia on ICU mortality and ICU discharge.
Methods: Data on ICU-acq bacteremias were collected in a large multi-center study in the Netherlands (NEJM 2009;360:20). ICU-acq bacteremia was defined as a positive blood culture >48 h after ICU-admission; patients with an ICU stay of <3 days were excluded. Only the first event of ICU-acq bacteremia was taken into account. Polymicrobial bacteremia was defined as isolation of >1 microorganism <48 h. Coagulase-negative staphylococci, Micrococcus spp, *Bacillus* spp, diphteroids and propionibacteria were considered contaminants.

Data were analysed using a competing risks model based on Cox proportional hazards models. Patients with an ICU stay >40 days were administratively censored.
Results: Of 5939 included patients, 5456 were eligible for analysis, of which 266 (5%) were censored at day 40. 769 patients had ICU-acq bacteremia (353 with a contaminant). The highest daily hazard ratio (HR) for mortality after ICU-acq bacteremia was obtained for non-fermenters (HR: 3.14, 95% CI 1.94–5.02) (table 1). In addition, the daily risk for discharge decreased (HR: 0.63, 95% CI 0.39–0.99), further enhancing the cumulative risk for dying in ICU. Similar prognostic effects were found for ICU-acq bacteremia with *Candida* spp, enterococci, Enterobacteriaceae and polymicrobial infection. ICU-acq bacteremia with streptococci or *S. aureus* was not significantly associated with changed mortality or discharge rates. Although bacteremia with contaminants did not increase mortality rate (HR:1.12, 95% CI 0.87–1.45), patients had an increased cumulative risk of dying in the ICU due to a prolonged ICU stay (HR: 0.79, 95% CI 0.69–0.91).
Conclusion: The prognosis of ICU-acq bacteremia differs markedly between pathogens and imposes, for some pathogens, a directly increased risk of dying per day as well as an indirect risk of dying in ICU because of a reduced daily probability of ICU-discharge.

Table 1

Group	n	HR	95% CI	p-value	HR discharge	95% CI	p-value	Onset, median (IQR)
Non-fermenters	47	3.12	1.94–5.02	<0.001	0.63	0.39–0.99	0.05	12 (13.5)
Candida spp./yeasts	28	2.74	1.55–4.87	<0.001	0.55	0.31–0.96	0.04	6.5 (5.25)
Polymicrobial	42	2.69	1.69–4.27	<0.001	0.45	0.27–0.74	0.001	11.5 (13)
Enterococci	114	2.31	1.68–3.16	<0.001	0.52	0.39–0.69	<0.001	12 (11)
Enterobacteriaceae	130	2.13	1.55–2.91	<0.001	0.49	0.38–0.66	<0.001	11.5 (14)
Streptococci	18	1.63	0.61–4.35	0.33	0.91	0.50–1.64	0.75	5 (7.5)
Contaminants	353	1.12	0.87–1.45	0.38	0.79	0.69–0.91	<0.001	9 (9)
S. aureus	37	0.94	0.42–2.11	0.88	0.71	0.47–1.09	0.12	7 (9)

n = number of patients; HR = hazard ratio; IOR = interquartile range.

P1439 Polymicrobial bacteraemias in ICU patients. Impact on outcome

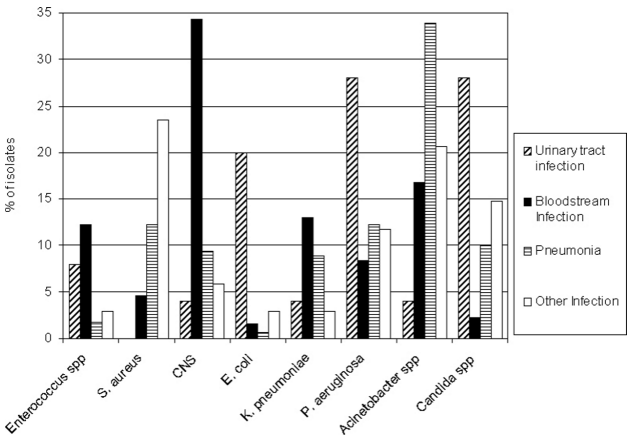
S. Sancho, R. Zaragoza, J. Camarena, A. Artero, R. González, J. Nogueira (Valencia, ES)*

Introduction: The aims of this study were to know the epidemiological and clinical features of polymicrobial bacteremias in UCI patients and to know their impact on mortality.
Methods: From January 2000 to Juny 2009 all clinically significant bacteremias in a teaching hospital were collected. Clinical and microbiological characteristics were recorded. Polymicrobial bacteremia was defined when two or more microorganisms were isolated in the same blood culture. Multivariate analysis was used to determine the differences between polymicrobial and monomicrobial bacteremias and to analyse the factors associated to related mortality to infection, using the SPSS package (13.0).
Results: Among 293 ICU-bacteremias, 45 (15.3%) were polymicrobial. The mean age of patients was 61.9 SD 14 years. Bacteremia was hospital-acquired in 93.3% of these cases. 26.7% developed septic shock and 57.8% severe sepsis. The main sources of bacteremia were: respiratory (31.1%), catheter (17.7%) and abdominal foci (13.3%). The microorganisms most frequently isolated were: *Acinetobacter baumannii* (35.5%), *Enterococcus* spp (24.4%) and *Pseudomonas aeruginosa* (22.2%). Empirical antimicrobial treatment was inadequate in 28.9% of these cases. The global mortality rate was 53.3% and the related mortality 22.2%. The isolation of *Enterococcus* spp. in blood culture was the only factor associated with polymicrobial bacteremias (p = 0.037; OR = 7.06; 95% CI: 1.12–44.51). The factors associated with related mortality were: *S. aureus* (p = 0.004; OR = 10.06; 95% CI: 2.07–48.94), the presence of septic shock (p = 0.0001; OR = 9.80; 95% CI: 3.01–31.89), the APACHE II during bacteremia (p = 0.0001; OR = 1.21; 95% CI: 1.11–1.31), and inadequate empirical antimicrobial treatment (p = 0.018; OR = 4.24; 95% CI: 1.28–14.11) but not polymicrobial bacteremias.
Conclusions: Polymicrobial bacteremias had a high prevalence in the ICU, were hospital-acquired, and their main sources of infection were repiratory and abdominal. *Enterococcus* spp were the unique microorganism associated with polymicrobial bacteremias. Although the rate of inadequate empirical antimicrobial treatment was high, mortality rates were not higher in polymicrobial bacteremias than in the group with monomicrobial bacteremias.

P1440 Incidence and microbiology of intensive care unit acquired infections in the region of Crete in Greece

C. Tsioutis, E.I. Kritsotakis, K. Chaniotaki, M. Roumbelaki, D. Babalis, A. Gikas on behalf of the Cretan Coordination Center for Nosocomial Infection Control*

Objectives: To determine the incidence of intensive care unit (ICU) acquired infections and to specify the microbiological and antibiotic resistance profiles of infecting organisms in the region of Crete in Greece.
Methods: An active surveillance protocol was introduced in the ICUs of five major public hospitals in the region. All patients who presented an infection at least 48 hours after ICU-admission were surveyed until discharge. Infections were defined according to the Centers for Disease and Prevention criteria. Infecting pathogens and antibiotic-susceptibility test results were obtained from each hospital's laboratory reports. Multi-drug resistant isolates were defined as those resistant to two or more classes of antibiotics.
Results: During February to December 2007, 1249 patients who were hospitalized in the ICUs for a mean length of stay of 7.5 days acquired 307 infections for an overall incidence rate of 32.8 infections per 1000 patient-days. Three infection sites represented 92.8% of all infections; lungs (43%), bloodstream (42.3%), and urinary tract (7.5%). Eighty-five percent of the recorded ICU-acquired infections were culture positive, 34% of which were polymicrobial. Of the 370 bacterial strains isolated, 55.7% were Gram-negative microorganisms, 34.8% Gram-positive microorganisms, 9.2% fungi, and 0.3% were anaerobes. Most frequently isolated pathogens included *Acinetobacter* spp (24.6% of all isolates), coagulase-negative *Staphylococcus* (17.6%), *Pseudomonas aeruginosa* (11.9%), *Staphylococcus aureus* (9.7%), *Klebsiella* spp (9.5%), *Candida* spp (8.9%), and *Enterococcus* spp (5.9%). The distribution of pathogens by site of infection is shown in the Figure. Overall, 81% of *Acinetobacter* spp, 25% of *P. aeruginosa*, and 53% of Enterobacteriaceae isolates were multi-drug resistant. Thirty-three percent of the *S. aureus* isolates were methicillin resistant, and 28.6% of *Enterococcus* isolates were vancomycin resistant.
Conclusion: ICU-acquired infections constitute a major problem in the region, complicated by the high rates of multi-drug resistant pathogens. Study results emphasize the need for comprehensive infection control programs to reduce the burden of nosocomial infections in the ICUs of the region.



P1441 High frequency of non-fermenters among respiratory isolates from hospital-acquired pneumonia and ventilator-associated pneumonia in Asian countries: an ANSORP study

J.H. Song (the Asian Network for Surveillance of Resistant Pathogens (ANSORP)*

Objectives: Pathogen distribution of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) is important to select appropriate empirical antimicrobial therapy. Although it might vary by country, few data have been available in Asian countries.
Methods: The Asian Network for Surveillance of Resistant Pathogens (ANSORP) performed a prospective surveillance study of respiratory isolates from HAP and VAP in adult patients from 75 hospitals in 10 Asian countries from 2008 to 2009. The bacterial isolates were classified into definite, probable and possible pathogens according to the type of specimens.
Results: A total of 2,640 cases of HAP or VAP were enrolled and 1,508 cases of HAP and 898 of VAP were evaluated. Cultures of respiratory specimens were positive in 69.4% of cases. In HAP, *Staphylococcus aureus* (15.8%) and *Pseudomonas aeruginosa* (15.5%) were the most frequent isolates followed by *Acinetobacter* spp. (12.7%), and *Klebsiella pneumoniae* (11.0%). In VAP, *Acinetobacter* spp. was the most frequently isolated (35.4%) followed by *P. aeruginosa* (26.0%), *K. pneumoniae* (17.0%) and *S. aureus* (12.9%). Among definite pathogens of HAP or VAP, *S. aureus* was most frequent (22.1%) followed by *Acinetobacter* spp. (13.2%), *P. aeruginosa* (13.2%), *K. pneumoniae* (11.8%), and *E. coli* (11.8%). *P. aeruginosa* and *Stenotrophomonas maltophilia* were more frequent in late-onset HAP than in early-onset HAP, while *Acinetobacter* and *P. aeruginosa* were more frequent in late-onset VAP than in early-onset VAP. The distribution of the bacterial isolates differed by country: *S. aureus* (Korea); *Acinetobacter* (China, Thailand, and Malaysia); *P. aeruginosa* (Hong Kong and Taiwan) and *K. pneumoniae* (Philippines, Indonesia, and Singapore) were the most common respiratory isolates.
Conclusion: *Acinetobacter*, *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* were the most frequent respiratory isolates from adults with HAP or VAP in Asian countries. *Acinetobacter* and *P. aeruginosa* were frequent pathogens in Asian countries, especially in cases of late-onset VAP.

P1442 High burden of *S. aureus* bloodstream infections in European hospitals, irrespective of methicillin susceptibility

M. de Kraker, M. Wolkewitz, H. Grundmann on behalf of the BURDEN Study Group*

Objective: Determine the excess mortality and the excess length of stay attributable to methicillin resistant *S. aureus* (MRSA) blood stream infections (BSI) in Europe using a novel approach.
Methods: BSI caused by MRSA affect patients that are older, more ill and have a longer hospital stay than BSI caused by methicillin susceptible *S. aureus* (MSSA), making direct comparisons difficult. In this study a parallel matched cohort design was used, where patients with a *S. aureus* BSI (exposed) were compared to patients without such a BSI (controls). Two parallel cohorts were constructed, comparing either MRSA or MSSA exposure. Matching was based on duration of admission prior to enrolment. Thirteen hospitals from as many countries participated in this study from July 2007 to July 2008. Hospital patients were routinely sampled and all patients above 18 years with a *S. aureus* BSI were included as exposed patients. Modern statistical methods were used for comparing mortality and length of stay within the two parallel cohorts: multivariate Cox's regression for competing events and a multivariate generalized linear model with gamma distribution.
Results: In total 2489 patients could be included into the study: 248 patients had a BSI caused by MRSA, 618 patients had MSSA BSI and 1623 were controls. Hospital mortality for MRSA patients was 36% compared to 9% for the controls. For MSSA patients 22% died and 7% of the controls died in the hospital. Cox's regression for competing events showed that MRSA patients died more often than the controls (hazard ratio (HR) 3.5, confidence interval (CI) 2.4–5.2). MRSA patients also stayed longer in hospital. The overall difference in length of stay was 9.2 days (interquartile range (IQR) 5.2–13.5). The impact of a MSSA BSI on in-hospital mortality was comparable to the HR found in the MRSA cohort (HR 3.1, CI 2.3–4.2). MSSA patients stayed 8.6 days longer in hospital than the controls (IQR 6.8–10.4). When combining the data of the two parallel cohorts, the HR for in-hospital mortality was 1.1 times larger (CI 0.7–1.8) for MRSA versus MSSA, the excess length of stay was 0.6 days (IQR –3.7–5.3).
Conclusions: The burden of *S. aureus* blood stream infections in the hospital is high, for MRSA as well as MSSA. Infection prevention should be aimed at decreasing the number of *S. aureus* blood stream infections, irrespective of methicillin susceptibility.

P1443 Catheter-related bloodstream infections among VINCat hospitals: the impact outside the ICU

F. Bella, N. Freixas, B. Almirante, M. Pujol, E. Limón, J. Vallés, F. Barcenilla, L. Matas, F. Gudiol on behalf of the VINCat Committee*

Background: Catheter-related bloodstream infections (CR-BSI) continue to be a cause of high morbidity and mortality rates despite they are the most preventable health-care associated infections. Surveillance of CR-BSI is useful for quality improvement, however, while there is a huge amount of information based on Intensive Care Units (ICU), only few data concerning non-ICU patients are available.
Objective: to determine the comparative frequency of CR-BSI in ICU and non-ICU patients admitted to VINCat hospitals during 2008.
Methods: VINCat is a nosocomial infection surveillance program in Catalonia, (6.5 million population), in Spain. Beginning 2007, 39 hospitals perform laboratory based prospective standardized surveillance for CR-BSI using CDC definitions. Rates of central venous catheter (CVC) BSI and short and midline peripheral venous catheter (PVC) BSI were expressed by 1.000 patient-days according to area of hospitalization.
Results: From January to December 2008, 608 episodes of CVC-BSI (0.20 ep/1.000 patient-days) and 117 episodes of PVC-BSI (0.04 ep/1.000 patient-days) were recorded. Among patients with CVC-BSI, 232 episodes were acquired in the ICU, 199 in medical wards and 177 in surgical wards. While the crude number of CR-BSI were higher in non-ICU areas, adjusted rates for ICU patients were: 1.9 ep/1.000 patient-days, for medical patients: 0.12/ep/1.000 patient-days and for surgical patients: 0.14/ep/1.000 patient-days. Among patients with PVC-BSI, 18 episodes (0.15 ep/1.000 patient-days) were observed in ICU patients, 30 (0.02 ep/1.000 patient-days) in surgical patients and 69 (0.05 ep/1.000 patient-days) in medical patients. Among patients with CVC-BSI, coagulase-negative staphylococci (CNS) were the causative microorganisms in 50% of cases, followed by *Staphylococcus aureus* (14%) and *Pseudomonas aeruginosa* (7%). In contrast, patients with PVC-BSI had more frequently *S. aureus* (53%) followed by CNS (30%).
Conclusions: The VINCat surveillance program has allowed us to determine that CR-BSI among non-ICU patients remains largely underestimated and are frequently caused by *S. aureus*. Targeted interventions in specific areas are needed to reduce this serious complication.

P1444 The clinical outcome of intravascular cathetertip colonization with Gram-negative micro-organisms in patients without preceding bacteraemia

A. van Eck van der Sluijs, J.J. Oosterheert, M. Ekkelenkamp, A. Hoepelman, E. Peters (Utrecht, NL)*

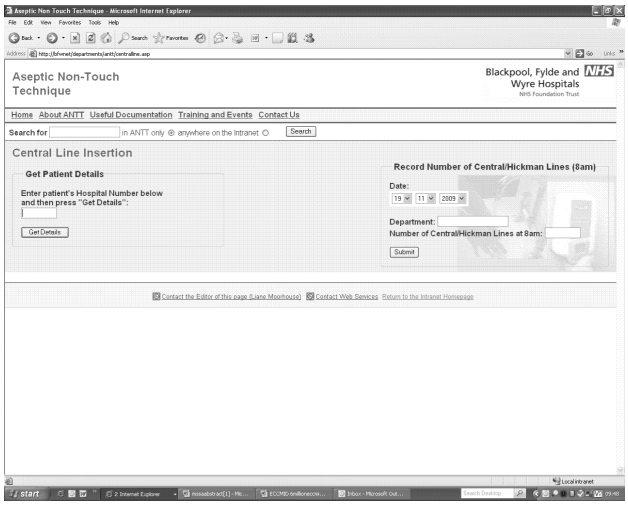
Introduction: Although Gram-negative micro-organisms are frequently associated with catheter-related bloodstream infections, the prognostic value and clinical implication of a positive cathetertip culture with Gram-negative micro-organisms remains unclear. The aim of this study was to determine the outcomes of patients with intravascular catheters colonized with these micro-organisms, without preceding positive blood cultures,

and to identify the risk factors for the development of subsequent bacteremia.
Methods: All patients with positive intravascular cathetertip cultures with Gram-negative micro-organisms between 2005 and 2009 were retrospectively studied at the University Medical Center Utrecht, the Netherlands. Sixty-seven patients with a positive blood culture in a period of 48 hours before catheter removal were excluded. Possible risk factors for Gram-negative bacteremia were recorded. The main outcome measure was bacteremia with Gram-negative micro-organisms. Other endpoints were duration of hospital admission, in-hospital mortality, secondary complications of Gram-negative bacteremia and duration of intensive care admission.
Results: Two-hundred-and-thirteen catheter tips from 181 patients were colonized with Gram-negative micro-organisms. Forty (19%) developed subsequent Gram-negative bacteremia. Multivariate analysis showed that arterial catheters were associated with the development of subsequent Gram-negative bacteremia (odds ratio (OR)=5.17, 95% confidence interval (95% CI): 1.24–21.60) and jugular catheters were a protective factor (OR = 0.21, 95% CI: 0.046–0.98). Mortality was significantly higher in the group with subsequent Gram-negative bacteremia (35% versus 20%, OR = 2.12, 95% CI: 1.00–4.49).
Conclusion: Intravascular cathetertip colonization with Gram-negative micro-organisms can predict a subsequent Gram-negative bacteremia. A bacteremia is more likely in patients with an arterial catheter and the patients with a Gram-negative bacteremia seemed to have a higher mortality. This may have clinical implications for the management of intravascular catheter tips colonized with Gram-negative micro-organisms in patients without preceding bacteremia.

P1445 Central venous catheter and catheter-related bloodstream infections surveillance: an innovative use of 20th century technology for 21st century solutions

M. Przybylo, L. Moorhouse, S. Staff, S. Hamid, A. Guleri (Blackpool, UK)*

Objectives: Catheter-related blood stream infections(CR-BSI) account for a third of all post-48 h BSI. A comprehensive HCAI containment programme(CHCP) was initiated in 2008 in Blackpool Victoria Hospital. The trust is investing in a cutting edge electronic patient record system (EPR) to modernize patient record system, enhance clinical quality and patient safety but the e-referral, e-prescribing, e-surveillance will be rolled out in 2nd phase of the project. Currently data capture on all central venous catheters (CVC) inserted, monitoring and CR-BSI was quite challenging with staff limited infection control team (ICT) working on other HCAI reducing initiatives. We present an innovative use of current and limited IT system in addressing a challenge – surveillance/audit of CVC bundles & CRBSI.
Methods: An innovative use of existing IT technology to create a facility from intranet page for e-registration of CVC data was discussed with hospital webpage planning department. The switchover to 'CVC e-register' began in May09. Hospital computers are password protected. A hyperlink for CVC e-register on trust intranet page, opens a simple input form. Medical profession inserting a CVC is required to enter basic essential data and submit it to ICT managed database. The head nurse of critical care & wards submit a daily tally of number of patients with CVCs. This database is used to obtain daily patient list, central collation of data from follow up of patients outside critical care, line care data, outcomes & CRBSI.
The database is cross referenced against positive blood cultures. This system alerts the microbiologist who can then clinically advice clinical team of the patient.
Results: Since May 2009 – 493 central venous procedures were recording using the CVC e-register. 31% (151) CVCs, 12% (59) Hickman lines, 56% (276) others and <1% femoral and Picc lines. Ward followup surveillance of CVCs reveals 33% in medical, 9% in surgical & 59% in critical care unit. Compliance has increased with time and feedback to clinical teams (11–91%). Details to be presented.
Discussion: 42% of CVCs inserted on critical care are managed on wards with limited expertise of CVC line care. Feeding back of results and raised awareness of the CHCP has increased compliance for using CVC e-register. Phase 2 of this project would be integration with the EPR in about 12-months. This innovative use of existing technology has been successful in gathering data on CRBSI and CVC use/care.



P1446 Catheter-related bacteraemia and infective endocarditis caused by *Kocuria* species

C.Y. Liu, C.C. Lai, C.H. Liao, P.R. Hsueh (Taipei City, TW)*

Objective: *Kocuria* species is a member of the Micrococcus family, most are normal flora of human skin and mucosa. Misidentification of coagulase-negative staphylococcus is not uncommon, which requires genomic sequencing for precise identification. We reported five patients with *Kocuria* spp. Bacteremia from a university hospital from 2006 to 2009.
Methods: We retrospectively review the clinical features of patients with *Kocuria* spp. bacteremia from January 2006 through June 2009 at National Taiwan University Hospital. The identification of *Kocuria* spp. was confirmed by 16S rRNA gene analysis. Demographic data, underlying diseases, clinical, microbiological, treatment and outcome were collected.
Results: A total of 21 blood isolates from five patients were identified as *Kocuria* spp. by conventional biochemical methods during the study period. Twenty isolates of *K. kristinae* were confirmed by 16S rRNA. One isolate initially identified as *K. varians* was finally confirmed to be *K. marina.* The clinical courses of four patients indicated that significant infections were caused by *K. kristinae,* while the other patient probably reflected contamination. Various underlying conditions, including gastric cancer, short bowel syndrome and Port-A catheter for total parental nutrition were found. One patient had clinically diagnosed infective endocarditis. All infections were successfully treated with antibiotic therapy and removal of catheters without infection-related mortality.
Conclusions: *Kocuria* species is an emerging cause of infection in immunocompromised patients. The clinical manifestations are protean, and include catheter related infection and infective endocarditis. Accurate identification with molecular methods is imperative for the diagnosis of these unusual pathogens.

P1447 Microbiological investigation in patient care with invasive devices

D. Platace (Riga, LV)*

Objective: In modern day medicine the invasive devices continue to be essential for the management of critically ill patients. Approximately 50% of health care-associated infections (HAIs) happen due to the

combined effect of the patient's flora and invasive devices. The risk for HAI is 11% to 38% with colonization by methicillin-resistant *S. aureus*, 25% with colonization by vancomycin-resistant enterococcus, and as high as 38% with colonization by *Candida*.
Methods: By means of microbiological investigation the contamination level of the ICUs and surgical units was determined: a) using a Count-Tact applicator and a culture medium specially selected for this method (n = 100), b) using the swab method (n = 90), c) using the urinary and peripheral venous catheters sedimentation method (n = 30), d) using air microbiological contamination test method with "Sas Super Iso 100" (n = 199). The samples taken with the aim of identifying bacterial species present were put on selective culture mediums. Interpretation of the Count-Tact method results was performed according to the risk level present and the colony forming unit (CFU) count on a 25 cm^2 surface.
Results: The amount of microorganisms on the nurses' hands exceeded acceptable level sixteen-fold, and medium to high levels of bio-contamination were discovered on patient's changed bed sheets as well as in nurses' hair and their workwear. *Mucor* fungi were detected in 78.5% (44/56) cases on nurses' workwear. *Aspergillus*, Penicillium, *Mucor* fungi were identified in all air samples. In 21.4% (3/14) of cases *Staphylococcus aureus* was discovered in the sterile zone of the peripheral venous catheter 72 hours and 96 hours after catheter's insertion. Both 72 hours and 7 days after the insertion of a urinary catheter 100% (16/16) of the cases revealed the presence of at least one and in some cases several of the following pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, β haemolytic *Streptococcus* and fungi, such as *Candida albicans* and *Candida tropicalis*.
Conclusions: The medium to high levels of microbiological contamination and urinary catheters' colonization with pathogens were determined in patients care with invasive devices. Although microbiological contamination does not necessarily lead to infection, it is nevertheless an indication that infection control measures can be improved.
This study has been supported by the project of Europen Social Fond (ESF).

P1448 Infections in patients undergoing craniotomy: first attempt to identify risk factors associated with post-craniotomy meningitis in Greece

I. Kourbeti, G. Bertsias, D. Karabetsos, C. Neophytou, M. Filippou, A. Ioannou, A. Vakis (Chalkida, Heraklion, GR)*

Objectives: Nosocomial meningitis is usually seen in neurosurgical patients after craniotomy and leads to prolonged length of hospital stay (LOS), surgical revisions, neurological impairment or death. We conducted a retrospective cohort study to determine the incidence, bacteriology and risk factors for post-craniotomy meningitis (PCM) in Greece and describe the infection rates in patients undergoing craniotomies.
Methods: Patients >18 years old, having non-stereotactic craniotomies between 1/1999 and 12/2005 in University of Crete Medical Center were eligible. Surgical Site infections (SSI) and other infections were defined using NNIS criteria. Data were analyzed with SPSS.
Results: 619 surgeries for 479 patients (64.6% male) were analyzed. The median age was 48. Most craniotomies were performed for trauma (41%). The median delay to surgery was 2 days. The median length of stay was 13 days. There were 38 cases of PCM (6.1%). The median interval to the diagnostic lumbar puncture was 13 days.5 patients had >1 episode. 74% of the episodes were culture confirmed. Univariate analysis revealed that meningitis was associated with concomitant infection outside the surgical field ($p < 0.001$), presence of another SSI ($p < 0.001$), low pre-op GCS ($p = 0.016$, emergency procedure ($p = 0.005$), ICP placement ($p = 0.009$) and duration of ICP monitoring ($p = 0.004$), presence of any drain and duration of drain placement ($p < 0.001$), presence of a ventricular and lumbar drains ($p < 0.001$), CSF leak ($p < 0.001$), revision surgery ($p = 0.017$), admission to ICU ($p = 0.007$), prolonged ICU stay ($p < 0.001$), length of stay from admission to surgery ($p = 0.012$) and use of dural substitute ($p = 0.003$). Multivariate analysis indicated that emergency procedure, concomitant infection, malignancy and drain presence, especially of lumbar and ventricular drains were independently associated with the risk of meningitis. The overall mortality in the cohort was 13.4% but it was 31.6% in patients who developed meningitis (OR 3.3). Ventilator associated pneumonia (VAP) was the most common infection outside the surgical field (13.1%). SSI other than meningitis/ventriculitis developed in 9%. 26% of the patients developed at least one infection.
Conclusion: A significant percentage of patients undergoing craniotomy developed at least one infection with VAP being the most prevalent. SSI were second. Continuing device-related perioperative communication of the CSF and the environment, concomitant infections and emergency surgery were independent risks factor for PCM development.

P1449 The influence of nutritional status on the emergence and evolution of nosocomial infections

M. Gamaletsou, K.A. Poulia, D. Karageorgou, M. Yannakoulia, N. Sipsas (Athens, GR)*

Objectives: The aim of the present study was to record the incidence of the emergence of the malnutrition in a sample of elderly patients and to look into a possible association of malnutrition with the emergence of nosocomial infections.
Methods: Patients over 60 years old admitted to a tertiary medical center of Athens from January until September 2009 were included in the study. On admission, anthropometric measurements and nutritional status was evaluated by the Subjective Global Assessment questionnaire (SGA). During hospitalization, nutritional status, nutritional interventions, laboratory results and applied treatment were recorded, while patients were observed for nosocomial infections development.
Results: 248 patients aged 75.22±8.459 years were included. According to statistical analysis of data, 54% of patients had mild to severe nutritional status on admission day. Poor nutritional status correlated with low albumin levels and increased weight loss in the recent past. The emergence of nosocomial infections was associated with the nutritional status of the patients, since 39.5% of those who had poor nutritional status developed a nosocomial infection, whereas only 11.8% of those with good nutritional status developed a nosocomial infection ($p = 0.016$). The emergence of nosocomial infections was associated with a greater weight loss in the recent past ($p = 0.022$), a longer hospitalization duration ($p < 0.001$) and a higher mortality rate ($p < 0.001$).
Conclusion: The results of this prospective clinical study underscore the necessity of nutritional status assessment on admission day and timely nutritional intervention, when indicated, in order to decrease the risk of nosocomial infections.

Surgical site infections

P1450 Are antibiotic impregnated cement spacers safe in the treatment of two-stage orthopaedic revision surgery?

M.L. Sorlí, R. Torres, L. Puig, A. Gonzalez, A. Alier, M. Montero, J. Villar, H. Knobel, J.P. Horcajada (Barcelona, ES)*

Background: In two-stage orthopedic revision surgery, the use of antibiotic-impregnated cement spacer is a common procedure. We hypothesized that culturing of samples obtained from the cement spacers would improve the microbiological diagnosis of subclinical prosthetic joint infections.
Methods: Prospective cohort study. Patients undergoing a second-stage orthopedic revision surgery from January 2007 to July 2008 were prospectively included. Before second-stage surgery patients received 6 weeks of guided antimicrobial therapy and had an antibiotic free period of 3.2 weeks (4.1 SD). Periprosthetic tissue (PT) specimens (5) were collected for culture. Removed spacers were sonicated during 5 min. (40Hz). Both PT and sonicate-fluid (SF), were inoculated in aerobic agar (Chocolate Polyvitex), anaerobic agar (Schaedler + 5% blood) and in thioglycolat for 7 days. Cut-off positive culture was defined as growing

of >5 CFU on either plate. Subclinical prosthetic joint infection (SPJI) was defined as having at least one positive culture. All patients with SPJI diagnosis were treated with guided antibiotic therapy. Microorganisms isolated were assessed and compared with those isolated in the first-stage surgery. Clinical status was evaluated after a median follow-up of 44 weeks. Clinical failure was defined when purulence in the synovial fluid or implant site, sinus tract and/or clinical signs of acute inflammation were present.
Results: 55 patients were included (37 knee, 17 hip, 1 shoulder). 3 patients dropped from follow up. 10 (19.2%) SPJI were detected. Clinical failure was found in 17 (32.7%) patients. 70% of these were patients previously diagnosed of SPJI (7 out of 10), and 23.8% were patients with negative cultures (10 out of 42). p*f* -0.009.
Conclusions: (1) Positive cultures of sonicated spacers or PTs (SPJI) were found in 19.2% of patients undergoing a second-stage orthopedic revision surgery. (2) SPJI is associated with a poor clinical outcome. (3) Periprosthetic tissue cultures and sonication should be done in order to rule out SPJI.

P1451 Reducing surgical site infection rates in orthopaedic implant surgery: results of a 6-year infection control programme

R. Finkelstein, O. Eluk, T. Mashiach, D. Levin, B. Peskin, G. Nirenberg, S. Karkabi, M. Soudry (Haifa, IL)*

Background: To report the results of an infection control program on surgical site infections (SSIs) complicating total hip and total knee replacement.
Methods: Prospective cohort study of patients undergoing total hip and total knee replacement. Interventions included prospective surveillance, chlorhexidine 4% showers, depilation before surgery, administration of preoperative antibiotic prophylaxis in the operating room and comprehensive postdischarge follow-up. Infections were evaluated using CDC's definitions. Logistic regression models were fitted to assess infection rates over time, adjusting for factors known to affect SSI rates (NNIS risk index category, type of operation, sex, age, emergency operation, administration of preoperative antibiotic prophylaxis, length of stay in hospital before surgery).
Results: 1720 consecutive procedures were evaluated from April 1, 2002 to May 31, 2008. Rates of organ/space infections remained low, but unchanged over the study period (mean 1.09%). The overall rate of infections, the rates of superficial (SUP) and deep incisional SSIs during the first two years were 7.90%, 5.78%, and 0.96%, respectively and they decreased to 1.96%, 1.06%, and 0.0, respectively by the end of the study ($p < 0.001$, <0.001, and 0.03, respectively). The rate of SSIs due to *S. aureus* decreased from 1.54% to 0.30% ($p = 0.02$). The adjusted odds ratios for these infections at the end of the study as compared to March 31, 2004, were as follow: total number of infections 0.23 (95% confidence interval [CI-95], 012–0.44), SUP 0.17 (CI-95, 0.07–0.39) and SSI due *S. aureus* 0.14 (CI-95, 0.03–0.64).
Conclusions: We observed significant reductions in infection rates in most types of infections, particularly in the overall rate of SSIs and infections due to *S. aureus*. These differences remained significant when adjusted for potential confounding variables.

P1452 The effect of hospital volume on surgical site infection rates following orthopaedic procedures: what seems to be the most appropriate threshold?

D. Weitzel-Kage, D. Sohr, M. Behnke, P. Gastmeier (Berlin, DE)*

Background: Surgical site infections (SSI) belong to the most common nosocomial infections. The association between the operation volume and the risk of SSI is an importend issue. We wanted to investigate if high volume hospitals have lower surgical site infection (SSI) rates following orthopaedic operations.
Objectives: We used 3 orthopaedic procedures to analyse the association between the annual volume of operations and the incidence of SSI.
Method: The German national nosocomial infection surveillance system (KISS) is focussing on surveillance of 29 selected indicator procedures. KISS is using the definitions of the Centers for Disease Control and Prevention and the method of the National Health Safety Network for recording SSI. We analysed the data for 3 orthopaedic procedures types performed between January 2001 and June 2006: 35.579 hip prosthesis procedures, 29.237 knee prosthesis procedures and 16.642 arthroscopic operations were included. Two limits were considered: $\geqslant 50$ and $\geqslant 100$ procedures per year.
Results: Higher SSI rates in low volume hospitals were found for all 3 three procedure types considering the limit of an annual number of 50 operations per year with significant differences for hip and knee prosthesis. (Table).
Using the limit of >100 operations per year the SSI rates were even lower in the group of high volume hospitals (Knee prosthesis SSI rate 0.91%, Hip prosthesis SSI rate 0.94%).
Conclusion: The annual volume of operations has a significant impact on SSI rates following orthopaedic procedures. The hospitals should perform at least 50 operations annually, but more than 100 operations per year is even better.

Type of operation	SSI rate in hospitals with an annual number of procedures		Odds ratio	p value
	⩽50 OPs	>50 OPs		
Hip prosthesis	1.73%	1.06%	1.65	0.017
Knee prosthesis	1.91%	1.00%	1.93	0.016
Arthroscopic operations	0.33%	0.27%	1.24	0.561

P1453 Clinical features and prognosis of prosthetic joint infection: a multicentre cohort in Andalusia, Spain

M.D. del Toro, I. Nieto, F. Guerrero, J. Palomino, E. Nuño, J.E. Corzo-Delgado, A. del Arco, J.M. Lomas, C. Natera, A. Ruíz, P. Rico, A. Romero, J. Delgado, J. Rodríguez-Baño, M.A. Muniain and the Prosthetic Joint Infection Group of SAEI*

Objective: To describe the more relevant clinical characteristics and prognosis of prosthetic joint infection (PJI) in 12 Andalusian hospitals.
Method: Prospective observational study of the cohort of PJI recruited from 1/10/06 to 1/10/09 in the participating hospitals using stantard criteria, with a follow-up of at least 12 months after the end of antimicrobial treatment. Infectious diseases physicians provided recommendations bases in a previously launched consensus management guideline.
Results: 273 patients were included: 108 (39.5%) hips (46 hemiarthroplasty), 143 (52.5%) knees and 22 (8%) elbow/ankle implants; 172 (64%) were women; median age was 72 years (range: 32–95); 63% (171) had comorbidities. 33% had peroperatory risk factors for infection (surgical site infección [SSI] 28, surgical hematoma/bleed 37), and 24% risk factors after the implantation. Types of infection according to Tsukayama's classification: 89 (32%) acute postsurgical (API), 133 (48%) chronic potsurgical (CPI), 37 (13%) acute haematogenous (AHI), and 14 (5%) culture positive in prosthesis exchange without previous infection data. Aetiology: *S. aureus* 76 (29%) (15 were methicillin-resistant), coagulase-negative staphylococci 73 (27%), Gram-negative bacilli 36 (13%), *Streptococcus* 27 (10%), polymicrobial 22 (8%), others 11 (4%), unknown 28 (10%). Initial treatment planned was surgical debridement with prosthesis retention in 114 (42%), prosthesis removal in 103 (38%), arthrodesis/resection arthroplasty in 34 (12%) and supressive antibiotic therapy in 22 (8%). After 12 months of follow-up, 48.5% were considered cured; 20.5% failure of inicial treatment; 19% were still using antibiotic; 3.6% died; and 8.4% were lost. The prosthesis was mantanied in 45%, exchanged in 35%, and resection arthoplasty was performed in 20% (1 patient was amputated). At the end of therapy, 28% walked without help, 54% needed 1–2 crutches, and 18% could not walk. On univariate analysis, factors associated with

failure of inicial treatment were: for API, to have an infection caused by a multi-drug resistant organism; for CPI, prosthetis retention and delay of surgical treatment; and for AHI, diabetes mellitus and infection due to MRSA.
Conclusion: Overall, unselected cases of PJI are associated with a high morbidity and high frequency of functional disability 12 months after the completion of therapy. PJI due to multidrug-resistant organisms are associated with treatment failure.

P1454 Improving the quality of prophylactic antibiotics in total knee arthroplasty

K.S. Chan (Yung-Kang, TW)*

Objectives: Infection of a total knee arthroplasty (TKA) remains a devastating complication associated with this procedure. It leads to prolonged hospital stay, readmissions to the hospital, increased morbidity and mortality, and excesses the healthcare cost. Prophylactic antibiotics have proven to be effective in reducing the incidence of infection after TKA. However, inappropriate use of antimicrobial prophylaxis could cause prolonged duration and use of broad-spectrum antibiotics, increase the incidence rate of resistant microorganism. The objectives of this study were to reduce the quantity of prophylactic antibiotics and improve the quality of surgical prophylaxis.
Methods: This was an intervention study in a 1311-bed tertiary hospital. The intervention program was initiated from October, 2008. All patients underwent TKA during the study period were collected. Intervention programs included education regarding current evidence-based guidelines, development of local guidelines, and implementation of reminders to facilitate adherence to the local guidelines. Antibiotic with 5 doses or more (Cefazolin 1 dose before and 3 doses after the procedure were suggested by the guideline) is defined as prolonged prophylaxis. The quality of prophylaxis was audited before and after the intervention during a six-month period. Outcome parameters were dosage and costs of antibiotic use, length of hospital stay, unplanned hospital readmission.
Results: There were 140 procedures before the intervention and 126 during the intervention period. A significant improvement in the adherence to the local guidelines was noted between the pre-intervention and intervention periods. Prolonged prophylaxis was observed in1.6% instead of 90.75% in the pre-intervention period. The costs of antibiotics reduced by 44.8% per procedure ($P < 0.001$). The length of hospital stay of pre-intervention period was significantly longer than the intervention period (7.7±1.9 days VS 7.2±2.1 days, $p = 0.023$). No patient was re-admitted to the hospital during the study period.
Conclusions: The intervention led to improved quality of surgical prophylaxis and to reduced antibiotic use. In addition, it shortened the hospital stays and reduced the costs without impairment of patient outcome.

P1455 No correlation between skin microbial counts after preoperative skin disinfection and surgical site infections

S. Tschudin, D. Egli-Gany, M. Dangel, D. Oertli, W. Weber, R. Frei, A.F. Widmer (Basel, CH)*

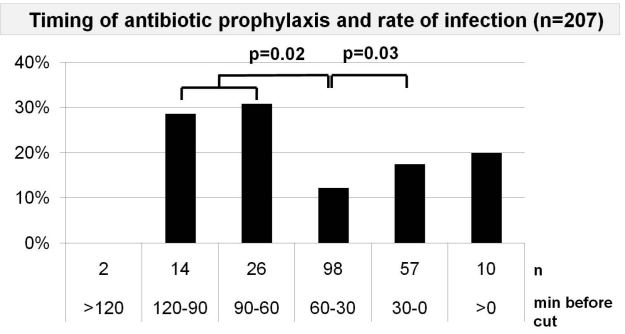
Objectives: Preoperative skin disinfection as outlined by WHO is standard practice for the prevention of surgical site infections (SSI), yet few studies examined the association between skin microbial flora after disinfection and the development of a SSI. No study examining an association between skin microbial counts and a subsequent infection has so far been completed in a large sample compromised of a variety of surgical patients.
Therefore we examined the correlation between total colony forming unit counts of skin flora after preoperative skin disinfection at surgical incision site and the development of a subsequent SSI in patients from different surgical specialties.
Methods: Prospective observational study in which 1014 skin cultures immediately were taken from patients after preoperative skin disinfection, before incision. Disinfection of the surgical site was performed according to the study protocol, using standardized procedure under supervision of a study nurse or the attending surgeons. Skin swabs were incubated for 48 hours and colony forming units (cfu) were enumerated and identified by means of standard laboratory identification methods. The finding of more than 10 cfu was regarded as significant colonization. The diagnosis of SSI was based on surveillance, full chart review and postdischarge surveillance (⩾30 days after surgery) using the Centers for Disease Control/National Nosocomial Infection Surveillance (CDC/NNIS) definitions.
Results: 1014 skin cultures from surgical sites were taken from a total of 1005 patients, mainly receiving orthopedic or cardiac surgery. 36 (3.6%) cultures revealed significant colonization of the preoperative site after skin disinfection. Coagulase-negative staphylococcus was the most common organism detected (83.3%), followed by *S. aureus* (8,3). 41 SSIs were detected accounting for a surgical site infection rate of 4.0%. Most SSIs occurred in cardiac surgery (34.2%). Significant cfu counts after skin disinfection were not correlated with SSI (Table).
Conclusion: This large study was not able to establish a correlation between residual colonization and SSI, indicating that a standardized three step disinfection is sufficient to prepare the surgical site.

Residual cfu after disinfection	Rate of SSI
<10	3, 8%
⩾10	0, 2%

P1456 Timing of antibiotic prophylaxis influences the incidence of surgical site infection rates after colon surgery

P. Rafeiner, U. Beutner, J. Celeiro, K. Di Salvo, G. Rettenmund, J. Lange, M. Zünd, M. Schlegel (St. Gallen, CH)*

Objectives: Surgical site infection (SSI) is a common adverse event after colon surgery leading to high morbidity and mortality. Published SSI rates for colon surgery (COLO SSI rate) range between 5 and 30%. In 2008 we performed a surveillance study to determine the SSI rate for colon surgery and to identify risk factors for SSI.
Methods: The study was performed at a tertiary care centre during 2008. Data from all patients with a colon surgery according to the National Nosocomial Infections Surveillance System (NNIS) COLO definition were entered prospectively into a database. The Centers of Disease Control (CDC) guidelines for diagnosis of SSI were followed. Follow-up lasted for 30 days. Discharged patients were contacted by phone 30 days after surgery.



- Pre-op antibiotic prophylaxis with cefamandole + metronidazole: 191/207 (92%)

Results: 227 cases have been surveyed. Age (median) was 66.4 years. The SSI rate for colon surgery was 20.7% (95% CI: 15.9–26.4%). There were found 49% superficial infections, 10.6% deep and 40.4% organ / space infections.
Out of 207 cases 98 cases (43%) got the antibiotic prophylaxis with correct timing (30–60min pre-op). SSI rate after properly timed antibiotic prophylaxis was 12.2% (CI 95%: 7.1–20.2%). Otherwise, the rate was significantly increased: 60–90min pre-op: 30.8% (CI 95%: 16.5–50%), 90–120min pre-op 28.6% (CI 95%: 11.7–54.6%), <30min

pre-op: 17.5% (CI 95%: 9.8–29.4%). Higher body surface was linked to a higher infection rate (body surface <1.7 m^2: 11.6% (95% CI: 6–21.2%), ⩾1.7 m^2: 24.7% (95% CI: 18.6–32%), p = 0.03).
Conclusions: Our SSI rate for COLO is higher than reported by NNIS (5.4%), but similar to published rates obtained under study conditions. Since data contribution to NNIS is voluntary, a selection bias or qualitative differences cannot be excluded. We identified incorrect timing of antibiotic prophylaxis as the key risk factor for surgical site infections. Efforts must be done for optimizing the processes to obtain an appropriate timing of pre-op antibiotic prophylaxis. Check lists (as the 'Surgical Safety Checklist', proposed by WHO) might contribute to achieve this aim.

P1457 Surveillance and epidemiology of surgical site infections after cardiothoracic surgery in the Netherlands, 2002–2007

J. Manniën, J.C. Wille, J.J. Kloek, B.H. van Benthem (Bilthoven, Amsterdam, NL)*

Objective: Surgical site infections (SSI) after cardiothoracic surgery substantially increase the risk for illness, mortality and costs. Surveillance of SSIs might assist in the prevention of these infections. This study describes the Dutch surveillance methods and results of data collected between 2002 and 2007.
Methods: Three cardiothoracic procedures were included: coronary arterial bypass grafts (CABG), valve surgery, and a combination of CABG with concomitant valve surgery. The SSIs were divided into sternal and harvest site SSIs. Postdischarge surveillance of SSIs was mandatory for sternal wounds and elective for harvest site wounds, with a follow-up period of 42 postoperative days. Multivariate logistic regression was used for risk factor analysis of CABG, with adjustment for random variation among hospitals.
Results: Eight of the sixteen Dutch cardiothoracic centers participated and collected data on 4066 procedures and 183 SSIs, revealing a SSI rate of 2.4% for sternal wounds and 3.2% for harvest sites. Sixty-one percent of all SSIs were recorded after discharge. For sternal SSI after CABG, the significant risk factors were rethoracotomy, diabetes, preoperative length of stay, and obesity; for harvest site SSI the most relevant risk factor was a long time on the extra-corporal circulation (Table 1). Adjusted SSI rates regarding CABG varied between hospitals from 0.0% to 9.7%. A culture result was available for 46 of the 53 (87%) deep sternal SSIs and for 26 of the 44 (59%) superficial SSIs. Fifty-eight percent (49 of 84) of the isolated micro-organisms were identified as Staphylococci (37 *Staphylococcus aureus*). For 41 of the 86 (48%) harvest site SSIs, a culture result was recorded. Thirty-one percent (19 of 61) of the isolated micro-organisms were identified as Staphylococci (15 *Staphylococcus aureus*).
Conclusions: Large differences were found in SSI rates between Dutch hospitals, which indicate room for improvement. The follow-up of patients after hospital discharge avoids underestimation of SSI rates.

P1458 Surgical site infections after orthopaedic surgery in Cyprus: incidence, risk factors, microbiology and impact

E.I. Kritsotakis, I. Dimitriadis, D. Bagatzouni, M. Alexandrou, V. Zinieri, C. Tsioutis, A. Gikas (Heraklion, GR; Larnaca, Nicosia, Limassol, Pafos, CY)*

Objectives: To determine the incidence of surgical site infections (SSI) after common orthopaedic surgery procedures in Cyprus, compare observed rates with international data, and specify main epidemiologic features including risk factors, predominant pathogens and resistance patterns, use of antibiotic prophylaxis, and impact of SSI on patient outcome.
Methods: The US National Nosocomial Infections Surveillance (NNIS) system protocols were employed to prospectively collect data for patients who underwent orthopaedic surgery during an 11-month period in the 4 public hospitals of Cyprus. Procedures surveyed included open reduction of fracture (FX), knee prosthesis (KPRO), and hip prosthesis (HPRO). Comparisons of procedure-specific SSI rates with international data were performed by means of standardized infection ratios after stratification by the NNIS risk index. Risk factors for SSI were evaluated by multivariate logistic regression.
Results: A total of 44 SSIs were detected in 1403 operations (3.1 per 100 operations), of which 27% were detected post-discharge. Among the recorded SSI, 61% were superficial, 23% were deep and 16% were organ-space infections. Most frequently isolated pathogens were: *Staphylococcus aureus* (29.4%; of which 60% methicillin-resistant), *Pseudomonas aeruginosa* (17.6%; none resistant to ciprofloxacin, ceftazidime or imipenem), *Enterococcus* spp. (11.8%; none resistant to vancomycin), and coagulase negative staphylococci (11.8%). Antibiotic prophylaxis was administrated for 98% of the procedures classified as clean and for 92% of clean-contaminated procedures, for a median duration of 2 days. Procedure specific SSI rates and risk-adjusted comparisons with international data are presented in the Table. SSI rates were significantly higher for KPRO and HPRO compared with US data, and for KPRO compared with Spanish data. Among 12 potential risk factors examined, significantly and independently associated with increased risk for SSI were: Charlson comorbidity index ⩾4 (odds ratio [OR]=3.1, p = 0.040), and wound class not clean (OR = 5.6, p < 0.001). The mean postoperative hospital stay was significantly longer for patients with SSI than those without SSI (20.1 vs 10.1 days, p < 0.001). SSI was not associated with mortality.
Conclusion: This study demonstrated the feasibility of implementing a standardized surveillance protocol of SSI in Cypriot public hospitals and generated data useful for designing targeted infection control interventions.

Procedure-specific SSI rates in Cyprus and risk-adjusted comparisons with international data

NNIS Operative Category	Study results		Risk-adjusted comparisons with:					
	No. of SSI/ procedures	SSI rate (%)	US rates [1] SIR	p	Spanish rates [2] SIR	p	UK rates [3] SIR	p
Knee prosthesis	14/288	4.9	4.5	<0.001	2.5	0.002	1.2	0.270
Open reduction of fracture	24/832	2.9	2.2	<0.001	0.9	0.408	0.8	0.240
Hip prosthesis	6/283	2.1	1.6	0.194	0.5	0.057	1.1	0.495

NNIS, National Nosocomial Infection Surveillance system; SSI, surgical site infection, SIR, standardized infection ratio. [1] Am J Infect Control 2004;32:470–85. [2] Am J Infect Control 2006;34:134–41. [3] UK Health Protection Agency 2006.

P1459 Surgical site infection after open heart surgery

C. Ezpeleta, E. Gomez, M.J. Unzaga, I. Atucha, J.A. Alava, A. Arias, R. Cisterna (Bilbao, ES)*

Objective: To know the rates of NI (Nosocomial Infection) and surgical site infections (SSI) in patients operated on open heart surgery in our hospital and to compare with those of HELICS (Hospital in Europe Link for Infection Control trough surveillance).
Patients and Methods: The 2422 patients (pt) operated on open-heart surgery in our hospital from Jan 2000 to Oct 2009 were included. Preoperative protocol regarding infection control includes: shower with 4% clorhexidine soap the night before surgery and repeated the day of surgery. Hair clipping just before surgery. Antibiotic prophylaxis: Cefuroxime 1.5 gr. IV 5 minutes before surgery. A second dose is administrated intraoperatively if the length of surgery is >6 hours or hemodilution >15 ml/kg or blood loss >1.5 l. All the pt are prospectively studied since the day they are operated until the end of the episode by the infection control team during admission and continued 1 year after discharge. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, ASA, NNIS risk index, antibiotic prophylaxis, nosocomial infections, microorganisms, length of hospital stay, treatment and outcome. CDC definitions of nosocomial infection are used.
Results: 1630 men, mean age 66.6 y. Nosocomial infections: 487 pt acquired 708 NI, 127 of them were surgical site infections (SSI), Respiratory 231, Urinary tract 187, local catheter site 62, bacteremia 64: primary 24, secondary 30, and catheter related 10. Cumulated incidence of pt with NI 20.11%. SSI: 32 incisional superficial, 39 deep incisional and 56 organ space. NNIS score 0: 156 pt, 1.9% SSI; Score 1: 1510 pt, 4.2% SSI; Score 2: 724 pt, 6.4% SSI, Score 3: 30 pt, 26.7% SSI. Antibiotic prophylaxis was administered in 99.5% of the cases. The

dosage, time, drug and duration of the prophylaxis were appropriated (99.9%, 99.5%, 99.8% and 95.2% respectively). Microorganisms in SSI: coagulase negative Staphylococci (CNS) 45, *P. aeruginosa* 12, *S. aureus* 18 (6 SAMR). Cumulated incidence of SSI 5.24%.
Conclusions: Comparing our data of SSI (5.24%) with the latest HELICS-SSI report 2004 (1043 cases) our results are lower than the total results (10%). Gram positive cocci are the most common isolated in SSIs in our hospital, similarly in the HELICS report Gram-positive cocci are recovered in 68.4% of cases. In our case series CNS are the most frequent recovered from SSI and in the HELICS report *S. aureus* predominates with 31.6% of isolates.

C. difficile infection

P1460 Molecular characterization and antimicrobial phenotypes in clinical isolates of *Clostridium difficile* in a tertiary care hospital, Mallorca

C. Déniz, E. Riera, J.L. Pérez, A. Oliver, A. Mena (Palma, ES)*

Objectives: There have been some changes in the epidemiology of the *Clostridium difficile* infection, mainly represented by the rapid spread of epidemic strains, including hypervirulent strain known as ribotype 027. In order to know the epidemiology of our hospital environment we performed molecular characterization and antimicrobial phenotypes of toxigenic *C. difficile* clinical isolates collected from August 2007 to August 2009, at the major public hospital of the island of Mallorca, Spain.
Methods: A total of 70 clinical isolates of *C. difficile* obtained from different patients with diarrhoea during 2 years were studied. Rapid ELISA detection of toxins A and B (Wampole®, Inverness medical), directly from the stools or from the culture, were performed for all the isolates. Molecular toxigenic profile of the strains was studied by PCR amplification of the tcdA (toxin A), tcdB (toxin B) and cdtA, cdtB (binary toxin) regions. Typing and clonal relation was studied by PCR ribotyping through amplification of the 16S-23S rRNA. *C. difficile* ribotypes 001, 078 and 027 were used as controls. Isolates with PCR ribotypes patterns different from those control strains were classified by letters. Finally, antimicrobial susceptibility patterns to metronidazole (MTR), vancomycin (VAN), clindamycin (CLIN), erythromycin (ERY) and moxifloxacin (MXF) were studied by determining the MIC using the Etest method.
Results: 96% of the isolates were toxigenic by PCR and 3 isolates were false positives ELISA results. All toxigenic isolates produced toxins A and B and 27% of the isolates also produced binary toxin. We found 30 different PCR ribotype patterns. The most prevalent ribotypes were A ribotype (A+B+, 21%), 078 ribotype (A+B+bin+, 19%), B ribotype (A+B+, 13%) and 001 ribotype (A+B+, 9%). All strains were susceptible to MTR and VAN, but resistance to CLIN, ERY and MXF was found in 61%, 23% and 23%, respectively. We also observed almost 100% CLIN resistance and 50% ERY resistance in ribotypes 078 and 001 and 50% MOXI resistance in ribotype 001.
Conclusion: The first epidemiological study of *C. difficile* in our hospital shows the presence of heterogeneous ribotyping population with binary toxin-producing strains, mainly represented by the hypervirulent ribotype 078. We found no clear association between antimicrobial profiles and ribotyping. Finally, there is no evidence of occurrence of clusters in our hospital or presence of the 027 epidemic ribotype and A-B+ strains.

P1461 Ribotyping and further subtyping of isolates from *Clostridium difficile* infection in a UK teaching hospital is effective in analysing transmission and assessing impact of enhanced measures to control spread

G. Jones, J.K. Sutton, S. Green (Southampton, UK)*

Objective: Standard infection control measures to reduce the incidence of *C. difficile* infection, (CDI) operate in our hospital. We assessed the impact of these measures on the distribution of *C. difficile* ribotypes over an 8 month period in 2009.
Methods: Ribotyping was performed on *C. difficile* isolates obtained from culture of toxin A+B enzyme immunoassay (EIA) positive faeces confirmed by polymerase chain reaction. Multi-locus variable number of tandem repeats analysis (MLVA) was performed on selected isolates of ribotype 027 during a period of increased incidence of CDI. Movements of patients with indistinguishable ribotypes and MLVA subtypes were tracked to determine transmission networks.
Results: 170 cases of CDI were identified and 166 *C. difficile* isolates were ribotyped. 65 (39%) were ribotype 027 and 21 (13%) were ribotype 106. 47 strains representing 9 other ribotypes were identified and 33 strains were of sporadic unidentifed ribotypes. MLVA identified 13 distinct subtypes from 49 027 isolates. Two dominant 027 subtypes comprising 16 and 14 isolates respectively, identified transmission between patients within wards A and B. MLVA also clearly demonstrated that a patient transfer from ward A to ward B introduced the first subtype to ward B to create a cluster of cases comprising both dominant 027 subtypes. A smaller ribotype 106 cluster was identified on another ward. Ribotyping and MLVA data combined with analysis of patient movements were used to track transmission between patients and wards. Enhanced control measures including introduction of adenosine triphosphate bioluminescence monitoring to improve cleaning reduced CDI from 95 cases in the first 3 months of the study to 36 in the final 3 months (61% reduction). Strikingly, the proportion of 027 cases fell from 51/95 (54%) in months 1–3 to 1/36 (3%) in months 5–8. In the final 3 months of the study, no single ribotype accounted for more than 6 of the 36 isolates.
Conclusions: Routine ribotyping and selective MLVA were effective in characterizing outbreaks and improving control of CDI. Enhanced measures to control spread of CDI were effective and resulted in the virtual elimination of 027 strains by the end of the study period. Overall the observations were compatible with a significant reduction in nosocomial transmission and highlight the need for other ongoing measures to reduce CDI including continued effective antibiotic stewardship.

Number of *C. difficile* ribotypes identified per month

	Month of study								Total
	1	2	3	4	5	6	7	8	
O27 ribotype (n)	8	24	19	9	3	1	0	0	65
All ribotypes (n)	22	40	29	23	15	12	11	13	166

P1462 Detection of hypervirulent strains PCR-ribotype 027 and 078 causing severe *Clostridium difficile* infection in northern Italy

A. Tuscano, R. Baldan, P. Cavallerio, C. Parlato, L. Fossati, M. Moro, R. Serra, D. Cirillo (Milan, Turin, IT)*

Objectives: *Clostridium difficile* (Cd) is recognized as the main cause of hospital acquired antibiotic-associated diarrhoea worldwide. Its virulence is attributed to the production of toxins A and B. From 2003 increasing incidence, severity and mortality of Cd infection (CDI) were reported and related to the emergence and spread of highly virulent strains, such as PCR-ribotype 027 and 078. These hypervirulent strains produce the binary toxin (CDT) and harbour mutations/deletions in the tcdC gene, encoding for an inactive negative regulator of toxins A/B production.
In order to investigate the presence and spread of hypervirulent strains and to characterize the main Cd clones, we examined at the molecular level all the toxigenic Cd strains isolated in 2 large Italian nosocomial settings, the San Raffaele (hSR) and the San Giovanni Battista hospitals (SGBh).
Methods: We characterized by PCR-ribotyping, detection of tcdA, tcdB, cdtA/cdtB genes and sequencing of the tcdC gene 129 SGBh Cd strains and 157 hSR Cd strains. All strains were investigated for the presence of ermB gene, responsible for the macrolide-lincosamide-streptogramin B resistance phenotype, and selected strains were tested for susceptibility to ciprofloxacin, moxifloxacin, metronidazole and vancomycin.
Results: 9 cases (8 at the SGBh (6.2% of the SGBh isolates) and 1 at the hSR) of severe CDI were caused by ribotype-027: strains were resistant to

moxifloxacin and susceptible to vancomycin and metronidazole; 55.5% harboured the ermB gene. 28 cases were caused by ribotype-078 (21 at the hSR, where it represents the second most commonly isolated ribotype (14%), 7 (5.4%) at the SGBh): 70.5% of these strains were resistant to moxifloxacin and all susceptible to vancomycin and metronidazole; 60.7% harboured the ermB gene. In both hospitals we identified also ribotype 018 as the main clone, accounting for half of the isolates, and ribotype 001.
Conclusion: The main clone detected in both hospitals was the ribotype 018, but we report here that the hypervirulent strains 027 and 078 are present in Northern Italy. Ribotype 078 represents the second most frequently identified ribotype at the hSR, while ribotype 027 accounts for 6.2% of the SGBh Cd isolates. We noticed that the antibiotic resistance pattern of ribotypes 027 and 078 is changing toward the development of high-level resistance to antibiotics.

P1463 Molecular epidemiology and antimicrobial resistance of *Clostridium difficile* isolates in Taiwan

*Y.C. Lin *, Y.T. Huang, N.Y. Lee, C.H. Liao, W. Ko, P.R. Hsueh (Taipei City, TW)*

Objectives: *Clostridium difficile* associated diarrhea is a global health problem and a highly virulent strain (ribotype 027) has disseminated throughout North America and European countries. Data regarding the molecular epidemiology and antimicrobial resistance of *C. difficile* isolates in Taiwan remained limited.
Methods: Eighty-six isolates of *C. difficile* obtained from patients treated at National Taiwan University Hospital (NTUH) and 25 from National Chen-Kung University Hospital (NCKUH) from August 2007 and June 2009 were analyzed. Molecular typing using a repetitive-element PCR typing (DiversiLab® kits for *C. difficile*) and antimicrobial susceptibility testing to 15 antimicrobial agents were performed. Another 14 isolates identified during 2001 and 2004 were also included for analysis. Isolates with a correlation of 95% determined by DiversiLab® software version 3.4 were considered to be within the same clone.
Results: Among these isolates, 12 clones were identified with a major clone (Clone A) covering 47.4% (N = 55) of isolates. Furthermore, clone A was distinct from North American pulsotype1 (NAP1, corresponding to ribotype 027), NAP7 and NAP8 (ribotype 078). Isolates of this clone were distributed equally among both centers and could be traced back to 2001. Clone C (N = 14) isolates, appeared since 2001, were only detected at NTUH and appeared to be similar to NAP7. No metronidazole- or vancomycin-resistant isolates were identified. Decreased susceptibility for moxifloxacin (MIC $\geqslant$8 mg/dL) were found in 18 isolates among different clones (9 isolates in clone A, 2 in clone B, 3 in clone C, 2 in clone E and 1 in clone F) and these isolates also exhibited decreased susceptibility to gemifloxacin and nemonoxacin. Partial sequence of gyrA and gyrB genes for the 19 quinolone resistance isolates revealed the substitution Thr82 to Ile (13 isolates), Asp81 to Asn (1 isolate), Asp71 to Gly (1 isolate) in GyrA, Asp426 to Val (1 isolate) in GyrB. Three isolates have substitution in both GyrA and GyrB: 2 with Thr82 to Ile in GyrA plus Ser 416 to Ile in GyrB and 1 with Thr82 to Ile in GyrA plus Asp426 to Val in GyrB.
Conclusions: Dissemination of fluoroquinolone-resistant clones of *C. difficile* occurred in Taiwan. Although no NAP1 (ribotype 027) isolates were detected in the current surveillance from Taiwan, further monitoring is mandated.

P1464 Mortality and attributable mortality of *C. difficile* infection in critical ill patients

J. Leitner, D. Schmid, H.W. Kuo, F. Allerberger, P. Tesik, V. Michl, C. Wenisch (Vienna, AT)*

Objectives: A prospective hospital-based cohort study was performed between November 2008 and August 2009 to measure the attributable mortality of *C. difficile* infection (CDI) in patients with CDI compared with non-diarrhoea patients admitted at the intensive care unit (ICU) with equal comorbidity score.
Methods: 90 CDI patients and 180 ICU non diarrhea patients were included. Death risk ratio and attributable risk of death (i.e. excess mortality) were used to measure the relative and absolute effect of CDI on the risk of pre-discharge mortality within the two comorbidity-severity categories low and moderate-severe comorbidity using the Charlson Comorbidity score. Death risk ratio was calculated by death risk in the CDI-group (exposed group) / death risk in the ICU-group (unexposed group) and the attributable risk of death (attributable mortality) was calculated death riske – death risku. The attributable risk percent, AR% (mortality fraction) was calculated by AR%= (death riske – death risku) / death riske X 100. Death risks were compared by using χ^2 test or the Fisher's exact test. The survival time between the CDI-patient-group and ICU-patient group within the two comorbidity-severity categories was analysed by using Kaplan–Meier survival analysis. For the CDI-patient group day zero corresponded to the date of disease onset, and for the IC-patient group day zero corresponded to the day of ICU admission. Study subjects with a follow up period of at least 30 days were included in this survival analysis. A total of 141 ICU-patients and all the 90 CDI patients remained for this analysis.
Results: Table 1 shows the risk of death in low compared to moderate & severe comorbidity in CDI and ICU non diarrhea patients.
Within 30 days CDI patients with low comorbitiy showed higher survival rate than the ICU patient-group ($p < 0.001$). No significant difference was observed between survival rates of the CDI patients and ICU patients in the moderate/severe comorbidity category.
Conclusion: *C. difficile* infection does neither result in increased mortality in patients with low nor with moderate/severe Charlson comorbidity score.

Table 1. Effect of CDI on the mortality risk; CDI-patient group compared with the ICU-patient group by comorbidity severity categories low and moderate/severe

Comorbidity severity	Risk of death in:		RR (95% CI)	Excess mortality	Mortality fraction
	CDI-patient-group	ICU-patient-group			
Sub-cohort I: Low comorbidity	4.2% (2/48)	39.8% (53/133)	0.1 (0.03–0.41)	n.a.	n.a.
Sub-cohort II: moderate & severe comorbidity	28.6% (12/42)	21.3% (10/47)	1.34 (0.65–2.78)	7.3%	25.5%

P1465 Community-associated *Clostridium difficile* infection in Denmark: clinical features and molecular characterization

L.M. Søes, M. Kemp, S. Persson, K.E. Olsen (Copenhagen, DK)*

Objective: The aim of this study is to clarify clinical features and risk factors in *Clostridium difficile* infections and to determine toxin gene profiles of isolates found in faecal specimens from patients attending their general practitioner on suspicion of gastroenteritis.
Methods: Stool samples submitted from general practitioners from eastern parts of Zealand on suspicion of gastroenteritis are analysed for bacterial, viral and parasitic gastrointestinal pathogens including *C. difficile*. The *C. difficile* isolates are analysed by PCR for detection of the toxin genes tcdA, tcdB and cdtA/cdtB. A case–control study is being conducted to reveal clinical features of disease, including severity of disease, prior use of antibiotics and co-morbidity and to investigate possible risk factors in terms of acquisition of disease, including exposure to animals and young children.
Results: Currently 60 patients have been enrolled and diagnosed with *C. difficile* by stool culture. Age ranges from 14 weeks to 93 years, with half of the patients being less than 24 month of age. Even when excluding these, mean age is 54 years. Preliminary data of toxin gene profile analysis are shown in Table 1. Concomitant bacteria are found in two in 21 stool samples in the group of patients less than 24 months of age with toxigenic strains. These two are attaching-and-effacing *E. coli*. According to present data duration of diarrhoea is approximately one month for patients more than 24 months of age and half a month for patients less than 24 months of age. Mean stool frequency is seven and

ten times a day for the two age groups, respectively. Weight loss during illness is reported by approximately seventy percent of the patients.
Conclusion: Preliminary data indicates average age in the group of the patients more than 2 years of age to be as low as 54 years. In the group of patients being younger than 24 months of age, approximately two thirds of the strains harbour genes encoding Toxin A and B. No concomitant bacterial pathogens of known importance are found in this group, suggesting *C. difficile* could be of importance for developing disease. Furthermore it may reflect the possibility of young children being a reservoir for *C. difficile*. For the patients already enrolled into the study current data indicates marked symptomatology with longlasting diarrhoea with high stool frequency often followed by loss of weight.

Table 1. Distribution of toxin profiles

Toxin profile	Patients <24 months of age (N = 33)	Patients >24 months of age (N = 27)
Toxin A, Toxin B, Binary toxin CDT[1]	1	9
Toxin A and Toxin B[2]	21	16
Non-toxigenic	11	2

[1]Strains PCR-positive for the toxin genes *tcdA*, *tcdB* and *cdtA*/*cdtB* encoding Toxin A, Toxin B and the binary toxin CDT. [2]Strains PCR-positive for the toxin genes *tcdA* and *tcdB* encoding Toxin A and Toxin B.

P1466 Impact of *Clostridium difficile* infection on resource utilization and clinical outcomes in hospitalized patients

M.F. Emons, B.H. Nathanson, H.T. Yu, T. Haidar, E. Alemao, D. Gardiner, R.K. Spoeri (Beverly Hills, Longmeadow, Collegeville, US)*

Objective: To evaluate the impact of *Clostridium difficile* infection (CDI) on resource utilisation and in-hospital mortality in patients with hospital-onset healthcare facility-associated (HO-HCFA) CDI.
Methods: This retrospective analysis utilised data from April 1, 2005 to December 31, 2008 within Cerner's Health Facts® database. Qualifying cases included adults with an inpatient encounter with a primary or secondary diagnosis of pseudomembranous colitis (ICD-9-CM code: 008.45) AND a positive *C. difficile* toxin test AND the first positive *C. difficile* test (index event) was ordered more than 72 hours after admission. Non-CDI controls were hospitalised for at least 72 hours with no diagnosis of pseudomembranous colitis AND no *C. difficile* culture or toxin assays during the study period. Primary outcomes were in-hospital mortality, hospital length-of-stay (LOS) and 90-day all-cause readmission. A propensity-score-matched sample and a non-parse regression model with the propensity score itself and all other covariates (eg, patient demographics, clinical markers of severity of illness, treatments of interest [ie, antibiotics, proton pump inhibitors, H2 antagonists, and non-steroidal anti-inflammatory drugs]) were both used to assess the effect of HO-HCFA CDI on outcomes.
Results: A 5:1 Greedy algorithm matched 1,312 cases to 1,312 controls. Patients with HO-HCFA CDI had significantly longer unadjusted hospital LOS than non-CDI patients (mean [SD]: 22.0 [19.0] versus 6.6 [5.8] days, $P < 0.001$). After matching, the LOS among survivors with HO-HCFA CDI was 10.8 days (95% CI, 9.7–12.0) longer than the non-CDI control group and LOS after the CDI event was equivalent to the entire LOS in the control group. No significant differences in mortality were found between matched HO-HCFA CDI and non-CDI groups (odds ratio = 0.89; 95% CI, 0.69–1.15) though the matched mortality rate of HO-HCFA CDI patients was high (8.7%). Thirty-three percent of all matched survivors required readmission within 90 days. The relative risk for readmission was significantly higher for HO-HCFA patients: 1.56 (95% CI, 1.38–1.76).
Conclusions: HO-HCFA CDI is a growing public health concern and is associated with significantly longer hospitalisations and higher risk of hospital readmission within 90 days. To reduce the higher resource utilisation among these patients, a better understanding is needed of risk factors and potential preventative measures.

P1467 Rapid and effective routine typing of *Clostridium difficile* isolates in a hospital setting using the repetitive sequence-based polymerase chain reaction DiversiLab® system

I. Khan, V. Garcia Arias, J. Laughlin (Slough, UK)*

Objectives: *Clostridium difficile* (*C. difficile*) infection (CDI) is the leading cause of nosocomial diarrhoea. Rapid and reproducible typing methods are essential for tracking and monitoring CDI. Our aim was to characterise the epidemiology and understand the underlying picture of CDI within our 500-bed hospital using the DiversiLab® repetitive sequence-based polymerase chain reaction (rep-PCR) system (bioMérieux) and integrate our findings within infection control practice.
Methods: CDI cases were detected by routine hospital surveillance using the VIDAS® *C. difficile* Toxin A & B assay (bioMérieux). Isolates were cultured and DNA was extracted, and amplified using the DiversiLab *Clostridium* Fingerprinting kit. PCR products were separated using microfluidics chips and analysed using the web-based software provided with the system.
Results: From April 2009 to July 2009 a total of 43 *C. difficile* toxin positive stool samples from 38 patients were detected. Forty-one isolates were available for typing. Eight isolates (19.5%) from six different patients showed >93.5% similarity in their fingerprint profiles, suggesting the existence of a predominant cluster. The isolates had >95% similarity to the *C. difficile* NAP1 strain in the DiversiLab database and all were confirmed to be ribotype 027 by the reference laboratory. Infection control investigations showed the six patients had been in the same ward at some point and enhanced surveillance and infection control precautions were implemented. Seven more clusters of two or more isolates with >90% similarity in their fingerprint patterns were observed with no epidemiological links and no similar strains found in the library database. A further five isolates (12.2%) showed a low discrimination fingerprint pattern with two bands common to NAP2, 4 and 6 strains. The remaining 10 isolates showed unique rep-PCR fingerprints. One patient had two recurrences of CDI with the same NAP1/027 strain and another had a second episode of CDI with a different fingerprint strain.
Conclusions: Routine in-house typing of *C. difficile* using the DiversiLab rep-PCR system enabled us to gain an understanding of the epidemiology of CDI within our hospital setting. The system offered us rapid, accurate and reproducible results to support the infection control team in routine *C. difficile* surveillance and CDI outbreak investigations.

P1468 *Clostridium difficile* resistant to fluoroquinolones in Italy: emergence of PCR-ribotype 018

P. Spigaglia, F. Barbanti, P. Mastrantonio (Rome, IT)*

Objectives: To investigate the susceptibility of Italian *Clostridium difficile* isolates to fluoroquinolones over time.
Methods: 147 toxigenic *C. difficile* isolates collected by the Istituto Superiore di Sanita, from 1985 to 2008, were investigated for susceptibility to fluoroquinolones and for a possible association between fluoroquinolone and MLSB-resistance. All strains were typed by PCR-ribotyping and analysed for susceptibility to moxifloxacin (MX), levofloxacin (LE), gatifloxacin (GA), ciprofloxacin (CI), erythromycin (EM) and clindamycin (CM) by the E-test method. All resistant strains were characterized for substitutions in GyrA and GyrB by real-time PCR and for the presence of an ermB gene by PCR assay.
Results: For convenience we divided the strains in two groups: the first group included 70 isolates from 1985 to 2001, the second group 77 isolates from 2002 to 2008. In total 50 isolates (34%) were found resistant to fluoroquinolones, with MICs between 8 and $\geqslant$32 mg/L for MX and GA, 24 and $\geqslant$32 mg/L for CI and $\geqslant$32 mg/L for LE. 10% (7/70) belonged to the first group, whereas 56% (43/77) to the second group. All strains had the substitution Thr82 to Ile in GyrA, except one isolate in the first group and one isolate in the second group that showed the substitution Asp426 to Asn in GyrB. 98% (49/50) of the *C. difficile* isolates resistant to fluoroquinolones were also resistant to EM and/or CM. Among the resistant strains isolated from 1985 to 2001, 57%

(4/6) had an erm(B) gene compared to 9% (4/43) among those isolated from 2002 to 2008. *C. difficile* strains resistant to fluoroquinolones and isolated from 1985 to 2001 belonged to type 001 (two strains), 126 (two strains) and 012, 020, 078 (one strain for each type). Fifty six percent (24/43) of the fluoroquinolone resistant strains isolated between 2002 and 2008 belonged to type 126, 40% (17/43) to type 018 and 2% (1/43) to type 020 and 001, respectively. Interestingly, all isolates resistant to fluoroquinolones isolated in 2007 and 2008 belonged to PCR-ribotype 018, that also represents the predominant PCR-ribotype in Italy at present.

Conclusions: The results indicate an increase of fluoroquinolone-resistance among Italian clinical isolates, recently associated to the spread of a clone PCR-ribotype 018.

P1469 A *Clostridium difficile* 027 epidemic in a hospital in Vienna, Austria, 2008–2009

S. Kasper, E.E. Kanitz, A. Indra, T. Watkins-Riedel, H. Masoud, S. Eberl, S. Huhulescu, F. Allerberger, D. Schmid (Vienna, AT)*

Objectives: From January to August 2009, 174 cases of *C. difficile* infections (CDI), including 38 cases of infection with *C. difficile* ribotype 027(027CDI) occurred in a Viennese hospital (HX). Previously, in 2008, 11 cases of 027CDI were identified in Austria. The objective was to describe the 027 CDI outbreak by time, place, person and to ascertain antibiotic exposure prior to diarrhoea onset, the frequency of recurrent CDI and the 30-day mortality.

Methods: Surveillance case definitions were given by the Study Group for *Clostridium difficile* (ESGCD). An outbreak case was defined as a patient with 027CDI, who became ill between December 2008 and August 2009 in HX or who was epidemiologically linked to HX. The epidemiological link was defined as stay at HX within 4 weeks prior to 027CDI occurrence or as contact to a 027CDI case of HX. A recurrent CDI was defined as the occurrence of a second episode of CDI within 60 days after the first episode. PCR ribotyping of *C. difficile* isolates was performed by the National Reference Laboratory for *C. difficile*, AGES. Medical charts of the 027 cases of HX were reviewed for recurrent episodes, antibiotic exposure prior to diarrhoea onset and outcome.

Results: A total of 38 027CDI outbreak cases occurred in HX and another 12 cases of 027 CDI in further 5 Viennese hospitals were epidemiologically linked to the 027CDI outbreak Twenty-four of 38 cases were male (60%), the median age was 80 years (range: 31–98 years). The proportion of recurrence was 15.8% (6/38) and the 30-day mortality was 34.2% (13/38). Medical records were available for 27 (66%) outbreak cases. All these received antibiotics prior to diarrhoea onset (β-lactam antibiotics: 55.6%, fluoroquinolones: 59.3%). ESGCD control measures were implemented in the third week of July.

Conclusion: Non-compliance to evidence based control measures as recommended by ESGCD resulted in a rapid spread of a high virulent CD strain, which caused a very high patient mortality. Following the measure implementation, the hospital-based CD surveillance indicated a decreasing trend of all CDI cases in HX within the past three months. The findings of the outbreak investigation underline the importance of ribotyping in elucidating links between separate hospital clusters.

P1470 CDI in England in 2008–09 versus 2007–08 as determined by The *Clostridium difficile* Ribotyping Network for England and N. Ireland (CDRN, formerly the CDRNE)

M. Wilcox, W. Fawley, M. Shemko, D. Fairley, A. Birtles, E. Bolton, S. Green, K. Hardy, P. Hawkey, P. Hawtin, J. Magee, B. Patel, A. Sails, N. Shetty, M. Wren for the C. difficile Ribotyping Network for England & N. Ireland CDRN), Regional Microbiology Network, Health Protection Agency*

Objectives: To describe the key epidemiological findings concerning CDI in England in 2008–09 versus 2007–08 as determined by The *Clostridium difficile* Ribotyping Network for England and N. Ireland (CDRN, formerly the CDRNE).

Methods: CDRN expanded in 2009 to 8 regional laboratories in England and N. Ireland. CDRN provides ribotyping and enhanced DNA fingerprinting to identify cross-infection, reduce transmission, optimise management of outbreaks and determine the epidemiology of *C. difficile*.

Results: In 2008/09 CDRN processed 4,682 faecal samples from 190 healthcare facilities (~100% increase over 2007/08); hence, about 1 out of every 8 or 9 *C. difficile* cases in England were examined by CDRN. Marked changes in ribotype prevalence in 2008/09 occurred, with a striking 19% decrease (from 55% to 36%) in CD 027, and 'compensatory' increases in other less common types. The prevalence of ribotypes 106 (13%) and 001 (8%) remained similar. There were clear regional differences in ribotype prevalence e.g. CD 027 was the commonest in each region except the North East (CD 001, 20.0% vs CD 027, 12.6%; $p < 0.001$). There have been shifting regional ribotype prevalences since CDRN(E) was introduced, most clearly demonstrated for 027. There was a significant association between all cause mortality and CD 027 (OR = 1.9; $p < 0.001$).

Notably, there was an increase from 9.6% to 12.5% in the proportion of (toxin positive) faecal samples that are *C. difficile* culture-negative. This may reflect more false positive samples, as CDRN examines samples that have tested locally as 'toxin positive'. Susceptibility testing of over 1000 *C. difficile* isolates has shown more evidence of emergence of reduced susceptibility to metronidazole with some institutional clustering.

Conclusions: Since CDRN was introduced in early 2007 there has been a marked decrease in incidence of CDI in England. During the same time frame recorded deaths due to CDI have decreased. These changes likely reflect successful control of ribotype 027. We believe that provision of timely ribotyping data by CDRN to hospitals has helped to control and reduce the prevalence of ribotype 027. Access to timely typing data is important for infection control teams to identify and control transmission of *C. difficile*.

P1471 Epidemiology of *Clostridium difficile* during norovirus outbreaks

C. Holmes, K. Hardy, S. Gossain, S. Manzoor, P. Hawkey (Leicester, Birmingham, UK)*

Objective: There have been reports noting an increase in the incidence of *Clostridium difficile* infection (CDI) during norovirus outbreaks. Hypotheses proposed for this increase include increased transmission of *C. difficile* due to norovirus diarrhoea, norovirus causing increased severity of CDI, or increased submission of diarrhoeal samples. The aim of this study was to document the incidence of CDI during hospital norovirus outbreaks.

Methods: The study was performed between December 2008 and April 2009 in 3 linked hospitals with 1,800 beds. All loose stools from patients on wards with confirmed norovirus outbreaks were examined using an RT-PCR norovirus assay. In addition all samples were examined for C difficile using an in-house and the BD GeneOhm PCR assay as well as the TechLab EIA toxin test. All *C. difficile* positive samples were cultured for that bacterium and PCR ribotyped.

Patient	Outbreak	Norovirus status	*C. difficile*			
			BD Gene Ohm PCR	In-house PCR	EIA	Culture (Ribotype)
A	3	+	+	+	+	+ (001)
B	3	+	+	Inhib	−	+ (062)
C	5	+	+	+	−	Insuf
D	14	−	+	+	−	+ (020)
E	15	+	+	+	−	+ (001)
F	16	+	+	+	+	+ (174)
G	16	+	+	−	−	−

Inhib: inhibitory PCR; Insuf: insufficient specimen for culture.

Results: One hundred and seven patient samples were included in the study from 21 norovirus outbreaks of which 70 (65%) were norovirus positive. Seven patients were positive for *C. difficile* by at least one of the

methods used; all but one of these patients were positive for norovirus (See table). Two instances of more than one *C. difficile* positive patient in the same norovirus outbreak were detected. In one of these, the patients had different *C. difficile* ribotypes, and in the other, one of the results may represent a false-positive PCR as culture was negative.
Conclusions: Only a small number (6) of patients with norovirus also had *C. difficile* in their stool, and no evidence of transmission of *C. difficile* during a norovirus outbreak was found, suggesting only a weak linkage between the two infectious agents. If EIA alone had been used to diagnose CDI only 2 of the 7 cases would have been detected. This demonstrates the low sensitivity of EIA compared to PCR for the detection of *C. difficile*.

P1472 Does linezolid protect against *Clostridium difficile* infection in major heart surgery patients?

*M. Valerio *, M. Pedromingo, P. Muñoz, M. Marin, L. Alcalá, E. Bouza (Madrid, ES)*

Objectives: *Clostridium difficile* infection (CDI) is one of the main causes of diarrhea associated to antimicrobial therapy. Antibiotics with good "*in vitro*" activity against *C. difficile*, other than metronidazole or vancomycin, could be protective of CDI. One of these drugs is linezolid. We tried to assess the potentially protective role of linezolid in the development of CDI in patients receiving linezolid therapy for Ventilator Associated Pneumonia (VAP).
Methods: During a four year period, we retrospectively analyzed a cohort of patients who developed VAP after major heart surgery (MHS) in our institution. Patients were divided in those who developed CDI in the postoperative period and those who did not. We analyzed the charts in order to determine variables associated to the development of CDI including the role of antimicrobial therapy. We collected data involving pre-surgical, intraoperative and post-surgical variables. Treatment with Daily Defined Doses (DDD's) of linezolid and other antimicrobials were carefully registered. Univariate and multivariate analysis were performed. All statistical tests were two-tailed.
Results: Overall, 1844 patients had MHS. 105 cases had VAP, of which complete clinical data were available in 91 cases. CDI occurred in 22 (24.2%) patients. When comparing VAP cases with and without CDI, EUROSCORE and overall antibiotics prescribed were not significantly different. Patients with chronic renal failure were more prone to develop CDI than patients without it (32% vs. 13%; $p = 0.04$) and patients with CDI had received more doses of linezolid than dose without CDI (DDD's 12.4 ± 9.7 vs 6.7 ± 4.0; $p = 0.007$). A multivariate analysis confirmed that receiving more DDD's of linezolid protects against developing CDI (HR 0.908, $p = 0.04$, CI 95% 0.83–0.99).
Conclusion: Our study shows that VAP patients treated with more DDD's of linezolid therapy may have a lower occurrence of CDI. Our work has some limitations; it is retrospective, has a limited number of cases and addresses a very particular population but it is the first to suggest on clinical bases the potential impact of linezolid in protecting against CDI.

P1473 Real-time monitoring and fine tuning of joint (PCT – acute hospital) *Clostridium difficile* infections containment programme: common root cause analysis of CDI in the community and hospital – the missing link

R. Kato, I. Maitland, M. Ibeto, M. Qulaghassi, A. Guleri (Blackpool, UK)*

Introduction: A dynamic passage exists for patients with healthcare associated infections between community and acute hospital. *Clostridium difficile* infections (CDI) is associated with significant morbidity, mortality, associated cost, compromise in clinical quality and patient safety. Reduction in CDI in community & hospitals is of high priority for Department of Health (DH). The joint (PCT-hospital) infection control teams (ICT) identified the need to develop a root cause analysis (RCA) tool that could be used in community and the hospital. We present a feasibility study of developing and using such a common CDI RCA tool (pre48 h and post48 h). The results of RCA were used to inform the joint CDI containment programme and fine tune action plans to address new challenges.
Method: In the setting of St Mary's Medical Practice and Blackpool, Victoria Hospital, data was collected, analysed and fed back to clinical/nursing teams on 20 CDI RCAs (10 each pre48 h & post48 h).
Results: Data from 20 RCAs (10 each pre48 h & post48 h) revealed that virtually all the patients were elderly (>70 years). The single most common association with CDI was non-compliance with (primary care / hospital) antibiotic formulary with prolonged, inappropriate and repeated antibiotic courses. 1.5% were on proton pump inhibitors. 5–15% patients were on laxatives or antimotility agents; 15% has recurrent episodes in last 12-months; Hand hygiene compliance was variable. There were lapses in infection control protocols in care homes / rehab hospital.
There was a delay in collection of sample in 30%; 25% each were in patients and care home residents; 15% rehab patients.
Conclusion: Management of healthcare-associated infections incl. CDI should be addressed as a continuum rather than a hypothetical divide between community-acquired and hospital-acquired. The importance of the applying the root cause analysis in both the hospital and the community is to attempt to eliminate the risk factors, as opposed to merely addressing the resulting problems. In so doing, we are able to identify areas for change, develop any recommendations and to look for new solutions. Nursing staff at care homes would benefit from training on management & monitoring patients with CDI. Primary care trusts should direct their local GP practices to put in place assurance systems which demonstrate how they are complying with good infection control practice through rigorous clinical audit and root cause analysis.

P1474 *C. difficile* strain dependency of sporocidal acitivity of disinfectants

M. Martin, I. Winterfeld, P. Goroncy-Bermes, F. Mattner (Lubeck, Hamburg, DE)*

Background: *C. difficile* infections (CDI) are recognized as the major causes of nosocomial gastroenteritis with an increasing morbidity and mortality. A considerable part of CDI is acquired by transmission through hand-hand contacts or by indirect contact to surfaces. Sporocidal activity of disinfectants is crucial for prevention of nosocomial transmission.
Objective: To compare sporocidal activity of different disinfectants using spores of different genotypical and epidemiological characterized *C. difficile* strains.
Methods: *B. subtilis* ATCC 6633, one *C. difficile* strain isolated form only one patient, one endemic strain, ribotyp 027, 126, and 078 (1.5 to 5 106 CFU per ml) were used to test glutaraldehyde (2%), peracetic acid (PAA) (0.05%), sodium hypochlorite (500 ppm), perform® (2%) and gigasept PAA concentrate ® (1 and 2%) for sporocidal activity according to DIN EN 13704. A reduction factor (Rf) of 3 or more was regarded as effective.
Results: Rf for glutaraldehyde (30 min) were 0.89 for *B. subtilis* and >4 for all *C. difficile* strains. Rf for PAA (15 min) were 0.17 for *B. subtilis* and between 0.1 and 3.94 for *C. difficile* strains. Sodium hypochlorite was effective after 15 min for *B. subtilis* and 5 min for all *C. difficile* strains. Perform was effective after >120 min for *B. subtilis* and between 60 and 180 min for *C. difficile*. Gigasept PAA concentrate® 2% (1%) was effective after 5 (5) min for *B. subtilis* and between 5 (15) and 15 (30) min for different *C. difficile* strains. The unique *C. difficile* strain and ribotyp 027 and 078 were less susceptible against most disinfectants compared to other *C. difficile* strains and *B. subtilis* ATCC 6633 used as surrogate for sporocidal activity.
Conclusion: In comparison to *B. subtilis, C. difficile* strains were less susceptible against PAA and gigasept PAA concentrate and more against sodium hypochlorite. Susceptibilities against disinfectants seem to be strain dependent. Our results question the usefulness of surrogate testing for the determination of sporocidal activity of different disinfectants.

P1475 Characterization of multi-resistant *Clostridium difficile* clinical strains isolated in Europe

P. Mastrantonio, F. Barbanti, P. Spigaglia on behalf of the ESGCD and the EACCAD*

Objectives: To investigate the multi-resistant *Clostridium difficile* clinical isolates collected in 14 different European countries during ESGCD Prospective Study performed in 2005.
Methods: 316 toxigenic strains were analysed for resistance to erythromycin (EM), clindamycin (CM), moxifloxacin (MX), tetracycline (TC), chloramphenicol (CL), rifampin (RIF) metronidazole (MZ) and vancomycin (VA) by the E-test method. Isolates resistant to MX were also analysed for their susceptibility to gatifloxacin (GA), levofloxacin (LE) and ciprofloxacin (CI). Multi-resistance was defined as resistance to at least 3 classes of antibiotics. The mechanisms of resistance were investigated by PCR and/or nucleotide sequencing. All strains were previously typed by PCR-ribotyping.
Results: 148 strains were resistant to at least one class of antibiotic and 82 of these strains (55%) resulted multi-resistant (M-R). M-R strains belonged to 24 different PCR-ribotypes and 5 of them, 001, 012, 017, 048 and 168, included the majority (91%) of the isolates. All strains were resistant to EM and CM. In particular, 39 strains (48%) were also resistant to MX and RIF, 24 (30%) to MX, 6 (7%) to TC, 6 (7%) to TC, MX, CL and RIF, 5 (6%) to MX, CL and RIF, 1 (1%) to TC and MX, 1 (1%) to TC, MX, RIF. All strains resistant to MX were also resistant to GA, LE and CI. As far as the mechanisms of resistance are concerned, 76 isolates (93%) were ermB-positive. The remaining strains were also negative for other important erm classes, for mutations in the 23SrRNA, L4 and L22 genes, and for the presence of efflux pumps. Among the ermB-positive strains, different genetic organizations of the ErmB determinant were observed. Only 3 strains resistant to MX showed a substitution in position 426 of GyrB, whereas the others had the substitution Thr82 to Ile in GyrA. All strains resistant to RIF had two substitutions, His502 to Asn and Arg505 to Lys in RpoB, except for two isolates showing the substitution Arg505 to Lys. All strains resistant to TC were tetM-positive and carried a Tn5397. Four isolates showed the co-presence of a tetM and a tetW gene. Finally, all strains resistant to CL had a catD gene.
Conclusions: The results indicate an increased tendency of *C. difficile* clinical isolates to acquire resistance to different classes of antibiotics and underline the importance of a constant monitoring of antibiotic susceptibility in this bacterium.

P1476 Community-onset *Clostridium difficile* infection in eastern London

J. Rangaiah, M. Wilks, M. Millar (London, UK)*

Objectives: *Clostridium difficile* infection (CDI) is increasingly being recognised as a problem in community in Europe and North America. We undertook this study to determine the clinical and epidemiological aspects of community-onset CDI (CO-CDI) in the East London community to which Microbiology Laboratory, Barts and The London NHS Trust provides diagnostic services to.
Methods: The data on faeces samples processed in microbiology laboratory between May 2007 and April 2008 and the patient details were obtained by searching the laboratory computer database. Patients with positive CDT result on admission and in community were identified based on patient location at the time of specimen collection. Details on clinical presentation, risk factors, clinical management and outcome of the CDT positive patients were obtained from the case notes and GPs.
Results: Of the 4036 specimens sent by GPs only 93 (2.30%) were requested for CDT as compared to 6281 of 15,084 (41.64%) by hospital doctors. Twelve of the 93 (12.90%) GP samples and 782 of the 6281 (12.45%) hospital samples tested positive. The 93 GP requests came from 35 of the 823 (4.25%) GP surgeries and a single GP practice accounted for 27.95% of all GP requests. Twelve patients tested by GP and another twelve presenting to A&E were tested positive resulting in a total 24 patients fulfilling the definition of CO-CDI. Previous hospitalisation (n = 20) and exposure to antibiotics (n = 22) were the most significant risk factors followed by exposure to proton pump inhibitors (n = 10) and previous CDI (n = 5). Four patients presenting to A&E with worsening diarrhoea had visited a GP surgery in the last week, where CDI was not considered as a possible diagnosis. In 3 patients death was attributable to CDI.
Conclusions: This study supports the view that there is considerable under-diagnosis of CDI in the community as evidenced by a wide variation in the testing practice for CDI among GPs. While the question of what are the other risk factors for community associated CDI remains to be investigated further, it is very important to have a robust mechanism for early diagnosis and adequate management of patients with known risk factors of CDI becoming symptomatic in community. It may be more cost-effective to manage these patients in the community and may also reduce the risk of further spread of infection in the hospital.

P1477 Risk factors for endemic *Clostridium difficile* infections in an academic hospital in the Netherlands

M. Hensgens, A. Goorhuis, C. van Kinschot, M.J. Crobach, E.J. Kuijper (Leiden, NL)*

Objectives: *Clostridium difficile* infection (CDI) is the most important cause of nosocomial diarrhoea. Risk factor analyses have almost exclusively been performed in outbreak situations, using retrospective data. We performed a prospective analysis of risk factors for CDI in an endemic setting.
Methods: In a prospective case–control study from July 2006 through May 2009, we compared demographic and clinical data of all consecutive CDI patients (n = 93) to the data of patients without diarrhoea (n = 76) and of patients with non-CDI diarrhoea (n = 63).
Antibiotic usage in the three months prior to CDI was assessed using Defined Daily Doses, to take into account both dosing and duration of the antibiotic therapy.
Results: The incidence of CDI in the LUMC was 2 per 10 000 in-patient days. In total 24 different PCR ribotypes were found, among which types 014 (16%), 078 (13%) and 015 (8%) were the most frequent. Independent risk factors for CDI were previous admission, recent endoscopy and the use of 2nd and 3rd generation cephalosporins. Previous endoscopy and previous use of 3rd generation cephalosporins, vancomycin and gentamicin were associated with diarrhoea in general. The use of fluoroquinolones and proton pump inhibitors was not associated with endemic CDI. During the study period, 38 outpatients were diagnosed with CDI. Compared to CDI case patients, they were younger (mean age 49 vs 56) and more often had community-associated CDI (52% vs 4%). Interestingly, second generation cephalosporins (15%) and immunosuppressive agents were frequently (63%) used by outpatients with CDI. Mortality rates after 60 days were 7.5% CDI in-patients and 5.3% for outpatients, compared to a low mortality rate in both control groups (1.6% and 0%).
Conclusion: Some previously described risk factors for CDI were found to be risk factors for diarrhoea in general. Furthermore, the use of fluoroquinolones and proton pump inhibitors did not influence the risk of CDI in this endemic setting. Outpatients with CDI represented a distinct group of patients, associated with a relatively high 60-day mortality rate.

P1478 Factors associated with *Clostridium difficile* infection in a cohort of patients

C. Micallef, V. Siva, L.D. Liebowitz (King's Lynn, UK)*

Introduction: In England, the Department of Health set specific targets to reduce hospital-acquired infections including *Clostridium difficile* infection (CDI). Known predisposing factors for developing CDI include: age $\geqslant$65 years, previous antibiotic therapy and proton pump inhibitors (PPIs).
Objectives:
– To establish whether a relationship exists between incidence of CDI and co-amoxiclav in a cohort of patients admitted to a CDI Isolation Unit with laboratory and clinically confirmed CDI.

– To identify additional key predisposing factors for developing CDI.
Methodology: The Queen Elizabeth Hospital (QEH) is a district general hospital in Norfolk, England, with ca. 500 beds, providing a service to 280,000 people. In March 2008, a ten-bedded isolation unit for CDI was inaugurated. Only patients with a laboratory-confirmed *Clostridium difficile* toxin A and B positive stool tested are admitted to the unit. All consecutive patients were reviewed retrospectively. The following parameters were recorded: age, sex, antibiotic history, PPIs, co-morbidities, positive Meticillin-resistant *Staphylococcus aureus* (MRSA) colonisation/infection status, renal dysfunction, white cell count, C-reactive protein (CRP) and albumin on diagnosis.
Results: Preliminary results revealed that 55.3% of patients who developed CDI were given co-amoxiclav prior to the infection. Females accounted for 68.4% of cases and 78.9% of patients were ⩾65 years of age. In addition: 68.4% of patients also had cardiovascular disease. PPIs were prescribed in 42.1% of cases. Previous/concomitant MRSA was found in 18.4% of patients. Renal dysfunction was present in 42.1% of patients. Albumin levels were low in 89.5% of cases with 32.3% of these, having levels <20g/l. A CRP of >5 mg/l was found in 97.3% of cases and 60.5% had WCC $> 10 \times 10^9$/l, with 56.5% of these having levels $>15 \times 10^9$/l. The study is on-going and we will be presenting the final data, with statistical analysis at ECCMID 2010.
Conclusions: At the QEH, after decreasing the use of cephalosporins and quinolones, co-amoxiclav is the antibiotic most frequently associated with CDI. Most CDI patients also suffer from cardiovascular diseases. The majority of patients had a low albumin level and increased CRP.

Hospital-treated paediatric infections

P1479 Inhaled colistin for the treatment of tracheobronchitis and pneumonia in critically ill children without cystic fibrosis

M. Falagas, G. Sideri, I. Korbila, E. Vouloumanou, J. Papadatos, D. Kafetzis (Athens, GR)*

Objective: The role of inhaled colistin in the treatment of chronic *Pseudomonas aeruginosa* infection in patients with cystic fibrosis has already been evaluated. Yet, data regarding the role of inhaled colistin in critically ill pediatric patients without cystic fibrosis are scarce.
Methods: We present a small case series of critically ill children without cystic fibrosis who received inhaled colistin (colistimethate sodium) treatment.
Results: Three children (1 female), admitted to the intensive care unit (ICU) of a tertiary-care pediatric hospital in Athens, Greece, during 2004–2009, were identified to have received inhaled colistin as monotherapy for tracheobronchitis (2 children), and as adjunctive therapy for necrotizing pneumonia (1 child). Colistin susceptible *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated from the cases' bronchial secretions specimens. All 3 children received inhaled colistin in a dosage of 75 mg diluted in 3ml of normal saline twice daily (1.875.000 IU of colistin daily), for a duration of 25, 32, and 15 days respectively. The infections improved in all 3 cases. Also, a gradual reduction, and finally total elimination of the microbial load in bronchial secretions was observed during inhaled colistin treatment in the reported cases. All 3 cases were discharged from the ICU. No bronchoconstriction or any other type of toxicity of colistin was observed.
Conclusions: Inhaled colistin was effective and safe for the treatment of 2 children with tracheobronchitis, and 1 child with necrotizing pneumonia. Further studies are needed to clarify further the role of inhaled colistin in pediatric critically ill patients without cystic fibrosis.

P1480 Differences in intestinal colonization of term and pre-term born neonates hospitalized at a neonatal intensive care unit

T. Gschliesser, V. Strenger, A.J. Grisold, G. Feierl, L. Masoud, B. Resch, W. Müller, B. Urlesberger (Graz, AT)*

Objectives: Whereas human gut is sterile at delivery, bacterial colonisation occurs within the first days/weeks thereafter. Intestinal flora is a possible source of late onset sepsis in neonates. The aim of the study was to describe intestinal colonisation with enterobacteria in neonatal intensive care unit (NICU) patients.
Methods: We included newborns hospitalised at the NICU Graz from June 2006 to May 2009. Stool samples were routinely taken at the NICU twice a week. All children received a prophylaxis to prevent necrotising enterocolitis with *Lactobacillus rhamnosus*, Gentamicin or Colistin and Nystatin. We retrospectively analysed the spectrum of enterobacteria for different groups of patients and performed statistical analysis by means of SPSS for Windows (Mann–Whitney U test, Fisher's exact test). Bacterial species that were found in less than 10 patients were excluded from statistical analysis.
Results: 808 patients (437 boys, 371 girls, 611 <36 weeks of gestation) were analysed. 232 had a birth weight <1.500g (very low birth weight – VLBW). The median age (in days) at first colonisation with enterobacteria was: term borns 6 (1–72), preterm borns 13 (1–109), VLBW 20 (1–109). Preterm and term borns were colonised with 1–8 species (median 1) and 1–5 (median 1), respectively. We found 29 different enterobacteria species and included 12 in statistical analyses. Further results see table 1.
Conclusion: Analysing the 12 most commonly detected enterobacteria species, VLBW were colonised significantly later. This may occur because of different feeding regimens in VLBW.

Table 1. The 12 most commonly detected species; number and age at first colonization

Species	Number colonised				Age (days) at colonisation, median (range)		
	All (n = 808)	<1,500 g (n = 232)	⩾1,500 g (n = 576)	p-value	<1,500 g	⩾1,500 g	p-value
Citrobacter amalonaticus	11	4	7	n.s.	62 (29–91)	26 (9–36)	0.023
Citrobacter freundii	53	23	30	n.s.	48 (12–202)	14.5 (1–38)	<0.001
Enterobacter cloacae	243	100	143	0.007	26.5 (5–112)	13 (1–72)	<0.001
Escherichia coli	301	69	232	n.s.	27 (1–202)	9 (1–63)	<0.001
Klebsiella oxytoca	277	104	173	n.s.	30 (5–202)	12 (1–73)	<0.001
ESBL pos.	10	3	7	n.s.	93 (67–102)	11 (5–32)	0.017
Klebsiella pneumoniae	203	77	126	n.s.	24 (2–121)	11 (1–78)	<0.001
ESBL pos.	34	17	17	n.s.	38 (9–71)	18 (3–36)	<0.001
Morganella morganii	74	34	40	n.s.	18 (2–58)	11.5 (1–52)	0.008
Pantoea species	10	3	7	n.s.	31 (25–328)	7 (2–18)	0.016
Proteus mirabilis	24	4	20	n.s.	16 (1–78)	11 (2–26)	n.s.
Serratia marcescens	47	22	25	n.s.	29 (5–91)	9 (2–26)	<0.001

P1481 Evaluation of *Streptococcus agalactiae* positive newborn cases

E. Hajdú, T. Szabó, Z. Reiger, P. Gál (Szeged, HU)*

Objectives: *Streptococcus agalactiae* (Group B *Streptococcus* – GBS) emerged as the leading infectious cause of neonatal morbidity and mortality. These bacteria can colonise the vagina of women without any infectious symptom. Infants can become infected with GBS during passage through the birth canal, although the majority of infants who are exposed to GBS during labour become colonized on skin or mucous membranes but remain asymptomatic. Our objective was to evaluate the GBS positivity among newborns.
Methods: The nasopharynx and stomach fluid surveillance samples of newborns admitted to the Neonatal Intensive Care Unit during 2007 and 2008 were cultured. We also used latex agglutination test (Pastorex Meningitis StreptoB, BioRad, Hercules, California US) for the detection of GBS antigen in urine and cerebrospinal fluid of newborns. Inflammatory parameters (C reactive protein, white blood cells and thrombocyte count) were used to confirm the presence of GBS infection. The vaginal samples of the GBS positive newborns' mothers were also analysed. The vaginal fluids of the pregnant women were cultured between the 35 and 37 gestational week according to CDC recommendations.
Results: Newborns in whom both the antigen test and the surveillance culture were positive for GBS all showed infectious signs (9 cases). Newborns with positive GBS latex urine samples only, had symptoms of infection in 44% (28 out of 63 cases), while those with only positive GBS surveillance cultures had clinical signs of infection in 38%. Vaginal culturing was performed in 51% of the affected women with 24% positivity rate (41/10). All GBS positive mothers received antibiotic prophylaxis during delivery. In the other 24 cases antibiotic was administered after the risk assessment for GBS infection.

Conclusion: The indication of antibiotic prophylaxis – for avoiding development of GBS caused infection in newborns – is based on risk-assessment or positive culturing. Among GBS positive newborns mothers had GBS positive vaginal culturing in low number, probably because of the improper sampling. Latex agglutination is a rapid test for detecting GBS but in itself it is not diagnostic.

	2007	2008	All
Number of positive GBS latex urine samples of newborns	26	37	63
Number of positive GBS culture of newborns	12	6	18
Number of cultured vaginal samples of mothers/GBS positive samples of mothers	16/7	25/3	41/10
Number of cases received antibiotic prophylaxis during delivery	13	21	34
Number of newborns with infectious symptoms	20	23	43

P1482 Bloodstream infections due to Gram-negative bacilli in a neonatal intensive care unit: a 6-year study

M. Papadimitriou, A. Alexaki, D. Kyriakou, N. Paleologou, K. Kallergi, J. Kapetanakis, E. Lebessi (Athens, GR)*

Objectives: Bloodstream infection (BSI) due to Gram-negative bacilli (GNB) is a frequent complication in neonatal intensive care units (NICUs). Moreover, multidrug resistant organisms are on the increase and pose a formidable clinical challenge. The aim of the present study was to assess the BSI due to GNB in a 30-bed, university-affiliated, level III-IV NICU at a large pediatric hospital in Athens.
Methods: The charts of all neonates with culture-proven BSI due to GNB admitted to our NICU over six years (Jan 2003 Dec 2008) were reviewed, in order to estimate epidemiological and clinical features, bacteriologic pattern and antimicrobial susceptibility.
Results: A total of 2815 neonates (58.5% males) were admitted during the study period. Among those, 57 episodes of BSI involving 59 GNB were diagnosed in 55 neonates (63.6% males, 50.9% preterms). Associated pathology in the above group included congenital anomalies (44.6%), necrotizing enterocolitis (21.4%), while meningitis was diagnosed in 10%. Mortality rate was 18.2% (10/55), while overall mortality in the NICU was 3.7% (104/2815). Average hospitalization in the NICU before infection was 39.4 days (0 to 322 d), and the mean NICU length of stay was 70.4 days (7 to 338 d). Thirty two cases were NICU-acquired. The incidence of NICU-acquired BSI due to GNB was 11.3/1000 admissions. No outbreaks or epidemics were observed. GNB accounted for 19.6% of organisms isolated from blood cultures. Twelve bacterial species were identified: *Escherichia coli* 42% (25), *Klebsiella pneumoniae* 23% (14), *Enterobacter cloacae* 12% (7), *Enterobacter aerogenes* 5% (3), *Klebsiella oxytoca* (2), *Acinetobacter baumannii* (2), *Serratia marcescens* (1), *Proteus mirabilis* (1), *Salmonella* non typhi (1), *Pantoea agglomerans* (1), *Pseudomonas aeruginosa* (1) and *Chryseomonas luteola* (1). Production of extended-spectrum β-lactamases (ESBLs) was detected in *Klebsiella* spp (63%), *E. coli* (16%) and *Enterobacter* spp (10%). Overproduction of AmpC β-lactamases was recognized in 20% of *Enterobacter* spp. Susceptibility to carbapenems was found to be 100%, to ciprofloxacin 98% and to aminoglycosides 85–90%.
Conclusion: Neonatal septicemia due to GNB is a major cause of morbidity and mortality. There was appreciable resistance to commonly used antimicrobials. Understanding the local epidemiology of neonatal BSI can lead to more appropriate initial antibiotic therapy and may contribute to improvement of infection control practices.

P1483 Congenital and neonatal cytomegalovirus infection in pre-term neonates

E.M. Gonzalez Barbera, B. Acosta Boga, M. Cernada Badia, A.W. Rosingh, J. Cordoba Cortijo, M. Gobernado (Valencia, ES)*

Objectives: To study the incidence of congenital or neonatal cytomegalovirus (CMV) infection children hospitalized in the Neonatology unit of a tertiary care hospital during the period January 2006–October 2009.
Methods: A prospective observational study was carried out in the Neonatology unit including neonates with a birth weight $\leqslant$1.500 g. or signs/symptoms compatible with CMV infection.
Congenital (CMV positive result in the two first weeks of life) and neonatal CMV infection were identified through urine shell-vial culture [MRC5 cell line (Vircell™), CMV monoclonal antibody stain (Chemicon™)] and/or plasma CMV viral load [artus CMV LC PCR Kit (Qiagen)]. We studied 1591 urine samples by shell-vial culture and 178 plasma samples by CMV viral load from 894 neonates. The same techniques were used for follow-up study (and in some cases we also carried out a CMV maternal study). We also collected data about blood transfusions in these patients.
Results: Fifty-three (5.93%) neonates were diagnosed with cytomegalovirus infection. Fourteen of them (26.41%) were considered congenital, in seven infants (13.20%) we couldn't distinguish between congenital or perinatal infection and thirty-two children (60.38%) were considered perinatal infection. Of them, in 16 cases the breast milk was studied for CMV presence (shell-vial/viral load) finding ten positive results.
All children except two with perinatal infection received blood products (one with shell-vial positive result of breast milk).
Conclusion: In our study neonatal CMV infection is frequent in preterm infants. The role of breast feeding is important in perinatal transmission (10 positive results/16 studied cases), but most of them (30/32) had been exposed to other transmission routes, such as the use of blood products. CMV urine shell-vial culture is a good and non invasive test for screening, but not for follow-up patients. CMV plasma viral load could be the best choice for following up on these patients.

P1484 Virulence factors and antibiotic resistance in uropathogenic *Klebsiella pneumoniae* isolates from paediatric units at a Portuguese hospital

S. Arriaga Cerqueira, P. Machado, J. Marto, A. Silva, L. Lito, J. Melo Cristino, A. Duarte (Lisbon, PT)*

Objectives: The aim of this study was to verify the relationship of *Klebsiella pneumoniae* isolates, as well as investigate their extended-spectrum β-lactamases (ESBLs) and virulence factors.
Methods: This study included thirty-nine *Klebsiella pneumoniae* isolates identified from urine culture collected from eight paediatric patients at Santa Maria Hospital, Lisbon, throughout 2008. Antibiograms were determined by disk diffusion method on Mueller-Hinton agar plates. Molecular typing was performed by M13-Polymerase Chain Reaction (PCR) fingerprinting and in representative strains by Multilocus Sequence Typing (MLST) to confirm the genotype. Genetic environment of blaCTX-M-type and virulence factors (iucC, magA, rmpA, khe, fimH, mrkD, capsular gene k2) were screened by PCR and the products were sequenced.
Results: M13-PCR fingerprinting identified two predominant clones among the isolates, which we designated M1 and M2. MLST confirmed this result, showing different sequence types (STs): M1 profile matched ST15 and ST326, which differed only in tonB allele sequence (98.1% of similarity); M2 corresponded to ST20. 40% of M1 isolates showed antibiotic resistance profile R1 (resistance to cefotaxime, CTX, ceftazidime, CAZ, gentamicin, GM, and ciprofloxacin, CIP) and 60% showed profile R2 (resistance to CTX, GM and CIP). 14.3% of M2 isolates exhibited profile R1, 71.4% was profile R3 (resistance to CTX, CAZ and GM) and 14.3% was profile R4 (resistance to CTX and CAZ). All the isolates of both M13-PCR fingerprinting profiles were imipenem-susceptible. We could also detect the gene for CTX-M-15 in 43.6% of all the isolates included in the study. None of the clones showed iucC, magA, rmpA or capsular gene k2, but they were positive for khe (50% in M1 isolates; 85.7% in M2), fimH (100% in M1; 92.9% in M2) and mrkD (80% in M1; 100% in M2).
Conclusion: The isolates included in this study have a high incidence of fimH and mrkD, which reinforces the fact that they are uropathogenic and might explain their persistence in some patients for periods as long as four months.

P1485 Antimicrobial resistance trends in β-haemolytic streptococci from throat infections in a paediatric hospital in Greece

K. Giannakopoulou, G. Antonaki, N. Paleologou, A. Kallimani, A. Margoni, S. Christopoulos, E. Lebessi (Athens, GR)*

Objectives: To evaluate the antimicrobial susceptibilities and macrolide – resistance phenotypes of large-colony-forming β-haemolytic streptococci (BHS) isolated from throat infections at a pediatric population in the area of Athens.
Methods: All BHS isolated from 7710 pharyngeal specimens obtained from patients referred to "P. & A. Kyriakou" Children's Hospital over a five-year period (Jan 2004 through Dec 2008) were reviewed using the laboratory archives. *Streptococcus agalactiae* isolates were not included. Susceptibility testing to penicillin, erythromycin, clindamycin, tetracycline, chloramphenicol, cefotaxime, rifampin and vancomycin was performed by disk diffusion test according to the CLSI guidelines. Macrolide resistance phenotypes were determined for strains isolated during 2006 to 2008, by double-disk diffusion test using erythromycin and clindamycin.
Results: During the study period a total of 1908 BHS were isolated. Of these, *Streptococcus pyogenes* was accounted for 95.2% (1817/1908) and *Streptococcus dysgalactiae* subsp. *equisimilis* (Lancefield group C, G) for 4.8% (91/1908). Among *S. pyogenes* isolates, resistance to erythromycin was found in 19% (annual resistance rates from 2004 to 2008: 13.1%, 15.8%, 21%, 19.8%, 26%), resistance to chloramphenicol and tetracyline was found in 0.4% and 7.9%, respectively. Sixty seven percent of tetracycline resistant strains were also resistant to erythromycin. Among *S. dysgalactiae* subsp. *equisimilis* isolates, higher resistance rates to erythromycin, chloramphenicol and tetracycline were found to be 23.1%, 1.1%, and 37.3%, respectively. The macrolide-resistance phenotype distribution (2006 through 2008) was as follows: M; 44.4%, 55.5%, 32.4%, MLSBi; 33.3%, 22.2%, 44.5%, MLSBc; 22.2%, 22.2%, 22.9%. The prevalent phenotype of MLSB resistance in *S. pyogenes* isolates have been changed from the M to the MLSBi. Resistance to antimicrobial agents other than macrolides, clindamycin, tetracycline, and chloramphenicol was not found.
Conclusions: There was a progressive increase of resistance to macrolides and a relative decrease in the M phenotype. This reduces the effectiveness of macrolides and clindamycin as an alternative treatment. Continual monitoring of antimicrobial resistance among large-colony-forming BHS is very important for the option of the empiric therapy.

P1486 Susceptibility rates, emm-types and endotoxin genes among *Streptococcus pyogenes* isolated from invasive paediatric infections

A. Makri, A. Stathi, J. Papaparaskevas, T. Guajardo, L. Zachariadou, A. Pangali, A. Voyatzi, A. Tsakris (Athens, GR)*

Objectives: The epidemiological investigation of *Streptococcus pyogenes* (Group A *Streptococcus* – GAS) strains isolated from invasive infections in a pediatric Greek hospital.
Materials and Methods: During a two year period (2007–2008), all invasive GAS isolates together with clinical and demographic data were collected prospectively. Antibiotic susceptibility was investigated using a gradient strip method (E-test, bioMérieux). Resistance phenotypes were assigned by the double disk approximation test, using erythromycin (E) and clindamycin (CL) disks. M-typing was performed with emm-gene sequencing. PCR was used for detection of speA/B/C endotoxin genes.
Results: Invasive GAS strains were collected from 50 patients. Specimens comprised deep soft tissue (54%), middle ear aspirates after myringodectomy (22%), blood (8%), BAL and pleural fluid (12%), and joint aspirates (2%). Emm-types 1, 77, 6, and 12 (14, 12, 10 and 8%, respectively) dominated (44% of all infections), whereas less frequent types were emm-type 5, 91, 28 and 11 (all with a rate of 6%). All emm-type 1 isolates were fully susceptible to all antibiotics tested. Overall E and CL resistance rates were 22% and 12% respectively. The Constitutive (CR-MLSB) and the Inducible Resistant (IR-MLSB) phenotypes were detected in 12% and 10% of all isolates, respectively, whereas the M-phenotype was detected in 2%. The speA toxin gene was detected mostly amongemm-type 1 isolates, whereas the speC was more common in several emm-types such as 6, 77, 12, 91 and 5. The IR-MLSB phenotype was related with emm-type 77, in contrast with CR phenotype which was associated with several other emm-types (11, 28, 77, and 12). There was a correlation between specific emm-types and clinical presentations. More specifically, emm-type 1 isolates were associated with sepsis and Streptococcal Toxic Shock Syndrome (STSS) cases.
Conclusions: Emm-type 1 susceptible isolates prevailed among invasive GAS pediatric isolates, were associated with speA toxin gene, and were detected mostly in cases of sepsis and STSS. The CR-MLSB phenotype was the main resistance phenotype, was detected in specific emm-type strains and was associated with the SpeC toxin gene. In contrast, the IR-MLSB phenotype was associated detected mostly among emm-type 77 strains.

P1487 Paediatric Chagas' disease in a non-endemic area

A.C. Aguilar, T. Juncosa, A. Gené, V. Fumadó (Barcelona, ES)*

Objectives: Until the middle of the 20th century Chagas' disease was confined to the rural area in Central and South America, but after industrialization country people moved to the cities taking with them culture and diseases, among others Chagas' disease.
Nowadays, the increase of migration from this countries to non-endemic areas such as USA and Europe has introduced among our paediatric population some patients infected by *T. cruzi,* either in their original country or in ours trough vertical transmission from their mothers.
The aim of this study was to describe the cases of children with Chagas' disease admitted in our Imported Pathology Unit during the last six years (2003–2008).
Methods: All patients coming from endemic areas, and newborns from Chagas' disease seropositive pregnant women were studied by two ELISA tests (Bioelisa Chagas BiokitR with recombinant antigens and in-house ELISA with complete antigens). Patients with available sample were also screened by nested PCR (TCZ3/Z4). In the seroreactive patients younger than one year old the ELISA test were done again up to 12 month old, in order to demonstrate vertical transmition if antibodies persist. Confirmed cases received Benznidazol as a treatment (8–10 mg/Kg/day, during 60 days). Both PCR and specific antibodies must be negative in order to demonstrate the effectiveness of treatment.
Results: Screening was performed in 202 patients from one day to 14 years old (157 were immigrants and 45 were born in Spain). Forty three of them (30 younger than one year old) were seroreactive in both ELISA tests (21.2%). We performed forty PCR tests, which were positive in ten cases. In 18 patients Chagas' disease were diagnosed. In seven of them, who were born in our country, vertical transmission was demonstrated. All 18 patients were treated. In five cases the specific antibodies become negative and demonstrated the healing. Follow up is in course in eleven of the seroreactive patients. Two patients didn't continue attending our hospital.
Conclusion: Taking into account the possible severity of Chagas' disease, the effectiveness of the treatment in the first years of live and the incidence in paediatric immigrate population from Central and South America found in our study, we suggest the systematical serologic screening in this population.

P1488 Antimicrobial resistance of pathogens in hospitalized children with urinary tract infection in Greece

S. Konstantopoulou, I. Daniil, I. Petraki, E. Nikolarou, E. Balampani, P. Ntoska, D. Papageorgiou, A. Charalampidou, D. Petropoulou (Athens, GR)*

Objectives: The manifestation of urinary tract infection (UTI) in children tends to be nonspecific, so the culture of urine collected appropriately is the method of choice for the diagnosis and management

of UTI, especially in infants and young children. Moreover, UTIs are among the most common infections with an increasing resistance to antimicrobials worldwide. The aim of this study was to indentify the causative pathogens in children hospitalized for community acquired febrile UTI in a tertiary hospital in Greece and to investigate their resistance to common antibiotics.
Methods: During three-years period (2007–2009), we studied a total of 117 urine culture specimens from children (65% female and 35% male, aged 0.08 to 13 years) hospitalized with symptoms and signs of UTI. Transurethral catheterization or bladder tap was the urine collection method in children <2 years old, while clean voided midstream urine specimens collected from older children. The urine specimens were examined by Gram stain and culture in appropriate media (blood agar and McConkey agar) for detection of uropathogens. Any isolated pathogen (after 24–48 hours incubation) was identified using BBL™ Enterotube™ II (BD Diagnostic Systems, Germany), Api System and Vitek 2 Compact (Biomerieux, France). Disk diffusion agar method was used according to the current CLSI guidelines for the antibiotic susceptibility test of isolated uropathogens.
Results: The most frequent UTI pathogens detected in children enrolled in the study were: *E. coli* (73.4%), *Klebsiella* spp. (8.9%), *P. mirabilis* (8.1%), *E. faecalis* (3.2%) and *Pseudomonas* spp. (1.6%). Antimicrobial resistance of *E. coli* isolates to commonly used antibiotics was: ampicillin 67%, trimethoprim-sulfamethoxazole (TMP-SMX) 27.3%, cefuroxime 11% and gentamicin 7.7%. Resistance to ampicillin, TMP-SMX, cefuroxime and gentamicin was noted for 67%, 31.5%, 12.1% and 8.1% of the total uropathogens, respectively.
Conclusion: *Escherichia coli* was the most prevalent pathogen in children hospitalized with UTI. Several of the first-line agents for empirical treatment of childhood UTI seem to have become ineffective, mainly ampicillin and TMP-SMX. Staining and culture examination of urine samples and antibiotic susceptibility test of detected uropathogens, along with the clinical information available at diagnosis, can help in treatment selection.

P1489 Bacterial flora in infected ingrown toenails in a paediatric population

A. Margoni, G. Schinas, K. Giannakopoulou, M. Matsas, G.L. Morassi, V. Soranoglou, N. Nikolaides, E. Lebessi (Athens, GR)*

Objectives: Ingrown toenails (IGTNs) is a common problem in pediatric population, especially adolescents. Conservative and surgical methods are available for treatment, but antibiotics necessity still remains controversial. The aim of this study was to define the bacteriological spectrum and the antimicrobial susceptibility patterns in healthy children with infected IGNTs, referred for care at "P. & A. Kyriakou" Children's Hospital.
Methods: Infected IGTNs treated at the outpatient clinic and the department of orthopedics from Jan 1, 2007 to Oct 31, 2009 were reviewed retrospectively, using the laboratory archives and patients charts. Antimicrobial susceptibility was tested by disk diffusion method according to CLSI criteria.
Results: Totally, 157 specimens from an equal number of children (56% boys), with a mean age 11.7±SD2.48 years, were recorded. Cefaclor or amoxicillin/clavulanate, were given in all cases as empiric therapy. The majority of infections were mixed with two to five organisms (76%). The most frequently isolated bacteria in mixed cases were *Staphylococcus aureus* (79%) and anaerobes (53%), followed by *Pseudomonas aeruginosa* (11%), Enterobacteriaceae (14%), *Streptococcus pyogenes* (9%), *Acinetobacter baumannii* (7%), *Eikenella corrodens* (6%), *Stenotrophomonas maltophilia* (5%), *Streptococcus agalactiae* (5%), *Streptococcus anginosus* group (3%), *Haemophilus* parainfluenza (3%), *Haemophilus* influenza (2%), *Candida* spp (2%). In monomicrobial cases (24%), *S. aureus* was also the most common (89%), followed by *S. pyogenes* (8%). Of all *S. aureus* isolates, 79% were found resistant to penicillin and 6% to methicillin (MRSA). All but one MRSA strains had the same resistant phenotype penicillin/oxacillin/fusidic acid/kanamycin/tetracycline, which commonly characterizes the community-acquired strains (CA-MRSA). Among *S. aureus* isolates the resistance to macrolides was 13.2% (17/128) [M-phenotype; 5, MLSBi (inducible resistance); 5, and MLSBc (constitutive resistance); 7]. Of *S. pyogenes* isolates, 14.2% (2/14) were resistant to macrolides. All other bacteria were wild strains.
Conclusions: A wide range of bacterial flora was observed in infected IGTNs, with the predominance of *S. aureus*. It is of note, that bacteria with intrinsic resistance, such as *P. aeruginosa*, *S. maltophilia, A. baumannii*, were isolated in significant frequencies. The identification and susceptibility testing of pathogens is useful, as pre-operative empirical treatment could be ineffective.

P1490 Enteroviruses linked to type 1 diabetes onset in young children

C.M. Mihai, R. Stoicescu, I. Durbala (Constanta, RO)*

Background: A rapid rise of T1DM (type 1 diabetes mellitus) among the youngest age group (1–9 years of age) is particularly marked for regions in Central and Eastern Europe. Despite a strong genetic component to the susceptibility of T1DM, this marked increase in incidence in different populations within a short period of time cannot be explained by increased transmission of T1DM susceptibility genes.
Objectives: To assess the link between the recent onset in T1D and the exposure to environmental triggers-Enteroviruses (EV) historically considered the prime suspects, in children with human leukocyte antigen (HLA)-conferred susceptibility or protection to T1DM at the onset, using direct detection of the EV genome by Reverse transcription polymerase chain reaction (RT-PCR) in peripheral blood.
Methods: Serum samples were collected from children with newly diagnosed type 1 diabetes (n = 21), aged 1–9 years; islet autoantibody-positive (n = 16) and -negative (n = 5) first-degree relatives of type 1 diabetic patients (n = 40); and control subjects (n = 64).
Results: 7 children newly diagnosed with T1DM tested positive for enterovirus (33.3%). Newly diagnosed T1DM children (7/21, 33.3%) and islet autoantibody-positive first-degree relatives (4/40, 10%) tested positive more than their corresponding matched controls (2/64, 3.1%), 95% confidence interval; OR 15.7, $P < 0.001$ and OR 21.4, $P < 0.01$, respectively). 2 children with low genetic risc, carring the HLA DQB1*0602 tested positive for enterovirus RT-PCR. All 7 children tested positive for enterovirus presented with severe diabetic ketoacidosis (pH < 7.1), 1 was negative for islet autoantibodies.
Conclusions: The presence of enteroviral infection appeared to be associated with severe diabetic ketoacidosis at onset, high circulating islet cell antibodies (ICA) titres. In patients with T1DM, who are at low genetic risk, enteroviruses could contribute to diabetes onset.

P1491 Prevalence and clinical features of respiratory syncytial virus and human metapneumovirus lower respiratory disease among children in Greece

F. Vagia, A. Makri, H. Iliadou, I. Mammas, T. Guajardo, E. Nika, N. Myriokefalitakis, A. Voyatzi (Athens, GR)*

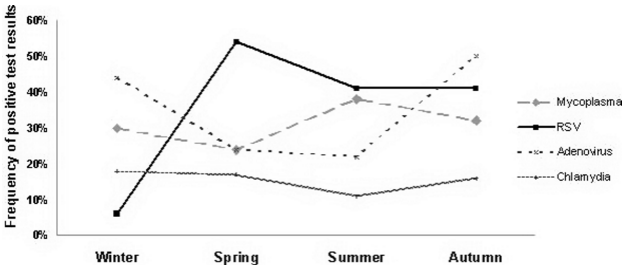
The clinical symptoms of human Metapneumovirus (hMPV) resemble those of respiratory syncytial virus (RSV) and influenza A/B (Flu A/B). The symptoms range from influenza like illnesses to severe respiratory tract infections as bronchiolitis and pneumonitis. A rapid diagnosis of these respiratory viral agents should be valuable for appropriate patient management and infections control measures. Objectives: of the present study were: 1) to identify and compare the incidence by age group of infections due to RSV, hMPV and flu A/B in infancy and early childhood, 2) to analyze clinical features and seasonality of the RSV, hMPV flu A/B during a two year period (2008–2009).
Material and Methods: A total of 606 children (male/female 2:1) below 5 years old (<2 y = 305), (2–5 y = 301) were hospitalized for lower respiratory tract illness (LRTi). Nasopharyngeal aspirates were tested for RSV, hMPV, fluA/B antigen using rapid and accurate immunoassays (Meridian Bioscience USA-Biotrin International Ltd, Ireland Quidel

Corporation, USA respectively). The patients clinical data were recorded. Disease severity was determined by the length of hospitalization and increased O2 requirement.
Results: RSV was detected in 28.5%, hMPV in 10.4% FluA 5% and FluB 3% in respiratory samples. Fourteen co-infections were also observed caused by hMPV+RSV (12) and RSV+FluA (2). Similar clinical symptoms (retractions, wheezing, crackles) for RSV and hMPV were found in 75%. No children required admission in intensive care unit. Oxygen requirement for RSV pneumonia was found 65% while for hMPV was decreased 30%. The remaining infants had uncomplicated clinical bronchiolitis and required a short hospital stay (<5 days). The mean age for hMPV was below 4 months and for RSV was 7 months while Flu A/B was detected as single infection in a group age among children for 2 to 5 years old. The significant RSV and hMPV incidence peak occurred during colder months while the occurrence of influenza followed seasonal patterns that become more expanded during the year.
Conclusions: (1) RSV (28.5%) remains the most frequent virus among neonates <2y. (2) The most prevalent diagnosis in children with positive sample for hMPV was bronchiolitis. (3) Our data confirm that rapid clinical diagnostic assays will be necessary to distinguish hMPV from RSV infection. (4) The burden of disease due to RSV, hMPV suggest that a vaccine should have significant health and socioeconomic benefits.

P1492 Seasonality of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, respiratory syncytial virus and adenovirus paediatric respiratory tract infections in northern Greece – a 2-year study

S. Prodromidou, C. Kanonidou, M. Pape, V. Anagnostou, K. Mandraveli-Chatzikosta, S. Dionysopoulou, E. Diza (Thessaloniki, GR)*

Objective: *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP), Respiratory syncytial virus (RSV), and Adenovirus (AV) are among the pathogens of respiratory tract infections in childhood. The aim of this retrospective study was to investigate the seasonal distribution of these infections in a paediatric population in northern Greece and their association with local temperature conditions.
Methods: The study population consisted of 572 patients (2 months-6 year old) admitted to AHEPA University Hospital of Thessaloniki with symptoms of respiratory tract infection from 11/07 until 10/09. The presence of specific antibodies (IgG, IgM, IgA) was quantitatively determined by ELISA (RIDASCREEN, r-biofarm) in paired serum samples from each patient: for MP in 462, CP 356, RSV 72 and AV 409. x2 test and x2 goodness of fit test were performed.



Seasonality of *M. pneumoniae*, RSV, Adenovirus, and *C. pneumoniae* respiratory tract infections in children.

Results: 136 (30%) of 461 paired samples tested for the above pathogens in winter were positive. AV was the most common pathogen (44%). From 405 cases in spring, 94 (24%) were positive and MP infection was the most frequent (35%). 76 (27%) of 278 samples were positive in summer and the number of MP infections was the highest (54%). In autumn 55 (35%) of 158 samples were positive and AV infection was diagnosed in 50% of cases. MP exhibited a peak of activity in summer (38%), although no significant difference was found between the 2 years of study and different seasons as well. A spring peak of RSV positive cases (54%) was observed. The prevalence of RSV infections was lower in summer (41%) and autumn (41%). A significant reduction of positive cases occurred from autumn to winter (6%) ($p < 0.05$). AV infections reached a peak in autumn (50%). A significant raise was observed from summer to autumn ($p < 0.01$) and a significant reduction from winter to spring ($p < 0.001$). Statistical difference among the years of study was also observed ($p < 0.01$). CP was detected all year round. A significant increase of positive cases was found during the second year ($p < 0.05$). The mean monthly local temperature value did not vary significantly between the 2 years.
Conclusion: The occurrence of respiratory tract infections in children was almost the same all year round. Each pathogen demonstrated distinct seasonal patterns. The peak of MP infections was observed in summer, of RSV in spring and AV in autumn. Studies on seasonality are valuable for optimizing medical management.

Antimicrobial stewardship, antibiotic policies and antibiotic consumption

P1493 Implementing antimicrobial stewardship and control of antibiotic prescription to decrease MRSA in the intensive care unit

L. Pagani, R. Aschbacher, N. Vernaz, M. Falciani, P. Mian, B. Moser, P. Zanon (Bolzano, IT; Geneva, CH)*

Objectives: to evaluate the impact of an Antimicrobial Management Team (AMT) on appropriateness of antimicrobial prescription and control of MRSA prevalence in the Intensive Care Unit (ICU) of a tertiary hospital.
Methods: after reviewing clinical, microbiological and pharmaceutical data of 2002 as historical comparison, a two-phase intervention by the AMT was implemented in our 18-bed general ICU. From January 2003 to March 2004, a regular on-site educational activity was carried out by infectious disease consultant, aimed at defining optimized antimicrobial therapy according to local epidemiology of MRSA and clinical pharmacology parameters. From April 2004 to December 2007, direct control or restriction on antimicrobial prescription by the AMT was added to, and mainly concerned broad-spectrum and last-generation drugs.
Results: the percentage of MRSA isolates recovered in ICU decreased progressively and significantly from 38% (95% CI, 29–47%) in 2002 to 15.6% (95% CI, 6–20%) in 2007 (–5.1% per year; $r = -0.99$; $P = 0.001$), which corresponds to a likewise significant decrease in resistance rates: 9.3 MRSA isolates per 1,000 patient-days in 2002 to 3.3 MRSA isolates per 1,000 patient-days in 2007 (–1.3 MRSA isolates per 1,000 patient-days; $r = -0.97$; $P = 0.001$). Variations in consumption of some antimicrobials, expressed in defined daily doses (DDD) per 100 patient-days, were assessed through time-series analysis and are shown in the table.
Conclusion: the interventional antibiotic policy carried out by the AMT has significantly decreased the prevalence of MRSA in this setting. These findings demonstrate that antimicrobial stewardship may help improve patient safety, while decreasing both antimicrobial resistance and aggregate economical costs.

Antimicrobials	January 2002 to December 2002		January 2003 to December 2007		Trend
	Average monthly use (min-max)	% of J01 use	Average monthly use (min-max)	% of J01 use	
Total antibiotic use	618 (398–810)	100%	513 (110–829)	100%	stable
Oxacillin	NA	0%	58 (0–200)	11%	stable
Ampicillin/sulbactam	327 (224–525)	53%	157 (50–368)	31%	downward*
Cefuroxime	10 (0–30)	2%	6 (0–37)	1%	downward*
III gen. Cephalosporins	80 (25–130)	13%	46 (0–140)	9%	downward*
Fluoroquinolones	126 (56–182)	20%	126 (8–274)	25%	stable
Vancomycin	35 (8–75)	6%	19 (0–73)	4%	downward*
Linezolid	NA	0%	22 (0–65)	4%	upward*

*Statistically significant at p-value <5%.

P1494 Antibiotic stewardship programmes: national recommendations, public release of structure and process indicator and practical implementation in south-western French hospitals, 2005–2008

C. Dumartin, A.M. Rogues, M. Pefau, B. Amadeo, A.G. Venier, P. Parneix, C. Maurain (Bordeaux, FR)*

Objectives: According to the national strategy launched in 2001 to improve antibiotic (AB) use, French hospitals are required to implement AB stewardship programmes (ABS). The Ministry of Health (MoH) issued recommendations regarding AB monitoring, organisation for prudent use (2006), and enforced mandatory reporting of a structure and process indicator (SPI) reflecting ABS, intented for public disclosure (2007). We performed yearly surveys to describe the development of hospital ABS in Southwestern France and to assess the compliance with national requirements.
Methods: Annual retrospective surveys were conducted in a convenience sample of 84 hospitals (22% of hospital beds in the region): 24 secondary/tertiary public hospitals, 31 acute care private hospitals, 14 rehabilitation centres, 8 local and 7 psychiatric hospitals. ABS was assessed by questions on structure and organisation; resources, guidelines, surveillance and evaluation. We described each measure, the SPI and their evolution for each hospital from 2005 to 2008.
Results: Improvements were seen in the content of ABS for each type of hospitals. SPI values were higher for private hospitals and rehabilitation centres than in others. In 2008, at least 98% of hospitals had implemented formularies, antibiotic committees, AB prophylaxis guidelines, and monitored AB use. AB advisors were appointed in 85% of hospitals in 2008 compared to 50% in 2005. Areas covered by official texts and included in SPI sharply improved from 2006: treatment guidelines (2006), computerised tools for AB management (2006), AB monitoring (2007), evaluation (2007). Little progress was made regarding time dedicated by pharmacist to AB management and restricted dispensation of AB using stop-orders.
Conclusion: Official texts and the public disclosure of a SPI have increased professionals and hospital managers' commitment to develop ABS, and resulted in improvements in most hospitals. However, even in hospitals involved in voluntary surveys, some measures still need to be reinforced, e.g. computerized tools for AB management, evaluation. It also appears crucial to consider practical implementation of regulatory required measures, as it will impact on their effectiveness: time spent by professionals, type of restrictive dispensation system, real use of evaluation and surveillance data, adaptation to hospital resources and needs.

P1495 Surveillance of antibiotic consumption in 861 French hospitals: lessons from a nationwide network, 2008

C. Dumartin, F. L'Heriteau, M. Pefau, P. Angora, X. Bertrand, S. Boussat, P. Jarno, L. Lacave, F. Nguyen, K. Saby, A. Savey, S. Alfandari, B. Coignard, B. Schlemmer, S. Touratier, A. Carbonne, A.M. Rogues (Bordeaux, Paris, Rennes, Nancy, Lyon, Tourcoing, Saint-Maurice, FR)*

Objectives: To improve antibiotic (AB) use, French hospitals are required to implement AB stewardship programmes including surveillance of AB consumption. A survey was performed by the regional centres for healthcare associated infections prevention and control, under the auspices of the French RAISIN-network, to describe AB consumption at hospital and ward level and provide tools for benchmarking.
Methods: Antibacterials for systemic use (J01 class of the WHO Anatomical Therapeutic Chemical classification (ATC)-Defined daily doses (DDD) system, 2008) were surveyed. Rifampin and oral imidazole derivatives were also included to better assess total AB exposure. AB consumption for inpatients, expressed in number of DDD per 1,000 patient-days (PD), and number of PD in 2008 were retrospectively collected by 861 voluntary hospitals: 35 teaching hospitals, 503 non-teaching hospitals, 11 cancer centres, 157 rehabilitation centres, 72 local hospitals, 83 long-term care (LTC) and psychiatric hospitals. They accounted for 42% of French hospitals beds and for about 58 million PD.
Results: AB use (pooled mean) was 370 DDD/1,000 PD. The most frequently used antibiotics were: amoxicillin–clavulanic acid (32% of total number of DDD), amoxicillin (17%) and ofloxacin (5.5%). Median consumption ranged from 56 DDD/1,000 PD in LTC and psychiatric hospitals to 610 in teaching hospitals, with wide variations among hospitals belonging to the same group. Median consumption differed according to hospital clinical wards: 59 in psychiatric wards, 71 in LTC wards, 199 in rehabilitation, 316 in gynaecology, 332 in paediatrics, 570 in surgery and medicine, 1,465 in intensive care units. Among similar wards, variations were seen in both AB volume and distribution.
Conclusion: AB use in French acute care hospitals did not seem higher than that reported in other European countries, when expressed in DDD/1,000 PD. This multicentre survey provided detailed information on AB use in a large sample of hospitals and wards. Its findings underscore 1) the relevance of data collection at ward level to foster consumption analysis and target AB use practice audits; 2) the usefulness of a nation-wide surveillance network, allowing relevant comparisons and benchmarking through sharing of experiences. Data from this network, together with information from practices audits, will enable to monitor trends in AB use in hospitals and to assess the impact of AB stewardship programmes.

P1496 The European Surveillance of Antimicrobial Consumption: point prevalence survey of antimicrobial prescriptions in 116 Belgian nursing homes

B. Jans, K. Latour, E. Broex, R. Stroobants, A. Muller, V. Vankerckhoven, H. Goossens on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Homes Sub-project Group*

Objectives: While rational antibiotic (AB) prescribing is an important measure to control the emergence of AB resistance, particularly in frail nursing home (NH) populations, European (EU) prevalence data on AB-use in NH are scarce. In April 2009, the European Surveillance of Antimicrobial Consumption (ESAC) NH sub-project, funded by the European Centre for Disease Prevention and Control, carried out a first point prevalence survey (PPS) in order to measure and describe AB prescriptions among residents living in European NH. We present the results for Belgian NH.
Methods: The PPS was carried out in April 2009. Inclusion criteria for residents were to be present in the NH for at least 24 hours and to receive systemic antibiotic(s) on the day of the PPS. Data were obtained from nursing notes, medication administration records and staff in relation to antimicrobial prescribing, characteristics, risk factors and determinants at NH- and at resident level.
Results: Data were available for 116 Belgian NH (11% of all Belgian NH). The mean number of beds by NH was 108 (25–324 beds). Among 12.085 eligible NH residents, 690 (median 5.1%, 0–16.3%) received an AB on the PPS-day. Residents with AB had a mean age of 84.3 years (35–109). In 7 NHs no residents received AB. In the total NH-population 2.2% (0–14.5%) had an urinary catheter, and of these, 21% received an AB. Wounds were present in 10.3% of the NH population (0–35%) and AB were prescribed in 13% of them. Vascular catheters were uncommon (0.13%). Among residents treated with an AB, 23% had a recent hospital stay (last 3 months). AB were administered orally in 94%, parenteral in 4% and nasally (decolonisation MRSA) in 2%. 46% of all treatments concerned the urinary tract (prophylactic: 43%) and 35% the respiratory tract (empirical: 92%). 55% of all prescribed regimens was an empirical treatment (RTI: 56%, UTI: 21%), 23% was prophylactic (UTI: 86%, RTI: 7%) and 20% was for a documented infection (UTI: 72%, GTI: 8%). The most frequently prescribed molecule was amoxicillin + enzyme inhibitor (17%) followed by nitrofurantoine (13%). Ciprofloxacin represented 8% of all treatments.
Conclusion: The Belgian NH AB-prevalence was only 5.1%. Notwithstanding the low urinary catheter use, AB-use in this group was important. This high proportion of AB-use for urinary tract and especially the high use of uroprophylaxis was surprising and needs to be explored.

P1497 Infection control resources in European nursing homes and their relation to antibiotic use: data of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Home subproject

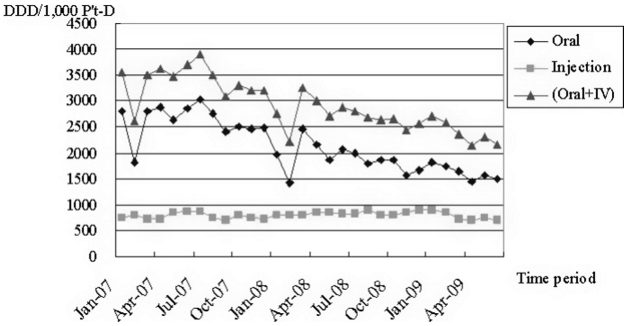
K. Latour, E. Broex, A. Muller, N. Drapier, V. Vankerckhoven, R. Stroobants, H. Goossens, B. Jans on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Home subproject group*

Objectives: The aim was to explore infection control (IC) resources in European nursing homes (NHs) and their relation to antibiotic (AB) use.
Methods: Within the framework of the European Surveillance of Antimicrobial Consumption (ESAC) NH subproject, NHs in European countries were asked to complete a standardised questionnaire on IC and AB policy.
Results: Across 16 countries 270 NHs returned the questionnaire. An IC practitioner was present in 25.3% of the NHs which responded to this question (n = 261). More public institutions had an IC expert appointed in comparison to private NHs (71.2% vs. 28.8%; p = 0.0035). Out of 66 NHs, 54% had a nurse as IC practitioner, 1.5% a physician and 43.9% both a nurse as well as a physician.
An IC committee was responsible for IC policies in 31.6% of the NHs (n = 253), while 56.4% of the NHs (n = 266) reported to have an official link with a hospital IC team.
The difference in prevalence of AB use was not significant between NHs with or without an IC practitioner (median 6.6% vs. 5.3%; p = 0.12) or IC committee (median 7.1% vs. 5.7%; p = 0.34). However, institutions officially collaborating with a hospital IC team showed a significant higher rate of AB prescriptions compared to those without a hospital link (median AB prevalence 6.7% vs. 5.1%; p = 0.0062).
In relation to AB use two important tasks of the IC practitioner were documented for 61 NHs. Formulation of recommendations on and advice for good AB use, including the development of an AB policy, was part of job responsibilities of 41.9% of the IC practitioners. Feedback to the GPs on AB consumption was given by 24.2% of the experts. However, NHs where the IC practitioners exercised these tasks showed no significant differences compared to NHs where IC experts did not do these tasks (p = 0.48 and p = 0.96, respectively).
Conclusion: A potential explanation for the higher median rate of AB use for NHs with an IC practitioner, an IC committee and especially with a link with a hospital IC team could be that these NHs experience a higher occurrence of infections, which justifies their need for internal or external IC expertise. However, further research on AB use and healthcare associated infections is needed to support this hypothesis.

P1498 Impacts on antimicrobial consumption in a community hospital by antibiotic stewardship and establishment of an effective informative system – a 30-month survey

W. Wang, J. Lu, H.L. Chen (Miao-Li, Taichung, TW)*

Introduction: Optimal antibiotic therapy has great impacts on patient prognosis and selection of resistant microorganisms in community and hospital settings. Data concerning antibiotic use in healthcare systems provide valuable information in designing antibiotic prescription program. We intended to illustrate trends of antibiotic use before and after implementation of several infection control maneuvers and an informative system for monitoring and control of antibiotic prescription in a community hospital.
Materials and Methods: Three stages were selected as following: stage I (from January 1st to December 31th, 2007-no infectious disease specialist nor protocol for rational antibiotic use available); stage II (from January 1st to August 31th, 2008 an infectious disease specialist on board with implementation of protocols and education for rational antibiotic use); stage III (from September 1st, 2008 to June 30th, 2009 establishing an information system to monitor and examine specific antibiotic prescriptions in the hospital). Data concerning amounts of antibiotic usage were searched from Pharmacology Database and were expressed as defined daily dose (DDD) per 1,000 in-patient hospitalization day according to WHO criteria. The differences and correlations of total and several categories of antibiotics between stages were determined by Manny-Whitney U test and Pearson's correlation test, respectively.
Results: The average of total antibiotic consumption in study period was 2,891 (2,100 in oral and 791 in parenteral antibiotics) DDD/1,000 patient-day. Cephalosporins (37%) and penicillins (20%) were two leading categories of antibiotic consumption. Decrease in total antibiotic use correlated significantly with decrease in oral antibiotic use in study period (r = 0.99). Trends in penicillins and cephalosporins showed moderately negative correlations with that of quinolones (r = –0.43 and –0.35, respectively). Oral antibiotic consumption in stage I was found to decrease significantly while comparing to that in stage III (p = 0.03). Amounts of antibiotics use showed decrease in most antibiotic categories during study period except quinolones (p < 0.05).
Conclusion: The trends of total and oral antibiotic consumptions showed decreased tendency and were significantly correlated with implementation of maneuvers and effective informative system. The impact of change in antibiotic consumption on antibiotic resistance patterns needs further studies to clarify.

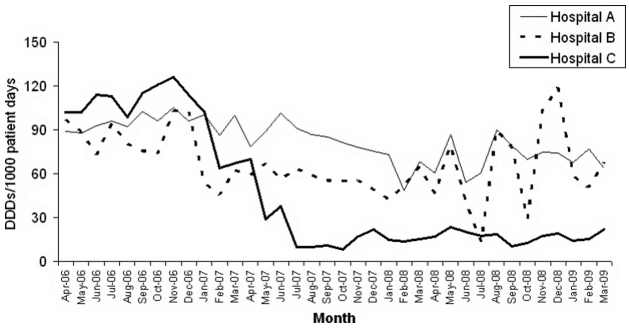


P1499 Comparison of antibiotic usage across hospitals in the north-central London sector using a standardized approach

P. Panesar, P. Coen, A. Lim, K. Patel, E. James (London, UK)*

Objectives: We set to explore the feasibility of using a standard data collection method to benchmark antibiotic consumption in terms of defined daily doses (DDDs) per 1000 occupied bed days, with the intention to compare it against hospital acquired infections such as *Clostridium difficile*. Antibiotic dispensing records, which are readily available from pharmacy stock control databases, were used to generate this information.
Methods: Antibiotic dispensing records were extracted for the period April 2006 to March 2009 from three North Central London (NCL) hospitals, which provide secondary and tertiary care to over 1000 residents. We focused on a subset of systemic antibiotics that have a higher propensity to cause *Clostridium difficile* infection, such as cephalosporins, clindamycin, quinolones and aminopenicillins. Dispensing records included antibiotic packs issued for hospitalised patients only, either as ward stock or dispensed to individual patients. Although antibiotic dispensing records were extracted from three different pharmacy stock management systems, these were all processed using the same methodology. The usage data were initially converted to DDDs by means of a standard conversion table and subsequently converted to rates per 1000 occupied bed days.
Results: We were able to produce usage data for all the antibiotics that were targeted. The results indicate variability in the use of antibiotics across the hospitals. An example of quinolone usage from the hospitals in the NCL sector is shown below.
Conclusion: We were able to present standardised antibiotic usage data in spite of it coming from different pharmacy systems. The method used facilitates benchmarking among different hospitals and has potential for identifying interventions to improve antibiotic use and to study its association with resistance in microorganisms. Not all current pharmacy

systems in the UK are designed for easy translation of records into standard usage data. We managed to develop working processes to generate compatible antibiotic usage data from the various existing pharmacy systems, which laid the groundwork for future epidemiological and surveillance studies.



P1500 A National Point Prevalence survey of antimicrobial prescribing for surgical prophylaxis

M.L. Heginbothom (Cardiff, UK)*

Objectives: An All-Wales Point Prevalence Survey (PPS) of antibacterial usage in secondary care was supported and carried out by pharmacists in twenty three hospitals across Wales during November/December 2008 to mark the first European Antibiotic Awareness Day. The data presented concentrates specifically on antimicrobial prescribing for surgical prophylaxis. The objective was to examine antimicrobial prescribing and duration of surgical prophylaxis.
Methods: Patients from 226 wards in 23 hospitals across Wales were included in the PPS. The number of patients surveyed on each ward, and the number prescribed antimicrobials at or before 8:00 am on the day of the PPS was recorded. Note: Not all hospitals surveyed all patients, and there was a bias towards surveying patients from medical wards. The auditors were asked to record the antimicrobial prescribed, site of infection, and duration of surgical prophylaxis based on the number of doses prescribed: Single dose; one day; >one day.
Results: Of the 4888 patients surveyed 1503 were prescribed systemic antimicrobial/s (31%); 206 patients were prescribed systemic antimicrobials for surgical prophylaxis (4.2%). Antimicrobial prescribing varied between hospitals dependant largely on specialties, with 22 different antimicrobials being prescribed. Cefuroxime was the most commonly prescribed antimicrobial (35.2% of total antimicrobials), which is of concern with regard to the association with *Clostridium* difficle infection. 85.2% of the antimicrobials prescribed for surgical prophylaxis were for IV administration. The proportion of patients receiving surgical prophylaxis for >one day ranged from 0–100% with an All-Wales average of 48.2%. Prophylaxis prior to urinary tract surgery or bone & joint surgery was less likely to be prescribed for more than one day than for the other sites.
Conclusions: The Scottish Intercollegiate Guidelines Network guidelines for antibiotic prophylaxis in surgery state that 'a single standard therapeutic dose of antibiotic is sufficient for prophylaxis under most circumstances'. Clearly, in some instances prescribing falls outside of the guidelines. It is hoped that short period audits with stakeholder feedback will reduce the number of patients receiving unnecessarily prolonged prophylaxis, and steer prescribers away from cephalosporin use. The PPS is being repeated in November 2009, and comparisons will be presented highlighting differences between the 2008 & 2009 PPS results.

P1501 Computerized surveillance of antibiotic usage and resistance: monitoring the impact of interventions

D. Lye, T.M. Ng, C.B. Teng, L.M. Ling, I.J. Hsieh, S.C. Yeak, H.Y. Tai, Y.S. Leo, B.S. Ang (Singapore, SG)*

Objectives: With high rates of multidrug-resistant (MDR) bacteria, we aimed to monitor antibiotic usage and resistance rapidly, at hospital and unit level, and in response to any intervention.
Methods: We built a computer programme downloading daily data on all positive cultures from Microbiology since January 2006, and antibiotic dispensing from Pharmacy since January 2007. Infection control (IC) and antimicrobial stewardship programme (ASP) interventions included: active MRSA surveillance and decolonisation from October 2006, enhanced with chlorhexidine wipes from November 2008 in medical Intensive Care Unit (ICU); hospital-wide MDR Gram-negative bacteria (GNB) isolation and hand hygiene campaign from January 2009; ertapenem to treat ESBL GNB from late 2007; an empiric antibiotic guideline from March 2009; and prospective review and feedback on all carbapenem orders from April 2009.
Results: Rise in amoxycillin–clavulanate and cefepime from March 2009 coincided with empiric guideline. Rise in ertapenem coincided with fall in imipenem from October 2007. Meropenem use fell from 27DDD/1000 bed-days in May 2008 to 17DDD/1000 bed-days in April 2009 and 10 in August 2009. Ceftriaxone fell from 141DDD/1000 bed-days in January 2007 to 93DDD/1000 bed-days in August 2009. Piperacillin–tazobactam rose from 40DDD/1000 bed-days in February 2008 to 54DDD/1000 bed-days in June 2009. Usage of vancomycin, ceftazidime and fluoroquinolones remained stably high. Incidence of MRSA fell from 3.1/1000 bed-days in January 2006 to 1.9/1000 bed-days in August 2009. MDR *Acinetobacter baumannii* (MDR AB) fell from 1.4/1000 bed-days in February 2006 to 0.16/1000 bed-days in February 2008, but rose to 1.8/1000 bed-days in August 2009; this rise was most marked in surgical and medical ICUs. ESBL *Escherichia coli* and *Klebsiella*, and carbapenem-resistant *Pseudomonas aeruginosa* remained stable. Active MRSA surveillance in medical ICU failed to reduce MRSA incidence but chlorhexidine wipes did. MDR GNB isolation was limited by lack of isolation rooms. Hand hygiene compliance did not correlate with MRSA or MDR AB incidence.
Conclusions: Computerised surveillance facilitates monitoring of impact of IC and ASP interventions. It highlighted high usage of fluoroquinolones and piperacillin–tazobactam, and a worsening problem of MDR AB. Prospective review and feedback on piperacillin–tazobactam, and a study on MDR AB are planned.

P1502 The rate of potentionally inappropriate antibiotic use in respiratory tract infections

M. Matuz, R. Benko, E. Hajdu, G. Soos (Szeged, HU)*

Objectives: To estimate the necessity of antibacterial therapy prescribed for respiratory tract infections.
Methods: The aggregated regional-level reimbursement data on systemic antibiotic prescriptions were purchased from the National Health Fund Administration. The study period was between 2007 January and June. All prescriptions claimed in the pharmacies of the region (n = 445 pharmacies) during this half year were included in the analysis. Antibiotic use was evaluated by means of the ATC/DDD methodology (version 2008). The indications of antibiotic therapies were determined according to the registered ICD (International Classification of Diseases version 10) codes. With the help of an infectologist consultant we classified indications (based on registered ICD-10 codes) into three categories: a) antibiotic therapy is probably required and useful b) antibiotic treatment is probably needless 3) undeterminable due to the inadequate nomenclature of the ICD code.
Results: Antibiotic therapy was judged to be probably required and useful in 33.3%, probably needless in 60.3% and undeterminable in 6.4%.

Table: Necessity of antibacterial use in different respiratory diseases according to the indicated ICD-10 codes

		DDD per 1000 inhabitant-days	Cum %
Probably required and useful Σ = 4.7 DDD per 1000 inhabitant-days (100%)			
J0390	Acute tonsillitis	1.7	36.9
J0200	Acute streptococcal pharyngitis	0.7	51.6
J0100	Acute sinusitis	0.5	61.8
J0300	Acute streptococcal tonsillitis	0.4	70.7
J2000	Acute bronchitis (*Mycoplasma pneumoniae*)	0.3	76.3
J1890	Pneumonia	0.2	81.4
J1800	Bronchopneumonia	0.2	86.1
J0190	Acute sinusitis	0.2	89.5
J40H0	Bronchitis, unspecified	0.1	91.6
Probably needless Σ = 8.5 DDD per 1000 inhabitant-days (100%)			
J0290	Acute pharyngitis	3.5	41.0
J2090	Acute bronchitis	2.8	73.8
J0690	Upper respiratory tract infection	1.0	85.4
J00H0	Common flu	0.5	91.2
Undeterminable Σ = 0.9 DDD per 1000 inhabitant-days (100%)			
J0410	Acute tracheitis	0.3	39.1
J0400	Acute laryngitis	0.2	64.7
J0420	Acute laryngotracheitis	0.2	86.1
J0600	Acute laryngopharyngitis	0.1	96.4

Conclusion: According to our estimation antibiotic therapies were prescribed for mainly viral diseases in high number. However this methodology gives upper estimation of antibiotic overuse (due to lack of clinical signs and knowledge on concomitant chronic diseases) the results are disappointing and alarming.

P1503 Modelling treatment strategies to contain resistance in hospital wards

P.K. Schulz zur Wiesch, R.D. Kouyos, S. Bonhoeffer (Zurich, CH)*

Nosocomial infections with drug-resistant bacteria are often associated with a worse prognosis and higher costs than those caused by their susceptible counterparts. Several strategies that coordinate the use of antibiotics in a population have been proposed to delay and minimize the rise of resistance, among them "cycling" (sequential use of different drugs), "mixing" (simultaneous use of different drugs in different patients), or population-wide combination therapy. Theoretical studies predict that combination therapy should in most cases be superior to the other strategies and that mixing should be generally preferable to cycling. The clinical evidence, however, does neither generally support combination therapy in nosocomial infections nor show a pronounced disadvantage for cycling. Thus, we currently do not have an understanding for the discrepant findings between theoretical and empirical studies. We developed a stochastic epidemiological model that describes a hospital ward, which is characterized by a small population size, a high population turnover and by the presence of both direct and environmental transmission. It considers four main compartments, in which the hosts are either protected by a microflora, susceptible, colonized or infected. Two different empirical therapies are available, and we monitor resistance to either or both of these antibiotics, which may arise via mutations or horizontal gene transfer. Both the total prevalence and the total amount of patients receiving inappropriate therapy are used as endpoints.

Compared to direct transmission, the dynamics differed profoundly when transmission occurred via environmental contamination, since the environmental reservoir impedes extinction and acts as a "memory". Cycling was sometimes beneficial, when the population size and prevalence of resistance allowed for an extinction of strains resistant to the previous antibiotic. The relative success of the three strategies was greatly influenced by the prevalences among the incoming patients, the costs and the rates at which resistance was acquired for each of the three resistant genotypes. Furthermore, short-term and long-term success were not always correlated.

This study shows that the success of treatment strategies in containing resistance may depend on inherent differences in the biology of the specific diseases.

P1504 Pharmacist interventions on antibiotic dosage performed trough a computer-assisted management programme with real-time alerts

J. Fernández-Morató, S. Grau, D. Conde-Estévez, M. Marin-Casino, J. Mateu-de Antonio, O. Ferrández, E. Salas (Barcelona, ES)*

Objectives: Inadequate antibiotic dosage (ID) may produce an increase in antimicrobial resistance, treatment failures and adverse effects. Computer-assisted management programs for antibiotic prescription linked to Computer Physician Order Entry-System (CMP-CPOE) have shown good results in antibiotic therapy optimization. The aim of this study was to assess a CMP-CPOE utility in pharmacist interventions on antimicrobial dosage adjustment (IADA), to compare clinical and antibiotic therapy features between patients with an IADA versus those with other interventions on antimicrobial therapy (OAI) and to identify predictive factors of ID.

Methods: Retrospective study in a 450-bed university hospital including all patients with antimicrobial interventions (AI) performed through a CMP-CPOE during 3 months (June-August 2008). This CMP-CPOE ran with real time alerts appearing on the computer screen. Pharmacists wrote AI in the computerized medical history. Data collected: demographics, SAPS-II at admission, type of admission, medical ward, lenght of hospital stay, crude mortality, creatinine clearence MDRD (CrCl), type of antibiotic, site of infection and empirical or directed antibiotic therapy. ID was considered when dose was not adjusted to patient characteristics or infectious foci.

Results: Total antibiotic prescriptions: 3599. Total AI: 175 (4.9% related of total antibiotic prescriptions). Patients with IADA: 89. IADA: 96 (54.9%)(1.1/patient): ID related to CrCl 55 (57.3%), infradosification 24 (25%), overdosification 12 (12.5%) and inadequate schedule 5 (5.2%). The clinical and antibiotic therapy features' differences observed according to IADA or OAI (univariate analysis) and the predective factors of ID (logistic regression) are presented in Table.

Conclusions: 1) More than half of AI were related to inadequate dosage. The real time CrCl reduction warnings allow to perform IADA focused in patients with severe renal impairment. Pharmacist intervention acceptance was remarkable in IADA, especially those related to overdosification, while in OAI was limited. The CMP-CPOE showed as a useful tool to optimize antimicrobial dosage. 2) Patients with IADA present higher severity of illness at admission and have more respiratory tract infections and empirical therapy with amoxicillin/clavulanate and levofloxacin. In multivariate analysis, patients with empirical antimicrobial therapy and severe renal impairment were associated independently with ID.

	IADA	OAI	p	OR (95% CI; p)
SAPS-II at admission	31.8	28.6	0.046	
95% CI	29.8–33.8	26–31.2		
Crude mortality	15 (15.6%)	5 (6.3%)	0.054	–
CrCl <30 ml/min/1.73 m^2	50 (52.1%)	16 (20.3%)	<0.001	4.5 (1.9–10.8; 0.001)
Nephrology admission	13 (13.5%)	4 (5.1%)	0.059	–
Amoxicillin/Clavulanate	20 (20.8%)	8 (10.1%)	0.055	–
Levofloxacin	9 (9.4%)	1 (1.3%)	0.024	–
Respiratory tract infection	36 (37.5%)	15 (19%)	0.007	–
Empirical antimicrobial therapy	61 (63.5%)	29 (29.1%)	<0.001	3.8 (1.8–8; <0.001)
Acceptance 24–48 h post IADA*	70 (72.9%)	42 (53.2%)	0.007	–

*All IADA related to overdosification were accepted: 12 (10.7%) vs 0 (0%) (p = 0.005).

P1505 Prevalence study on antibiotic consumption in Latvian general practitioners

U. Dumpis, E. Tirans, M. Akermanis, S. Veide (Riga, LV)*

Introduction: The largest proportion of antibiotics is used in ambulatory care. Despite general consumption data are calculated, they don't provide information on indications for use and demographics of the patients. This information is essential to analyze resistance selection pressure. Easy and cheap methodology was designed to benchmark ambulatory antibiotic use.

Methods: GPs where asked to record data on each patient that received antibiotics during one week in November, 2008. The questionnaire was

handed out on registration for the annual conference. Explanation on the methodology was given during the presentation in conference and written instructions. Questionnaire contained information of an antibiotic, dose and dosing interval, indication for use and general demographic data. Participation was voluntary and did not contain financial incentives.
Results: Two hundred forty eight questionnaires out of 600 (41%) where returned by post to the investigators. During one week one GP made mean 113 consultations and antibiotic was prescribed in mean 7 patients (0–33). Children younger than 10 years received most the prescriptions (24.8%). Patients older than 60 years accounted for 13% of the treated. All together, 1763 antibiotics were prescribed during the study period. Most of the patients received monotherapy, only 22 (1.3%) patients where prescribed two antibiotics. Most commonly prescribed antibiotic was amoxicillin (34% of prescribed), amoxicillin/clavulanate (19%) and clarithromycin (8%). Most commonly treated infections where pharingitis (29.1%), acute bronchitis (24.7%) and rhinosinusitis (9.9%). Pneumonia was mostly treated with amoxicillin/clavulanate(25%) amoxycillin (16%) and clarithromycin (19%). Uncomplicated urinary tract infection was mostly treated with oral furagine (27.5%), ciprofloxacin (22%) and norfloxacin (19%).
Conclusions: Methodology employed was cheap and easy to use and provided information on antibiotic use pattern in general practice. Despite rather low return rate and possible bias, we consider obtained information extremely important. Future interventions to reduce ambulatory antibiotic prescriptions should be focused on treatment of acute pharyngitis and bronchitis in children. Another point of significant concern was the high use of fluoroquinolones for uncomplicated urinary tract infection.

P1506 Attitude of healthcare professionals on antibiotic use in Lithuanian general and nursing hospitals – qualitative study

A. Jurkeviciene, A. Berzanskyte, R. Valinteliene, J. Miciuleviciene, A. Ambrozaitis (Vilnius, LT)*

Objectives: Survey in Lithuania showed huge variation of antibiotic consumption (11.5–79.5 DDD/100-bed days in general and 0–26.6 DDD/100-bed days in nursing hospitals) and their structure between hospitals in 2007. The main aim of following qualitative study was to enlighten health care professionals' attitude to antibiotic use.
Methods: Discussions with focus groups were organized inviting different staff. Questions about guidelines, antibiotic prescribing control, consumption surveillance, collaboration with microbiology laboratories, impact of pharmaceutical companies, needs of knowledge and teaching were discussed. Content analysis was applied working up the conversation material in several steps: multiple listening/reading of discussions; search of semantically close answers and keywords; summarising by forming categories, subcategories; interpretation.
Results: Focus group discussions showed that due to antibiotics obtained by patients antibiotic consumption in general hospitals should be higher than the one found in quantitative study in 2007. Representatives from nursing hospitals confirmed low antibiotic use. The general hospitals most often have guidelines approved by hospital chief, but seldom control on antibiotic prescribing. Guidelines for antibiotic use are available in larger nursing hospitals. Sometimes they are established locally as procedures, algorithms. Almost all participants expressed wish to have national guidelines. Antibiotic prescribing is generally based on doctor's decision, most often empirically in both types of hospitals. However in regional or smaller hospitals the staff prefers not to change any routine. According to physicians affirmation pharmaceutical companies don't influence antibiotic choice and prescribing. That is even more evident in nursing hospitals, as because of low consumption pharmaceutical companies are not interested in advertising, presenting antibiotics there. The need of knowledge and teaching on antibiotic subject was expressed in general hospitals and not prioritized in nursing hospitals, where other topics were considered to be more relevant.
Conclusions: The study revealed the absence of strict national policy on antibiotic use in Lithuanian hospitals in general. Lack of stewardship was enlightened as the main problem to work on.

P1507 The impact of educational campaign on public attitudes towards antibiotics

N. Radosevic, B. Popovic, G. Palcevski, V. Vlahovic-Palcevski (Rijeka, HR)*

Objective: Education of patients on inappropriate use and importance of compliance with instructions on taking antibiotics is an important strategy for improvement of antimicrobial use and containing emergence of resistance. The objective of this study was to assess the impact of a public campaign about rational use of antibiotics by perceiving the alterations in patients' attitudes towards antibiotics reviewed prior and following the campaign.
Methods: The public campaign about the rational antibiotic use and treatment of mild upper respiratory tract infections was conducted among the population in Primorsko-Goranska county during February 2009 through local daily newspapers, radio and TV spots and programmes, booklets and handouts for patients, posters and organised lectures.
Before the campaign anonymous questionnaires were dispensed to patients in primary care, who visited pharmacies and general practitioners. The questions concerned patients' knowledge on antibiotics, their expectations from physicians regarding prescribing an antibiotic and their awareness of the risks associated with the over consumption and misuse of antibiotics.
After the end of the campaign, another questionnaire was presented to the patients in the primary care. They were asked the same questions as they were before the campaign, with additional questions on the impact of the campaign.
Results: A total of 461 qustionnaires were filled in before the campaign, and 345 patients completed the questionnaire after the campaign. A significant difference was noted in responses concerning the appropriateness of taking antibiotics for typical viral symptoms (sore throat, fever, cold, flue) (Fig. 1.). About 2/3 of the respondents (62%) were informed about the campaign, mostly through TV spots (56%) and posters (43%). The majority remembered the inappropriateness of selfmedication (85%) and ineffectivenes of antibiotic treatment for viral infections (50%) as the main messages of the campaign.
Conclusion: A short term effect of educational campaign about the rational antibiotic use was a considerable impact on public knowledge on appropriate antibiotic usage. A long term effect and our future goal is continuous patient education as an important measure in decreasing unnecessary antibiotic prescribing and even decreasing bacterial resistance.

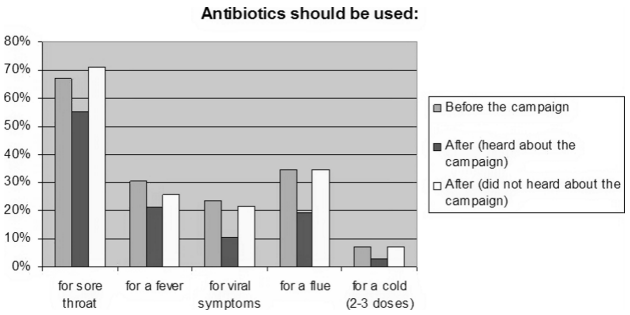


Figure 1. Attitudes towards appropriateness of taking antibiotics for typical viral symptoms.

P1508 Patients' perception about rational use of antibiotics

E. Keuleyan, R. Benchev, P. Yovchevski, N. Smilov, M. Valentinova, S. Tete, T. Anakieva (Sofia, BG)*

Objectives: On the occasion of 18 November 2009 – the day of antibiotics, an inquiry was performed at the Medical Institute – Ministry of the Interior, aimed to know the current patients' perception about antibiotic usage.

Methods: A questionnaire of 11 questions with multiple choice answers was developed by a multi-disciplinary equip and was distributed on 10 and 11 November 2009 among in-patients and out-patients of our 350-bed national multi-profile hospital. The inquiry was anonymous. Correct answers were announced on 18 November 2009.
Results: Ninety four forms were returned out of 149 distributed (63%). Probably, because of coincidence with grippe epidemics, 71% of patients (Ps) responded that antibiotics (AB) were created both against bacterial and viral infections. Majority (84%) considered AB indispensable to treat severe infections, e.g. pneumonia, peritonitis, but only 40% mentioned AB important in severe pandemics, e.g. plague, anthrax. Only 2% considered AB should be used in common cold and uncomplicated diarrhea. Top answers to the question: "What are the most frequent reasons for failure of AB therapy, were: "the duration of treatment was not respected" (60%) and "microorganisms are not susceptible" (53%). Replies to the question "Why AB should be prescribed only upon special considerations" showed that Ps are informed about AB resistance (83% responded, because the overuse of AB is followed by development of AB resistance, and 27% were aware about emergence of pan-resistant bacteria). Almost all (96%) responded that one should not take AB without prescription. Patients' perception about possibilities doctors to limit AB resistance was revealed as follow: "to prescribe the most appropriate AB in most appropriate regimen" – 64% and "to prescribe AB on specific occasions and after antibiogramme" – 62%. As to the control of infections, majority of Ps acknowledged the role of hand washing (78%), but only 36% considered the importance of vaccines.
Conclusions: This investigation revealed that majority of patients was aware about antibiotic resistance and was against the self-medication. At the same time it showed area for further education, e.g. accent on the importance of prophylaxis of infections through immunizations.

P1509 What do pharmacists recommend for men with acute urethritis symptoms: 2009 vs. 2003–2004

S. Korolev, I. Andreeva, S. Kozlov, A. Fokin, E. Shal, D. Iliin, A. Muraviov, P. Bortulev (Tula, Smolensk, RU)*

Objectives: In Russian Federation antibiotics are not officially included on the list of OTC medications, which was approved by the Ministry of Health. Nevertheless people can freely purchase antimicrobials from drug stores. We aimed our study to compare pharmacists' recommendations for men with acute urethritis symptoms in 2003–2004 vs. 2009.
Methods: Young male doctors (25–30 years old) – "simulated patients" – were trained to present complaints of acute urethritis to drug store personnel. In 2004 in 4 Russian cities (Moscow, Smolensk, Kaluga, Kaliningrad) and in 2009 in 5 cities (Moscow, Smolensk, Kaluga, Kalinigrad, Tula) pharmacists were asked for recommendation according to standard questionnaire.
Results: In 2004 and 2009 360 and 409 pharmacists were questioned, respectively (139/117 – in Moscow, 78/83 – in Smolensk, 72/64 – in Kaluga, 71/73 – in Kalinigrad, respectively, and 72 – in Tula in 2009 only). 208/168 (57.8%/41.1%) of pharmacists recommended to buy medications, 74/103 (20.6%/25.2%) advised to visit a doctor, 76/138 (21.1%/33.7%) recommended both in 2004 and 2009, respectively. In 273/291 cases (96.1%/95.1% from pharmacists who recommended treatment) drug store personnel advised antimicrobials in 2004 and 2009, respectively: nitroxoline (23.6%), norfloxacin (14.8%), nitrofurantoin (12.4%), ciprofloxacin (8.1%), pipemidic acid (7.4%), co-trimoxazole (4.3%), fosfomycin (3.8%) in 2004 and norfloxacin (24.3%), nitroxoline (13.0%), fosfomycin (12.4%), pipemidic acid (11.0%), ciprofloxacin (8.2%), furazidine (7.8%), nitrofurantoin (5.8%), amoxicillin/clavulanic acid (4.0%), azithromycin (2.0%) in 2009. Duration of recommended treatment course was 6.0±1.5 and 5.6±3.0 days in 2004 and 2009, respectively. In 16.5%/17.6% of cases in 2004 and 2009, respectively, drug store personnel recommended non-antimicrobials (the most commonly herbal remedies).
Conclusions:
1. Pharmacists are responsible for substantial number of OTC prescriptions of antimicrobials.
2. In general pharmacists' behavior has not changed over 5 year's period and antimicrobials remain the most frequently prescribed medications for acute urethritis in men.
3. Special programs should still be developed for drug store personnel to discontinue sales of antimicrobials without prescription.

Handwashing

P1510 Quantifying use of alcohol handrub and soap by non-healthcare workers in hand hygiene intervention studies

J. Savage, C. Fuller, S. Besser, S.P. Stone (London, UK)*

Objectives: The volumes of alcohol hand rub & soap procured by wards provide a proxy measure of hand hygiene compliance. This may be distorted by use of alcohol handrub (AHR) at ward entrances & bedside use of AHR/soap by patients & visitors. In England & Wales, the national cleanyourhands campaign promoted use of bedside AHR by healthcare workers (HCW). An unintended consequence of the campaign was that many hospitals had AHR at ward entrances & encouraged staff, patients & visitors to use it. An evaluation of the campaign 2004–8 showed procurement of soap & AHR had tripled but it is not clear how much of the increase was due to non-HCW use of consumables at ward entrances & the bedside. Such use has not been studied previously. The objectives of this study were to estimate the proportion of ward procurement of AHR and soap attributable to use of AHR at ward entrances, and to use of soap & AHR by non-HCWs at the bedside.
Methods: Proportion of AHR & soap used by visitors/patients inside the ward: Thirty six hours of direct observation of bedside hand hygiene behaviours by HCWs, patients and visitors were performed during weekday ward visiting hours (13.00–20.00hrs) on 27 acute medical wards and ITUs in 9 English trusts.
Proportion of AHR used at ward entrances: Each bag/bottle of AHR was collected from ward entrance dispensers for four consecutive days on 10 wards in 4 hospitals. Mean daily volume used was compared with mean daily ward procurement for the previous 12 months.
Results: At the bedside, only 6 (3.95%) out of 152 observed uses of AHR and 6 (4.35%) of 138 uses of soap were by visitors. No patient used soap or AHR. Mean daily use of AHR at ward entrances was 21.4% (range 7.8–32.8%) of mean daily procurement.
Conclusions: In this multicentre study, 96% of all bedside soap/AHR use appeared to be by HCWs. Although the proportion of AHR used at ward entrances varies widely, much of its use occurs outside visiting hours, so it seems reasonable to assume at least half of such use is by HCWs. This implies that at least 85% of ward AHR procurement represents use by HCWs. The rise in consumables reported during the cleanyourhands campaign is largely attributable to their use by HCWs, rather than visitors & patients. We suggest that hand hygiene intervention studies using consumables as a proxy measure should assess & adjust for such use.

P1511 Assessment of blinding of hand hygiene observers in randomized controlled trials of hand hygiene interventions

C. Fuller, J. Savage, S. Besser, S.P. Stone, E. Fragaszy, J. McAteer, S. Michie, B.D. Cookson, J. Gardiner (London, UK)*

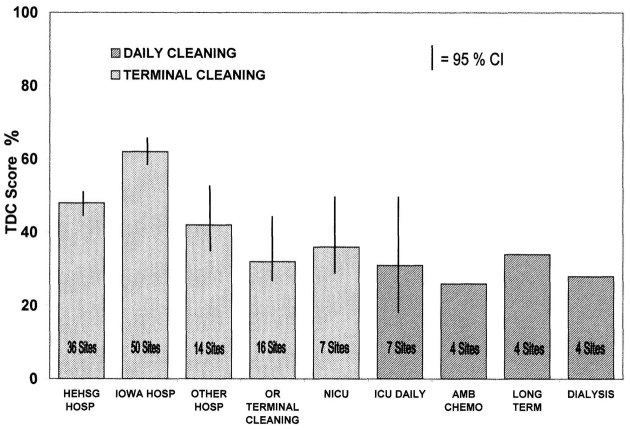
Objectives: Trials evaluating interventions to improve healthcare workers' hand hygiene compliance use directly observed compliance as a primary outcome measure. Observers should be blinded to the intervention, that is, have no knowledge of the allocation of wards or units to the intervention, and the effectiveness of blinding assessed, to prevent systematic bias with compliance over-estimated in intervention wards and underestimated in non-intervention wards. This issue has not been addressed in the literature.

The study aimed to design a method of assessing blinding, use it to determine the effectiveness of blinding in a current randomised controlled trial, and thus inform future hand hygiene trial methodology.
Methods: A method of assessing blinding was designed and used to assess blinding in a current randomised controlled trial, the Feedback Intervention Trial (NRR website N0256159318). The study hand hygiene observer, trained another researcher, blinded to the intervention, in use of a robustly standardised, valid, reliable and sensitive measure of hand hygiene compliance, the Hand Hygiene Observation Tool (HHOT) (www.idrn.org/nosec.php). 1030 simultaneous observations were carried out over 20 hours, on 7 intervention and 6 non-intervention wards in the trial. Between observer differences were compared for both types of ward using Cohen's kappa (individual hand hygiene behaviours) and the Mann–Whitney U (overall compliance).
Results: Raw agreement between observers for individual hand hygiene behaviours was excellent in both intervention wards (91.5%, kappa = 0.886 [CI 95% 0.792–0.98]) and non-intervention wards (92.4%, kappa = 0.894 [CI 95% 0.845–0.987]). There was no statistically significant difference between observers for overall compliance.
Conclusions: This study describes a robust and pragmatic method for assessing the adequacy of blinding in hand hygiene intervention trials. It demonstrates that blinding of the study hand hygiene observer to ward allocation was effective in a current trial, and that assessments of hand hygiene compliance were not biased. All hand hygiene studies should blind observers to the intervention and assess the effectiveness of blinding.

P1512 Do widespread shortcomings in hygienic cleaning limit the impact of other healthcare interventions to prevent pathogen transmision?

P. Carling for the Healthcare Environmental Hygiene Study Group Hospitals*

Objective: Despite improved hand hygiene compliance and the use of active surveillance cultures to optimize isolation practice, there is limited evidence that these interventions have led to widespread or sustained decreases in the overall burden of hospital associated infections. In view of the documented increased risk of acquisition of MRSA, VRE, *C. difficile* and *A. baumannii* from prior room occupants and recent studies prospectively demonstrating decreased transmission of MRSA, VRE and *A. baumannii* as a result of improved hygienic cleaning in the context of our previous finding of suboptimal discharge cleaning in 36 acute care hospitals, a broader evaluation of thoroughness of hygienic cleaning was undertaken by the Environmental Hygiene Study Group hospitals.
Methods: A novel fluorescent targeting system was covertly used to objectively evaluate if the thoroughness of disinfection cleaning (TDC) of standardized sets of high risk objects was being performed in a manner consistent with previously established guidelines in a range of healthcare settings.
Results: Terminal TDC in the first 36 acute care hospitals studied (48%) was similar to that found in 50 hospitals participating in the Iowa MRSA Reduction Project (62%), 14 other test hospitals (42%), 16 hospitals' operating rooms (32%) and 7 hospitals' neonatal intensive care units (36%) (Figure). Daily TDC in ICU isolation rooms in 7 hospitals (31%), in 4 ambulatory chemotherapy suites (26%), in 4 dialysis units (28%) and in 4 long-term care facilities (34%) was also suboptimal (Figure). Overall the mean TDC was 47.9 (Range 3–88; 95% CI 44.8–50.9).
Conclusion: The evaluation of hygienic cleaning of >62,500 high touch surfaces in 103 different institutions and 142 study sites confirmed the existence of similar opportunities to improve such cleaning in all venues. While it will remain for future studies to further quantify the favorable impact of more thorough hygienic cleaning in many of these settings, the realization of substantial improvement in cleaning using a structured objective process improvement program in 36 hospitals (ICHE 2008; 29: 1035–41) suggests that such interventions have significant potential for improving hygienic practice in many settings.



P1513 Hand hygiene in paediatric and neonatological intensive care unit patients: determination of daily indications and indication- and profession-specific analysis of the compliance

S. Scheithauer, J. Oude-Aost, T. Schwanz, K. Heimann, H. Haefner, B. Waitschies, T. Orlikowsky, S. Lemmen (Aachen, DE)*

Objectives: Hand hygiene (HH) is considered to be the single most effective tool to prevent healthcare-associated infections (HAIs). Intensive Care Units (ICUs), especially those caring for neonates represent a centre for HAIs with great impact on morbidity and mortality. Although there have been several reports on compliance rates (CRs), data on daily hand hygiene indications (HHI) and CRs for pediatric/neonatological ICUs (P/NICUs) are still lacking.
Methods: Therefore we conducted a prospective observational study to determine HHIs for and CR with HH according to the WHO definition in P/NICU-patients. The observation was carried out patient-directed by one observer only over 192 hours. Observation periods were distributed equally over the day (0:00–24:00). Analysis was performed P/NICU-, indication-, profession-, and shift-specifically. Observed CRs were correlated with disinfectant usage (DU), data were obtained from the spending records.
Results: HHI were significantly higher for PICU (316/24 h) than NICU (194/24 h) patients ($p = 0.004$). Observed CR resulted in 56% (PICU) and 61% (NICU) being significantly higher in nurses (61 and 66%) than in physicians (29 and 52%; $p < 0.001$). CRs were significantly higher before patient contact and aseptic task than after contact with patients and their surroundings ($p = 0.009$). Shift-specific CR was significantly highest during night shift (64%; $p = 0.05$). Glove usage instead of disinfection before patient contact /aseptic task was more common in PICU-(39%) than in NICU-patients (20%). Worrisomly, compared to direct observed results calculation by DU reveiled a 3-fold lower CR (20%).

Table 1: Hand hygiene characteristics for representative pediatric (PICU) and neonatological (NICU) ICU-patient-days (24 h)

	Hand hygiene indications		Hand hygiene activities		Compliance rate (%)	
	PICU	NICU	PICU	NICU	PICU	NICU
Early shift	109	78	58	40	54	51
Late shift	104	69	59	41	56	60
Night shift	103	47	59	37	57	79
Indication 1	72	54	36	42	50	78
Indication 2	64	24	48	16	75	67
Indication 3	46	23	22	11	48	49
Indication 4	64	56	38	35	59	62
Indication 5	79	37	39	16	49	44
Nurses (ICU-associated)	260	145	149	96	57	66
Physicians (ICU-associated)	35	14	10	7	29	52
Others	24	35	10	15	41	41
Sum	316	194	176	118	56	61

Legend: Indication 1: before patient contact; Indication 2: before aseptic task; Indication 3: after contact with body fluids; Indication 4: after patient contact; Indication 5: after contact with the patients' surroundings.

Conclusion: This study provides the first data on daily HH in N/PICU-patients including comparison of observed and calculated CR. Fortunately, direct observation reveiled high CRs especially in nurses and in situations of greatest impact. Unfortunately, influence of direct observation seems to be high. Thus, these data allow focusing future efforts in hand hygiene to reduce HAIs and improve patients' safety.

P1514 Hand hygiene promotion worldwide: strategies and achievements of the WHO First Global Patient Safety Challenge

B. Allegranzi, C. Kilpatrick, W. Graafmans, S. Bagheri Nejad, M. Chraïti, E. Mathai, H. Richet, D. Pittet (Geneva, CH)*

Objective: The WHO First Global Patient Safety Challenge (GPSC), launched in October 2005, aims at reducing healthcare-associated infections (HAI) worldwide, in particular by promoting hand hygiene (HH) and infection control (IC) best practices at the point of care. We evaluated the strategies and achievements of the First GPSC related to this objective.
Methods: The following components were assessed: 1) development and testing of guidelines and accompanying implementation strategies and technical tools; 2) evaluation of test results; 3) finalization and dissemination of implementation strategies and tools. Main indicators of success were HH compliance measured by a validated direct observation tool and the number of healthcare settings implementing HH promotion worldwide.
Results: The advanced draft of the WHO Guidelines on HH and the WHO multimodal HH improvement strategy and tools were developed with the contribution of more than 100 international experts and were tested in 2007–2008 in 8 pilot sites and in over 250 health-care settings worldwide. Main results from pilot sites were: a significant ($p < 0.01$) increase in the weighted mean of HH compliance from 34.0% at baseline to 55.7% after a three-month intervention period in 7/8 sites; preferred recourse to alcohol-based handrub for HH in all sites with local production of a WHO formulation at low cost in 6/8 sites and national scale-up in 4/6 sites; and improvement in healthcare workers' perception and knowledge about the importance of HAI and HH. Following evaluation of lessons learned, the final Guidelines and Implementation Toolkit were issued in May 2009. To catalyze their dissemination and to support long-term HH improvement worldwide, a new initiative, "Save Lives: Clean Your Hands", was launched on 5 May 2009. As of 5 November 2009, 5801 hospitals from 125 countries have registered to be part of this initiative using the WHO Guidelines and tools for HH promotion.
Conclusions: The feasibility and reliability of the WHO Guidelines and tools and their impact on HH procedures were demonstrated through testing in settings with different cultures and resource availability. The First GPSC has generated an unprecedented global momentum and mobilized thousands of healthcare settings to achieve HH improvement. By making IC a priority in healthcare everywhere, with HH as its solid and essential basis, the First GPSC efforts have the potential to save millions of lives and engender major cost savings.

P1515 Hand-hygiene champion junior doctor picks up the gauntlet: a randomized prospective study of 2,500 minutes of hand hygiene audit conducted by 17 junior doctors in a large district hospital of northwest England

M. Przybylo, M. Qulaghassi, A. Guleri (Blackpool, UK)*

Objectives: "Scientists estimate that people are not washing their hands often or well enough & may transmit up to 80% of all infections by their hands. Hand washing may be the single most important act to help stop the spread of infection and stay healthy (CDC)". Several Dept health high profile hand hygiene(HH) campaigns have made HH compliance results a standard item of trust board, divisional, hospital infection control meetings, trust induction day, etc. HH audits are conventionally conducted monthly by infection control/link nurse. We present an innovative project by a junior doctor(JD). The aim was to collect prospective, unbiased, sizeable data on HH compliance. Raise awareness and engage JDs in the hospital hand hygiene programme (HHP).
Methods: Data from 2500 minutes (125×20-min observational (HHA)) performed by randomly selected 17-JDs across 18-wards. Standard HHA tool (as used by nurses for monthly HHA) used. Consultant microbiologist explained the method of conducting the audit. The observer could choose any 1–3-clinical areas and conduct 4×20-min observational HHA at different times/different days (to eliminate 'bad-day' effect) in each area. 'Fly-on-the-wall' [un-noticed] technique was used during observations (to eliminate observation related change in behaviour). Definitions: Opportunity (O) for HH – touching patient or immediate surrounding (bed frame, med equipment, curtains, notes/trolley on bedside, etc). Hand hygiene (H) with alcohol gel or washing-soap & water. HH compliance = (total H ÷ total O) × 100. Overall and grade (staff nurse, jr nurses, jr docs, consultants, registrar/staff grade, pharmacist, dietician/domestics, etc).
Results: From 2500 mins (125 observations) data: Clinical areas – surgical (64 obs), medical (38), ITU (23 obs). Overall compliance was 37% (667/1787). Consultants 58% (O = 55), sr doctors 55% (O = 157), JDs 44% (O = 271), staff nurses 30% (O = 507), etc. Compliance was highest in intensive care 58% (O = 334), Surgical wards 35% (O = 860) and medical wards 30% (O = 582).
Conclusion: Poor hand hygiene compliance can potentially undermine hospital HCAI progam. This study has revealed detailed picture of hand hygiene compliance. The project has engaged and raised awareness about HH in junior doctors. The HHA will be repeated after 6-months to compare the change. It is stipulated that announced HH observations may potentially change HH behavious and single 20-min observation/month in each ward may fail to provide a complete picture.

P1516 Increase of alcoholic handrub consumption in 1261 German non-ICU units within one year

N. Mönch, M. Behnke, P. Gastmeier, C. Reichardt, C. Geffers (Berlin, DE)*

Objective: Hand hygiene is the essential method to prevent hospital acquired infections. HAND-KISS, a module of the German Krankenhaus-Infektions-Surveillance-System (KISS), established a new module for the systematic surveillance of alcohol-based hand rub consumption (AHC) in 2007. It is mandatory for all hospitals participating in the national hand hygiene campaign "AKTION Saubere Hände", which was started in January 2008 and is supported by the German ministry for health, to feed their AHC data into the HAND-KISS module.
We compared the consumption data for the years 2007 and 2008 of those hospitals, participating in HAND-KISS and the national hand hygiene campaign since 2008.
Methods: The following data are provided annually per unit: AHC, the number of annual patient days per unit and the unit characteristics. HAND-KISS calculates the consumption in ml per patient day (PD) for individual units and provides reference data stratified according to the specialty of the unit.
We examined the data of those units providing consecutive data for the years 2007 and 2008. Differences of consumption were tested by a Wilcoxon rank sum test for significance.
Results: 181 hospitals provided consumption data of 2041 non-ICUs in 2007, and 330 hospitals of 3328 non-ICUs in 2008. 1261 non-ICUs out of 134 hospitals provided data for both years. The median overall consumption rate for 2007 is 14.6 ml/PD (interquartile range (IQR) 10.6–20.8 ml/PD) and 16.9 ml/PD (IQR 12.2–23.8 ml/PD) in 2008. There was a significant increase between 2007 and 2008 in all types of units (table). The maximum increase of consumption is found in medical and medical-surgical units representing an increase of 14%.
Conclusion: The consumption of AHC in participating units is increasing. Since it is a mandatory measurement, all hospitals participating in the national hand hygiene campaign are also providing data to HAND-KISS. Among other interventions (e.g. lectures, increase availability of alcohol-based hand rub, reminders, national hand hygiene days, yearly meeting of campaigning hospitals, system change), the

annual measurement of unit based AHC and feed back of data to staff is one of the key elements of the campaign. Moreover, the placement of local consumption data in comparison to national reference consumption data provides a benchmarking system. This is the basis for ongoing discussions about hand hygiene and sustained sensibility for this topic in health care workers.

Table 1. AHC in ml/PD per unit type in 2007 compared to 2008

Type of unit	No. of units	AHC (ml/PD) Median 2007	Median 2008	Difference 2008–2007 (ml)	Difference 2008–2007 (%)	p-value
Surgical	276	15	16.8	2	13	<0.05
Medical	359	15.1	17.8	2.1	14	<0.05
Medical–surgical	110	14.4	17.2	2	14	<0.05
Other	439	12.8	14.4	1.6	13	<0.05
Paediatric	77	30.6	36	3	10	<0.05
All	1261	14.6	16.9	1.9	13	<0.05

P1517 Performance of and knowledge about hand hygiene in medical students

S. Scheithauer, T. Schwanz, H. Haefner, M. Weishoff-Houben, S. Lemmen (Aachen, DE)*

Objectives: Hand hygiene (HH) is considered to be the single most effective tool to prevent healthcare-associated infections (HAIs). Despite several recommendations available, compliance especially in physicians remains low. In order to evaluate knowledge about hand hygiene and their performance of hand hygiene in medical students, we conducted an observational study at the University Hospital / RWTH Aachen, Germany in 2009.
Methods: Performance of hand disinfection was evaluated in first (N = 28), third (N = 193), and fifth (N = 45) year medical students by using a black box. Several groups were compared: before and after instructions concerning HH, with and without information about measurement of results. Knowledge about HH was assessed using a standardized questionnaire.

Table 1: Performance of hand disinfection in medical students

	First year		Third year			Fifth year
	Before teaching (N = 14) (%)	After teaching (N = 14) (%)	Before teaching, no information about result control (N = 63) (%)	Before teaching, with information about result control (N = 64) (%)	After teaching (N = 66) (%)	No teaching, no information about result control (N = 45) (%)
Disinfection gap	12 (86)	10 (71)	58 (92)	45 (70)	12 (18)	33 (73)
1 gap	2 (14)	6 (43)	8 (13)	6 (9)	9 (14)	17 (38)
2–3 gaps	3 (21)	2 (14)	21 (33)	19 (30)	3 (5)	12 (27)
>3 gaps	7 (50)	2 (14)	29 (46)	20 (31)	0 (0)	4 (9)
Right hand gaps only	2 (14)	1 (7)	8 (13)	3 (5)	8 (12)	4 (9)
Left hand gaps only	0 (0)	4 (29)	4 (6)	3 (5)	1 (1)	3 (7)
Right and left hand gaps	10 (71)	5 (36)	46 (73)	39 (60)	3 (5)	26 (58)
Localization of the gap:						
Back of the hand	5 (36)	1 (7)	13 (21)	18 (28)	0 (0)	3 (7)
Palm of the hand	4 (29)	1 (7)	6 (10)	3 (5)	0 (0)	8 (18)
Thumb	3 (21)	2 (14)	33 (52)	14 (22)	8 (12)	4 (9)
Forefinger	6 (43)	5 (36)	14 (22)	9 (14)	0 (0)	3 (7)
Middle finger	5 (36)	2 (14)	8 (13)	7 (11)	0 (0)	1 (2)
Ring finger	3 (21)	1 (7)	12 (19)	10 (16)	0 (0)	0 (0)
Little finger	4 (29)	0 (0)	5 (8)	7 (11)	0 (0)	5 (11)
Fingertips	0 (0)	0 (0)	3 (5)	1 (2)	0 (0)	2 (4)
Finger nails	5 (36)	2 (14)	33 (52)	22 (34)	0 (0)	16 (36)
Knuckles	0 (0)	0 (0)	8 (13)	0 (0)	0 (0)	2 (4)
Interstitial finger sides	5 (36)	3 (21)	2 (3)	0 (0)	0 (0)	5 (11)
Wrist	2 (14)	0 (0)	22 (35)	16 (25)	2 (3)	9 (20)
Rings	3 (21)	1 (7)	2 (3)	2 (3)	0 (0)	0 (0)

Results: Disinfectant gaps were observed significantly more often in beginners (22/28; 79%) than in third year students (115/193; 60%; OR: 2.5 (1.0–6.4); p = 0.04). In third year students disinfectant gaps were seen significantly more often without information about result control (58/63; 92%) than with information about result control (45/64; 70%; OR: 5.0 (1.7–14.1); p = 0.001) and than after giving instructions on HH (12/66; 18%; OR: 52.2 (17.2–158.0); p < 0.001). Without instructions and without information about result control third year students (58/63; 92%) showed significantly more disinfectant gaps than last year students (33/45; 73%; OR: 4.2 (1.4–13.0); p = 0.007). More than 3 disinfection gaps were documented more often in first than in fifth year students (9/28 (32%) versus 4/45 (9%); OR: 4.9 (1.3–17.8)). Gaps on both hands were seen more often before teaching (46/63 and 39/64 vs. 3/66; OR: 34.0 (10–120) and OR: 56.8 (15.7–205.4)). Detailed results were shown in Table 1. All students knew about the importance of hand hygiene, no association to the level of education was observed.
Conclusion: Performance of hand hygiene was influenced by information about result control, and raised by teaching. Best performance was documented immediately after teaching, a training effect during studies could be documented. Thus we suggest implementing training on hand hygiene on a regular basis to improve quality of care in order to prevent HAIs.

Infection control

P1518 Evaluation of asymptomatic transmission and impact of control measures in contacts of a patient with Marburg haemorrhagic fever in 2008 in the Netherlands

A. Timen, M.P. Koopmans, L. Isken, S. Dittrich, P. Willemse, F. van den Berkmortel, P. Emmerich, D. Croonen-van Oudheusden, H.P. Daemen, M.E. Hulscher, A.E. Brouwer, J.T. van Dissel (Bilthoven, Helmond, Nijmegen, NL; Hamburg, DE; Leiden, NL)*

Objectives: In July 2008 an import case of Marburg hemorrhagic fever (MHF) was diagnosed in the Netherlands. We studied asymptomatic transmission among contacts with exposure and evaluated the impact of (restrictive) control measures.
Methods: Retrospective cohort study involving 130 contacts in the hospital and household setting. Blood samples were collected from December 2008 through February 2009, 5–7 months after possible exposure and were tested for the presence of MARV antibodies using an immunofluorescent antibody (IFA) assay. A questionnaire was designed addressing understanding of the rationale for the control measures, clarity of instructions, reported compliance with the measures and perceived impact (based on the Impact of Event Scale/IES). Data were analyzed using SPSS v.15. Results were stratified by risk (high risk meaning unprotected exposure, low risk meaning exposure after institution of strict hygienic measures). Differences in means were assessed by Student's t test. Logistic and linear regression methods were used to model variables associated with IES and risk level, respectively. Overall scales were composed for impact, compliance and clarity of instructions.
Results: Of the 130 contacts, 85 (65%; 50 high-risk, 35 low-risk) provided a blood sample and 78 (60%) completed the questionnaire. All sera tested were negative for IgG and IgM antibodies to MARV. Compliance with measuring temperature twice a day was seen in 81%, and for daily temperature reporting in 60%. 14% Felt restricted in the social contacts during the temperature monitoring, 58% was afraid to develop MHF, 40% was afraid to infect family or other people, 45% of the contacts were afraid that a colleague might be infected, 33% experienced stress and 20% cancelled a planned holiday abroad. The contacts with low-risk experienced more difficulties with measuring and reporting fever (p < 0.001) and with the prohibition to leave the country (p < 0.001). Contacts with high-risk had significantly higher scores on the impact scales (p < 0.001), both during as after the monitoring period.
Conclusions: The serosurvey confirms that no secondary transmission took place between the patient and any contact who provided a blood sample. Being a contact of a patient with MHF is a stressful event and restrictive control measures have a significant impact on the daily life of the contacts. Outbreak control teams in charge of the follow up of contacts need to be aware of the potential impact of the measures.

P1519 Evaluation of silver-based disinfectant designed for use on non-porous hard surfaces in healthcare facilities

R. Rennie, L. Turnbull, B. Bennett (Edmonton, CA)*

Objectives: Materials that have incorporated silver have achieved wide acceptance in advanced wound dressings to reduce the development

of infections. In this study we have examined a silver-based liquid disinfectant that would be utilized on non-porous surfaces to reduce organism loads and prevent the transmission of resistant bacterial species.
Methods: SILVAsan™ (Exciton Inc. and Ostrem Chemical, Edmonton, AB) was provided by the company in three proprietary formulations; SIL-0, SIL-1 and SIL-2. Each disinfectant contained a different concentration of silver diluted 1:16. Two strains of bacteria were tested; *S. aureus* ATCC 33591 (MRSA) and *P. aeruginosa* ATCC 27853. The samples were tested using American Organization of Analytical Chemists (AOAC) Official Method 955.15 Use-dilution Method. Sterile carriers were placed into inoculated broth and then removed and dried. The dried carriers were then incubated with the disinfectant for 1, 5 and 10 min at room temp. Samples were neutralized and then incubated for 48 hr at 350C and examined for growth.
Results: The sterility and viable controls each performed as expected. For these tests secondary neutralization was required and was confirmed with 5 replicates for each micro-organism. The carrier colony count for the *S. aureus* strain was 4.3×10^6 CFU/ml. The count for *P. aeruginosa* was 3.6×10^6 CFU/ml. The SIL-0 formulation failed at 10 minutes; 3 of 60 samples were positive for both *S. aureus* and *P. aeruginosa*. SIL-1 failed for the *S. aureus* strain. 20 of 60 tests were positive after 1 min, and 1 of 60 tests was positive after 5 min. *P. aeruginosa* was therefore not tested. The SIL-2 formulation passed the AOAC test parameters. For both species, none of 60 tests was positive after a 1 min. exposure. As a quality control check these same results were confirmed after 5 and 10 min. exposure.
Conclusion: Disinfectants containing silver in the correct form and concentration are effective at rapidly inhibiting the growth of relatively high concentrations of bacterial pathogens. The results presented show that a disinfectant such as SILVAsan™ can be used on inanimate surfaces to effectively prevent the spread of both MRSA and *P. aeruginosa*, common isolates in intensive and chronic care units, and burn units. The formulations presented have very low concentrations of silver (25 ppm), are present in a silver citrate complex form, and do not present any environmental, worker or patient health issues.

P1520 Survey on the use of multidose vials and fingerstick blood sampling devices in emergency departments compared with intensive care units in Korea

Y.G. Kwak, S.O. Lee, T.H. Kim, E.J. Choo, M. Jeon, J.B. Jun, K. Kim, J. Jeong, Y.S. Kim, J.W. Chung, M.S. Lee (Goyang, Seoul, Asan, Ulsan, Jecheon, KR)*

Objective: Parenteral medications are commonly dispensed in multidose formulations that might be used for prolonged periods for one or more patients, and fingerstick blood sampling devices are widely used for capillary-blood sampling for glucose monitoring in patients with diabetes mellitus. This study investigated the status of use of multidose vials (MDVs) and fingerstick blood sampling devices in emergency departments (EDs) in comparison with intensive care units (ICUs).
Methods: The study population was 389 nurses working at either ICUs or EDs in 6 university-affiliated hospitals in Korea, with routine patient contact through clinical care. A questionnaire on the use of 4 MDVs, such as heparin, insulin, lidocaine, and normal saline as well as fingerstick devices was administered to each of these nurses between December 2007 and February 2008. Questionnaire items involving the use of infection control practices were derived from previous reports.
Results: Surveys were completed by 338 (86.9%) of 389 nurses, including 159 (86.4%) of 184 ED nurses and 179 (87.3%) of 205 ICU nurses. When comparing the use of MDVs between EDs and ICUs, significant difference existed only in preparing heparin lock solution. When using heparin lock solution, 70.4% of the ED and 63.7% of the ICU nurses stated they always made heparin lock solution each time ($P=0.005$). On the contrary, 21.3% of ED and 33.5% of ICU nurses reported that they batched the heparin solution in syringes for multiple heparin locks ($P=0.019$). There were no other significant differences in using the 4 MDV medications between EDs and ICUs. For the fingerstick device, the rate of using separate fingerstick devices for each patient (71.7% vs 54.3%, $P=0.001$) and cleaning the devices between patients (36.5% vs 26.0%, $P=0.038$) were higher in ED, and the rate of hand hygiene was lower in EDs, both before (43.7% vs 74.3%) and after (64.6% vs 91.6%) using the device ($P<0.001$, respectively).
Conclusions: Findings from this investigation underscore the need for education, training, adherence to standard precautions, and specific infection-control recommendations targeting MDVs and diabetes-care procedures in both EDs and ICUs. Strategies for improving compliance with fundamental methods of infection control, especially hand hygiene in EDs and the use of heparin lock solution in ICUs, need to be explored.

P1521 Control of multidrug-resistant *Acinetobacter baumannii* epidemic nosocomial outbreaks

M. Galiè, E. Pistella, L. Emili, C. Santini (Rome, IT)*

Objectives: Epidemic outbreaks due to multidrug resistant microrganisms significantly increase the amount of hospital-acquired infections and the related morbidity and mortality. The results of standard infection control interventions are often disappointing and new, more complex strategies are probably needed. Clusters of infection due to multidrug resistant *Acinetobacter baumannii* (MRAB) have been frequently described in Rome. We report our experience of controlling two MRAB outbreaks occurred in our hospital.
Methods: Since 2002 in our tertiary care hospital is active a program for nosocomial infection control, mainly aimed to increase the hand-washing compliance. Since 2004, weekly microbiologic surveillance of long term (>1 week) ICU patients is performed and colonized patients are isolated.
Results: From June 2008 to March 2009 we observed two outbreaks of MRAB infections. Both clusters originated from patients admitted in ICU from other hospitals. The first cluster lasted 80 days and involved 17 patients, 11 before and 6 after the adoption of the follow specific precautions: contact cohort isolation of infected-colonized patients in a restrict area with specifically dedicated nurses, hand washing priority and use of disposable gloves and gowns before and between patient contact for health care personnel and for all visitors. No MRAB was detected in the following 3 months. On December 2008 a second outbreak involved 16 patients (4 early and12 late) in 6 distinct wards of 3 departments (ICU, Surgery and Internal Medicine). The infection control program was further implemented and extended to all the wards, with the following precautions: environmental disinfection and routine surveillance cultures from environmental sources and patients, daily ward round of the infection control nurse, surveillance cultures from patients coming from other hospitals at the admission. Protocol violations (mainly in hand washing compliance and availability of dedicated nurses) were registered and reported to the Infection Control Committee. Overall 33 patients were involved (18 late, despite the adoption of implemented precautions);14 died for infection (mortality: 42.4%).
Conclusions: MRAB can enter in hospital by patients admitted from other hospitals and rapidly spreads across the wards representing a marker of the level of compliance with routine isolation precautions. An aggressive infection control strategy decrease the diffusion rate and ultimately allow the eradication.

P1522 Point-of-use water filters reduce contamination by opportunistic Gram-negative bacteria present in the water distribution system of a haematology ward

C.L. Wright, L. Newton, K.G. Kerr, A.M. Snelling (Bradford, Harrogate, UK)*

Objectives: Studies have identified hospital water supplies as a source of outbreaks of Gram-negative infection, yet little attention has been paid to hospital water as a reservoir of the opportunistic pathogens *Pseudomonas* spp. and *Stenotrophomonas maltophilia* in non-outbreak settings. We investigated the longitudinal prevalence of these species, as well as overall microbiological quality of water, in water outlets of

a haematology ward before and after the introduction of point-of-use (POU) water filters.
Methods: Over 13 months, bi-weekly 100ml water samples from 39 outlets, including kitchen taps, hand-wash stations and 3 outlets per patient room (2 sinks and shower) were obtained and filtrates incubated on R2A agar for 48 h before sub-culture for identification of *Pseudomonas* spp. and *S. maltophilia.* In Phase 1, water was not filtered. In Phase 2, all outlets had POU devices (Pall Aquasafe 14-day filters). Incidences of bacteraemia were recorded in both phases.
Results: Phase 1 (06/08–01/09): 786 samples were collected of which 99% yielded bacterial growth, which was too numerous to count (TNTC) in 90%. Presumptive *Pseudomonas* spp. and *S. maltophilia* were isolated from 16% and 10% of samples, respectively; often in association with specific outlets. Phase 2 (01/09–07/09): there was a significant and immediate reduction in the frequency and density of isolation. Of 690 samples, only 136 (20%) yielded any bacterial growth with a mean of just 8.2 CFU per growth-positive filter. *Pseudomonas* spp and *S. maltophilia* were isolated from one (0.14%) and 10 (1.4%) filtered samples, respectively. Of 11 Gram-negative bacteraemias in Phase 1, 3 were associated with *P. aeruginosa*. In Phase 2, 9 Gram-negative bacteraemias occurred, with only one episode caused by *P. aeruginosa*.
Conclusions: The water supply on the ward represented a persistent source of *Pseudomonas* spp and *S. maltophilia.* Although a reduction in *P. aeruginosa* bacteraemias was observed with POU filtration, the overall numbers were small. Nevertheless, filtration was associated with a drastic reduction in both the frequency and numbers of these species isolated from every outlet. Accordingly this study has been extended with further filtration/no-filtration phases and will use molecular typing to compare *P. aeruginosa* and *S. maltophilia* isolates from water with those from clinical specimens, to further examine the role of water filtration as a technique to prevent infection of immunocompromised patients.

P1523 Air flow modellization by computational fluid dynamics for optimizing control for airborne microbial contamination in an intensive care room for severely burned patients

C. Beauchêne, N. Laudinet, F. Choukri, J. Rousset, J. Larbre, V. Bergeron, M. Chaouat, M. Benbunan, M. Mimoun, J. Lajonchère, F. Derouin (Chatou, Montigny, Paris, Lyon, FR)*

Objective: Controlling airborne contamination is of major importance in burn units because of the high susceptibility of burned patients to infections. In this study, Computational Fluid Dynamics (CFD) modeling was used to optimize the design of an intensive care room for severely burned patients.
Methods: The study was carried out in 4 steps: 1) patient room design, 2) CFD simulations to model air flows throughout the patient room, adjacent anterooms and the corridor, 3) construction of a prototype room that simulates the hospital environment 4) validation experiments.
The free access computational program Code_Saturne® program was used to mesh the rooms, the medical staff and the equipment into 330 000 cells and to simulate airflows and diffusion of microbial contaminants originating from different sources. Experiments with inert aerosol particles followed by time resolved particle counting were conducted in the prototype room for testing the CFD observations.
Results: CFD studies allowed testing several scenarii simulating the consequences of introduced contaminations. When the surgical zone was artificially contaminated, most particles dispersed toward the peripheral part of the room and were rapidly cleared, but part of them was trained by convection currents to the upper part of the room. This resulted in delayed particulate clearance times and justified the increase of upper exhaust grilles flow rates. Simulating health care workers' traffic between rooms revealed that opening the door between rooms that are at different temperatures resulted in turbulent air exchanges between the rooms. This phenomenon bypassed the pressure differences between the rooms. Experiment conducted in a full-scale pilot room validated CFD findings with respect to levels of confinement, protection of the patient's zone, decontamination kinetics and particle transfer at door opening.
Conclusion: CFD analysis was an effective tool for the design of intensive care rooms for burn patients. The combination of a 15 Pa positive pressure, high air exchange rates with an efficient filtration (or inactivation) were efficient in preventing entry and spread of microbial contaminants in the room. The major protective role of negatively pressured anterooms was also confirmed, although opening between rooms that are at different temperature could bypass this protection. Spiking experiments realized in a prototype room confirmed these findings, thereby validating the CFD prediction.

P1524 Effect of disinfection and drying on biofilm formation by bacteria and yeasts isolated from contaminated endoscopes

J. Kovaleva, J.E. Degener, H.C. van der Mei (Groningen, NL)*

Objective: Biofilm formation within endoscope channels can result in failure of the endoscope reprocessing and is an important factor in the pathogenesis of endoscopy-related infections. The ability for biofilm formation and the effects of the peracetic acid disinfectant and drying procedure on *Candida albicans*, *Candida parapsilosis*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, isolated from contaminated flexible endoscopes, in single- and dual-species biofilms were studied.
Methods: The biofilms were prepared in sterile tissue culture polystyrene 96-well microtiter plates. After 72 h incubation, single- and dual-species biofilms were treated for 10 min with the disinfectant at concentrations conform the minimal bactericidal concentration (MBC) of the strains tested and at 1% concentration, recommended for disinfection of flexible endoscopes by the company. In order to mimic the biofilm formation and to establish a possible regrowth of biofilms inside the endoscope channels after reprocessing, we developed the *in vitro* biofilm model which underwent the different steps of the disinfection and drying procedures applied for reprocessing of flexible endoscopes. The viability of the biofilm was quantified by using the tetrazolium salt (MTT) reduction assay and by counting colony-forming yeasts and bacteria of the 10-fold serial biofilm dilutions on agar plates.
Results: A low MTT formazan signal was demonstrated in all single- and dual-species biofilms directly after 10 min treatment with the MBC and 1% disinfectant. A total inhibition of microbial growth in all biofilms on agar plates occurred after treatment with 1% disinfectant. MBC caused a marked inhibition of microbial growth of all biofilms but not a 100% bactericidal and fungicidal efficacy. Regrowth of all biofilms occurred following 48 h incubation with trypticase soy broth directly after treatment with the MBC and 1% disinfectant if the drying procedure was skipped. No microbial growth and a low formazan signal was found in all biofilms after the disinfection procedure followed by drying for 1, 3 and 5 days.
Conclusion: Routine cleaning procedures do not remove biofilm reliably from endoscope channels. This study demonstrated the high efficacy of the drying procedure after the disinfection step against yeasts and bacteria in all single- and dual-species biofilms. Failure of decontamination endoscopes can be explained by an invalid drying procedure.

P1525 Infection control at a tertiary care centre: success depends on available head count

T. Bregenzer, C. Mohr, E. Bucheli, R. Laffer, M. Buehlmann (Aarau, Langenthal, CH)*

Background: Infection control (IC) teams frequently are understaffed. Up to four 100%-jobs per 500 patient beds are recommended for IC. Costs for IC teams can be calculated easily, whereas savings due to a reduction in the nosocomial infection (NI) rate are difficult to calculate. Therefore, it is difficult to quantify the returns of IC programs. Hand hygiene (HH), preferably with 70% alcohol hand rub, is probably the most effective measure to reduce NI. Repetitive HH instructions are necessary to maintain high HH compliance rates but are personnel-intensive and time-consuming.

Methods: NI prevalence was recorded in our hospital in 1999, 2002–2004, and 2006–2009 according to the Swiss Nosocomial Infection Prevalence study protocol. All healthcare workers (HCW) were trained in HH and repeated instructions were performed in 2005, 2007 and annually thereafter (indications and procedure according to EN 1500). HH compliance was measured according to the protocol of the Swiss Hand Hygiene Campaign in 2005 and 2007–2009, and was reported to HCW and hospital administration annually. Statistical analysis was done by χ^2-test.

Results: During the pre-intervention period the median NI rate was 11.74%. Additional IC staff was recruited in 2005. The first HH instruction in 2005 reduced the NI rate by approximately 40%. After temporary cessation of the HH instruction due to manpower shortage in the IC team, the NI rate rose to previous levels. After regular HH instruction was re-established, NI rates declined again and stayed continuously below 8% (correlation coefficient –0.83 (CI 95% –0.29, –0.97), p = 0.0117).

Conclusion: NI rates at our institution correlate with HH compliance and head count of the IC team. This intervention is only feasible with at least 1.5 full time IC-staff member per 500 beds. To maintain a low rate of NI, continuous HH promotion with repeated instructions is necessary.

Intervention	Pre-intervention						Established		
period	1999	2002	2003	2004	2005	2006	2007	2008	2009
IC-staff/500 beds	0.89	0.95	0.92	0.93	1.39	0.46	1.86	1.74	2.19
HH training for all HCW	ND	ND	ND	ND	+	ND	+	ND	+
HH promotion campaign	ND	ND	ND	ND	+	ND	+	+	+
HH compliance (%)	ND	ND	ND	ND	59	79	ND	58	74
NI rate*	12.9	10.7	10.5	12.8	ND	6.8	12.1	7.2	7.9*

*Chi-square for trend: 4.356, p = 0.0369, ND: not done.

P1526 Policies for healthcare workers safety in European facilities designed to deal with HIDs: EuroNHID data from a survey of 44 isolation facilities in 14 European countries

G. Thomson, B. Bannister, H. Brodt, P. Brouqui, F.M. Fusco, R. Gottschalk, H.C. Maltezou, S. Schilling, V. Puro, G. Ippolito for the European Network for Highly Infectious Diseases Group*

Objective: Highly Infectious Diseases (HIDs, e.g. Viral Haemorrhagic Fevers and SARS) are life-threatening, human-to-human transmissible diseases that may cause Public Health emergencies, requiring special procedures for their containment. To review health-care workers (HCWs) safety policies, the European Network for Highly Infectious Diseases project conducted, through a specifically developed checklist, a survey in the facilities designed to deal with HIDs. Data from 44 facilities in 14 European Countries are described.

Methods: The checklist, including 7 items and 27 questions, was developed through a "networking strategy": a project partner with specific expertise sent drafts for comments and amendments. Final agreement had been reached during a meeting involving all partners. Facilities to be surveyed were selected by national authorities, and are those planned for giving care to patients affected by HIDs. In site surveys were conducted from March to November 2009.

Results: Specific services for HCWs safety (e.g. occupational health service, risk manager, infection control team) are operating on 24 hours-basis in almost all surveyed facilities, and general protocols for the management of the main type of incidents are available, too. Special insurance and special compensation for HCWs working with HID patients are planned in 2 facilities only. Psychological aspects in HCWs safety were explored: assessment of HCWs fears and concerns about HID, such as assessment of safety climate and safety culture, were performed in about 50% of surveyed facilities. HCWs working with HIDs are not selected on the basis of a pshycological attitude. A pshycological support for HCWs is available in 85% of facilities. The availability of specific medical procedures on HCWs safety, that include the monitoring of health status, policies for vaccination and chemoprophylaxis, and post-exposure evaluation and management, differs among participating countries, but generally these aspects are appropriately considered and addressed. Only 25% of facilities have special training requirements to be completed before working with HID patients, and about 75% have specific programs of continuous education, that include also infection control issues.

Conclusion: According to these data, administrative and medical aspects are generally well addressed. Specific training programs should be implemented, while the psychological aspects in HCWs safety are still poorly attended.

P1527 Surveillance of nosocomial bloodstream infections and pneumonia in patients with haematopoetic cell transplantation (ONKO-KISS data) – do patient numbers matter?

M. Dettenkofer, R. Babikir, D. Luft, P. Gastmeier, W. Kern, A.F. Widmer, H. Bertz and the ONKO-KISS Study Group*

Objectives: For surveillance of nosocomial bloodstream infections (BSI) and pneumonia during neutropenia in adult patients undergoing hematopoetic cell transplantation (HCT), an ongoing multicenter surveillance project was initiated by the German National Reference Centre for Surveillance of Nosocomial Infections in 2000 (ONKO-KISS). Differences between centers with regard to the number of patients treated are analysed.

Methods: Nosocomial infections are identified using CDC definitions for laboratory-confirmed BSI and modified criteria for pneumonia in neutropenic patients [for detailed information see: CID 2005; 40: 926, or in German language: http://www.nrz-hygiene.de/surveillance/onko.htm]. In addition infection rates were compared between 15 continuously participating centers (2004–2008) divided in two subgroups with either >300 patients treated/5 years (subgroup a) or <300 patients treated/5 years (subgroup b).

Results: Over the 60-month period from July 2004 up to June 2009 23 centers participated. Altogether 5,853 patients with 86,340 neutropenic days were investigated. Of these, 3,391 (58%) had undergone allogeneic and 2,462 (42%) autologous HCT. The mean length of neutropenia was 14.8 days (9.0 d after autologous and 18.9 d after allogeneic transplantation). In total, 904 bloodstream infections and 490 cases of pneumonia were identified. Incidence densities for allogeneic transplantation were 8.7 BSI/1,000 neutropenic days (ND) and for pneumonia 5.9/1,000 NDs (for autologous transplantation: 15.5/5.1). Following allogeneic transplantation, 16.6 BSI/100 patients and 11.1 cases of pneumonia/100 pts. occurred whereas following autologous transplantation 13.9 cases of BSI/100 pts. and 4.6 cases of pneumonia/100 pts. were observed. The main pathogens associated with BSI were coagulase-negative staphylococci (42.3%). For results of the subgroup analysis see Table.

Conclusion: The ongoing ONKO-KISS project adds to the improvement of quality of care in HCT-patients by providing sound reference data on the occurrence of BSI and pneumonia during neutropenia. Comparison of centers with regard to the numbers of patients treated revealed a higher BSI rate in centers with smaller patient numbers. The difference was notably high in patients undergoing autologous transplantation.

Results within subgroups

	a) 7 centers transplanting >300 patients/5 years Range 45–267		b) 8 centers transplanting <300 patients/5 years Range 341–778	
Type of transplantation	Allogeneic	Autologous	Allogeneic	Autologous
Patients	2,297	1,147	621	724
Neutropenic days	4,343	10,978	13,183	6,375
Mean length of neutropenia (days)	18.9	9.6	21.2	8.8
BSI incidence density	8.3	12.9	9.2	17.9
Pneumonia incidence density	5.8	5.2	5.7	5.5
BSI cases per 100 patients	15.7	12.4	19.5	15.7
Pneumonia cases per 100 patients	11.0	5.0	12.1	4.8

Incidence densities per 1,000 neutropenic days, BSI: Bloodstream infection.

P1528 Effect of different antimicrobial agents on the antibacterial activity of soap formulations and their acceptability by healthcare staff

O. Lozhkina, E. Vorobyeva, A. Savinov (St. Petersburg, RU)*

Objectives: The aim of the present work was to examine how different antimicrobial agents added to the same soap base effect the antibacterial

properties of soap formulations and their acceptance by personnel of healthcare facilities.

Methods: We have prepared six antimicrobial soap compositions having the same base (anionic surfactants and emollients) and different commercially available antimicrobial agents: (1) triclosan, (2) methylisothiazolinone and methylisothiazoline, (3) imidazolidinyl urea and parabens, (4) diazolidinyl urea and parabens, (5) phenoxyethanol and parabens, (6) methylisothiazolinone, methylisothiazoline, phenoxyethanol and parabens, which were introduced into the base soap at concentrations recommended by their producers. The antimicrobial activity of the soaps was evaluated using a volunteer method according to the European Standard EN 1500 and *in vitro* tube dilution method. Afterwards, we studied the acceptance of the formulations with a bacterial reduction factor $\geqslant 3 \log_{10}$ by 98 healthcare workers in 5 hospitals in St. Petersburg, examining such factors as skin irritating and dryness, smell and washing off quickness.

Results: The *in vitro* study has shown that the compositions 1, 4 and 6 produced higher $\log_{10}$ reduction in colony forming units of tested organisms *Escherichia coli* and *Staphylococcus aureus* (3.5–5.58) than did samples 2, 3 and 5 (0.29–4.4). The formulations 1, 4, and 6 demonstrated better antimicrobial activity in respect to Gram-positive than Gram-negative bacteria. In the test using volunteer method, the results seemed to be the same. The formulations 1, 4 and 6 showed better reduction factor in colony forming units (3.2–3.4) than the compositions 2, 3 and 5 (2.4–2.7). The investigations of the acceptance of the formulations 1, 4 and 6 by healthcare facilities personnel revealed that the samples 1 and 6 caused moderate skin irritation and dryness at frequent use, the composition 6 was also poorly accepted because of the specific smell, and only the formulation 4 met compliance with the requirements of the staff.

Conclusions: The antibacterial soaps 1, 4 and 6 provided a greater bacterial reduction than the plain soap, but only the soap 4 was well accepted by healthcare workers. So, when choosing antimicrobial agents for soap, producers should pay a particular attention not only to their biocidal activity but also to skin irritation effect because it is a key factor that influences acceptance and ultimate usage.

P1529 Fungal spores in the air of a hospital environment: seasonal variability of baseline data

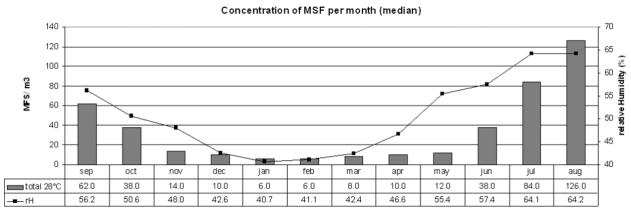
E. Bucheli, M. Reichmuth, M. Oberle, C. Ottiger, R. Laffer, H. Fankhauser (Aarau, Lucerne, Langenthal, CH)*

Background: The soil of plants is a source of mould fungus spores (MFS). Cases have been described in which human pulmonary mould infections in neutropenic patients had their source in plant soil. There are only few studies comparing air MFS count of an environment with and without plants. Therefore, when it was decided to install plants in the staircase of the building of our medical department (including a 4 bed protective haematologic isolation unit) we started measuring MFS concentration in the air during one year before the installation. It is our intention to compare air MFS count in a hospital environment with and without plants. Baseline data will be presented from the pre-installation era.

Methods: During 12 months (September 2008 – August 2009, pre-installation era), fungal spore count in the air was measured twice weekly at 6 standardized locations by sampling 0.5 m^3 air (MBV mas100 Microbiological air sampler) directly over a selective culture medium (sabouraud-agar). Air temperature and humidity were recorded in addition. The plates were incubated for pathogenic (thermo-tolerant) moulds at 35ºC for 2 days and at 28ºC for 4 days. Growing moulds were microscopically identified to genus level (*Aspergillus*, *Mucor*/*Rhizopus*, Cladosporium, Penicillium, Alternaria, *Fusarium*, Trichoderma, Scopulariopsis, Paecilomyces, Chrysonilia sitophila).

Results: Between December and May (relative humidity (RH) of 44.8%, SD±5.1) the mean concentration of MFS at 28ºC was 11.7/m^3 (SD±2.1), during the remaining months (RH of 56.8%, SD±6.1), it was 101.7/m^3 (SD±61.9). The MFS concentration on plates incubated at 35ºC did not show seasonal fluctuation (mean 3.3/m^3, SD±1.2). A peak, mainly of the concentration of *Aspergillus* spores, up to 110/m^3, was documented when Christmas trees were removed in early January 2009.

Conclusions: Baseline count of pathogenic MFS is low and evenly distributed within the building. MFS count on culture plates correlates with humidity and shows seasonal variability. Dust dispersing activities may dramatically rise the concentration of MFS. These baseline data will be compared with results from the second interval (post-installation era) after the installation of the plants in one year.



P1530 Effect of relative humidity on the survival of airborne opportunistic Gram-negative pathogens

P.J. Airey, C.B. Beggs, K.G. Kerr, A.M. Snelling (Bradford, Harrogate, UK)*

Objectives: There is increasing evidence that the hospital environment plays a major role in nosocomial infections. Bacteria can be disseminated to both patients and surfaces via the air, but it is not clear how readily different opportunistic pathogens persist in an airborne state. We investigated the ability of 4 such Gram negative species to survive in air under conditions typical of a ward environment, and looked at the effect of changing relative humidity (RH) as a potential control measure.

Methods: Representative isolates of *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *Pseudomonas aeruginosa* were nebulised (in water) into a 72m^3 aerobiological chamber, in which temperature, relative humidity (RH) and ventilation rate was controlled. Air was sampled at 10–15 min intervals using a 6-stage Anderson sampler, and surviving bacteria recorded as cfu/m^3 of room air. Firstly survival rates under ambient (50% RH, 23ºC) conditions were determined in a series of decay-curve experiments. Then, under steady-state conditions, the effects of RH under two ramping humidity profiles were investigated. Bacteria were nebulised into the chamber for 240 min with RH held at 30% (LOW) for the first 80 min, 50% (AMBIENT) for the central third (80–160 min) and 70% (HIGH) for the final 80 minute phase (160–240 min). In repeat experiments the humidity profile was reversed (i.e. HIGH-AMBIENT-LOW for consecutive 80min periods).

Results: Bacteria were nebulised into the chamber to a concentration of ~10^4 cfu/m^3. There was an immediate 3-log decrease in counts observed with each species on the decay curves. However, viable cells were still recovered from the air after 90mins, for all species except *P. aeruginosa*. In the steady-state experiments (bacterial numbers maintained at an input of ~10^4 cfu/m^3) the number of cfus recovered was 1–2 log higher at 70% RH than 50% RH. Few, or in most cases no, cfu/m^3 were recovered in the dry, 30% RH phases for three of the species. In contrast, larger numbers of B. cepacia were always recovered (~100 cfu/m^3) at low RH.

Conclusion: Low levels of three of these Gram negative nosocomial pathogens can survive in an airborne state for at least 90 min, which would facilitate their dissemination around a ward space. These results suggest that, by reducing the RH, even for a short period, such dissemination could be limited, thereby helping to reduce cross-infection of patients and contamination of surfaces.

P1531 Presence of pathogens on doctors' neckties

B. McGovern, E. Doyle, S.F. FitzGerald, L. Fenelon (Dublin, IE)*

Objectives: To assess whether potential pathogens – Staphylococcoccus aureus, vancomycin-resistant enterococci (VRE) and Gram-negative bacilli (GNB) – were present on the neckties of the male doctor population at an Irish tertiary care hospital.

Methods: Each participant completed an anonymous questionnaire detailing their grade, speciality, frequency of laundering their tie and their preference for wearing a tie at work. The front portion of the necktie was sampled using a Columbia blood agar contact plate which was then incubated in air at 37ºC. Bacterial growth was quantified and organisms identified after 48 hours.
Results: 95 doctors participated. 55% of ties belonged to doctors working in the medical specialities, 32% in surgical specialities and the remainder in other specialities e.g. radiology. 61% of the total male NCHD population working in the hospital at the time were sampled.
50% of ties sampled cultured >50 colony-forming units (cfu) of bacteria and 18% cultured >100cfu. Skin commensals such as coagulase-negative staphylococci, micrococcus and diptheroids were found on all ties sampled. In addition, *Staphylococcus aureus* was isolated from 10 ties (12%); 8 of which were resistant to methicillin (8.5% of ties were MRSA-colonized). GNB were isolated from 12% of ties. VRE was not isolated. Potential pathogens were present in low numbers, 2–10cfu per plate.
55% of participants had never laundered their tie. 80% stated that they would have no objection to abandoning the tie as part of their work attire. Doctors who stated they would prefer to wear a tie at work were not significantly more likely to have laundered their tie (x2=0.618, dF=1, p = 0.4097). The magnitude of growth did not correlate with laundering of ties. There was no correlation between the presence of pathogens and the laundering of ties (x2=2.244, dF=1, p = 0.1341). No grade of staff was more likely to have laundered their tie.
Conclusion: 1 in 5 neckties worn by doctors were colonized with potential pathogens. Almost 1 in 10 ties sampled were found to be colonized with MRSA (8.4%). 4 out of 5 doctors would be happy not to wear a tie to work. The presence of skin flora on all ties sampled suggests that they are being handled regularly. This implies that neckties worn by doctors may act as a potential reservoir of organisms which could be harmful to patients if transmitted.

P1532 Implementing peripheral vascular catheter care bundle in a tertiary referral hospital in Dublin, Ireland

M.R. Finan, A. Fetalento, J. Walsh, V. Caffrey, A. Morris, E. Smyth, F. Fitzpatrick (Dublin, IE)*

Background: The 2006 hospital infection society survey of health care associated infection found that two thirds of patients in this hospital had a peripheral vascular catheter (PVC) in situ.
An increased incidence of PVC-related *Staphylococcus aureus* bacteraemia was noted in this institution since 2006. Compliance with hospital policy for documentation of PVC is poor. In August 2009, the national PVC care bundle was implemented in two medical and one surgical ward. The aim was to improve PVC documentation, increase staff awareness and reduce PVC-associated infections.
Methods: Initial introduction and educational sessions on the PVC bundle were carried out with ward nursing staff. Compliance with the care bundle was assessed initially on a daily basis for two weeks and weekly thereafter for ten weeks. The following were assessed; were PVC's in situ still required, was there evidence of extravasation or inflammation, maintenance of dressing, the duration of insertion, and whether hand hygiene was performed with all PVC procedures. Immediate feedback was given to staff in regards to non-compliance and advice on the appropriate action.
Results: A mean of 60 patients were assessed and 18–35 peripheral lines reviewed each time PVC care bundle compliance was audited in the 3 wards. Compliance was 86% on the medical wards (range 55–100%) and 72% on the surgical ward (range 50–100%). The target of 100% compliance was reached on 10 occasions and 3 occasions on the medical and surgical wards respectively. The failure to comply occurred with PVCs remaining in situ, when not clinically indicated, duration of use in excess of 72 hours and poor maintenance of PVC dressings. There were no PVC-related *S. aureus* infections during the study period, in contrast to 3 episodes in the previous six months of this year.
Conclusion: PVC care bundle is basic best practice to prevent PVC-related infections. Major non-compliances have been identified. Ownership by ward staff and immediate feedback and education to staff is essential for success. This is reflected in compliance reaching 100% on both wards when PVCs were audited and feedback given daily, rather than weekly. While the study period was short, it is encouraging that no PVC-related bacteraemia occurred by comparison with the period prior to care bundle introduction. We hope to implement the PVC care bundles in all wards with the ward staff taking ownership of auditing compliance in order to maintain optimal care.

P1533 Shedding of enterococci and environment contamination in patients with diarrhoea

M. Röttig, E-M. Zeitlberger, R. Bliem, C. Sebesta, O. Janata (Vienna, AT)*

Objectives: Vancomycin-resistant Enterococci (VRE) are emerging pathogens especially in hospitalized patients. Consumption of glycopeptides and transmission from patient to patient are the major risk factors for the acquisition of these pathogens. Enterococci (EC) are part of the human gastrointestinal flora; they are shed with the stool and can contaminate the patient bedside environment. Aim of our study was to determine whether patients with nosocomial diarrhea shed EC or not and what are the risk factors for dissemination.
Methods: In 2009 we quantified the EC-load in the bedside environment of 81 patients using sedimentation-plates (2 plates per patients' bed-surroundings) with selective media. After 2 hours exposure time the plates were incubated at 37ºC for 5 days. Colonies were counted and defined as colony forming units (cfu). Patient data collected included patient mobility, duration of hospitalization and the presence or absence of diarrhea. Diarrhoea was defined as nosocomial if symptoms started later then 72 hours after admission.
Results: Sedimentation-plates of 46 patients with diarrhea and 35 controls without diarrhoea were investigated. Patients with diarrhea spread EC more often and to a larger degree than the controls. Given that a baseline level of EC contamination is to be expected, we defined a threshold level of 2 cfu for the control. We observed at least 2 cfu per plate in 21 surroundings of 46 patients with diarrhoea (46%) but only in 7 of 35 patients without (20%). In addressing the issue of patient mobility, we observed EC with 57% of the bedridden non-mobile patients with diarrhea, and 27% of the patients with diarrhea were mobile and could use the restroom (toilet), whereas partially mobile patients indicated a value 43%.Regarding the control-group EC grew on 36% of the plates positioned around bedridden patients and only in 17% around mobile patients. It must be pointed out that 4 of 7 EC-positive patients without diarrhoea were located next to EC-positive Patients with diarrhea.
Conclusion: Using sedimentation-plates positioned around the beds of patients with or without diarrhea we found that the spread of EC into the immediate bedside environment was markedly higher in patients with diarrhea, especially if they were bedridden, mobility being a significant factor. Furthermore, the environmental contamination around patients without diarrhea was clearly higher in rooms also occupied by patients with diarrhea.

P1534 Nursing home-associated infections in France: Delphi consensus guidelines for prevention

G. Gavazzi, K. Chami, R. Derbal, B. De Wazières, B. Lejeune, F. Piette, J. Hajjar, M. Rothan-Tondeur for the Observatoire du Risque Infectieux en Geriatrie Association (ORIG)*

Objectives: Because the prevalence of nursing home associated Infection (NHAI) is high in France (14.5%) and because infection control measures for hospital can not be applied in nursing home (NH), we elaborated a survey aiming to establish consensus guidelines for prevention of NHAI for elderly living in French NH.
Methods: A nationwide survey using a two-round modified Delphi procedure (Rand Corporation) has been performed by the "Observatoire

du Risque Infectieux en Gériatrie". An investigative group of 5 specialists in the field of infectious disease, geriatrics and infection control conducted a comprehensive literature search and review based on English or French published practice guidelines, systematic review and articles (or abstracts when articles were not available) on the topic of infection prevention in Long-term care Facilities, nursing homes or hospitals. The literature search has been fulfilled by 4 research analysts. The literature search has been examined by 25 specialists who compiled a preliminary list of guidelines. Using a modified Delphi survey online instrument developed by INSERM UMR-S 707 "Epidemiology, Information Systems, Modelling" and modified by the Geriatric Infection Risk Monitoring Organization (ORIG), 80 experts of all relevant medical specialities in the field of prevention and infection control or geriatrics and randomly selected medical coordinator in nursing homes, rated their agreement with each of these 301 guidelines using a 9-point scale (1=strongly disagree to 9=strongly agree). Recommendation-specific median were estimated. The consensus was defined if the recommendation was rated by at least 90% of the experts and if the median score was greater than 7.

Results: The response rate was 93% for the first round, and 99% for the second round. The expert panel reached consensus on 264 recommendations and 37 recommendations were rejected. The different relevant topics were organisational procedures (outbreak, surveillance), basic hygiene measures, antibiotic policies, general measures linked to patient (vaccination) and multiltidrug resistant pathogens. The difference between first and second round emphasizes the issue of implementation in NH.

Conclusions: The guidelines for NHAI prevention in French Nursing Homes will be published nationwide. A cluster-randomized controlled trial has been launched with the purpose of evaluating the effectiveness of an infection prevention program, using these consensus guidelines.

MRSA control

P1535 Integrated MRSA management – a novel approach combining in-house and outpatient MRSA decolonization

A. Stroh, B. Jahn (Wiesbaden, DE)*

Objectives: Eradication of methicilline resistant *Staphylococcus aureus* (MRSA) represents a key element to abolish the risk of subsequent MRSA infection. Therefore, decolonization to eradicate MRSA is recommended. In many patients, length of stay (LOS) is to short to successfully eradicate MRSA in the hospital setting. To optimize and economize the MRSA decolonization related input, the concept of an Integrated MRSA Management (IMM) was developed. IMM adapts the principles of in house decolonization schemes to the outpatient setting and MRSA decolonization is continued immediately after hospital discharge.

Material and Methods: All elements of MRSA decolonization including treatment of nose, throat, skin, hair and wounds were adapted to be performed by a specially trained nursing team or by the patient himself. Within 72 h after discharge and according to the patient's situation and colonization state, requirements regarding nursing and decolonization, cleaning and disinfection of patient surroundings as well as domestic services were evaluated. An IMM decolonization plan was set up and decolonization, based on mupirocine for topical antibiotic treatment of the anterior nares and polyhexanide as antiseptic compound for body washings, mucosa and wound care was performed, concomitant with the supporting procedures. The colonization state was assessed by microbiological controls. Communication and all activities were documented by pda.

Results: Within IMM in 73% of patients without skin alteration MRSA eradication was successful according to german national guide lines, compared to 34% success rate in patients bearing this risk factor (e.g. wounds, entry sites). Overall MRSA eradication success was 47%.

Conclusion: The concept of IMM allows complete MRSA decolonization even with short LOS. In perspective, IMM will minimize patients' risk of MRSA infection and decrease the MRSA load for hospitals as former MRSA patients can be readmitted as MRSA negative. The needs of caMRSA carriers in the outpatient setting can also be addressed within IMM.

P1536 MRSA screening in cardiac intensive care – the Achilles' heel: a clinical audit and survey of outcomes at Lancashire cardiac centre

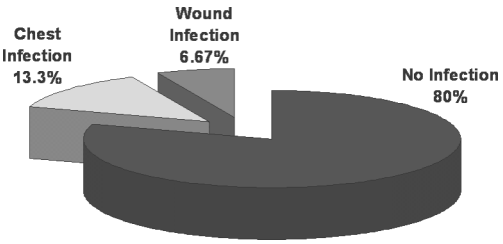
S. Summers, M. Przybylo, M. Purohit, A. Guleri (Blackpool, UK)*

Objectives: MRSA PCR screening of patients on admission to cardiac intensive care and then weekly thereafter (by culture) for long stay patients was introduced in Apr08 at Lancashire cardiac centre (LCC), Blackpool Victoria Hospital (BVH). Rapid detection of MRSA carriage permits timely decisions on optimal treatment, isolationbio-burden reduction, reducing the potential for cross transmissionself infections and cost containment. All patients for elective cardiac surgery get screened for MRSA in preassessment clinic (PAC). This clinical audit was performed following a case of PAC MRSA screen negative, postoperative (POp) MRSA PCR positive developing post-op MRSA infection. The results of this audit were used to inform the HAI containment programme for LCC.

Methods: Case notes data extraction & root cause analysis on cardiac patients identified as MRSA screen (PAC) culture negative and MRSA PCR (POp) positive [MCNPP] between April 08 – 09 from pathology data system. Review of literature on antibiotic prophylaxis (cardiac surgery) & MRSA and trust MRSA policy & antibiotic formulary.

Results: Between April 08–09, 15 MCNPP patients age 56–84yrs were identified. 80% (12/15) were males; length of stay 5–29 d. Cardiac surgeries included coronary artery bypass graft (CABG) 60% (9/15); CABG & aortic valve replacement (AVR) 20% (3/15); 7% (1/15) each CABG revision, AVR and decortication. 20% (3/15) were PAC screened negative >12 weeks pre-op while 80% (12/15) 3-weeks preop. All MCNPP patients received topical bio-burden reducing regime (TBRR) within 24-hrs of MRSA PCR result. The cardiac surgeons vary in choice & duration of antibiotic prophylaxis (cefuroxime ± glycopeptides). Hence 47% (7/15) managed to receive glycopeptides at induction. 73% (11/15) had no documented healthcare contacts between PAC screen and operation, while remaining included occupational (nurse), angiograms, cataract operation & day case. POp infections included pneumonia (13.3%); wound infection (6.7%) and none (80%).

Discussion: BVH won HCAI innovation technology award 2009 (78% reduction in MRSA using PCR for emergency admissions). In this audit MRSAPCR picked up 100% carriage & TBRR was offered on same postop day. 20% POp infections were seen in this cluster. The recommendations from this clinical audit included 3-week validity of PAC MRSA negative screen; considering a MSSA/MRSA PCR preop screening; introduction of consistent evidence based antibiotic prophylaxis policy; Details to be presented.



P1537 MRSA PCR screening: is it still a cost-effective option during times of financial turbulence in NHS hospitals?

A. Guleri, B. Lunt, J. Carter Lyndsay, N. Harper, R. Palmer, A. Jones (Blackpool, Malton, UK)*

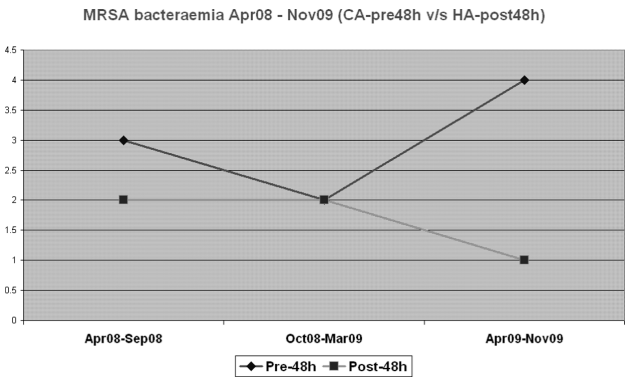
Background: Rapid and accurate identification of meticillin-resistant *Staphylococcus aureus* (MRSA) in hospital admissions is essential for timely decisions on optimal treatment, isolation/bio-burden reduction,

reducing the potential for cross transmission/self acquisition of healthcare-associated infections (HCAI) and cost containment. The NHS is expected to face a £15bn budget shortfall due to effects of recession. Here we present the cost effectiveness of the second year of a rapid MRSA screening programme which was implemented at Blackpool Victoria Hospital and the economic advantages of continuing the service despite a 15% cut in overall budget.

Methods: The clinico-economic re-evaluation included various aspects including cost analyses (total cost of the service [equipment, consumables, salaries), estimated savings from reduction in total MRSA infections including bacteraemia, savings from collateral benefits of the associated MRSA programme). Clinical implications, patient and staff feedback were also collected.

Results: In the first year (08–09) a 78% reduction (9 bacteraemia against 40 in 07–08) was recorded, 45% (4/9) of which were HCAIs. In the second year (09–10) 5 bacteraemias were recorded between Apr – Nov 09, of which 20% (1/5) were post-48hrs (Fig 1). The cost of service over 18 mths was approx £591,332. Estimated savings from the overall 30% reduction in all MRSA infections over the previous 18 mths ranged from £806,000 (using £2,000/infection including bacteraemia) to £2,014,597 (using £4,999/infection including bacteraemia) models. A 51% reduction in glycopeptide unit-days and spend was also recorded from £251,168 (06–07) to £124,060 (08–09). A total of 31,867 tests were performed and 96% of patients could be re-assured of their negative MRSA status within an average 5hr turn-around-time.

Conclusions: Rapidly available MRSA results are routinely used to complement clinical decision making and optimise treatment in our hospital. The cost effectiveness of a HCAI programme is proportional to its success. The key to success in this model was the teamwork between CEO, ICT & clinical teams (med & nursing)in the hospital. The body of evidence which supports the efficacy of rapid screening is growing. A sound business case and return on investment for the use of rapid diagnostic methods in our hospital can be made. These technologies also enhance clinical quality, improve patient safety and reduce the overall cost of MRSA infections.



P1538 Natural history of MRSA colonization – a 4-year experience from southern Sweden

A.K. Larsson, E. Gustafsson, H. Ringberg, E. Melander (Helsingborg, Malmö, SE)*

Objective: The natural history of MRSA colonization is not well studied and the duration of colonization varies among existing studies. Most studies deals with healthcare associated MRSA and carriage after hospitalization. In Skåne County, southern Sweden, all known MRSA cases have been followed up the same way since 2003 enabling a good base for studying the natural history of MRSA colonization.

Methods: All notified MRSA cases in Skåne County are followed with cultures from the nares, throat, perineum and possible skin lesions as long as they are colonized and at least for a year with at least five consecutive negative cultures. They are examined for risk factors. Contact tracing among household contacts and possible health care contacts is performed.

Results: 442 of the 578 notified MRSA cases during 2003–2006 were correctly followed up and included. 91% cleared their MRSA colonization during the followup-time. The median duration of MRSA colonization was 4.6 months. Having household contacts with MRSA, young age, colonization with spa-type t002 or colonization in more than 2 locations was significantly associated with a longer duration of colonization. Receiving treatment for a clinical infection (as compared to asymptomatic carriage) and spa-type t355 was significantly associated with shorter duration of colonization.

Conclusions: The study of our MRSA cohort, a mix of healthcare- and community associated MRSA cases, indicates that MRSA coloniazation can be cleared in less than 6 months and that MRSA cases can be defined as "MRSA negative" with a thorough follow-up with consecutive negative cultures, no MRSA among house hold contacts and absence of risk factors. This may have implications for the future handling of MRSA cases in our country.

P1539 MRSA admission screening – much ado about nothing?

A. Witteck, G. Rettenmund, M. Schlegel (St. Gallen, CH)*

Objectives: In 2007, the number of patients with newly detected MRSA doubled at the cantonal hospital St. Gallen (MRSA rate 2.5% in isolates). In order to early diagnose MRSA and prevent its spreading within the hospital, new guidelines for screening/preemptive isolation were implemented. Aim of this surveillance was to evaluate these guidelines and to compare MRSA patients detected by admission screening with those diagnosed by clinically indicated cultures during hospitalization.

Methods: The surveillance was performed from 04/2008 to 03/2009 in patients admitted to the tertiary-care hospital St. Gallen and 10 affiliated public hospitals in Eastern Switzerland. All MRSA admission screenings in accordance with the guidelines and all MRSA newly detected by clinical sample were included. The screening guidelines considered epidemiological factors (stay in a foreign hospital, a Swiss hospital with known high MRSA prevalence or a chronic care facility during the past six months) and personal risk factors (wound, tracheostoma, urinary catheter and IVDU).

The MRSA admission screening consisted of nasal, throat and axillary/inguinal swabs as well as wound swabs, urine in the case of urinary catheter and respiratory secretion provided the patient was intubated or a tracheostoma present.

Results: During the one-year observation period, 161 patients were screened for MRSA (111 in St. Gallen, 50 in the affiliated hospitals). Only 6/161 (3.7%) MRSA were detected by screening (number needed to screen: 27). During the same time period 21 patients were diagnosed by clinically indicated samples. Patient characteristics are shown in the table.

	MRSA screening		MRSA detection
	Negative (n = 155)	Positive (n = 6)	by clinical sample (n = 21)
Age (mean, 95%-CI)	61.3±2.9	60.8±13.3	56.1±8.6
Sex, % female	43.2 (67/155)	33.3 (2/6)	57.1 (12/21)
Personal RF %			
Skin leason	39.2 (47/120)	83.3 (5/6)§	80.9 (17/21)
Tracheostoma	10.5 (13/124)	0 (0/6)	5.3 (1/19)
Urine catheter	32.2 (38/118)	33.3 (2/6)	21.1 (4/19)
PEG	4.8 (6/124)	0 (0/6)	0 (0/19)
IVDU	3.9 (5/127)	0 (0/6)	0 (0/18)
Immunosuppression	1.7 (2/118)	16.7 (1/6)§	11.1 (2/18)
Dialysis	9.5 (12/126)	0 (0/6)	5.0 (1/20)
Diabetes	13.6 (16/118)	0 (0/6)	26.3 (5/19)
Epidemiological RF %			
Foreign hospital	65.8 (75/114)	83.3 (5/6)	0 (0/18)*
CH high prevalence hospital	9.5 (11/116)	0 (0/6)	0 (0/18)
Chronic care facility	20.7 (25/121)	16.7 (1/6)	9.5 (2/21)

CI: Confidence Interval, RF: Risk factor, PEG: Percutaneous endoscopic gastrostomy, IVDU: Intravenous drug use, CH: Switzerland.
*<0.001, Screening vs clinical pos. §p = 0.08, §p = 0.13, Screening neg vs pos.

All MRSA-positive patients would have been detected either by nasopharyngeal or wound swabs (except by one with only positive urine). In the patients with positive MRSA screen, all swabs of axilla/inguina were negative.
Conclusions: In our setting with low MRSA-prevalence, the majority (77.8%) of patients with newly detected MRSA was diagnosed by clinically indicated samples. Several well-accepted risk factors seem not to be relevant (tracheostoma, IVDU, dialysis). Wounds and immunosuppression tend to be more frequent in positive versus negative screened patients. Screening of axilla/inguina did not increase the sensitivity of the screening (no positive results).
However, due to small numbers (probably because of not performed, but indicated screenings) results must be considered with caution and may differ in other settings.

P1540 Effect of search-and-destroy policy on *Staphylococcus aureus* bacteraemia and overall methicillin-resistant *S. aureus* rates: experience from neurosurgery clinic of a tertiary-care educational university hospital with endemic MRSA

O. Sipahi, B. Arda, A. Bayram, S. Aydemir, T. Turhan, F. Cilli, H. Sipahi, S. Ulusoy (Izmir, TR)*

Objectives: *S. aureus* is one of the most important etiologic agents in nosocomial infections. Search&destroy policy is effective for preventing *S. aureus* infections in settings with low MRSA rates. The aim of this study was to evaluate the effect of search&destroy policy on *S. aureus* bacteremia, meningitis and overall MRSA rates in neurosurgery clinic of a developing world tertiary-care educational university hospital where MRSA is endemic.
Method: This study was performed at a tertiary care general teaching hospital with an active neurosurgery ward with 78 beds, 16 of which are in intensive care unit. After 1 July 2008, patients admitting to neurosurgery intensive care unit of our setting were sampled for nasal *S. aureus* carriage. Bacteriologic culture and identification of *S. aureus* from any sample was performed with conventional methods. Antibacterial susceptibility testing was performed with disk-diffusion test according to CLSI criteria. Cases who had nasal *S. aureus* carriage were treated with nasal bacitracin ointment (three times a day, for three days, control culture performed on day 5–6). Cases with MRSA carriage were put under contact isolation. Resistance patterns of the samples, total number of patients and patient days in the overall neurosurgery clinic (including both ICU beds and normal beds) were retrieved from hospital records database. The preintervention period was considered as January 2007–2008 June and post intervention period was considered as July 2008-October 2009.
Results: Overall *S. aureus* bacteremia rate decreased significantly (20/3651 cases and 0.41/1000 patient days vs 6/2959 and 0.2/1000 patient days, $p = 0.02$). Overall *S. aureus* meningitis rate did not change significantly (4/3651 vs 8/2959, $p < 0.05$). MRSA rate in any clinical sample (only one sample from each case) did not change significantly (55/99 versus 38/61 $p > 0.05$).
Conclusion: Despite the fact that the search&destroy policy was performed in only ICU cases, overall bacteremia rate decreased significantly. However overall MRSA rate did not change. Our findings suggest that this strategy may be beneficial in resource-limited countries where bacteremia is a significant problem.

P1541 Regional surveillance of MRSA and benchmarking in the EUREGIO MRSA net-project

A. Jurke, R. Köck, I. Daniels-Haardt, A.W. Friedrich (Münster, DE)*

Objectives: MRSA is a major cause of Healthcare-associated infections (HCAI). Since MRSA can be disseminated between hospitals serving one regional patient catchment area, successful prevention and control of MRSA-HCAI requires concerted efforts. Therefore, regional benchmarking of hospital MRSA surveillance data might open up opportunities to reduce infection rates.
Methods: 35 hospitals (92% of all hospitals) covering 10.851 patient beds and comprising 343.000 admissions in 2008 in the German part of the EUREGIO Twente/Münsterland have established a network for combating MRSA infections. In 2007 they have agreed upon a synchronized strategy for screening of risk patients at admission and are reporting MRSA data. In 2007 and 2008 the following indicators were assessed: rate of nasal swabs/100 patients admitted, MRSA incidence, nosocomial MRSA incidence density, nosocomial MRSA-cases/1,000 MRSA days and the number of MRSA blood cultures. Wilcoxon signed-rank test was used.
Results: In average, the rate of screening cultures/100 patients in the 35 hospitals was 22.2% in 2008 (Q(25)=8.2%, Median 17.0%, Q(75)=39.2%. There was a significant ($p < 0.05$) increase of this rate from 2007 to 2008. Alltogether 88,464 patient were screened in 2008.
The MRSA incidence in 2008 was in average 1.47 MRSA cases per 100 admissions (Q(25)=0.68, Median = 1.03, Q(75)=1.40), which also increased significantly ($p < 0.05$) since 2007. The number of MRSA-positive blood cultures (MRSAB) decreased from 2007 (2.8 per 100,000 patient days) to 2008 (1.32 per 100,000 patient days).
The mean nosocomial MRSA incidence density in 2008 was 0.19 nosocomial MRSA-cases per 1,000 patient days (Q(25) = 0.02, Median = 0.16, Q(75) = 0.24) and remained stable from 2007 to 2008. In 2008, there were an average of 8.6 nosocomial MRSA-cases per 1,000 MRSA-days (Q(25) = 4.3, Median = 8.7, Q(75) = 12.4) with a slight decrease ($p > 0.05$) compared to 2007.
Conclusion: Successful adherence to the synchronized regional screening strategy was measurable by continued increased performance of screening in hospitals. The enhanced screening of risk patients resulted in a considerably higher MRSA-incidence, since an increased number of carriers were detected at admission. Nevertheless, the number of MRSAB decreased in the study period. All surveillance data is given as feedback to the regional hospitals and health authorities. This should encourage regional benchmarking and improve prevention and control of MRSA HCAI in the long run.

P1542 Is it worthwhile to control methicillin-resistant *Staphylococcus aureus* in a highly endemic country? A 9-year experience in a Portuguese hospital

M.L. Gonçalves, A. Costa, J. Narciso, L. Afonso, C. Hipolito, O. Neto, A.B. Correia, F. Fernandes, A. Canudo, A. Santos Costa, P. Pedro, C. Gasparinho, F. Ferreira, R. Mato, I. Sanches, H. de Lencastre (Lisbon, Oeiras, Caparica, PT; New York, US)*

Objective: To evaluate the impact of a prolonged comprehensive control program for methicillin-resistant *Staphylococcus aureus* (MRSA) infection and colonization, in a Hospital located in a country with a very high MRSA prevalence.
Methods: Hospital SAMS is a 121-bed acute-care private hospital in Lisbon, Portugal, serving a population of 122,000 that also uses other healthcare institutions. MRSA has been endemic in SAMS since at least 1997, representing almost 50% of all *S. aureus* isolates. Medical and surgical wards and medical-surgical intensive care unit (ICU) were the most affected. After a baseline study period (1997–1999), an infection control program was launched in 2000 in these units. It included screening cultures (universal screening in ICU and screening of high-risk patients in the selected wards), use of contact-droplets precautions, appropriate hand hygiene and decolonization of colonized patients.
The program was available for evaluation during three time periods: Period 1 (2000–2003) – full implementation of the program; Period 2 (2004–2005) – adherence to the program was progressively discontinued; Period 3 (2006-June 09) – reimplementation of the full-program plus screening of high-risk patients at hospital admission. An analysis comparing the incidence rate of hospital-acquired MRSA infections per 1,000 patient-days in the different intervention periods was carried out.
Results: Patients infected or colonized with MRSA have been admitted to the Hospital since the beginning of the program. Of the 2,327 patients screened at admission in ICU (2000-Jun2009) 2.8% were colonized. In Period 3 from the 833 high-risk patients screened at Hospital admission

14.4% were colonized. The majority came from nursing homes or other Hospitals. The baseline rate of hospital-acquired MRSA infections in ICU was 4.56 infections per 1,000 patient-days and decreased to 0.83 ($P = 0.02$) and to 0 ($P < 0.001$) in periods 1 and 3 respectively. The global baseline rate of hospital-acquired MRSA infections in the three units was 0.57 and decreased to 0.15 in periods 1 and 3 ($P < 0.001$). In contrast, there was an increase of hospital-acquired MRSA infections in all units during Period 2.
Conclusions: A comprehensive program to control MRSA infection and colonization can be successful even in a highly endemic area and can be sustained for a prolonged period. Motivation, education and feedback are some of the most important tools for the sustainability of such a control policy.

P1543 Resistance proportion, incidence density and the number of isolation days – where lies the real burden of MRSA?

K. Kaier, R. Babikir, M. Dettenkofer, U. Frank, M. Kist, C. Schneider, A. Conrad (Freiburg, DE)*

Objectives: Knowledge of the burden of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital level is important for infection control. The aim was to compare how the monthly MRSA proportion and incidences of MRSA differ (magnitude, trends). In addition, MRSA-related isolation days were assessed describing the monthly burden of isolating patients with MRSA.
Methods: Data were collected retrospectively (01/2003–12/2008) at University Medical Center Freiburg, Germany, a 1,600-bed tertiary care hospital. The MRSA proportion was defined as the number of non-duplicate MRSA positive strains relative to all non-duplicate *S. aureus* isolates, excluding screening results. MRSA incidences were determined (MRSA-cases/1000 patient days (pd)) and were grouped in colonisations, infections, hospital acquired cases (MRSA detected >48 h after admission) and patients admitted with MRSA. Additionally, the length patients were isolated was assessed and incidences of isolation days/1000 pd were calculated.
Results: The MRSA proportion did not follow any trend ($p = 0.1$) showing a mean proportion of 0.09. A slight increase ($p < 0.001$) in the incidence of all MRSA cases was identified (mean: 0.5/1000 pd). In detail, a decreasing ($p = 0.0012$) incidence of hospital acquired MRSA (mean: 0.15/1000 pd) faces an increasing ($p < 0.001$) incidence of patients admitted with MRSA (mean: 0.35/1000 pd). The incidence of MRSA-related isolation days for hospital acquired MRSA (mean: 2.54/1000 pd) decreased ($p = 0.034$) whereas the incidence of isolation days related to patients admitted with MRSA (mean: 4.1/1000 pd) increased ($p < 0.001$). The average length patients with hospital acquired MRSA-infections were isolated (mean: 23.1 days) was longer than the length of isolation of patients with hospital acquired MRSA-colonisations (mean: 17.0 days). Similarly, the average length of isolation for patients admitted with MRSA-infections (mean: 17.5 days) was longer than that of patients colonized with MRSA at admission (mean: 11.6 days).
Conclusion: Both the MRSA incidences based on isolation events and based on isolation days show comparable dynamics with decreasing trends for hospital acquired MRSA and increasing trends regarding patients admitted with MRSA. Furthermore, the average length of isolation is longer for hospital acquired MRSA rather than for patients admitted with MRSA and, within these two groups, longer for patients infected with MRSA rather than patients colonized with MRSA.

Clinical trials of antimicrobial agents

P1544 Patients included in randomized controlled trials do not represent those seen in clinical practice

M. Falagas, E. Vouloumanou, C. Sgouros, S. Athanasiou, G. Peppas, I. Siempos (Athens, GR)*

Objective: Clinicians rely on findings of randomized controlled trials (RCTs) to formulate clinical decisions regarding individual patients. To examine whether patients included in RCTs are representative of those encountered in real life clinical situations.
Methods: PubMed was searched for RCTs referring to the field of infectious diseases. Data regarding the exclusion criteria of the identified RCTs were extracted and critically evaluated.
Results: Thirty trials (seventeen referring to respiratory tract, five to skin and soft tissue, four to intra-abdominal, two to gynecologic, and two to bloodstream infections) were included in our study. All retrieved RCTs reported extensive exclusion criteria. After comparing in a qualitative manner (based on our clinical experience) the eligible patient population in the identified RCTs with the respective population that would be encountered in general practice, we noted that the abovementioned patient populations differ considerably.
Conclusions: RCTs in the field of infectious diseases use extensive and stringent exclusion criteria; a fact that may lead to considerable difference between the patient populations of RCTs and those viewed in clinical practice. The application of the findings of RCTs to the care of individual patients should be performed cautiously.

P1545 Aerosolized in combination with intravenous colistin vs. intravenous colistin in the treatment of ventilator-associated pneumonia: a matched case–control study

D. Kofteridis, A. Valachis, C. Alexopoulou, S. Maraki, D. Dimopoulou, F. Economidou, E. Mavrogeni, M. Christofaki, L. Sifaki, D. Georgopoulos, G. Samonis (Heraklion, GR)*

Objectives: The incidence of ventilator-associated pneumonia (VAP) caused by multidrug-resistant (MDR) strains is increasing. Intravenous (IV) or aerosolized (AS) plus IV colistin have been recently used to treat these life threatening infections. However, little is known for the beneficial role of AS colistin in VAP patients. The purpose of this study was to compare the efficacy and safety of AS plus IV colistin vs only IV colistin in patients with Gram-negative MDR VAP.
Methods: A retrospective, matched case–control study was performed at the Intensive Care Unit of the University Hospital of Heraklion, Greece, from January 2005 through December 2008. Forty-three patients suffering of VAP diagnosed by standard criteria, caused by Gram-negative MDR as documented by positive cultures of bronchial secretions or bronchoalveolar lavage fluid, received AS plus IV colistin and were matched based on age, and APACHE II score to 43 control patients who had received only IV colistin. Patients who received at least 6 doses of AS or >3 days of IV therapy were eligible for evaluation.
Results: Demographic characteristics, clinical status, and Gram-negative isolated pathogens were similar between the two treatment groups. *A. baumannii* (66 cases; 77%) was the most common pathogen, followed by *K. pneumoniae* (12; 14%) and *P. aeruginosa* (8; 9.3%). One patient in the IV group had concurrent *A. baumannii* bacteremia, while in the AS plus IV group 2 had *A. baumannii* and 1 *K. pneumoniae* bacteremia. No colistin resistant strain was isolated from patients of either group.
No significant differences between the two groups were observed regarding eradication of pathogens ($P = 0.679$) clinical cure ($P = 0.10$) and mortality ($P = 0.289$). Eight patients (19%) in each treatment group developed reversible renal dysfunction. No AS colistin adverse events were recorded.
Conclusion: Addition of AS to IV colistin did not provide additional therapeutic benefit to patients with Gram-negative MDR VAP.

P1546 Activity of doripenem in patients with baseline bacteraemia in 6 pooled phase III pivotal studies

O. Umeh, K. Kaniga, M. Lee, N. Ketter, A. Quintana, R. Redman (Raritan, US)*

Objectives: Doripenem (DOR) 500 mg given every 8 hours as a 1-hour infusion was studied and is approved in the US & Europe (EU) for complicated intra-abdominal infections (cIAI) and complicated urinary tract infections in adults, and in the EU as a 1 or 4-hour infusion for nosocomial pneumonia (NP) including ventilator-associated pneumonia

(VAP). DOR is active *in vitro* against most bacteria commonly isolated from bacteremic patients (pts). To date, outcomes in the subgroup of DOR-treated pts with concurrent bacteremia and either cIAI, cUTI or NP has not been reported. Here we report the clinical and microbiological (micro) outcomes in a subgroup of pts with bacteremia at baseline (BL) from 6 pooled DOR clinical studies.

Methods: Micro outcomes for all micro evaluable (ME) subjects with BL bacteremia in DOR studies 05–10 were obtained. The ME analysis set excluded pts who received concomitant non study antibiotics. Micro eradication was either defined as the presence of $\geqslant$1 post-BL negative blood culture or presumed if a patient had no post-BL blood culture but recovered from the index infection. The overall response to treatment in bacteremic and non bacteremic pts was compared.

Results: The table below summarizes the findings.

Overall, DOR was effective in clearing baseline bacteremia. The response to treatment in DOR-treated pts with bacteremia was similar to pts without bacteremia. In contrast, in the comparator arm, there was a trend towards lower clinical cure rates in bacteremic versus non-bacteremic pts; however this comparison is limited by the small sample size.

Conclusion: DOR is a good therapeutic option for subjects with cIAI, cUTI or NP who have concomitant bacteremia at diagnosis.

Table: Summary of clinical cure at TOC and clearance of baseline blood pathogen in ME patients

	Doripenem			Comparator			Diff[a]	
	N	n	%	N	n	%	(%)	95% CI
Clearance of blood pathogen(s)								
Overall	80	76	95.0	77	71	92.2	2.8	(−6.1; 11.7)
cUTI	47	47	100.0	23	23	100.0	0.0	
cIAI	14	13	92.9	23	22	95.7	−2.8	(−24.4; 18.8)
NP (non-VAP)	3	3	100.0	11	9	81.8	18.2	(−25.8; 62.2)
VAP	16	13	81.3	20	17	85.0	−3.7	(−34.1; 26.6)
Had both clinical cure at TOC and clearance of blood pathogen for subjects *with* bacteremia at baseline								
Overall	80	68	85.0	77	57	74.0	11.0	(−2.8; 24.8)
cUTI	47	44	93.6	23	23	100.0	−6.4	(−16.6; 3.8)
cIAI	14	12	85.7	23	17	73.9	11.8	(−19.6; 43.2)
NP (non-VAP)	3	3	100.0	11	7	63.6	36.4	(−13.3; 86.0)
VAP	16	9	56.3	20	10	50.0	6.3	(−32.1; 44.6)
Clinical cure rates at TOC for subjects *without* bacteremia								
Overall	984	858	87.2	706	584	82.7	4.5	(0.9; 8.1)
cUTI	474	449	94.7	231	206	89.2	5.5	(0.7; 10.4)
cIAI	311	263	84.6	285	242	84.9	−0.3	(−6.5; 5.8)
NP (non-VAP)	68	57	83.8	66	58	87.9	−4.1	(−17.3; 9.2)
VAP	131	89	67.9	124	78	62.9	5.0	(−7.4; 17.5)

[a]Doripenem minus Comparator.

P1547 Efficacy of tigecycline versus ampicillin–sulbactam or amoxicillin–clavulanate to treat complicated skin and skin structure infections – study 900

P. McGovern, D. Rill, E. Zito, T. Babinchak on behalf of the 900 study group*

Background: Tigecycline (TGC) has demonstrated clinical efficacy and safety versus vancomycin plus aztreonam in previous complicated skin and skin structure infection (cSSSI) trials. The current study was a global, multicenter, open label, randomized, comparative study of tigecycline (TGC) versus ampicillin-sulbactam (AS) or amoxicillin–clavulanate (AC) for the treatment of hospitalized subjects with cSSSI conducted in 19 countries.

Methods: Subjects meeting the definition of cSSSI (i.e. infections involving deeper soft tissue, or requiring significant surgical intervention, or a significant underlying disease state such as deep or extensive cellulitis, infected ulcers, major abscess, burns, or infected bites) were randomized to receive TGC 100 mg loading dose followed by 50 mg q 12 hours or AS 1.5–3 gms q 6 hours or AC 1.2 gms q 6–8 hours for 4 to 14 days. The protocol allowed the addition of vancomycin 1 gm q 12 hours to the AS or AC-treated subjects if infection with methicillin-resistant *Staphylococcus aureus* (MRSA) was suspected or confirmed. Subjects were not allowed to switch to oral therapy. The primary efficacy endpoint was the clinical response in the clinically evaluable (CE) population at the test of cure (TOC) assessment which occurred 8–50 days after the last dose of test article.

Results: For the CE population, clinical cure rates were 77.5% (162/209) for TGC versus 77.6% (152/196) for AS or AC (95% CI −8.7, 8.6; $p \leqslant 0.001$ for non-inferiority). Microbiologic efficacy in the ME population was similar between the two treatment arms with 79.2% (95/120) of TGC treated organisms and 76.8% (76/99) of AS or AC treated organisms considered eradicated at the TOC. Clinical cure rates in the ME population for subjects with MRSA infection were 69.4% (25/36) in TGC treated subjects and 75.0% (18/24) in AS or AC treated subjects who received concomitant vancomycin. The most frequently reported adverse events were nausea (43.7% in TGC; 17.1% in AS or AC) and vomiting (23.9% in TGC; 5.7% in AS or AC).

Conclusion: Tigecycline monotherapy was found to be non-inferior to AS or AC with or without vancomycin in subjects with complicated skin and skin structure infections.

P1548 Efficacy of tigecycline versus ceftriaxone and metronidazole for complicated intra-abdominal infections – analysis of pooled clinical trial data

P. McGovern, H. Leister, E. Zito, H. Tucker, D. Mansfield, T. Babinchak (Collegeville, US)*

Objectives: Tigecycline (TGC) has demonstrated clinical efficacy and safety versus imipenem-cilastatin and ceftriaxone (CTX) plus metronidazole (MET) in previous phase III and phase IV clinical trials. The current analysis is a pooled analysis of 2 phase IV multicenter, open-label, randomized, comparative studies of TGC versus CTX/MET for the treatment of hospitalized subjects with complicated intra-abdominal infection conducted in 23 countries world-wide.

Methods: 946 subjects with complicated intra-abdominal infection were randomized to receive TGC 100 mg loading dose followed by 50 mg q 12 hours or CTX 2 grams once daily, plus MET 1 gram to 2 grams daily, for a minimum of 4 days and not more than 14 days. Subjects were stratified at randomization based on APACHE II scores $\leqslant$10 or $>$10. Subjects were not allowed to receive oral therapy. The primary efficacy endpoint was the clinical response in the clinically evaluable (CE) population at the test of cure (TOC) assessment which occurred 8–44 days after the last dose of test article.

Results: For the CE population, clinical cure rates were 76.2% (295/387) for TGC versus 76.9% (289/376) for CTX/MET (95% CI −6.8, 5.3; $p < 0.001$ for non-inferiority). Clinical response for subjects with APACHE II scores $\geqslant$10 were 69.2% (54/78) for TGC versus 70.7% (53/75) for CTX/MET. Microbiologic efficacy in the ME population was similar between the two treatment arms with 74.7% (192/257) of TGC-treated subjects and 75.1% (184/245) of CTX/MET-treated subjects considered eradicated at the TOC. One CTX/MET subject with a *Bacteroides* thetaiotamicron isolate developed decreased susceptibility to metronidazole while on therapy. Nausea (30.1% TGC, 25.1% CTX/MET), vomiting (20.5% TGC, 15.7% CTX/MET), and diarrhoea (14.7% TGC, 12.7% CTX/MET) were the most frequently reported adverse events. Overall discontinuation rates due to adverse events were similar in each treatment arm, 8.3% TGC and 5.6% CTX/MET.

Conclusion: This pooled analysis demonstrates that tigecycline monotherapy was efficacious and well-tolerated when compared to a combination regimen of CTX/MET in subjects with complicated intra-abdominal infection.

P1549 Efficacy and safety of moxifloxacin vs. ertapenem in complicated intra-abdominal infections: results of the PROMISE study

J. De Waele, J. Tellado, J. Alder, P. Reimnitz, M. Jensen, B. Hampel, P. Arvis (Ghent, BE; Madrid, ES; Montville, US; Wuppertal, Leverkusen, Berlin, DE; Loos, FR)*

Introduction: Source control and initiation of optimal antimicrobial therapy are the cornerstones of the management of complicated intra-abdominal infections (cIAIs). Moxifloxacin (MXF) is an important treatment option for cIAI as it has proven clinical efficacy, and activity against the vast majority of causative organisms. The current study was carried out to compare the efficacy and safety of MXF and ertapenem (ERTA) in the treatment of patients with cIAI.

Methods: PROMISE was a prospective, randomised, double-dummy, double-blind, multinational trial in patients with cIAIs. Patients were treated for 5–14 days with MXF, 400 mg IV qd, or ERTA, 1 g IV qd. The primary efficacy variable was clinical response 21–28 days after the end of therapy. Non-inferiority of MXF was demonstrated if the lower limit of the 95% confidence interval (CI) was above −10%.

Results: Of 804 patients randomised (two-thirds from European countries), 798 were valid for the ITT/safety analyses (MXF 408, ERTA 390). Demographics and baseline characteristics were similar in both treatment arms. In the PP population (MXF 352, ERTA 347), the mean (±SD) APACHE II score was 6.8 (±4.4). The mean (±SD) POSSUM (35.1[±7.6]) and Mannheim Peritonitis Index (19.0[±7.2]) scores demonstrate that patients with severe peritonitis were included. The most common cIAI diagnosis was diffuse, secondary peritonitis (MXF 181, ERTA 185). For the primary efficacy variable, MXF was non-inferior to ERTA (Table). This included good efficacy in the more seriously ill patients (APACHE II >10: MXF 55/66, 83.3%; ERTA 53/62, 85.5%; 95% CI −14.8, 10.5), patients with diffuse, secondary peritonitis (MXF 162/181, 89.5%; ERTA 174/185, 94.1%; 95% CI −9.2, 1.9), and patients with non-appendicitis (MXF 160/180, 88.9%; ERTA 157/171, 91.8%; 95% CI −8.8, 3.5). Good bacteriological efficacy was also seen overall (Table) and in patients with polymicrobial infections (MBV population: MXF 212/250, 84.8%; ERTA 205/231, 88.7%). Similar numbers of patients in both arms experienced drug-related treatment-emergent adverse events (ITT/safety population: MXF 77/408, 18.9%; ERTA 74/390, 19.0%).

Conclusions: MXF, the only fluoroquinolone currently marketed for monotherapy of cIAI, was as effective and well tolerated as ERTA. This included good efficacy in the most severely ill patients.

Table 1. Clinical and bacteriological response in the different patient populations of the PROMISE study

Populations	MXF, n/N (%)	ERTA, n/N (%)	95% CI
Clinical response			
PP	315/352 (89.5)	324/347 (93.4)	−7.9, 0.4
MBV	265/297 (89.2)	254/276 (92.0)	−7.6, 1.9
ITT	334/408 (81.9)	339/390 (86.9)	−9.9, 0.0
ITT with organisms	280/340 (82.4)	263/308 (85.4)	−8.8, 2.2
Bacteriological response			
MBV	257/297 (86.5)	249/276 (90.2)	−9.0, 1.5
ITT with organisms	271/340 (79.7)	258/308 (83.8)	−10.0, 1.5

PP: per protocol, MBV: microbiologically valid, ITT: intent-to-treat.

P1550 Efficacy of IV/PO moxifloxacin and IV piperacillin/tazobactam followed by PO amoxicillin–clavulanate in the treatment of diabetic foot infections: results of the RELIEF study

N. Schaper, M. Dryden, P. Kujath, D. Nathwani, P. Arvis, P. Reimnitz, J. Alder, B. Hampel, I. Gyssens (Maastricht, NL; Winchester, UK; Luebeck, DE; Dundee, UK; Loos, FR; Wuppertal, DE; Montville, US; Berlin, DE; Nijmegen, NL)*

Objectives: Diabetic foot infections (DFIs) cause substantial morbidity and are a leading cause of lower-extremity amputations. As DFIs are usually polymicrobial, broad-spectrum antibiotics – as well as proper wound care – play an important role. Due to their broad spectrum of activity and pharmacodynamic properties, fluoroquinolones, such as moxifloxacin (MXF), have several potential advantages over other antimicrobial classes. The RELIEF study was conducted to provide further data on the efficacy of MXF in specific complicated skin and skin structure diagnoses. Data on DFIs are presented.

Methods: This was a double-dummy, double-blind, randomised, controlled, multinational trial. Patients with a DFI requiring antimicrobials were stratified according to infection severity and the requirement for surgery, and received either IV/PO MXF 400 mg qd or IV piperacillin/tazobactam 4.0/0.5g tds followed by PO amoxicillin/clavulanate 875/125 mg bd (PIP/TAZ-AMC), for 7–21 days. The DFI diagnosis was based on predetermined criteria, documented by repeated photographs and confirmed by an independent data review committee (DRC). The primary efficacy variable was clinical response 14–28 days after completion of therapy (test-of-cure, TOC) as determined by the DRC.

Table 1. Clinical and bacteriological success rates at TOC

	MXF n/N (%)	PIP/TAZ-AMC n/N (%)	P-value‡
Clinical cure			
PP	84/110 (76.4)	75/96 (78.1)	0.65
MBV	69/92 (75.0)	64/85 (75.3)	0.70
ITT	86/123 (69.9)	76/110 (69.1)	0.98
ITT with organisms	71/102 (69.6)	65/96 (67.7)	0.93
Bacteriological success§			
MBV	66/92 (71.7)	61/85 (71.8)	–
ITT with organisms	69/102 (67.6)	62/96 (64.5)	
Bacteriological success by key organism (MBV population)§			
Staphylococcus aureus			–
Methicillin-susceptible	43/53 (81.1)	39/57 (68.4)	
Methicillin-resistant	8/11 (72.7)	10/12 (83.3)	
Streptococcus pyogenes	3/3 (100)	2/2 (100)	–
Enterococcus faecalis	19/30 (63.3)	20/29 (69.0)	–
Escherichia coli			–
ESBL-producing	1/1 (100)	1/1 (100)	
Non ESBL-producing	6/8 (75.0)	8/11 (72.7)	
Bacteroides fragilis	3/3 (100)	3/4 (75.0)	–

‡Cochran–Mantel–Haenszel test. §n/N = number of patients experiencing eradication or presumed eradication/number of patients with pathogen isolated. ITT: intent-to-treat; PP: per-protocol; MBV: microbiologically valid.

Results: A total of 206 patients were valid for the PP analysis (MXF=110, PIP/TAZ-AMC=96). There were fewer men in the MXF vs the PIP/TAZ-AMC arm (55.5% vs 71.9%; P = 0.02) and mean HbA1c levels were higher in the MXF vs the PIP/TAZ-AMC arm (9.7% vs 9.0%; P = 0.04). Most patients had moderate-to-severe DFIs with a PEDIS score of 3 (MXF 87/107, 81.3%; PIP/TAZ-AMC 81/94, 86.2%). In the MBV population, polymicrobial infections were common (MXF: 56/92, 60.9%; PIP/TAZ-AMC: 53/85, 62.3%); the most frequently isolated organism overall was *S. aureus* (MXF 64/92, 69.6%; PIP/TAZ-AMC 69/85, 81.2%). MRSA was isolated from relatively few patients overall (23/206; 11.1%). Initial surgeries were carried out on 150 patients (MXF

78/110, 70.9%; PIP/TAZ 72/96, 75.0%). A total of 23/110 (20.9%) MXF- and 24/96 (25.0%) PIP/TAZ-AMC-treated patients had additional surgeries after the start of therapy. MXF and PIP/TAZ-AMC had similar efficacy with respect to clinical cure at TOC (Table). Bacteriological success rates were also comparable (Table).
Conclusion: In this large randomised trial IV/PO MXF had similar efficacy to IV PIP/TAZ-AMC in the subset of patients with DFI. MXF can be considered a valuable option for the treatment of DFI.

P1551 Efficacy and safety of daptomycin versus vancomycin or teicoplanin for the treatment of complicated skin and soft tissue infections: a multicentre, randomized, assessor-blind trial

H. Gollnick, G. Fierlbeck, R.A. Seaton, H.J. Thurston, R.L. Chaves (Magdeburg, Tubingen, DE; Glasgow, UK; Basel, CH)*

Objectives: Daptomycin (DAP) is effective in treatment of complicated skin and soft tissue infections (cSSTI) caused by non-resistant and multidrug-resistant Gram-positive bacteria. Only limited comparative data is available regarding cSSTI treatment in elderly. Furthermore, no comparative data for DAP vs. teicoplanin in cSSTI has been published to date. The primary objective of this study was to compare DAP vs. vancomycin or teicoplanin (comparator) at test of cure visit (TOC, Day 7–14) for Clinical Success (CS, complete resolution of signs and symptoms or improvement requiring no additional therapy).
Methods: This phase IIIb, comparative, assessor-blinded study included adult patients with cSSTI who were randomised (1:1) and stratified by age (⩾65 yrs) and by presence of systemic inflammatory response syndrome. Intravenous therapy with study drugs was to be given for at least 4 days before oral therapy switch, if needed (total therapy duration of 5–14 days).
Results: Of 189 patients exposed to treatment, 97 were in the DAP group and 92 in the comparator group (vancomycin 46; teicoplanin 46 patients). Treatment exposure was similar in the two groups, and 1/3 of patients were elderly. Descriptive efficacy was comparable in DAP and the comparator group (Table). Discontinuation for adverse events (AEs) or death occurred more frequently in the comparator group (n = 9, 9.8%) than in the DAP group (n = 3, 3.1%). CPK increase was more often reported as an AE in the comparator group vs. DAP (5 vs. 1); 1 patient discontinued the study in comparator group due to this AE. 55 (56.7%) patients in the DAP group and 51 (55.4%) patients in the comparator group reported AEs. The majority of AEs were mild or moderate in intensity. 33 patients experienced serious adverse events (SAE), 17 (17.5%) in the DAP group and 16 (17.4%) in the comparator group. Discontinuations due to SAE were few in both arms (3.1% and 2.2%).
Conclusions: DAP appeared to be at least as effective with a trend to superior efficacy relative to vancomycin or teicoplanin, especially in the elderly and in the microbiologically evaluable populations. The safety of all study drugs was similar and good.

	Daptomycin	Pooled comparator	Vancomycin	Teicoplanin	95% CI
Primary efficacy criterion at TOC: Clinical success in clinically evaluable population					
Number of patients	58	47	22	25	
Clinical success	53 (91.4)	41 (87.2)	19 (86.4)	22 (88.0)	−10.0–13.5
age <65 years	37 (92.5)	28 (93.3)	16 (94.1)	12 (92.3)	−12.4–11.7
age ⩾65 years	16 (88.9)	13 (76.5)	3 (60.0)	10 (83.3)	−22.4–45.1
Clinical success in ITT population					
Number of patients	97	92	46	46	
Clinical success	65 (67.0)	58 (63.0)	31 (67.4)	27 (58.7)	−9.2–17.6
age <65 years	45 (72.6)	41 (67.2)	26 (72.2)	15 (60.0)	−11.4–20.7
age ⩾65 years	20 (57.1)	17 (54.8)	5 (50.0)	12 (57.1)	−21.4–26.2
Microbiological success in microbiologically evaluable population					
Number of patients	57	43	–	–	–
Microbiological success	56 (98.2)	39 (90.7)	–	–	−20.8–32.0
age <65 years	38 (97.4)	26 (96.3)	–	–	−30.2–36.8
age ⩾65 years	18 (100.0)	13 (81.3)	–	–	NA

All values expressed as n (%) unless otherwise specified.

P1552 Daptomycin in the treatment of osteomyelitis caused by MRSA. A comparative observational study

T. Ascione, M.D. Iannece, P. Rosario, M. Conte, S. Quaranta, R. Pempinello (Naples, IT)*

New antibiotics have recently been shown to be effective in cases of serious infections sustained by Meticillin-resistant *Staphylococcus aureus* (MRSA) strains. Objectives: to compare the efficacy of Teicoplanin in respect to Linezolid and Daptomycin in patients with chronic osteomyelitis sustained by MRSA.
Materials and Methods: In an observational study we included all cases of osteomyelitis sustained by MRSA treated with Teicoplanin, Linezolid or Daptomycin during the last 3 years in our division. Epidemiological, laboratory and microbiological findings were considered. Patients were evaluated for clinical efficacy, safety and tolerability. Cure was defined by the clinical and radiologic evidences, coupled with a negative Magnetic Resonance Imaging study or by the absence of captation by scintigraphic examination with leukocytes labelled *in vitro* with 111In, as was assessed 3 months after the end of treatment.
Results: Fifty-three patients with post-traumatic chronic osteomyelits (median age 58 [range 19–70], 66% males) were included. Fracture fixators were present in 10 (19%) cases. Median (range) time from diagnosis of chronic osteomyelitis to our observation was 9 months (range 3–12). Ineffective antimicrobial treatment before our observation was reported in 32 patients. We treated 29 patients with Teicoplanin, 13 with Linezolid and 11 with Daptomycin. Median (range) length of antibiotic treatment was 16 (12–24) weeks for cases treated with Teicoplanin, 12 (8–12) weeks for cases treated with Linezolid, and 11 (6–12) weeks for cases treated with Daptomycin. Cure rates (intention to treat evaluation) were 83%, 77% and 91%, respectively, for cases receiving Teicoplanin, Linezolid and Daptomycin. Antibiotic treatment was discontinued in 3 patients receiving Linezolid because of side effects (2 cases had optic neuritis, 1 had moderate anemia). Three patients who failed after Teicoplanin treatment had MIC to Teicoplanin between 1 and 2 microg/ml.
Conclusion: Teicoplanin is largely employed in the daily practice for patients with osteomyelitis. However, it needs to be administered for a longer period and it is not always effective because of the emergence of glycopeptides tolerant MRSA. Many concerns arise in suggesting the long term administration of Linezolid because of side effects on the nervous and haemopoietic systems. Daptomycin whose administration was safe and effective represents an effective choice for all cases with osteomyelitis sustained by MRSA.

P1553 Predictors of clinical failure in patients with complicated skin and skin structure infections caused by methicillin-resistant *Staphylococcus aureus* by diabetes mellitus status: results from 3 randomized controlled trials of linezolid and vancomycin

B. Lipsky, A. Reisman, D. Ruzzi, D. Huang (Seattle, New York, US)*

Objective: Complicated skin and skin structure infections (cSSSI) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are common and often difficult to treat. The rate of treatment failure appears to be higher for diabetic compared with nondiabetic patients. Knowing which demographic or clinical factors, especially in diabetics, may be associated with clinical failure of treatment of patients with MRSA cSSSI could help improve outcomes.
Methods: Using data from 3 prospective, open-label, randomised phase 3b/4 clinical trials comparing the efficacy of linezolid (LZD) and vancomycin (VAN) for treating cSSSI, we conducted a post hoc analysis of factors associated with outcome. In the analysis cohort, we included only patients who received at least 1 dose of study medication and had culture-confirmed MRSA infection. We defined clinical failure as persistence or progression of clinical signs and symptoms of infection after receiving at least 2 days of treatment. Using a multivariate logistic regression model, we examined baseline demographic and clinical

variables to identify significant independent predictors of clinical failure at end of study (EOS, 6 to 28 d after last dose of study drug) for diabetics and nondiabetics. We selected predictors by χ^2-df, stepwise selection by Akaike Information Criterion, and validated the model with the Hosmer-Lemeshow goodness of fit test. Because of the exploratory nature of the analysis, we included variables significant at $\leqslant 0.10$ in the model.

Results: Among 1038 patients (LZD 539, VAN 544) eligible for inclusion in these analyses, 845 (81%) had observations present for all variables included in the model, including 287 (34%) diabetics and 558 (66%) nondiabetics. By stepwise multivariate regression analysis, we found 4 variables significantly associated with clinical failure in the diabetic population, and 3 associated with clinical failure in the nondiabetic population (see below).

Conclusions: Factors significantly independently associated with a higher likelihood of clinical failure of treatment of cSSSI (other than randomisation to 1 particular study) among diabetics were the presence of a polymicrobial infection, or peripheral vascular disease, or decreased weight; and among nondiabetics, they were treatment with VAN, or the presence of a polymicrobial infection. The presence of these factors, especially in diabetics, should alert physicians to patients possibly at higher risk of poor outcomes.

Table 1. Predictors of clinical failure at EOS

Population	Predictor of clinical failure at EOS	Odds Ratio[a]	95% CI
Diabetic	Presence of a polymicrobial infection[b]	3.10	1.52, 6.33
	Randomised in the Itani study[c]	3.03	1.74, 6.25
	Presence of PVD	1.81	1.03, 3.17
	Decreased weight	1.01	1.00, 1.02
Nondiabetic	Treatment with VAN	2.22	1.34, 3.66
	Presence of a polymicrobial infection[b]	2.04	1.16, 3.62
	Randomised in the Itani study[c]	1.68	0.96, 2.95

[a]Odds ratios greater than 1.0 indicate excess failure.
[b]Polymicrobial infection was defined as culture-confirmed MRSA + any other pathogen. [c]The Itani study excluded patients with a primary baseline diagnosis of cellulitis. CI, confidence interval; PVD, peripheral vascular disease.

P1554 Safety profile of oral torezolid phosphate 200, 300, or 400 mg once daily in an acute complicated skin and skin structure infection, phase 2 study

C. De Anda, P. Bien, P. Prokocimer (San Diego, US)*

Objective: Torezolid is the active moiety of the prodrug torezolid phosphate (TP), an oxazolidinone with 4- to 16-fold greater activity than linezolid against Gram-positive species including methicillin-resistant *Staphylococcus aureus* (MRSA). Our objective is to report the TP safety profile in patients with complicated skin and skin structure infections (cSSSI) enrolled in the Phase 2 dose-ranging study.

Methods: Patients diagnosed with cSSSI were randomized to receive 200, 300, or 400 mg oral TP once-daily (QD) for 5 to 7 days. Treatment emergent adverse events (TEAE) were assessed from first dose through the late follow-up (LFU) visit. Safety laboratory tests including a chemistry panel and complete blood count (CBC) with differential were to be obtained from patients at screening/day 1, day 3, day 5, end of therapy (EOT), and test of cure (TOC) visits.

Results: There were 188 patients that received at least one dose of study drug, of which 69% experienced at least one TEAE. TEAEs were overwhelmingly mild (72%) to moderate (25%). The most common study drug-related TEAEs (>5%) reported were nausea, diarrhoea, vomiting, and headache. No patients discontinued study drug due to an adverse event. Five patients reported a serious adverse event (SAE) during the study, only one of which was reported as related. Five patients had a substantially abnormal value (defined using standard regulatory criteria) in ALT, 3 in AST, 1 in alkaline phosphatase, and 1 in serum creatinine. Of the 8 patients with substantially abnormal ALT or AST, none had elevated levels of both, 3 were Hepatitis C positive (3 were negative and 2 were not tested), and 5 have an admitted history of IV drug or alcohol abuse. No patients had substantially abnormal values in platelet count, absolute neutrophil count, or hemoglobin levels.

Conclusion: TP proved to be well tolerated in all dosage levels with no significant difference in TEAE occurrences between the doses over a 5–7 day period of administration. These data support the usage of 200 mg QD for further development into the treatment of severe cSSSI.

P1555 Linezolid use in the treatment of vancomycin-resistant *Enterococcus* in paediatric patients

J. Deville, J. Goldfarb, S. Kaplan, O. Equils, D. Huang, J. Ang, J. Salazar (Los Angeles, Cleveland, Houston, New York, Detroit, Hartford, US)*

Objective: Vancomycin-resistant *Enterococcus* (VRE) is a difficult to treat pathogen in hospitalised paediatric patients. Our objective was to assess the efficacy and safety of linezolid (LZD) for treatment of VRE infections in paediatric patients.

Methods: Hospitalised children with VRE infection were enrolled in a multicentre, open-label, noncomparator study from 2002–2004, as an extension to the parent study of LZD vs vancomycin for treatment of resistant Gram-positive infections in children. Patients less than 11 y received LZD 10 mg/kg (up to 600 mg) every 8 h; all others received LZD 600 mg every 12 h. Intravenous (IV) treatment was required for the first 3 d, and then oral LZD could be used, at the discretion of the investigator. The primary efficacy measures were the patients' clinical outcomes at test of cure (TOC; 12–28 d after treatment completion). Safety measures included reported adverse events (AE).

Results: Thirteen seriously ill children aged 4 m to 17 y (mean±SD: 7 y±6.4 y) were enrolled. Primary baseline infections were vascular associated bacteraemia (n = 4), bacteraemia of unknown source (n = 3), urinary tract infection (n = 3), skin and skin structure infection (SSI, n = 1), pyelonephritis (n = 1) and intra-abdominal abscess (n = 1). In 69% (9/13) of the patients, vancomycin-resistant (VR) *E. faecium* was isolated, 1 had VR *E. faecalis*, 1 had vancomycin-intermediate (VI) *E. faecium* and VI *E. gallinarum,* 2 had vancomycin-susceptible *E. faecium*, 1 also had *E. gallinarum;* however, no susceptibility testing was performed on the latter because of insufficient culture sample. Mean treatment duration of LZD was 17.1±7.2 d. At the TOC, the clinical cure rate was 8/12 (66.7%) for the intent-to-treat population (1 was withdrawn from the study per protocol exclusionary criteria) and 5/7 (71.4%) for the microbiologically evaluable group (culture-confirmed VRE infections). Three patients had drug-related AE (diarrhoea, anaemia, decreased cyclosporine level). One patient discontinued LZD due to AE (abdominal distention and pain, diarrhoea, and anaemia). Two patients died during the study but neither death was related to LZD (end-stage liver disease and end-stage renal disease waiting for a heart transplant). Changes in haematology and chemistry parameters were consistent with the underlying disease states and were largely transient.

Conclusion: LZD was effective and well tolerated in treating severely ill children with VRE infections.

P1556 Oral torezolid phosphate in the treatment of severe complicated skin and skin structure infections in a phase 2 dose-ranging study

C. De Anda, P. Bien, P. Prokocimer (San Diego, US)*

Objective: Torezolid (T) is the active moiety of the prodrug torezolid phosphate (TP), a second generation oxazolidinone with 4- to 16-fold greater activity than linezolid against Gram-positive species. The objective of this analysis was to review the clinical and microbiological outcome rates of the more severe cases of complicated skin and skin structure infections (cSSSI) including large abscesses, cellulitis and wound infections with oral TP once daily (QD), 200 mg, 300 mg, or 400 mg at the end of therapy (EOT) and test of cure (TOC) visits in the modified intent-to-treat (MITT) and the clinically evaluable (CE) patient populations. In line with latest regulatory recommendations, severe cSSSI was defined as having systemic signs or symptoms of infection (>38ºC oral temperature, >10,000/mm^3 white blood cells, or

>10% bands) or lymphatic involvement adjacent to the primary lesion, and a lesion measurement of at least 10 cm or greater at baseline.
Methods: Patients were randomized to receive 200, 300, or 400 mg oral TP QD for 5 to 7 days. Patients were evaluated for clinical response by the investigator at EOT and TOC. Microbiological samples were to be obtained from the cSSSI site at baseline and then any follow-up visits if medically indicated.
Results: A total of 80 of the 188 patients that received study drug met the criteria for severe cSSSI as defined above. Of these 80 patients, 85% had systemic signs of infection and 15% had lymphatic involvement in the absence of systemic signs of infection. Lesion sizes ranged from 10 cm to 46 cm. The clinical cure rates at EOT and TOC were as follows: A total of 59 patients of this sub-group had *S. aureus* as the baseline pathogen (15% MSSA, 85% MRSA). The microbiologic outcome was 100% eradication for all pathogens.
Conclusion: TP demonstrated high clinical cure rates and high microbiological eradication in all dosage levels tested in patients with severe cSSSI. These data support the selection of 200 mg QD for further development towards the treatment of severe cSSSI.

	200 mg	300 mg	400 mg
MITT population			
EOT	24/26 (92%)	25/25 (100%)	27/29 (93%)
TOC	23/26 (88%)	25/25 (100%)	26/29 (90%)
CE population			
EOT	23/24 (96%)	24/24 (100%)	27/28 (96%)
TOC	22/23 (96%)	23/23 (100%)	25/26 (96%)

P1557 Vancomycin for the treatment of patients with Gram-positive infections: a meta-analysis of randomized controlled trials

K. Vardakas, M. Mavros, N. Roussos, M. Falagas (Athens, GR)*

Objective: To clarify whether the use of vancomycin could be associated with improved outcomes in comparison with other approved or in phase III trials investigational antibiotics for the treatment of infections caused by Gram-positive cocci.
Methods: Data from randomized controlled trials were pooled using the methodology of meta-analysis.
Results: Fifty trials comparing vancomycin with linezolid, daptomycin, quinupristin-dalfopristin, tigecycline, ceftaroline, ceftobiprole, telavancin, teicoplanin and dalbavancin were included in the meta-analysis. In the ITT population vancomycin was equally effective to comparator antibiotics [1.08 (0.98–1.19)]. In the clinically evaluable populations, comparators were more effective than vancomycin when all infections [1.16 (1.4–1.36)] and skin and soft tissue infections [SSTIs, 1.18 (1.00–1.38)] were studied. No differences were noted when patients with febrile neutropenia [1.07 (0.82–1.39)], pneumonia [1.08 (0.82–1.43)], and bacteremia [1.10 (0.79–1.53)] were studied. Comparators were more effective in open label trials [1.28 (1.08–1.50)], but not in double-blind trials [1.06 (0.90–1.25)]. Total adverse effects attributed to studied antibiotics were more common in comparators [1.12 (1.01–1.24)], but fewer patients were withdrawn from trials in the comparator arm [0.78 (0.63–0.98)]. Mortality was not different between vancomycin and comparator antibiotics when all trials were included in the analysis [OR 1.08 (95% CI 0.93–1.25)]. In double blind trials, vancomycin was not associated with lower mortality [0.83 (0.65–1.05)], but in open label trials comparators were associated with higher mortality [1.28 (1.05–1.55)].
Conclusion: Based mainly on data from open label trials, vancomycin is an effective treatment choice for patients with Gram positive infections other than SSTIs.

P1558 Treatment with ciprofloxacin for 7 or 14 days in women with acute pyelonephritis. A randomized, double-blind, placebo-controlled multicentre trial with parallel groups

T. Sandberg, G. Kahlmeter, N. Kuylenstierna, A. Lannergård, G. Otto, B. Settergren, G. Skoog, G. Stridh (Göteborg, Växjö, Falun, Uppsala, Lund, Kristianstad, Solna, SE)*

Objectives: To compare the bacteriological and clinical efficacy and safety of 7 and 14 days treatment with ciprofloxacin in women with community-acquired acute pyelonephritis.
Methods: 251 non-pregnant women, 18 years of age or older, with a presumptive diagnosis of acute pyelonephritis were randomly assigned to treatment with ciprofloxacin 500 mg twice daily for 7 or 14 days. The study was double blind and placebo-controlled during the second week of treatment. To be included in the per protocol analysis, patients must have demonstrated a positive urine culture with a strain susceptible to ciprofloxacin. The main endpoint was the bacteriological and clinical outcome 10 to 14 days after completion of treatment with active drug. Long-term outcome was assessed 5 to 8 weeks post-treatment. Recruitment of study patients took place at 21 departments of infectious diseases in Sweden.
Results: A total of 156 patients with a median age of 43 years (range 18–86) could be evaluated according to the protocol. *Escherichia coli* was the predominant pathogen (92%). 42 (27%) patients had positive blood cultures. Short-term cure rate for patients treated with ciprofloxacin for 7 days (n = 73) and 14 days (n = 83) was 97.3% and 96.4% (p = 0.004; one-sided non-inferiority test), respectively. Cumulative efficacy at long-term follow-up was 93.2% and 92.9% (p = 0.015; one-sided non-inferiority test), respectively. Both regimens were well tolerated.
Conclusion: Treatment of acute pyelonephritis in women with ciprofloxacin for 7 days was not inferior to a 14 day-course.

Animal models – antimicrobials

P1559 *In vivo* assessment of activity of calcium-deficient apatite linezolid drug delivery system versus systemic administration of linezolid in a rabbit osteomyelitis experimental model due to MRSA

A. Gaudin, G. Amador, H. Gautier, V. Le Mabecque, A.F. Miegeville, G. Potel, J.M. Bouler, P. Weiss, J. Caillon, C. Jacqueline (Nantes, FR)*

Background: Linezolid is considered as an alternative to vancomycin for severe osseous infections. Calcium-deficient apatites (CDA) can be associated with therapeutic agents such as linezolid to form drug-delivery systems. The aim of this work was to evaluate the *in vivo* activity of linezolid adsorbed onto CDA microparticles in addition to standard treatments.
Methods: Femoral trepanation of rabbits was performed, followed by injection of 109 CFU MRSA (linezolid MIC = 2 μg/mL) suspension into the knee cavity. A surgical debridement of the infected tissues was performed 3 days later and animals were randomly assigned to: V(iv) (vancomycin constant IV infusion to reach a 20×MIC serum steady-state concentration), L(iv) (10 mg/kg/12 h IV infusion), CDA (osseous gap filled with 100 mg CDA), L(CDA) (100 mg CDA with linezolid 10 μg/mg) and L(CDA)+ L(iv) (100 mg CDA with linezolid 10 μg/mg filling in addition to 10 mg/kg/12 h IV infusion). Surviving bacteria were counted in joint fluid (JF), bone marrow (BM) and bone (BO) at days 3 and 7 (4-day treatment).
Results: See graphic.
Conclusions: (1) V(iv) was ineffective against MRSA after a 4-day treatment. (2) CDA alone showed no *in vivo* antibacterial activity. (3) CDA as linezolid drug delivery (L(CDA)) system demonstrated significant *in vivo* activity in BM and BO, in this model, as compared to vancomycin. (4) L(CDA) + L(iv) treatment did not exhibit a greater efficacy in the three compartments than L(CDA) alone.

Treatment	n	Mean±SD delta $\log_{10}$ CFU/g of tissue (day 7 – day 3)		
		JF	BM	BO
$V_{(iv)}$	5	–0.03±0.93	–0.73±1.84	–0.61±0.76
$L_{(iv)}$	5	–0.77±1.32	–2.69±2.00*	–2.25±1.63*
CDA	5	–0.49±0.32	0.56±0.31	0.22±0.25
$L_{(CDA)}$	5	–0.80±1.11	–2.56±1.48*	–1.18±0.86**
$L_{(CDA)}$ + $L_{(iv)}$	5	–1.21±0.30	–3.51±0.43*	–1.85±0.95**

n: number of animals; *$P < 0.05$ vs $V_{(iv)}$ and CDA; **$P < 0.05$ vs CDA. Student–Newman–Keuls test after ANOVA.

P1560 Comparative efficacy of moxifloxacin and the influence of therapy withdrawal in a murine uterus infection caused by *Bacteroides fragilis*

M. Glenschek-Sieberth, K. Merfort, S. Obertegger, R. Endermann (Wuppertal, DE)*

Objectives: Female reproductive organs are often affected by severe complications following complicated intra-abdominal infections (cIAIs). Such complications, including uterine tube infections, can result in infertility. The efficacy of moxifloxacin (MXF), piperacillin/tazobactam (PIP/TAZ), ertapenem (ERTA) or metronidazole (MET) plus ceftriaxone (CRO) or ciprofloxacin (CIP) was assessed in a murine model of chronic uterus infection caused by *Bacteroides fragilis*.
Methods: C57BL/6JOla mice were treated with β-estradiol. After 4 days, the left uterine tube was infected with a suspension of *B. fragilis* O6688 (3×10^8 colony forming units [CFU] per mouse). To evaluate therapeutic efficacy, mice were treated b.d. IV for 4 days starting at day 21 post-infection (p.i.) with MXF 100 mg/kg, PIP/TAZ 400 mg/kg, ERTA 25 mg/kg, or MET 100 mg/kg plus CRO 30 mg/kg or CIP 90 mg/kg. These doses simulate the pharmacokinetic/pharmacodynamic drivers of the human IV doses. CFUs in the uterine tubes were determined after hysterectomy. Therapeutic efficacy was assessed as the reduction in CFUs in the uterine tube homogenates on day 25 p.i. To evaluate the influence of therapy withdrawal on efficacy, CFUs in uterine tube homogenates were also assessed on day 30 p.i.
Results: CFU reductions in the uterine tubes on days 25 and 30 p.i. are shown in the table. In the murine chronic uterus infection model:

- MXF monotherapy had significantly higher efficacy than PIP/TAZ or ERTA and comparable activity to combination therapy with MET/CRO or MET/CIP in terms of CFU reduction one day after end of therapy.
- Six days after the cessation of treatment, the mean *B. fragilis* count reached the level of the untreated control animals for all treatment regimens except MXF and MET/CIP.
- MXF also displayed the highest CFU reductions on day 1 and day 6 after the withdrawal of therapy.
- MXF was the only regimen that reduced *B. fragilis* CFUs to below the detection limit in some animals at six days after the end of treatment.

Conclusions: A four-day treatment duration was probably too short to effectively eliminate *B. fragilis* from site of infection. Nonetheless, MXF was the only treatment option that eradicated *B. fragilis* to below the detection limit in some animals at six days after the end of treatment. MXF monotherapy seems to be a valuable treatment option for infections caused by *B. fragilis*.

Comparison of CFU reduction in uterine tubes on days 25 and 30 post-infection

Day 25 post-infection (1 day after therapy withdrawal)				Day 30 post-infection (6 days after therapy withdrawal)			
Treatment	Median $\Delta\log_{10}$ CFU reduction compared to untreated control (n = 30)	*P*-value† (active treatment vs untreated control)	*P*-value† (MXF vs comparator)	Treatment	Median $\Delta\log_{10}$ CFU reduction compared to untreated control (n = 23)	*P*-value† (active treatment vs untreated control)	*P*-value† (MXF vs comparator)
MXF (n = 33)	–4.78	<0.0001	–	MXF (n = 22)	–2.40	0.0031	–
PIP/TAZ (n = 20)	–1.25	0.0004	<0.0001	PIP/TAZ (n = 10)	–0.36	0.3573	0.0770
ERTA (n = 15)	–1.62	0.237	0.0003	ERTA (n = 10)	+0.40	0.6808	0.0047
MET/CRO (n = 10)	–4.19	0.0001	0.2684	MET/CRO (n = 10)	+0.23	0.5969	0.0078
MET/CIP (n = 10)	–4.69	0.0001	0.4292	MET/CIP (n = 10)	–2.21	0.0031	0.8869

† Mann–Whitney test.

P1561 Efficacy of garenoxacin, a des-F (6) quinolone, in an experimental skin infection model caused by methicillin-resistant *Staphylococcus aureus* in diabetic mice

Y. Fukuda, M. Takahata, K. Hashimoto-Hasegawa, Y. Ito, Y. Sugiura, K. Kizawa, I. Nozawa, J. Mitsuyama (Toyama, JP)*

Objectives: Garenoxacin (GRN), a des-F (6) quinolone, exhibits potent antibacterial activity against *S. aureus*, including MRSA, which causes severe skin infection, especially in diabetic patients. The efficacy of GRN in experimental skin infection models with MRSA in diabetic mice was evaluated and compared to levofloxacin (LVX) and moxifloxacin (MXF).
Methods: Male C57BLKS/J Iar-+Leprdb/+Leprdb mice were used as skin infection models caused by MRSA in diabetic mice. Under anesthesia, mice were infected by placing a 5 uL droplet containing ca. 10^5 CFU of MRSA F-3410 (MIC: GRN 0.0313 mg/L, LVX 0.25 mg/L, MXF 0.0625 mg/L) on the thigh skin, from which hair had been shaved. At 2 h after infection, 5 mg/kg of each quinolone was administered orally once. The viable cells counts in skin were measured at 24 h after infection. At the same time, histopathological observation was performed. Drug concentrations in the serum and thigh skin of mice infected with MRSA F-3410 were also measured by HPLC with a single oral dose of 20 mg/kg, and pharmacokinetic evaluations were performed by non-compartment analysis.
Results: The viable cell count in the skin (Log10 CFU/skin) of the GRN-treated group (3.41±0.51) was significantly less than LVX- (6.18±0.39*), MXF- (4.44±0.31*) and non-treated (6.34±0.40*) groups, (N = 10, Mean±S.D,, *p < 0.001 vs. GRN). In the non-treated group, 24 h after infection, infiltration of inflammatory cells surrounding bacterial colonies reached subcutaneous adipose tissue. After GRN treatment, although cell debris derived from inflammatory cells persisted on the skin surface, the inflammatory reaction was very slight compared to non-treated mice. The AUC0–24 of GRN in serum and thigh skin at 20 mg/kg was 12.7 and 7.51 ug-h/mL or g (N = 3, Mean), and the AUC0–24/MIC ratios were 405 and 240, respectively, which were greater than LVX and MXF. The greater AUC/MIC ratios of GRN in both serum and skin reflected its favorable therapeutic effect.
Conclusions: GRN is considered a valuable quinolone in the treatment of skin infections caused by *S. aureus*, including MRSA in diabetes.

P1562 Evaluation of oritavancin efficacy and pharmacokinetic profile in the hamster model of *Clostridium difficile* infection

I. Fadhil, H. Deng, O. Rozborskaya, J. Gagné, O. Bélanger, V. Ostiguy, I. Bédard, C. Cadieux, M. Malouin, S. Fournier, A. Rafai Far, T. Parr Jr., D. Lehoux (St. Laurent, CA)*

Objectives: Oritavancin (ORI) is a novel lipoglycopeptide which demonstrates activity against *Clostridium difficile* (CD) and prevents spore outgrowth. It is under preclinical evaluation for the treatment of infections caused by CD. We report here, the pharmacokinetic (PK) profile of ORI and its efficacy in a hamster model of *Clostridium difficile* infection (CDI).
Methods: CDI was established in Golden Syrian hamsters with a subcutaneous dose of clindamycin at 100 mg/kg one day before infection followed by oral gavage with 10^5 colony-forming units (CFU) of spores of CD ATCC 43255 on Day 0. On Day 1 post-infection (PI), either ORI at 10, 50 or 100 mg/kg/day (in hydroxypropyl β-cyclodextrin), vancomycin (VA) (in phosphate buffered saline) at 50 mg/kg/day (n = 10 hamsters/group) or ORI in PEG400 at 100 mg/kg/day (n = 5/group) were administered orally (PO) once daily for 5 days. Animals were observed daily for clinical signs during 20 days. The hamsters were also dosed intravenously (IV) at 10, 25 and 50 mg/kg of ORI or PO at 10, 50 and 100 mg/kg. Blood samples and cecal contents were harvested at 8 time points ranging from 15 min to 120 h (n = 3/time point), and ORI levels were determined by liquid chromatography/mass spectrometry.
Results: ORI at 10, 50 and 100 mg/kg prolonged survival by 9, 13 and 17 days over untreated controls, respectively. On Day 12 PI, ORI at 100 mg/kg exhibited superior efficacy to VA with 100% survival for

animals treated with ORI compared to 0% survival with VA. In PEG400, ORI yielded 100% survival at Day 20. After PO administration, the maximal concentration (Cmax) in plasma was ⩽0.12 mg/L at 2 h. The Cmax in cecal matter was between 196 mcg/g and 1922 mcg/g after PO administration, and below 1 mcg/g for all doses by 72 h. After IV administration, the concentration in plasma was between 59 and 279 mg/L at 15 min and decreased to 0.13–0.52 mg/L at 48 h.
Conclusion: Oritavancin is maintained at high levels in the hamster cecum, where the CD infection is. As such, ORI displayed efficacy in the hamster model of CDI, preventing CDI relapse longer than VA, and preventing relapse when it was formulated in PEG400.

P1563 Comparison of the efficacies of silver-containing dressing materials for treating a full-thickness rodent wound infected by methicillin-resistant *Staphylococcus aureus*

J. Chae, J.H. Lee, D.G. Kim, S.H. Hong, W.M. Lee, M. Ki (Seoul, Daejeon, KR)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) may cause infections during wound dressing. We aimed to compare the antibacterial activities and wound-healing effects of commercially available silver-coated or silver-impregnated wound dressings on MRSA-infected wounds.
Methods: Full-thickness skin defects were made on the back of rats (N = 108) and were infected with MRSA. The rats were divided into the following 6 groups according to the dressing used for the wounds: nanocrystalline silver (Acticoat®, silver carboxymethylcellulose (Aquacel® Ag), silver sulfadiazine (Medifoam silver®, silver sulfadiazine (Polymen silver®, silver sulfadiazine (Ilvadon®, and povidone iodide (Betadine). We analyzed the wound sizes, histological findings, and bacterial colony counts for the groups. We also inoculated the silver materials on Mueller-Hinton agar plates containing MRSA and compared the inhibition zones in the agar plates.
Results: The order of the rate of wound-size decrease was Acticoat® > Aquacel® Ag > Polymen silver® > Medifoam silver® > Ilvadon® > Betadine. The histological findings revealed that the Acticoat showed more reepithelialization and granulation tissue formation and less inflammatory cell infiltration than the other materials. The order of the time required for wound healing was Acticoat® > Aquacel® Ag > Polymen silver® > Ilvadon® > Medifoam silver® > Betadine. The bacterial colony counts reduced in all the groups, except in the Medifoam silver® group. The order of the size of the inhibition zone was Acticoat® > Aquacel® Ag > Ilvadon® > Polymen silver® > Betadine > Medifoam silver®.
Conclusion: Silver-coated or silver-impregnated wound dressings can be used for treating MRSA-infected wounds. Considering its superior efficacy in comparison to the efficacies of other silver-coated or silver-impregnated wound dressings, Acticoat should be preferentially used for the treatment of MRSA-infected skin wounds.

P1564 Efficacy of ceftazidime/NXL104 combination in murine septicaemia caused by extended-spectrum β-lactamases and AmpC-producing Enterobacteriaceae sp.

P. Levasseur, A.M. Girard, L. Lavallade, C. Miossec, D. Shlaes, M. Black, J. Pace, K. Coleman (Romainville, FR; Connecticut, US; Philadelphia, US)*

Objective: NXL104 is a novel β-lactamase inhibitor that has been shown *in vitro* to inhibit both class A and class C enzymes. It is in phase 2 of clinical development in combination with ceftazidime (CAZ). In this study, the efficacy of CAZ/NXL104 was evaluated against CAZ-resistant (CAZ-R) Enterobacteriaceae bearing extended spectrum and AmpC β-lactamases in a murine septicaemia model.
Methods: MICs were determined with NXL104 at 4 microgram/mL fixed concentration and variable concentrations of CAZ. Septicaemia: Groups of mice (n = 10 to 20) were intraperitoneally infected with one of 12 CAZ-R strains (MICs 32–>128 microgram/mL) expressing multiple β-lactamases including CTX-M, SHV, TEM, and AmpC: five strains of *Klebsiella pneumoniae*; three strains of *Escherichia coli*; two strains each of *Enterobacter cloacae* and *Citrobacter freundii*. Sub-cutaneous treatments were given one- and four-hours post-infection. CAZ/NXL104 was administered at 4/1 weight ratio; reference treatments were CAZ alone, cefotaxime (CTX) and piperacillin/tazobactam (PTZ). The 50% effective dose (ED50) was calculated by the Probit method.
Results: MICs of CAZ/NXL104 for the 12 CAZ-R strains were ⩽0.125–1 microgram/mL. *In vivo*, CAZ/NXL104 was significantly more effective compared to CAZ, CTX, or PTZ against all strains ($p < 0.05$). ED50 ranges of CAZ/NXL104 were 2–27 mg/kg for these strains compared to >60 mg/kg, >90 mg/kg, and >90 mg/kg respectively for CAZ, CTX, and PTZ.
Conclusion: The potent *in vitro* activity of NXL104 when combined with CAZ, against CAZ-R Enterobacteriaceae bearing class A ESBLs and class C β-lactamases, translates into good parenteral efficacy in the mouse septicaemia model.

P1565 *In vivo* synergism between cefditoren and specific antibodies against a penicillin-resistant serotype 6B *Streptococcus pneumoniae* in a mice sepsis model

F. Cafini, M.J. Gimenez, J. Yuste, D. Sevillano, L. Aguilar, L. Alou, M. Torrico, N. Gonzalez, J. Prieto, P. Coronel (Madrid, ES)*

Objective: To explore the *in vivo* effect of the presence of specific antibodies on the efficacy of cefditoren (CDN) treatment of sepsis caused by a serotype 6B *S. pneumoniae* isolate non-susceptible to β-lactams (penicillin and cefotaxime MIC of 2 mg/l, amoxicillin MIC of 4 mg/l).
Methods: CDN MIC for the infecting strain was 1 mg/l. Eight to 12 week old female BALB/c mice weighing 19–22 g were used. Hyperimmune serum (HS) was obtained from mice weekly inoculated with whole cell heat-inactivated inoculum for 5 weeks. All experiments were performed in groups of 5 animals inoculated with 2×10^7 cfu by intraperitoneal (i.p.) route. Animals were observed and deaths recorded for 7 days. The highest double dilution (1/2, 1/4, 1/6) of HS producing 0% survival (when single administered 1 h before inoculation of the infecting strain) and the highest CDN dose (6.25, 12.5, 25 and 50 mg/kg; three times daily for 48 h initiating subcutaneous treatment 1 h after inoculation) showing 0% survival of infected animals were determined in separate dose-ranging studies versus placebo. The HS dilution and CDN dose showing 0% survival of infected animals in separate experiments were combined to explore synergism: animals received an i.p. single administration of the HS dilution 1 h prior to inoculation, and CDN treatment (three times daily for 48 h) was initiated 1 h after inoculation.
In addition, for pharmacodynamic purposes, serum concentrations of CDN were measured in non-inoculated mice at 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 8 h after a single dose administration of the minimal dose obtaining 100% survival in experiments with antibiotic administration alone. Two animals per timepoint were used. The percentage of the dosing interval that concentrations exceeded the MIC (T>MIC) was determined.
Results: The table shows %survival over time.

Experimental arm	% Survival		
	24 h	48 h	Day 7
Control (placebo)	40	0	0
HS 1/2 dilution	80	40	40
HS 1/4 dilution	60	20	0
CDN 50	100	100	100
CDN 25	40	40	40
CDN 12.5	60	0	0
CDN 12.5 + HS 1/4	100	100	100

CDN 50 mg/kg provided a T>MIC of 33.4% of the dosing interval (8 h).
Conclusions: CDN showed 100% efficacy at doses of 50 mg/kg, with T>MIC of 33.4%. In the presence of antibodies, sub-therapeutic doses

of CDN produced 100% survival in mice infected by a β-lactam non-susceptible serotype 6B strain, due to the synergistic action of CDN 12.5 mg/kg (0% survival alone) and HS 1/4 dilution (0% survival alone).

P1566 Synergistic antimicrobial efficacy of furanone C30 and tobramycin against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model

L. Christensen, M. van Gennip, M. Alhede, N. Kumar, S. Kjelleberg, N. Høiby, T. Bjarnsholt, M. Givskov (Copenhagen, DK; Sydney, AU)*

Objective: *Pseudomonas aeruginosa* biofilms are frequently linked to infections on foreign-body implants, which can cause serious complications. *P. aeruginosa* uses quorum sensing (QS) to regulate its virulence, and the biofilm mode of growth contributes to *P. aeruginosa*'s tolerance towards the immune system and numerous antibiotics. Previously, we have shown that *in vitro* wild-type (WT) *P. aeruginosa* biofilms treated with either the QS inhibitor (QSI) furanone C30 or tobramycin alone had little effect on the eradication, but treatment with both C30 and tobramycin showed a synergistic effect and killed the biofilm. This synergistic effect was also tested in an *in vivo* biofilm model.
Methods: The pharmacokinetics of 30 mg/kg body weight (BW) tobramycin was estimated in non-infected BALB/c mice and the calculated half-life ($t^1/_2$) was 0.37 hours. We used a modified version of the *in vivo* foreign-body infection model introduced in 2007 and inserted silicone tube implants (id 4 mm, od 6 mm) instead of square implants in the mice. The implants were colonized with WT *P. aeruginosa* and inserted in the peritoneal cavity of BALB/c mice. Treatments consisted of i.p. injection of: (a) C30 (1 mg/kg BW, dissolved in 2.6% ethanol) every 8-hour, (b) 2.6% ethanol every 8 hour, (c) 30 mg/kg BW tobramycin, and (d) 0.9% NaCl. Treatment with C30 and tobramycin was initiated 1-hour and 24-hours post-insertion, respectively. After insertion the mice were divided into four groups: Gr. 1 (combination treatment) injection (a and c) (n = 11); Gr. 2 (C30 group) injection (a and d) (n = 8); Gr. 3 (tobramycin group) injection (b and c) (n = 10); Gr. 4 (placebo) injection (b and d) (n = 9). Implants were removed 48-hours post-insertion and the CFUs per implant were determined.
Results: Combination treatment of WT *P. aeruginosa* (Gr. 1) resulted in a significant clearing of the implants as compared to both the placebo and the single treatments groups (Gr. 2, Gr. 3) ($p = 0.0002$, $p = 0.0003$ and $p = 0.001$, respectively). A significant difference in clearing was also observed between the placebo group and the single treatment groups ($p = 0.006$ and $p = 0.0003$). We also found a significant difference in clearing between the two single treatment groups ($p = 0.01$).
Conclusion: The present results showed that a synergistic antimicrobial efficacy can be achieved when treating with a combination of furanone C30 and tobramycin, resulting in an increased clearance of *P. aeruginosa* during a foreign-body infection in mice.

P1567 Effect of qnrA, qnrB and qnrS on the *in vivo* activity of fluoroquinolones

J. Domínguez-Herrera, C. Velasco, F. Docobo-Pérez, J.M. Rodríguez-Martínez, R. López-Rojas, A. Briales, C. Pichardo, P. Díaz-de-Alba, J. Pachón, A. Pascual (Seville, ES)*

Objectives: Since the introduction of the fluoroquinolones, resistance of the Enterobacteriaceae to these agents has become common. The main mechanism of quinolone resistance is the accumulation of mutations in the Type II Topoisomerases. However, plasmid-mediated quinolone resistance, mainly qnr genes, contributes to reduced susceptibility to those agents allowing the emerging of high level resistant mutants *in vitro*. The aim of the study was to investigate the *in vivo* implication of qnrA, qnrB or qnrS, using transformed strains of *Escherichia coli*, in order to clarify the clinical implication of these genes in the treatment of infections with fluoroquinolones.
Methods: The bacteria strain *E. coli* ATCC 25922 was transformed with the pBK-CMV plasmid alone or the same plasmid with the qnrA, qnrB or qnrS genes. Pharmacokinetic studies were performed after a single intraperitoneal dose of ciprofloxacin (CPX) (20 mg/Kg) or levofloxacin (LVX) (25 mg/Kg), in female immunocompetent mice C57BL/6. Using an agar diffusion bioassay the pharmacokinetic parameters, area under curve (AUC) and serum peak concentration (Cmax) were determined. To evaluate the efficacy of different treatments, we used an experimental pneumonia model in female immunocompetent mice C57BL/6 during 72 hours. The animals were included in three different therapeutic groups (n = 15): control (without treatment), CPX (40 mg/Kg·day) or LVX (50 mg/Kg·day), for the four strains. After this, viable bacteria in lungs (log10 CFU/g of lung), mortality (%) and qualitative blood culture (%) were measured. Results were statistically analyzed with the ANOVA (with post-hoc test of Tukey and Dunnett) or χ^2 tests.
Results: Pharmacokinetic parameters: CPX (AUC 8.21 ug·h/mL y Cmax 13.22 ug/mL) and LVX (AUC 5.39 ug·h/mL and Cmax 8.71 ug/mL). Pharmacodynamic parameters (PD) (AUC/MIC 0–24 h) for each strain are shown in the attached table.
The results in the pneumonia model are shown in the table.
Conclusions: The presence of the qnrA, qnrB or qnrS genes, in *E. coli* strains reduces the therapeutic efficacy of ciprofloxacin and levofloxacin in an experimental pneumonia model in mice.

E. coli strain	Group	MIC (mg/L)	PD parameter (AUC/MIC 0–24 h)	Log_{10} CFU/g of lung (mean±sd)	Mortality (%)	Positive blood culture (%)
ATCC 25922 (pBK-CMV)	Control	–	–	9.37±0.48	100	100
	CPX	0.002	8210	**1.87**[a] ±2.07	**53.33**[a]	**0**[a]
	LVX	0.004	2695.21	**1.67**[a] ±2.50	**28.57**[a]	**0**[a]
ATCC 25922 (pBK-Qnr A)	Control	–	–	9.40±0.25	100	92.86
	CPX	0.125	131.36	**5.71**[ab] ±0.86	**53.33**[a]	**26.67**[a]
	LVX	0.5	21.56	**5.71**[ac] ±0.75	**71.43**[a]	**21.40**[a]
ATCC 25922 (pBK-Qnr B)	Control	–	–	8.84±0.75	100	100
	CPX	0.125	131.36	**4.95**[ab] ±1.71	**57.14**[a]	**35.71**[ab]
	LVX	0.125	86.25	**4.54**[ac] ±1.49	**50**[a]	**28.57**[ac]
ATCC 25922 (pBK-Qnr S)	Control	–	–	8.36±1.26	93.33	93.33
	CPX	0.125	131.36	**5.38**[ab] ±1.75	**42.86**[a]	**28.57**[ab]
	LVX	0.5	21.56	**4.99**[ac] ±1.63	**35.71**[a]	**21.43**[a]

Statistical significance ($p \leq 0.05$): [a] vs. internal control group; [b] vs. *E. coli* ATCC 25922 (pBK-CMV) CPX treatment; [c] vs. *E. coli* ATCC 25922 (pBK-CMV) LVX treatment.

P1568 A single 1200 mg human equivalent dose of oritavancin is highly efficacious in the neutropenic mouse thigh infection model

D. Lehoux, K. Laquerre, V. Ostiguy, I. Fadhil, M. Malouin, C. Cadieux, N. Goyette, S. Fournier, I. Sarmiento, F. Arhin, G. Moeck, T. Parr Jr. (St. Laurent, CA)*

Objectives: Oritavancin (ORI) is a lipoglycopeptide active against Gram-positive bacteria including streptococci, staphylococci, enterococci, and clostridia irrespective of their vancomycin- or oxacillin-resistance phenotype. Previous animal studies and a Phase 2 clinical study demonstrated that single and infrequent doses of ORI are efficacious dosing regimens. In this study, we compared the efficacy of several ORI human equivalent (HEQ) doses including a single dose in a neutropenic murine thigh infection model using 14 *Staphylococcus aureus* (SA) clinical isolates.
Methods: Thigh infection was established in neutropenic female CD-1 mice (19–21g; n = 3/group). Mice were made neutropenic by intraperitoneal injection of cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) before the infection. Each inoculum consisted of one of 14 clinical isolates (3 methicillin-susceptible SA [MSSA], 11 methicillin-resistant SA [MRSA]; ORI MICs 0.015 to 2 mg/L). The mice were infected with the inoculum containing 10^5 colony forming units (CFU) into each thigh. Efficacy of ORI doses simulating human exposure (i.e. 24 h AUC-matched) of 100, 200, or 400 mg daily x 3 days, or a single 1200 mg HEQ dose was evaluated. Both thighs were harvested and CFU were counted after 72 h treatment. Mean Log CFU/thigh changes from baseline were calculated, and correlation between the efficacy and MIC of tested strains was evaluated.
Results: ORI efficacy was HEQ dose-dependent. Treatment with 100, 200, or 400 mg daily x 3 days, or a single 1200 mg HEQ dose generated 1.5, −0.1, −1.6, and −2.7 log CFU/thigh changes from baseline against

MSSA, and 0.8, –0.9, –2.8, –2.8 Log CFU/thigh changes from baseline against MRSA, respectively. Bacterial load reduction was significantly greater ($p \leqslant 0.05$) for the single 1200 mg dose compared to the 200 mg daily dose for both MRSA and MSSA. The log CFU reduction observed with the 200 mg daily dose x 3 remained similar for all tested strains independent of their MIC, as it did with the single 1200 mg HEQ dose.
Conclusion: Front loading of ORI exposure was the most effective dosing regimen in this model. The more rapid and profound bacterial killing that was achieved by the 1200 mg HEQ dose of ORI is consistent with its concentration-dependent bactericidal activity *in vitro*. The apparent lack of impact of ORI MIC on *in vivo* efficacy, for the strains evaluated here, suggests that the test strains fall within the wild-type ORI susceptibility distribution.

P1569 Ceftaroline is superior to cefepime against a *Klebsiella pneumoniae* strain an experimental rabbit meningitis model

P. Cottagnoud, A. Stucki, F. Accosta, U. Eggerman, D. Biek, M. Cottagnoud (Berne, CH; Oakland, US)*

Objectives: In the present study we tested ceftaroline versus cefepime as monotherapy against a *Klebsiella pneumoniae* strain in the rabbit meningitis model and determined the penetration of ceftaroline into inflamed meninges and uniflamed meninges. Ceftaroline is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), as well as common Gram-negative pathogens.
Methods: We used the rabbit meningitis model as described by Dacey and Sande. The *Klebsiella pneumoniae* strain has been kindly provided by Dr. S. Droz, Inst. Inf. Dis., Bern. The MICs (mg/L) were the following: cefepime: 0.25 mg/L, ceftaroline: 1 mg/L. Meningitis was induced by intracisternal inoculation of 10^6 CFU. Treatments were administered 8 h after inoculation and evaluations were performed over a period of 8 h. Ceftaroline (40 mg/kg) was injected at h 0 and 4 in order to mimic kinetics in humans. Cefepime (100 mg/kg) was injected at hour 0 and 4, as a standard dose previously used in this model and corresponding to high doses in humans. CSF samples were repeatedly collected during therapy in order to determine killing rates. Results of the bactericidal activity are expressed in delta log10 CFU/ml·h and delta log10 CFU/ml over 8 h. Penetration of ceftaroline into inflamed meninges was measured by comparison of CSF AUC/serum AUC (Area Under the Curve). Killing rates of the different regimens are presented in the following table.
Results: Penetration of ceftaroline into inflamed meninges: 15.1%±9.7, and into uninflamed: 3.17%±1.3.
Conclusions: 1. Ceftaroline was superior to cefepime against *Klebsiella pneumoniae* in this experimental rabbit meningitis model. The penetration of ceftaroline into inflamed meninges was 15% and 3% into uninflamed meninges.

Groups (N)	Inoculum (log_{10} CFU/ml)	Killing rates/h (delta log_{10} CFU/ml·h)	Killing rates/8 h (delta log_{10} CFU/ml·8 h)
Controls (10)	6.13±0.24	+0.12±0.02	+1.0.8±0.22
Cefepime (10)	5.82±0.88	−0.48±0.09	−3.54±0.94
Ceftaroline (10)	6.18±0.70	−0.70±0.17*	−5.61±1.08**

*P < 0.01, ceftaroline versus cefepime; **P < 0.01, ceftaroline versus cefepime.

P1570 Protective effect of xylitol and lactobacilli against *Clostridium difficile* infection in hamster model

P. Naaber, K. Truusalu, I. Smidt, S. Kõljalg, E. Sepp, P. Hütt, R. Mändar, J. Stsepetova, M. Mikelsaar (Stavanger, NO; Tartu, EE)*

Objectives: To optimize *Clostridium difficile* infection model in hamsters and to evaluate the protective effect of lactobacilli, xylitol and their combinations.
Methods: To find an optimal *C. difficile* infection model, the hamsters were treated with ceftriaxone (n = 10) or ampicillin (n = 20) prior inoculation with *C. difficile* VPI 10463. Intestinal lactobacilli, *C. difficile* and total anaerobes were counted in the intestine of dead or sacrificed (on Day 5 after challenge with *C. difficile*) animals. To evaluate protective effect hamsters were treated with lactobacilli *(L. rhamnosus* GG, n = 5; mixture of lactobacilli – *L. paracasei* B472 and *L. rhamnosus* B11, n = 5), xylitol (n = 6) and their combination (n = 12 and n = 5) before and after ampicillin and after *C. difficile* administration.
Results: (1) Ceftriaxone (60 mg/kg) and *C. difficile* spores (10^5) did not induce infection in hamster model. (2) The optimal model was achieved applying ampicillin 30 mg/kg and 10–30 *C. difficile* spores. The lethal infection was registered during 1–3 days with high *C. difficile* counts in small intestine (6–8.3 log CFU/g). (3) *Lactobacillus* GG or mixture of lactobacilli (10^8 CFU/ml) administered a day before and daily after challenge with *C. difficile* did not prevent lethal *C. difficile* infection. (4) Xylitol (1 ml 20% solution) alone or in combinations with lactobacilli (10^{10} CFU/ml) administered daily starting 5 days before *C. difficile* challenge significantly delayed lethal infection (median of survival days 4–5 vs 1, $p \leqslant 0.001$). Xylitol in combination with applied lactobacilli significantly increased survival of animals on Day 5 after challenge with *C. difficile*: xylitol 2/6, p = 0.1; xylitol + GG 7/12, p = 0.005; xylitol + mixture of lactobacilli 3/5, p = 0.02 vs control 0/10. At the same time, lactobacilli in combination with xylitol enhanced significantly 5-Day survival when compared to xylitol mono-treatment (OR 0.1 [CI 95%; 0.010–0.975]). All survived animals were *C. difficile* and its toxin negative. No differences in lactobacilli, anaerobes and C difficile counts were found in different settings.
Conclusion: In our hamster model prophylactic administration of xylitol delayed lethal infection and reduced mortality. Co-administration of xylitol with lactobacilli had synergistic effect. The protective effect may be due to the inhibition of *C. difficile* adhesion by xylitol and modulation of intestinal microflora by xylitol and lactobacilli.

Animal models – pathophysiology

P1571 Non-invasive imaging technologies to monitor bacterial skin infection in rodent models

S. Kimber, C. DeBank, R. Garland, N. Williams (Bristol, UK)*

Objective: *Staphylococcus aureus* is the most prevalent bacterial pathogen isolated from skin and soft tissue infection in human patients. Increasing methicillin-resistance necessitates the identification of alternative treatments. Testing potential treatments requires the use of predictive models of infection and these should generate high quality data, while at the same time considering the 3Rs. The aim of our studies is to assess the benefits of non-invasive luminescent and thermographic *in vivo* imaging techniques in monitoring bacterial skin infection.
Methods: BALB/c mice were anaesthetised and subjected to tape-stripping to disrupt the epidermis. Stationary growth phase *S. aureus* (either a luciferase expressing strain or a wild type) were inoculated onto the area. At 2 or 4 hours post-infection, separate groups of mice were treated with vehicle or topical antimicrobial treatment. Mice were observed regularly for symptoms of clinical disease throughout the timecourse of infection, and the luminescence imaging used to monitor the extent and spread of bacterial growth. In addition, thermal imaging was used to measure the local temperature at the wound site as a marker of inflammatory changes. At termination, the wound area was excised, homogenised and plated out to assess bacterial numbers.
Results: The optimal dose of bacteria leading to clinical infection was identified. At these doses, cutaneous lesions were established without systemic clinical signs of infection over the timecourse. Positive control treatment was effective in reducing bacterial counts. Thermal imaging allowed a comparison of skin spot temperature with core temperature (using rectal thermometer/transponders). This was compared between infected, non-treated animals; infected, treated animals and uninfected (shaved only) animals. Bioluminescent strains allowed the quantification of infection, as superficial infections are not associated with the loss of signal which can occur in deep tissue infections.
Conclusions: Non-invasive imaging can allow additional real-time information to be obtained from cutaneous infection models. The data

obtained correlates closely to recovered bacterial numbers, but has the advantage of allowing kinetic measurements to be made on many fewer animals. Thermal imaging may also prove useful (provided controls are employed to clarify whether temperature changes simply reflect core temperatures, responses to shaving, or the extent of bacteria infection).

P1572 Pharmacological modification of iron homeostasis by nifedipine affects the course of *Salmonella Typhimurium* infection

S. Mair, M. Nairz, T. Muehlbacher, A. Schroll, T. Sonnweber, G. Weiss (Innsbruck, AT)*

Objective: Iron overload aggravates the clinical course of infections by negatively affecting cell mediated immune mechanisms of macrophages and T helper cell type 1 effector pathways. Recently, we have shown that the calcium antagonist nifedipine enhances DMT1 mediated iron transport *in vitro* and causes subsequent depletion from liver and the circulation in iron overloaded mice. Herein, we thus investigated whether nifedipine may impact on the clinical course of an invasive infection.
Methods: Mice were fed with a control diet or iron enriched diet for three weeks, infected intraperitoneally with *Salmonella Typhimurium* and treated with solvent control or nifedipine for three consecutive days. 24 hours later mice were sacrificed, the bacterial loads were quantified in the liver and the spleen, and the expression of iron and innate immunity genes were determined by means of RT-PCR and Western blot analysis.
Results: Nifedipine treated mice, independently of dietary iron overload, showed an improved survival and presented with reduced numbers of *Salmonella* in the liver and the spleen as compared to solvent injected animals. Even though these effects were more pronounced in the iron diet fed group. This was paralleled by reduced iron content and decreased ferritin expression in the liver in the nifedipine treated group. Interestingly, we did not observe differences in the expression of a panel of pro- and anti-inflammatory cytokines according to nifedipine treatment.
Conclusion: Although, detrimental effects of nifedipine could have been anticipated in this sepsis model, our data provide evidence that nifedipine has a beneficial effect and may be a promising adjunct therapy by mobilizing iron and decreasing the availability of this essential nutrient for bacteria.

P1573 Gene expression studies on the effect of dexamethasone in infant rats with pneumococcal meningitis

C. Blaser, M. Wittwer, S.L. Leib (Berne, CH)*

Objectives: An intensive inflammatory reaction of the host contributes to the pathophysiology of bacterial meningitis (BM). In consequence, the anti-inflammatory dexamethasone (DXM) has been advocated as adjuvant therapy to antibiotics. Recent meta-analyses have demonstrated that adjunctive DXM is beneficial in the treatment of adults with BM but showed no beneficial effect in neonates and children. In an unbiased approach, differential gene expression by dexamethasone was assessed in infant rats with pneumococcal meningitis.
Methods: Eleven days old rats were infected intracisternally with 10 μL saline containing *Streptococcus pneumoniae* (serogroup 3, 6.18 log10 cfu/ml; n = 8) or sham-infected with sterile saline (n = 6). All animals were treated with ceftriaxone (100 mg/kg body weight s.c.). Animals of both groups were then randomized to either receive placebo (0.85% sterile saline s.c., q8 h, n = 4 for meningitis and n = 3 for controls) or an equal volume of saline containing DXM (0.7 mg/kg body weight s.c., q8 h, n = 4 for meningitis and n = 3 for controls). The animals were sacrificed at 72 h after infection and the hippocampus and cortex were dissected. Total RNA was isolated and processed for analysis of gene expression based on Rat Exon 1.0 ST Microarrays of Affymetrix and quantitative real-time PCR. Based on the results of a previous microarray study in experimental pneumococcal meningitis, 47 genes such as neuronal markers, components of neurogenetic pathways (wnt, TGF), microglial markers, on and off signals for chemotaxis were selected for real-time PCR analysis.
Results: A significant correlation between the results of microarray analysis and quantitative PCR was observed ($r = 0.842$, $p < 0.0001$). In ongoing analysis of the microarray and PCR results, Aif1, Cxcl10 and P2ry6 were found to be up-regulated by the infection and down-regulated under DXM treatment. In contrast, P2ry12 was not regulated by the infection but was down-regulated in infected DXM-treated animals below the level found in sham-infected, saline-treated animals.
Conclusions: Aif1, Cxcl10, P2ry6 and P2ry12 are associated with microglial activation and/or induction of chemotaxis. In summary, infection with *S. pneumoniae* and the subsequent inflammatory reaction activates microglia and chemotaxis. The anti-inflammatory DXM can reduce these processes.

P1574 The impact of Panton-Valentine leukocidin on the patogenicity of *Staphylococcus aureus* USA300 in rabbit skin infection model

U. Lipinska, O. Dumitrescu, C. Badiou, A. Tristan, Y. Benito, F. Vandenesch, J. Etienne, K. Hermans, G. Lina (Ghent, BE; Lyon, FR)*

Background: *S. aureus* encoding Panton Valentine leukocidin (PVL) have been epidemiologically linked to primary skin and soft tissue infection in humans. This link, as well as the detection of PVL in human samples, suggests that PVL plays a role in the pathophysiology of primary skin and soft tissue infections. Conflicting data were obtained in experimental studies using mice, a species poorly sensitive to PVL. However, to date, the role of PVL in skin infection has not been investigated using rabbits, a species highly sensitive to PVL.
Methods: USA300 PVL positive, its isogenic deltaPVL and USA300 deltaPVL PVL-complemented strains were cultured until mid-exponential growth phase and washed with sterile PBS. The bacteria were resuspended in PBS and approximately 10^8 cfu in 50 microliters was immediately used for intradermal injection into the rabbits (11 animals per group). The size of the lesions and the area of dermonecrosis were recorded on a daily basis, until day 21. On day 21, the animals were euthanized and skin lesions were sampled for bacteriology and sera were investigated for the presence of antibodies against PVL.
Results: The surface of the skin lesions due to PVL positive strains (USA300 and USA300 deltaPVL PVL-complemented) were significantly higher compared to those induced by the PVL negative strain, from day 1 to 7 for the USA300 wild strain and from days 1 to 3 for the USA300 deltaPVL PVL-complemented strain. Dermonecrosis records yielded similar results, but significant difference was observed only the first day. Only rabbits challenged with PVL positive strains developed antibodies against PVL, attesting for the *in vivo* PVL production by the PVL positive strains. The median anti-PVL antibody titer increased 8.6-fold after inoculation of USA300 strain and 20.7-fold after inoculation of the USA300 deltaPVL PVL-complemented strain.
Conclusions: Rabbit model of skin abscess showed that PVL has an impact at the early phase of the constitution of *S. aureus* USA300 skin infection.

P1575 The choice of murine infection model and the immune status of the mice has an impact on the pharmacodynamics of dicloxacillin against *Staphylococcus aureus* infection

A. Sandberg-Shaal, C. Vingsbo Lundberg, N. Frimodt-Møller (Copenhagen, DK)*

Objectives: Murine infection models are useful tools in pharmacokinetic (PK) and pharmacodynamic (PD) studies of antibiotics. In this study we compared the peritonitis model to the thigh infection model and the impact of immunosuppression on the sensitivity of the infection models in determining PD parameters for dicloxacillin.
Methods: NMRI mice were immunosuppressed with cyclophosphamide at day 4 and 1 before inoculation. Immunosuppressed mice or immunocompetent mice were then inoculated with *S. aureus* (clinical isolate E19977) either intraperitoneally or intramuscularly. At one hour post infection, mice were treated subcutaneously with a single dose of

dicloxacillin ranging from 0.1 to 400 mg/kg (n = 3 for each treatment group). The bacterial loads in the peritoneum or the thigh were determined at 4 hours post treatment.
Results: The log10 CFU level at start of treatment, the total increase of log10 CFU levels (Emax) and the half max effective dose (ED50) for the four infection models, immunocompetent peritonitis model (IC peritonitis), immunosuppressed peritonitis model (IS peritonitis), immunocompetent thigh model (IC thigh) and immunosuppressed thigh model (IS thigh) is shown in table 1. The ED50 for dicloxacillin was in the IC peritonitis model 6.7 mg/kg, in the IS peritonitis model 10.6 mg/kg, in the IC thigh model 20.6 mg/kg and the IS thigh model 28.2 mg/kg.
Conclusions: The murine peritonitis model resulted in a lower ED50 value for dicloxacillin than the thigh model regardless of immune status of the mice, indicating that the peritonitis model is more sensitive to antibiotic treatment of *S. aureus* infections. Further, the immunocompetent variants resulted in lower ED50 values than the corresponding immunosuppressed model, indicating that an intact immune system contributes to the effect of the antibiotic. Thus, the choice of infection model will have an impact in the PKPD results, and this may especially be of importance when extrapolating PKPD parameters to human infection.

Table 1. PD parameters for dicloxacillin in four infection models

	CFU at start	Emax (log_{10})	ED50 (mg/kg)
IC peritonitis	5.75	2.43	6.75±1.55
IS peritonitis	5.30	3.63	10.59±1.32
IC thigh	6.74	1.94	20.62±1.35
IS thigh	6.79	2.91	28.24±1.63

P1576 Eradication of *E. coli* associated with inflammatory bowel disease in a mouse model using ciprofloxacin and the probiotic strain *E. coli* Nissle

A.M. Petersen, S. Schjørring, S.C. Gerstrøm, K. Krogfelt (Copenhagen, DK)*

Objectives: Specific *E. coli* of the phylogenetic group B2 has been linked to active Inflammatory Bowel Disease (IBD). In some studies antibiotics have shown some, although shortlived, effect in the treatment of IBD, and it has been demonstrated that *E. coli* Nissle has prophylactic abilities comparable to 5-ASA in IBD. Our objective was to test treatment of IBD associated *E. coli* combining Ciprofloxacin with *E. coli* Nissle in a mouse model.
Methods: Two B2 *E. coli* strains from IBD patients and one *E. coli* isolate from a healthy person were investigated. Colonisation was achieved in streptomycin treated mice for 5 days with all tested *E. coli* strains, and subsequently 3 different treatment setups were tested, either treatment with inoculation for 3 days with *E. coli* Nissle alone, treatment with 3 days of Ciprofloxacin alone or treatment with 3 days of Ciprofloxacin followed by 3 or 9 days of treatment with *E. coli* Nissle.
Results: After colonisation of the mouse intestine with the tested strains introduction of *E. coli* Nissle did not result in eradication of neither IBD associated *E. coli* nor the *E. coli* from a healthy control, however co-colonisation was obtained. Treatment with Ciprofloxacin for three days alone resulted in effective eradication of tested *E. coli*, without any detection of tested *E. coli* for the following nine days. Combination of Ciprofloxacin for 3 days followed by *E. coli* Nissle for 3 days resulted in a lack of colonisation with *E. coli* Nissle, however introduction of *E. coli* Nissle every day for the rest of the study made it possible to achieve an increase in colonisation with *E. coli* Nissle again. In one set of experiments the tested IBD strain were surprisingly able to recolonise after Ciprofloxacin treatment when *E. coli* Nissle were introduced, however at a level 3 logs under *E. coli* Nissle.
Conclusion:, In the mouse model *E. coli* Nissle can not be used alone as treatment of IBD associated *E. coli*, however 3 days of Ciprofloxacin are efficient in eradicating these strains. After Ciprofloxacin treatment colonisation with *E. coli* Nissle is not possible with only 3 days of inoculation. Furthermore, the introduction of *E. coli* Nissle may support recolonisation with IBD associated *E. coli* after a treatment with ciprofloxacin.

Clinical and experimental studies

P1577 Tolerance to prolonged treatment with linezolid

E. Martinez Alfaro, M. García Sanchez, J. Blanch Sancho, F. Mateos Rodriguez (Albacete, ES)*

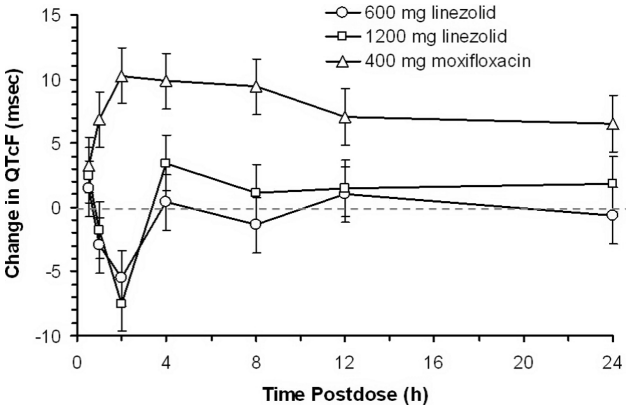
Background: The goal of this study was to asses de tolerance to prolonged treatment with linezolid, describe the adverse reactions and consequences.
Methods: We reviewed retrospectively all patients attended at the infectious disease unit between July 2002 until December 2008, who received linezolid for more than one month.
Results: We included 30 patients. Fifty percent were women. The mean age of patients was 58.5 years (range 16–85). The mean duration of the treatment was 6.4 months (range 1.5–23 months). The main indication for prolonged treatment with linezolid was prosthetic joint infection in 11 of the 30 patients (36.36%) caused for both multidrug resistant microorganism or either patients with allergic reactions to other antibiotic, sixteen percent of patients had chronic ostheomielytis. Other indications were diabetic foot infections, vascular devices infections, multidrug tuberculosis and chronic ostheoarthritis. Twenty patients did not present adverse reactions (66%), five patients presented severe anaemia, three patients polyneuropathy (10%), 3 patients dizziness, headache and insomnia (10%) and just one patient (3%) deafness. Linezolid was stopped for the adverse reactions in ten patients (33%). The patient with deafness and two patients with polyneuropathy were recovered at 6 months. Just one patient have severe polyneurophaty, after two years without treatment with linezolid.
Conclusions: Linezolid had adverse reactions when it was used for a long time, so, it is important monitoring their effects. Almost all adverse reaction disappear when the treatment is stopped. Polyneuropathy can produce severe consequences and disablement for a long time.

P1578 Lack of an effect of linezolid on QTc interval prolongation

B. Damle, R. LaBadie, C. Cuozzo, C. Alvey, C. Chong, H. Choo, D. Kirby (New York, New London, US; Singapore, SG)*

Objective: To demonstrate the lack of an effect of single intravenous (IV) infusion doses of linezolid (600 and 1200 mg), relative to placebo, on the QTc interval.
Methods: This was a randomized, 4-way crossover, double-blind (for linezolid/placebo; open label for moxifloxacin) study in 40 healthy subjects. Treatments included 1-h IV infusion of linezolid 600 and 1200 mg, placebo, and oral moxifloxacin 400 mg (positive control), each separated by a washout of 96 h. Pharmacokinetic (PK) samples and triplicate 12-lead ECG were collected as follows: predose [–1 and –0.5 h for ECG, and 0 h for ECG and PK], and 30 min, and at 1 (end of infusion), 2, 4, 8, 12 and 24 h after dosing. PK were determined by noncompartmental analyses. Time-matched, placebo-subtracted change in QT intervals with Fridericia's (QTcF) correction were determined for linezolid and moxifloxacin using a repeated measure mixed effect model with appropriate terms for the given design and baseline as a covariate.
Results: The figure provides time-matched adjusted differences of QTcF for each treatment compared to placebo values as obtained from the statistical model. For both linezolid doses, the upper bound of the 90% CI at every time point postdose was below 10 msec, thus satisfying the criteria for a negative thorough QT/QTc study. Using the same repeated-measures model, at population Tmax for moxifloxacin (between 2 and 4 h) the 2-sided 90% CI when comparing moxifloxacin to placebo was greater than 5 msec indicating that the study was sensitive to assess QTc prolongation. An increase in linezolid dose from 600 to 1200 mg resulted in an increase in mean Cmax and AUCinf values of 104% and 134%, respectively. Mean clearance of linezolid was 13.7% lower

for the 1200 mg compared with the 600 mg dose. Median Tmax values and mean Vss values were comparable across doses but mean half-life slightly increased with dose. A total of 29 treatment-emergent adverse events (AEs) were reported by 24 subjects. The most common AE was nausea occurring in 4 subjects receiving 1200 mg linezolid. Other AEs were headache and dysmenorrhoea, reported by 2 subjects each.
Conclusions: Therapeutic and supratherapeutic doses of linezolid did not have a clinically relevant effect on QT/QTc interval. Linezolid exposure increased in a more than dose proportional manner from 600 to 1200 mg dose. Single doses of linezolid up to 1200 mg were well tolerated.



P1579 Safety, tolerability and pharmacokinetics of intravaginal pentamycin

B. Frey Tirri, J. Bitzer, B. Geudelin, J. Drewe, C. Winnips (Basel, CH)*

Objectives: Pentamycin is a polyene macrolide with a wide spectrum of antimicrobial activity. The 3-mg dose strength of intravaginal pentamycin is already registered in Switzerland for the treatment of vaginal candidiasis, trichomoniasis and mixed infections. The currently approved treatment regimen is 3 to 6 mg daily for 5–10 days. A higher-dosed vaginal tablet of pentamycin (10-mg dose strength) is being developed for global registration and the objective of this study was to evaluate the safety, tolerability and pharmacokinetics of doses of intravaginal pentamycin up to 10 times the new daily therapeutic dose in healthy volunteers.
Methods: This study was a double-blind, randomised (3:1), vehicle-controlled, escalating-dose study in 19 healthy women (age range: 21–36 years), who applied daily either one of 5 doses of intravaginal pentamycin (3, 10, 30, 60 or 100 mg) or the corresponding dose of pentamycin vehicle alone for 6 days. Safety and tolerability were assessed by physical examination, complete gynaecological examination, monitoring of vital signs and electrocardiogram, recording of adverse events, and laboratory tests. Plasma concentrations of pentamycin were measured pre-dose and then at 30 minutes, 1, 2, 4, 6, 12 and 24 hours post-dose on days 1 and 6 and pre-dose on days 2–5. The lower limit of quantification of the assay (high-performance liquid chromatography and mass spectrometry) was 5 ng/ml.
Results: There were no major differences in the occurrence of adverse events across the dose groups and between the subjects treated with the active compound ($n = 15$) and the vehicle-treated subjects ($n = 5$). The most frequently reported adverse events were mild or moderate vaginal discharge and mild symptoms of vaginal irritation, which also occurred in women who applied the vehicle. The gynaecological examination performed after treatment completion did not reveal any abnormality. Blood pressure, electrocardiographic recordings and laboratory parameters did not show significant changes attributable to the type of treatment or to the dose of active ingredient applied during the study. The plasma levels of pentamycin were below the limit of quantification in all samples.
Conclusion: The intravaginal administration of pentamycin at a dose as high as 100 mg daily for up to 6 days does not increase the occurrence of adverse reactions versus vehicle alone, and is not associated with systemic absorption of the active ingredient through the vagina.

P1580 Antimicrobial effect of doxycycline on control of glycaemia in diabetic patients with moderate periodontitis after phase 1 periodontal treatment

M. Zangeneh, N. Zangeneh, M. Naseh, J. Poursamimi, S. Golbon (Tehran, Qazvin, IR)*

Background: There are contradictory results of different studies about the effect of periodontal treatment on metabolic control of diabetes. Due to the importance of management of these patients, the purpose of this study was to examine the effect of periodontal treatment (scaling and root planning [SRP]) with doxycycline on glycemic control of type 2 diabetic patients.
Material and Methods: In this double blind randomized controlled clinical trial, 40 patients with type 2 diabetes mellitus who had moderate periodontitis were treated with SRP+ placebo (SRP; $N = 20$) or with SRP + doxycycline (SRP+Doxy; $N = 20$), 100 mg/day, for 21 days. Periodontal parameters including bleeding on probing (BOP), clinical attachment loss (CAL), plaque index (PI), probing depth (PD) and also metabolic parameter (HbA1c) were recorded at baseline and 3 month after treatment.
Results: After 3 month, all periodontal parameters and HbA1c showed significant improvement in each group. ($p < 0.001$) The reduction of all periodontal parameters and HbA1c was not significant between two groups in this study ($pBOP = 0.799$), ($pCAL=0.667$), ($pPI=0.253$), ($pPD=0.989$), ($pHbA1c = 0.301$).
Conclusion: Periodontal treatment with the adjunctive systemic doxycycline therapy may influence the systemic conditions of patients with type 2 diabetes mellitus, but no statistical difference was observed between control and treatment groups. Therefore, confirmmatory studies with a larger sample and controlled diabetic patients is necessary.
Research supported by the Islamic Azad University, Tehran Medical Branch, Tehran, Iran.

P1581 Adult native septic arthritis: 10 years in review in order to establish local guidelines on empiric antibiotic therapy

O. Clerc, L. Senn, G. Prod'hom, G. Greub, G. Zanetti (Lausanne, CH)*

Objective: Antibiotic stewardship includes development of practice guidelines incorporating local microbiology and resistance patterns. In case of septic arthritis (SA), addition of vancomycin to the empiric therapy and broad-spectrum antibiotherapy in some clinical settings are subjects of discussion. Our objective was to review the local epidemiology of native septic arthritis in adults, in order to establish local guidelines for empiric therapy.
Methods: Retrospective study based on positive synovial fluid cultures and hospital discharge diagnoses of SA obtained from 1999 to 2008 in patients $\geqslant$16 years. Medical records were reviewed to assess the diagnosis and complete relevant clinical information.
Results: During this ten-year period, we identified 233 SA on native joints in 231 patients. 107 episodes (46%) were obtained through positive synovial fluid cultures, and 126 episodes (54%) through the discharge diagnosis. 147 SA (63%) were large joint infections (LJI). 35 SA (15%) occurred in intravenous drug users. Preexisting arthropathy was present in 51% of cases. 42% of patients with small joint infection (SJI) were diabetic, vs. 23% with LJI ($p = 0.003$). When available, synovial fluid direct examination was positive in 35% of cases. Etiologic agents are reported in the table. Five of the 11 MRSA SA (45%) occurred in known carriers. SJI were more frequently polymicrobial (24% vs. 1%, $p < 0.001$).
For LJI, an empiric treatment with amoxicillin/clavulanate (A/C) would have been appropriate in 85% of cases. MRSA (8 cases) and tuberculous (7 cases) arthritis would have been the most frequently untreated pathogens. Addition of vancomycin to A/C in MRSA carriers would rise the adequacy to 87%. In contrast, A/C would cover only 75% of SJI (82% if restricted to non-diabetic patients). MRSA (3 cases) and *P. aeruginosa* (9 cases, 7 monomicrobial) would be the main untreated pathogens. An anti-pseudomonal penicillin would have been appropriate in 94% of cases of SJI ($P = 0.002$ vs. A/C, $p = 0.19$ if diabetic patients not included).

Conclusions: Treatment with A/C seems adequate for empiric coverage of LJI in our setting. Broad-spectrum antibiotherapy was significantly superior for SJI in diabetic patients, due to different causative bacteria. In an area of low MRSA incidence, our results do not justify a systematic empiric therapy for MRSA, which should be considered in a known carrier.

Etiologic agent	Large joints No. of cases (%) N = 147	Small joints No. of cases (%) N = 86
Polymicrobial	2 (1.4%)	21 (24.4%)
Staphylococcus aureus		
– MSSA	78 (53%)	26 (30.2%)
– MRSA	8 (5.4%)	3 (3.5%)
Streptococcus spp.	20 (13.6%)	13 (15.1%)
P. aeruginosa	4 (2.7%)	7 (8.1%)
Other Gram-negative	14 (9.5%)	6 (7%)
Coagulase-negative staphylococci	3 (2%)	3 (3.5%)
Others	13 (8.8%)	2 (2.3%)
Unknown	5 (3.4%)	5 (5.8%)

P1582 Short parenteral antibiotic treatment for native septic arthritis

L. Tovmirzaeva, J. Garbino, A. Moldovan, F. Jaques, I. Uçkay (Geneva, CH)*

Objectives: The ideal duration of antibiotic treatment in the therapy of septic native joint arthritis is unknown. To assess the epidemiology of septic arthritis at Geneva University Hospitals. To assess risk factors for recurrence with emphasis on surgical and medical treatment parameters.
Methods: Case–control study.
Results: A total of 169 episodes in 157 patients (median age 63 years, 65 females) were retrieved. The infected joints were: knee (n = 51), hip (n = 21), shoulder (n = 32), ankle (n = 9), sterno-clavicular (n = 2), elbow (n = 2), sacroiliac (n = 1), and interdigital (n = 43). In 21 episodes (21/169, 12%), arthritis recurred after the end of antibiotic treatment. In multivariate analysis, lack of surgical intervention (odds ratio 11.3, 95% confidence interval 2.7–46.2), Gram-negative infection (OR 5.9, 1.4–25.3), and immunosuppression (OR 5.3, 1.3–22) were significantly associated with recurrence, while open arthrotomy vs. arthroscopic drainage (OR 0.5, 0.2–1.8), total duration of antibiotic therapy (OR 1.0, 1.0–1.0), or duration of intravenous antibiotic therapy (OR 1.0, 1.0–1.0) were not. Seven days of intravenous therapy had the same effect than 8 to 15 days (OR 0.4, 0.1–1.7) or <21 days of intravenous treatment (OR 1.1, 0.4–3.1). 2 weeks of total antibiotic treatment had the same outcome as a therapy of 2 to 4 weeks (OR 0.4, 0.1–2.3) or >4 weeks (OR 0.4, 0.1–1.6).
Conclusions: Among modifiable parameters, at least one surgical intervention is of utmost importance in the treatment of septic native joint arthritis. The modalities of concomitant antibiotic therapy are secondary. Selected antibiotics might be administered orally after few days of parenteral regimen for a total duration of two weeks.

P1583 Initial empirical antimicrobial therapy and clinical outcome of hospital-acquired pneumonia and ventilator-associated pneumonia in Asian countries: an ANSORP study

D. Chung, J.H. Song for the Asian Network for Surveillance of Resistant Pathogens (ANSORP)*

Objectives: We evaluated the empirical antimicrobial therapy and the clinical outcome of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) among adults in Asian countries.
Methods: A total of 1,508 cases of HAP and 898 of VAP collected from a prospective surveillance study performed by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) in 10 Asian countries from 2008 to 2009 were analyzed. Clinical outcome was assessed by crude mortality, pneumonia-related mortality at 30 days, and the length of hospital stay.
Results: Late-onset pneumonia accounted for 83.1% of HAP and 73.7% of VAP. *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* were the most frequent respiratory isolates. Among culture-positive cases of HAP and VAP, polymicrobial infection accounted for 23.3% and 31.2%, respectively. Initial empirical antimicrobials included penicillins (48.0%), cephalosporins (41.9%), fluoroquinolones (27.7%), carbapenems (22.5%) and glycopeptides (12.1%) in HAP, whereas cephalosporins (47.6%), penicillins (44.5%), carbapenems (35.7%), fluoroquinolones (19.0%) and glycopeptides (15.2%) in VAP. Due to the emergence of *Acinetobacter* spp. in many countries, colistin was initially used in 3.0% of HAP and 14.3% of VAP cases. Initial empirical antimicrobial regimen was concordant in 73.7% of HAP and 67.6% of VAP. Crude mortality at 30-day was 34.9% (14.3–57.4%) in HAP and 44.8% (11.1–66.7%) in VAP. Pneumonia-related mortality at 30-day was 22.8% for HAP and 30.2% for VAP. Pneumonia-related mortality in those receiving concordant empirical therapy was lower than in those receiving discordant therapy (17.6% vs. 23.9% in HAP, P = 0.045; 26.6% vs. 37.1% in VAP, P = 0.014). The length of hospital stay in HAP and VAP was 30.5 days (SD 36.0) and 28.5 days (SD 34.4) respectively.
Conclusion: Anti-pseudomonal β-lactams and glycopeptides were frequently used as initial empirical antimicrobial regimens for HAP and VAP in Asian countries. Colistin was also frequently used for treatment of VAP for the treatment of *Acinetobacter* pneumonia. Antimicrobial resistance in major pathogens negatively affected the clinical outcome of HAP and VAP.

P1584 Clinical efficacy of oral vancomycin compared to oral and to intravenous metronidazole in *Clostridium difficile* infection, 2009

J. Leitner, D. Schmid, H.W. Kuo, F. Allerberger, V. Michl, P. Tesik, C. Wenisch (Vienna, AT)*

Objectives: Recent reports showed frequent treatment failure of metronidazole for the treatment of *Clostridium difficile* infection (CDI). Therefore we wanted to compare the efficacy of oral vancomycin with oral and intravenous metronidazole in inpatients with CDI.
Methods: The source population comprised CDI patients of a Viennese hospital fulfilling the case definition given by ESCD with the need of antimicrobial therapy. Study subjects were included consecutively according to their hospital admission between November 2008 and August 2009. Exclusion criterion was the lack of 30-day follow-up. The treatment regimes oral metronidazole (group I), intravenous metronidazole (group II) and oral vancomycin (group III) were compared with regards to duration of diarrhoea following the start of antibiotic therapy, treatment failure of the first chosen antibiotic therapy regime (defined by change to an alternative antibiotic regime), frequency of recurrent CDI (i.e. defined by recurrent episode following ⩾2 weeks previous episode) and 30-day mortality. For univariable analyses χ^2 test, χ^2 test for contingency tables or Fisher's exact test and the t-test were used. The Charlson Index was used for co-morbidity scoring.

Univariable analyses

Outcome	Therapy regime								
	Metronidazole p.o. N = 47		χ^2-test p	Metronidazole i.v. N = 11		χ^2-test p	Vancomycin N = 5	
	n (%)	RR (95%CI)		n (%)	RR (95%CI)		n (%)	RR (95%CI)
No response to first choice antibiotic(s)	8 (17%)	0.43 (0.12–1.48)	0.24	1 (9.1%)	0.23 (0.03–1.96)	0.21	2 (40%)	1
Recurrent episode of diarrhoea (⩾2 weeks since the onset of the previous episode)	2 (4.3%)	n.c.	1	1 (9.1%)	n.c.	1	0	1
30-day mortality	3 (6.4%)	n.c.	1	4 (36.4%)	n.c.	0.24	0	1
	Metronidazole p.o. N = 44		t-test p	Metronidazole i.v. N = 11		t-test p	Vancomycin N = 5	
	Mean	Range		Mean	Range		Mean	Range
Duration of diarrhoea (days)	14	1–82	0.86	8.4	3–24	0.12	13.4	7–21

Results: After the exclusion of 25 patients due to missing follow up data, a total of 63 study subjects remained for analyses. The patient characteristics of the three therapy groups did not reveal relevant imbalances in age, sex or comorbidities. There were no significant differences regarding therapy response, recurrences, and duration of diarrhoea between the treatment regimes (Table 1). In contrast, 30-day mortality was significantly higher in patients on intravenous

metronidazole compared to the two oral treatment regimens ($p = 0.01$ using χ^2 test for contingency tables).
Conclusion: Intravenous metronidazol was associated with a poor outcome in *C. difficile* infection compared to oral vancomycin and oral metronidazole.

P1585 Phagocytic activity and production of reactive oxygen species in J774 mouse macrophages exposed to increasing concentrations of oritavancin: comparison with vancomycin and azithromycin

S. Lemaire, P. de Galard, P.M. Tulkens, M.P. Mingeot-Leclercq, F. Van Bambeke (Brussels, BE)*

Objectives: Oritavancin (ORI) is a lipoglycopeptide that accumulates to high levels in macrophages (up to 300-fold) and localizes in lysosomes. Although ORI shows extensive activity against intraphagocytic forms of *S. aureus* (Barcia-Macay et al, AAC, 2006), concerns have been raised about the possibility for the drug to also impair critical macrophage functions. Our aim was to assess the effect of ORI *in vitro* on phagocytosis of infectious organisms and production of reactive oxygen species (ROS) in macrophages at clinically-relevant conc. Azithromycin (AZI, accumulating to high levels in lysosomes) and van (VAN, low cellular accumulation) were used as comparators.
Methods: J774 mouse macrophages were incubated for 3 h with increasing concentrations of ORI (0–50 mg/L; Cmax in patients: 25 mg/L) or VAN or AZI (0–100 mg/L) and then used to measure (i) the phagocytosis of serum-opsonized *P. aeruginosa* PAO1 (CFU counting [MIC of ORI >32 mg/L]) and fluorescent red latex beads [2 μm diameter] (fluorimetry; subcellular localization assessed by confocal microscopy in comparison with the lysosomal tracer lysotracker green), and (ii) production of ROS by monitoring the cell permeant indicator 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester in the presence of 0.5% H_2O_2.
Results: As shown in the table, exposure of J774 macrophages to ORI (i) did not significantly inhibit their ability to engulf *P. aeruginosa* over the whole range of conc., or phagocytize latex beads (for conc. <25 mg/L; these localized in lysosomes as in control cells [no change in latex beads engulfment seen for cells incubated with VAN or AZI up to 100 mg/L]); (ii) did not impair the production of ROS (for conc. <40 mg/L).
Conclusions: At extracellular conc. up to its human Cmax, ORI did not affect two critical functions of macrophages related to the handling and killing of infectious organism *in vitro*. Differences in conc. needed to affect phagocytosis of latex beads and *P. aeruginosa* cells may be related to opsonization of bacteria (receptor-mediated phagocytosis). Together with our previous observation that ORI at Cmax is bactericidal against *S. aureus* phagocytized by macrophages, the data presented here suggest that no untoward effect is to be expected from extensive accumulation of ORI in macrophages in a context of intracellular infection and handling of particulate matters, as long as its concentration in serum remains in this range or lower.

Parameter studied	Values at increasing oritavancin extracellular concentrations					
	0 mg/L	1 mg/L	10 mg/L	25 mg/L	40 mg/L	50 mg/L
P. aeruginosa phagocytosis[a]	100±0.1	99.9±0.1	99.9±0.1	99.9±0.1	nd	99.9±0.1
Latex beads phagocytosis[b]	100±7.6	125.3±8.4	106.5±7.1	40.6±17.9	nd	45.3±19.4
ROS production[c]	117±5.3	120.7±8.5	118.2±4.1	118.1±1.5[d]	146.8±1.8	nd

[a]percentage of control (10^6 CFU/mg cell prot.); [b]percentage of control (10^6 beads/mg cell prot.); [c]fluorescent signal at 25 min (in % of value measured at time 0 min); [d]tested at 20 mg/L; nd: not determined.

P1586 The modulatory effect of rifampicin and gentamicin on expression of virulence factors in *Escherichia coli* O157: H7

E.A. Rahal, A. Kanbar, G.M. Matar (Beirut, LB)*

Objectives: *Escherichia coli* O157: H7 is the agent of food poisoning outbreaks that may result in diarrhea, hemorrhagic colitis and the potentially fatal hemolytic uremic syndrome. The pathogenic process of *E. coli* O157: H7 mainly relies on intimin, a bacterial outer membrane attachment protein, and the production of two cytotoxins: shiga-like toxin (SLT) I and II. Our goal was to assess the effects of antibiotic exposure on the expression and production of toxins in this bacterium.
Methods: We assessed the *in vitro* effects of rifampicin and gentamicin on the expression of intimin, SLTI and SLT II in *E. coli* O157: H7 strains obtained from outbreaks in the United States. Toxin gene expression at the RNA level was assessed by reverse transcription polymerase chain reaction (RT-PCR); toxin protein levels were semiquantitatively examined by reverse passive latex agglutination (RPLA).
Results: Minimal inhibitory concentrations (MIC) of these antibiotics were capable of inhibiting detection of transcription of the genes encoding these virulence factors. Moreover, MIC concentrations also resulted in decreasing toxin release from bacteria by more than 10 fold. On the other hand, minimal bactericidal concentrations (MBC) of these antibiotics were incapable of decreasing expression and subsequent release of these toxins. Expression and release of toxins was inhibited when bacteria were exposed to the MIC concentration of either rifampicin or gentamicin and then incubated in an MBC concentration of the same antibiotic.
Conclusions: These data have implications on treatment of *E. coli* O157: H7 and indicate that inhibition of toxin synthesis and release prior to administration of a bactericidal dose of an antimicrobial agent might be more appropriate. Such a regimen would circumvent toxin release and hence prevent potentially fatal sequelae.

P1587 Protective effect of daptomycin and rifampicin on osteoblast-like cells bearing intracellular *Staphylococcus aureus*

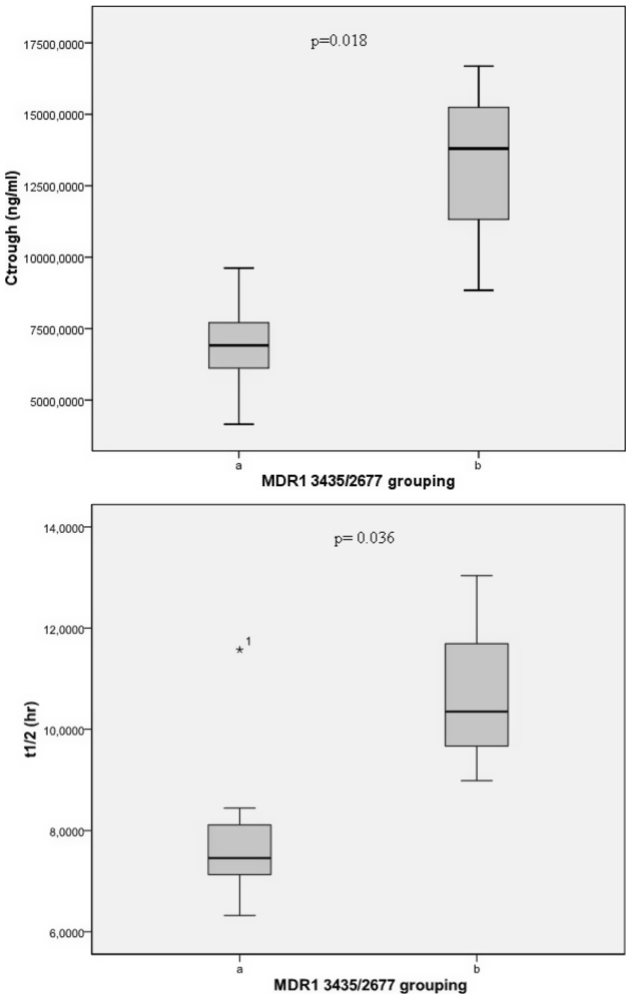
J-P. Rasigade, S. Trouillet, T. Ferry, F. Vandenesch, S. Tigaud, J. Etienne, F. Laurent (Lyon, FR)*

Objective: *Staphylococcus aureus* is the most frequent pathogen in osteomyelitis. It can be internalized by and survive inside osteoblasts, thus evading from the host immune response and most antibiotics. Intracellular persistence of *S. aureus* has been proposed as an explanation for the recurrent nature of osteomyelitis. Among the antibiotics used in treating bone infections, daptomycin and rifampicin are effective against intracellular pathogens including *S. aureus*. We aimed at investigating the yet unknown influence of these antibiotics on the survival of osteoblasts bearing intracellular *S. aureus*.
Methods: Osteoblast-like MG-63 cells were infected with ATCC 9144 *S. aureus* reference strain for 2 hours. Cultures were then incubated for 2 hours with 100 mg/l gentamicin, alone or in combination with daptomycin (6 mg/l or 60 mg/l) or rifampicin (5 mg/l or 10 mg/l). Gentamicin has no intracellular bactericidal activity and was solely used to eliminate the remaining extracellular *S. aureus*. Cultures incubated with gentamicin alone served as control. After 2 h, cultures were washed and incubated in 1% penicillin-streptomycin medium to avoid survival of bacteria released by cell lysis. After 18 h, cell viability was assessed using trypan blue exclusion and the remaining intracellular *S. aureus* were quantified by plating Triton X100 cell lysates on agar.
Results: The effects of daptomycin and rifampicin on the survival of *S. aureus*-infected MG-63 cells were expressed as the mean fold increase in cell viability after 18 h, as compared with that of cultures incubated with gentamicin alone. Results were ranked as follows: 10 mg/l rifampicin (2.68-fold, $p < 0.02$), 60 mg/l daptomycin (2.51-fold, $p < 0.02$), 6 mg/l daptomycin (2.25-fold, $p < 0.02$) and 5 mg/l rifampicin (1.64-fold, non-significant). Plate counting of the remaining viable intracellular *S. aureus* after 18 h showed no significant difference between wells initially incubated with gentamicin, daptomycin and rifampicin (mean 10^4 *S. aureus* CFU/well).
Conclusions: Daptomycin and high concentration rifampicin significantly improved survival of *S. aureus*-infected osteoblast-like cells in culture. The maintained effect of daptomycin at low concentration is of interest in clinical practice. Further studies are ongoing to determine if sustained incubation of *S. aureus*-infected cells with daptomycin or rifampicin eventually leads to the complete elimination of intracellular bacteria.

P1588 Effect of multidrug-resistant gene polymorphisms on daptomycin concentrations: preliminary results

L. Baietto, A. D'Avolio, F.G. De Rosa, M. Dominici, S. Garazzino, M. Siccardi, G. Di Perri (Turin, IT)*

Objectives: Daptomycin (DAPT) is a new antibiotic with acitivity against Gram-positive bacteria and approved for complicated skin and skin structure infections (cSSSI) and right-side infective endocarditis due to *S. aureus*. DAPT is a substrate of P-glycoprotein (MDR1), an efflux transporter involved in the removal of metabolites and xenobiotics from cells into urine, bile, and the intestinal lumen. Because renal excretion is the primary route of elimination of DAPT and dosage reduction is mandatory when the Clcr is <30 ml/min, we aimed to evaluate the effect of MDR1 C3435T, C1236T and G2677T single nucleotide polymorphisms (SNPs) on DAPT pharmacokinetic (PK) parameters in patients with cSSSI.
Methods: The study involved 12 patients with normal renal function treated with DAPT (4 mg/kg, OD) administered by a 30 min IV infusion. Blood samples were collected at 0, 0.5, 1.5, 5, 9, 24 hour post dose at steady state. Plasma DAPT concentrations were determined by an HPLC-MS method. DAPT PK parameters (AUC0–24, Cmax, Ctrough, t1/2, V, Cl) were determined using a non-compartmental method. Patients were genotyped for C3435T, C1236T and G2677T SNPs using RT-PCR. Statistical analysis was performed using the Mann Whitney test.



Results: Medians [IQR] of AUC0–24, Cmax, Ctrough, t1/2, V and CL were, respectively, 634.2 [599.1–1058.2] μg*h/ml, 72.0 [31.7–93.5] μg/ml, 7.6 [3.2–12.5] μg/ml, 7.8 [2.8–6.7] h, 0.083 [0.060–2.38] L/kg and 6.4 [4.1–12.8] ml/h/kg. Patients homozygous for MDR1 3435T (N = 3) had Ctrough and t1/2 significantly higher (p = 0.018 and p = 0.036). Patients with MDR1 2677T (N=:8) had lower Ctrough (p = 0.048) and t1/2, (not statistically significant). No correlation was observed for C1236T SNP. Consistent with the results of genotyping analyses, patients were divided into two groups: a) 3435TT/2677GG and 3435TT/2677GT (N = 3); b) 3435CT/2677TT, 3435CC/2677TT and 3435CT/2677GT (n = 9). We observed that the first group of patients had a statistically significant higher Ctrough and t1/2 compared with the second group (p = 0.018 and p = 0.036).
Conclusion: PK parameters obtained were similar to available data in healthy volunteers. We observed a relationship between DAPT Ctrough and t1/2 and the SNPs C3435T and G2677T probably due to different renal excretion of DAPT. Further studies are needed to confirm these data and to understand their clinical significance, especially for dosage adjustment in renal diseases, DAPT-related muscular toxicity and clinical outcome.

PK/PD studies and clinical efficacy

P1589 Pharmacokinetics of azithromycin in serum after administration of extended-release and immediate-release formulations in paediatric patients with acute otitis media

A. Fang, P. Liu, R. LaBadie, P. Crownover, A. Arguedas (New York, New London, US; San Jose, CR)*

Objectives: A 30 mg/kg single dose of AZ-IR has been used in paediatrics to treat AOM. AZ-ER formulation provides higher dose in one administration and has been used in adults. To provide exposure bridging between the two formulations, this study was to determine whether a 60 mg/kg single dose of ER provides similar or greater AZ systemic exposure compared to the 30 mg/kg IR in paediatric patients with AOM, by demonstrating that the lower boundary of the 90% confidence interval (CI) of the primary endpoint (ER/IR ratio of area under the curve over 72 hours AUC72) and the secondary endpoint (point-to-point concentration ratios) are ⩾80% and ⩾70% respectively.
Methods: This was a randomized open-label single-centre study. Thirty-eight AOM patients (6 months to 6 years old) received a single dose of AZ-ER 60 mg/kg or AZ-IR 30 mg/kg (n = 19 per group). Serum samples were collected at 1, 2, 3, 4, 8, 24, 48 and 72 hours after dosing. AZ levels were analyzed by validated HPLC/MS/MS method. PK analysis was done by non-compartmental method. Log transformed PK data were analyzed by one-way analysis of variance.

PK endpoint	Adjusted geometric mean			
	AZ-ER N = 18	AZ-IR N = 18	ER/IR Ratio of adjusted means (%)	90% confidence interval for ratio (%)
Parameter				
AUC_{72} (ng·h/mL)	9848	6234	157.98	(98.87, 252.44)
C_{max} (ng/mL)	611.5	667.3	91.63	(56.21, 149.38)
T_{max} (h)*	3 (2–8)	2 (1–4)	N/A	N/A
Concentration (ng/mL)				
C_1	100.0	152.8	65.44	(23.56, 181.77)
C_2	293.1	579.7	50.57	(25.24, 101.30)
C_3	382.0	381.7	100.07	(57.91, 172.92)
C_4	441.8	267.9	164.93	(103.78, 262.12)
C_8	244.8	140.3	174.41	(110.07, 276.36)
C_{24}	142.4	82.3	173.01	(111.45, 268.55)
C_{48}	94.8	50.1	189.35	(129.76, 276.30)
C_{72}	56.0	30.6	183.14	(124.61, 269.14)

AUC_{72}: area under the curve over 72 hours; C_{max}: the maximum concentration; T_{max}: the time to reach C_{max}; N/A: not applicable; *: median (range).

Results: Thirty-six Hispanic patients completed the study (61% male). Summary statistics of the PK parameters and concentrations at each time point are shown in the table.

- Primary Comparison of PK parameters: The ratio of the adjusted mean AUC72 (ER/IR) was 157.98% with the lower boundary of the 90% CI >80%.
- Secondary Comparisons of concentrations: The lower boundaries of the 90% CIs of ER/IR AZ concentration ratios for the first 3 hours were <70%, which was expected for ER formulation since it was designed to slow down the absorption (delayed Tmax and lower Cmax) to improve the tolerability. AZ ER concentrations at all remaining time

points after 3 hours were higher than those from IR with the lower boundary of the 90% CI >70%.
- Safety: No serious adverse events or clinically significant safety labs other than from AOM were observed.

Conclusions: AZ-ER 60 mg/kg single dose provides similar or greater systemic exposure in paediatrics compared with the AZ-IR 30 mg/kg single dose. Both ER and IR were well tolerated.

P1590 Pulmonary pharmacokinetics of the prodrug colistin methanesulphonate and formed colistin following pulmonary administration of the prodrug in rats

S. Ws Yapa, J. Li, R. Nation, C.J. Porter, M.P. McIntosh (Melbourne, AU)*

Objectives: Colistin methanesulphonate (CMS), an inactive prodrug of colistin, is being used increasingly by inhalation to treat respiratory infections caused by multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. However, very limited pulmonary pharmacokinetic (PK) information on CMS and generated colistin is available. The objective was to determine the pulmonary PK of CMS and generated colistin following pulmonary instillation of CMS to rats.
Methods: The study was conducted in conscious, male Sprague-Dawley rats (n = 3 per time point). For pulmonary administration (15 mg/kg), rats received an intratracheal instillation of 100 μL CMS solution. Following blood sample collection, rats were sacrificed at pre-determined time points up to 12 hr. The lungs were lavaged with phosphate buffered saline (4ºC). CMS and colistin concentrations in lavage fluid were quantified by validated HPLC assays. Urea concentrations in lavage fluid and plasma were determined using a commercially available assay kit to allow determination of the volume of epithelial lining fluid (ELF).
Results: The ELF volumes were 82±14 μL. Initial CMS concentrations in ELF were in the vicinity of 20,000 mg/L and declined with a half life (t1/2) of 1.8 hr to approximately 100 mg/L at 12 hr (Figure 1). Colistin concentrations of 59±5 mg/L were evident in ELF 5 min post dose with a maximum concentration of 803±322 mg/L achieved at 4 hr declining to 262 mg/L at 12 hr. The t1/2 in the terminal phase for colistin was 4.8 hr. The area under the ELF concentration versus time curve (0–12 hr) for CMS and colistin was 33,056 mg·hr/L and 5,608 mg·hr/L, respectively.
Conclusion: To the best of our knowledge, this is the first study to report the time-course of CMS and generated colistin in ELF after pulmonary administration of CMS in rats. Extended exposure to CMS and, importantly, colistin in ELF was achieved. This may be beneficial given that the minimum inhibitory concentrations for colistin against susceptible Gram-negative pathogens are ⩽2 mg/L. This study provides important information on the pulmonary PK of CMS and formed colistin after pulmonary administration of the prodrug.

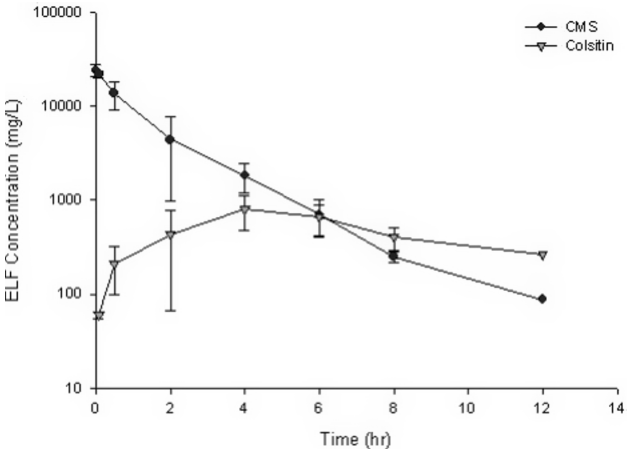


Figure 1. Time course of the concentration of CMS and colistin in ELF.

P1591 Rationale for the therapeutic dose selection of oral torezolid phosphate in complicated skin infections

P. Bien, K. Bartizal, A. Louie, G. Drusano, P. Prokocimer (San Diego, Albany, US)*

Objectives: Dose selection for use in pivotal Phase 3 trials is a critical milestone in any drug development program. In the torezolid phosphate (TP) program, the objective of selecting an optimal Phase 3 therapeutic dose was based on PK/PD modeling, Phase 1 safety/PK, and Phase 2 dose-ranging trial results in complicated skin and skin structure infections (cSSSI). TP is 4–16 fold more potent *in vitro* and 4–8 fold more potent *in vivo* than linezolid, the only currently marketed oxazolidinone antibiotic.
Methods: Nonclinical PK/PD mouse thigh models of infection identified the dose and schedule of TP linked with optimal antimicrobial effect. Target attainment analyses were performed by MIC. The population PK analysis (3 stage Hierarchical Bayesian approach) was used to perform a 10,000 subject simulation for AUC from the Phase 2 clinical study (n = 188) results where oral doses of 200, 300, or 400 mg torezolid phosphate once-daily for 5 to 7 days were administered to patients with cSSSI.
Results: AUC/MIC was found to be the PK/PD parameter best correlated with efficacy. Once-daily dosing provided the same efficacy as equally divided doses. Single-dose 72-hour studies in mouse thigh models of MRSA infection demonstrate the pivotal role of granulocytes in amplifying torezolid antibacterial effect. This granulocyte-mediated amplification of torezolid bacterial killing is of a magnitude unprecedented for antibacterials. The overall expectation analysis showed that on Day 1, 98.5% of the population achieved stasis; on Day 3 this was 99.8%, where 89.3% of the population achieved maximal cell kill. Population PK analysis and PD simulations demonstrated that 165 mg of torezolid (equivalent to a 200 mg TP dose), is expected to provide maximal bacterial killing in cSSSI in human. Results from the Phase 2 cSSSI clinical trial (mean duration of treatment 6.4 dosing days) showed that TP was equally safe, well tolerated, and efficacious at all three dose levels tested, with an overall clinical cure rate of 95.7%.
Conclusion: Both the PK/PD investigation and the Phase 2 study results point towards the selection of a 200 mg QD dose of TP for the Phase 3 pivotal studies in cSSSI.

P1592 Microbiological efficacy of torezolid phosphate in patients with complicated skin and skin structure infections

P. Bien, C. De Anda, P. Prokocimer (San Diego, US)*

Objective: Torezolid (T) is the active moiety of the prodrug torezolid phosphate (TP), an oxazolidinone with 4- to 16-fold greater activity than linezolid against Gram-positive species including methicillin-resistant and linezolid-resistant *Staphylococcus aureus* (MRSA). The objective of this analysis was to evaluate the microbiological efficacy of TP in patients with complicated skin and skin structure infections (cSSSI) enrolled in the oral Phase 2 dose-ranging study.
Methods: Patients diagnosed with cSSSI were randomized to receive 200, 300, or 400 mg oral TP once-daily (QD) for 5 to 7 days. Microbiological samples were to be obtained from the cSSSI site at baseline and then any follow-up visits if medically indicated.
Results: There were 188 patients that received at least one dose of study drug, of which 82% had a skin pathogen isolated at baseline. Of the 154 patients with a baseline pathogen isolated, *S. aureus* was the most common pathogen isolated (90%) of which 19% were MSSA and 81% were MRSA. The MIC range for MRSA was 0.12–0.5 μg/mL with a MIC90 of 0.25 μg/mL. The microbiological eradication at Test of Cure (TOC) in the Microbiological Evaluable (ME) patient population was 97.7% for all pathogens, 97.9% for MRSA, and 95.7% for MSSA for all dosage groups.
Clinical Response rates at TOC in the ME patient population are presented in the table.

Conclusion: TP demonstrated high microbiological efficacy in all dosage levels tested in patients with severe cSSSI, consistent with the clinical success rates reported at all doses investigated in this study. These data support the selection of 200 mg QD for further development for the treatment of severe cSSSI.

	200 mg		300 mg		400 mg	
	Cure	Failure	Cure	Failure	Cure	Failure
Aerobic Gram-positive organisms	43 (100%)	0 (0.0%)	41 (93.2%)	3 (6.8%)	44 (95.7%)	2 (4.3%)
Staphylococcus aureus	39 (100%)	0 (0.0%)	33 (91.7%)	3 (8.3%)	43 (97.7%)	1 (2.3%)
MRSA	32 (100%)	0 (0.0%)	25 (92.6%)	2 (7.4%)	36 (97.3%)	1 (2.9%)
MSSA	7 (100%)	0 (0.0%)	8 (88.9%)	1 (11.1%)	7 (100%)	0 (0.0%)

P1593 Bioavailability and pharmacokinetics of torezolid phosphate after intravenous administration in healthy subjects

K.A. Muñoz, P. Bien, C. Bethune, R. Wright, P. Prokocimer (San Diego, Madison, US)*

Objectives: Torezolid phosphate (TP) is a novel oxazolidinone phosphate prodrug antibiotic that is rapidly converted to the microbiologically active molecule torezolid. Torezolid is active against Gram-positive bacteria, including linezolid- and methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. To further characterize intravenous TP, a randomized, double-blind, placebo-controlled, single-dose escalation, bio-availability, safety, tolerability, and pharmacokinetic study was performed in healthy subjects. Doses were escalated from 100 to 200 to 400 mg/day. Absolute bioavailability was assessed following a single 200 mg dose of TP in tablet and infusion formulations.
Methods: Three cohorts of 12 subjects each (9 active and 3 placebo) received a single dose of placebo or 100, 200, or 400 mg intravenous TP in one of three possible regimens: 1) in 500 mL of saline infused over 2 hours, 2) in 250 mL of saline infused over 2 hours or, 3) in 250 mL of saline infused over 1 hour. For the absolute bioavailability determination, a cohort of 8 subjects received the following treatments in a cross-over design: a single 1-hour infusion of 200 mg TP in 250 mL saline and, 2) a single dose of one 200 mg TP tablet administered with 240 mL water.
Results: After single dose administration, preliminary estimates indicated mean Cmax and AUC_{0-inf} values for torezolid increased in a linear and proportional manner to TP dose levels ranging from 100 to 400 mg (1.16 to 4.98 μg/mL and 16.34 to 56.96 μg·hr/mL, respectively). Torezolid systemic clearance (CL) and volume of distribution (Vss) were not affected by dose levels. The prodrug was only detected above the limit of quantitation (5 ng/mL) during the infusion, and for a maximum of 4 hours after infusion. The absolute bioavailability of torezolid from the TP tablet was approximately 100%.
Conclusions: The absolute bioavailability of torezolid from TP tablets was approximately 100%, demonstrating TP is rapidly and completely converted to the active moiety and absorbed. Single dose escalation from 100 to 400 mg TP by intravenous administration resulted in dose proportional exposure and linear pharmacokinetics for torezolid.

P1594 Improved pharmacokinetics of the novel oxazolidinone antibiotic torezolid phosphate compared to linezolid in healthy subjects

K.A. Muñoz, C. Bethune, J. Bohn, R. Wright, P. Bien, P. Prokocimer (San Diego, Madison, US)*

Objectives: To compare the pharmacokinetics and safety of the therapeutic dose of torezolid phosphate (TP) and the prescribed dose of linezolid after multiple dosing in healthy adults.
Methods: A multiple-dose study was performed to determine the pharmacokinetics of the prodrug TP and its active moiety, torezolid, compared to linezolid in healthy adults. Each cohort of 10 subjects (8 active and 2 placebo) received oral doses of 200 mg TP once a day or twice daily oral doses of 600 mg linezolid for 21 days.
Results: Following oral administration of TP capsules at 200 mg QD for 21 days, the pharmacokinetics of torezolid appeared to be generally independent of duration of dosing. Steady-state concentrations of torezolid were achieved after 3 days administration of TP and there was no evidence of accumulation. The torezolid oral clearance (CL/F) values ranged from 6.63 to 7.48 L/hr on Days 1 and 21. Mean apparent volume of distribution (Vz/F) values for torezolid were independent of duration of dosing, as well. Torezolid was eliminated with terminal mean half-life (t1/2) values ranging from 11.1 to 14.7 hours. In contrast, linezolid mean Cmax and AUC values increased after multiple dosing as a function of the duration of dosing, with the highest values observed on Day 21. Linezolid mean CL/F values decreased approximately 34% from Day 1 to Day 21, with values on Day 1 of 8.73 L/hr and decreasing to 5.76 L/hr on Day 21. The mean t1/2 values for linezolid increased from 3.8 to 5.8 hours for Days 1 and 21, respectively. Mean linezolid Vz/F values were comparable on Days 1 and 21 with observed values of 42.9 and 46.1 L, respectively. Multiple doses of 200 mg QD TP and 600 BID linezolid through 21 days were well-tolerated with similar numbers of adverse events and treatment-related adverse events reported in each cohort. There were no serious adverse events reported in either cohort.
Conclusions: After multiple dosing with TP or linezolid for 21 days, linezolid exposure increased and was nonlinear with respect to time while torezolid exhibited consistent exposure without accumulation. Both drugs were safe and well-tolerated.

P1595 *In vitro* pharmacodynamic assessment of efficacy and resistance potential for a novel non-quinolone DNA gyrase/topoisomerase inhibitor

M. Kuhn, D. Hanna, J. Hardink, J. Hamel, M. Maloney, M. Mitton-Fry, D. Girard (Groton, US)*

Objectives: The clinical need for new antimicrobials to combat hospital and community acquired Gram-positive infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) remains important. The objective of this work was to assess the clinical durability of novel (non-quinolone) lead compounds targeting DNA gyrase and topoisomerase IV utilizing *in vitro* static and *in vitro* dynamic (IVDM) and *in vivo* pharmacology models. The efficacy of the lead compound and its relationship to resistance emergence was tested by simulating projected human dosing parameters in the *in vitro* models. The *in vitro* results were compared with the *in vivo* infection model data.
Methods: Static concentration time kills (SCTK) were performed following CLSI guidelines against FQR MRSA 01A1095. A previously described *in vitro* PKPD hollow fiber model was employed to simulate human PK parameters in a multi-day q24 h dosing regimen at inoculum of 10^6 and 10^9. Single dose rat systemic and thigh infection models were used to test mutant suppression.
Results: SCTK exhibited >3-log kill at 4X MIC. AUC/MIC 50 and above completely eradicated 10^6 burden of 01A1095 in the hollow fiber. AUC/MIC 50 resulted in a 1-log kill at 24 h at 10^9 in the hollow fiber, but was unable to suppress the emergence of gyrA (D83N) mutant sub-population upon additional dosing. To examine suppression of first step mutant *in vivo*, rats were challenged with 1:5 log ratio of gyrA (D83N) mutant to sensitive parent 01A1095. In both *in vivo* models, bacterial burden was reduced after single dose. Animals were rarely cleared of infection by the lead agents with cultures at 24 h showing all gyrA (D83N) mutant at high doses of 200 mg/kg, 100 mg/kg and 50 mg/kg.
Conclusions: SCTK, IVDM and *in vivo* efficacy models reveal a potent bactericidal agent targeting FQR MRSA. Multi-day q24 h dosing in the hollow fiber showed the predicted human efficacious dose exhibited >2-log decrease in CFU. Inoculum studies simulating relevant infection burden in the in the hollow fiber showed pre-existing sub-populations, in particular gyrA (D83N), was able to persist at AUC/MIC ratio of 50 and confirmed in the *in vivo* setting. Chemical attributes to increase the TI or topoisomerase activity, as well as looking at combination therapy would greatly enhance the clinical durability of this novel series against serious Gram-positive infections.

P1596 Penetration of moxifloxacin and levofloxacin into human cerebrospinal fluid and brain tissue

A. Tsona, S. Metallidis, P. Selviaridis, P. Kollaras, N. Foroglou, P. Nikolaides (Thessaloniki, GR)*

Objectives: The aim of the present study was to determine the penetration of moxifloxacin and levofloxacin into cerebrospinal fluid(CSF) and brain tissue of humans.
Methods: A total of 38 patients undergoing hydrocephalus drainage or brain tumor excision were evaluated. 20 patients received a single intravenous dose of 400 mg moxifloxacin and 18 patients received a single intravenous dose of 500 mg levofloxacin. Samples of blood, CSF and brain(brain-adjacent tumour tissue) were collected during surgery 2 h after drug administration. Moxifloxacin and levofloxacin concentrations in serum, CSF and brain homogenate were analysed by means of a validated HPLC method.
Results: Mean concentrations of moxifloxacin in serum and CSF, respectively, were 3.67 μg/ml and 0.90 μg/ml. Mean concentrations of moxifloxacin in serum and brain, respectively, were 4.94 μg/ml and 9.90 μg/gr. Penetration index was 23% in cerebrospinal fluid and 216% in brain samples. Mean concentrations of levofloxacin in serum and CSF, respectively, were 3.49 μg/ml and 1.45 μg/ml. Mean concentrations of levofloxacin in serum and brain, respectively, were 3.53 μg/ml and 1.8 μg/gr. Penetration index was 37% in cerebrospinal fluid and 55% in brain samples.
Conclusions: Concentrations of moxifloxacin in CSF were lower than those in serum, in contrast to brain tissue concentrations that exceeded serum concentrations. Concentrations of moxifloxacin in brain were ten fold higher from concentrations in CSF. Concentrations of levofloxacin in CSF and brain were lower than those in serum.
Concentrations of levofloxacin in brain were higher from concentrations in CSF. Levofloxacin achieved better penetration in CSF and moxifloxacin in brain. These findings suggest that valuable informations on brain tissue penetration can be obtained only from brain material. Data from CSF penetration cannot be extrapolated to the brain since the blood: CSF barrier differs from the blood:brain barrier. Both moxifloxacin and levofloxacin are effective for the treatment of meningitis, postneurosurgical meningitis and brain abscesses caused by *H. influenzae, N. meningitidis*, *S. pneumoniae*, staphylococcus, streptococcus, Enterobacteriaceae, anaerobes because they achieve concentrations higher than the MIC90 in serum, CSF and brain tissue.

P1597 Monte Carlo simulations in support of NXL103 dose selection

M. Felices, V. Gualano, A. Tarral, C. Sable, H. Merdjan (Evry, Romainville, FR; Plymouth Meeting, US)*

Objectives: NXL103 is a novel oral streptogramin, a combination of linopristin (PI) and flopristin (PII) with activity against *S. aureus* (SA), including methicillin-resistant (MR) strains, *S. pneumoniae* (SP), including multidrug resistant strains, *H. influenzae*, and atypical pathogens. The study objective was to predict the probability of target attainment (PTA) for various combinations of dose regimen and MIC values, by using Monte Carlo (MC) simulations.
Methods: In murine models of thigh and lung infections, NXL103 efficacy demonstrated to be best correlated with the AUC24/MIC ratios of 14 and 32 for SA and SP, respectively. The MIC90 values for a contemporary population of SA and SP isolates were 0.25 and 0.5 microg/ml, respectively. As a result, the selected MIC values were 0.25, 0.5 and 1 microg/ml. The simulated dose regimens were 300, 500, 600 or 1000 mg BID, and 250, 500 or 600 mg TID. PI and PII were in a 5:7 dose ratio in the above NXL103 doses. Given the beneficial food-effect on NXL103 bioavailability, it was assumed that either all doses were given with food, or either one, or two, or three daily doses (TID only) were given fasted. For each dosing condition, 10,000 replicates of PI and PII AUCs were simulated from validated population pharmacokinetic models. The steady state AUC24 of NXL103 was derived, together with its distribution. Ultimately, the PTA was calculated as the proportion of the AUC24/MIC distribution above a given target. All calculations were done with SAS.
Results: The PTA is tabulated for the various dose regimens given in fed conditions and for both target AUC24/MIC values. Efficacious daily doses should not be changed if a maximum of one dose per day is taken fasted. These results specifically apply to the current tablet formulation of oral NXL103 containing PI and PII in a 5:7 dose ratio (ca 42:58).
Conclusion: A 500 mg BID dose regimen should be efficacious against SA with MIC values of 0.25 and 0.50 microg/ml, including CA-MRSA and HA-MRSA strains. Higher doses may be required to treat infections caused by SP strains.

	Target AUC_{24}/MIC = 14			Target AUC_{24}/MIC = 32		
Dose regimen	MIC = 0.25	MIC = 0.5	MIC = 1	MIC = 0.25	MIC = 0.5	MIC = 1
300 mg BID	0.93	0.53	0.11	0.43	0.07	0.00
250 mg TID	0.98	0.69	0.20	0.59	0.13	0.01
500 mg BID	1.00	0.89	0.40	0.82	0.31	0.04
600 mg BID	1.00	0.95	0.56	0.91	0.44	0.07
500 mg TID	1.00	0.98	0.72	0.96	0.61	0.14
600 mg TID	1.00	0.99	0.84	0.99	0.76	0.24
1000 mg BID	1.00	1.00	0.91	0.99	0.85	0.34

P1598 Pharmacokinetics and tolerability of NXL104 in normal subjects and patients with varying degrees of renal insufficiency

H. Merdjan, A. Tarral, W. Haazen, E. Evene, M. Robertson, C. Sable (Romainville, FR; Antwerp, BE; Paris, FR; Fordham, UK; Plymouth Meeting, US)*

Objectives: NXL104 is a broad spectrum class A and C β-lactamase inhibitor currently developed by Novexel in combination with ceftazidime (CAZ) for the treatment of serious infections due to resistant Gram-negative pathogens. Most of NXL104 elimination occurs by renal excretion of unchanged drug in urine, making NXL104 potentially sensitive to changes in renal function. The objectives were to investigate the pharmacokinetics (PK) and tolerability of a single 100 mg dose of NXL104 (30-min intravenous infusion) in normal subjects and in patients with varying degrees of renal impairment.
Methods: This was an open-label study conducted in 6 healthy male subjects (creatinine clearance CrCL >80 mL/min), and in patients of either sex (n = 6 per group) with mild (CrCL 50 to 79 mL/min), moderate (CrCL 30 to 49 mL/min), or severe renal impairment (CrCL <30 mL/min non anuric), and in anuric patients requiring hemodialysis (HD). Anuric patients participated in 2 randomised sessions 7 to 14 days apart: within and between 4-hour HD sessions. Blood samples for PK were collected for up to 44 h after the infusion start. During HD, arterial and venous blood was collected every 30 min for the determination of HD clearance (CL). Whenever possible, urine fractions were collected for measuring NXL104 renal CL. NXL104 was analysed in plasma and urine by using validated LC-MS/MS methods. PK interpretation was by non-compartmental analysis. Tolerability was assessed by physical and safety laboratory examinations, ECG, vital signs and adverse events (AEs) recording.
Results: Four mild episodes of possibly related AEs (general discomfort, stomach pain, ructus, transient symptoms of hypoglycaemia in an insulin-dependent patient) were recorded in 3 anuric patients. NXL104 total CL was found to decrease as a function of CrCL: 14.6±1.2 L/h in normal subjects, 5.8±1.6 L/h in mild renal impairment, 3.8±0.6 L/h in moderate impairment, 2.2±0.9 L/h in severe impairment, and 1.0±0.8 L/h in anuric patients. Based on published data quantitatively similar changes are seen in CAZ CL. NXL104 HD CL was 9.3±0.1 L/h resulting in 54% of the dose being removed by a 4-hour HD session.
Conclusion: NXL104 was well tolerated in renally impaired patients. CrCL was a major determinant of NXL104 PK. The findings suggested that CAZ and NXL104 doses should be adjusted to the same proportions in renally impaired patients. NXL104 should preferably be administered after the end of an HD session.

P1599 Combined population pharmacokinetic analysis of four phase 1 studies with NXL104

M. Felices, V. Gualano, A. Tarral, C. Sable, H. Merdjan (Evry, Romainville, FR; Plymouth Meeting, US)*

Objectives: NXL104 is a broad spectrum class A and C β-lactamase inhibitor currently developed by Novexel in combination with ceftazidime for the treatment of serious infections due to resistant Gram-negative pathogens. This analysis was conducted to characterise the pharmacokinetics (PK) of NXL104 in healthy subjects and renally impaired patients, and to identify the covariates that influence its PK parameters.
Methods: A population (Pop) PK analysis was performed by combining the data from 4 Phase 1 studies totalling 136 healthy subjects and 24 patients with varying degrees of renal impairment. The dataset included some diversity with regard to age, sex, unit doses, dosing duration, concomitant medications and creatinine clearance (CrCL). Two basic models were built: one based on plasma concentrations only, the other one incorporating both plasma and urine concentrations of NXL104. A distinction was made a priori between primary and exploratory covariates. Data were analysed using the NONMEM software.
Results: Based on plasma data only, the structural PK model was a two-compartment model parameterised in terms of total (CL) and intercompartment (Q) clearances, central (V1) and peripheral (V2) volumes of distribution. The typical values for non-renal clearance, Q, V1 and V2 were 0.456 L/h, 6.27 L/h, 13.8 L and 7.20 L, respectively. The between-subject variability on CL, Q and V1 was 20%, 30%, and 31%, respectively. The residual error model was mixed, with proportional and constant terms of 26% and 0.2 ng/mL, respectively. The most significant primary covariate was CrCL (+9.65 L/h on CL per 100 mL/min increment of CrCL). In addition, a significant age-effect was found on CL (+0.21 L/h per 10-yr increment) and was of low enough magnitude not to warrant any dose adjustment as a function of age in adults. No primary covariate was found to influence V1 or V2. The effects of unit dose and treatment duration were not significant, suggesting that NXL104 PK is directly dose proportional and time-invariant. The model based on joint plasma and urine data led to essentially similar results. Validation tests demonstrated the descriptive performance of these Pop PK models.
Conclusion: This Pop PK analysis done with Phase I data allows the use of a sparse sampling approach for estimation of individual PK parameters in further studies. It demonstrated the strong relationship between CrCL and NXL104 CL.

P1600 Rationale of combined antibiotic therapy derived from *in vitro* PK/PD studies: role of treatment duration simulated in a dynamic model

S. Zinner, M. Smirnova, E. Strukova, Y. Portnoy, A. Firsov (Cambridge, US; Moscow, RU)*

Objective: Duration of simulated treatments with antibiotics may be critical in searching for optimal antibiotic combinations. To study the possible impact of this factor on the delineation of the pattern of interaction between linezolid (LZD) and doxycycline (DOX), the effects of LZD+DOX on *Staphylococcus aureus* were examined at different times after the start of treatment at varying antibiotic exposures.
Methods: *S. aureus* ATCC 43300 (MIC of LZD 2 mg/L, MIC of DOX 0.25 mg/L) was exposed to twice-daily LZD (ratios of 24-hour area under the curve (AUC) to the MIC 25, 50 and 150 h), once-daily DOX (AUC/MICs 50, 100 and 270 h) or their combinations (LZD/DOX 25 h/50 h, 50 h/100 h and 150 h/270 h) for 5 days. Mono-exponential concentration-time profiles of LZD and DOX given alone and in combination were simulated with half-lives of 6 and 15 h, respectively. The central compartment of the model was multiply sampled to count surviving organisms on antibiotic-free media. The antimicrobial effects were expressed by areas between the bacterial concentration – time curves with and without antibiotic (ABBCs) calculated from time zero to 1, 2, 3, 4 and 5 days after the start of treatment.
Results: Regardless of the observation period, the effects of LZD+DOX at the minimal and intermediate AUC/MIC ratios were greater than those of LZD and DOX given alone at the respective AUC/MICs throughout the entire time courses of the ABBC. At the maximal AUC/MIC ratios that approach clinically achievable values, the advantages of the combination over monotherapy with LZD were seen only on the 4th day, when the ABBC with LZD+DOX was 30% greater than the ABBC of LZD. A more pronounced (43%) increase in the antimicrobial effect of LZD by DOX was observed at the end of treatment.
Conclusions: These data suggest that (1) combined treatment with LZD+DOX is more beneficial at relatively small AUC/MIC ratios, and (2) synergistic interactions between LZD and DOX were revealed only in long-term simulations.

P1601 Bacterial resistance studies in an *in vitro* dynamic model: use of antibiotic-susceptible organisms supplemented with their resistant mutants

A. Firsov, M. Smirnova, E. Strukova, Y. Portnoy, S. Zinner (Moscow, RU; Cambridge, US)*

Objective: Bacterial resistance studies using *in vitro* dynamic models are highly dependent on the starting inoculum (SI) that might or might not contain spontaneous resistant mutants (RM)s. To standardize the initial experimental conditions, SI can be reconstructed using antibiotic-susceptible bacteria supplemented with its RM. This method was examined with linezolid-exposed *Staphylococcus aureus*.
Methods: To obtain RMs, a clinical isolate of *S. aureus* (MIC of linezolid 2 mg/L) was serially passaged in Mueller-Hinton broth containing successively increasing concentrations of linezolid (from 1 to 512 mg/L). To optimize the relative content of the parent strain and its RM in the SI, the mutant prevention concentrations (MPCs) were determined with and without RMs. Using an optimized inoculum (parent strain/RM), five-day treatments with twice daily linezolid were simulated at concentrations either between the MIC and MPC or above the MPC for most of the dosing interval. Selection of RMs was detected by population analysis and susceptibility testing (culture MICs).
Results: RMs were enriched starting with the 5th-6th passage, with continued loss in susceptibility up to the 15th passage. RMs obtained after the 8th (RM8) and 15th (RM15) passages had MICs of 8 and 128 mg/L, respectively, and these MICs were stable after 15 passages on antibiotic-free plates. The presence of RM15 (2 and 4 log CFU/ml added to 10 log CFU/ml of the parent strain) dramatically increased the MPC. Unlike RM15, the MPCs determined with and without 2 log (but not 4 log) CFU/ml of RM8 were similar (14 mg/L). At a SI of 8 log CFU/ml (parent strain) plus 2 log CFU/ml (RM8) exposed to linezolid concentrations between the MIC and MPC but not at concentrations above the MPC, RMs were enriched over the 5 days. This enrichment was accompanied by the concomitant loss in susceptibility.
Conclusions: Data obtained with the reconstructed SI support the mutant selection window hypothesis. This method provides a standardization of resistance studies using *in vitro* models. However, the relative amounts of susceptible and resistant subpopulations are critical for the prediction of anti-mutant dosing regimens.

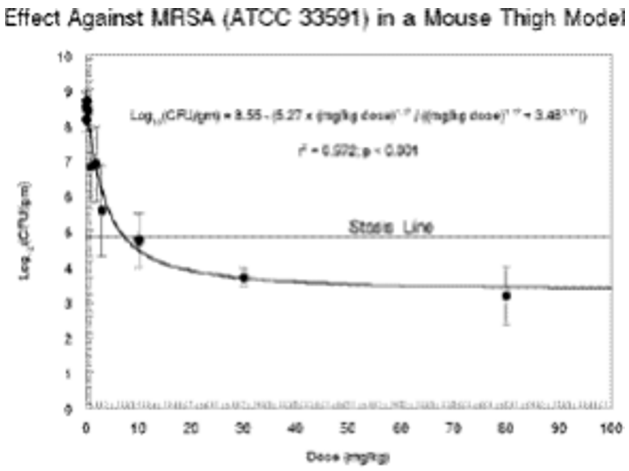
P1602 Mouse thigh MRSA infection model data and mathematical modelling to determine telavancin dosing for complicated skin and skin structure infection trials

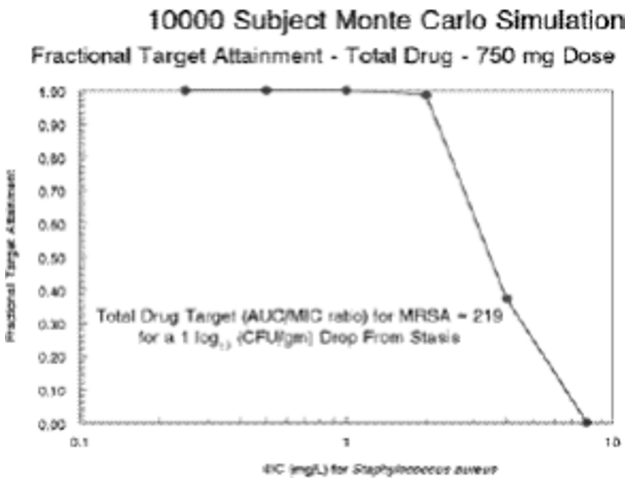
T. Lodise, N. Patel, S. Hegde, S. Barriere, J. Shaw, G. Drusano (Albany, San Francisco, US)*

Objectives: Telavancin is a lipoglycopeptide approved in the US and Canada for complicated skin and skin structure infection (cSSSI) due to Gram-positive bacteria, including MRSA. The objectives were to (1) identify the pharmacodynamic (PD) target using a mouse thigh infection model against MRSA and (2) conduct population (pop) PK modeling

and Monte Carlo simulation (MCS) to determine an optimal telavancin dose for cSSSI trials. Specifically, the goal was to identify a dosing regimen that had >90% probability of achieving the desired PD target derived from the mouse thigh infection model for organisms with MICs <2 mg/L. An MIC value of 2 mg/L was selected for the probability of target attainment (PTA) analysis because it is 4-fold higher than the telavancin MIC90 for MRSA.

Methods: The neutropenic mouse thigh infection model was used to identify the PD linked variable for telavancin. Mouse thigh infection studies were performed for a single MRSA strain (ATCC 33591; MIC = 1 mg/L). A number of dosing regimens (10–16 mice per dosing cohort) were evaluated. An inhibitory sigmoid-Emax model was fit to the data using ADAPT II and PK parameters were identified using BigNPAG. Total drug exposure target associated with a −1 log10 (CFU/gm) drop from stasis served as the PD target for the MCS analysis. Telavancin plasma concentration-time profiles from 73 volunteers were modeled using a 2-compartment model with zero-order infusion & 1st order elimination & transfer. Model parameters were identified in a pop PK analysis (NPAG). MCS (ADAPT II) of 9999 subjects was used to identify a telavancin dose that provided a >90% probability of achieving the PD target for MICs ⩽2 mg/L.





Results: The PD target associated with −1 log10 (CFU/gm) drop from stasis in the mouse thigh infection model was a total AUC/MIC ratio of 219 (figure). In the pop PK analysis, mean (SD) values for volume, clearance, K12 and K21 were: 4.79 (2.46) L, 0.99 (0.28) L/hr, 1.51 (0.74) hr-1, and 1.14 (0.52) hr-1 respectively. In the PTA analysis, telavancin doses of 750 mg IV Q24H provided a >90% probability of achieving a total AUC/MIC of 219 for organisms with MIC values ⩽2 mg/L (figure).

Conclusions: Based on the animal data and mathematical modeling, the telavancin dose selected for cSSSI trials was 10 mg/kg/day (~750 mg daily for normal weight subjects). This regimen had a >95% probability of achieving an AUC/MIC ratio of 219 for organisms with MICs less than or equal to 2 mg/L.

P1603 Aerosol therapy with colistin: a biopharmaceutical concern as illustrated in rats

S. Marchand, D. Thi Viet Phuong, P. Gobin, J. Olivier, O. Mimoz, W. Couet (Poitiers, FR)*

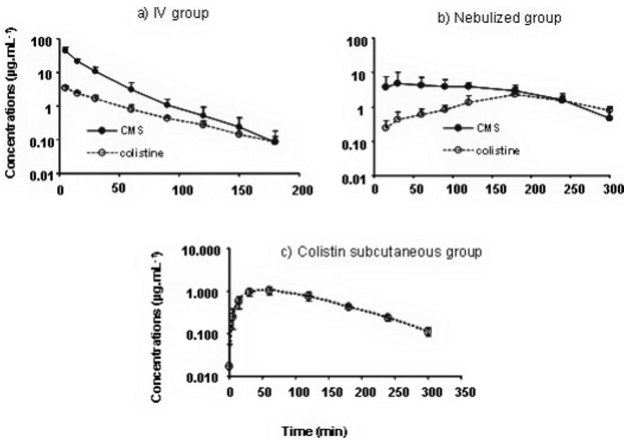
Objective: The aim of this study was to investigate the pharmacokinetics of colistin methanesulphonate (CMS) and colistin following CMS nebulisation in rats.

Methods: Male rats (280–320g) received (i) a CMS dose of 15 $mg \cdot kg^{-1}$ either as an intravenous (IV) bolus (n = 6) or (ii) by nebulisation using a MicroSprayer IA-1B® system (Penn Century Inc, Philadelphia, US) (n = 6), or (iii) colistin subcutaneously (SC) at a dose of 1.5 $mg \cdot kg^{-1}$. Multiple blood samples were drawn from a catheter implanted the day before through anaesthesia in a femoral artery, for up to 300 min. CMS and colistin plasma concentrations were determined by a new validated LC-MS/MS assay. Pharmacokinetics parameters were estimated using a non-compartmental analysis.

Results: Mean±SD concentrations of CMS and or colistin are illustrated below (TABLE).

Colistin clearance was estimated to 8.5±1.0 mL/min/kg after SC administration, assuming complete bioavailability and allowing to estimate that the fraction of CMS converted to colistin after CMS IV administration was only equal to 13% on average. It could then be estimated that after CMS nebulisation 69% of the dose reached directly the systemic circulation and that 30% was converted within the lung before being absorbed. Therefore colistin area under curve was higher after CMS nebulisation (486±170 μg·mL/min) than after IV administration of CMS at the same dose (160±20 μg·mL/min), meaning that colsitin exposure was 3 folds greater after nebulisation than IV administration of CMS.

Conclusion: Because colistin is nebulised as a prodrug (CMS), conversion to the active moiety within the lung is necessary to provide efficacy. Yet because systemic conversion of CMS into colistin is limited to 13% on average, and because colistin formed within lungs (30% of the dose) is eventually totally absorbed, colistin systemic exposure is greater after nebulisation and therefore toxicity may also be greater. Although this observation should not be directly extrapolated to humans, this issue deserves consideration.



P1604 **Impact of area-under-the-inhibitory-curve on clinical and microbiologic outcomes in fluconazole treated candidaemia**

B. Footer, S. Monte, L. Podkulski, G. Prescott, C. Rotstein, J. Brown, J. Paladino, J. Schentag (Buffalo, US; Toronto, CA)*

Objectives: The goal of this case series was to identify an AUIC breakpoint for fluconazole that links pharmacokinetics and pharmacodynamics with microbiologic and clinical outcomes as well as time to organism eradication in adults with candidemia.
Methods: This study was a retrospective, case series of adult patients (>18 years)with an index *Candida* bloodstream infection and sufficient demographic, laboratory and microbiologic data to calculate serial AUICs and determine clinical and microbiologic outcome. Patients had to have received at least three days of fluconazole. Because failures and successes were enrolled, time to eradication for both groups in relation to PK/PD parameters could be examined. Clinical and pharmacokinetic information was used to determine the optimal AUIC breakpoints (25, 50, 75, 100, 250, and 400) that were predictive of microbiologic and clinical outcomes, as well as more rapid fungal eradication.
Results: For all *Candida* species, the AUIC 100 breakpoint was descriptively and statistically superior to breakpoints <100 for microbiologic and clinical outcomes as an AUIC >100 versus those <100 demonstrated greater microbiologic eradication (72% vs. 56%; p = 0.122) and clinical success (61% vs. 39%; p = 0.067). Similar to all species, the AUIC 100 breakpoint was descriptively and statistically superior to other breakpoints <100 for both outcomes in non-*albicans* species. The AUIC >100 breakpoint was significantly associated with greater microbiologic eradication (78% vs. 44%, p = 0.035) and clinical success (81% vs. 28%, p = 0.05). Higher AUICs did not provide additional microbiologic or clinical benefit for both the all *Candida* and non-*albicans* species. In terms of time to eradication, an AUIC >100 tended to be associated with a more rapid time to eradication versus those <100 in the all *Candida* species group (p = 0.095). For non-*albicans* species a statistically significant more rapid time to eradication in those patients with an AUIC >100 was achieved (p = 0.004).
Conclusion: An AUIC >100 appears predictive of greater microbiologic eradication, positive clinical outcomes, and time to eradiation for all known *Candida* species. A statistical relationship existed for microbiologic eradication and time to eradication in non-*albicans* species at the AUIC >100 breakpoint. Further research is needed to correlate these findings with venous access catheter removal to assess that effect on microbiologic, clinical, and time to eradication outcomes.

P1605 **The effect of posaconazole on the pharmacokinetics of midazolam and simvastatin in healthy volunteers**

G. Krishna, L. Ma, P. Prasad, A. Moton, M. Martinho, G. Weiner, E. O'Mara (Kenilworth, Miami Gardens, US)*

Objectives: To determine the effect of posaconazole (POS) on the pharmacokinetics (PK) of simvastatin (SMV) and characterize the CYP3A4 inhibitory potential of POS for a range of doses with concomitant administration with midazolam (MDZ).
Methods: This was a randomized, fixed-sequence, parallel-group, single-site, open-label study. Healthy male and female subjects (n = 35) received POS 50 mg, 100 mg, or 200 mg once daily (QD) for 13 days. In all groups, subjects received POS plus 1 dose of MDZ 2 mg on day 8 and POS plus 1 dose of SMV 40 mg on day 11. Subjects also received 1 dose of MDZ 2 mg alone on day −9 and SMV 40 mg alone on day −6. Blood samples for assessment of MDZ, SMV and SMV acid were collected at predetermined time points. Log-transformed PK parameters for POS, MDZ, SMV and SMV acid were analysed separately for each treatment group using ANOVA model to extract the effect due to treatment and subject.
Results: Coadministration of multiple oral administrations of POS significantly increased MDZ, SMV and SMV acid exposure (Table). MDZ Cmax increased by 2.0-, 2.4- and 2.7-fold and AUC by 3.1-, 4.0- and 5.7-fold, respectively, after POS 50 mg, 100 mg and 200 mg QD, compared with MDZ alone. MDZ median Tmax ranged from 0.5 to 1 h. MDZ terminal phase half-life ($t^1/_2$) significantly increased from 4.1 h with SMV alone to 6.8, 8.7 and 10.7 h, respectively, after POS 50 mg, 100 mg or 200 mg QD. The variability (%CV) for MDZ Cmax and AUC ranged from 18% to 46%. SMV Cmax increased by 7.4-, 9.4- and 11.4-fold and AUC by 5.7-, 10.3- and 10.6-fold, respectively, after POS 50 mg, 100 mg and 200 mg QD, compared with SMV alone. SMV median Tmax ranged from 1 to 2 h; $t^1/_2$ was slightly lower with coadministered POS than with SMV alone. SMV %CV for Cmax and AUC ranged from 42% to 89%.
SMV acid Cmax increased by 5.5-, 9.2- and 9.5-fold and AUC by 5.4-, 7.3- and 8.5-fold, respectively, after POS 50 mg, 100 mg and 200 mg QD, compared with SMV alone. SMV acid median Tmax ranged from 3 to 4 h; $t^1/_2$ was slightly lower with coadministered POS than with SMV alone. SMV acid %CV for Cmax and AUC ranged from 46% to 67%.
POS 50 mg, 100 mg and 200 mg was safe and well tolerated when coadministered with MDZ and SMV.
Conclusions: Coadministration with POS increased plasma concentration of MDZ, SMV and SMV acid, which is consistent with inhibition of CYP3A4-mediated metabolism of MDZ, SMV and SMV acid by POS.

Analyte	Treatment comparison	POS dose (mg)	Ratio estimate (%)	90% CI
MDZ	MDZ + POS vs MDZ	50	305	257–362
		100	399	306–519
		200	570	482–674
SMV	SMV + POS vs SMV	50	565	443–722
		100	1031	840–1267
		200	1060	863–1302
SMV acid	SMV + POS vs SMV	50	535	418–684
		100	734	582–925
		200	848	704–1023

Gram-negative infections: clinical and PK/PD studies

P1606 **What is the impact of a rapid-diagnostic test (E-test) in the treatment of patients with Gram-negative bacteraemia?**

E. Moral-Escudero, E. García-Vázquez, A. Fernández-Rufete, A. Hernández-Torres, J. Ruiz, G. Yagüe, H. Albendín-Iglesias, J.A. Herrero, J. Gómez (Murcia, ES)*

Objectives: To evaluate influence of a rapid-diagnostic test (RT) in antibiotic (AB) therapeutic decisions in non-paediatric patients with Gram negative bacteraemia (GNB. Patients and Methods: A RT (validated in 5 hours)consisting in direct antibiogram (Mueller-Hinton agar) was done in blood isolates of GNB. AB used in the E-test were ciprofloxacin, cefotaxime, cefepim, cefepim-clavulanic, and imipenem. GNB were also identified and sensitivity test performed by standard criteria (McFarland). RT information was provided at the physician in a 24 hour-working routine (<24 hours after blood cultures were obtained). Data collection has been done according to a standard protocol (epidemiological, clinical, microbiological and laboratory data). Information about empirical treatment was registered (T1) as well as the AB administered once the information of RT was provided (T2) and on the ideal AB the Infectious Diseases consultant would have prescribed (considering not only sensitivity results but also clinical characteristics, localization of infection and minimum "ecological" impact) (T3). Decision about T2 was always taken by the physician in charge or the physician on duty. The economic cost of 72 hours (mean "won time" by RT compared to standard sensitivity test) of T1, T2 and T3 was calculated according to the price of AB provided by our Pharmacy Department; RT cost was 14€.
Results: RT was performed in 99 patients; one blood culture yield 2 different GNB; 4 patients died before RT results were available (excluded for the analysis); 5 patients died due to non-infectious complications and 9 died with septic shock (mortality 18.2%). Microbiological isolates

were: *E. coli* (60%; 19.3% ESBL), *K. pneumoniae* (10.5%; 2% ESBL), *P. aeruginosa* (10.5%) and other enterobacteria 13.7%. Main AB used in T1 were quinolones (14%), 3rd generation cephalosporins (16%), carbapenems (15%) and piperacillin–tazobactam (28.3%). T1 was considered adequate in 26.3% and T2 in 60%. Economical cost of 72 hours of T1, T2 and T3 per patient was 70.6€, 69.9€ and 44.2€; the economical cost of T3 including 14€ of the RT is 58.2€.

Conclusions: In an era of increasing MDR-GNB, RT provides early and useful information about AB treatment options; this data should be interpreted by an Infectious Diseases specialised physician to make information economically and "ecologically" profitable. New diagnostic techniques are not cost-effective if they are not properly interpreted.

P1607 Colistin plus rifampin or other antibiotics in 90 patients with serious MDR *Ps. aeruginosa* or *A. baumannii* infection

S. Fondelli, C. Tascini, P. Malacarne, F. Giunta, B. Viagi, F. Forfori, P. Lambelet, F. Fallani, A. Novelli, E. Tagliaferri, A. Leonildi, F. Menichetti (Pisa, Viareggio, Florence, IT)*

Background: Best Colistin (C) combination therapy for infections due to MDR non fermenter Gram-negative bacilli has not been established as well as the best dose-schedule (tid vs. od).

Methods: We reviewed patients (pts) treated with C at Pisa Hospital from 2001 to 2009 analyzing clinical and microbiological outcome, adverse events and dose schedule.

C was administered as 3 M tid or 9 M od. Agents used in combination were Rifampin (R) (600 mg/day) or other antibiotics (OA) (mostly carbapenem).

Cure was defined as resolution of signs of infection (SI); improvement as reduction of SI; and failure as death or increasing of SI. Microbiological eradication was considered only when culture control resulted negative; microbiological failure was defined a persistence of the causative pathogen. Renal and neurological toxicity was studied. Serum calcium concentration was recorded in all pts and correlated with clinical outcome.

Results: In the study period 90 pts (males 67, females 23, mean age 57.6) with 98 infections caused by MDR/PDR Gram-negative rods (*P. aeruginosa* 58%; *A. baumannii* 35%) were treated with C in combination. Infections were represented by: VAP 36%, SSTI 27%, Sepsis 15%, Pneumonia 12%, Osteomyelitis 5%, other infections 5%. C was administered intravenously for 26±12 days (D). The overall clinical response was obtained in 69% of patients (38% C, 31% I) and microbiological eradication was obtained in 55% of cases. Clinical and microbiological response in pts treated with C+R or C+OA are shown in the table. C od (9 M) was administered in 11 pts for a mean period of 17 days without adverse events or emergence of resistant strains. Mean Cmax±SD was 79±39 mg/L. All pts with favourable clinical outcome had serum calcium concentration >8.6 mg/dL. Renal toxicity was noted in 3% of pts.

Conclusions: C+R or C+OA seems to have similar clinical efficacy but C+R seems to be most effective in sepsis and in microbiological eradication in VAP. Colistin od provide Cmax concentration well above the breakpoint for MDR Gram-negative rods. Serum calcium concentration seems to influence the clinical outcome.

Table 1. Clinical and microbiological response in pts treated with C+R or C+OA

	C+R		C+OA	
	CR	MR	CR	MR
Overall	32/45 (71%)	27/45 (60%)	35/53 (66%)	26/53 (50%)
VAP (35)	7/13 (54%)	9/13 (69%)	16/22 (73%)	8/22 (36%)
Sepsis (15)	5/5 (100%)	4/5 (80%)	3/10 (30%)	5/10 (50%)
SSTI (26)	10/16 (63%)	9/16 (57%)	9/10 (90%)	8/10 (80%)

P1608 Targeting multidrug-resistant *Pseudomonas aeruginosa*: pharmacodynamics of the combination of colistin and ciprofloxacin

J. Li, A. Poudyal, H. Yu, R. Owen, J.B. Bulitta, A. Forrest, B. Tsuji, R. Nation (Melbourne, AU; Albany, Buffalo, US)*

Objective: Multidrug-resistant (MDR) *Pseudomonas aeruginosa* (Pa) is presenting a global medical challenge due to lack of new antibiotics. Rational combinations (combos) must be considered to minimize development of resistance particularly to colistin (COL), a last-line therapy. This study evaluated the pharmacodynamics (PD) of COL and ciprofloxacin (CIP), both alone and in combo, using a rational approach.

Methods: MICs of COL and CIP were measured with microbroth dilution. Against 12 strains (3 COL susceptible (COL^S), 6 heteroresistant (COL^{HR}) & 3 resistant (COL^R)), checkerboard synergy panel studies were conducted with COL/CIP and viable counting was performed at 24 h to enhance detection of synergy for translation to static time-kill (STK) studies. COL/CIP were evaluated in STK studies against 1 COL^S, 4 COL^{HR} and 1 COL^R strains with an inoculum of ~10^6 CFU/mL: 8 concentrations for COL alone & CIP alone, and 9 combos of COL/CIP (low, mid & high for each) up to 64 × MIC for S and HR strains or 16 mg/L for R strains. Viable counting was done up to 48 h and COL population analysis profiles (PAPs) were examined at 48 h.

Results: MICs of COL and CIP were 0.5 to >128 mg/L and <0.125 to 32 mg/L, respectively. COL/CIP fractional inhibitory concentrations were >0.5 for most strains. However, COL 2 mg/L/CIP 2 mg/L (clinically achievable for COL and CIP) inhibited visible growth of all 12 strains; for the strain resistant to both COL and CIP, viable counting revealed COL 2 mg/L/CIP 2 mg/L achieved substantial killing (to 2.45 log CFU/mL at 24 h). In STK, COL alone showed rapid initial killing (up to ~6 log) against all strains except COL^R Pa. Good killing was also observed with CIP alone against CIP^S strains. For COL or CIP alone, substantial regrowth occurred at 48 h for some strains even at 64 × MIC. Generally the COL/CIP combos with mid and high COL showed synergistic killing (up to ~6 to 7 log kill within 4 h or at 48 h) against most strains. PAPs showed emergence of resistance in 3 COL^{HR} strains with low COL alone and in only 1 COL^{HR} strain with the low COL/low CIP combo.

Conclusions: A COL/CIP combo is increasingly being used against MDR Pa, in particular in cystic fibrosis clinics. This study highlights the importance of optimizing the absolute and relative concentrations of COL and CIP.

P1609 A semi-mechanistic pharmacokinetic–pharmacodynamic model with adaptation development for *in vitro* activity of ciprofloxacin against *Pseudomonas aeruginosa*

N. Grégoire, S. Raherison, C. Grignon, E. Comets, M. Marliat, M.C. Ploy, W. Couet (Poitiers, Limoges, Paris, FR)*

Objective: To implement pharmacokinetic–pharmacodynamic (PK-PD) model to describe over time the effects of ciprofloxacin (CIP) against *P. aeruginosa in vitro*.

Methods: Time-kill curves were generated over 24 h with an inoculum of 5×10^6 CFU/mL at concentrations from 0.5 to 16×MIC. *P. aeruginosa* susceptibility to CIP was assessed before and after 24 h exposure to CIP: (i) modifications of the DNA gyrase and topoisomerase IV enzymes caused by mutations in Quinolone Resistance Determining Region of gyrA and parC subunit genes, were investigated using PCR, (ii) CIP efflux pumps activation was tested by addition of Phenyl Arginine β-naphthylamide, a known inhibitor of Mex multidrug efflux system implicated in fluoroquinolones active efflux in *P. aeruginosa*. A previously described PK-PD model (1), in which the concentration necessary to achieve 50% maximal kill rate (EC50) increased as a function of antibiotic concentration and time, to account for bacteria adaptation, was fitted to the time-kill data. A population approach was used with Nonmem® software.

Results: At intermediate CIP concentrations, microbial regrowth was observed after initial killing. Sub-inhibitory CIP concentrations (⩽ MIC) favoured the emergence of mutants with increased but moderate resistance to CIP (MIC 8 fold increase), only by over-expression of Mex efflux pumps. The selected PK-PD model with adaptation adequately described the microbial response to CIP in the range of concentrations investigated.
Conclusion: Susceptibility of *P. aeruginosa* to CIP may rapidly decline after multidrug efflux pump activation. This phenomenon was adequately described by a PK-PD model with adaptation. Resistance development over longer period of time should now be investigated using dynamic approaches (hollow-fiber model) to mimic multiple dosing treatments.
Reference(s)
[1] V.H. Tam et al., J. Antimicrob. Chemother. 55: 699–706 (2005)

P1610 Pharmacokinetic/pharmacodynamic analysis using Monte Carlo simulation to evaluate cumulative fraction of response of doripenem against *Pseudomonas aeruginosa* and Enterobacteriaceae

A. Canut, A. Isla, M.A. Solinís, A. Labora, A. Rodríguez-Gascón (Vitoria, ES)*

Objective: Carbapenems are widely used therapies for serious infections involving *Pseudomonas aeruginosa* and multidrug-resistant Enterobacteriaceae. The main objective of this study is to calculate the probability of attaining targeted pharmacodynamic exposure for two intermittent infusion rates of doripenem against *P. aeruginosa* and ESBL phenotype Enterobacteriaceae or Enterobacteriaceae with stably depressed AmpC production.
Methods: Susceptibility data to doripenem of clinical *P. aeruginosa* isolates; ESBL phenotype *E. coli*, K, pneumoniae, K, oxyotca and *P. mirabilis;* and *E. cloacae*, *E. aerogenes, C. freundii, S. marcescens* and *M. morganii* with stably depressed AmpC production were obtained from recently published data (Castanheira et al, 2009, Mendes et al, 2009). Four dosing regimens consisting on 500 mg every 8 h or 1000 mg every 12 h with short (1 h) or prolonged (4 h) duration of infusion were evaluated. Percentage of the dosing interval duration for which concentrations of the antibiotic were above the MIC of the pathogen (T>MIC) was used as the pharmacodynamic parameter to predict doripenem efficacy (target of 40% of the dose interval). For each infusion rate 20,000 patients were simulated based upon a log-normal distribution of volume of distribution and elimination half-life. Mean pharmacokinetic parameters and their distribution were extrapolated from published studies. Cumulative fraction of response (CFR) for the requisite pharmacodynamic target was calculated considering the distribution of MICs for each of the species under the current study.
Results: Against *P. aeruginosa*, at least 1000 mg every 12 h, administered as a short infusion were necessary to obtain a CFR⩾90%, obtaining lower success rates with the 500 mg dosage regimens, principally due to the high frequency of strains with MIC ⩾4 mg/L. Calculated CFRs were higher than 90% against all Enterobacteriaceae with all perfusion rates (the MIC90 values were <1 for all species).
Conclusion: Taking into account the latest published susceptibility patterns, the probability of success against *P. aeruginosa* is only higher than 90% when 1000 mg are administered every 12 h. In contrast, doripenem 500 mg administered as 1 h perfusion is enough to achieve a high likelihood of obtaining the pharmacodynamic target against multidrug-resistant Enterobacteriaceae isolates.

P1611 Rational design of colistin and ciprofloxacin combination regimens against *Pseudomonas aeruginosa* using mechanism-based models

J.B. Bulitta, A. Forrest, A. Poudyal, H. Yu, R. Owen, J. Li, B. Tsuji, R. Nation (Albany, Buffalo, US; Melbourne, AU)*

Objectives: The present lack of efficacious treatment options against severe infections by multidrug-resistant (MDR) *Pseudomonas aeruginosa* (Pa) causes a global healthcare problem. Our aim was to develop a rational approach to optimize combination regimens with colistin (COL) and ciprofloxacin (CIP) against Pa using mechanism-based models (MBM).
Methods: Population pharmacodynamic (PD) modelling using the MC-PEM algorithm in S-ADAPT (V1.56) was applied to evaluate killing of the predominant subpopulation by COL & CIP alone or in combination. We modelled quantitative viable counts and turbidity data from checkerboard synergy panel studies at 24 h for 12 Pa strains (see abstract number 2063). Time-kill (TK) data (inoculum: 10^6 CFU/mL; 8 samples over 48 h per profile) on one COL-susceptible (COL^S), one COL-heteroresistant (COL^{HR}) and one COL-resistant (COL^R) strain with 8 concentrations of COL or CIP alone and 9 concentrations of COL/CIP in combination were modelled in NONMEM VI. MBM with up to 6 subpopulations were developed and fitted to all data of one strain simultaneously. These MBM assessed the degree and potential mechanisms of interaction.
Results: MBM of the synergy panel data indicated additive killing by CIP & COL for 10 strains, synergy for one COL^S strain, and antagonism for one COL^S strain. MBM of TK data yielded unbiased and reasonably precise population predictions (slopes: 0.99 to 1.00, r^2: 0.79 to 0.96). Killing was described by a Hill-function for CIP and by a 2nd-order function for COL. For the mechanism of synergy, COL effectively reduced the EC50 of CIP to 10–13% of that in the absence of COL for the CIP^R subpopulation against the COL^S and COL^R strain. A reduction of CIP-EC50 to 35% was achieved against the CIP^S subpopulation of the COL^R strain. No reduction was seen for the CIP^S and $CIP^{intermediate}$ subpopulation of the COL^S strain. A half-maximal reduction of CIP-EC50 was achieved by 0.88 mg/L COL. Killing of the COL^{HR} strain by COL and CIP was additive.
Conclusions: MBM of the synergy panel data correctly predicted largely additive effects of COL & CIP against the predominant susceptible subpopulation. For 2 of 3 strains, the time-kill modelling identified a reduction of the CIP-EC50 at low COL concentrations against the CIP^R population as the most likely mechanism of synergy. This may be explained by COL increasing intracellular concentrations of CIP.

P1612 Carbapenem-resistant *Acinetobacter baumannii* pneumonia: meropenem–rifampicin combination versus colistin

J.Y. Song, W. Choi, J. Heo, J. Noh, D.W. Park, J.W. Sohn, W.J. Kim, M.J. Kim, H.J. Cheong (Seoul, KR)*

Background: Carbapenem-resistant *Acinetobacter baumannii* has become a major nosocomial pathogen during the past years, and pneumonia is the most serious nosocomial infection with limited therapeutic options.
Methods: A retrospective study was performed on 51 adult patients with carbapenem-resistant *A. baumannii* pneumonia admitted to three Korea University Hospitals from September 2006 through August 2009. Clinical and microbiological outcomes of meropenem-rifampicin treatment were compared with those of colistin treatment.
Results: Out of 51 patients with carbapenem-resistant *A. baumannii* pneumonia, 22 were treated with meropenem-rifampicin and 29 with colistin. No significant difference was found between two groups regarding the baseline characteristics (demographic findings, co-morbidities, severity of illness, etc) and laboratory findings. Meropenem-rifampicin showed better clinical cure rates (77.3% versus 20.7%, $p < 0.01$) and lower 30-day mortality rates (13.6% versus 65.5%, $p < 0.01$); mean intervals from antibiotic start to death were 15.0±3.4 days and 15.9±8.1days respectively ($p = 0.86$). Microbiological eradication rates were 68.2% for the meropenem-rifampicin group and 20.6% for the colistin group ($p < 0.01$).
Conclusion: Meropenem-rifampicin was better effective than colistin in treating carbapenem-resistant *A. baumannii* pneumonia. Considering poor microbiological eradication rates, new antimicrobial agents and combinations need to be investigated.

Antimicrobial resistance in Gram-negative bacteria

P1613 Clinico-epidemiologic and molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. causing infections at five Indian hospitals, 2007–2009

D. Mathai, A. Kapil, K. Radhakrishnan, S. Parija, D. Mendiratta, K. Prasad, J. Hepsibah, K. Mayank, A. Manoharan on behalf of the ICMR-ESBL Study Group*

Objective: The association with community acquisition (CA), nosocomial (NO) occurrence, clinical risk factor and treatment outcome of blood stream (BSI), Respiratory tract (RTI), urinary tract (UTI) and Skin and soft tissue including surgical site (SSTI) infections caused by ESBL producing Gram-negative bacilli (GNB) that are endemic in Indian hospitals remains poorly characterized.
Methods: We determined ESBL prevalence, clinical risk factor and treatment outcome among 1849 [*Escherichia coli* (EC) (n = 984), *Klebsiella* spp., (KS) (710) and EC (155)] isolates that were consecutively collected at five tertiary care hospital laboratories causing: 636 UTI; 522 BSI; 348 RTI and 343 SSTI. During May 2007 to September 2009 (28 month) these isolates were forwarded to BMPLIII for susceptibility testing against 13 antimicrobials (five classes) by disk diffusion and Etest methods and using CLSI interpretive criteria. Multidrug resistant (MDR), if resistant ⩾2 classes. PCR testing for blaTEM, blaSHV, and blaCTX-M was done on 416 blood isolates Infection was clinically defined as CA when acquired as outpatient with no records of hospitalization up to 1 year and patients did not have any features of health care associated (HA) or NO (seen >72 hours after admissions). Outcome (death within 14 days was evaluated.
Results: Overall, ESBL prevalence ranged 67.2% to 90.4%. NO and CA rates were 43.6% and 21.9% respectively. Prior hospitalization and male were significant risk factor for ESBL, with 163 post surgical SSTIs. Among ESBLs, resistance to levofloxacin was 83.4%, Pipercillin/tazobactum 61.5%, amikacin 50% and carbapenems and tigecycline 13.5%. Overall, MDR among ESBLs was 37% and in CA isolates 7.4%. CTX-M genotype was(67.1%)Overall 7.4% patients died; 79.0% with ESBL. Intial choice of antimicrobials was inappropriate in 75.7%.
Conclusion: ESBL and MDR isolates continues to be high in Indian hospitals and their emergence in the community is alarming. There is a six fold greater mortality. Surgical SSTI should be largely preventable via good infection control practice Carbapenem and tigecycline resistance mechanism is to be elucidated. CTX-M seems to be the predominant genotype. Measures to limit further escalation into community and limit spread inter and intra in hospitals are urgently needed.

P1614 Antibiotic resistance in *Escherichia coli* infection in the Valencian community, Spain

J.M. Sahuquillo-Arce, M. Selva-San Adrián, J. Beltrán-Heras, E. González-Barberá, M. Gobernado (Valencia, ES)*

Objective: To analyze the distribution of *Escherichia coli* resistance to cefuroxime (CFX), ceftriaxone (CTO), ciprofloxacin (CIP) and co-trimazol (STX), according to sex and age in a period time of 33 months in the Valencian Community, a Spanish region with a population of almost five million people.
Methods: Retrospective analysis of data compiled by the Valencian Community Microbiological Watch Network (RedMIVA) referring to *E. coli* infections from January 2007 to August 2009. This network registers information from 22 hospitals. All isolates where identified according to standard procedures and the regarding information is transmitted daily via the internet to the Public Health Centre server, where it is automatically treated and recorded.
Data was analyzed by means of the Deviation Information Criteria method (DIC) and logistic regression (LR).
Results: The number of isolates studied was above 100,000 in all four antimicrobials. Data from the three years was added together for each antibiotic since it displayed similar patterns and trends. Men had higher levels of resistance and exhibited an abrupt increase at the age of puberty, while resistance development in women seemed inhibited until the pre-menopause age. Statistical analysis proved that resistance in women and men presented with different patterns. Data is shown in table 1.
Conclusions: This work exposes the distribution of resistance to four antimicrobials with a widespread use. Surprisingly, men and women exhibited different patterns in the acquisition of resistance. Thus, age and sex materialize as crucial factors to be taken into account when the time comes to establish antibiotic use guides.
Whether these results are repeated in different regions is still to be confirmed; but, the idea of a hormonal influence is very appealing.

Table 1. Percentages of antibiotic resistance in women and men

	Resistance (%) by age group																	Total n
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	⩾85	
CIP																			
Men	5.6	7.1	14.2	34.9	28.0	32.1	37.1	29.3	34.1	32.0	36.0	40.7	43.4	46.6	52.8	55.8	59.4	66.8	36828
Women	8.3	9.5	13.1	12.0	12.5	13.2	14.2	16.0	19.5	24.1	25.2	31.8	34.5	38.7	41.6	44.3	46.7	49.1	90655
CTO																			
Men	5.2	4.4	6.8	10.5	8.9	10.3	9.5	8.1	10.8	11.5	13.2	13.5	14.7	13.3	16.2	16.2	17.1	15.4	30510
Women	5.0	3.6	3.8	3.8	3.3	3.7	3.1	5.0	5.6	6.4	7.8	7.9	9.8	11.3	11.1	11.9	11.9	12.7	72951
CFX																			
Men	7.2	5.2	9.4	17.7	14.7	11.8	14.1	12.1	15.7	16.1	16.8	18.4	19.6	18.1	20.5	21.6	22.5	23.7	41580
Women	6.7	5.8	6.0	5.3	4.9	4.8	4.6	6.7	7.0	9.0	10.3	10.6	12.5	14.3	14.7	15.8	15.5	17.0	96893
STX																			
Men	26.4	27.9	35.8	36.9	33.6	36.0	40.4	35.7	39.1	40.5	38.7	42.1	41.9	38.6	41.7	43.1	41.2	44.0	30666
Women	29.1	30.3	32.5	25.7	26.9	25.3	25.3	27.0	28.5	30.4	31.7	35.4	35.7	39.1	38.5	39.0	39.2	38.0	77563

P1615 Antimicrobial resistance surveillance in Germany: first results for *E. coli*, *K. pneumoniae* and *P. mirabilis* from urine samples in ambulatory care, 2008–2009

I. Noll, B. Schweickert, G. Krause, T. Eckmanns (Berlin, DE)*

Objectives: In 2008, Germany expanded the scope of its national antimicrobial resistance surveillance system to the sector of ambulatory care. This presentation gives a first insight into resistance in the most frequent Gram-negative pathogens isolated from urine samples from outpatients in 2008/2009. Data will be stratified by age groups and selected geographical regions.
Methods: The dataset is taken from the German Antimicrobial Resistance Surveillance (ARS) System. Analysis is based on non-duplicate isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis* from urine samples in four laboratories with continuous data collection from January 2008 to October 2009. Species identification and antimicrobial susceptibility testing is performed by VITEK 2, results are evaluated according to CLSI guidelines. Proportions of susceptible isolates were calculated for the following antibiotics: ampicillin (AMP), ampicillin/sulbactam (AMS), piperacillin (PIP), piperacillin/tazobactam (PIT), cefotaxime (CTX), ciprofloxacin (CIP), co-trimoxazole (SXT).
Results: The most frequent Gram-negative pathogens isolated from a total of 87.873 urine samples were *E. coli* (n = 49,179; 47.2% out of 104,248 isolates), *P. mirabilis* (n = 5,065; 4.9%) and *K. pneumoniae* (n = 4,393; 4.2%). Results of susceptibility testing are displayed as percentages of susceptible isolates of all non-duplicate isolates tested in table 1. In *E. coli* overall proportions of susceptibility for the most frequently used antimicrobials in urinary tract infections as AMP, AMS, SXT and CIP vary from 54.6% (AMP) to 84.2% (CIP), in *P. mirabilis* the corresponding range spans from 64% (SXT) to 92.3% (AMS) and in *K. pneumoniae* proportions for selected antibiotics are all above 80%. Stratification by age groups shows significant lower levels of susceptibility for patients older than 60 for AMP, PIP, CIP and SXT in *E. coli* as well as in *P. mirabilis.* Regional differences with similar patterns are observed for CIP, SXT and PIP across species. Significant changes in resistance between 2008 and 2009 did not occur.
Conclusions: These first large-scale data from ambulatory care indicate that non-susceptibility of *E. coli* and to a lesser degree of *P. mirabilis* from urines to first-line antibiotics is highly prevalent, that it is even higher in patients older than 60 and that there are some regional variations. Interpretation of the data should consider that in ambulatory care settings specimens are mainly taken from pre-treated patients.

Table 1. Susceptibility of *E. coli*, *P. mirabilis*, *K. pneumoniae* from urine samples of outpatients in Germany 2008/09

Pathogen	Stratification[a]	Susceptibility[b]							n
		AMP	AMS	PIP	PIT	CTX	CIP	SXT	
E. coli	Total	54.6	69.2	57.2	87.3	97.0	84.2	70.8	41,449
	by agegroup								
	⩽15	57.7	72.9	59.6	90.1	98.2	96.6	75.3	4,391
	16–59	58.2	71.1	60.3	88.7	97.6	89.3	74.6	15,555
	⩾60	51.4	67.2	54.5	85.7	96.3	78.1	67.1	21,503
	by region								
	BE	–[c]	–[c]	60.1	88.3	97.0	88.8	73.8	8,944
	BW	–[c]	70.1	60.9	86.2	94.9	83.8	74.3	3,677
	NW	53.7	68.4	54.4	87.2	97.9	82.6	67.9	17,667
	SH	56.6	71.5	57.7	88.2	96.9	84.6	70.4	6,519
P. mirabilis	Total	68.2	92.3	71.4	96.1	99.0	87.9	64.0	4,577
	by agegroup								
	⩽15	79.7	93.9	79.1	96.8	99.4	96.1	74.6	727
	16–59	72.8	93.6	74.4	96.7	99.3	90.9	65.2	1,150
	⩾60	63.5	91.4	68.0	95.6	98.8	84.4	60.6	2,700
	by region								
	BE	–[c]	–[c]	77.1	97.9	99.7	93.5	70.4	1,059
	BW	–[c]	92.7	73.1	96.6	99.1	89.0	68.6	328
	NW	66.8	90.7	67.0	94.6	98.7	81.7	57.9	1,844
	SH	71.2	95.0	71.7	96.9	98.8	93.5	63.2	804
K. pneumoniae	Total	R*	82.8	R*	89.1	96.2	92.4	86.4	3,975
	by agegroup								
	⩽15	R*	82.5	R*	90.6	99.4	97.7	89.8	177
	16–59	R*	83.7	R*	89.2	97.2	94.6	86.7	1,245
	⩾60	R*	82.3	R*	88.9	95.5	90.9	86.1	2,553
	by region								
	BE	R*	–	R*	91.9	96.8	96.4	90.4	788
	BW	R*	82.7	R*	89.1	95.7	91.0	84.8	376
	NW	R*	83.6	R*	88.0	96.1	90.4	86.2	1,680
	SH	R*	80.2	R*	88.5	95.7	95.0	80.9	575

[a] BE, Berlin; BW, Baden-Wuerttemberg; NW, North Rhine-Westfalia; SH, Schleswig-Holstein.
[b] Percentages of susceptible isolates from non-duplicate isolates tested (n) against AMP, ampicillin; AMS, ampicillin/sulbactam; PIP, piperacillin; PIT, piperacillin/tazobactam; CTX, cefotaxime; CIP, ciprofloxacin; SXT, co-trimoxazole.
[c] Not tested.
*Intrinsic resistance.

P1616 Imported raw chicken meat as a potential reservoir for ESBL-producing *Escherichia coli* in the United Kingdom

H. Dhanji, N.M. Murphy, S. Durmus, S. Lee, J. Iverns, R. Hope, N. Woodford, D. Livermore (London, UK)*

Objective: Clinical *E. coli* isolates with group 1 CTX-M ESBLs have become frequent in the UK since 2003. Most have CTX-M-15 or (less often) CTX-M-3 enzyme encoded by IncFII or IncI1 plasmids and many of these isolates belong to the international O25b:H4 ST131 lineage (phylogenetic group B2). It was postulated that the spread of this clone might be facilitated either (i) by human-to-human contact, along with travel, or (ii) via a food source disseminated by the global market. To investigate the latter possibility we examined the antibiotic profiles, phylogenetic grouping, ESBL genes and plasmid types of 141 oxyimino-cephalosporin-resistant *E. coli* isolated from raw chicken meat imported into the UK from South America.
Methods: MICs were determined by the BSAC agar dilution method. Multiplex PCR was used to seek antibiotic resistance genes and to assign isolates to phylogenetic groups. CTX-M ESBL-encoding plasmids were transferred by conjugation to *E. coli* K12 J53–2 (rifR). Plasmids were extracted and rep typed by PCR. A real-time PCR assay was used to identify ST131 isolates.
Results: Among the 141 isolates, 44 had CTX-M group 2 enzymes, 38 had CTX-M group 8 and 59 had CMY-type AmpC; none had group 1 CTX-M ESBLs. No isolates with CTX-M group 8 ESBLs belonged to phylogenetic group B2, however 5/44 (11%) CTX-M group 2-positive isolates and 2/59 (3%) CMY-positive isolates belonged to this extraintestinal virulent group; none of the isolates belonged to the O25b:H4 ST131 clone. Phylogenetic group D was dominant among CTX-M ESBL-producing isolates and group B1 was dominant among isolates producing CMY-type AmpC. In contrast with most clinical *E. coli* with CTX-M ESBLs, all isolates with CTX-M group 8, 39/44 (89%) with CTX-M group 2 and 54/59 (92%) with CMY enzymes were susceptible to ciprofloxacin. CTX-M group 2 genes were found on IncFIB plasmids and CTX-M group 8 genes on IncI1 plasmids.
Conclusion: Imported raw poultry from South America is a reservoir for *E. coli* with CTX-M group 2 and 8 ESBLs, which are extremely rare in UK clinical isolates, and for those with CMY-type AmpC enzymes. However, it is not a reservoir for the clinically dominant group 1 CTX-M ESBLs nor for the *E. coli* O25b:H4 ST131 lineage.

P1617 Prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* rapidly increasing in Denmark

D.S. Hansen, H. Schumacher, K. Schønning, F. Hansen, M. Stegger, F.B. Hertz, N. Frimodt-Møller for the DANRES working group*

Objectives: Escherichia (EC) and *Klebsiella pneumoniae* (KPN) producing ESBL have been on the increase in Northern Europe during the last years. Our aim was to conduct a national prevalence study of nosocomial and community acquired ESBL-producing EC, KPN and *Proteus mirabilis* (PM) isolates from blood and urine.
Methods: During September-October 2007, 13 of 15 laboratories (covering 95% of the country) screened all EC, KPN, and PM isolates from blood and urine for ESBL-production. Confirmatory ESBL-test positive isolates were sent to a central laboratory for further susceptibility testing and for ESBL genotyping by PCR and sequencing. Phylogenetic grouping of the EC isolates was done using PCR.
Results: 18.259 patients were blood cultured and 47.504 patients had urine cultures taken. Among blood or urine cultures positive with EC (N = 12.382), KPN (N = 1.751) or PM (N = 541), 257 (2.1%), 93 (5.3%) and one (0.2%) isolate(s), respectively, were reported ESBL positive. The prevalence of ESBL-producing EC from nosocomial and community acquired urine samples were 2.3% and 1.5%, for KPN 6.6% and 5.0%, respectively. Among 280 isolates with ESBL phenotype CTX-M enzymes dominated among the 205 EC (92%), 74 KPN (89%) and one PM isolate(s). CTX-M-15 was the most prevalent among EC (60%) and KPN (76%) isolates. The distribution of the 205 EC isolates among phylogroup A, -B1, -B2 (78% were CTX-M15), D, and NT, were 41 (20%), 31 (15%), 54 (26%), 65 (32%), and 14 (7%) isolates, respectively. Extended susceptibility testing showed susceptibility towards meropenem (100%), fosfomycin (98%), and cefoxitin (91%).
Conclusion: The ESBL is now quite prevalent in Denmark, mainly due to CTX-M-15. This development challenges the empiric sepsis treatment with cephalosporin monotherapy.

P1618 Carbapenem resistance in extended-spectrum β-lactamase producing *Klebsiella* spp. and *Escherichia coli* blood isolates

O. Koseoglu Eser, H. Uludag Altun, B. Sener, G. Hasçelik (Ankara, TR)*

Objectives: Carbapenem resistance in Enterobacteriaeceae is usually associated with treatment failure in life-threatening infections. This study was conducted to test the *in vitro* effect of imipenem (IMP), meropenem (MER) and ertapenem (ERT) on *Klebsiella* spp. and *Escherichia coli* blood isolates and to evaluate the underlying carbapenem resistance mechanism.
Methods: A total of 210 non-duplicated *K. pneumoniae* (n = 47), *K. oxytoca* (n = 10) and *E. coli* (n = 153) isolates which were all ESBL positive with BD Phoenix system, were tested for IMP, MER and ERT susceptibility by microdilution test. All carbapenem non-susceptible isolates were then screened for metallo β-lactamase (MBL) with IMP-EDTA E-test, for AmpC β-lactamase with cefoxitin-boronic acid (BA) and for ESBL with CTX/clavulanate (CLA) and CAZ/CLA and also with a modification using CLA together with BA. KPC-type carbapenamases were screened with the modified Hodge test and combination disk method with MER/MER-BA and ERT/ERT-BA.
Results: The resistance rates of IMP, MER and ERT were 5.7%, 1.9% and 2.4%, respectively. All the isolates susceptible to IMP and MER, were also susceptible to ERT, except one. Twenty-three isolates were non-susceptible to any of the carbapenems. Seven of these yielded negative ESBL result with CTX/CLA and CAZ/CLA, however, six of them were found ESBL positive with the addition of BA to CTX/CLA. Three isolates which were found to be KPC producers with one of the screening tests, were all ERT resistant *K. pneumoniae*. AmpC activity was present in three isolates of which two were also KPC producers. Only one isolate, resistant to all carbapenems, showed phenotypic MBL production and was also a producer of KPC and AmpC.
Conclusion: Laboratories should consider testing for ertapenem susceptibility since it is one of the indicators of KPC activity. However,

alternative mechanism of carbapenem resistance may be a combination of ESBL or AmpC with porin loss. Therefore more reliable ESBL confirmatory tests such as use of CLA together with BA seems to be mandatory to detect ESBL co-presence with AmpC. Carbapenem non-susceptible ESBL isolates may pose a new problem in the future due to limited treatment choices and hospital infection control.

P1619 Plasmid mediated antimicrobial resistance. Characterizing extended-spectrum β-lactamases in *E. coli* isolated from cattle

C.J. Boinett, N. Coldham, M.F. Anjum, M. Kirchner, A. Carattoli, M.J. Woodward (Surrey, UK; Rome, IT)*

Objectives: The aim of this study was to characterize plasmids harbouring CTX-M genes and other antimicrobial resistance markers, with respect to their relative plasticity and ease of dissemination.
Methods: Bovine *Escherichia coli* isolates (n = 52) found to posses the CTX-M gene, which encodes for a β-lactamase conferring resistance to extended spectrum β-lactams were examined. Resistance phenotypes were determined using the disk diffusion assay. Isolates were also subjected to analysis by pulse field gel electrophoresis, plasmid profiling, miniaturized DNA microarray analysis and PCR based replicon typing.
Results: All 52 isolates were found to be resistant to cefotaxime (CTX), however when analysed by PFGE, their macro-restriction profiles varied considerably with similarities between 25–100%. Analysis of the plasmid content showed that all 52 isolates harboured multiple large plasmids (50–100kb). DNA microarray analysis was used to identify genes that confer antimicrobial resistance. All the isolates were found to encode either CTX-M group 1 (CTX-M-1, -15 and -32) or CTX-M group 9 (CTX-M-14 and -14b) variants, with 3 isolates encoding both CTX-M-14 and -15 genes and the majority encoding multiple antimicrobial resistance genes. Plasmid transfer rates were determined for 26 / 52 strains by *in vitro* conjugation studies. The isolates for conjugation were selected on the basis of distinct plasmid profiles and also considering their antimicrobial resistance profiles. Twelve of these isolates were able to transfer plasmids conferring cefotaxime resistance to recipient *E. coli* K12 strains with frequency of transfer rates between 3.75×10^{-3} and 3.69×10^{-7}. The majority of the isolates harboured multi-replicon plasmids, with 39 / 52 having an IncF variant.
Conclusion: These data confirm the highly variable nature of the CTX-M ESBL *E. coli* strains found on different farms although these were found to carry only limited CTX-M types. Interestingly 20 isolates encoded the CTX-M-15 gene, the most prevalent β-lactamase worldwide. FIA, FIB, FII replicons in *E. coli* have been linked with the dissemination of CTX-M-15 genes and therefore the success of plasmids harbouring this gene. It is proposed that complex methods of gene acquisition may have given rise to these CTX-M encoding strains and that the plasmids are likely to be promiscuous.

P1620 *In vitro* activity of doripenem, a new carbapenem, against recent Gram-negative clinical isolates from Belgium

C. Lammens, Y. Glupczynski, J. Van Eldere, U. Thomson, H. De Beenhouwer, E. De Laere, P. De Mol, A. Dediste, D. Govaerts, H. Jansens, S. Lauwers, K. Magerman, E. Nulens, M.J. Struelens, M. Van De Vyvere, G. Verschraegen, H. Goossens (Wilrijk, Yvoir, Leuven, Aalst, Roeselare, Liege, Brussels, Montigny-le-Tilleul, Edegem, Hasselt, Bruges, Antwerp, Ghent, BE; Stockholm, SE)*

Objective: The objectives were to document the susceptibility of Gram-negative clinical isolates to doripenem and competitor antimicrobial antibacterials in Belgium, and to document the prevalence of resistant phenotypes and genotypes.
Method: Non-duplicate, consecutive Gram-negative aerobic species isolates were collected from patients admitted in ICU and hospitalized for minimum of 48 hours and potentially associated with deep seated infection in which a deep specimen was collected, during a survey carried out in 14 Belgian hospitals, in 2008–2009. MICs were determined by microbroth dilution method according to CLSI guidelines. Testing included doripenem (DOR), meropenem (MER), imipenem (IMI), as well as ceftazidime, cefepime, piperacillin–tazobactam, amikacin, ciprofloxacin, levofloxacin and tigecycline. ESBLs and MBLs were detected and characterized phenotypically and genotypically.
Results: MICs obtained are listed in the table.
Resistance rates of Enterobacteriaceae and *Pseudomonas* spp to DOR, MER and IMI were 1.0/14.4, 1.3/31.5 and 4.4/42.5%, respectively. ESBLs were detected by double combination discs in 55/571 (9.6%) Enterobactericaceae (range of ESBL by centre: 2.1–23.8%), mainly of the CTX-M-group. Four plasmidic ampC cephalosporinases were detected in 3 *E. coli* and 1 *K. pneumoniae* strains. MBLs were detected by PCR-sequencing in 5/174 (2.9%) *P. aeruginosa* and class D OXA-carbapenemases 3/21 (14.3%) *A. baumannii*.
Conclusion: The *in vitro* activity of doripenem was comparable to that of meropenem and superior to that of imipenem against Enterobacteriaceae. Doripenem was the most active carbapenem tested against *Pseudomonas* spp, regardless of β-lactam resistance. Overall carbapenems retained excellent activity against Enterobacteriaceae including all ESBL-and AmpC-producing isolates. However, the emergence of MBLs and oxa-carbapenemases in *Pseudomonas* spp. and in *Acinetobacter* spp. in several Belgian ICUs is a matter of concern and warrants close epidemiologic surveillance.

		Doripenem			Meropenem			Imipenem		
Organism	n	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Acinetobacter spp.	21	0.06–⩾32	2	⩾32	0.06–⩾32	2	⩾32	0.06–⩾32	4	⩾32
Citrobacter spp.	20	0.03–0.25	0.125	0.25	0.03–0.25	0.06	0.125	0.125–16	0.25	8
E. coli	178	0.03–0.5	0.06	0.125	⩽0.015–1	0.06	0.125	0.125–⩾32	0.25	1
Enterobacter spp.	143	⩽0.015–⩾32	0.25	1	0.03–⩾32	0.125	1	⩽0.015–⩾32	1	4
Haemophilus spp.	29	⩽0.015–1	0.25	0.5	⩽0.015–0.5	0.25	0.5	0.125–4	2	4
Klebsiella spp.	145	⩽0.015–8	0.125	0.25	⩽0.015–⩾32	0.06	0.125	⩽0.015–⩾32	0.5	2
Morganella spp.	31	⩽0.015–4	1	2	⩽0.015–1	0.25	1	0.5–16	2	8
Proteus spp.	43	0.125–8	1	2	0.06–2	0.25	1	0.25–⩾32	4	16
Pseudomonas spp.	181	0.06–⩾32	2	16	0.25–⩾32	4	⩾32	1–⩾32	8	⩾32
Serratia spp.	51	⩽0.015–⩾32	0.25	1	0.03–⩾32	0.125	1	0.125–⩾32	1	8
Stenotrophomonas spp.	56	0.5–⩾32	⩾32	⩾32	0.5–⩾32	⩾32	⩾32	2–⩾32	⩾32	⩾32
Other										
Enterobacteriaceae	11	⩽0.015–16	0.06	0.125	⩽0.015–⩾32	0.06	0.25	⩽0.015–0.5	0.25	0.5
Non-Enterobacteriaceae	13	0.25–⩾32	16	⩾32	0.25–16	4	16	0.5–16	4	16

P1621 Intestinal colonization by carbapenemase-producing enterobacteria among patients in an intensive care unit

L.N. Andrade, J.C. Ferreira, M.A. Evaristo, F. Bellissimo-Rodrigues, A. Basile-Filho, R. Martinez, A.L. Darini (Ribeirão Preto, BR)*

Objective: The aim of this study was to investigate the intestinal colonization by carbapenemase-producing enterobacteria among patients in the intensive care unit (ICU) at the University Hospital of the Faculty of Medicine of Ribeirao Preto-University of Sao Paulo (HCFMRP-USP) at Ribeirao Preto, Brazil after an outbreak of KPC-2-producing *Klebsiella pneumoniae*.
Methods: Ninety-four patients admitted at the ICU from June to September of 2009 were submitted to a selective culture of rectal swab specimens collected on admission and at discharge or death. However, only 135 rectal swab specimens were collected, 71 on admission and 64 at discharge from the hospital or death. Carbapenemase-producing bacteria were selected in MacConkey medium with imipenem (8 mcg/mL) or ceftazidime (32 mcg/mL). Identification and antimicrobial susceptibility profile of the isolates were performed using the Vitek® 2 System (bioMérieux). The Modifed Hodge test was used to detect carbapenemase production. PCR and sequencing was performed to investigate carbapenemases-encoding genes.
Results: Sixty-five bacterial species (48.1%) were isolated out of the 135 rectal swabs specimens evaluated. Among these, 46.1% (30/65) were identified as enterobacteria: 66.6% (20/30) *K. pneumoniae*, 13.3% (4/30) *Enterobacter cloacae*, 6.6% (2/30) *Citrobacter freundii*, 3.3% (1/30) *Enterobacter aerogenes*, 3.3% (1/30) Pantoea sp., 3.3% (1/30) *Escherichia coli* and 3.3% (1/30) *Citrobacter* youngae. All enterobacteria showed a multiresistance profile. The Modifed Hodge test was positive for 43.3% (13/20) *K. pneumoniae*, indicating carbapenemase production and negative to other enterobacteria. PCR amplification and sequencing identified blaKPC-2 gene in the 13 *K. pneumoniae* detected as carbapenemase producers but no carbapenemase-enconding gene was

detected in the other 17 enterobacteria. Thus, other resistance genes may be responsible for the multiresistance profile in the last ones.
Conclusion: After control of the KPC-2-producing *K. pneumoniae* outbreak, isolation of these bacteria in infections was not frequent. However, this investigation showed that KPC-2-producing *K. pneumoniae* and other multiresistant bacteria are present in intestinal colonization of patients at the ICU and can be source of infections and dissemination of resistance genes.

P1622 Emergence of *Escherichia coli* isolates producing metallo-β-lactamase VIM-1 in Italy

M.M. D'Andrea, T. Giani, C. Venturelli, E. Giacobazzi, F. Arena, G.M. Rossolini (Siena, Modena, IT)*

Background: The increasing rate of resistance to oxyimino-cephalosporins and fluoroquinolones in clinical isolates of Enterobacteriaceae prevents the use of these drugs. Given this fact carbapenems are increasingly used in therapy to eradicate infections due to such strains. The appearance of carbapenem resistant isolates of Enterebacteriaceae is worrisome because of the limited therapeutic options that remain for the treatment of infections caused by such strains. In this work we report the appearance and persistence of VIM-positive *Escherichia coli* strains in an Italian hospital.
Methods: 5 bacterial carbapenem resistant isolates of *E. coli* were collected during November 2008 – October 2009 from the Modena University Hospital. Identification at the species level and antimicrobial susceptibility tests were performed using the Vitek automated system. MIC for carbapenems were confirmed using Etest and interpreted according to the criteria of CLSI. Genotyping was performed by RAPD and PFGE. β-lactamases content was investigated by PCR using primers specific to blaVIM and blaIMP alleles. Presence of class I integrons was assessed by PCR using primers specific to Int1 integrase gene and the 3′CS sequences. Amplification products were sequenced on double strand using amplification primers.
Results: Analyzed isolates were susceptible only to aminoglicoside, gentamicin and tigecycline. Etest analysis showed a lowering of the MIC values in the presence of EDTA, suggesting the production of a metalllo-β-lactamase. PCR analysis were positive for the blaVIM allele and negative when performed using primers for the blaIMP alleles. Genotyping techniques showed that at least two isolates, obtained from the same ward, were related to each other. PCR investigations showed that the blaVIM-1 allele was part of a class I integron. Sequencing demonstrated that all isolates carried the blaVIM-1 allele.
Conclusions: This study demonstrate the appearance and persistence of VIM-producing clones of *E. coli*. Such isolates were detected from samples obtained from 2 different wards, suggesting the persistence of such strains in the hospital. Resistance to carbapenems in strains of Enterobacteriaceae is quite uncommon in Italy, and this particular phenotype has mainly to be related to alteration of outer membrane permeability.

P1623 Prevalence of multi-resistant micro-organisms in the ambulatory setting in a Swiss region

A. Kronenberg, S. König, S. Droz, K. Mühlemann (Berne, CH)*

Objectives: To determine the prevalence of Methicillin-resistance in *Staphylococcus aureus* (MRSA) causing skin infections and extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in urinary tract infections in the ambulatory setting in the canton of Berne, Switzerland. Resistance rates were compared with resistance rates of the passive Swiss surveillance system ANRESIS (www.anresis.ch).
Methods: From September 2008 to February 2009 all primary care physicians residing in the canton of Berne (n = 1284) were asked to collect skin swabs or urine samples from consecutive patients with newly diagnosed purulent skin infection or urinary tract infection (UTI). For analysis samples were categorized as "routine" (if the physician indicated that this diagnostic procedure would have been performed also outside the study) and solicited samples (if samples were taken for study purpose only). All samples were analyzed at the Institute for Infectious Diseases, University of Berne, following local laboratory standards. Basic demographic data were collected for all patients, in case of multiresistance detailed data on risk factors were collected in addition.
Results: A total of 1018 urine samples were collected, 68% of which were culture positive. 87% were monobacterial infections. *E. coli* was the most prevalent microorganism isolated (71%), followed by enterococci (17.3%), *Klebsiella* spp. (5.3%), *S. saprophyticus* (4.1%) and *Proteus mirabilis* (3.7%). Susceptibility rates in solicited samples were higher than in routine samples for all antibiotics tested with the exception of fosfomycin (Table 1). Antibiotic use during three months before sampling was a significant risk factor for antibiotic resistance for all antibiotics tested except fosfomycin ($p < 0.01$). 5 (1%) ESBL producing *E. coli* were confirmed, 4 of the 5 patients had known risk factors, but none had a history of ESBL infection or carriage.
A total of 213 wound swabs were analyzed, of which 138 (65%) were culture positive. The prevalence of MRSA was 2.1% (2 of 94 *S. aureus* isolates).
Conclusion: Resistance prevalence among *E. coli* causing UTI in the ambulatory setting are higher among patients with complicated UTI than those with uncomplicated UTI. Therefore, passive resistance surveillance based on routine microbiological data do not reflect resistance rates in all patient groups. The prevalence of community-associated multi-resistance (MRSA and ESBL) is still rare in this region.

Table 1. Susceptibility rates (%) and n tested of *E. coli* in solicited and routine samples

Antibiotic	solicited	routine	p-value
Amoxicillin–clavulanic acid (amc)	82.7 (231)	69.8 (232)	<0.01
Ampicillin (amp)	66.2 (231)	54.7 (232)	0.02
Cefuroxime axetil (cxa)	77.9 (231)	69.8 (232)	0.06
Fosfomycin (fos)	100 (31)	100 (31)	–
Nitrofurantoin (nfu)	97.5 (40)	87.5 (48)	0.12
Norfloxacin (nor)	93.5 (231)	82.3 (232)	<0.01
Trimethoprim–sulfamethoxazole (sxt)	79.2 (231)	73.3 (232)	0.16
Multiresistance*	8.2 (231)	15.9 (232)	0.02

*Defined as resistance to at least 3 out of amc, cxa, nor or sxt.

P1624 Molecular and phenotypic characterization of enteroaggregative *Escherichia coli* clinical isolates and their antibiotic resistance pattern

M.Y. Alikhani, M.M. Aslani, A. Zavari, A.R. Zamani (Hamadan, Tehran, IR)*

Objective(s): Enteroaggregative *Escherichia coli* (EAEC) is an emerging category of diarrheagenic *E. coli*. EAEC can induce growth impairment and malnutrition in children, even in children without diarrhea. The aim of the present study was to investigate the frequency of EAEC in children with diarrhea and their antibiotic resistance patterns.
Methods: Stool specimens from 140 children under 12 years of age with acute diarrhea were collected. The specimens were cultured for *E. coli* using standard methods, and then screened for EAEC using a PCR and HeLa cell adherence. PCR to detect gene of the EAEC-associated plasmid pCVD432 were performed. Adherence of EAEC was examined by a method described by Scaletsky et al. Antimicrobial susceptibility testing was performed using the Bauer-Kirby method according to protocols of CLSI. The reference strains, *E. coli* 17-2, *E. coli* K12 and *E. coli* ATCC 25922 were included as a quality control in all assays.
Results: 15 (10.8%) EAEC were detected in 700 *E. coli* colonies tested with pCVD432 PCR. Of the EAEC isolates 13 (86.7%) showed AA pattern. In overall, 73.3% of the EAEC isolates were resistant to at least one of 14 antimicrobials tested. The isolates were resistant to ampicillin (100%), erythromycin (100%), Cephalothin (78.6%), co-trimoxazole (71.4%), tetracycline (64.2) and Nalidixic acid (57.1%), cefexim (50%), amoxiclav (50%), ceftriaxone and cefotaxime (42.8%). Considerable numbers of isolates were also reduced susceptible to

ciprofloxacin (42.8%) and norfloxacin (7.1%). Multidrug resistance to antibiotics was observed in 8 cases (53.3%) and the major resistance profile was ampicillin – erythromycin – Cephalothin.
Conclusions: EAEC is a diarrheal pathogen of emerging importance. The pCVD432 PCR showed good correlation with the HeLa cell adhesion assay, as 86.7% of pCVD432 positive isolates were confirmed as EAEC. However, compared to the assay for AA adherence the PCR has been found to be simple and specific in epidemiological studies. This study showed that EAEC isolates were highly resistance to tetracycline, co-trimoxazole and ampicillin, which are commonly used antibiotics in our area. It has been explained that the increased antibiotic resistance was due to a readiness for transfer of antibiotic resistance via conjugation probably encoded by the pCVD plasmid. Guidelines for appropriate use of antibiotics in developing countries need updating.

P1625 Activity of colistin against *Klebsiella pneumoniae* from 2007 to 2009, including isolates resistant to carbapenems

S. Hawser, S. Bouchillon, D. Hoban, M. Dowzicky (Epalinges, CH; Schaumburg, Collegeville, US)*

Background: Due to significant resistance to currently available therapies, successful treatment of infections due to *Klebsiella pneumoniae* is becoming limited. Moreover, a recent increase in the numbers of carbapenem-resistant isolates has been documented. The emergence of drug-resistant isolates has led in more recent years to the re-introduction of "salvage" therapies such as colistin (polymyxin E). This study investigated the activity of colistin against clinical isolates of *K. pneumoniae* originating from multiple infection sources that were collected during 2007–2009.
Methods: All isolates were collected during the T.E.S.T surveillance program (Tigecycline Evaluation and Surveillance Trial) and included infection sources such as blood or other normally sterile fluids, pneumonia, and non-catheter related urinary tract specimens. MICs were performed according to CLSI guidelines and interpreted using EUCAST breakpoints. A total of 280 clinical isolates were tested for their susceptibilities to colistin and additional comparator antimicrobial agents.
Results: See table.
Conclusions: These data show that colistin exhibited good *in vitro* activity against the majority of isolates, including carbapenem-resistant isolates. Only 8 isolates (2.9%) were resistant to colistin, though there was no apparent relationship between colistin resistance and resistance to other antimicrobials.

Antimicrobial	Mode	MIC_{50}	MIC_{90}	%S	MIN	MAX
Ceftazidime	=0.5	=0.5	16	86.1	=0.5	>16
Colistin	0.25	0.25	0.5	97.1	=0.12	>4
Ertapenem	=0.03	=0.03	=0.03	96.8	=0.03	>32
Gentamicin	=0.25	=0.25	1	90.7	=0.25	>8
Imipenem	0.25	0.25	0.5	98.9	=0.03	4
Levofloxacin	=0.25	=0.25	4	86.4	=0.25	>4
Piperacillin/Tazobactam	4	4	>64	78.9	=0.5	>64
Tigecycline	=0.5	=0.5	1	93.6	=0.5	>2

%S, percent susceptible using EUC AST breakpoints

P1626 Global surveillance update of Enterobacteriaceae from the TEST programme, 2004–2009

B. Johnson, S. Bouchillon, J. Johnson, D. Hoban, R. Badal, M. Dowzicky (Schaumburg, Collegeville, US)*

Background: Cross-resistance to several classes of antimicrobials is often seen in nosocomial pathogens. The T.E.S.T. program determined the *in vitro* activity of tigecycline and comparators against strains of Enterobacteriaceae cross-resistant to one or more of the following antimicrobials: amoxicillin–clavulanic acid, piperacillin–tazobactam, levofloxacin, ceftriaxone, cefepime, ampicillin, amikacin, minocycline, ceftazidime, meropenem and imipenem. The isolates were collected from 1,334 cumulative investigational sties in 58 countries throughout 2004–2009.
Methods: A total of 67,130 clinical Enterobacteriaceae were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI or FDA (tigecycline) breakpoints, where available.
Results: 5,401/41,112 (13.1%) *E. coli* and *Klebsiella* spp were ESBL producers. Of the Enterobacteriaceae, 17.2% were resistance to levofloxacin, 12.1% to minocycline, 2.1% to amikacin, 0.2% to imipenem, 1.4% to meropenem and 0.6% to tigecycline. Of the 26,018 *Enterobacter* spp. and *S. marcescens* collected, 1,778 (6.8%) presented resistance to ceftriaxone and ceftazidime but susceptible to cefepime suggestive of AmpC phenotype. Only 1,992 (3.0%) Enterobacteriaceae showed any degree of non-susceptibility against tigecycline. Tigecycline also showed excellent inhibitory activity against members of Enterobacteriaceae that were resistant to amikacin, levofloxacin, minocycline and meropenem inhibiting 92%, 94%, 82% and 88% of isolates, respectively.
Conclusion: The presented data suggest that tigecycline is little affected by cross-resistance and may be an effective therapeutic option against nosocomial or community pathogens regardless to the resistance patterns.

P1627 CROMagar KPC evaluation for detection of carbapenemase-producing Enterobacteriaceae

F. Panayea, I. Galani, P. Adamou, M. Souli, A. Antoniadou, H. Giamarellou (Athens, GR)*

Objectives: Our aim was to compare CROMagar KPC with MacConkey agar with imipenem 1μg/mL for the detection of KPC and VIM producing Enterobacteriaceae strains from surveillance cultures.
Methods: 135 rectal swabs from 120 patients (78 ICU and 42 pathology/surgery wards) were tested. Swabs were plated on both MacConkey agar No3 + imipenem 1μg/mL (MC) and CROMagar KPC (Hy labs)(CR) and were incubated at 35ºC, O2 for 48 h. Identification and antimicrobial susceptibility testing of all different colonies from MC and all different blue colonies from CR was performed by Phoenix (BD). Strains were screened for KPC and VIM by merop-merop+boronic acid and merop-EDTA, ceftaz-EDTA disc respectively and confirmed by PCR methodology. Isolation of Enterobacteriaceae on CR was also tested with known VIM + and KPC+ strains.
Results: Carbapenem resistant strains recovered from MC were: *Kl. pneumoniae* 44 (36 KPC+, 8 VIM+), *Ps. aeruginosa* 20, *Pr. mirabilis* 4, *E. cloacae* 1 (KPC+), *E. aerogenes* 1 (KPC+) and *A. baumannii* 21. CR recovered 54 carbapenemase producing *Kl. pneumoniae* strains (41 KPC+, 13 VIM+), isolated the first day of incubation. KPC+ strains were 100% non-susceptible to imipenem (MIC⩾8) and 95.3% to meropenem (⩾8) and VIM+ strains were 93.3% non-susceptible to imipenem and 46.7% to meropenem. *Enterobacter* spp. strains were not isolated, most probably due to the resemblance of their colonies to the coexisting *Kl. pneumoniae* strains. *Ps. aeruginosa* and *A. baumannii* strains exhibited white colonies. *Pr. mirabilis* strains were not recovered. Collectively, *Kl. pneumoniae* isolated strains were 43 KPC+ (34 both plates, 7 only CR, 2 only MC) and 15 VIM+ (6 both plates, 7 only CR, 2 only MC). Sensitivity of MC and CR for *Kl. pneumoniae* KPC+ strains was 83.7 and 95.3 and for VIM+ strains 53.3 and 86.6 respectively. All false (–) results on CR were due to the coexistence of VIM+ and KPC+ *Kl. pneumoniae* strains in the sample (same colonies). On the contrary, in 10 out of 14 false (–) results on MC there was no growth of lac(+) colonies, although strains' imipenem MIC was within non-susceptible range. MC and CR detected carbapenemase producing *Kl. pneumoniae*strains with an overall sensitivity 81.5, 100 and specificity 100, 100 respectively.
Conclusion: CR detects with high sensitivity and specificity within 24 h either KPC or VIM producing Enterobacteriaceae strains in surveillance cultures, allowing immediate implementation of infection control measures to avoid spread of resistant clones.

P1628 Activity of colistin against Enterobacteriaceae from 2007 to 2009, including drug-resistant isolates

S. Hawser, S. Bouchillon, D. Hoban, M. Dowzicky (Epalinges, CH; Schaumburg, Collegeville, US)*

Background: Due to significant resistance to currently available therapies, successful treatment of infections due to Enterobacteriaceae is becoming limited. Moreover, a recent increase in the numbers of carbapenem-resistant isolates has been documented. The emergence of drug-resistant isolates has led in more recent years to the re-introduction of old "salvage" therapies such as colistin (polymyxin E). This study investigated the activity of colistin against clinical isolates of Enterobacteriaceae originating from multiple infection sources that were collected during 2007–2009.
Methods: All isolates were collected during the T.E.S.T surveillance program (Tigecycline Evaluation and Surveillance Trial) and included infection sources such as blood or other normally sterile fluids, pneumonia and non-catheter related urinary tract specimens. Colistin MICs were performed on a selection of clinical isolates and tested according to CLSI guidelines and interpreted using EUCAST breakpoints. A total of 1,360 clinical isolates were tested for their susceptibilities to colistin and comparator antimicrobial agents.
Results: See table.
Conclusions: These data show that colistin exhibited good *in vitro* activity against the majority of isolates, many of which were resistant to other antimicrobials. Overall, the percent susceptibilities for colistin ranged from 85.3% (*E. cloacae*) to 98.8% (*E. coli*).

Organism (n)	MIC_{50}	MIC_{90}	MIC Range	%S*
Enterobacter aerogenes (292)	0.5	1	0.25–>4	95.2
Enterobacter cloacae (258)	0.5	>4	=0.12–>4	85.3
Escherichia coli (254)	0.5	0.5	=0.12–>4	98.8
Klebsiella oxytoca (276)	0.25	0.5	=0.12–>4	97.5
Klebsiella pneumoniae (280)	0.25	0.5	=0.12–>4	97.1

*%S, percent of isolates susceptible using EUCAST breakpoints.

P1629 Ineffectiveness of carbapenems against *Klebsiella pneumoniae* clinical isolates and increasing resistance to colistin. A 4-year study from a Greek university hospital

I. Neonakis, S. Maraki, H. Messaritakis, S. Baritaki, A. Georgiladakis, D. Spandidos (Heraklion, GR)*

Objective: *Klebsiella pneumoniae* has emerged as one of the most problematic pathogens as treatment has largely been limited to only a few antimicrobials. Usually, carbapenems are the molecules used and in cases of non-susceptibility to these, colistin is the antibiotic that routinely is administered. The aim of the present study was to determine the rates and trends of non-susceptibility to cabapenems and colistin in our Hospital.
Methods: A total of 959 *K. pneumoniae* strains from different patients isolated in our hospital over the last four years (2005–2008) were analyzed. The numbers of isolates per year were: 2005, 143; 2006, 194; 2007, 293; 2008, 329. Identification and susceptibility testing were performed using the Vitek II system (Biomerieux, France). Isolates with either intermediate or full resistance to an antibiotic were characterized as non-susceptible to this agent. The statistical significance of the differences in non-susceptibility observed among the years for each antimicrobial was determined by the Pearson Chi-Square test [Asymp. Sig. (2-tailed)]. Probability (P) was set significant at the level of 0.05. Statistical analysis was performed using the SPSS 11.5 software (SPSS, Chicago, IL).
Results: The non-susceptibility rates found for each year (2005, 2006, 2007 and 2008) were: A) Imipenem (IMP): 14.0%, 25.8%, 28.7% and 49.5%; B) Meropenem (MER): 9.2%, 27.3%, 32.8% and 51.7% and C) Colistin (COL): 1.4%, 4.1%, 9.6% and 18.5%. The increase of non-susceptibility was statistically significant ($P < 0.0001$) for all three antimicrobials. The overall non-susceptibility rates found over the four-year period were: IMP: 33.1%, MER: 36.5% and COL: 10.3%. Among the IMP-non-susceptible isolates almost one out of four (23.8%) was also non-susceptible to COL.
Conclusion: The results of the present study indicate that the effectiveness of carbapenems has been dramatically compromised over the last years and the increase of non-susceptibility to COL is rapid and steep. Unless effective policies will be designed and implemented, carbapenems and COL will no more be therapeutic options against *K. pneumoniae* isolates within the next few years.

P1630 Fluoroquinolone resistance of *Pseudomonas aeruginosa* isolates causing nosocomial infection is correlated with levofloxacin but not ciprofloxacin use

H. Liu, Y.J. Lee, Y.C. Lin, K. Sun, C.L. Chun, P.R. Hsueh (Taipei City, TW)*

Objective: This study investigated the correlation between fluoroquinolone (ciprofloxacin or levofloxacin) use and the rates of fluoroquinolone-resistance in *Pseudomonas aeruginosa* isolates from patients with nosocomial infection at a teaching hospital in Taiwan.
Methods: Antibiotic utilization data were extracted on a monthly basis from the inpatient pharmacy computer system records from January 2003 through December 2008. Fluoroquinolone use was expressed as defined daily dose per 1,000 patient-days (DDDs/1,000PDs) and correlated with the rates of fluoroquinolone-resistant *P. aeruginosa* every six months. Regression analysis was performed to explore the relationship between ciprofloxacin and levofloxacin use (both parenteral and oral forms) and the resistance of *P. aeruginosa* isolates.
Results: During the study period, the susceptibility of *P. aeruginosa* to fluoroquinolones decreased after increasing use of fluoroquinolones, and increased after decreasing use of levofloxacin. Parenteral levofloxacin use was significantly positively correlated with resistance of *P. aeruginosa* to ciprofloxacin ($P = 0.015$) and fluoroquinolones (either ciprofloxacin or levofloxacin, $P = 0.014$). Use of both parenteral and oral forms of levofloxacin were also significantly positively correlated with resistance of *P. aeruginosa* isolates to ciprofloxacin ($P = 0.029$), levofloxacin ($P = 0.031$), and fluoroquinolones ($P = 0.010$). The total amount of ciprofloxacin (oral and parenteral) and parenteral ciprofloxacin use were negatively correlated with resistance of *P. aeruginosa* isolates to fluoroquinolones. However, the amounts of oral ciprofloxacin and oral levofloxacin were each positively correlated with resistance of *P. aeruginosa* to fluoroquinolones.
Conclusions: Levofloxacin use was associated with increased resistance of *P. aeruginosa* to fluoroquinolones, whereas ciprofloxacin use did not have a significant impact on fluoroquinolone resistance rates.

P1631 Antimicrobial susceptibility among *Pseudomonas aeruginosa* isolates from a central hospital in the centre of Portugal: a 6-year surveillance

S. Pereira, L. Albuquerque, H. Oliveira, R. Leitão, O. Cardoso (Coimbra, PT)*

Objectives: *Pseudomonas aeruginosa* (PA) is an important nosocomial pathogen causing a wide spectrum of infections and leading to substantial morbidity and mortality. In order to apply optimal therapeutic guidelines physicians must be aware of recent resistance surveillance, and epidemiological data. The main objective was to determine antimicrobial susceptibility of PA isolates in order to assist the guidelines for empirical regimens and infection control measures.
Methods: From April 2003 to April 2009, 2210 PA isolates were collected in Centro Hospitalar de Coimbra. Nosocomial isolates ($N = 1411$) and ambulatory ones ($N = 799$) were obtained from sputum (40.2%), urine (23.7%), exudates (13.8%), blood (5.1%) and other sources (12.2%). They were identified with API32GN (bioMérieux) and MicroScan WalkAway (Dadebehring) and susceptibility patterns were determined with these panels. Susceptibilities to Piperacillin (PIP),

Piperacillin plus Tazobactam (TZP), Aztreonam (AZT), Ceftazidime (CAZ), Imipenem (IP), Meropenem (MP), Amikacin (AMK), Gentamicin (GN), and Ciprofloxacin (CIP) were guideline by CLSI.
Results: Overall, AMK was the best agent (87.1%), followed by TZP (86.5%), MP (83.6%), PIP (83.4%), CAZ (80.0%), AZT (76.4%), IP (75.3%), GN (70.4%) and CIP (63.1%). Nosocomial isolates responded to AMK (83.4%), TZP (81.1%), PIP (76.8%), MP (75.3%), CAZ (72.5%), AZT (67.5%), IP (64.8%), GN (64.0%), and CIP (56.0%). Ambulatory isolates presented susceptibility superior to 90%, except for GN (81.6%) and CIP (75.7%). During these years the susceptibilities to most important β lactams (IP, MP and CAZ) had diminished; activity of IP decreased 10.5%, MP 8.0%, CAZ 7.3%, and among nosocomial isolates the decrease was greater: IP 15.4%, MP 13.0% and CAZ 10.3%. TZP fortunately sustained the activity along time.
Conclusion: Appropriate empirical treatments based on knowledge of particular resistance patterns are important determinants of the success of therapeutics, therefore studies of surveillance can be helpful in fighting the development and spread of resistance.

P1632 Multidrug-resistant *Klebsiella pneumoniae* isolates in a tertiary hospital

S. Tsiplakou, V. Papaioannou, A. Stylianakis, D. Paraskevopoulou, F. Zouboulоglou, P. Petraki, P. Papanikolaou, A. Koutsoukou (Athens, GR)*

Objectives: Multidrug resistant (MDR) *Klebsiella pneumoniae* isolates are increasingly encountered as nosocomial pathogens. The aim of the present study was to define the resistance profile of MDR *K. pneumoniae* for a period of three years in a tertiary hospital.
Methods: From November 2006 to October 2009 a total of 1215 *K. pneumoniae* isolates, one per patient, were collected from patients hospitalized in ICUs (441 isolates) and non-ICU wards (774 isolates) of our hospital. Identification and susceptibility testing were performed using the Vitek 2 system (Biomerieux) according to CLSI guidelines. The antimicrobials tested were aztreonam (AZT), amoxicillin–clavulanic acid (AMC), cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), imipenem (IMP), meropenem (MER), piperacillin–tazobactam (TZP), amikacin (AN), gentamicin (GM), netilmicin (NET), ciprofloxacin (CIP), colistin (CS), and tetracycline (TE). Additionally E-test strips (AB Biodisk) were used to determine susceptibility to IMP, MER and CS. Susceptibility to tigecycline (TG) was determined the last year of the study. Isolates with MIC values for imipenem and/or meropenem >1 mg/L were screened for metallo-β-lactamase (MBL) production by E-test MBL strips.
Results: A total of 805 isolates (66.2%) were multidrug resistant 387 (87.7%) isolates were collected from ICU patients and 418 (54%) from non-ICU patients. Among MDR isolates high resistance levels were observed for β-lactams, reaching up to 91% for CAZ, 92% for FEP, 95% for FOX and TZP, 93% for AZT, AMC and CRO, 82% for IMP and 78% for MER. Resistance rates to aminoglycosides varied from 23% for GM, to 52% for AN and to 88% for NET. Resistance rates were as high as 89% for CIP, 33% for TE and 16% for CS. Seventy five isolates were found to be resistant to TG. According to E-test MBL results 68% of the carbapenem non-susceptible isolates were MBL producers.
Conclusions: The majority of *Klebsiella pneumoniae* isolates from ICUs and more than half the isolates from non-ICU wards are multidrug resistant. Commonly used antimicrobials such as lactams, carbapenems included, quinolones and aminoglycosides cannot be used in empiric treatment as resistance rates are high. Susceptibility to colistin at 84% shows that it remains active for MDR *K. pneumoniae* isolates. Tigecycline may also be used for the management of infections due to MDR isolates although emergence of resistance is already documented.

P1633 Emergence of KPC-producing *Klebsiella pneumoniae* isolates in a Greek hospital

E. Vagiakou, G. Ganteris, A. Kyratsa, M. Orfanidou, E. Marda, P. Giakkoupi, A. Vatopoulos, E. Malamou-Lada (Athens, GR)*

Objectives: To evaluate the frequency and phenotype resistance of *Klebsiella pneumoniae* carbapenemase producing (KPC-producing) strains isolated from clinical samples, during one year period (October 2008–October 2009), in the General Hospital of Athens "G. Gennimatas".
Methods: During one year period a total of 412 *Klebsiella pneumoniae* strains were isolated from an equal number of patients. Susceptibility tests were performed by Kirby-Bauer method, MICs determination by automated system (Vitek 2-Biomerieux) and E-test method (AB Biodisk, Solna, Sweden), according CLSI standards. Preliminary phenotypic detection of KPC enzymes was based on the combination of a bioassay test – Hodge (cloverleaf) test – and the CD synergy test (CD test with meropenem and boronic acid). The presence of the bla-KPC gene was confirmed by PCR and molecular typing was performed by pulsed field gel electrophoresis (PFGE).
Results: Eighty-one from a total of 412 *Klebsiella pneumoniae* strains were KPC-producers (81/412, 19.7%) and isolated from cultures of blood (n: 24), bronchial secretions (n: 11), urine (n: 2), wounds (n: 10), catheter tips (n: 15), cerebrospinal fluid (n: 1), peritoneal fluid (n: 1), nasal (n: 2) and enteric colonisation (n: 15). The distribution of isolates was as follows: ICU (38), Haematology unit (20), Surgical units (15), Neurology (6), Neurosurgery (1), Nephrology (1). Hodge test was positive for production of carbapenemases and CD test was positive for KPC production. All strains had reduced susceptibility to carbapenems (MIC range: 2–⩾16 mg/L) and were sensitive to gentamicin. Eleven were resistant to tetracycline (11/81, 13.6%), 10 to tigecycline (10/81, 12.5%) and 8 to colistin (8/81, 10%). Most of the isolates belonged to PFGE type-1.
Conclusions:

1. KPC producers are rapidly disseminating in several units of our hospital environment.
2. Phenotypic detection of carbapenemase-producing *K. pneumoniae* isolates is critical for limiting the spread of the multi-resistant strains.
3. All *K. pneumoniae* strains with reduced susceptibility to carbapenems should be monitored for KPC enzymes by simple screening methods confirmed by molecular assays.
4. Most of the *K. pneumoniae* strains of our hospital belonged to PFGE type-1.

P1634 Increasing occurrence of CMY-producing *Proteus mirabilis* causing bloodstream infections

G. Brigante, M.M. D'Andrea, C. Pianetta, T. Giani, C. Comi, S. Bracco, N. Corbo, G.M. Rossolini, F. Luzzaro (Lecco, Siena, IT)*

Objectives: Plasmid-mediated AmpC β-lactamases (CBLs) have been increasingly reported over the last few years. However, prevalence and distribution of CBLs among enterobacteria are still largely unknown. The aim of this study was to evaluate the CBL production in blood culture isolates in comparison with the production of extended-spectrum β-lactamases (ESBLs).
Methods: Enterobacteria obtained from blood cultures performed at the Manzoni Hospital (Lecco, Italy) during a 5-year period (2004 to 2008) were included in the study. Identification and antimicrobial susceptibility testing were carried out using the VITEK2 automated system (bioMérieux, Marcy l'Etoile, France). ESBL and/or CBL production were assessed according to CLSI criteria (when available) and confirmed by PCR and gene sequencing.
Results: Overall, 45 ESBL-positive isolates were detected, including *Escherichia coli* (n = 29), *Proteus mirabilis* (n = 5), *Klebsiella pneumoniae* (n = 5), *Enterobacter cloacae* (n = 2), *Enterobacter aerogenes* (n = 2) and *Serratia marcescens* (n = 2). No significant differences were observed over the years (2004, n = 5; 2005, n = 10; 2006, n = 11; 2007, n = 11; 2008, n = 8). On the contrary, CBL production was detected solely in

Proteus mirabilis isolates (n = 6), starting from February 2007 from a neurosurgical patient. The remaining 5 isolates were collected from February to August 2008 from patients admitted to different wards (nephrology, n = 1; infectious diseases, n = 2; and intensive care unit, n = 2). All CBL-producing isolates were positive for blaCMY genes (blaCMY-2, n = 1, and blaCMY-16, n = 5). Carbapenems and amikacin were consistently active against both ESBL- and CBL-positive isolates. With respect to CBL-producing isolates, bacteremia was associated with urinary tract infection in 5/6 cases. Partial or complete response was obtained in the above patients treated with meropenem (n = 4) or cefepime (n = 1). The remaining patient died for causes non attributable to infection (therapy was not assessable).
Conclusions: *Proteus mirabilis* isolates producing CBLs have emerged among enterobacteria causing bloodstream infections in our hospital. Together with ESBL-producing enterobacteria, they represent a new challenge for physicians and microbiologists. Carbapenems and amikacin show potent *in vitro* activity against both ESBL- and CBL-positive isolates thus representing a valid therapeutic option for treating infections caused by these MDR pathogens.

P1635 Multidrug-resistant *Pseudomonas aeruginosa*: associated epidemiological trends and clinical risk factors

R. Ngo, M. Kanatani (Los Angeles, US)*

Objective: The purpose of this study was to determine the incidence of multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) at the Ronald Reagan University of California, Los Angeles Medical Centre (RRUCLAMC) and identify contributing clinical risk factors. Antibiotic resistance patterns, antibiotic utilization trends, and outcomes associated with MDRPA were also evaluated.
Methods: Between August 2004 and December 2008, all patients with positive *Pseudomonas aeruginosa* (PA) blood cultures and their associated antibiotic susceptibilities were retrospectively identified. PA strains resistant to >3 classes of anti-pseudomonal antibiotics (β-lactams, fluoroquinolones, carbapenems, and/or aminoglycosides) were classified as MDRPA. Clinical characteristics, such as underlying disease states, medications received during hospitalization, and patient outcome data were also analyzed.
Results: Over the study period, 187 PA blood isolates were identified, of which 53 were MDRPA. Patients who received transplant immunosuppressants, corticosteroids, or total parenteral nutrition (TPN) were two times more likely to develop MDRPA bacteremia than those who did not receive these medications. The cumulative percentages of PA (and MDRPA) isolates resistant to each of the antibiotic classes were as follows: 16.6% aminoglycosides (96.8% MDRPA), 32.6% β-lactams (75.4% MDRPA), 37.4% carbapenems (68.5% MDRPA), 45% fluoroquinolones (38.1% MDRPA). Carbapenem utilization increased by 50%, and PA resistance to carbapenems increased by over 40%. During the specified time frame, the incidence of MDRPA decreased by 25%. Patient outcome data illustrated that 25% of patients with non-MDRPA expired, while 51% of patients with MDRPA expired.
Conclusions: Over a 3-year period, almost 30% of all PA blood isolates identified were MDRPA. Patients receiving immunosuppressants and TPN were two times more likely to develop MDRPA. Cumulative resistance of PA isolates was highest with fluoroquinolones and lowest with aminoglycosides, indicating the low susceptibility of PA to fluoroquinolones and high susceptibility to aminoglycosides. Increased utilization of carbapenems may be associated with increased resistance towards this anti-pseudomonal antibiotic class and the decreased incidence of MDRPA. Thus, a fine balance exists between the judicious use of anti-pseudomonal antibiotics to avoid resistance and appropriate therapy, making the treatment of MDRPA challenging.

P1636 Imipenem and ciprofloxacin consumption are risk factors for selection of resistant *Pseudomonas aeruginosa*: results of a multicentre observational study in French hospitals

K. Miliani, F. L'Heriteau, L. Lacave, A. Carbonne, P. Astagneau (Paris, FR)*

Objective: To determine whether high consumption of specific antibiotics (ABs) could be independent risk factors for high incidence rates of key resistant *P. aeruginosa*: Ceftazidime-, Imipenem-, Ciprofloxacin-, or, Amikacin-resistant.
Methods: Data collected were collected from the antimicrobial surveillance network in Northern France and the public reporting system of infection control indicators. The collected data were related to hospital characteristics (hospital size, type, proportion of long-term care beds), annual AB consumption expressed in defined daily doses per 1000 patient-days (PD), annual incidence rates of key *P. aeruginosa* pathogens expressed in number of non-susceptible isolates/1000 PD and quality indicators of nosocomial infection control including hydro-alcoholic hand-rub products consumption. The study sample were the volunteer healthcare facilities participating in the Network for the 2007 period, which have performed AB susceptibility test in more than 10 non-duplicate *P. aeruginosa* isolates during the year. Univariate and multivariate logistic regression analyses were carried out to determine risk factors of being HCF with at least one key resistant *P. aeruginosa* pathogens with incidence rate >75th percentile (p75).
Results: Of 102 HCF participating in the survey, 84 had tested >10 isolates. Among those, 11 were public teaching hospitals, 39 public non-teaching, and 34 private. Overall, 51.3% were HCFs <300 beds and 67.9% had a proportion of long-term care beds ⩾25%. In the univariate analysis, total and specific ABs (except imidazoles) consumption higher than p75 were associated with at least one *P. aeruginosa* key resistance rates >p75 ($P < 0.1$). In the multivariate analysis, after adjustment for hospital type and proportion of long-term care beds, high *P. aeruginosa* resistance rates were related to highly imipenem and ciprofloxacin consumption (adjusted OR [95% CI]: 14.6 [2.8–77.2] and 5.6 [1.3–23.2], $P < 0.05$ respectively). In contrast, *P. aeruginosa* resistance was not significantly related to alcohol-based hand-rub consumption ($P = 0.20$).
Conclusion: These findings suggest that imipenem and ciprofloxacin could play a major role in a high incidence of resistant *P. aeruginosa*, independently of cross-transmission.

P1637 Longitudinal analysis of the activity profile of tigecycline and comparators against important Gram-negative pathogens in Europe, 2000–2009

M.K. Torres, N.P. Brown, C.M. Pillar, C. Thornsberry, D.F. Sahm, M. Dowzicky (Chantilly, Collegeville, US)*

Objective: TIG, a broad spectrum glycylcycline, is utilized in the hospital where infections can be difficult to treat due to antibiotic resistance (R) because of its potency against prevalent R pathogens. In this setting, R among Gram-negatives (GN) is of concern and it is important to understand both the changes in R patterns among common agents and the potential emergence of R to recently approved agents. This study analyzes the activity (ACT) profile of TIG and comparator agents against Enterobacteriaceae (EN) and *Acinetobacter* spp. (AC) in Europe (EU) for the past 10 years, a period spanning TIG's approval for use in Europe in 2006.
Methods: A total of 2,379 EN (*E. coli*, *K. pneumoniae*, *Citrobacter* spp., *Enterobacter* spp. and *S. marcescens*) and 322 AC isolated between 2000–2009 were collected from 12 countries across EU. Isolates (ISO) were centrally tested by broth microdilution (CLSI M7) against TIG and comparator agents. TIG was analyzed according to relevant R phenotypes including MDR status (concurrent R to ⩾3 different classes of agents). EUCAST breakpoints (BPs) were used to interpret TIG MICs and CLSI (M100-S19) BPs for all other agents, where applicable.
Results: The ACT profile of TIG against EN was consistent from year to year, with MIC50s of 0.25 or 0.5 mg/L and MIC90s of 0.5 or 1 mg/L.

TIG R among EN remained <1%. R to ceftriaxone (7.6% in '05; 14.2% in '09) and MDR (9.4% in '05; 14.3% in '09) fluctuated over time for EN. In contrast to other agents, TIG and imipenem ACT was not notably affected against cephalosporin R and MDR populations of EN. Against AC, TIG was the most potent agent with a consistent MIC50 of 0.25 or 0.5 mg/L and an MIC90 of 1 or 2 mg/L. Among AC, MDR rates increased from 15% to 52% over the evaluated period. In contrast to other agents where little ACT was observed against MDR ISO, TIG maintained MIC50s of 0.5–2 mg/L and MIC90s of 1–4 mg/L, though MIC distributions showed a slight elevation in TIG MICs against MDR ISO relative to non-MDR ISO.

Conclusions: Over the period spanning early development and three years beyond the approval of TIG for use in EU, TIG has maintained a consistent ACT profile against targeted GN pathogens from across EU. In contrast to the majority of agents evaluated, the potency of TIG is maintained over this period against R subpopulations, including MDR ISO. Continued surveillance is warranted to monitor for changes in patterns of R and for any changes in TIG ACT as usage increases.

P1638 Susceptibility of Gram-negative pathogens to colistin and comparators in Germany, 2007

M. Kresken, D. Hafner on behalf of the Antimicrobial Resistance Working Party*

Objectives: The emergence of healthcare associated infections caused by multidrug-resistant Gram-negative bacteria (MDR GN) has become an increasing problem over the past 20 years. Colistin (COL), a "forgotten" antibiotic, has attracted attention, because of its activity against MDR GN. It is, however, not active against Proteeae and *Serratia* spp. The objective of this study was to evaluate the susceptibilities of clinical isolates of *Enterobacter cloacae* (ECL), *Escherichia coli* (ECO), *Klebsiella pneumoniae* (KPN), *Klebsiella oxytoca* (KOX), *Pseudomonas aeruginosa* (PAE) and *Acinetobacter baumannii* group (ABA) to COL and comparators.

Methods: A total of 1,529 isolates were prospectively collected from 21 microbiology laboratories across Germany, which participated in the surveillance study conducted by the Paul-Ehrlich-Society in November 2007. MICs of COL, ciprofloxacin (CIP), ceftazidime (CAZ), gentamicin (GEN) and meropenem (MEM) were determined by the broth microdilution procedure according to the standard DIN ISO. MICs were interpreted by EUCAST criteria, if available. Breakpoints of COL were $\leqslant$2 mg/L (susceptible) and >2 mg/L (resistant). The CLSI MIC method using CAZ±clavulanic acid (CLA) and cefotaxime±CLA was employed as screening test for ESBL-producing isolates.

Results: Isolates were primarily recovered from wounds (26%), respiratory specimens (26%) urine (20%) and blood (9%). There were 460 ICU isolates and 1,069 non-ICU isolates. Of the ECO, KPN and KOX isolates, 11.5%, 10.5% and 15.8% showed an ESBL-phenotype. MIC-50 and MIC-90 values (mg/L) are displayed in the table. Of the ECO isolates, 100% were susceptible (S) to COL and MEM, while 72% were S to CIP and 87% to GEN. Of the ECL isolates, 91% and 9% were S and resistant to COL, respectively. Susceptibilities of ECL to CAZ, CIP, GEN and MEM were 52%, 89%, 88%, and >99%, respectively. Among KPN and KOX isolates, susceptibilities to COL and MEM were 98–100%, while susceptibilities to CIP and GEN were 82–85% and 94–96%, respectively. COL was the most active drug against ABA and PAE, with susceptibility rates of 100% for either species. For ABA, susceptibilities of comparators varied from 78% (CIP) to 95% (MEM). Susceptibility rates of PAE for comparative agents were as follows: GEN (91%) followed by CAZ (87%), MEM (85%) and CIP (75%).

	ECO	ECL	KPN	KOX	ABA	PAE
n	418	191	190	101	113	516
COL	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/2	$\leqslant$0.25/0.5	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/0.5	0.5/1
CAZ	$\leqslant$0.25/2	1/$\geqslant$64	$\leqslant$0.25/4	$\leqslant$0.25/1	4/32	2/16
CIP	$\leqslant$0.06/$\geqslant$16	$\leqslant$0.06/1	$\leqslant$0.06/4	$\leqslant$0.06/4	0.25/$\geqslant$16	0.25/4
GEN	1/$\geqslant$32	0.5/4	0.5/1	0.5/1	1/16	2/4
MEM	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/$\leqslant$0.25	$\leqslant$0.25/1	0.5/8

Conclusions: Overall, susceptibility to COL seems to be high among GN pathogens in Germany. COL may be considered as an important treatment option.

P1639 Distribution of SGI1 among clinical multidrug-resistant strains of *Salmonella enterica* serotype Typhimurium from Southern Italy, 2006–2008

M. Legretto, R. Monno, A. Pupillo, D. De Vito, G. Rizzo, C. Pazzani (Bari, IT)*

Objectives: The objective of this study was to investigate and characterize the prevalence of *Salmonella* Genomic Island (SGI) among clinical multidrug-resistant (MDR) strains of *Salmonella enterica* subspecies enterica serovar Typhimurium isolated from Apulia (Southern Italy) in 2006–2008. The genetic basis of antimicrobial resistance and clonal relatedness was also assessed in this study.

Methods: Antimicrobial susceptibility was determined by the disc diffusion method. Detection of SGI, class 1 integrons and antimicrobial resistance genes was performed by PCR. Primers complementary to tdhF-int and SO44-yidY were employed to amplify the left and right junction of SGI, respectively. Class 1 integrons, gene cassettes, sulfamethoxazole, chloramphenicol and tetracycline resistance genes were detected by primers complementary to intl1, 5′ and 3′ conserved regions, sul1, floR and tetG, respectively. Genomic relatedness was established by random amplified polymorphic DNA patterns.

Results: A total of 255 S. Typhimurium strains from clinical cases were investigated. Eight-eight strains were multidrug-resistant (exhibiting resistance to three o more antimicrobials). A major pattern of resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline was detected in 56 isolates which accounted for 64% of all multidrug-resistant strains. The presence of SGI was detected in 86% of strains which exhibited the major resistance pattern. None of the other multidrug-resistant strains, except for one isolate, was found PCR positive for SGI. The isolates PCR positive for SGI were further analysed for the presence of class 1 integrons, sul1, floR and tetG resistance genes. The resistance pattern to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline was encoded by the blaPSE-1, floR, aadA1, sul1 and tetG genes, respectively; which were organized in a gene array indistinguishable to that found in SGI1. All isolates PCR positive for detection of SGI were clustered into a unique amplified DNA pattern.

Conclusion: This study has highlighted the widespread of SGI among S. Typhimurium clinical strains isolated from Southern Italy in three years of study. The presence of SGI was detected in 21% of all isolates and accounted for the most prevalent identified multidrug resistance pattern. Additionally, all strains PCR positive for SGI were clonally related and characterized by a unique RAPD cluster type.

P1640 Surveillance of antimicrobial resistance of human non-typhoid Salmonellae in Bulgaria

G. Asseva, P. Petrov, T. Kantardjiev (Sofia, BG)*

Objectives: (1) To study the etiology of human salmonelloses in Bulgaria, (2) to perform screening for resistance to antibiotics and to establish characteristic groups for each serotype typical for the country, (3) to study with genetic methods resistant isolates.

Methods: Conventional microbiological methods for identification and serotyping of salmonellae. Resistance to 16 antimicrobial agents was studied with Bauer-Kirby disc diffusion method. Screening for ESBLs was performed using the double-disc synergy method. Plasmid analysis, PCR, sequencing, PFGE were applied for investigation of resistant isolates.

Results: For the period 1999–2006 2626 *Salmonella enterica* isolates belonging to non-typhoid serotypes have been studied with conventional microbiological and genetic methods. Three hundred twenty nine *Salmonella* isolates were resistant to antimicrobial agents. For the three leading serotypes in Bulgaria: S. Enteritidis, S. Typhimurium, S. Corvallis a statistically significant increase of resistance to all

antimicrobial groups was proved. The most frequent mechanisms of resistance were as follows: S. Enteritidis – to quinolones (65.8% of isolates), S. Typhimurium – to ampicillin, carbenicillin, tetracycline, chloramphenicol (48% of isolates), S. Corvallis – to cefotaxime, ampicillin, carbenicillin, ceftazidime, cefuroxime, cephalothin, gentamicin, chloramphenicol (18.9% of isolates). Extended-spectrum β-lactamases (ESBLs) of several types: CTX-M, TEM, SHV were proved to be the main mechanism of resistance in clinical S. Corvallis isolates and were found in clinical isolates of S. Enteritidis, S. Typhimurium, S. Isangi, S. Brandenburg, S. Virchow as well.

Conclusions:

1. Clinical *Salmonella* isolates from nontyphoid serotypes, distrtibuted in Bulgaria demonstrated a definitive increase in resistance to all groups of anitibiotics.
2. Different mechanisms of resistance were proved for the leading serotypes causing human salmonelloses in Bulgaria
3. ESBLs from several types: TX-M, TEM, SHV were discovered to be the main mechanism of resistance in S. Corvallis; these enzymes were found in clinical isolates of S. Enteritidis, S. Typhimurium, S. Isangi, S. Brandenburg, S. Virchow.
4. The genetic method PFGE and cluster anaslysis demonstrated distribution of 4 clones of S. Corvallis (3 of them clustering resistant isolates) 2 clones of S. Brandenburg (1 of them clustering resistant isolates).

P1641 *Salmonella enterica* bloodstream infections in eastern London: trends in antimicrobial resistance

J. Rangaiah, S. Reddy, A. Sefton (London, UK)*

Objectives: Invasive *Salmonella* infections are severe and can be life threatening. Antimicrobial susceptibility patterns are constantly changing in clinically important serotypes. Microbiology department, Barts and The London NHS Trust provides diagnostic services to an ethnically diverse community in East London including a large immigrant population from Indian Subcontinent. We undertook this retrospective surveillance study to determine relative proportions of serotypes and recent trends in antimicrobial resistance in SALMONELLA ENTERICA bacteremia isolates in this setting.

Methods: All SALMONELLA ENTERICA serotypes isolated from blood cultures between 2005 and 2008 were included in the study. Duplicate isolates from the same patient excluded. Antibiotic susceptibility testing was performed according to national standard methods. Ciprofloxacin and ceftriaxone MIC were determined using E-test on relevant isolates. All isolates were sent to the national reference laboratory for confirmation of identification and phage typing.

Results: One hundred thirty-three SALMONELLA ENTERICA including serotypes S. TYPHI (n = 66), S. PARATYPHI A (n = 32), S. PARATYPHI B (n = 1), S. ENTERITIDIS (n = 16), S. TYHPIMURIUM (n = 5) and others (n = 13). Incidence of overall infections and by S. TYPHI, S. PARATYPHI A and S. ENTERITIDIS peaked in 2006 (47, 20, 17 and 7 respectively) and a clear trend in their fall through 2007 till 2008 was observed (26, 14, 5 and 2 respectively). E1 was the commonest phage type (n = 20) followed by E9 (n = 9). Multidrug resistance was observed in 38.4% of S. TYPHI. Reduced ciprofloxacin susceptibility (MIC $\geqslant$0.125–1 mg/l) was noted in 77.6% (38/49) of S. TYPHI and 84% (21/25) of S. PARATYPHI A isolates. This increased by 9.6% from 2006 (72.2%) to 2008 (81.8%) for S. TYPHI. In all but 1 S. TYPHI and 3 S. PARATYPHI A isolates reduced ciprofloxacin susceptibility was associated with resistance to Nalidixic acid. All isolates were sensitive to 3rd generation cephalosporins and ceftriaxone MIC was <1 mg/l in all tested (n = 79).

Conclusions: Though Enteric fever is less common in the developed world, it is a significant problem (74.4% of all salmonella bacteremia in this study) in communities who have a higher risk of exposure. Therefore, it is important to determine on a regular basis, the incidence of these infections and antimicrobial susceptibility patterns which would in turn help in devising preventive strategies and empirical antimicrobial guidelines.

P1642 Molecular characterization of *Shigella sonnei* biotype G strains harbouring class 2 integrons isolated from faecal samples in Mexico City

F. Tuz-Dzib, L. Reyes-Gonzalez, P. Lopez-Rivas, G. Ruiz-Palacios (Mexico City, MX)*

Objective: To characterize the presence of *Shigella sonnei* biotype g strains, multiresistant and carrying a typical class 2 integron (gMR-Int2) isolated from fecal specimens in a tertiary hospital in Mexico City, 1999 to 2007.

Methods: A total of 53 *S. sonnei* strains isolated from 8,607 fecal samples during 1999 January to December 2007 in Mexico City were evaluated. All isolates were tested for resistance to a panel of 22 antimicrobial agents by microdilution method according to the CLSI guidelines. Also, biotyping was carried out by fermentation profile to rhamnose, xilose and ONPG and detection of class 1 and 2 integrons was investigated by PCR using primers for integrases and conserved region of integrons. Molecular typing was performed by pulsed-field gel electrophoresis (PFGE), enterobacterial repetitive intergenetic consensus-sequenced based PCR and plasmid profile.

Results: *S. sonnei* isolates were resistant to: trime-sulphamethoxazole (66%), spectinomycin (86.7%), tetracycline (71.7%) streptomycin (85%). No isolates were resistant to quinolones and spectrum-extended cephalosporins. Class 1 integrons were detected in 2 isolates only. From 2003 to 2007, 97% (38/39) were classified as biotype g with the presence of typical class 2 integron (2.2 Kb) and a resistance phenotype to streptromycin-trimethoprim, while only 2 (14%) sporadic cases of *S. sonnei* gMR-Int2 during 1999 to 2002 ($p < 0.001$) were identified. Plasmid analysis resulted in 7 different plasmid profiles with two a five DNA bands. Based on PFGE analysis, all the more recent *Shigella* isolates showed closely related pulsotypes.

Conclusions: Our data showed that *S. sonnei* gMR-Int2 strains are emerging in Mexico since 2003. Molecular typing, indeed, suggest the spread of *S. sonnei* gMR-Int2 in our country, could be attributed to a few clones. These findings support the evidence seen in many countries to consider *S. sonnei* gMR-Int2 isolates as a pandemic strain.

P1643 Antimicrobial resistance in faecal *Escherichia coli* isolated from calves

V. Kmet, D. Bujnakova, S. Novak, M. Kmetova (Kosice, Zvolen, SK)*

Objectives: Commensal animal *Escherichia coli* may act as reservoir of antibiotic resistance genes (βlactamase and quinolone) and virulence factors (verotoxins) for human population. Animal *Escherichia coli* fluoroquinolone resistance is increasing (Slovak Antimicrobial Resistance Veterinary Database, http://www2.saske.sk/atbres/). The aim of study was to characterize antibiotic resistance of faecal *Escherichia coli*, resistance mechanisms and verotoxin production.

Methods: During 2009 year a 123 faecal *Escherichia coli* isolates from calves from various regions in Slovakia were isolated and analyzed. For phenotypic antibiotic resistance determination, CLSI (2008) criteria were used. Verotoxin genes (vt1 and vt2), ESBL CTX-M, plasmid quinolone resistance (qnrA, B, S), integron 1, replicon typing of plasmids and phylogenetic analysis were determined by PCR.

Results: The most frequently occurences were resistance to tetracycline (86%), streptomycin (78%) and ampicillin (79%). Among tested *Escherichia coli* isolates also high resistance rates to neomycin (50%), chloramphenicol (59%) and florfenicol (30%), cotrimoxazole (49%) and spectinomycine (21%) could be recorded. Resistance to ampicillin+sulbactam (10.6%), ceftiofur (1.6%), gentamicin (14%) were lower. The high level of fluoroquinolone MICs (CIP $\geqslant$4 mg/L and ENR $\geqslant$16 mg/L) reached 42%, however plasmid quionolone resistance was not detected. Majority of strains contained vt1 or vt2 genes, integron 1 and plasmid replicon types B/O, FIC, FIB or F1B. Strains belonged to pathogen phylogenetic group B2 a D and commensal group A and B1 also. Only one strain with ESBL CTX-M1 was detected in *Escherichia coli* from one farm.

Conclusions:
1. The high level of fluoroquinolone MICs in *Escherichia coli* was recorded and food animals could be a reservoir of quinolone resistance for human population.
2. ESBL CTX-M1 was detected in *Escherichia coli* only from one farm.
3. Majority of faecal *Escherichia coli* strains were verotoxigenic and belonged to pathogen and commensal phylogenetic groups.

P1644 Low prevalence of plasmid-mediated quinolone resistance determinants from farm animal faeces in a cattle-rearing area of France

F. Garnier, D. Chainier, O. Barraud, F. Denis, M.C. Ploy (Limoges, FR)*

Objectives: We conducted a retrospective study to determine the prevalence of plasmid-mediated quinolone resistance determinants (PMDR), qnr-like, aac(6′)-Ib-cr and qepA genes from farm animal faeces after enrichment infusion of the samples.
Method: We screened 258 stool samples from bovines (n = 131), pigs (n = 45), poultry (n = 42) and ovines (n = 40). Two hundred milligrams of stool were enriched by an overnight culture in a 5 milliliters Brain Heart Infusion (BHI) broth, at 37ºC without agitation. One milliliter and a half of the BHI broth was then centrifuged and DNA was extracted from pellets by using the QIAamp DNA Minikit (Qiagen®). The qnrA, qnrB, qnrS, aac(6′)-Ib-cr and qepA genes were then screened by simplex PCR-based techniques. Amplification products obtained were sequenced and analyzed with the GenBank of the ncbi website (www.ncbi.nlm.nih.gov).
Results: Out of 258 farm animal faeces, only 5 samples contained a qnrB gene (1.3%) and 1 a qnrS gene (0.38%). The qnrB-like genes detected were 1 qnrB2 gene from a poultry, 3 qnrB6 genes from one bovine and two poultry and 1 qnrB17 from a bovine. The qnrS1 gene was detected from a poultry. The qnrA-like, aac(6′)-Ib-cr and qepA genes were not found.
Conclusion: To our knowledge, this is the first time that screening of PMDR was realized directly from faeces after an enrichment step. Our results showed a low prevalence of PMDR in farm animal faeces in a cattle-rearing area of France.

Antimicrobial resistance

P1645 Activity of tigecycline against levofloxacin-resistant pathogens from 2008

S. Hawser, B. Johnson, S. Bouchillon, R. Badal, M. Dowzicky (Epalinges, CH; Schaumburg, Collegeville, US)*

Background: Levofloxacin (LVX) is indicated for use in multiple therapeutic indications including urinary tract infections, skin and skin structure infections, pneumonia, sinusitis, pyelonephritis amnongst others. Resistance to this agent has risen since its introduction in clinical use and cross-resistance with other antibacterial classes has been reported. This report documents the activity of tigecycline and other antimicrobial agents against a range of LVX-resistant organisms isolated worldwide during 2008 as part of the Tigecycline Evaluation Surveillance Trial (T.E.S.T.) study.
Methods: MICs were performed and interpreted according to CLSI and EUCAST / FDA guidelines where appropriate. A total of 32,468 clinical isolates were collected in this study of which 8,060 isolates (24.8%) exhibited resistance to LVX.
Results: The majority of LVX-resistant isolates were from Europe (48.6%), Latin America (24.5%) and North America (15.4%). The susceptibility of the most commonly detected LVX-resistant organisms for which tigecycline is indicated for use are shown in the table.
Conclusions: Taken together, this substantial body of data shows that LVX resistance is common though resistance to tigecycline was very low. There was no relationship between LVX-resistance and resistance to tigecycline. Tigecycline continues to exhibit excellent activity, including against isolates that are resistant to the widely used antibiotic LVX.

Organism (n)	MIC_{90}	%S	%I	%R
Gram-positives				
Staphylococcus aureus MRSA (1101)	0.5	99.9	0	0.1
Staphylococcus aureus MSSA (118)	0.25	100	0	0
Enterococcus faecalis (612)	0.25	99	0	1
Enterococcus faecium (606)	0.25	100	0	0
Gram-negatives				
Enterobacter aerogenes (129)	2	92.2	7.8	0
Enterobacter cloacae (434)	4	83	13	4
Escherichia coli ESBL+ (621)	1	99.7	0.24	0.06
Escherichia coli ESBL− (1063)	1	99.6	0.4	0
Klebsiella oxytoca ESBL+ (16)	4	87.5	6.2	6.3
Klebsiella oxytoca ESBL− (42)	4	88.1	11.9	0
Klebsiella pneumoniae ESBL+ (520)	4	89.6	8.1	2.3
Klebsiella pneumoniae ESBL− (327)	2	90.2	8	1.8

P1646 Antimicrobial activity of tigecycline and comparator agents tested against clinical bacterial strains from the Asia-Pacific region, 2008–2009

J.M. Bell, H. Sader, D. Farrell, R. Jones, J. Turnidge (North Adelaide, AU; North Liberty, US)*

Background: To assess the activity of tigecycline and comparator agents against strains collected in various countries in the Asia-Pacific (APAC) region. Tigecycline is the first glycylcycline approved for clinical use and has demonstrated activity against key Gram-positive and -negative bacterial pathogens worldwide, including multidrug-resistant (MDR) *Acinetobacter* spp., ESBL-producing Enterobacteriaceae (ENT), methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant enterococci (VRE); pathogens frequently isolated in the APAC region.
Methods: As part of the SENTRY Antimicrobial Surveillance Program, 35 institutions from 8 nations contributed 9,804 strains as follows (no. of medical centres/strains): Australia (7/2,494), China (CH; 17/4,098), Hong Kong China (1/395), South Korea (KOR; 3/960), New Zealand (NZ; 3/634), Singapore (SIN; 1/433), Taiwan (TW; 2/590) and Thailand (1/200). All isolates were forwarded to a central laboratory (USA or Australia) where they were tested against tigecycline and comparators by CLSI broth microdilution methods. CLSI and EUCAST interpretations were applied for comparison agents. Tigecycline breakpoints published by the USA-FDA were applied for indicated species.

Table 1

Organism (no. tested)	Cumulative % inhibited at tigecycline MIC (mg/L) of:						
	⩽0.06	0.12	0.25	0.5	1	2	4
S. aureus (3,142)	2.0	29.7	92.1	99.5	100.0	–	–
CoNS (214)	4.7	31.3	86.9	99.1	100.0	–	–
Enterococcus spp. (1,129)	11.6	51.1	97.9	99.8	100.0	–	–
β-haemolytic strep. (362)	93.4	98.6	100.0	–	–	–	–
Viridans group strep. (96)	90.6	96.9	99.0	100.0	–	–	–
S. pneumoniae (907)	84.2	93.7	100.0	–	–	–	–
E. coli (1,056)	1.6	29.7	91.1	99.1	99.8	100.0	–
Klebsiella spp. (714)	0.0	1.7	32.1	80.5	95.9	98.3	99.9
Enterobacter spp. (405)	0.0	0.5	21.7	85.2	94.3	97.8	99.0
Acinetobacter spp. (533)	4.7	19.7	32.5	49.2	89.7	99.1	100.0
P. aeruginosa (731)	0.0	0.0	0.3	0.6	1.1	4.2	22.2

Results: MRSA rate was 43.4% overall, ranging from 8.7% in NZ to 70.1% in SIN and 77.3% in KOR. 83.6% of coagulase-negative staphylococci were resistant to oxacillin. VRE rates (10.1% overall) were highest in TW (47.4%) and KOR (32.6%). Prevalences of ESBL phenotype among *E. coli*/*Klebsiella* spp. were 35.4/33.7% overall, highest in TW (41.9/54.2%) and CH (62.0/42.6%). Imipenem (IMI) resistance (R) among *Acinetobacter* spp. was 49.5% overall, highest in KOR (78.7%) and SIN (87.5%). Tigecycline was very active against the most frequently isolated organisms, except *P. aeruginosa* (Table). >99% of staphylococci and enterococci were inhibited at

⩽0.5 mg/L of tigecycline. Among ENT (including ESBL-producing strains) and *Acinetobacter* spp. (including IMI-R strains) 97.8–100.0% were inhibited at ⩽2 mg/L of tigecycline.
Conclusion: Almost 10,000 clinical strains from the APAC region were tested and tigecycline was very active against the organisms most frequently recovered from the hospitals evaluated, except *P. aeruginosa*. Tigecycline spectrum included MRSA, VRE, ENT with ESBL phenotype and IMI-R *Acinetobacter* spp. Tigecycline appears to be a valuable option for the treatment of infections caused by MDR organisms frequently isolated in the APAC region.

P1647 Multicentre evaluation of tigecycline activity in Europe: report from the SENTRY Antimicrobial Surveillance Programme, 2009

D. Farrell, D. Biedenbach, R. Jones, H. Sader (North Liberty, US)*

Objectives: To assess the contemporary potency and spectrum of tigecycline and comparator antimicrobials against recent (2009) Gram-positive (GP) and -negative (GN) pathogens from Europe. Tigecycline, the first glycylcycline, presents a therapy option for emerging multidrug-resistant (MDR) GP and GN pathogens and is approved by the European Medicines Agency (EMEA) for the treatment of complicated skin and skin structure (cSSSI) as well as intra-abdominal infections (IAI).
Methods: A total of 4,284 GP and GN clinically-significant non-duplicate isolates from multiple types of infections were collected from 13 European (EU) countries and Israel that participated in the tigecycline surveillance program during 2009. Susceptibility (S) testing was performed by a central monitoring laboratory (JMI Laboratories) against a large panel of antimicrobials using CLSI methods (M07-A8, 2009). Identifications were confirmed and interpretive/screening criteria were also by CLSI guidelines (M100-S19, 2009). All quality control tests were within published ranges.
Results: Tigecycline inhibited all *S. aureus* isolates at ⩽0.5 mg/L (MIC90 at 0.25 mg/L) and coagulase-negative staphylococci (CoNS) at ⩽1 mg/L (MIC90 at 0.5 mg/L) regardless of S to oxacillin. Tigecycline also had potent activity against all enterococci (MIC90, 0.25 mg/L), including vancomycin-resistant (VRE) strains, and β-haemolytic streptococci (BHS; MIC90, 0.06 mg/L). The MIC90 for Enterobacteriaceae was 0.25, 1, and 1 mg/L for *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., respectively. Tigecycline was active against *Acinetobacter* spp. (MIC90, 2 mg/L), but less potent against isolates of *P. aeruginosa* (MIC50/90, 4/>4 mg/L). Tigecycline activity is summarized in the Table against nine organism groups.
Conclusions: In 2009, tigecycline continued to demonstrate broad antimicrobial activity against common pathogens associated with cSSSI and IAI occurring in the EU. Tigecycline was active against antimicrobial-resistant as well as MDR strains including MRSA, VRE, and Enterobacteriaceae (including ESBLs). Based on the potency and spectrum shown here, tigecycline has a role in empiric therapy for treating cSSSI and IAI bacterial pathogens in these EU nations. Tigecycline exhibited spectrum/potency generally exceeding currently available agents against sampled isolates from the EU.

Table 1. Activity of tigecycline tested against 4,284 isolates of Gram-positive and -negative pathogens from European medical centers (2009)

Organism (no. tested)	Cumulative % inhibited at MIC (mg/L)							
	⩽0.03	0.06	0.12	0.25	0.5	1	2	⩾4
S. aureus (1,403)	0.0	9.6	41.7	97.6	100.0	–	–	–
CoNS (462)	1.3	12.8	34.4	89.2	99.6	100.0	–	–
Enterococcus spp. (614)	1.5	19.4	48.9	96.4	100.00	–	–	–
BHS (214)	55.6	95.3	99.1	100.0	–	–	–	–
E. coli (795)	0.0	9.8	49.9	94.2	99.9	100.0	–	–
Klebsiella spp. (297)	0.0	0.0	4.0	57.9	87.9	94.6	100.0	–
Enterobacter spp. (147)	0.0	0.0	0.0	39.5	86.4	92.5	96.6	100.0
P. aeruginosa (287)	0.0	0.4	0.4	0.7	0.7	3.1	11.2	100.0
Acinetobacter spp. (65)	0.0	1.5	18.5	36.9	53.9	75.4	96.9	100.0

P1648 Results of the Finnish national resistance surveillance FiRe network from 1996 to 2008

M. Bergman, A. Nissinen, P. Huovinen, A. Hakanen and the Finnish Study Group for Antimicrobial Resistance (FiRe network)*

Objectives: Nationwide laboratory networks are essential when monitoring trends in antimicrobial susceptibility in different bacteria. Susceptibility testing standards and data collecting systems are in a key role when producing reliable and uniform data. In this study, we present the structure and operating model and main results of the Finnish Study Group for Antimicrobial Resistance (FiRe).
Methods: FiRe is a coalition of the Finnish clinical microbiology laboratories and the bacteriology units of the National Institute for Health and Welfare (THL). FiRe was founded in 1992 and one of its primary goals was to standardise the antimicrobial susceptibility testing methodology in the country. In 1996, the CLSI (NCCLS) standard method was accepted as the basis for the standard methodology to be recommended in Finland. However, from the beginning of 2011, FiRe will adapt its susceptibility testing to the EUCAST standard. Since 2006, resistance data has been collected into a centralized database using WHONET program in the data processing. WHONET is a Windows-based database software developed for the management of antimicrobial susceptibility test results.
Results: The resistance of *Escherichia coli* (N >750,000) has changed during the last years from 23.7% to 16.6% to trimethoprim, from 3.5% to 5.1% to fluoroquinolones and from 3.2% to 1.0% to nitrofurantoin. The resistance of *Streptococcus pneumoniae* (N >50,000) has increased during the same period from 1.4% to 3.1% to penicillins, from 5.3% to 24.6% to macrolides and from 3.3% to 11.5% to clindamycin. In 2008, susceptibility data from the whole of Finland was obtained electronically for the first time. The database was able to automatically produce a report for different bacteria (Finres). In 2008, the number of tested isolates was 402 727 including 2 860 334 susceptibility test results. The data collected covered 18 clinically most important bacterial species.
Conclusions: According to our experience, resistance surveillance benefits of a centralised organization with a uniform nationwide susceptibility testing standard. This kind of structure enables efficient and timely national resistance surveillance.

P1649 Resistance patterns of selected respiratory tract pathogens in Poland (continuation of the Alexander Project)

I. Wasko, A. Skoczynska, A. Kuch, A. Golebiewska, M. Forys, W. Hryniewicz (Warsaw, PL)*

Objective: To obtain the antimicrobial susceptibility data on major pathogens responsible for community acquired respiratory tract infections (RTI) in Poland, 2007–2009.
Methods: From January 2007 until September 2009, 1041 isolates were collected: 424 *S. pneumoniae*, 345 *H. influenzae*, and 272 *S. pyogenes*. The first two species were isolated from patients with community acquired lower RTIs. *S. pyogenes* was isolated from patients with pharyngitis/tonsilitis. The isolates were identified to the species level by standard procedures and MICs were determined by the broth microdilution method and interpreted according to the CLSI guidelines.
Results: Among *S. pneumoniae* isolates 26.7% had penicillin MICs >0.06 mg/l whereas 9.7% of them had MIC >2 mg/l. The proportion of isolates with MIC >2 mg/l has increased from 3.9% (only intermediate-resistant) in the first year to 12.0% and 15.9% in the second and third year of the study, respectively. The susceptibility of *S. pneumoniae* was as follow: amoxicillin (94.3%), cefaclor (72.0%), cefprozil (76.5%), 3rd generation cephalosporins (90.3%), macrolides (68.9%), ciprofloxacin (91.0%), levofloxacin (99.0%), clindamycin (75.2%), tetracyclines (70.8%), trimethoprim/sulfamethoxazole (49.3%), rifampicin (99.8%) and telithromycin (98.7%).
Among *H. influenzae* isolates 4.4% were resistant to ampicillin, via production of β-lactamases and 13.4% had changes in PBP3 being low-BLNAR. All *H. influenzae* isolates were susceptible *in vitro* to

amoxicillin/clavulanic acid, 3rd generation cephalosporins, azithromycin, fluoroquinolones and chloramphenicol (except one isolate), whereas 93.9%, 97.7%, 99.4% and 72.8% were susceptible to cefaclor, cefprozil, tetracyclines and trimethoprim/sulfamethoxazole, respectively.
A significant number of *S. pyogenes* isolates was non-susceptible to tetracycline (22.8%) and erythromycin (14.7%). Among isolates resistant to erythromycin half represented cMLSB and the other half iMLSB phenotype.
Conclusions: The percentage of pneumococci with higher penicillin G MICs have been increasing during the study including highly resistant isolates, what may eliminate this drug from empirical RTIs therapy caused by this pathogen. A trend of quickly growing percentage of *S. pyogenes* isolates resistant to erythromycin and tetracycline was observed.

P1650 Trends of resistance in *Helicobacter pylori* isolates in Austria, 2004–2008

E. Jülg, G. Zarfel, M. Hönigl, G. Feierl, E. Leitner, L. Masoud, U. Wagner-Eibel, E. Marth, A.J. Grisold (Graz, AT)*

Objectives: Incidence of *Helicobacter pylori* infection has increased worldwide, with *H. pylori* being the primary cause of peptic ulcer disease and an etiologic agent in the development of gastric cancer. Eradication of *H. pylori* infection requires a proton pump inhibitor (PPI) and a combination of two or more antibiotics depending on the antibiotic-susceptibility of isolates. As the infection is primarily treated with a standard therapeutic regimen, it is important to know the local antibiotic-susceptibility patterns. Aim of this study was to analyze the susceptibility trends of *H. pylori* strains isolated from gastric biopsies.
Methods: In this retrospective study 546 *H. pylori* strains, isolated from 2004–2008 at the Institute of Hygiene, Medical University of Graz, Austria, were investigated. The isolates were characterised by standard laboratory methods. Susceptibility testing was performed by minimum inhibitory concentration (MIC) determination by the E-test method (AB biodisk, Solna Sweden) for amoxicillin, clarithormycin, metronidazole, tetracycline and ciprofloxacin according to the recommendations of CLSI.
Results: From 2004–2008 a total of 546 *H. pylori* isolates were investigated (2004: 64; 2005: 94; 2006: 132; 2007: 109; 2008: 147). All isolates, except one in 2006 were susceptible to amoxicillin, and all except one in 2008 susceptible to tetracycline, respectively. For ciprofloxacin resistance rates (%/year) were 5.4 /2004; 8.0/2005; 15.0/2006; 15.8/2007; 13.3/ 2008, for clarithromycin: 53.6/2004; 66.6/2005; 65.4/2006; 66.4/ 2007; and 68.8/2008 and for metronidazole: 35.7/2004; 53.3%/ 2005; 35.5/ 2006; 48.4/ 2007, and 50.4/2008, respectively.
Conclusion: In this retrospective study of *H. pylori* isolates 99.8% susceptibility was found for amoxicillin and tetracycline. For clarithromycin resistance rates were found to be constantly high at 65.4% to 68.8%. Varying resistance rates were found for metronidazole, ranging from 35.5% (2006) to 53.9% (2005), whereas rates of resistance for ciprofloxacin were at a low but increasing rate from 5.4% in 2004 to 13.3% in 2008. These data underline once more the usefulness of individual testing and local surveillance in *H. pylori*.

P1651 Primary *Helicobacter pylori* resistance to antimicrobial agents in southern Poland

E. Karczewska, I. Wojtas, E. Sito, A. Budak (Cracow, PL)*

Objectives: Common consumption of antibiotics and chemioterapeutics contributed to the development of resistance among *Helicobacter pylori* clinical strains that is the main factor compromising the efficacy of eradication therapy. Therefore, the aim of this study was to determine the current state of amoxicillin, metronidazole and clarithromycin primary resistance prevalence in *H. pylori* clinical isolates obtained from symptomatic adult patients over a period of one year (from January 2008 to February 2009). According to the appearance of alternative regimens including newer fluoroquinolones, the susceptibility to levofloxacin was also assessed.
Methods: Fifty eight *H. pylori* strains isolated from gastric biopsies of patients who underwent upper gastrointestinal endoscopy at gastrological clinic in Krakow were examined. Only patients with no previous *H. pylori* eradication attempt were enrolled in the study. Minimal inhibitory concentration (MIC) values of amoxicillin, metronidazole, clarithromycin and levofloxacin were determined by Etest method. Resistance breakpoints to amoxicillin, metronidazole, clarithromycin and levofloxacin were defined as MIC values: >1 μg/ml, >4μg/ml, ⩾1 μg/ml and >1 μg/ml respectively.
Results: All strains were susceptible to amoxicillin which showed the highest *in vitro* activity (MIC = 0.016 μg/ml). Resistance to metronidazole was 55%, to clarithromycin 26% and 19% of isolates were simultaneously resistant to clarithromycin and metronidazole. High level of resistance to metronidazole (MIC >256 μg/ml) was observed in the majority of resistant strains. Resistance to levofloxacin was detected in 7% of *H. pylori* strains. Two of fifty eight *H. pylori* strains (3%) were multi-resistant (to clarithromycin, metronidazole and levofloxacin).
Conclusion: Our results revealed high rates of *H. pylori* primary resistance to metronidazole, clarithromycin and to both: clarithromycin and metronidazole in adults patients in the southern part of Poland. It was shown that nitroimidazole resistance is less clinically relevant than clarithromycin resistance. Therefore clarithromycin should not be recommended for empirical eradication therapy. To avoid the increasing clarithromycin resistance and treatment failure, pretreatment susceptibility testing should be performed routinely. Amoxicillin is still very effective against *H. pylori* strains. Low rate of *H. pylori* resistance to levofloxacin allows to reserve this fluoroquinolone to rescue treatment.

P1652 Antimicrobial susceptibilities of *Propionibacterium acnes* isolated from patients with acne lesions

M. Hui, L.H. Fu, Z.H. Liu, L.Y. Lam, M. Eastel, N.M. Luk, A.K. Chan, K.K. Lo, C.Y. Chan (Shatin, Hong Kong, HK)*

Objectives: Prolonged antibiotic use is sometimes required for patient with acne. In the absence of routine anaerobic susceptibility testing, the choice of antibiotics is often empirical. Therefore we aimed to determine the *Propionibacterium acnes* susceptibility profiles from patients with acne lesions.
Methods: Patients presented to the dermatology clinic with acne lesions were recruited. Skin swabs were taken from the lesions and transported in Amies medium. These were then cultured anaerobically on horse blood agar supplemented with vitamin K for 14 days at 37ºC. Identities of the organisms were confirmed by Gram's staining, biochemical reactions and short chain fatty acid production. MIC testing was performed with agar dilution method according to the CLSI M11-A7. Ampicillin, doxycycline, minocycline, erythromycin, clarithromycin, tetracycline and clindamycin were tested. Interpretive criteria for ampicillin, tetracycline and clindamycin were adopted from CLSI. Descriptive data was recorded for other antibiotics.
Results: A total of 57 isolates were collected. All of them were susceptible to ampicillin. Tetracycline and clindamycin susceptibilities were 53 (93.0%) 46 (80.7%) respectively. MIC50 and MIC90 for doxycyline were 0.25 mg/L and 2 mg/L respectively; while that of minocycline were 0.12 mg/L and 1 mg/L respectively. Erythromycin and clarithromycin MICs frequency distribution showed bimodal distribution. For erythromycin, a cluster of 46 isolates had MIC of 0.06 mg/L, while the remaining 11 isolates had MIC of ⩾128 mg/L. These two clusters of isolates exhibited the same bimodal distribution with clarithromycin (at <0.03 mg/L and ⩾125 mg/L).
Conclusion: Antibiotic resistance against ampicillin, tetracycline were uncommon. However, clindamycin resistance appeared to be emerging. Cross resistance with erythromycin and clarithromycin was observed, suggesting a common resistant mechanism. Minocycline and doxycycline MICs were still low when compared with previously reported data. Overall, antibiotic resistance in *Propionibacterium acnes* appears to be emerging and requires continue surveillance.

P1653 Increasing resistance of *Bacteroides fragilis* and related species to carbapenems: a US survey, 2006–2008

D.R. Snydman, N.V. Jacobus, L. McDermott, R. Ruthazer, Y. Golan, D.W. Hecht, E.J. Goldstein, L. Harrell, S. Jenkins, D. Newton, J.E. Rosenblatt (Boston, Chicago, Culver City, Durham, New York, Ann Arbor, Rochester, US)*

Objectives: Since recognition of transferable clindamycin and tetracycline resistance in *Bacteroides* in 1980, we have coordinated a US national survey on the susceptibility of *B. fragilis* to assess trends in resistance and to provide guidance on susceptibility, since these species are not routinely tested in clinical laboratories.
Methods: Agar dilution MICs were determined for 1314 isolates from 2006–2008 for *B. fragilis* and related species from 7 geographically diverse centers in the US. Antibiotics included 4 carbapenems, 2 b-lactam/b-lactamase inhibitors, 1 quinolone, 1 glycylcycline, clindamycin, metronidazole, and chloramphenicol. Isolate identity was confirmed by API 20ATM.
Results: Analysis of resistance trends from 2006–2008 for *Bacteroides fragilis* showed an increase in resistance to the carbapenems, with resistance rates rising from 1%, 2% and 1.5% in 2006 for imipenem, meropenem and doripenem rose to 2.7%, 5.4%, and 5.4% in 2008. Ertapenem resistance rose from 2% in 2006 to 4.5% in 2008. Cefoxitin resistance rose from 3.5% to 9.4%. Clindamycin resistance remained unchanged with rates of 29.5%. Moxifloxacin resistance rose from 29.8% to 34.8% in 2008. Tigecycline resistance was 3% in 2006 compared to 5.3% in 2008. For the b-lactamase inhibitors, piperacillin–tazobactam resistance rose from 1% to 2.7%, while ampicillin-sulbactam remained unchanged (3.5%). For non fragilis species such as *B. ovatus, B. thetaiotaomicron,* and *B. vulgatus* moxifloxacin resistance rose from approximately 35% to over 60% in 2008. Clindamycin resistance was over 40%. Chloramphicol remained 100% active, however, isolates from geographically diverse areas had MIC's to chloramphenicol of 16 mcg/ml. No metronidazole resistance was observed in 2008 although 4 isolates had MIC of 8 mcg/ml.
Conclusion: In 2008 resistance of *B. fragilis* to the carbapenems increased dramatically. However, even with the rise in resistance. the rates are still low and represent only a handful of isolates from geographically diverse areas. The βlactamase inhibitors also remain active. Clindamycin and moxifloxacin resistance is very high, and these agents can not be regarded as useful as monotherapy for seriously ill patients with intraabdominal mixed infections. Metronidazole and chroramphenicol resistance, although not detected, shows a shift to a few strains with higher MIC's. Whether these trends will continue will be assessed in future surveillance studies.

P1654 Identification and sensitivity testing of *Bacteroides* species from blood cultures

A. Smith, A. Berry, A. Lennon, A. Tyers, M. Jackson, M. Riggio (Glasgow, UK)*

Objective: Routine identification and sensitivity testing of anaerobes in routine diagnostic microbiology is usually undertaken using phenotypic testing with a limited range of antimicrobial agents. Inadequate identification and sensitivity testing compromises surveillance of antimicrobial susceptibility patterns and empiric choice of antibiotics. The aim of this study was to pilot the surveillance of antimicrobial resistance in *Bacteroides* species from blood cultures using molecular identification (16S rRNA gene sequencing) and antimicrobial susceptibility testing using E-test methodology.
Method: Blood cultures collected at Glasgow Royal Infirmary over a 4-year period (n = 73,862) were reviewed for the number of anaerobes identified over this study period. We identified 151 strict anaerobes from these records and selected 28 consecutive isolates provisionally identified as Gram negative anaerobic rods for further study. Isolates were retrieved from frozen cultures and sub-cultured for purity. Isolates were subsequently identified by PCR and sequencing of the 16S rRNA gene and compared to phenotypic methods of Mast ring, Rapid Ana II and Rapid32A.
Results: 16S rRNA gene sequencing identified isolates as *Bacteroides fragilis* (n = 16), 4 *Bacteroides thetaiotaomicron* (n = 4), *Bacteroides ovatus* (n = 2), *B. vulgatus* (n = 2), Parabacteroides distasonis (n = 1) and uncultured bacterial clones (n = 3). Concordance between genotypic and phenotypic identification was 50% for the Mast ID and Rapid Ana II systems and 66% for the Rapid 32A system. The antimicrobial susceptibility of the *B. fragilis* isolates comprised MIC50 = 16ug/ml;, MIC90 ⩾ 32 for penicillin; MIC50 = 0.125, MIC90 = 0.5 for augmentin; MIC50 = 0.75, MIC90 = 2.0 for erythromycin; MIC50 = 0.19, MIC90 = 4 for clindamycin and MIC50 = 4, MIC90 = 12 for tetracycline.
Discussion: Use of 16sRNA sequencing of Gram negative anaerobes from blood cultures has proved useful in accurate identification of isolates to species level, although 3 isolates were identified as previously uncultured clones. Antimicrobial susceptibility testing suggests that augmentin has a useful spectrum of activity against *B. fragilis* isolates. Phenotypic assays have a limited role in accurate species identification and should not be used in surveillance schemes for antimicrobial resistance in anaerobes. Molecular sequencing techniques provide an accurate method for identification and should be considered as the gold standard for identification of septicaemic isolates.

P1655 Prevalence of multidrug-resistant bacteria and *Clostridium difficile* in Dutch hospitals, 2007–2009

T.I. van der Kooi, J. Manniën, J.C. Wille, B.H. van Benthem (Bilthoven, Utrecht, NL)*

Objectives: To assess the prevalence of presence of colonization or infection of meticillin resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamase producing bacteria (ESBL), vancomycin resistant enterococci (VRE) and *Clostridium difficile* in Dutch hospitals and possible trends therein.
Methods: Since 2007 the PREZIES network for the surveillance of nosocomial infections (NI) in the Netherlands organizes biannual national prevalence surveys. Data on the presence of multidrug resistant (MDR) bacteria and *C. difficile* were collected from admission through the survey day. All inpatients of one year and older, that have been admitted before the survey day were included, with the exception of patients in psychiatry and (haemo)dialysis. The surveillance took place on several days in March and/or October, but patients of one ward had to be included on the same day.
Results: In total, 37,601 patients of 50 hospitals were included from March 2007 through March 2009. 53% of all Dutch hopsitals participated and 76% of them participated more than once. The average prevalence of NI was 6.6% and the proportion of patients with NI 5.8%. Table 1 shows the prevalence of the MDR micro-organisms and *C. difficile* over time.

Table 1. Percentage of patients (and range between hospitals) positive for multidrug resistant organisms and *Clostridium difficile* per survey

	March 2007	October 2007	March 2008	October 2008	March 2009
Number of patients	8,424	3,497	9,449	5,760	10,471
Number of hospitals	30	12	29	21	37
MRSA	0.2 (0.0–1.2)	0.2 (0.0–0.6)	0.2 (0.0–12)	0.1 (0.0–13)	0.3 (0.0–3.8)
ESBL	0.5 (0.0–2.2)	0.4 (0.0–1.5)	0.6 (0.0–3.2)	0.6 (0.0–1.7)	1.0 (0.0–3.0)
VRE	0.04 (0.0–0.6)	0.03 (0.0–0.3)	0.03 (0.0–0.3)	0.02 (0.0–0.8)	0.2 (0.0–1.7)
Proportion of hospitals with VRE	6.7	8.3	10.3	4.8	16.2
C. difficile	0.5 (0.0–1.9)	0.3 (0.0–0.8)	0.6 (0.0–4.7)	0.1 (0.0–1.3)	0.3 (0.0–0.9)

The prevalence of MRSA and *C. difficile* remained constant. The prevalence of ESBL showed a significant increase (Spearman's rank correlation coefficient 0.02, $p < 0.0001$) over time. In the seven hospitals that participated in all five surveys, the increase was significant too (0.2, 0.3, 0.4, 0.4 and 0.8 respectively). The high prevalence of patients with VRE in March 2009 could partly be contributed to an outbreak in one hospital. However, when only the number of hospitals with VRE was considered the increase was significant too.
In the Netherlands, routine screening of MRSA occurs in patients that havebeen admitted to foreign hospitals, in calf and pig farmers and

their family members, and in contacts of MRSA patients. Therefore, the proportion cannot be interpreted as the prevalence of MRSA infection. For VRE only contact screening, in specific wards, is recommended. For the other organisms there are no screening recommendations.
Conclusion: The prevalence of *C. difficile* infection and MRSA colonization or infection did not increase during 5 successive surveys. The prevalence of ESBL and VRE increased significantly over the last 2 years. This increase might be partly explained by increased awareness for ESBL and VRE in the last year.

P1656 Heteroresistance to imipenem observed for clinical *Clostridium difficile* isolates using E-test susceptibility testing

T. Norén, I. Alriksson, J. Andersson, M. Unemo (Örebro, SE)*

Objective: Carbapenems are frequently used in critically ill hospital patients who are at high risk of *Clostridium difficile* infection (CDI). The resistance rate in *C. difficile*, measured by minimal inhibitory concentration (MIC), is generally found low by standard agar dilution (imipenem 4–8, meropenem 1–4 and ertapenem 4–8 mg/L), thus presumably a low risk of inciting CDI. In contrast, using the Etest (Biodisk AB, Solna, Sweden), a high level of imipenem resistance (97% of 606 isolates, MIC > 32 mg/L) was observed in 1993–2008 required further evaluation.
Method: The Etest was performed on 21 toxigenic *C. difficile* isolates recovered consecutively during January 2008 belonging to 11 different PCR-ribotypes. Recommended IsoSensitest agar media.was used for four different concentrations of *C. difficile* inoculum (McFarland 0.5, 1, 2 and 4). The MIC was read at the intersection on the antimicrobial gradient strip after 48 h anaerobic incubation at 37ºC. Imipenem, ertapenem and meropenem were tested. *Brucella* and Müeller-Hinton agar were compared and finally agar dilution was performed according to CLSI to confirm our result. Susceptible controls *C. difficile* (ATCC 9689), *Bacteroides fragilis* (ATCC 25285) and *C. perfringens* (ATCC13124) were included.
Results: Out of the samples investigated 14/21 had initially MICs of >32 mg/L by Etest. Double zones were read on 10 of these 14 (>32 and 2–4 mg/L). Microcolonies sub-cultured from the resistant zone gave similar results of double zones. Diluted concentrations of the inoculum (McFarland 4 to 0.5) still read±one step of MIC on the E-test strip. Müeller-Hinton or *Brucella* agar did not alter the MIC interpretation. Agar dilution in *Brucella* agar, however, lowered MICs of all but one isolate to expected levels of 1–4 mg/L. Two isolates were ertapenem resistant using the Etest while all were susceptible to meropenem according to CLSI guidelines.
Conclusion: Etest results from testing *C. difficile* against imipenem may show incorrect high MICs or may result the selection of a heteroresistant subpopulation within the inhibition zone of the E-strip. Accordingly caution should be made evaluating Etest results for imipenem when testing *C. difficile* and possibly if encountering unexpected imipenem Etest related resistance in other pathogens.

P1657 Prevalence and antimicrobial susceptibility of mycoplasmas isolated from male urethral exudates in a Spanish hospital

A. Beteta, L. Vega, S. Martínez, L. Gómez-Chacón, M. Gil (Talavera de la Reina, ES)*

Objectives: *Mycoplasma hominis* and *Ureaplasma urealyticum* can cause various pathologies such as urethritis, prostatitis, epididymitis, urethral syndrome and urolithiasis. The aim of this study was to describe the antibiotic susceptibility profile of *M. hominis* and *U. urealyticum* isolated from male urethral exudates in patients with urethritis, from 2000–2009 at the Ntra. Sra. del Prado Hospital in Toledo.
Methods: The samples obtained from urethral exudates from men with symptoms of urethritis were collected by standard procedures from January 2000 to October 2009. Detection, quantification and antimicrobial susceptibility testing of *U. urealyticum* and *M. hominis* were performed by *Mycoplasma* IST2 test (bioMérieux SA).
Results: A total of 736 patients were recruited, and 383 (52%) samples were considered of microbiological value and informed to the clinician. *Ureaplasma urealyticum* was isolated in 11.5% patients, and *Mycoplasma hominis* in 2.6% of them.
For *Ureaplasma urealyticum* isolates the susceptibility rate was 10.2% for ciprofloxacin and 30.8% for ofloxacin. The susceptibility rates for azithromycin, clarithromycin and erithromycin were 89%, 83% and 87.2% respectively. For josamycin and pristinamycin were 97% and 93.1%. For both doxycycline and tetracycline were 100%.
For *Mycoplasma hominis* isolates, the susceptibility rates for ciprofloxacin and ofloxacin were 66.7% and 62.5% respectively. All the isolates were resistant to azithromycin, clarithromycin and erithromycin. For josamycin, pristinamycin, doxycycline and tetracycline the susceptibility rate was 100%.
Conclusions: Fluoroquinolones resistance was very high among *U. urealyticum* and *M. hominis* isolates. Macrolides resitance was low in *U. urealyticum,* while *M. hominis* was resistant to all of them.
Both *U. urealyticum* and *M. hominis* indicated high rates of susceptibility to doxycycline and tetracycline, so they may be used in empirical treatment of Mycoplasmas genital infections.

P1658 Prevalence rate and antimicrobial susceptibilities of *Ureaplasma urealyticum* and *Mycoplasma hominis* in pregnant women residing in Jinju, Korea

I. Bae, E. Koh, S. Kim, N.Y. Lee, H. Jung (Jinju, Seoul, Masan, KR)*

Objectives: *Ureaplasma urealyticum* and *Mycoplasma hominis* are associated with an increased risk of developing pregnancy complications, such as a preterm birth and premature rupture of membranes. The distribution of antimicrobial susceptibility of genital mycoplasmas has changed over time and geographic area. The purpose of this study was to determine the isolation rates and antimicrobial susceptibilities of *U. urealyticum* and *M. hominis* in pregnant women who reside in Jinju, Korea.
Methods: Vaginal swabs were obtained from 258 pregnant women from the years 2004 and 2008. The swabs were tested for the presence of *U. urealyticum* and *M. hominis* at Gyeongsang National University Hospital. The identification and antimicrobial susceptibilities were determined with commercially available kits, *Mycoplasma* IST2 (bioMérieux, Marcy-l'Etoile, France) and evaluated according to the CLSI.
Results: *U. urealyticum* and *M. hominis* were detected in 114 of 258 specimens (44.2%). *U. urealyticum* was detected in 105 specimens (38.6%) and *M. hominis* was detected only in two specimens (1.8%). Seven specimens (6.7%) were positive both for *U. urealyticum* and *M. hominis.* Susceptibility of *U. urealyticum* to azithromycin, erythromycin, and clarithromycin were 75.2%, 82.9% and 88.6%, respectively, while almost all of the isolates were susceptible to josamycin (99.0%) and pristinamycin (100%). Susceptibility of ofloxacin and ciprofloxacin were shown to be 56.2% and 15.2%.
Conclusion: The isolation rates of genital mycoplasma in pregnant women were 44.2% in Jinju; most of the mycoplasmas were identified as *U. urealyticum. U. urealyticum* were highly resistant to quinolones, but susceptible to josamycin and doxycyline. Characteristically, susceptibility to azithromycin (the empirical treatment for pregnant women in our region), was not as high as expected. Therefore, empirical treatment without the isolation of genital mycoplasma will fail in many cases. *In vitro* determination of the antimicrobial susceptibility of the genital mycoplasmas in each clinical case is required to avoid therapeutic failures.

P1659 Prevalence of micro-organisms responsible for ventilator-associated bacterial pneumonia among patients in intensive care unit of a hospital in Madrid. Comparison with a multicentre study

M.C. Martínez, B. Buendía, M.J. Moreno, S. Agudo, M. López-Brea (Madrid, ES)*

Objective: To study the most prevalent microorganisms isolated in patients with mechanical ventilation of ICUs in Hospital Universitario

de la Princesa during a period of six month and compare it with a multicentric epidemiological study (ENVIN-UCI: Estudio Nacional de Vigilancia de Infección Nosocomial en Servicios de Medicina Intensiva) realized in more than one hundred ICUs from hundreds of Spanish hospitals in the years 2006, 2007 and 2008.
Methods:149 purulent tracheobronquial secretion removed by bronchoaspirate from patients with ventilator-assiated pneumonia (VAP) – in accordance with the criterias of ventilator-associated pneumonia – were processed by quantitative culture techniques in blood agar and chocolate agar and incubated at 37ºC in O2 and CO2 atmosphere respectively until 48hs. Gram stain was realized to begin an empirical antibiotic therapy. Identification of microorganism was performed by MicroScan of Siemens and antibiotic susceptibility by microdilution.
Results: From 365 patients admitted in ICU from March to August of Hospital Universitario de la Princesa, we had 149 isolated from 60 patients with ventilator-associated pneumonia. The most prevalent microorganisms in ICU of Hospital Universitario de la Princesa, compared with the multicentric epidemiological study, are listed in the table.
The most prevalent microorganism isolated in bronchoaspirate has been MSSA, with a percentage similar to the multicentric study, followed by *Pseudomonas aeruginosa*. We can probe *Pseudomonas aeruginosa* has a significant reduction in his prevalence, compared to multicentric study, and this percentage is slightly lower than MSSA. Furthermore, the rest of the microorganisms isolated are so different from the total epidemiological study.
Conclusion: The primary organisms responsible for VAP include *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Enterobacteriaceae. However, precentage of aetiologies differs considerably between intensive care units.
We have proved *Acinetobacter baumannii* is lower in our hospital than in the rest of Spanish hospital included in ENVIN-UCI, where this microorganism is one of the most prevalent Gram-negative rod isolated. In our hospital *Acinetobacter baumannii* has suffered a considerable reduction in its prevalence in the last years. The significant morbidity and mortality associated with VAP require early konwledge of the prevalence of the microrganisms responsible of it in our ICUs to select appropriate empirical therapy.

Microorganism	H.U.de la Princesa's UCI	ENVIN-UCI		
		2006	2007	2008
MSSA	12.75%	11.81%	12.50%	11.27%
Pseudomonas aeruginosa	12.08%	16.90%	17.55%	18.63%
Stenotrophomonas maltophilia	8.05%	2.34%	4.21%	4.98%
MRSA	7.38%	7.42%	4.09%	3.58%
Haemophilus influenzae	7.38%	5.91%	5.17%	6.28%
Enterobacter aerogenes	6.71%	1.37%	1.44%	1.63%
Escherichia coli	6.04%	6.73%	7.21%	7.58%
Streptococcus pneumoniae	4.03%	2.34%	2.64%	2.23%
Acinetobacter baumannii	2.68%	12.77%	11.78%	8.99%

P1660 High rates of multidrug resistance in major bacterial isolates from adult patients with hospital-acquired pneumonia and ventilator-associated pneumonia in Asian countries: an ANSORP study

J.H. Song for the Asian Network for Surveillance of Resistant Pathogens (ANSORP)*

Objectives: Antimicrobial resistance which has prominently emerged in major respiratory pathogens in many parts of the world can lead to inappropriate empirical antimicrobial treatment in patients with pneumonia. *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most common respiratory isolates from HAP or VAP cases in Asian countries.
Methods: The Asian Network for Surveillance of Resistant Pathogens (ANSORP) performed a prospective surveillance study of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) in 10 Asian countries from 2008 to 2009. Among respiratory isolates, *Acinetobacter* spp. (330 isolates), *Pseudomonas aeruginosa* (335), *Klebsiella pneumoniae* (225), and *Staphylococcus aureus* (215) were tested for antimicrobial susceptibility according to CLSI guidelines.
Results: *Acinetobacter* spp. showed a very high resistance rate to imipenem which was 70.0% (MIC90 >64 mg/L), especially in Malaysia, Thailand, and China. Resistance rates to ceftazidime, ciprofloxacin, ampicillin/sulbactam, and piperacillin/tazobactam were 77.9%, 82.1%, 76.4%, and 77.9%, respectively. Resistance rate to colistin was 0.6%. Resistance rates of *P. aeruginosa* to ceftazidime, cefepime, piperacillin–tazobactam, imipenem, and ciprofloxacin were 31.9%, 24.8%, 33.7%, 26.3%, and 26.0%, respectively. None was resistant to colistin. Resistance rate of *P. aeruginosa* to imipenem was the highest in China (64.1%). MIC90 of imipenem and doripenem for *P. aeruginosa* were 32 mg/L and 8 mg/L, respectively. Among *K. pneumoniae* isolates, 36.0% were resistant to cefotaxime, 29.3% to ciprofloxacin, and 2.2% to imipenem. MIC90 of doripenem for *K. pneumoniae* was 0.12 mg/L. In Asian countries, *S. aureus* isolates showed high resistance rates to methicillin (77.2%), ciprofloxacin (73.0%), clindamycin (56.7%), erythromycin (71.6%), and tetracycline (64.2%). MIC90 of doripenem for *S. aureus* was 2 mg/L.
Conclusion: Major respiratory isolates from HAP or VAP in Asian countries showed very high resistance rates to major antimicrobial agents. Particularly, *Acinetobacter* spp. and *P. aeruginosa* showed high resistance rates to imipenem in Asian countries, which could limit the therapeutic options in the clinical practice.

Antimicrobial resistance in Gram-positive bacteria

P1661 Characterization of *Staphylococcus aureus* clinical isolates from skin and soft tissue infections collected between 2006 and 2009 in Poland

D. Zabicka, A. Kozinska, K. Bojarska, W. Hryniewicz (Warsaw, PL)*

Objectives: The study was performed to evaluate the antibiotic susceptibility of *Staphylococcus aureus* skin and soft tissue isolates, both methicillin sensitive (MSSA) and methicillin resistant (MRSA), collected in hospitals from various locations in Poland between 2006 and 2009 and to characterize on molecular level the MRSA isolates from this group.
Methods: Study was performed on a group of 340 *S. aureus* clinical isolates from skin and soft tissue infections in hospitalized patients from various locations in Poland. Minimal inhibitory concentration (MIC) for daptomycin, tigecycline and linezolid was done by using Etest method, MIC of oxacillin, vancomycin, teicoplanin, gentamicin, rifampicin, trimethoprim-sulfametoxazole, tetracycline, doxycycline and fusidic acid was done by using broth microdilution method. Susceptibility to erythromycin and clindamycin and evaluation of MLSB resistance mechanism was done by disc diffusion method. Methicillin resistance was detected by cefoxitin disc diffusion test and the presence of mecA gene was confirmed by PCR. All MRSA strains were characterized by SCCmec typing and detection of eta, etb, tst and lukPV toxins genes by PCR based methods.
Results: Among the examined strains 87 (25%) were methicillin resistant. The presence of five SCCmec types was detected, with type IV or V in 44 isolates. lukPV gene was detected only in the isolates of SCCmec type IV (n = 2) or V (n = 14). None of the examined MRSA isolates contained eta or etb genes, two isolates contained tst gene. All examined *S. aureus* strains were susceptible to daptomycin with MIC50 = 0.25 mg/L and MIC90 = 0.5 mg/L. There was no significant difference of daptomycin MIC values observed between methicillin MSSA and MRSA. All examined isolates were also susceptible to linezolid and tygecycline. Approximately 50% of MRSA were susceptible to most of the examined antibiotics. However, 39% of MRSA were resistant to gentmicin, 39% to tetracycline and 29.2% to doxycycline. Most of the MRSA (69.1%) strains were resistant to erythromycin and clindamicin in MLSB mechanism. In the group of MRSA 4 isolates (2.3%) had vancomycin MIC $\leqslant$ mg/L.
Conclusion: The study enabled to evaluate the baseline susceptibility to daptomycin of *S. aureus* strains isolated from skin and soft tissue infections in Poland between 2006 and 2009 on the level of

MIC50 = 0.25 mg/L and MIC90 = 0.5 mg/L. High prevalence of strains with SCCmec type IV and V was observed in analysed population of clinical isolates.

P1662 Antimicrobial drug susceptibility and mecA finding in clinical isolates of *Staphylococcus* spp. from canine pyoderma

M.C. Emanuele, R. Bosco, C. Piraino, M. La Giglia, M. Bivona, M. Vitale (Palermo, IT)*

Objectives: The susceptibility to selected antimicrobial agents of *Staphylococcus* spp isolates from dogs affected by recurrent canine pyoderma was tested. The methicillin resistance was also investigated by conventional and molecular methods, since companion animals can act as reservoirs for MRSA/MRSI spreading for human infections.
Methods: 15 strains of *Staphylococcus* (3 *S. aureus* and 12 *S. intermedius)* were tested for their antimicrobial susceptibility by disk diffusion test (DDT) against the following agents: ampicillin (AMP), amoxicillin+clavulanate (AMC), cefotaxime (CTX), clindamycin (CL), enrofloxacin (ENR), eritromycin (E), penicillin (P), tetracycline (TE), vancomycin (VA). Oxacillin (OX) susceptibility was tested by DDT and by microdilution test, according to NCCLS guidelines. The strains were also analyzed for the presence of mecA gene by PCR on DNA extracted from the isolates.
The reference *S. aureus* strains: ATCC29213 and ATCC43300 were included as controls.
Results: All strains showed resistance to at least three of the antimicrobials tested whereas are susceptible to cefotaxime, enrofloxacin and vancomycin. Frequently the strains exhibited associations and the resistance phenotypes are showed in the table. 5 of them showed eritromycin resistance, in 2 strains together with clindamycin. The mecA gene was found in 3 multiresistant *S. intermedius* strains and 1 *S. aureus*, showing heterogeneous characteristics, phenotipically susceptible or with low levels of MIC. Because of the heterogeneous expression of methicillin resistance in *Staphylococcus*, the finding of mecA phenotipically susceptible strains suggests a repressed mec A expression.
Conclusion: The finding of antibiotic resistance in all the isolates is likely to be due to the selective pressure exerted by previous and repeated antimicrobial treatment since the dogs were affected from recurrent pyoderma. Multiresistant *S. intermedius* mecA-positive from dog have been reported in Europe and their finding implies the risk of the carriage to human.
Monitoring of antibiotic resistance in animals can elucidate the possible contribution of pet to the spread of staphylococcus methicillin resistant in the community and surveillance activity worldwide contributes to acquire updated epidemiological data.

N	Strains	Resistance phenotype	OX 35ºC	MIC OX 35ºC	MecA
1	*S. aureus*	AMP-AMC-P	S	0.5	NEG
2	*S. aureus*	AMP-AMC-P-TE	R	4	POS
3	*S. intermedius*	AMP-AMC-P-E-TE	S	2	POS
4	*S. intermedius*	AMP-AMC-P-E-TE	S	<0.5	NEG
5	*S. intermedius*	AMP-AMC-P	I	<0.5	NEG
6	*S. intermedius*	AMP-AMC-P	S	<0.5	NEG
7	*S. intermedius*	AMP-AMC-P-E-TE	S	<0.5	NEG
8	*S. intermedius*	AMP-AMC-P	S	<0.5	NEG
9	*S. intermedius*	AMP-AMC-P	I	0.5	POS
10	*S. aureus*	AMP-AMC-P-TE	S	2	NEG
11	*S. intermedius*	AMP-AMC-P	S	<0.5	POS
12	*S .intermedius*	AMP-AMC-P	S	<0.5	NEG
13	*S. intermedius*	AMP-AMC-P	S	<0.5	NEG
14	*S. intermedius*	AMP-AMC-P-E-CL-TE	S	0.5	NEG
15	*S. intermedius*	AMP-AMC-P-E-CL-TE	S	0.5	NEG

P1663 Detection of reduced glycopeptide susceptibility and heterogeneous resistance among *Staphylococcus epidermidis* isolated from blood cultures of neonates using the E-test macromethod and the glycopeptide resistance detection method

K. Svensson, B. Söderquist (Örebro, SE)*

Objectives: Coagulase-negative staphylococci (CoNS) are today the most common finding in blood cultures obtained from neonates. Since the prevalence of methicillin resistance as well as multi drug resistance is high among CoNS, vancomycin is one of few remaining therapeutic alternatives. During the 1990s staphylococci with decreased susceptibility to vancomycin has been recognized. However, these bacteria often constitute a limited fraction of the bacterial population (i.e. a heterogeneous bacterial population). In addition, slowly increasing minimum inhibitory concentration (MIC) values (MIC creep) for glycopeptides among *S. aureus* has been reported. The aim of this study was to investigate if the MIC values of *S. epidermidis* isolated from patients with neonatal bacteraemia have changed (MIC creep) regarding glycopeptide resistance during the three last decades. In addition, the presence of heterogeneous glycopeptide resistance (HGR) was investigated.
Material and Methods: All isolates (n = 341) of CoNS isolated from blood cultures obtained from neonates (<28 days of age) from 1984 to 2008 that have been stored at −70ºC were screened by TMB and finally determined to species level by API32STAPH or by sequencing the rpoB gene. Antibiotic susceptibility test of *S. epidermidis* isolates (n = 236) was performed by Etest (bioMérieux, Sweden) by the standard method (Müller-Hinton agar, 0.5 McFarland) as well as by the macromethod (Brain heart infusion agar, 2.0 McFarland) and the newly developed glycopeptides resistance determination (GRD)-test (bioMérieux).
Results: All isolates were susceptible to vancomycin (VA) but 20 isolates showed decreased susceptibility to teicoplanin (TP). The mean values for the MIC (mg/ml) for VA for the three decades were 1.67, 1.71, and 1.76, respectively, and for TP 3.78, 5.01, and 4.33, respectively. The percentage of isolates with MIC ^{3} 1.5 for VA increased from 85% during the 1980s to 94% during the first decade of the 21st century and for TP with MIC ^{3} 2 the percentage were 63% and 83%, respectively. HGR was shown among 45 isolates using the macromethod and among 128 by the GRD method.
Conclusion: A MIC creep regarding glycopeptides among *S. epidermidis* isolated from blood cultures from neonates is present during the three last decades in Sweden. In addition, HGR among *S. epidermidis* was found. However, discordance between the two methods used was found, but the GRD test has not been evaluated for CoNS previously, only for *S. aureus*.

P1664 Identification of the first clinical glycopeptide-intermediate *Staphylococcus aureus* in Switzerland

A. Bizzini, M. Oriol, O. Sakwinska, P. Moreillon, J. Bille, J.M. Entenza (Lausanne, CH)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) are a leading cause of morbidity and mortality. Invasive MRSA infections frequently require the use of glycopeptides-based regimens, vancomycin (VAN) or teicoplanin (TEC) being the drugs of choice. MRSA with a reduced susceptibility to glycopeptides (GISA) have been associated with treatment failures and detected in various countries. Here we describe the development of the first clinical GISA isolated in Switzerland, in a patient on a ventricular assist device chronically colonized by MRSA.
Methods: MRSA were isolated from the patient at various timepoints during the hospital stay. Antibiotic susceptibility testing was performed by E-test according to the CLSI recommendations. The presence of resistant subpopulations was determined by population analysis profile (PAP) using *S. aureus* ATCC 29213, Mu3 and Mu50 as VAN-suceptible, hetero-GISA and GISA controls, respectively. Molecular typing was done by amplified fragment length polymorphism analysis (AFLP).

Results: 4 representative MRSA isolates were isolated in november 2008 (MRSA 1), march 2009 (MRSA 2), april 2009 (MRSA 3) and june 2009 (MRSA 4). VAN and TEC MICs (in mg/L) were, respectively, MRSA 1: 2 and 3, MRSA 2: 2 and 2, MRSA 3: 4 and 12, MRSA 4: 8 and 16. PAP for the tested strains are represented in Figure 1. All strains were genetically related as determined by AFLP.
Conclusions: Because of persistent colonisation of the ventricular assist device by MRSA, and serial exposure to various antibiotic treatments (including VAN for MRSA bacteremia episodes) the original glycopeptide-susceptible MRSA gradually evolved towards a hetero-GISA then a GISA phenotype. Relatively to other countries, MRSA prevalence is relatively low in Switzerland (ca. 10%) and GISA strains have not been detected thus far. Our results therefore represent the identification of the first clinical glycopeptide-intermediate *S. aureus* in Switzerland. Ongoing work with the VAN-susceptible, hetero-GISA and GISA organisms aims at defining the genetic evolution of the tested strains.

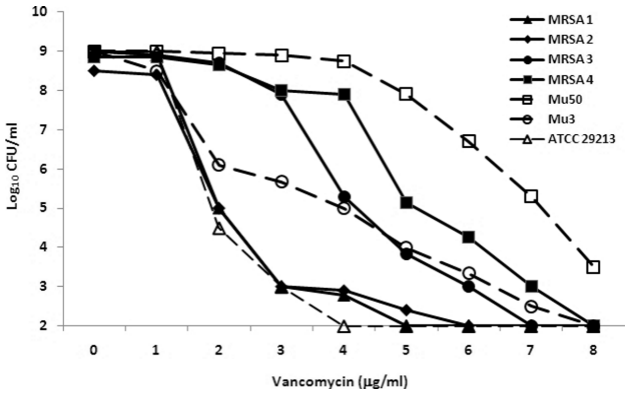


Figure 1. PAP of the tested strains.

P1665 The evaluation of VISA and VRSA in MRSA strains isolated from patients hospitalized at intensive care units in Turkey

H. Irmak, S. Cesur, H. Simsek, N. Coplu, H. Kilic, U. Arslan, G. Bayramoglu, B. Ozhak Baysan, Z. Gulay, S. Hosoglu, M. Berktas, S. Gencer, A. Pekcan Demiroz, B. Esen, N. Karabiber, F. Aydin, A. Nevzat Yalcin (Ankara, Konya, Trabzon, Antalya, Izmir, Diyarbakir, Van, Istanbul, TR)*

Aim: The aim of this study is to determine whether VISA and VRSA strains are present among MRSA strains isolated from patients hospitalised at intensive care units in 8 cities in 7 geographical regions representative of Turkey and to establish the MIC values of isolated strains for vancomycin, teicoplanin, linezolid, tigecycline, quinupristin-dalfopristin and daptomycin.
Material and Methods: 260 MRSA strains from 8 cities (Ankara, Konya, Antalya, istanbul, Izmir, Diyarbakir, Van and Trabzon) which are representative of 7 geographical regions of Turkey. Wheteher VISA and VRSA are present on MRSA was investigated using vancomycine screening agar method and E-test methods.
The sensitivity of MRSA strains to vancomycin, teicoplanin, linezolid, tigecycline, quinupristin/dalfopristin and daptomycin was determined using E-test method. In statistical evaluation, Kruskal–Wallis test was used. $p < 0.05$ was considered statistically significant.
Results: VRSA and VISA was detected in none of overall 260 strains. All MRSA strains were 100% sensitive to vancomycin, teicoplanin and linezoide, 99.6% (259/260) was sensitive to daptomycin, 96.9% (252/260) to tigecycline and 95% (246/259) to quinupristin-dalfopristin. The MIC values of MRSA strains for vancomycin, teicoplanin, linezolid, tigecycline, quinupristin-dalfopristin and daptomycin were significantly different betweeen different cities ($p < 0.05$).
Conclusion: In the present study, no VRSA and VISA strains were detected in the MRSA strains isolated form intensive care units. The establishment of 3% (8/260) resistance to tigecycline, 0.4% (1/260) resistance to daptomycin and 5% (13/259) resistance to quinupristin/dalfopristin.was striking.
It is our opinion that screening of antibiotic surveillance data in our country at certain intervals will be benefical in preventing the spread of these strains and determining the correct antibiotic treatment.

P1666 Staphylococci resistance in specialized hospitals

V. Goik, N. Kozlova, E. Barantsevich, N. Barantsevich, E. Schlyakhto (St. Petersburg, RU)*

Objectives: The aim of the present study was to determine the resistance to widely used antibiotics of airborn staphylococci from hospitals of different specialization in St. Petersburg.
Methods: Serial dilution method with the use of Muller-Hinton agar was used to determine the sensitivity of 473 Staphylococci strains (169 *S. aureus* strains – SA and 304 coagulase negative Staphylococci strains – CNS) isolated from the air of six St-Petersburg hospitals to seven antibiotics.
Results: The antibiotic resistant strains ratio of Staphylococci in the air was the highest in the burn trauma hospital – 100%. All SA and 78.3% of CNS strains were polyresistant and 92.3% of SA and 64.9% of CNS were meticillin resistant.
In haematologic hospital resistant strains ratio equaled 76.4%, with 85.7% among SA and 73.2% in CNS. Polyresistant strains of SA equaled 71.4%, in CNS-14.6%. The ratio of meticillin resistant strains was 47.3%.
In the operation room of the cardiosurgical hospital 62.6% of Staphylococci were resistant to antibiotics. Meticillin resistant strains equaled 23.4% and 14.3% in SA and CNS respectively. The ratio of polyresistant strains was 23.3% in SA and 9.5% in CNS.
In the air of general surgery operation room the number of resistant strains in CNS was higher (85.7%) that in *S. aureus* (66.7%). The absence of polyresistant and meticillin resistant SA strains and presence of 19.0% meticillin resistant CNS was detected there.
In the operation room of traumatological hospital resistant strains of Staphylococci equaled 83.1% with the absence of the polyresistant strains. Only 1.7% meticillin resistant strains were discovered in this hospital and all of them were CNS.
In the delivery room 84.0% of resistant strains were revealed, but the polyresistant and meticillin resistant strains were found only among the CNS (2.6%). In general surgical and traumatological hospitals and the delivery hospital, strains resistant to 1 or 2 antimicrobial agents prevailed among resistant *Staphylococcus* spp.
Conclusions:
1. The presence of polyresistant and meticillin resistant strains of *Staphylococcus* spp. in the air of hospitals depended upon the type of the hospital.
2. The ratio of these strains was higher in the hospitals with immunosupressed, severily ill patients and widely used antibiotic agents (burn, haemathological, cardiosurgical hospitals), than in traumatological, general surgical and delivery hospitals, where the selective factors were lacking.

P1667 Resistance of airborne hospital *Staphylococcus* strains in St. Petersburg

V. Goik, N. Kozlova, E. Barantsevich, N. Barantsevich, E. Schlyakhto (St. Petersburg, RU)*

Objectives: The present study was aimed to determine the sensitivity of Staphylococci, isolated from the air of 6 St-Petersburg hospitals, to the antimicrobial agents.
Methods: Airborn *Staphylococcus* strains from 6 St-Petersburg hospitals (burn trauma, haematological, cardiosurgical, traumatological, general surgical and birth centers) were obtained by aspiration method with 4-cascade impactor BP 50/100/200. Serial dilution method with the use of Muller-Hinton agar was used to determine the sensitivity of

473 *Staphylococcus* strains (169 *S. aureus* – SA and 304 coagulase negative *Staphylococcus* – CNS) to 7 antibiotics: Penicillin (Pn), Oxacillin (Ox), Gentamicin (Gm), Erythromycin (Er), Tetracycline (Tc), Rifampicin (Rf), Vancomycin (Van). Oxacillin was used to determine meticillin resistant strains of *S. aureus* (MRSA) and coagulase negative Staphylococci (MRCNS).

Results: The study revealed, that 76.0% of *Staphylococcus* strains were resistant to one or more antibiotics, the ratio of resistant strains among SA and CNS was comparable: 74.6% and 78.0% respectively. About a half of the strains appeared to be resistant to Pn (49.5%) and Er (44.0%), less than 1/3 – to Ox (26.2%), Tc (26.0%) and Gm (24.4%) and only 1.1% showed resistance to Rf. All strains were sensitive to Vancomycin. The ratio of SA strains, resistant to Pn, Ox, Gm, Tc, versus all the isolated ones (64.7%, 37.3%, 32.5%, 33.1%) was almost twice exceeding the same ratio in CNS (39.5%, 20.0%, 19.7%, 22.0%). On the contrary, the number of strains, resistant to Er, among CNS exceeded the one of SA: 52.3% and 30.0% respectively. The number of strains resistant to Rf in SA and CNS was almost the same: (1.2% and 1.0%). 33 spectrums of antibiotic resistance were discovered in Staphylococci, with polyresistant strains (resistant to 4 and more antibiotics) comprising more than $^1/_4$ of the number of the strains (29.9%). SA showed 36.0% of polyresistant strains and CNS – 23.3%.

Conclusions:

1. The majority of airborn *Staphylococcus* spp., obtained from the hospital environment in St. Petersburg, demonstrated resistance to antibiotics.
2. Polyresistant *Staphylococcus* strains comprised 29.9% of the airborn Staphylococci in hospitals of St. Petersburg.
3. The resistance to most antibiotics tested in *S. aureus* strains exceeded the one in coagulase negative Staphylococci.

P1668 Daptomycin activity and spectrum when tested against contemporary (2009) Gram-positive strains collected in European medical centres

S. Putnam, R. Jones, G. Moet, H. Sader (North Liberty, US)*

Objectives: To evaluate the *in vitro* activity and spectrum of daptomycin (DAP) tested against clinical isolates collected in European (EU) hospitals. Gram-positive antimicrobial resistance (R) continues to pose healthcare concerns worldwide. DAP is a cyclic lipopeptide approved in the United States (2003) and EU countries (2006) for the treatment of complicated skin and skin structure infections (cSSSI) and *S. aureus* (SA)-associated bacteremia (BSI) and right-sided endocarditis.

Methods: 2,775 consecutive strains were collected in 2009 from 24 medical centers located in EU countries (Belgium [BE], France [FR], Germany [GE], Ireland [IR], Italy [IT], Spain [SP], Sweden [SD], Switzerland [SL], Turkey [TU], and UK) and Israel [IS]. The collection included: SA (1,398; 22.7% oxacillin-resistant [MRSA]); coagulase-negative staphylococci ([CoNS] 454; 82.8% oxacillin-resistant); *Enterococcus* spp. ([ESP] 613, 15.0% vancomycin [VAN]-R); β-haemolytic (BHS; 212) and viridans group streptococci (VGS; 98). Organisms were isolated mainly from BSI (48.6%) and cSSSI (23.3%). The strains were susceptibility tested against DAP and comparators by CLSI broth microdilution methods in cation-adjusted Mueller-Hinton broth supplemented to 50 mg/L of calcium for DAP tests.

Results: DAP was very active against SA and CoNS (100.0% susceptible [S]; MIC50/90, 0.5/0.5 mg/L for both). MRSA rates ranged from a low of 0.0% (SD) to a high of 48.1% (IR); seven countries had an MRSA rate >25.0%. DAP was highly active against MRSA (MIC50/90, 0.5/0.5 mg/L) as was linezolid (MIC50/90, 2/2 mg/L; 100% S) and vancomycin (MIC50/90, 1/1 mg/L, 100% S). All ESP were S to DAP (MIC50/90, 1/2 mg/L) and linezolid (MIC50/90, 2/2 mg/L). Tigecycline and ampicillin were active against 97.2 and 62.2% of ESP, respectively. The overall prevalence of vancomycin-resistant (VR) ESP was low at 15.0%, ranging from 0.0% (BE, IS, SP, SD and SL) to 35.9% (IR) and 51.0% (TU). VAN-R did not adversely influence DAP activity against ESP and all VAN-R *E. faecalis* (MIC50, 1 mg/L; 7 strains) and *E. faecium* (MIC50/90, 2/2 mg/L; 86 strains) were S to DAP. DAP was also active against BHS (MIC90, 0.25 mg/L) and VGS (MIC90, 0.5 mg/L).

Conclusions: DAP showed significant, sustained potency against recent (2009) clinical Gram-positive organisms isolated in EU medical centers. All organisms presented here were DAP-S based on CLSI and EUCAST breakpoints.

Organism (no. tested)	Cumulative % inhibited at daptomycin MIC (mg/L) of:						%S	
	⩽0.12	0.25	0.5	1	2	4	CLSI	EUCAST
S. aureus (1,398)	1.3	56.4	99.2	100.0			100.0	100.0
OXA-S (1,080)	1.4	59.0	99.4	100.0			100.0	100.0
OXA-R (318)	0.9	47.5	98.7	100.0			100.0	100.0
CoNS (454)	9.3	52.9	95.4	100.0			100.0	100.0
Enterococcus spp. (613)	0.2	1.6	11.9	58.1	91.4	100.0	100.0	–
BHS (212)	67.0	98.1	100.0				100.0	100.0
VGS (98)	24.5	54.1	91.8	100			100.0	–

P1669 Molecular characterization of clinical isolates of vancomycin- and teicoplanin-resistant *Enterococcus faecium* and *Enterococcus faecalis* from any hospitals located in the Picardie region, France

M. Biendo, C. Adjide, S. Castelain, M. Belmekki, F. Rousseau, M. Slama, O. Ganry, F. Eb (Amiens, FR)*

Objectives: The aim of the present study was to use pulsed-field gel electrophoresis (PFGE) to characterise glycopeptide resistant *E. faecium* (GREfm) and glycopeptide resistant *E. faecalis* (GREfs) isolates from clinical samples. The van genotypes of the GRE isolates and the virulence factor genes, gel E, hyl, asa1, esp and cyl A were detected by multiplex PCR, hybridisation and sequenced.

Methods and Results: A total of 138 GRE recovered from 127 patients were collected between April 2004 and January 2009 from five Picardie area hospitals (France). The patients were 67 (52.7%) men and 60 (47.3%) women. The distribution of patients according to the positive samples for GRE showed that, 94 patients had one rectal swab each positive, and 1 patient presented 3 positive rectal swabs; 24 patients had one positive clinical sample each, and 8 patients had 11 positive clinical samples + 6 rectal swabs. The molecular identification showed that, 131 enterococci isolates belonged to the species *E. faecium* (94.9%) and 7 (5.1%) isolates to the species *E. faecalis*. All these GRE isolates had only the van A gene. Multiplex PCR showed that, the genotype efm A+ hyl+ esp+ and the genotype efm A+ hyl+ were found in 89, 67.9% and 42, 32.1% of the 131 clinical strains GREfmA respectively, where there were: rectal swabs, 76.4% (100/131) and clinical samples, 23.6% (31/131) of whom: deep pus, urine, blood, bile, sputum, catheter, and urethral swab. The efs A gene is frequently associated with gel E or/and asa1 genes while the efmA gene is frequently associated with hyl or/and esp. The cyl A gene was not detected in this study (Table 1). PFGE revealed seven different pulsotypes, designated A to G. Pulsotype A included 131 GREfm A genetically indistinguishable. The pulsotypes B, C, D, E, F and G included each one GREfs A isolate, which were considered as unrelated.

Conclusion: Our results show high GRE fecal carriage rate, Efm A being the mainly encountered GRE. An emerging Efm A clone has been identified during hospital Picardie area outbreaks. In contrast, only 7 Efs A have been isolated in our institutions. Molecular analysis showed the intra-hospital spread of gel E-, asa1-, hyl-, esp-positive GRE clones.

Isolate type	Phenotype	Susceptibility data (MIC – μg/mL)				PFGE	Virulence factor
	van	Penicillin	Ampicillin	Vancomycin	Teicoplanin	type	genes
E. faecalis GRE n = 7	A	3	1.5	>256	32	B	gel E^+ $asa1^+$
	A	3	1.5	>256	32	C	gel E^+ $asa1^+$
	A	3	0.50	>256	32	D	gel E^+
	A	3	0.75	>256	32	E	gel E^+
	A	1.5	1.5	>256	>256	F	gel E^+
	A	2	1.5	>256	32	G	$asa1^+$
	A	4	1	>256	32	G	gel E^+ $asa1^+$
E. faecium GRE n = 131	42 (32%) A	96–>256	48–>256	>256	32–>256	A	hyl^+
	89 (67%) A	96–>256	48–>256	>256	32–>256	A	hyl^+ esp^+

asa1 = aggregation substance; gel E = gelatinase; cylA = cytolysin; esp = enterococcal surface protein; hyl = hyaluronidase.

P1670 First report of confirmed cases of vancomycin-resistant enterococcal infection in Thailand

A. Leelaporn, S. Pattanachaiwit, S. Limsrivanichakorn, T. Yungyuen, T. Wensanthia (Bangkok, TH)*

Objective: A confirmed case of infection caused by vancomycin-resistant enterococci (VRE) had never been reported in Thailand. Therefore, the aim of this study was to identify vanA-VRE, and vanB-VRE from various specimens collected from patients at Siriraj Hospital, Mahidol University, Bangkok, Thailand.
Methods: All enterococci isolated from clinical specimens sent to the Diagnostic Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital, between January 2004 and October 2009, were screened for VRE by vancomycin (Vm) disk diffusion and/or Vm agar screen methods. Suspected VRE isolates were confirmed as VRE by multiplex polymerase chain reaction technique targeting vanA, vanB, vanC1, vanC2, *Enterococcus faecalis* and *E. faecium* specific sequences. Species identification of enterococci was also performed by biochemical method and automated Vitek 2 system. Susceptibility to other antimicrobial agents was determined by disk diffusion method.
Results: Approximately 10,200 enterococcal isolates were detected in various clinical specimens studied. Among those isolates, 16 VRE were identified from urine (13 isolates), pus (2 isolates) and blood (1 isolate). All VRE isolates were *E. faecium*. The majority of them (12/16 isolates, 75%) were vanA VRE whereas the remaining isolates were vanB VRE. All VRE isolates were still susceptible to linezolid.
Conclusion: A total of 12 isolates of vanA-VRE and 4 isolates of vanB-VRE have been identified from clinical specimens in Siriraj Hospital since the first isolate was detected in 2004. All VRE isolates were *E. faecium*. This is the first report of genetically confirmed clinical VRE isolates in Thailand.

P1671 Antimicrobial susceptibility of daptomycin and comparator agents tested against bloodstream isolates of *Staphylococcus aureus*: analysis of a 5-year trend in European medical centres (2005–2009)

D. Biedenbach, R. Jones, H. Sader (North Liberty, US)*

Objectives: To evaluate daptomycin activity against *S. aureus* collected from patients with bloodstream infections (BSI) in European (EU) hospitals. Daptomycin is a natural lipopeptide derived from Streptomyces roseosporus that is rapidly bactericidal against Gram-positive pathogens. Daptomycin is approved by the European Medicine Agency (EMEA) for treating complicated skin and skin structure infections and *S. aureus*-associated bacteremia and right-sided endocarditis, including those caused by methicillin-resistant *S. aureus* (MRSA).
Methods: *S. aureus* BSI isolates (4,886) were consecutively collected from 29 sites in 13 EU countries. Susceptibility (S) was determined by the CLSI broth microdilution method. Cation-adjusted Mueller-Hinton broth was used for testing all agents and was supplemented to 50 mg/L of calcium for testing daptomycin as recommended by the CLSI and EUCAST.
Results: Between 2005 and 2009, the MRSA rate declined nearly 9% in EU, with an overall rate of 27.4% during the five year period. The lowest MRSA rate was observed in Sweden (two sites, 1.1%) and the highest rate was in Greece (two sites, 53.8%). Resistance (R) to erythromycin and clindamycin also declined from 33.6 and 18.1% in 2005 to 26.4 and 10.1% in 2009, respectively. The highest MIC value for daptomycin was 1 mg/L (100.0% S using CLSI and EUCAST breakpoints) with MIC50 and MIC90 values of 0.25 and 0.5 mg/L, respectively. Vancomycin (MIC50/90, 1/1 mg/L; 100.0% S) and linezolid (MIC50/90, 1/2 mg/L; >99.9% S) were two- to four-fold less active than daptomycin. Only one linezolid-R was observed and quinupristin/dalfopristin-R isolates were only observed in France. Daptomycin potency was very uniform among the countries evaluated and daptomycin MIC distributions did not vary significantly overtime (Table).
Conclusions: Daptomycin showed consistent potency against an extensive collection of clinical isolates of *S. aureus*, including MRSA, from numerous EU medical centres over the last five years. All isolates were S to daptomycin, which was more potent compared to vancomycin and linezolid and has excellent activity against *S. aureus* isolates with co-R to other antimicrobial classes.

Year	Organism (no.)	Cumulative % inhibited at daptomycin MIC (mg/L) of:				
		⩽0.06	0.12	0.25	0.5	1
2005	MSSA (701)	0.0	3.7	80.0	99.7	100.0
	MRSA (323)	0.0	1.9	59.1	99.1	100.0
2006	MSSA (826)	0.0	7.1	92.3	99.8	100.0
	MRSA (284)	0.4	2.8	73.6	99.7	100.0
2007	MSSA (823)	0.0	5.0	89.3	99.8	100.0
	MRSA (321)	0.3	3.7	77.9	98.8	100.0
2008	MSSA (814)	0.1	2.7	66.7	99.6	100.0
	MRSA (298)	0.0	2.7	48.3	98.3	100.0
2009	MSSA (384)	0.0	1.0	58.3	100.0	–
	MRSA (112)	0.0	2.7	50.0	97.3	100.0

P1672 Eight-year (2002–2009) summary of the Zyvox Annual Appraisal of Potency and Spectrum Programme in European countries

R. Jones, J. Ross, M. Stilwell, R. Mendes (North Liberty, US)*

Objectives: To document the rates of susceptibility (S) for the oxazolidinone, linezolid (LZD), when tested against a longitudinal resistance (R) surveillance sample of European (EU) medical center isolates (ZAAPS; 2002–2009). Samples from 12–24 sites annually in 11 countries were monitored by a central laboratory design using reference methods (CLSI) and regional interpretations (EUCAST).
Methods: A total of 13,966 Gram-positive pathogens were tested from 6 pathogen groups: *S. aureus* (SA; 6,096), coagulase-negative staphylococci (CoNS; 2,073), enterococci (2,055), *S. pneumoniae* (2,267), β-haemolytic (BHS; 947) and viridans gr. (VGS; 528) streptococci. CLSI (M07-A8, 2009) methods and interpretations (M100-S19, 2009) were used, supplemented by EUCAST (2009) breakpoints. At least 15 comparator agents were tested. LZD-R strains (MIC, ⩾8 mg/L) were confirmed by a second method (disk, Etest) and then by molecular tests to determine R-mechanisms (cfr, target mutations) and clonality by PFGE and/or automated riboprints for perceived clusters.
Results: LZD generally remained without documented R from 2002–2005, but beginning in 2006 LZD-R strains emerged at very low rates ⩽1.1% among SA (G2576T mutant in Ireland, 2006), CoNS (usually *S. epidermidis*; France and Italy, 2006–2008) and enterococci (*E. faecium* in Germany [2006, 2008, 2009], *E. faecalis* in Sweden and UK [2008]), each strain having a target mutation. A mobile cfr was detected in an Italian CoNS strain (2008), and clonal spread was noted for LZD-R strains at that site (PFGE results). Overall the LZD-S rates were >99.9, 99.7 and 99.8% for SA, CoNS and enterococci, respectively. All LZD MIC90 results ranged from 1 to 2 mg/L. All streptococci were LZD-S (⩽2 mg/L), but penicillin-R was 26.7% in pneumococci and fluoroquinolone-R was >1% in BHS and VGS. Other resistances noted were: MRSA and MRCoNS ranging from 20.0–30.1% and 37.5–83.8%, respectively, without trends toward greater R. VRE rates increased from 6.9% (2002) to 16.9% (2009), with 79.3% having VanA phenotype in 2009.

Organism (no. of strains)	Cumulative % inhibited at ceftaroline MIC (mg/L) of:						
	⩽0.06	0.12	0.25	0.5	1	2	4
MSSA							
Europe (2895)	0.9	5.9	88.3	99.9	100.0	–	–
USA (2169)	0.7	4.5	88.4	99.9	100.0	–	–
MRSA							
Europe (1004)	0.0	0.0	2.2	31.6	83.9	99.5	100.0[a]
USA (2674)	0.0	0.1	1.1	34.3	94.7	100.0	–

[a]Only 5 strains (Greece and Italy).

Conclusions: ZAAPS surveillance for LZD-S rates confirmed high levels (⩾99.7% S) for staphylococci and enterococci from 2002–2009 and without R among streptococci. No trends toward LZD MIC creep or escalating R rates were detected in this multi-year post-marketing surveillance program for the EU (see Table).

P1673 Report of linezolid resistance from the Zyvox® Annual Appraisal of Potency and Spectrum Programme (Europe, Latin America, Asia-Pacific)

J. Ross, R. Jones, H. Sader, U. Uchino, F. Ikeda, Y. Fukui, I. Kobayashi (North Liberty, US; Tokyo, Kochi, JP)*

Objectives: To monitor the *in vitro* activity and to detect resistance (R) to linezolid (LZD) in various geographic areas of the world, excluding the United States (USA), the Zyvox® Annual Appraisal of Potency and Spectrum Program (ZAAPS) surveillance program was established in 2002. LZD, the first oxazolidinone agent clinically applied, is an important therapeutic option for infections caused by antimicrobial-R Gram-positive (GP) pathogens. Although rare, LZD-R has been observed particularly in enterococci (ENT) and recently among coagulase-negative staphylococci (CoNS). Resistance rates remain extremely low for indicated *S. aureus* (SA) and streptococci.
Methods: 5,367 isolates were collected from 64 sites in 21 countries in 2009. Isolates were received from the following organism groups (n): SA (2,767), CoNS (802), ENT (668), *Streptococcus pneumoniae* (SPN; 597), viridians group streptococci (VGS; 207) and β-haemolytic streptococci (BHS; 326). At least 200 isolates from each country (expect China [n = 800] and the United Kingdom [n = 400]) were requested to be sent to a reference laboratory. CLSI broth microdilution susceptibility testing was performed using TREK Diagnostic (Cleveland, OH, USA) panels. LZD-R isolates were confirmed with Etest (bioMérieux, North Carolina, USA) and disk diffusion. PCR and sequencing were performed to detect mutations in 23S rRNA, L3, L4, and L22 proteins, and acquired gene (cfr).
Results: Overall LZD-susceptibility (S) in the ZAAPS study was >99.9% with only 3 LZD-R ENT isolates identified. Non-susceptible (NS) staphylococci strains were not detected in ZAAPS 2009 compared to the 2008 program where 3 NS CoNS strains were found. MRSA rates varied from 0.0% (Sweden) to 82.0% (Korea) with an overall rate of 35.6%. Vancomycin-R ENT rates ranged from 0.0% (Japan, Belgium, Mexico, Spain, Sweden) to 50.0% (Taiwan). Three ENT isolates were LZD-R (0.45%; see table). SPN had overall penicillin and erythromycin R rates of 33.4% (MIC, ⩾2 mg/L) and 55.9%, respectively. All streptococci had LZD MIC values of ⩽2 mg/L.
Conclusions: LZD-R was considered very low (<1%) among contemporary pathogens from indicated organism groups in this worldwide surveillance study. As LZD use continues to evolve, the need for close monitoring of the *in vitro* activity of LZD versus Gram-positive pathogens and for the emergence of R is apparent.

Table 1. Linezolid-R isolates found in the 2009 ZAAPS Program

Species	City/Country	LZD MIC (mg/L)	R- mechanism[a] (23S mutation)
E. faecium	Frankfurt/Germany	>8	G2576T
E. faecium	Frankfurt/Germany	8	G2576T
E. faecalis	Shenzhen/China	8	Not found

[a]All isolates were screened for *cfr* and were negative.

P1674 A longitudinal perspective of tigecycline activity in the EU against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus* spp. including resistant and multidrug-resistant phenotypes

D. Draghi, C.M. Pillar, M. Dowzicky, D.F. Sahm (Chantilly, Collegeville, US)*

Background: Due to the prevalence of methicillin resistant *S. aureus* (MRSA), penicillin resistant *S. pneumoniae* (PRSP), and erythromycin resistant *S. pyogenes* (SPY), agents used to treat Gram-positive (GP) infections should maintain activity against these resistant subpopulations. Tigecycline (TIG) is primarily intended for treatment of infections encountered in healthcare settings where resistance (R) is a continuous threat. It is important to understand current activity and trends in R to utilized agents, in particular those recently approved for use. This study reports the activity profile of TIG over 10 years spanning its development and approval for use against target GP organisms in Europe (EU).
Methods: TIG activity was profiled from 2000 until 2009 against a collection of 2195 *S. aureus* (SA), 88 vancomycin-susceptible *E. faecalis* (VSEf), 611 *S. pneumoniae* (SP), and 493 *S. pyogenes* (SPY) collected from 12 countries in EU. Isolates were tested by broth microdilution against TIG and comparators utilizing CLSI guidelines (M7-A9). EUCAST breakpoints (BPs) were applied to TIG results. CLSI (M100-S19) BPs were used to interpret all comparators (where applicable).
Results: Against SA, TIG maintained consistent activity from year to year with MIC50s of 0.06–0.12 mg/L and MIC90s of 0.12–0.25 mg/L, and >99% susceptibility. The TIG activity profile was not altered against MRSA or MDR subpopulations. SA also remained >99% susceptible to linezolid, vancomycin and daptomycin in contrast to other evaluated agents (e.g. clindamycin [CLI], erythromycin [ERY], levofloxacin). Similar to SA, TIG had a potent and consistent activity profile against both SP (MIC50s 0.015–0.03 mg/L and MIC90s 0.015–0.06 mg/L; 100%S) and SPY (MIC50s 0.015–0.03 mg/L and MIC90s 0.03–0.06 mg/L; 100%S) including PRSP and macrolide R SPY subpopulations. Excluding CLI and ERY, there was little observed R to the evaluated agents among the tested streptococci. TIG also was potent year to year against VSEf, with MIC50s 0.06–0.25 mg/L and MIC90s 0.12–0.5 mg/L. No TIG R was detected among VSEf across the evaluated period.
Conclusions: Over the years studied, TIG has maintained potent *in vitro* activity overall against target GP cocci with >99% susceptibility rate, including R and MDR isolates. Notable trends in decreased activity for TIG were not observed despite increased use since approval in 2006; however, continued surveillance is warranted as the threat of R development is ongoing.

P1675 Limited variety of genetic lineages drive the increase of multidrug resistance among non-invasive pneumococci in southern Finland

L. Siira, J. Jalava, P. Tissari, M. Vaara, T. Kaijalainen, A. Virolainen (Helsinki, Turku, Oulu, FI)*

Objectives: The proportion of multidrug resistant *Streptococcus pneumoniae* isolates has increased in the Southern Finnish Helsinki and Uusimaa districts from 1.6% (15 of 959 isolates) in 2007 to 3.6% (34 of 949 isolates) in 2008. Multidrug resistance was defined as non-susceptibility to penicillin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, and doxicycline. The aim of this study was to combine our antimicrobial susceptibility results with sero- and genotyping to find out whether the increase in multidrug resistance is clonal.
Methods: Twelve non-invasive multidrug resistant isolates collected in year 2008 were selected for further characterization in this study. Antimicrobial susceptibility was tested by the agar plate dilution method at 5% CO2. Serotyping was performed with latex agglutination for the neutral serogroups/types 7 and 14, followed by counterimmunoelectrophoresis with pneumococcal antisera. The capsular reaction test Quellung was used for confirmation where needed. Genotyping was carried out by

multilocus sequence typing and the resulting sequence types (STs) were assigned to genetic lineages.
Results: The serotype distribution of the isolates was limited to five serotypes, 19F (n = 5), 19A (n = 3), 6B (n = 2), 23F (n = 1), and 14 (n = 1), while nine different STs and four genetic clonal complexes (CCs) were represented in the material. The CCs were CC271 (n = 8), CC90 (n = 2), CC81 (n = 1), and CC156 (n = 1). CC271 claimed all serotype 19F and 19A isolates.
Conclusion: The proportional increase of multidrug resistance among pneumococci seems to have a clonal component, which is indicated by the limited variety of genetic lineages, dominated by CC271. Furthermore, the CC271 isolates expressed both serotypes 19F and 19A. Similarly, on the serotype level, serogroup 19 is overrepresented and the serotype distribution of our subset of multidrug resistant isolates differs both from that of the invasive pneumococci collected in Helsinki and Uusimaa districts in year 2008 and the national serotype distribution of invasive resistant pneumococci. CC271 has previously been noted to include drug resistant serogroup 19 isolates, such as the international Taiwan19F-ST236 strain, and some members of this CC display capsular switching within serogroup 19.

P1676 Trends in antimicrobial resistance in Canadian strains of *S. pneumoniae*, 1993 to 2009

K. Green, A. McGeer, A. Plevneshi, S. Pong-Porter, K. Hassan, W. Rudnick, D. Low on behalf the Canadian Bacterial Surveillance Network*

Background: The Canadian Bacterial Surveillance Network (CBSN) has been monitoring resistance trends in Canadian isolates of *S. pneumoniae* (SP) since 1993.
Methods: CBSN is a collaborative network of microbiology laboratories from across Canada that submit bacterial isolates to a central laboratory for broth microdilution antimicrobial susceptibility testing performed according to CLSI standards.
Results: Of the 32, 868 SPN isolates submitted and tested between 1993 and November of 2009, 36% were from blood/CSF (blood: 35%; CSF: 1%), 33% from sputum, 31% from other sites. The trends in antimicrobial susceptibility are expressed below as percentage resistant. Preliminary results from 2009 suggest increasing resistance in all classes of antibiotics except fluoroquinolones. From 2008 to 2009, erythromycin resistance and tetracycline resistance increased from 21.2% to. 25.3% (p = 0.02) and from 9.8% to. 12.7% (p = 0.03), respectively. Resistance to clindamycin increased from 8.5% in 2008 to 11.3% in 2009 (p = 0.02). Penicillin resistance increased from 6.5% in 2008 to 8.8% in 2009 (p = 0.04). Ceftriaxone resistance (meningeal breakpoint MIC ⩾2) increased from 3.3% in 2008 to 5.4% in 2009 (p = 0.01). Amoxicillin resistance continued to increase: to 3.5% in 2009 from 2.1% in 2008 and 1.6% in 2007 (p = 0.04). In contrast, resistance to ciprofloxacin and levofloxacin did not change significantly from 2008 to 2009 (2.2% vs. 2.3% and 1.5% vs. 1.6% respectively; however, between 2008 and 2009, moxifloxacin resistance decreased slightly (0.7 vs. 0.5 (p NS)).
Conclusion: In 2009, antimicrobial resistance increased to penicillin, amoxicillin, ceftriaxone, erythromycin, trimethoprim/sulfa and tetracycline. Resistance to ciprofloxacin and levofloxacin remained stable/increased slightly; resistance to moxifloxacin did not change. Moxifloxacin remains the most active agent against pneumococci in Canada.

	Resistance (%)																	
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Pen NS	5.7	8.1	8.8	11.9	13.6	15.0	13.4	12.3	14.4	15.2	14.9	14.8	15.3	15.3	17.2	16.6	22.0
Pen R	0.9	1.3	2.2	4.1	6.6	5.7	5.9	5.9	6.8	6.6	6.2	5.4	4.8	6.3	4.6	6.5	8.8
Amox R	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.5	0.6	1.1	1.1	1.1	1.4	1.6	2.1	3.5
Eryth R	1.9	3.4	3.2	4.8	6.8	10.5	9.8	11.2	12.8	14.2	15.9	18.0	19.1	19.4	22.5	21.2	25.3
Clinda R	0.0	1.7	1.3	2.4	3.6	5.1	4.4	5.5	5.8	6.5	7.4	8.1	8.2	8.6	9.7	8.5	11.3
T/S R	3.8	4.7	9.7	12.7	14.6	12.3	12.0	11.3	12.0	13.2	13.3	13.5	12.2	11.8	12.0	11.3	14.1
Tet R	1.4	2.3	3.4	2.5	6.4	9.1	7.1	5.5	9.1	9.6	9.7	10.9	10.4	11.2	13.1	9.8	12.7
Ceftrx R (M)*	0.0	0.2	0.1	0.7	1.3	2.5	1.5	2.0	2.4	1.5	1.8	2.5	1.9	3.7	2.9	3.3	5.4
Ceftrx R (NM)**	0.0	0.0	0.0	0.2	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.2	0.9
Cipro R¶	0.5	0.8	0.7	0.8	1.8	1.8	1.6	1.4	2.1	2.7	1.8	2.0	2.4	2.7	2.0	2.2	2.3
Levo R	0.0	0.4	0.1	0.2	0.5	0.3	0.4	0.9	1.2	1.9	1.2	1.5	1.5	1.7	1.2	1.5	1.6
Moxi R	NT	NT	0.0	0.0	0.3	0.2	0.2	0.4	0.4	0.3	0.4	0.6	0.7	0.9	0.7	0.7	0.5

*M = meningeal breakpoint; **NM = non-meningeal breakpoint; ¶breakpoint used for reduced susceptibility ⩾4 μg/mL; NT = not tested.

P1677 Antibacterial resistance among *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* from Kuwait and 5 centres in Turkey: results from the Survey of Antibiotic Resistance (SOAR), 2007–2009

D. Gur, A. Tunger, S. Aydemir, G. Soyletir, G. Altinkanat, G. Bayramoglu, F. Aydin, C. Kayacan, Z. Aktas, E. Mokaddas, A. Acar, D. O'Brien (Ankara, Izmir, Istanbul, Trabzon, TR; Kuwait City, KW; London, UK)*

Objectives: Antibiotic resistance among community-acquired respiratory tract pathogens such as *Streptococcus pneumoniae* (SP) and *Haemophilus influenzae* (HI) is a worldwide problem. *Moraxella catarrhalis* (MC) remains one of the three leading causes of acute otitis media (AOM) in children. SP, HI and MC data from the SOAR programme are reported.
Methods: MICs for various antimicrobials were determined using Etest; disk susceptibility testing was performed according to CLSI guidelines. Susceptibility was assessed using CLSI interpretive criteria.
Results: 524 SP, 418 HI and 67 MC strains were collected from five centres in Turkey. Overall, 47.2% (247/524) of SP were penicillin-susceptible and 52.8% (277/524) were penicillin-nonsusceptible (PNSP) based on CLSI 2009 oral penicillin breakpoints. Based on CLSI 2009 IV penicillin breakpoints, 99.2% (520/524) were susceptible to penicillin. PNSP prevalence was highest (64.8%; 59/91) in Istanbul but lowest (42.7%;35/82) in Trabzon in Turkey. Overall, susceptibility to amoxicillin/clavulanate (XL), cefuroxime (XM), and clarithromycin (CH) was 97.7% (512/524), 64.7% (339/524), and 61.9% (324/524), respectively. The level of non-susceptibility to ofloxacin (OF) was 11.1% (58/524). A total of 418 HI isolates were collected of which 2.6% (11/418) produced β-lactamase (BL). Overall, 9 isolates (2.2%) were ampicillin resistant but BL negative (BLNAR). Overall, susceptibility to XL, XM and OF was 100% (418/418), 97.1% (406/418) and 99.8% (417/418), respectively. All MC strains were β-lactamase positive but amoxicillin/clavulanate susceptible. Also, 97 SP strains were collected in Kuwait. The prevalence of PNSP was 60.8% (59/97) and erythromycin-nonsusceptible *S. pneumoniae* (ENSP) was 42.3% (41/97) in Kuwait. The level of susceptibility to XL, XM, and OF was 100% (97/97), 63.9% (62/97), and 96.9% (94/97), respectively.
Conclusion: XL and XM retained good *in vitro* activity against SP and HI strains. Among SP strains, the prevalence of quinolone non-susceptibility such as OF is increasing.

P1678 Antibacterial resistance among *Streptococcus pneumoniae* from 4 centres in Thailand: results from the Survey of Antibiotic Resistance (SOAR), 2007–2009

B. Khantawa, V. Baosount, B. Warachit, C. Yodsawat, K. Malatham, P. Santanirand, W. Reechaipichitkul, A. Lulitanond, P. Chaimance, S. Sawatwipachai, D. O'Brien (Chiangmai, Hat Yai, Ramathibodi, Khon Kaen, Bangkok, TH; London, UK)*

Objectives: *Streptococcus pneumoniae* is the main bacterial pathogens implicated in community-acquired respiratory tract infections (CARTIs), which occur frequently and account for significant morbidity and mortality. The emergence of drug resistance in these organisms has complicated empirical treatment of such infections. It is therefore imperative to know the susceptibility pattern of CARTI pathogens when selecting an antimicrobial agent for empirical therapy.
Methods: The study was split into three phases. Phase 1: disk diffusion to determine susceptibilities to key therapeutic drugs (oxacillin, erythromycin, clindamycin, co-trimoxazole. tetracycline, chloramphenicol, ofloxacin) Phase 2: Strains that were non-susceptible to oxacillin and/or erythromycin were tested against other antibiotics (penicillin, amoxicillin/clavulanate, cefprozil, ceftriaxone, azithromycin, clarithromycin, levofloxacin) by using Etest. Phase 3: Erythromycin-clindamycin double-disk test was used to determine the resistance phenotypes of erythromycin-resistant *S. pneumoniae* isolates. Prior to the start of each phase of the survey, a quality control four-day run-

in period test was performed. Susceptibility was assessed using CLSI interpretive criteria.
Results: In total 281 (Phase 1), 159 (Phase 2) and 97 (Phase 3) *S. pneumoniae* strains were tested from four centres in Thailand. 49.8% (140/281) of SP were penicillin-susceptible and 50.2% (141/281) were penicillin-nonsusceptible based on oxacillin resistance (Phase 1) and CLSI 2009 oral penicillin breakpoints (Phase 2). Overall, resistance to erythromycin was 34.6% (97/281). Among erythromycin and/or penicillin resistant strains, the level of susceptibility to amoxicillin/clavulanate and azithromycin/clarithromycin was 98.7% (157/159) and 25.8% (41/159) respectively. Among 97 erythromycin resistant strains, 57.7% (56/97) were MLSB types and 42.3% (41/97) were M types.
Conclusion: Amoxicillin/clavulanate retained excellent *in vitro* activity against *S. pneumoniae* strains. Among *S. pneumonaie* isolates, the prevalence of macrolide resistance was high.

P1679 Oral β-lactam activity against recent (November 2008–June 2009) penicillin non-susceptible serotype 19A *Streptococcus pneumoniae* isolates in Spain

A. Fenoll, L. Aguilar, M.J. Gimenez, M.D. Vicioso, O. Robledo, J.J. Granizo, P. Coronel (Madrid, ES)*

Objective: To explore susceptibility to oral β-lactams vs. cefotaxime of penicillin (PEN) non-susceptible serotype 19A isolates since in Spain this serotype has significantly increased in last years after introduction of the 7-valent pneumococcal conjugate vaccine, with significant reduction in PEN susceptibility.
Methods: 268 successive PEN non-susceptible (MIC $\geqslant$0.12 mg/l) serotype 19A isolates received from November 2008 to June 2009 in the Spanish Reference Pneumococcal Laboratory were tested. Susceptibility was determined by agar dilution following CLSI, using Mueller-Hinton agar supplemented with 5% sheep blood, incubating under 5% CO2 atmosphere. CLSI breakpoints (oral for PEN and cefuroxime; non-meningitis for amoxicillin and cefotaxime) were used.
Results: MIC50, MIC90 (mg/l), range and percentage (%) of susceptible (S), intermediate (I) and resistant (R) isolates are shown in the table.
Conclusions: Cefditoren exhibited the highest intrinsic activity in terms of MIC50 and MIC90, with values four times lower than those of cefotaxime. Non-susceptibility (intermediate + resistant) rates to oral β-lactams with current CLSI breakpoints were high, ranging from 33.2% for amoxicillin to 98.9% for cefaclor.

	MIC_{50}	MIC_{90}	Range	%S	%I	%R
PEN	1	2	0.12–8	–	75.0	25.0
Amoxicillin	1	8	$\leqslant$0.06–32	66.8	8.6	24.6
Cefaclor	$\geqslant$32	$\geqslant$32	1–$\geqslant$32	1.1	1.1	97.8
Cefuroxime	4	8	0.12–$\geqslant$32	25.4	17.9	56.7
Cefpodoxime	2	4	0.06–16	25.4	10.1	64.6
Cefdinir	4	8	0.06–16	24.6	1.5	73.9
Cefditoren	0.25	0.5	0.03–2	*	*	*
Cefotaxime	1	2	0.06–8	85.1	11.9	3.0

*No CLSI breakpoints available.

P1680 Molecular epidemiological surveillance of *Streptococcus pneumoniae*: 1998–2009 in Monterrey, Mexico

R.M. Hinojosa-Robles, H. Martinez-Rodriguez, M.E. Velazquez-Meza, L.G. Rivera-Morales, I.M. Rivera-Morales, G.M. Gonzalez-Gonzalez, J. Ramos-Jimenez, F. Rositas-Noriega, M. De la Cruz-Valdez, R. Tijerina-Menchaca (Monterrey, MX)*

Objectives: To Identify resistance patterns of *Streptococcus pneumoniae*, determine the serotypes prevalence, to identify the presence of drug resistant clones and estimed the coverage of the 7-valent pneumococcal conjugate vaccine and the PPV-23 polysaccharide vaccine in patients with pneumococcal infections.
Methods: Strains were identified by standard methods, serotyped by the Quellung reaction, and the molecular analysis was done by PFGE. Antimicrobial susceptibility testing was performed by the broth microdilution technique and the E-test method and interpreted according to CLSI guidelines.
Results: A total of 466 pneumococcal clinical isolates were recovered from 1998 to 2009 from paediatric and adult patiens with invasive diseases;29%, pneumonia;37%, and no-invasive diseases;34%.The most frequent capsular types were: 19F, 23F, 6B, 6A, 14, 19A, 9V, 4, 35B, 3, 11, and 18C accounting for 83% of the isolates. Resistance to penicillin was observed in 77% of meningeal isolates and 37% in non-meningeal isolates. Resistance to cefotaxime was 49% to meningeal isolates and 33% to non-meningeal isolates. Resistance to macrolide was 46%, imipenem 31%. Resistance to chloramphenicol, tetracycline, trimethoprim/sulphamethoxazole was 14%, 45% 72% respectively. Levofloxacine, linezolid and quinupristin/dalfopristin were active against 99.8% and vancomycin 100% of the isolates tested. Molecular analysis showed the presence of 5 international resistance clones: Spain 23F-1, Cleveland 23F-2, Tennessee 23F-4, Taiwan 19F-14 y Spain 9V-3. The coverage to 7-valent vaccine was 70% and for the PPV-23 was 68%. The Mortality observed in this study was 10%.
Conclusions: Is important to determine the antimicrobial with 99–100% activity in order to select the most appropriated treatment for our patients. The clonal relationship between mexican strains and the international clones indicates its imminent spread to northeastern Mexico. The vaccination coverage is within expected ranges for the mexican population. These results encourage us to continue this surveillance system.

P1681 Changing patterns of antibiotic resistance and serotype distribution in group B streptococcus isolated in a maternity hospital in Kuwait

S. Boswihi, N. Al-Sweih, E. Udo (Kuwait, KW)*

Objectives: Group B streptococcus (GBS) is a common cause of infections in neonates and pregnant women. Although still susceptible to penicillin, there are reports of reduced susceptibility to penicillin in some GBS isolates. In this study GBS isolates obtained from patients at the Maternity hospital in Kuwait were studied for their serotypes and susceptibility to antibiotics, and the results were compared to those of a previous study conducted in 2001.
Methods: A total of 154 GBS isolates were collected from 1 July to 31 October 2007 from HVS, urine, blood and miscellaneous sources. All isolates were serotyped using latex agglutination test. Antibiotic susceptibility testing was performed by the disk diffusion method. MIC for penicillin, erythromycin and clindamycin was performed by E-test or agar dilution methods.
Results: Most of the isolates belonged to serotype V (38.3%), serotype III (19.5%), serotype Ia (10.4%), serotype II (10.4%). Seventeen isolates (11.0%) were nontypeable. Eighteen (11.68%) and 10 (6.5%) isolates were resistant to erythromycin (MIC: >1 mg/L) and clindamycin (MIC: >2 mg/L), respectively. They were susceptible to penicillin. However MIC determination showed that 31.2% of them had elevated penicillin MIC values of 0.064 mg/L and 13.6% had MIC of - 0.094 mg/L. Eight isolates (5.2%) expressed reduced susceptibility to penicillin (MIC: 0.125–0.19 mg/Ll).
Conclusion: The result showed that serotype V detected in 38.3% of the isolates became the dominant serotype in 2007 whereas serotype III was the dominant serotype in 2001. The proportion of GBS isolates resistant to erythromycin and clindamycin increased from 0.7% and 1.7% in 2001 to 11.6% and 6.5% in 2007. There was a significant shift in the number of isolates expressing penicillin MIC of 0.064 mg/L from 4.4% in 2001 to 44.8% in 2007. Furthermore, whereas none of the 2001 isolates had MIC 0.125–0.19 mg/L, this was detected in 5.2% of the 2007 isolates. The result demonstrated a trend towards increasing antibiotic resistance in GBS isolates in a Kuwait hospital.

P1682 Antimicrobial susceptibility profiles in *Streptococcus agalactiae* invasive and colonizing strains from Greece: a 6-year study

S. Vourli, A. Tarpatzi, K. Tsiveriotis, M. Bompola, N. Bournas, G. Salamalekis, L. Zerva (Athens, GR)*

Objectives: *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is an important pathogen during the perinatal period, but it is also responsible for various invasive infections, most frequently skin and soft tissue infections, in non-pregnant adults. Most GBS strains are susceptible to penicillin, but resistance to erythromycin and clindamycin seems to be increasing. Few data on antimicrobial resistance of strains isolated in Greece are available. We present antimicrobial susceptibility profiles of GBS isolates from a 730-bed tertiary hospital of Athens.
Material: We studied 189 GBS isolates collected in the Clinical Microbiology Laboratory of our hospital from 2004 to 2009. Ninety-three of these were consecutively isolated from routine clinical specimens (urine, vaginal swabs, blood, synovial fluid and pus), and the remaining 96 from the colonisation screening of pregnant women (performed from 2008 to 2009).
Identification and antimicrobial susceptibility testing were carried out by the Phoenix ™100 automated system. Medical files of GBS-positive patients were retrieved from the hospital records and all information concerning the clinical significance of the isolates was collected.
Results: Overall, antimicrobial susceptibility results were available for 145 out of 189 GBS isolates. No penicillin, ampicillin, cefazolin or vancomycin resistance was observed in any of the examined strains. Twenty-eight strains (19.3%) were found to be erythromycin resistant. Only 9 out of the 93 clinical isolates (2 isolated from blood, 1 from synovial fluid and 6 from pus) were considered as invasive, after review of the medical records, and they were susceptible to all antibiotics tested.
Conclusions: Taking into account the size of our hospital, the rate of GBS invasive infections in non-pregnant adults appears to be low, according to our findings. A high percentage of erythromycin resistant was noticed, which is consistent with results coming from studies in other countries. Notably, invasive isolates were more susceptible than non-invasive ones.

P1683 Impact of new CLSI *S. pneumoniae* penicillin susceptibility testing breakpoints on reported resistance changes over time

R.M. Mera, L.A. Miller, H.A. Madsen, D.F. Sahm (Durham, Upper Providence, Hendon, US)*

Objectives: To describe and contrast the differences over time in *S. pneumoniae* penicillin susceptibility rates when applying either the old or the new CLSI (Clinical & Laboratory Standards Institute) penicillin breakpoints using a large surveillance database.
Methods: The analysis comprised a total of 93,661 US isolates from the TSN Network® surveillance database (Eurofins Medinet) during the period 1996 to 2008. Under the former CLSI criteria, *S. pneumoniae* penicillin resistance was defined as MICs $\geqslant$2 μg/ml irrespective of clinical syndrome. The new CLSI guidelines are identical for oral penicillin use and non-meningitis sources, with a resistant breakpoint of $\geqslant$2 μg/ml. For non-meningitis and parenteral penicillin, the revised resistant breakpoint is $\geqslant$8 μg/ml; while for meningitis, the revised resistant breakpoint is $\geqslant$0.12 μg/ml.
Results: Penicillin resistance, when defined using the old CLSI breakpoint of MICs $\geqslant$2 μg/ml, had a very distinctive pattern over time, with an initial rise that starts in 1996, a peak in 2000, decline until 2005 and rebound through 2008 (15.6%, 23.2%, 15.2% and 17.0%, respectively). Using the new CLSI criteria and applying a resistance breakpoint of $\geqslant$8 μg/ml to blood and sputum isolates, the average resistance levels were 1.9% and 2.6%, without any appreciable changes over time. Using the new meningitis criteria of $\geqslant$0.12 μg/ml, resistance prevalence was 34.8% in 2008, while it was 12.3% using the old criteria of $\geqslant$2 μg/ml. Evaluating MIC levels separately, the percent of non-meningitis isolates with MICs of 1 μg/ml had a continuous decline from 14.3% in 1996 to a low of 6.3% in 2008, while the percent of isolates with MICs of 2 μg/ml showed a rise from 12.2% in 1996 to a peak of 16.1% in 2001 and a decline to 8.3% in 2008. The percent of isolates with MICs of 4 and 8 μg/ml show no significant changes from their average levels of 5.3% and 1.4% respectively.
Conclusion: The rise, fall and subsequent rebound of penicillin resistance in the US presumably influenced by the introduction of the conjugate pneumococcal vaccine is not observed when the new $\geqslant$8 μg/ml resistance breakpoint is applied. In the post-vaccine period, isolates with MICs of 1 and 2 μg/ml decline while those with MICs of 0.12 to 0.5 increase, which may signal the loss of resistant vaccine serotypes and the acquisition of resistance by non-vaccine serotypes.

P1684 Analysis of quinolone-resistant *Streptococcus pneumoniae* collected from PROTEKT studies between 2000 and 2007

J. Blackman Northwood, C. Couturier, S. Brown, I. Morrissey (Fordham, UK; Paris, FR; Portland, US)*

Objective: The genetic & geographical relationship between quinolone-resistant (QR) *S. pneumoniae* (SP) collected as part of PROTEKT Global & PROTEKT US was determined.
Methods: SP were collected from PROTEKT Global (47 countries worldwide; N = 41,693) & PROTEKT US (49 states; N = 55,729). MICs were determined using CLSI guidelines. Quinolone resistance determining regions (QRDR) within gyrA, gyrB, parC & parE were sequenced & multi locus sequence typing determined for QRSP by previously published methods.
Results: QR rates for both studies remained stable ~1% each year (Table). However, Italy had a steadily increasing rate of QR. Hong Kong had a very high QR rate while Massachusetts (MA), Colorado (CO), New Jersey (NJ) & New York (NY) had higher than the US average (0.9% [SD0.6]) QR rates.
Globally, QRSP were from a wide range of sequence types (ST) & clonal complexes (CC). QRSP from Hong Kong were mainly ST81 (58/71), which was found in numerous locations worldwide. ST66 was the dominant clone in Italy (58/81), most collected from a single centre. There were 3 dominant CC in the US: CC81 (67/527), CC271 (37/527) & CC439 (34/527). QRSP were represented by >23 CC & were evenly distributed across the US except for CC81 which peaked in NY & CC439 which peaked in MA. QR was due to typical QRDR mutations as previously reported.
Conclusion: QR in Hong Kong was due to the dominance of CC81, as previously reported. QR in Italy was due to the emerging resistant clone ST66, although this could be a localized outbreak as most came from 1 centre in Catania over a short period of time. QRSP in the US was due to the presence of numerous clones including CC81 & CC439. The presence of CC271 in QRSP is also important as this clone is often associated with resistance to macrolides, penicillin, tetracycline & cotrimoxazole.
This study shows QR remained very low during both studies & was caused by multiple CC in most countries.

Table 1. Total number of isolates collected (N) & %QR by Year [NT; not tested]

Region		2000–01	01–02	02–03	03–04	04–05	05–06	06–07	Average
Hong Kong	N	70	58	74	70	62	73	93	71
	%QR	14.3	15.5	10.8	11.4	17.7	17.9	12.9	14.2
Italy	N	119	284	267	285	228	192	590	281
	%QR	0.0	0.7	1.5	3.9	4.4	5.2	7.5	4.1
MA	N	353	268	246	205	137	106	NT	219
	%QR	4.8	1.9	1.2	2.4	2.2	0.0	NT	2.5
CO	N	65	239	265	108	226	167	NT	178
	%QR	4.6	4.2	2.6	0.9	0.9	0.6	NT	2.2
NJ	N	191	193	160	35	81	NT	NT	132
	%QR	1.0	3.1	1.9	2.9	0.0	NT	NT	1.8
NY	N	542	529	597	493	626	548	NT	556
	%QR	1.7	1.1	3.0	1.6	1.4	1.3	NT	1.7
Global average	N	3435	4256	6320	6739	7083	6395	7465	5956
	%QR	1.4	1.1	0.9	1.0	1.0	1.4	1.4	1.1
US Average	N	10,103	10,012	10,886	8494	9487	6747	NT	9288
	%QR	0.9	1.2	0.8	1.1	0.9	0.8	NT	0.9

P1685 Trends in antimicrobial resistance in invasive isolates of *Streptococcus pneumoniae* in Turkey, 2003–2008

D. Gülmez, D. Gür, G. Hasçelik on behalf of EARSS-Turkish Study Group*

Objectives: Antimicrobial susceptibility test results of blood and cerebrospinal fluid (CSF) isolates of *S. pneumoniae* collected between 2003–2008 from 16 centers in Turkey were evaluated.
Methods: Antimicrobial susceptibility tests were performed in individual laboratories following CLSI guidelines (2009) according to the European Antimicrobial Resistance Surveillance System (EARSS). *In vitro* susceptibilities were interpreted for penicillin, erythromycin, tetracycline and fluoroquinolones.
Results: A total number of 683 isolates were evaluated. Of these, 547 were blood, and 136 were CSF isolates. In CSF isolates, overall resistance to penicillin was 26.5%; which has increased from 7.7% in 2003 to 57.1% in 2008. In the blood isolates, the results for penicillin interpreted according to the oral and parenteral breakpoints, erythromycin, tetracycline and fluoroquinolones are shown in the Table.
Conclusions: The level of resistance to penicillin in CSF isolates is high in Turkish isolates and has increased over the years. In the blood isolates, the rate of resistance to erythromycin has increased gradually and has not shown a steady increase in tetracycline and fluoroquinolones. These results should be taken into account in the empirical therapy of meningitis and other invasive infections due to *S. pneumoniae.*

Table 1. *In vitro* resistance rates to antimicrobial agents in blood isolates of *S. pneumoniae* (2003–2008)

	I + R (%)				
	Penicillin oral	Penicillin parenteral	Erythromycin	Tetracycline	Fluoroquinolones
2003	14.3	1.1	7.5	25.0	0
2004	23.0	0	8.9	9.7	1.8
2005	21.6	0	8.4	6.7	2.4
2006	20.7	2.4	14.9	10.8	0
2007	22.7	4.5	14.3	20.9	2.3
2008	31.6	2.6	23.7	10.5	12.0
Total	22.1	1.6	12.5	14.0	2.2

I: Intermediate, R: Resistant.

P1686 Susceptibility to respiratory fluoroquinolones in *Streptococcus pneumoniae* in Toronto, Canada

K. Green, D. Low, A. McGeer on behalf of the Toronto Invasive Bacterial Diseases Network*

Background: FQ resistance (FQR) emerged in *S. pneumoniae* (SPN) shortly after the introduction of ciprofloxacin. FQR increased from 1995 to 2002, and by 2002 was clinically significant in the elderly, and those with recent exposure to FQ and healthcare institutions. We examine trends in FQR in SPN in at-risk populations in Toronto since 2002.
Methods: Since 1995, TIBDN has collected all SPN isolated from sterile sites in residents of Toronto/Peel (pop 4M); from 2002, respiratory tract isolates were collected and the population area expanded. Broth microdilution susceptibility testing to CLSI standards is performed on all isolates. Pop'n FQ use is obtained from IMS Health. Demographic and medical data were collected from patients.
Results: From 1995–2008, FQ use increased from 67.3–97.3 scripts/1000pop/yr; Levo use from 0 to 12.3 scripts/1000pop/yr, and Moxi use from 0 to 17.7 scripts/1000pop/year. 8645/9796 (88%) of SPN isolates were available for testing. After 2002, FQR decreased significantly for isolates in all subgroups (see Table), with the exception of isolates from respiratory specimens in patients currently receiving FQ. In 2008/9, fluoroquinolone resistance appeared to increased marginally, but the differences are not statistically significant. In 2009, moxifloxacin resistance rates were less than 2% in all patients except those patients failing fluoroquinolone therapy, and residents of nursing homes.
Conclusion: Despite increased use of respiratory fluoroquinolones, fluoroquinolone resistance in pneumococcal isolates is not increasing. Only nursing home patients, and patients failing treatment with fluoroquinolones at presentation are at risk of fluoroquinolone resistance.

	Lev R	Mox R	Lev R	Mox R	Lev R	Mox R	Lev R	Mox R
Invasive disease	**2000–2 (N = 857)**		**2003–5 (N = 943)**		**2006–7 (N = 775)**		**2008–9 (N = 746)**	
All adults	1.8%	0.50%	1.3%	0.33%	0.30%	0	1.1%	0.32%
Adults >65 y	3.2%	1.0%	1.2%	0.41%	0.27%	0	1.4%	0.72%
Hospital-acquired	7.4%	0	3.9%	0	0	0	0	0
Nursing home acquired	8.6%	2.5%	2.5%	0	2.1%	0	7.9%	2.6%
Failing FQ therapy	21%	7.1%	27%	9.1%	0	0	9.1%	9.1%
FQ prior 3 months	4.8%	1.6%	1.9%	0.93%	1.5%	0	1.9%	1.9%
Respiratory isolates	**2002 (N = 352)**		**2003–5 (N = 1317)**		**2006–7 (N = 935)**		**2008–9 (N = 868)**	
All adults	5.7%	1.2%	3.4%	1.6%	2.7%	1.4%	3.0%	1.5%
Adults >65 y	8.6%	1.7%	4.0%	1.8%	3.9%	2.6%	9.6%	2.3%
Hospital-acquired	8.7%	4.4%	0.8%	0.4%	3.2%	2.1%	1.9%	0
Nursing home acquired	9.1%	0	11%	7.1%	3.9%	0	18%	9.1%
Current FQ therapy	60%	20%	25%	9.4%	38%	25%	50%	50%
FQ prior 3 months	15.4%	0	7.4%	3.7%	3.8%	1.9%	6.1%	1.5%

P1687 Current profile of respiratory tract infection agents against *Streptococcus pneumoniae* and *Haemophilus influenzae* in Europe: impact of patient population

D. Draghi, N.P. Brown, C.M. Pillar, C. Thornsberry, D.F. Sahm (Chantilly, US)*

Objective: The impact of comorbidities, including patient age, on patterns of resistance among respiratory pathogens is an important consideration for the empiric therapy of respiratory tract infections (RTI). This study reports the current susceptibility (S) profile in Europe (EU) of agents commonly used to treat RTI infections caused by *S. pneumoniae* (SP) or *H. influenzae* (HI), including isolates with multi-drug resistance (MDR), and also evaluates the overall impact of patient age on resistance patterns.
Methods: From 2006–2009, the GLOBAL surveillance program collected 1790 SP and 1715 HI isolates from 6 countries across EU. All isolates were centrally tested by broth microdilution in accordance with CLSI (M7-A8). Current CLSI (M100-S19) and FDA breakpoints (BP) were applied as appropriate. MDR was defined as resistant (R) to $\geqslant$2 of the following: penicillin (PEN), cefuroxime (CFX), azithromycin (AZI), trimethoprim-sulfamethoxazole (SXT), and tetracycline (TET). Patient age was defined by years; pediatric $\leqslant$17 (PED); adult 18–64 (ADT); elderly $\geqslant$65 (ELD).
Results: Against SP overall, S among common RTI agents ranged from 98.3% (levofloxacin [LVX]) and 96.4% (AMC) down to 68% (clarithromycin [CLA]/AZI). PEN-R and MDR rates among SP varied by patient age (PED/ADT/ELD): %PEN-R (11.6/11.1 /9.2); %MDR (35.2/28.2/25.0). % susceptible to TET, macrolides/ketolides, and CFX also varied by age (PED/ADT/ELD): TET (68.7/75.7/79.9); AZI (60.1/67.7/71.3); CLA (60.1/67.2/71.0); CFX (78.5/83.7/84.5). LVX MIC90s remained at 1 mg/L and S exceeded 98% against SP, regardless of age or R phenotypes. Though rare, LVX-R was generally restricted to MDR isolates where it was most common among the ELD (4.2%) and ADT (2.5%). Among HI, ampicillin R varied from 11.2% for PED to 13.8% for ELD. For HI regardless of age, S rates were >99% for LVX, CIP, AZI, AMC, CFX and 77–78% for CLA and SXT.
Conclusions: Among SP, decreased S to CLA, CFX, and TET and increased PEN-R and MDR was observed among the PED population. Regardless of patient age, SP remained susceptible to LVX (>98%S) and AMC (>95%S). The evaluated fluoroquinolones (FQ; LVX/CIP), macrolide (AZI), and β-lactams (CFX, AMC) were potent against HI (>99%S) regardless of age. Variation in the S of SP to β-lactams and macrolides among subpopulations and the consistent activity of FQ across these populations are important factors to consider when choosing an empiric agent for the treatment of respiratory infections.

Intestinal microbiota and detection of enteropathogens

P1688 Molecular diagnostics of gastroenteritis in clinical samples: a multicentre, quality control study in the Netherlands

T.A. Schuurs, H. Nguyen, A.J. Stellingwerff, A.M. Kooistra-Smid, G.T. Noordhoek (Leeuwarden, Groningen, NL)*

Objectives: Molecular diagnostics on samples from patients with gastroenteritis is a new development in medical microbiology laboratories. However, at this moment there are no proficiency panels available to evaluate the performance of these laboratories for detection of gastroenteritis in clinical samples. Therefore, as an initiative of the gastroenteritis study-group in The Netherlands, in August 2009 a quality control panel was organised.
Methods: The panel consisted of 11 duplicated and randomized faeces samples (total 22). The samples contained, in varying loads, none, one or several of the following bacteria and/or parasites: *Salmonella enterica*, *Campylobacter jejuni*, *Giardia lamblia* and/or *Cryptosporidium parvum*. The performances of the laboratories were anonymously evaluated in combination with a questionnaire on the molecular methods used.
Results: Of twenty laboratories participating, 9 tested both for bacteria and parasites, 3 bacteria only and 8 parasites only. Four out of 12 labs, reporting bacterial targets, submitted results corresponding with the expected outcome. For the parasite targets this was 9 out of 17 labs. Two false-positives were submitted by 2 different labs. However, incorrect results were mainly false-negatives and were on samples with low target-loads. Average delta Ct-values of high-positive samples were significantly higher for labs that missed low-positives. In general, no differences existed between laboratories with correct and incorrect results, regarding pre-treatment, DNA extraction and real-time PCR methods used. However, laboratories with false-negatives added higher percentage of the sample to the extraction procedure than those with correct results (33% vs. 18%; $p = 0.065$). Furthermore, laboratories that missed *G. lamblia,* pre-treated samples by heating (80–100ºC) in lysis buffer whereas flawless laboratories pre-treated by vigorously shaking in lysis buffer.
Conclusion: Labs performed better for parasitic than for bacterial targets. Decreased sensitivity was reflected by false-negative results on weak-positive samples and by increased Ct-values on samples with higher loads, correctly reported by those laboratories. Large differences in applied methods suggest that the underlying factor(s) explaining decreased sensitivity are probably lab-specific. In order to improve sensitivity we recommend to optimize the amount of faeces added to the extraction procedure and to avoid excessive heating during pre-treatment.

P1689 Development and clinical evaluation of a multiplex real-time PCR for the detection of bacterial gastroenteritis

J.H. van de Bovenkamp, C.M. van Herk, I.O. Op den Buijs, A. van den Brule, H.T. Tjhie (Veldhoven, NL)*

Objectives: A multiplex real-time PCR was developed to detect simultaneously *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica* and *Campylobacter* spp. (SSYC) and was internally controlled with phocid herpesvirus (PhHv). A new screening strategy was chosen for the detection of gastroenteritis causing pathogens. Except for the internal control, labelled with Vic, all different probes used in the multiplex real-time PCR were labelled with FAM.
Methods: This multiplex PCR was evaluated in a six week summer period in which most positive samples were expected. In parallel with PCR also culture, as "the gold" standard, was tested. Faeces was cultured on specific plates for *Campylobacter*, *Yersinia* and *Salmonella*. In addition, a selinite broth was used for *Salmonella*. DNA was extracted the next day with the easyMag from a mixture of overnight cultured selinite broth and faecal suspension and subsequently used for the multiplex PCR. Multiplex positive samples were directly retested in a single real-time PCR for *Salmonella* and *Campylobacter*. *Shigella* and *Yersinia* were tested the next day if the single real-time PCR was negative.
Results: From the 507 consecutive samples, 63 (12.4%) were positive for both culture and PCR; 423 both negative; 3 (0.6%) were culture positive and PCR negative; and 18 (3.6%) culture negative and PCR positive. All 18 PCR positives and culture negative samples could be confirmed as positive in separate PCR's.
Of the 81 PCR positive samples 53 were positive for *Campylobacter* spp., 9 were positive for *Salmonella* spp., 9 for *Shigella* spp., 8 for *Yersinia*, and two combined *Campylobacter* and *Shigella*, and one was positive for both *Campylobacter* and *Yersinia* infections.
Conclusions: This multiplex real-time PCR strategy can be used as a screening method for bacterial faecal pathogens which could reduce the workload for culture. Furthermore, this approach enables the inclusion of other pathogens such as Shiga toxin producing *E. coli* (STEC) or separately labelled viral and parasite infections.

P1690 IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota

A.E. Budding, M.E. Grasman, A.A. van Bodegraven, P. Savelkoul (Amsterdam, NL)*

Introduction: The composition of the human intestinal microbiota is currently receiving much attention. While large scale sequencing efforts are effectively being employed in its characterization, the complex and expensive nature of these techniques restricts implementation to a small number of highly specialized laboratories. We have developed a profiling technique, IS-pro, for high-throughput analysis of the human intestinal microbiota. This technique combines species identification by 16S-23S interspace length with phylum identification by colour labelling and provides an instant overview of the Bacteroidetes/Firmicutes composition of samples. We validated it in silico, *in vitro* and *in vivo*, for colonic mucosal biopsies.
Methods: The potential of IS-pro was evaluated in-silico with a database containing sequences of 342 bacterial species. *In vitro* validation was performed with monocultures and mixes of cultured bacteria. Finally, we performed *in vivo* validation with 100 colonic mucosal biopsies of 20 healthy individuals obtained from 5 locations throughout the colon: Caecum, hepatic flexure, splenic flexure, sigmoid and rectum. Bands that were commonly found in multiple individuals were sequenced.
Results: In silico evaluation showed that IS-pro can theoretically discriminate >50,000 bacterial species. *In vitro* testing showed lower detection limit to be 10 bacteria/µl. No interactions were found between species within or between different phyla. *In vivo* validation on duplicate samples showed excellent reproducibility of IS-pro. A high level of correlation of mucosal samples throughout the colon was identified by IS-pro, corresponding well with current knowledge and further underlining the reproducibility of the technique. All sequenced bands could be traced back to gut microbes. Phylum coloring was correct for all identified species.
Conclusion: IS-pro has a very high discriminatory potential, and is well suited for analysis of the complex microbiota of the human gut. The test is not influenced by interactions between different bacteria in complex mixtures and is highly reproducible on clinical samples. Because of its simplicity, IS-pro can be performed in general clinical microbiological laboratories. As such it can make analysis of the human intestinal microbiota broadly accessible to clinical practitioners. The high-throughput nature of the test makes analysis of large numbers of samples feasible and as such it can play a critical role in research efforts.

P1691 Impact of antenatal antibiotic or corticosteroid treatment on the implantation of the neonatal intestinal microbiota

E. Montassier, N. Molinari, P. Loisel, C. Gras-Leguen, D. Darmaun, C. Rougé, J.C. Rozé, G. Potel, M.F. de La Cochetière (Nantes, Toulouse, Montpellier, FR)*

Objective: At birth, the gastrointestinal tract is essential germ free. Initial colonisation occurs during birth and shortly afterwards until a dense, complex microbiota develops. Intestinal microbiota has a profound effect on the development of the gastrointestinal tract and in the maintenance of the integrity of the mucosal surface. Establishment of normal microbiota is important for physiologic, nutritional, and immune development. Perturbations of the intestinal ecosystem, especially during early development, may have consequences that extend well beyond the neonatal period. Study of the implantation of the intestinal microbiota in newborn is thus of utmost significance. Our work stands in this context. The aim is to study the impact of antenatal antibiotic or corticosteroid treatment on the implantation of the neonatal intestinal microbiota.
Methods: Criteria for enrolment were gestational age <32 weeks, birth weight <1500 grams, postnatal age no greater than 2 weeks and absence of any disease other than those linked to prematurity. Faecal samples were collected weekly from birth up to 11 weeks. After DNA isolation, V6 to V8 region of the 16S rRNA gene amplification, samples were analysed using a fingerprinting method (Temporal Temperature Gradient Gel Electrophoresis). Each dominant microbiota was converted into electrophoretic distances, digitized and further analyzed. A bivariable model was used to study correlations between antenatal treatment and neonatal microbiota.
Results: 47 neonates born to 34 women were included. 242 faecal samples were analyzed. Results are summarized in Table 1: significant correlations have been found between (1) corticosteroid treatment and the implantation of phylum Firmicutes – family Lachnospiraceae, and phylum Proteobacteria – family Enterobacteriaceae; (2) antibiotic treatment and the implantation of phylum Firmicutes – family Clostridiaceae, phylum Firmicutes – family Staphylococcaceae, Phylum Actinobacteria – family Bifidobacteriaceae. No link has been found when the treatment was given during labour or delivery.
Conclusion: To our knowledge it is the first time that correlations are highlighted between antenatal treatment and specific intestinal microbiota implantation. Although this pilot study stands at a very early stage in the impact of antenatal treatment analysis, it suggests microbiota modulation possibilities.

Antenatal treatment	Neonatal microbiota						
	Multivariate analysis			Phylogenetic signature			
	electro-phoretic distances	OR	p-value	phylum	class	order	family
Corticosteroids (28 mothers)	110	39.42 [2.915–533.373]	0.0057	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae
	211	22.41 [2.124–236.445]	0.0097	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
Antibiotics (18 mothers)	43	18.33 [1.918–175.174]	0.0116	Firmicutes	Clostridia	Bacillales	Staphylococcaceae
	97	0.048 [0.0005–0.482]	0.0099	Firmicutes	Clostridia	Clostridiales	Clostridiaceae
	322	39.30 [1.693–900.072]	0.0221	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae

P1692 Increased risk of gastric cancer associated with combined effect of *Helicobacter pylori* virulence determinants

M. Douraghi, Y. Talebkhan, H. Zeraati, M. Eshagh Hosseini, M. Mohagheghi, M. Mohammadi (Tehran, IR)*

Objectives: Persistent colonization and maintenance of *H. pylori* in harsh conditions of stomach are attributed to a series of virulence determinants. Two potent polymorphic secretory toxins; vacA and cagA and the functionally active adhesin babA2 are identified as the more ubiquitous virulence markers in disease associated strains. This study was undertaken to determine whether *H. pylori* virulence markers as screening determinants affect the risk of gastric cancer independently or cooperatively.
Methods: Collectively, 212 *H. pylori* infected patients including 48 gastric cancer (GC) and 164 non gastric cancer patients were assessed for Hp vacA polymorphic alleles, cagA polymorphism and functional babA2 using PCR.
Results: Our study revealed that each gene individually or cooperatively, were significantly associated with GC (Table 1). The OR for GC risk was further increased significantly with the number of studied genes. As indicated in Table 1, the s1 or i1 allele individually affect the GC risk up to more than 8-fold which was higher than that of concurrent carriage of s1i1m1/babA2+/ABCC CagA. However, the proportion of those who actually have disease (PPV) of the latter as triple-positive genotypes is higher than any other studied genes singly or synergistically.
Conclusion: This analysis thus reveals that specific virulence gene clusters are associated with an increasing risk of GC. Therefore, the current study provides evidence of cooperative activity of *H. pylori* virulence genes in the development of gastric malignancy. We propose the exploration of the screening power of multiple *H. pylori* virulence determinants for improved population screening for the risk of GC development in high prevalent geographic regions. These efforts should focus on joint screening powers of host–pathogen-environment susceptibility factors.

Table 1. Association of *H. pylori* genotype(s) with gastric cancer

Genotype (Positive vs. others)	Cases, n (%)	Controls, n (%)	*P* value	OR	95% CI	PPV	NPV
s1	46 (95.8)	119 (72.6)	0.004	8.697	2.027–37.326	27.8	95.7
i1	42 (87.5)	75 (45.7)	0.001	8.307	3.347–20.614	35.8	93.6
m1	29 (60.4)	42 (25.6)	0.001	4.434	2.254–8.720	40.8	86.5
babA2	41 (85.4)	104 (63.4)	0.006	3.379	1.427–8.003	28.2	89.5
ABCC CagA	20 (41.7)	36 (22)	0.007	2.540	1.283–5.026	35.7	82
s1i1m1	29 (60.4)	39 (23.8)	0.001	4.892	2.47–9.667	42.6	86.8
s1i1m1/ABCC CagA	13 (27.1)	11 (6.7)	0.001	5.1	2.137–12.492	54.1	81.3
s1i1m1/*babA2+*	27 (56.3)	29 (17.7)	0.001	5.985	2.980–12.019	48.2	86.5
s1i1m1/*babA2+*/ABCC CagA	13 (27.1)	9 (5.5)	0.001	6.397	2.535–16.143	59	81.5

OR, odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Molecular typing: streptococci and staphylococci

P1693 PBPs and MurM variations in invasive β-lactam-resistant 19A *Streptococcus pneumoniae* ST320, ST81 and ST276 clones from Spain, 1997–2007

R. del Campo, C. Ardanuy, D. Rolo, E. De la Pedrosa, R. Cantón, A. Fenoll, F. Baquero, J. Liñares on behalf of the CIBERESP*

Background: Since PCV-7 was introduced in 2002 in Spain, a significant increase in invasive infections caused by 19A *S. pneumoniae* was observed. Sequential alterations in PBP sequences are the classical mechanism to acquire penicillin resistance, whereas murM mutations are required for high penicillin and cefotaxime resistance. The aim of this study was to analyze the PBPs and murM genetic variants in a 19A *S. pneumoniae* collection recovered before and after of the PCV introduction (1997–2007).
Material and Methods: Forty-two invasive 19 A *S. pneumoniae* isolates with both penicillin and cefotaxime resistance were collected at the Spanish Reference Laboratory for Pneumococci. Population structure was evaluated by PFGE-SmaI and MLST. Internal fragments of the PBP1a, PBP2b, PBP2x and MurM encoding genes were amplified and sequenced.
Results: The proportion of serotype 19A among penicillin resistant strains progressively increased from 2% in 1997 to 12.1% in 2007. This increase was especially noticeable in the 2005–2007 period. Resistance rates were as follows: tetracycline, 100%; co-trimoxazole, 88%; erythromycin, 74%; clindamycin, 74% and chloramphenicol, 38%; amoxicillin, 33% resistant and 10% intermediate resistant (IR) (MIC range 0.5–8). Eight different PFGE patterns were observed, and three major clonal groups were identified by MLST: ST81-Spain23F (17 isolates); ST320-Taiwan19F (16 isolates); and ST276-Denmark14 (9 isolates). Identical DNA and protein sequences were detected in all isolates for the murM gene. Two different DNA/protein alleles were

observed in the pbp1a sequences, one of them related to the ST320 isolates. Four different alleles were found for the pbp2b gene, clustering two of them to ST320 isolates, another one only in ST81 isolates, and the last one present in ST81 and ST276 isolates. For the pbp2x gene two different DNA sequences were obtained, corresponding one of them to the ST320 isolates, but PBP2x aminoacid sequences were identical in all isolates.
Conclusion: In recent years, incidence of 19A invasive multirresistant *S. pneumoniae* isolates is increasing in Spain, mainly due to the spread of the clones ST81-Spain23F, ST276-Denmark14 and ST320-Taiwan19F. PBP1a, PBP2x, and MurM proteins are almost identical in ST81 and ST276, suggesting association between 19A capsular transformation and modified PBPs. In ST320 isolates, specific alleles were found for PBP1a and PBP2x, indicating a possible 19F/19A cps recombination event.

P1694 Genotypic analysis of invasive resistant *Streptococcus pneumoniae* by semi-automated repetitive-element PCR

F. Pardo, M.L. Pérez del Molino, L. Martínez-Lamas, M. Hernández, B.J. Regueiro (Santiago de Compostela, ES)*

Objective: To determine by using rep-PCR (DiversiLab System) the degree of clonal diversity among invasive resistant *S. pneumoniae* isolates, related with results obtained by serotyping.
Methods: We analized all invasive resistant strains of *S. pneumoniae* (2004–2007). Serotyping was carried out by the quellung reaction with specific antisera. Minimal inhibitory concentration (MIC) was obtained by the agar microdilution method. Clonality dispersion was obtained by rep-PCR. In the phylogenetic analysis, were considered indistinguishable isolates or those with the same genotype had a similarity index (SI) of $\geqslant$97% and no obvious band differences. Isolates were characterized as being similar or subtypes if they had $\geqslant$97% similarity and one band different.
Results: The 97 resistant strains, classified into eleven different serotypes (48.5% included in 7-valent pneumococcal conjugate vaccine). The strains could be grouped in 55 genotypes, belonged to 14 clusters, 9 of these overlapping between serogroup and genotype and 4 grouped strains of different serogroups. The strains belonging to serotype 19A, all erythromycin-resistant, were genetically similar, 83.3% had a SI above 93%. Only 18.2% of 11 isolates of serotype 19F can be clustered (SI > 97%). In the serogroup 14 a 90.9% had a SI below 92%. About 75% strains belonging to serotype 15A (all resistant to penicillin, tetracycline and erythromycin) had a SI > 95.
Conclusion: There is clear greater clonal diversity among vaccine serotypes. The rep-PCR, have proven to have a greater discriminatory power in the phenomena of genetic exchange than serogroup. Our data indicate a need for continued monitoring of invasive pneumococccci, to detect changes in emergence of serogroups and to determine the effects of the vaccine its genetic struture. The Diversilab System has the potential to increase the speed of analysis and to obtain compared results.

P1695 The presence of pilus and clade types among invasive pneumococci recovered in the Czech Republic

H. Zemlickova, V. Jakubu, M. Fridrichova, M. Trojanek, M. Musilek, J. Motlova (Prague, CZ)*

Objectives: *Streptococcus pneumoniae* is a major cause of meningitis and pneumonia. Although at least 91 serotypes have been identified, only a limited number of them are more frequently detected in disease. The effective spread of the given serotypes represented by a few clones has not been fully elucidated yet and several virulence factors have been suggested to influence the success of particular clones. One of the identified virulence factors is adhesive pilus that can be organized into 3 clade variants. The aim of the study was to determine the prevalence of the pilus and clade type among the pilus positive isolates from a collection of invasive strains of serotypes 4, 6B, 9V, 14, and 19F.
Methods: A total of 256 invasive isolates of serotypes 4 (65), 6B (34), 14(57), 9V (48), and 19F (52) recovered from 1996 to 2003 were studied. Serotyping was performed by the Quellung reaction and all isolates were analyzed by MLST. MICs of antibiotics were determined by the broth microdilution method. The presence of pilus was detected by PCR targeting the sortase B, C, and D genes which are structural parts of the pilus. Confirmation of pilus absence was done by amplification of the entire pilus islet. For the pilus positive strains, the clade type (I, II, and III) was determined using PCR.
Results: The pilus was found in all serotypes, although the proportion of pilus positive isolates varied between serotypes from 1.7% in serotype 14 to 77% in serotype 9V. Out of 88 pilus positive isolates, 26.1% were penicillin non-susceptible. The STs were homogenous for the presence of the pilus and clade type. The pilus was found in all isolates of serotypes 9V, 6B, and 4 representing Spain9V-3, Poland6B-20, and Sweden4–38 clones, respectively. Of the piliated isolates, 67% were classified into clade type I, 19.3% into clade type II, and 13.6% into clade type III. Clade I was found in serotypes 4, 14, 9V, and 19F; clade II in serotype 6B; and clade III in serotypes 4 and 6B. The prevalence of serotype 4 among the invasive isolates increased from 1.7% in 1996 to 11.1% in 2003 and an upward trend from 3.3% in 1996 to 5.3% in 2003 was also observed in serotype 9V.
Conclusions: The expresion of the pilus could constitute a selective advantage in competition with other serotypes and could contribute to the increase of serotype 4 among the invasive pneumococcal isolates.
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P1696 Association of group A streptococcal emm types with virulence traits and macrolide resistance

G. Blandino, S. Puglisi, M. Minutolo, R. Musumeci, A. Speciale (Catania, Monza, IT)*

Objectives: Aim of this study was to determine M (emm) subtypes of *Streptococcus pyogenes* strains isolated from asymptomatic carriers and children with pharyngitis, and to investigate the correlation between emm types, the presence of fibronectin binding protein genes, and phenotypes of antibiotic resistance.
Methods: We detected and characterized emm subtypes in 59 strains of *S. pyogenes* isolated in Sicily (Italy) from asymptomatic carriers (29) and children with pharyngitis (30) previously characterized for the presence of prtF1 and pfbpI genes and erythromycin resistance phenotypes (Musumeci et al., Clin. Infect. Dis. 2003; 37:173–179). emm gene specific PCR and emm sequence typing were performed as described by Beall et al. (J. Med. Microbiol. 1998; 47:893–898). In addition, to confirm the relationship between emm pattern type and SOF (serum opacity factor), sof gene was detected and sequenced basing upon PCR and sequence analysis of a variable length 450–650-base PCR fragment.
Results: The most frequent emm subtypes detected in isolates from pharyngitis were: emm1.0 (30.0%), emm89.0 (23.3%), emm2.0 (13.3%) and emm28.0 (10.0%). One new emm12 subtype (emm12.40) was detected. In isolates from asymptomatic carriers (enrolled from different classes of many primary schools) the most frequent emm subtypes were: emm12.0 (51.7%) and emm94.0 (10.3%); while there were no emm1.0 strains. The most significant differences between the two groups concerned the presence of the two more common emm subtypes, 1.0 ($P = 0.001$) and 12.0 ($P < 0.001$). The prtF1 and prtF2 genes were simultaneously present in 14 of 16 (87.5%) emm12.0 and 8 of 9 (88.9%) emm89.0 isolates. Correlation with phenotypes of resistance showed that 9 of 11 (81.0%) M phenotype strains belonged to emm12.0 subtype and all 3 iMLS-A phenotype strains to emm89.0.
Conclusion: This study suggests that *S. pyogenes* emm12 and emm89 isolates, mainly positive for prtF1 and prtF2 genes, are more virulent than the others, and that emm12 isolates may have a role in the asymptomatic carrier status.

P1697 Distribution of PFGE types of group B streptococci originating from Polish pregnant women in relation to capsular polysaccharides and surface protein genes

M. Brzychczy-Wloch, T. Gosiewski, M. Bodaszewska, W. Pabian, D. Ochonska, P.B. Heczko (Cracow, PL)*

Objectives: Capsular serotyping, based on capsular polysaccharides (CPS), is the classic method for typing *Streptococcus agalactiae*. Group B streptocopcci (GBS) can also be classified on the basis of surface protein antigens. Genotyping methods, like pulsed field gel electrophoresis (PFGE) are used to characterize and distinguish specific clones among GBS isolates. The study tried to find out the correlation between PFGE pulsotypes and serotypes or surface protein genes in GBS isolates.
Methods: Research was carried out from August 2007 till July 2009. 1176 women in the 3rd trimester of pregnancy from Southern Poland region underwent GBS colonization screening according to CDC recommendations. A multiplex PCR method was used to determine the genes coding particular capsular polysaccharides (CPS) and to detect the surface protein genes bca, rib, ε, alp 2, alp 3 and alp4. Isolates were genotyped by pulsed field gel electrophoresis (PFGE) after macrorestriction with SmaI enzymes. The PFGE banding patterns were analyzed with the Jaccard coefficient and UPGMA by using Molecular Analyst (Applied Maths) software. Into detailed genetic analysis we chose to include 100 *S. agalactiae* isolates originating from vaginal and rectal carriage, representing serotypes: Ia (n = 25), Ib (n = 7), II (n = 14), III (n = 29), IV (n = 7) and V (n = 18).
Results: The PFGE method showed high genetic diversity between *S. agalactiae* strains originating from pregnant women. In 100 strains a total of 63 restriction profiles were identified using PFGE. At an 80 per cent similarity level, 56 different PFGE patterns were established. We showed the exact correlation between strains belonging to specific pulsotypes and serotypes. Within each serotype, PFGE patterns differed considerably, the largest degree of heterogeneity was observed among type Ib, II and III strains. Serotype Ia, IV and especially V were more homogeneous. Strains with identical macrorestriction profiles belonged to the same CPS type, but varied with regard to serosubtypes, based on surface protein genes.
Conclusion: For studies of epidemiology and pathogenesis of GBS, it is important to identify as many phenotypic or molecular markers as possible to increase the discriminatory power of typing systems. Serotypes distribution of *Streptococcus agalactiae* varies with geographical region, ethnic origin and the virulence of clinical isolates. This is the first study dealing with the molecular characterization of GBS isolates in Poland.

P1698 Molecular typing of *Streptococcus agalactiae* by multiple locus variable-number of tandem-repeat analysis

E. Haguenoer, G. Baty, C. Pourcel, M.F. Lartigue, A.S. Domelier, A. Rosenau, R. Quentin, L. Mereghetti, P. Lanotte (Tours, Orsay, FR)*

Background: Multilocus Sequence Typing (MLST) is presently the reference method for genotyping *Streptococcus agalactiae* strains, the leading infectious cause of morbidity in newborns which also causes substantial diseases in immunocompromised children and adults. Multiple Locus Variable Number of Tandem repeats (VNTR) Analysis (MLVA), a PCR method based on the study of tandem repeats polymorphism, could represent an alternative to type bacterial strains.
Objectives: The purpose of this study was to describe a MLVA scheme for the genotyping of *S. agalactiae* and to compare its performances with those of MLST.
Methods: A collection of 186 strains isolated from human and cattle was studied. Among the 152 human strains, 71 were from vaginal carriage, 59 were from cerebral-spinal fluid, 17 were from endocarditis (bacteremia) and 5 were from gastric fluid of newborns. The 35 bovine strains were linked to clinical evidence of mastitis. Seven VNTR loci were selected for their polymorphism and used to genotype the bacterial population. The MLVA profile is composed of a string of allele numbers, corresponding to the number of repeats at each VNTR Locus. The relationships between the strains were evaluated with the UPGMA algorithm by comparison of allelic profiles. The results were compared to those obtained by MLST based on the analysis of seven housekeeping genes.
Results: The results showed a good concordance between the two techniques and demonstrated the higher discriminatory power of MLVA as compared to MLST. One hundred and twelve genotypes were discriminated with the panel of 7 VNTR loci (diversity index: 0.96 versus 0.88 for MLST). The analysis with the UPGMA algorithm showed a clonal distribution of the population similar to the one obtained by MLST, except for the strains of clonal complex CC23 divided into two groups according to the serotype and for the strains of clonal complex CC17 divided into two groups according to the human or bovine origin.
Conclusion: The MLVA scheme proposed here is a rapid, cheap and easy to perform genotyping method with exchangeable results between laboratories, applicable to epidemiologic surveillance of *S. agalactiae* and to outbreak situations.

P1699 Phenotypic and molecular characterization of *Streptococcus pyogenes* isolated from scarlet fever patients

C. Silva-Costa, M. Ramirez, J. Melo-Cristino (Lisbon, PT)*

Objectives: *Streptococcus pyogenes* (GAS) is a human pathogen responsible for a number of disease presentations, including scarlet fever. The aims of this study were the phenotypic and molecular characterization of 101 GAS isolated from cases of scarlet fever collected in 2002–2008 in a teaching hospital in Lisbon.
Methods: Antimicrobial susceptibility testing and macrolide resistance phenotype were determined by disk diffusion and macrolide resistance genotypes were determined by PCR. The exotoxin profile was determined by PCR for the detection of genes speA, speB, speC, speF, speH, and ssa. A combination of T typing, emm typing, pulsed-field gel electrophoresis (PFGE) was used to define clones. Bacitracin susceptibility was determined for all the isolates by disk diffusion.
Results: during the seven years of the study, the overall rate of erythromycin resistance was 9.9% (n = 10) and all the macrolide resistant isolates presented the M-phenotype carrying the mef(A) gene. All the 101 isolates included in this study were typeable by PFGE and seven major clones accounted for 81% of the isolates. With minor exceptions, each clone was associated with a T type, emm type and an exotoxin profile. All the isolates carried the chromosomal speB and speF genes, whereas the phage-encoded speA, speC, speF, and ssa were variable. The genes speA and speC, often associated with scarlet fever, were present together in 7.9% (n = 8), and alone in 28.7% (n = 29) and 54.5% (n = 55) of the isolates, respectively.
Conclusion: There was a large diversity among GAS strains isolated from scarlet fever paients, although seven clones accounted for the majority of the isolates. Only 8.9% of the isolates lacked the two scarlet fever associated toxins SpeA and SpeC, although only a fraction carried both.

P1700 Molecular typing of methicillin-resistant *Staphylococcus aureus* in animal origin foods

Z. Salehipour, M.M. Soltan Dallal, S. Eshraghi, T. Zahraei Salehi, A. Rahimi Forushani, T.P. Abedi Mohtasab, J. Fallah Mehrabadi (Tehran, IR)*

Objective: Methicillin-resistant staphylococcus aureus (MRSA) strains are common pathogens in serious infections and nosocomial outbreaks. These strains might be transmitted to humans via consumption of animal origin foods. Aim of this study is prevalence and molecular characterization of these strains in different foods.
Methods: 913 food samples of animal origin were collected from July 2006 to November 2007 and cultured on Baird Parker agar. All suspected colonies were characterized by biochemical tests. presence of mecA gene was confirmed by PCR method. MRSA isolates were evaluated by the disk diffusion method for different antimicrobial agents along with

minimum inhibitory concentration (MIC) determination of oxacillin. Presence of tst gene in MRSA isolates was assessed via PCR and reverse passive latex agglutination test and followed by biotyping and spa typing.
Results: Among 93 *S. aureus* isolates, five (5.37%) harbored the mecA gene. 60% of the five MRSA isolates were resistant to tetracycline, while 40% were resistant to Ceftriaxone. Four out of five MRSA isolates (80%) produced SEs and TSST1. Three MRSA isolates belonged to human biotype, whereas the remaining two isolates belonged to non-host-specific (NHS) biotype. Amplification of spa gene showed three different fragments. Applying HindIII and HaeII, 4 and 3 distinct RFLP patterns were yielded, respectively.
Discussion: In present study, prevalence of MRSA strains in foods is worrying relatively. Bacterial strain typing for MRSA can be considered a tool of importance to investigate suspected outbreaks together with their control and surveillance, and PCR-RFLP is a reliable method for typing.

P1701 Probing *Staphylococcus aureus* diversity through a novel fluorescent amplified fragment length polymorphism assay

D. Desai, M.J. Ellington, C. Arnold, M. Desai (London, UK)*

Objectives: FAFLP is a robust, high-resolution, PCR-based methodology used for the analysis of genetic diversity within bacterial genomes, without prior knowledge of genome sequence. It combines the principle of restriction fragment length polymorphism with selective PCR amplification of subsets of fragments generated by restriction endonucleases. Here we sought to evaluate and establish a novel fluorescent amplified fragment length polymorphism (FAFLP) assay to assess the genetic diversity within *Staphylococcus aureus* including major methicillin resistant clones.
Methods: A total of 53 *S. aureus* isolates, from 11 major multilocus sequence types, including 15 isolates of the Epidemic MRSA-15 clone were used for comparative analysis using FAFLP. Genomic DNA was restricted using the endonuclease combination Csp6I and BglII. A combination of four one-base selective fluorescently-labelled BglII primers (selective base +A, T, G & C) with a non-selective Csp6I primer (+0) were evaluated for the development of a multiplex assay. FAFLP products were analysed using the ABI 3730 sequencer and GeneMapper software (v.4.0) for data analysis. Clusters from the generated profiles were compared with staphylococcal cassette chromosome mec (SCCmec) and multilocus sequence typing (MLST) data.
Results: FAFLP generated 240 to 290 amplified fragments ranging in size from 60–600bp: these fragments constituted an FAFLP profile. Thirty-five unique profiles were exhibited amongst the 53 isolates. The multiplex assay differentiated EMRSA-15 (ST22) from EMRSA-1, 3 and 16 (ST36) isolates as well as isolates that were ST30 and ST5. Genetic heterogeneity within the EMRSA-15's was also revealed. Comparison of FAFLP data with SCCmec data showed that FAFLP data differentiated isolates harbouring type I and II as well as IV; it also discriminated between isolates harbouring different sub-types of SCCmecIV.
Conclusions: The established multiplex FAFLP assay increased the discriminatory power by approximately four-fold compared to a singleplex, as the number of data points spanning the genome increased. This approach provides a cost-effective and high-throughput method applicable to outbreak analysis through to global epidemiological studies and has the potential for use in hospital infection control. The identification of differential fragments between different lineages of MRSA will be incorporated into a PCR-based diagnostic assay.

P1702 Development of an automated method for high-throughput multilocus sequence typing of *Staphylococcus aureus*

T.M. Pryce, G.W. Coombs, L. Lim, F. O'Brien, L.K. Smith (Perth, AU)*

Objectives: To develop and implement automated and high-throughput DNA extraction and PCR amplification methods for multilocus sequence typing (MLST) of *Staphylococcus aureus* using existing laboratory automation.
Methods: A protocol using the MagNA Pure robotic instrument was developed for all pre-PCR steps. These steps include genomic DNA extraction from *S. aureus* and pipetting prepared DNA templates into the separate master mixes representing the seven MLST housekeeping genes (alleles). Following DNA extraction, PCR amplification of all seven alleles was performed using BIORAD MyCycler. Subsequent PCR products were purified with a magnetic bead system (Ampure) using the Perkin Elmer EP3 robotic instrument. Sequencing reactions (forward and reverse) and sequencing clean-up steps were performed using the Perkin Elmer Multi-Probe II and the Perkin Elmer EP3 instruments respectively. All sequencing reactions were analysed by capillary electrophoresis using the ABI 3730XL Genetic Analyser. Twenty control strains of *S. aureus* of known sequence type were used as controls to develop the automated test method.
Results: The MagNA Pure robotic instrument produced consistent DNA quality and high yield from *S. aureus* that was suitable for PCR. The mean DNA concentration was 17.8 ng/μL (SD 2.5 ng/μL). The OD 260/280 ratio was 1.805 (SD 0.04). The PCR sensitivity for six of the seven housekeeping gene alleles was approximately 5 pg/ul. A 10-fold decrease in PCR sensitivity was observed for the tpi housekeeping allele (approximately 50 pg/uL) and therefore the primer concentration for this allele was increased to ensure adequate and comparable sequencing signal strengths to the other six housekeeping gene alleles. The PCR amplification produced consistent PCR product amplification. Sequence analysis of *S. aureus* GPBTU ST22 control from 35 consecutive MLST runs demonstrated high reproducibility and high sequence quality.
Conclusions: The assay was highly reproducible and produced consistent and expected results for all controls tested. The method has minimal pipetting steps and is less labour-intensive than other manual molecular methods and has improved sequence quality and streamlined workflow. This automated method has now been used for more than 300 clinical isolates identifying 56 *S. aureus* sequence types. The method is well-suited to reference laboratories performing high-throughput *S. aureus* MLST.

P1703 Comparison of two multi-locus variable-number tandem repeat analysis methods for genotyping highly clonal methicillin-resistant *Staphylococcus aureus* in Scotland

A. Holmes, W. Hannant, K. Girvan, G. Edwards, K. Templeton (Edinburgh, Glasgow, UK)*

Objectives: The aim of this study was to investigate the usefulness of two different multi-locus variable number tandem repeat analysis (MLVA) methods for subtyping the major Scottish and UK lineages of methicillin-resistant *Staphylococcus aureus* (MRSA), EMRSA-15 (ST22) and EMRSA-16 (ST36), which account for 70% and 20% of isolates referred to the Scottish MRSA Reference Laboratory respectively.
Method: A gel-based MLVA method, MLVA-gel (Sabat et al. JCM 41:1801–1804, 2003), based on seven loci, was compared to an automated capillary electrophoresis-based method, MLVA-frag (Schouls et al. PLoS ONE 4(4):e5082, 2009), based on eight loci; the methods shared two common loci, sspa and spa. A total of 60 MRSA strains that had previously been analysed by pulsed-field gel electrophoresis (PFGE; 21 EMRSA-15, 16 EMRSA-16, and 23 MRSA strains with diverse PFGE patterns) were investigated. In addition, 30 EMRSA-15 strains with identical PFGE patterns (PF15 h) from two geographically linked but epidemiologically distinct outbreaks and several sporadic cases were analysed.
Results: All isolates were typeable by both MLVA methods. The MLVA-gel method was more discriminatory resolving 16 EMRSA-15 and 10 EMRSA-16 types, compared with 10 EMRSA-15 and 4 EMRSA-16 types using the MLVA-frag method; both methods were equally able to distinguish between the 23 diverse PFGE types. Analysis of the PF15 h outbreak and sporadic isolates revealed the MLVA-gel and MLVA-frag methods were able to resolve them into 7 and 6 subtypes, respectively. Importantly, both assays were able to confirm that the two geographically related outbreaks were not genetically linked.
Conclusion: The MLVA-gel method was better able to distinguish closely-related variants of EMRSA-15 and EMRSA-16, most likely

a reflection of the faster molecular clock of some of the loci used. Importantly, both methods were able to subtype strains indistinguishable by PFGE, suggesting these assays would be useful epidemiological tools for identifying and tracking specific subtypes of highly clonal MRSA lineages in Scotland.

P1704 Genetic homogeneity within the methicillin-resistant *Staphylococcus aureus* lineage ST398

T. Bosch, X.W. Huijsdens, M.E. Heck, A.J. de Neeling, H. Grundmann, L.M. Schouls (Bilthoven, NL)*

Objective: In 2003, the first isolates of livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) belonging to sequence type (ST) 398 were reported. Since then, the LA-MRSA ST398 lineage has frequently been isolated in the Netherlands and other countries. In 2008, 37% of all MRSA isolates analyzed in the Dutch national surveillance program belonged to this lineage. In this study, the genetic diversity of ST398 lineage isolates was investigated using three molecular typing tools.
Methods: All MRSA (n = 5510) submitted from January 2008 till October 2009 for the national surveillance were characterized using staphylococcal protein A (spa) typing and multiple-locus variable number of tandem repeat analysis (MLVA). Many of the isolates belonged to the MLVA complex (MC) 398, representing the ST398 lineage. Pulsed-field gel electrophoresis (PFGE) using restriction enzyme Cfr9I was performed on a random selection (n = 93) of the MC398 isolates.
Results: Spa-typing of 2257 MC398 isolates revealed 48 different spa-types. Spa-types t011 (n = 1315) and t108 (n = 578) were predominant, accounting for 84% of all isolates. MLVA-typing yielded 42 different MLVA types (MTs) with MT398 (n = 1267) and MT572 (n = 559) as the predominant MTs which made up 81% of all MC398 isolates. PFGE typing of 93 MC398 isolates resulted in 48 distinct patterns which could be sub-divided in 11 PFGE clusters (clusters A-K, cut off value 80%). However, isolates from cluster A (n = 16) and E (n = 49) accounted for >70% of all 93 isolates. No match was found when comparing the PFGE profiles of the MC398 isolates with the national MRSA PFGE database containing profiles from over 4000 isolates. The Simpson diversity indices for spa, MLVA and PFGE were 59.3%, 62.0%, and 95.5%, respectively.
Conclusions: MC398 isolates in the Netherlands represent a genotypic homogeneous group comprising two genogroups that make up more than 80% of all isolates. PFGE using restriction enzyme Cfr9I enabled us to further differentiate MC398 and yielded profiles that were distinct from those of all other MRSA. However, the division into 2 predominant groups was retained. The results indicate that LA-MRSA MC398 is a distinct clonal MRSA lineage. Whole genome sequencing will be required to elucidate the origin and evolution of these isolates.

P1705 SCCmec type VII (former VT) found in Danish ST8-t024-PVLneg CA-MRSA

K. Boye, S. Pulis, L. Hansen, S. Sørensen, H. Westh (Hvidovre, Copenhagen, DK)*

Objective: In Copenhagen, Denmark, the second-most common SCCmec cassette found in MRSA is type V based on a multiplex PCR targeting ccrC and the IS431 next to the truncated mecR that is characteristic of type V (Boye et al, 2007). One of the type V cassettes from a MRSA-ST8 was sequenced as part of a full-genome sequencing project. Comparison with GenBank sequences revealed that the cassette was most homologue to the SCCmec type VII (former VT) found in MRSA-ST59 from Taiwan (Takano et al, 2008). The cassette was annotated and described.
Methods: The MRSA-isolate M253 was recovered in 2005 from a 90-year old woman living in a nursing home in Copenhagen. The isolate was characterized by PCR for mecA, spa, PVL, AMCE and by spa-typing and SCCmec typing. Whole genome sequencing was performed on a GS FLX (454 Life Sciences, a Roche company, CT, USA). Reads were aligned and assembled using the Newbler assembler software provided with the GS FLX instrument. The contigs were compared to GenBank sequences using BLAST and the detailed alignment optimized by visual inspection.
Results: The SCCmec cassette of M253 was 41,500 bp in length and carried two ccrC-genes, ccrC2 and ccrC8, as has been described in SCCmec type VII from Taiwan (Higuchi et al, 2008). It contained the IS431 next to the truncated mecR as SCCmec type V does. The Taiwan SCCmec VII contained a Sau-like IS not found in the Danish cassette. While the Taiwan MRSA was isolated from children, belonged to sequence type ST59 and carried the PVL-genes, the Danish type VII came from an old woman, belonged to ST8 and was PVL-negative. SCCmec VII is misclassified as SSCmec V in our multiplex PCR (Boye et al, 2007).
Discussion: CC8-MRSA t024 is very common in Denmark especially in nursing homes. It is, however, an interesting question where the SCCmec VII originated. All other MRSA found in the patients nursing home have been t024 SCCmec IVa. This patient has been screened yearly since 2005 and later isolates have been t024 SCCmec IVa. Both MRSA are ST8-t024-PVLneg. Based on the full genome sequence the two isolates are unrelated and the patient must have had two different MRSAs.
Boye et al (2007): Clin Microbiol Infect 13: 725–727. Takano et al (2008): Antimicrob Agents Chemother 52: 837–845.

P1706 Laboratory identification of healthcare-associated and community-associated MRSA strains using SCCmec Evigene

J. Lamson, B. Raaby, J. Villadsen, D. Houg, A. Rasmussen, H. Stender (Roskilde, Kogens Lyngby, Vedbaek, DK; Woburn, US)*

Objective: Healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) and community-associated-MRSA (CA-MRSA) have traditionally been identified by the onset of the infection but today HA-MRSA strains are emerging outside health-care settings and CA-MRSA strains are emerging within hospitals. HA-MRSA strains are linked to increased resistance to a wide range of antibiotics, including higher vancomycin MIC, whereas CA-MRSA strains remains susceptible to non-β-lactams but linked with invasive necrotizing infections. There is therefore a need for diagnostic tools to differentiate HA-MRSA and CA-MRSA strains to ensure optimal patient therapy and management. In this study we used SCCmec EVIGENE, a signal-amplified, sandwich hybridization format, with probe sets for each of the five SCCmec (staphylococcal cassette chromosome mec) types for the laboratory identification of HA-MRSA strains by the presence of SCCmec types I, II or III and identification of CA-MRSA strains by the presence of SCCmec types IV or V.
Methods: The five SCCmec type specific probe sets used in the EVIGENE assay were verified on PCR SCCmec typed strains (Oliviera and de Lencastre, 2002) determined by Statens Serum Institut and a SCCmec type V strain provided by Ito et al. The SCCmec EVIGENE was tested on 175 clinical MRSA isolates obtained from Europe (152), Middle East (2), North America (8) and South America (13) for the presence of each of the five SCCmec types. In addition, mecA EVIGENE® was performed to confirm the presence of the mecA gene.
Results: All (175/175) were mecA positive. 50.9% (89/175) were identified as HA-MRSA strains by the presence of SCCmec type I (40), II (27) or III (22) and 44.0% (77/175) were identified as CA-MRSA strains by the presence of SCCmec type IV (61) or V (16). 5.1% (9/175) were unidentified due to detection of multiple SCCmec types (9).
Conclusions: Unequivocal identification as HA-MRSA or CA-MRSA was obtained for 94.9% of the MRSA strains. SCCmec EVIGENE with probe sets for each of the five SCCmec types therefore offers laboratory identification to supplement the clinical diagnosis of HA-MRSA and CA-MRSA infections.

P1707 ***In vivo*** **evolution of a single ST247-MRSA-I clone recovered over 13 years from a cystic fibrosis patient: variation in the spa-type and immunogenicity**

R. del Campo, V. Gómez-Piña, M. Pérez-Vázquez, A. Vindel, A. Fernández-Olmos, M. García-Castillo, A. García-Sánchez, L. Máiz, R. Cantón, E. López-Collazo, A. Lamas (Madrid, ES)*

Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant cause of chronic pulmonary infection in Cystic Fibrosis (CF) patients. The aim of this study was to analyze the *in vivo* evolution of MRSA in a single CF-patient, analyzing the microbiological aspects as well as the inmunogenicity particularities.
Material and Methods: MRSA isolates were obtained from single CF-patient during 13 years (1996–2009). Antibiotics susceptibility was performed by agar dilution method and PFGE-Sma I was applied to analyze genetic relatedness among the isolates. SCCmec, spa, pvl, and MLST typing methods were carried out by specific PCR reactions. Immunogenicity ability was determined in parallel in a CF-MRSA isolate and in the HARMONY ST245-MRSA reference strain with different settings: planktonic or biofilm growth, and presence of subinhibitory ciprofloxacin concentrations. Sonic crude extract of both strains were added to human monocytes, incubating for 3, and 6 hours. TREM-1, TNF-α, IL-6, IL-12 and IL-10 expression levels were analyzed by real-time quantitative PCR.
Results: A total of 30 ST247-MRSA-I isolates with related PFGE patterns were finally selected. Most of them presented a multirresistant phenotype including aminoglycosides, quinolones, and macrolides. During the first eight years, t051 spa type was the predominant one, whereas six different spa types were detected (t051, t052, t303, t2849, t2482 and t5125) along the time, five of them belonging to the same spa-clonal complex (spa-CC051). In the last isolates we identify mainly the spa type t303. All isolates were negative for pvl gene. A particular mode of growth was identified only able to growth around tetracycline and erythromycin disks. When human circulating monocytes were exposed *in vitro* to the different strains and settings, the inflammatory response was lower in the CF-MRSA isolate comparing with the HARMONY strain, and also in the biofilm formation comparing with the planktonic one. However, the presence of ciprofloxacin exerted no effect on the inflammatory response.
Conclusion: A single ST247-SCCmecI-MRSA clone was detected during 13 years in the sputum of a CF-patient, presenting six different spa-types and a singular mode of growth. The bacterial adaptations in these patients may shift the response of the monocytes from the classical response to an alternative or attenuated response. This could be reflected in the altered immune response is observed in these patients.

P1708 **Comparison of the DiversiLab system with spa typing and pulsed-field gel electrophoresis for the characterization of methicillin-resistant *Staphylococcus aureus* isolates**

D. Goldenberger, E. Schultheiss, A.F. Widmer, R. Frei (Basel, CH)*

Objectives: Evaluation of the performance of the DiversiLab system (DL), a commercially available repetitive sequence-based PCR, compared to pulsed-field gel electrophoresis (PFGE) and spa typing. Typing methods were applied to a well-defined set of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from the University Hospital Basel evaluating discriminatory power and feasibility in a diagnostic routine setting.
Methods: By use of the DL system, we analyzed a total of 109 MRSA isolates which had been previously characterized by PFGE, spa typing, and spa clonal complex analysis. To get significant results, only non-repetitive isolates differing by at least one band in the PFGE pattern were selected. The DL method was performed according to the manufacturer's guidelines including DNA extraction, PCR amplification and capillary electrophoresis using micro-fluidic chips. The definition for DL clustering comprised >95% similarity or a fingerprint pattern with one band difference. The results of DL, PFGE, spa typing and spa clonal complex analysis were compared and analyzed using the DL on-line software tool as well as the GelCompar software.
Results: DL typing separated the 109 MRSA isolates in 12 distinct clusters and 6 singleton patterns. The 5 largest DL clusters represented 91 (83%) strains. spa typing differentiated the collection into 49 spa types. Clustering analysis into spa clonal complexes resulted in 3 clusters: spa-CC 067/548 including spa types t001, t002, t041, t067, t548 (n = 29); spa-CC 008 including t008, t024, t051, t064, t121, t400 (n = 28); and spa-CC 012 including t012, t018, t030, t037, t074 (n = 9). spa-CC 008 and spa-CC012 share 2 DL clusters, while spa-CC 067/548 strains mainly clustered in 2 distinct DL patterns. DL typing grouped the strains similarly as spa typing and spa clonal complex analysis, but PFGE had the highest resolution.
Conclusion: The DL method is rapid and easy to perform in contrast to spa typing and PFGE. However, the DNA extraction step is rather labor-intensive and might be replaced by a shorter, preferentially automated technique. DL typing shows good correlation with spa typing and spa clonal clustering, while PFGE remains the most discriminatory technique.

P1709 **The impact of strain typing method on assessing the relationship of paired nares and wound isolates of methicillin-resistant *Staphylococcus aureus***

J. Clarridge, A. Harrington, M. Roberts, K. Maquelin (Seattle, US; Rotterdam, NL)*

Objectives: Although the anterior nares is the site of choice for the Veterans Administration methicillin-resistant *Staphylococcus aureus* (MRSA) surveillance program, a close correlation between nares colonization and wound infections has not been established. The purpose of this study is two-fold: to determine the relatedness of paired wound and nares specimens from 40 patients by four different methods of strain typing and to compare the methods.
Methods: MRSA from wounds and nares were identified by routine methods. Specimens categorized as wound included abscess aspirates, joint fluids, pleural fluids and superficial and deep wound swabs. Nares isolates were paired with wound isolates if the nares isolate was obtained within 48 hours of the wound isolate. Eighty strains from 40 patients were studied. Isolates were typed by rep-PCR using the DiversiLab System (DL) (bioMérieux, Durham, NC), pulsed-field gel electrophoresis and the SpectraCell Raman typing (SCR) system (River Diagnostics). Additionally an antibiotic profile on each strain was obtained, thus four major measures were available for strain typing.
Results: PFGE analysis divided the 80 isolates into 5, SCR into 12 groups and DL into 9 different groups. Overall by combining the four measures the 80 strains could be divided into 19 unique groups. Comparing only the pairs of wound and nasal MRSA strains, 27 (67.5%) were grouped together by all the methods used, indicating that the isolates from both sites were the same. Four (10%) pairs of strains differed by all methods and represent different strains at the two different sites. The remaining 9 pairs (23%) differed by only one method: the outliers were: SCR, 7; DL, 1; antibiotic profile, 1.
Conclusions: In this study the four typing methods agreed about the pairing for 77.5% of the MRSA isolate pairs: 67.5% were indistinguishable and 10% differed. The remaining 22.5% of the pairs were indistinguishable with 3 of 4 typing methods. However, a problem in establishing an exact or causal linkage between the stains from individual patients and not just an epidemiological association is that by PFGE and DL about 70% of strains fall into only 2 large groups. It is hoped that a more discriminatory method such as SCR if robust might fill such a role.

P1710 Comparison of high-resolution melting curves analysis of the short-sequence repeat region of the protein A, spa sequencing and pulsed-field gel electrophoresis for the investigation of an outbreak due to MRSA

J.I. Stuart, R.C. Reyes, A. Stuart, M. McGavin, P. Ainsworth, M.A. John, Z. Hussain (London/ON, CA)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infection world-wide. In an outbreak situation, rapid identification and typing is an important epidemiological tool in determining clonal relatedness. Several molecular techniques are available for differentiating *S. aureus* but no method is superior under all conditions. Pulse field gel electrophoresis (PFGE) has been widely used and is the gold standard. More recently sequencing of the polymorphic X or short sequence repeat region of the protein a gene (spa) has been used as an alternative to current techniques. We here describe the comparison of spa sequencing, PFGE and a method using high resolution melting curve analysis (HRM) of the polymorphic X region in the investigation of an outbreak due to MRSA.
Methods: Nineteen outbreak-related MRSA strains were typed by spa sequencing, PFGE and HRM. The DNA sequences of the spa repeat region in both directions were imported as ABI files and analyzed by automated spa typing (tools.egenomics.com), using Kreiswirth nomenclature. PFGE was performed using standard techniques. MRSA types (CMRSA) were defined using Canadian MRSA PFGE typing guidelines. Amplicons of the spa region X were analyzed by HRM utilizing a difference graph format. *S. aureus* ATTC 43300 was included with each for standardization of curves.
Results: Strains from seventeen patients were identical by all three methods, corresponding to spa type 2 and CMRSA 2 PFGE type. One strain was closely related to the 17 strains by spa typing. It was spa-type 407. The only difference is the 6th cassette, which is G1 in spa-type 407 and D1 in spa-type 2. The D1 and G1 repeats differ by 2 nucleotides. PGFE and HRM failed to differentiate this strain from outbreak strains. One strains classified as CMRSA 9 and Spa-type 1 was unrelated to other strains, and was also deemed as unrelated by HRM analysis.
Conclusions: There was an excellent correlation clustering of strains by HRM compared to PFGE. The spa sequencing was most discriminatory, because it identified a strain, which was different though closely related to outbreak-causing strains. PFGE and HRM failed to discern this difference. HRM potentially represents a quick and inexpensive screening tool to rule out similarity of strains.

P1711 New typing assay based on PCR amplification of IS257-ileS2 junctions for tracking high-level mupirocin resistance dissemination in staphylococci

E. Pérez Roth, J. Alcoba Florez, B. Rivero Pérez, S. Méndez Álvarez (Santa Cruz de Tenerife, ES)*

Objectives: Transfer of ileS2-carrying plasmids plays a critical role in the dispersion of mupirocin resistance. Here we have investigated the presence, location, and orientation of insertion sequence IS257 flanking the ileS2 gene and we have evaluated the utility of PCR amplification of IS257-ileS2 junctions for tracking high-level mupirocin resistance.
Methods: Forty-eight MRSA clinical isolates each carrying one of nine well characterized ileS2-carrying plasmids encoding high-level mupirocin resistance belonging to four structural groups was included. Specific primers were designed and used for the amplification of the upstream and downstream IS257-ileS2 junctions. IS257-ileS2 junctions confirmation was carried out by Southern blotting and hybridization with two probes specific for the ileS2 and an IS257 internal probe. Filter mating and curing experiments, as well as hybridization with specific DNA probes were performed to investigate the antibiotic resistance gene content of the plasmids.
Results: Using the same amplification conditions four single PCRs were performed for each *S. aureus* clinical isolate harbouring an ileS2-encoding plasmid to detect the existence and orientation of IS257 copies flanking the ileS2 gene. In all nine plasmids the ileS2 gene was found to be flanked by IS257 copies. Considering upstream and downstream regions, plasmids belonging to different groups have distinc IS257-ileS2 organizations. Isolates carrying plasmids of the same group showed an identical organization of the IS257-ileS2 spacers and thus the same amplification patterns. As in the case of pGO400, pUSA03, and pV030–8 ileS2-encoding plasmids, S1 and S2 plasmids showed directly repeated copies of IS257 but upstream amplification patterns differed in size. S3 group plasmids have inverted copies of IS257, while in S4 plasmids, both IS257 copies were in opposite orientation to ileS2. Interestingly, IS257-ileS2 amplification patterns correlate with the ileS2-hybiridizaton polymorphs. However, the antibiotic resistance gene content vary for plasmids of the same group.
Conclusion: Our study highlights the value of IS257-ileS2 junctions heterogeneity for distinguishing ileS2-carrying plasmids through PCR amplification. This novel assay offers a rapid, simple, and feasible method for typing ileS2-carrying plasmids, and hopefully it could serve as a useful tool for clinicians and epidemiologists in their efforts to prevent and control high-level mupirocin resistance.

P1712 Molecular characterization of sporadically-occurring nosocomial methicillin-resistant *Staphylococcus aureus* isolates from Ireland

P. Kinnevey, A. Shore, A. Rossney, D. Coleman (Dublin, IE)*

Objective: Ireland has a high prevalence of MRSA and the predominant strains from Irish hospitals since the 1970s have been well characterised. However, there are no published data on sporadically-occurring MRSA isolates from Ireland. This study aimed to undertake detailed molecular characterisation of representatives of such sporadically-occurring MRSA.
Methods: Forty-seven MRSA isolates recovered from patients in Irish hospitals between 2000 and 2006 with unfamiliar antibiogram-resistogram or pulsed-field gel electrophoresis typing patterns underwent spa and SCCmec typing. Representatives of each spa and SCCmec type combination underwent multilocus-sequence typing (MLST).
Results: The 47 MRSA isolates yielded 21 spa types; the most prevalent of which were t032 (10/47, 21%), t190 (5/47, 11%), t242 (5/47, 11%) and t008 (4/47, 9%). Thirty-one isolates (66%) harboured SCCmec II, III or IV, or previously described subtypes, while 26% (12/47) yielded additional SCCmec amplimers indicative of novel SCCmec I, II, IV and V subtypes. Four isolates (9%) harboured potentially novel SCCmec elements and failed to yield ccr and/or mec amplimers. The 33 spa and SCCmec type combinations identified belonged to 22 sequence types (STs), with ST8 predominating (8/33, 24%). Four STs were represented by more than one spa type: (a) ST8; t190, t064, t1209 and t008 (b) ST22; t032, t022 and t2951 (c) ST36; t012 and t018 (d) ST5; t109 and t242. Conversely, six spa types were represented by more than one ST; in three instances the STs belonged to the same clonal complex (CC) (a) t008; CC8 (ST8, ST983, ST985 and ST1337), (b) t242; CC5 (ST5 and ST1435), (c) t127; CC1 (ST1115 and ST1336) but in three instances the STs belonged to different CCs (a) t002; CC6 (ST979) and CC5 (ST930 and ST100) (b) t032; CC616 (ST984) and CC22 (ST22) (c) t018; CC30 (ST36) and CC731 (ST982). spa, SCCmec and MLST typing yielded 36 type combinations, belonging to 12 CCs, among the 47 isolates, with CC8 predominating (28%; 13/47), followed by CC22 (11/47, 23%), CC5 (10/47, 21%), CC1 (3/47, 6%), CC30 (2/47, 4%) and CC45 (2/47, 4%). Six CCs (CC731, CC6, CC862, CC779, CC361 and CC616) were represented by single isolates.
Conclusions: This study revealed extensive genetic diversity among Irish 'sporadic' nosocomial MRSA. The considerable variability within SCCmec of these isolates further highlights the diversity of SCCmec and represents a significant gene pool for recombination events with other SCCmec elements.

P1713 Raman spectroscopy to determine endogenous or exogenous *Staphylococcus aureus* surgical-site infection of dermatology patients

R. te Witt, E. Hopman, K. Munte, D. Willemse-Erix, G. Puppels, K. Maquelin, W. van Leeuwen, A. van Belkum (Rotterdam, NL)*

Background: Surgical site infections (SSI) are one of the most common hospital-acquired infections, frequently caused by endogenous *Staphylococcus aureus*. At the department of Dermatology a study has been performed, to investigate whether nasal and wound screening for *S. aureus* during dermatological surgery could predict SSI.
Methods: A prospective pilot study was conducted involving 106 patients with 133 lesions between March 1 2009 and July 10 2009. Intra-operative and postoperative, culture swabs were taken from dermatological surgery wounds. Moreover, nasal swabs of patients and staff were examined to determine the carrier state for *S. aureus*. Bacterial isolates were characterized in triplicate using Raman spectroscopy to discriminate endogenous from exogenous infection.
Results: Sixteen percent of the patients were persistent carrier of *S. aureus*. A SSI occurred in 14% of the persistent carriers, compared to 3.5% in the non-persistent carriers, which was not statistically significant ($p = 0.09$). All SSI's of the persistent carriers were shown to be endogenous infections, i.e. strain type from nose matched that of the lesion. For the non-persistent carriers, all infections were proven to be exogenous. The isolate obtained from 1 patient matched that found in a colonized staff member.
Discussion: Nasal carriers of *S. aureus* may have an elevated risk for developing SSI in a dermatologic setting. However, due to a low incidence rate of infections in dermatologic surgery, peri-operative screening for *S. aureus* carriage might not be useful. Raman spectroscopy proved to be a rapid and reproducible typing technique to discriminate endogenous from exogenous infection.

P1714 Characteristics of Panton-Valentine leukocidin positive *Staphylococcus aureus* strains isolated from abscessing pneumonias in the Czech Republic

I. Machova, J. Kahankova, M. Fridrichova, L. Kuntová, M. Dvoráková, J. Doskar, H. Zemlickova, R. Pantucek, P. Petras (Prague, Brno, CZ)*

Objectives: Description of phenotypic and genotypic characteristics of Panton – Valentine leukocidin (PVL) positive strains isolated from eight abscessing pneumonias in the Czech Republic between December 2007 and May 2009. Short case reports are described too. Five of them were lethal.
Methods: Carriage of the lukS-PV and lukF-PV genes for PVL, and mecA gene was tested by a multiplex PCR assay. The phenotypic and genotypic methods were used for detection of superantigens (enterotoxin A – J, TSST-1, and exfoliatins A and B), and antibiotic resistance to 12 antibiotics by microdilution method. The PVL positive MRSA strains were characterized by PFGE, spa typing, SCCmec typing, arcA gene detection, and agr typing.
Results: Four PVL positive strains from the collection of 8 strains were MRSA. Those strains were characterized by molecular methods and correlated with the known European MRSA types. Three isolates were classified into SCCmec type IV, which is ordinarily found in community acquired methicillin resistant *S. aureus* strains (CA-MRSA). One strain was composite type IV + V. The last 4 strains were MSSA.
Conclusions: Abscessing pneumonias in Czech Republic are caused by CA-MRSA and by methicillin sensitive *S. aureus* producing PVL. Early detection of PVL production is very important for treating patients. All three survived patients have been treated by linezolid.
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Molecular typing: other bacteria

P1715 Ages-Webribo – a new deterministic identification algorithm for the characterization of *Clostridium difficile* isolates by capillary gel electrophoresis-based PCR-ribotyping

A. Indra, M. Blaschitz, M. Schneewe, S. Huhulescu, S. Kernbichler, A. Fiedler, F. Allerberger (Vienna, AT)*

Objectives: *Clostridium difficile* is a frequently identified cause of hospital-acquired infections. Identification of newly emerging hypervirulent *C. difficile* strains – like 027, 078 and 053 is mostly done by PCR-ribotyping. Although ribotyping is widely used the method lacks an interlaboratory interchangeable format. In order to overcome capillary-sequencer-dependent result variations of different types of sequencing machines a newly developed deterministic identification algorithm – AGES-WEBRIBO (webribo.ages.at) – was applied for capillary gel electrophoresis based *Clostridium difficile*-PCR ribotyping.
Methods: 2600 isolates collected between the years 2006 to 2009 at Austrian healthcare facilities were primarily analysed with an Applied Biosystems (ABI) 310 Genetic analyzer yielding 303 different PCR-ribotypes. 65 of the 303 ribotypes were randomly chosen and retested with on an ABI 3130 Genetic Analyzer. Quality control of data gained was done using the AGES-WEBRIBO internet platform.
Results: There was no increase in discrimatory power using different sequencer machines, although there was better narrow double-peak resolution with the ABI-3130 system. Three (4.5%) of the 65 on the ABI-3130 sequencer examined samples showed no correct identification after analysing the data with AGES-WEBRIBO.
Conclusion: 95.5% correct identifications of *C. difficile* ribotypes were achieved between different ABI-Sequencer models with the newly developed deterministic-data-analysis approach (AGES-WEBRIBO). This result clearly shows that every user is able to determine even rare PCR-ribotypes without a respective reference strain at hand. Further tests will show the ability of AGES-WEBRIBO (webribo.ages.at) to correctly identify data gained with capilliary sequencer models from other suppliers than ABI. We consider the updated version of AGES-WEBRIBO in combination with capillary gel electrophoresis based PCR ribotyping to be a universal tool for inter-laboratory comparability of *C. difficile* ribotyping.

P1716 Epidemiological study of *Clostridium difficile* isolates in a French university hospital, during a 4 year-period from 2006 to 2009

I. Poilane, C. Rousseau, P. Cruaud, A. Collignon (Bondy, FR)*

Objectives: Recently, the epidemiology of *Clostridium difficile* infections have changed with the emergence of a hypervirulent strain PCR-ribotype 027. After Canada and the North of Europe, an outbreak due to this strain was described in France in 2006. The aim of this study was to perform a phenotypic and molecular characterization of *C. difficile* strains in order to follow the epidemiology of *C. difficile* infections from 2006 to 2009 in Jean Verdier-René Muret hospitals.
Methods: From 2006 to 2009, 95 strains isolated from symptomatic patients were studied. For the diagnostic of *C. difficile* infections, a two step algorithm was performed: toxin A and B were detected on stools with an immunoenzymatic method (ICTAB, Meridian) and a toxigenic culture was performed with detection of toxins A and B on colonies with the same immunoenzymatic method. The agar diffusion method was performed for screening antibiotic resistance to metronidazole, vancomycin, erythromycin and moxifloxacin. The E-test was performed for determination of metronidazole and vancomycin MICs.
For molecular characterization, PCR-ribotyping was performed according to Bidet et al. In addition, binary toxin genes cdtA and cdtB were detected by PCR.
Results: From 2006 to 2009, all the isolates studied were susceptible to vancomycin and metronidazole with no significant increase of MICs. The susceptibility to erythromycin and moxifloxacin increases from 2007

to 2009 respectively 14% to 100% for erythromycin and 28.5% to 100% for moxifloxacin. In 2007, 8% of the isolates were positive for cdtA and cdtB genes and 11.7% in 2008. PCR-ribotype JV18 was the most prevalent PCR-ribotype in 2007 and 2008 but disappeared in 2009. This PCR-ribotype corresponds to the PCR-ribotype 001 according to Stubbs et al. The isolates displaying this PCR-ribotype were resistant to erythromycin and moxifloxacin and were principally isolated in the same ward, suggesting a cross infection.
Conclusion: This study showed that the susceptibility to metronidazole and vancomycin remained stable on a 4 years period. Different clones of *C. difficile* circulated with emergence of an epidemic strain resistant to erythromycin and moxifloxacin displaying the PCR ribotype 001. This isolate emerged in the gastroenterology unit where fluoroquinolones are frequently used demonstrating the role of antibiotic selection pressure. During the period of the study, no isolate with a PCR-ribotype 027 was detected.

P1717 Comparison of PCR ribotyping and an optimized random amplification of polymorphic DNA protocols for genotyping of *Clostridium difficile* produces profiles similar in discriminatory power and reproducibility

L.M. Green, T. Worthington, A.C. Hilton, P.A. Lambert (Birmingham, UK)*

Objectives: To determine if a newly optimised random amplification of polymorphic DNA (RAPD) PCR protocol provides comparable discriminatory power and reproducibility to PCR ribotyping for genotyping isolates of *Clostridium difficile*.
Methods: An optimised RAPD PCR protocol was used to type a panel of eleven known control PCR ribotypes incuding epidemic UK strains. DNA preparations of all eleven isolates were prepared at different times in order to determine the reproducibility of the method. PCR ribotyping was also performed on isolates in order to obtain amplification profiles for the known ribotypes.
RAPD PCR was performed using two 10 base pair primers in independent reactions performed in 25µl reaction volumes. The amplification cycles were as follows: 5 cycles of 4.5 minutes at 94ºC, 30 seconds at 94ºC, 2 minutes at 20ºC and 1 minute at 72ºC; this was followed by 30 cycles of 5 seconds at 94ºC, 30 seconds at 36ºC and 1 minute at 72ºC. Following amplification, PCR products were size separated on a 2% agarose gel in 1 x TAE buffer (40mM Tris-HCl, 1mM EDTA, 0.1% (v/v) glacial acetic acid, pH 8). Following electrophoresis, amplicons were detected by Ethidium Bromide staining and viewed under UV light. PCR Ribotyping was performed as previously described by Stubbs et al., (1999) with modifications. Following characterisation, Gelcompar II (Applied Maths, Belgium) was used to analyse the discriminatory capacity of both methods and also determine the reproducibility of profiles produced by this RAPD protocol.
Results: Eleven distinct profiles were produced from the eleven ribotypes investigated; both for PCR ribotyping technique and RAPD protocols. PCR ribotyping produced profiles that were grouped into four distinct clones at a 70% similarity level when analysed by Gelcompar; similar results were produced in the RAPD reactions. RAPD amplification profiles for each ribotype were reproducible when repeated in independent reactions at different times; these profiles displayed 100% similarity when analysed with Gelcompar.
Conclusion: The RAPD protocol described here produces amplification profiles that are equivalent in reproducibility and discriminatory power to the widely used PCR ribotyping. RAPD is considerably quicker than PCR ribotyping protocols and profiles produced uncomplicated and easy to compare and analyse. RAPD genotyping of *C. difficile* as described in this study may provide a rapid, clinically relevant, easily adopted characterisation method for local epidemiological mapping of *C. difficile*.

P1718 Genotypic characterization of *Clostridium difficile* strains isolated from patients with *C. difficile* infection in a tertiary hospital in Spain

M. Marin, A. Martin, L. Alcala, R. Insa, E. Cercenado, M. Sanchez-Somolinos, E. Bouza (Madrid, ES)*

Objectives: *Clostridium difficile* (CD) is the main aetiological agent of antibiotic-associated diarrhoea and a common nosocomial pathogen. In recent years, epidemic strains belonging to the 027 and 078 ribotypes have emerged (both binary toxin+). Genotypic characterization of isolates is essential for recognition of these strains and complements epidemiological investigations. Data on the genotypic characteristics of CD strains circulating in Spain are scarce. We genotyped CD strains circulating in our hospital and isolated from patients with CD infection (CDI) during the year 2007.
Methods: Only strains from patients with a new episode of CDI at least 60 days after a previous episode were considered for study. Strains were cultured and identified by conventional microbiological methods. DNA was obtained from pure cultures using Chelex resin (Instagene matrix, BioRad). The tcdA gene (toxin A), tcdB gene (toxin B), and binary-toxin genes cdtA and cdtB were detected by multiplex PCR (Persson, 2008). Isolates were characterized by PCR-ribotyping (Stubbs, 1999). Phylogenetic analysis of ribotyping profiles was conducted using Bionumerics software 5.0. tcdC gene PCR and sequencing were performed only for clustered strains as previously described (Rupnik, 1998 and Spigaglia, 2002).
Results: Seven hundred and fifty-six CD strains were isolated during 2007. Non-toxigenic CD isolates (96 strains) and those belonging to the same episode (212 strains) were excluded from the study. Overall, 448 CD strains from 435 patients with CD-associated diarrhoea were studied. Two toxigenic profiles were detected: tox A+B+bin– (386 isolates, 86.2%, 45 ribotypes) and tox A+B+bin+ (62 strains, 13.8%; 7 ribotypes). CD strains were clustered in 22 different ribotypes (418 strains, 93.3%) and 30 ribotypes were non-shared. The most frequent ribotypes were 001 (222 strains, 49.5%) and 014 (75 strains, 16.7%). Ribotype 078 was the third most frequent (48 strains, 10.7%). Only 6 isolates (2 patients) belonged to ribotype 027. Analysis of the tcdC gene revealed deletions of 18 bp to 54 bp in 62 strains. All but 2 strains were tox A+B+bin+.
Conclusions: Most of our toxigenic CD isolates were distributed in two ribotypes. Ribotype 078, which is a frequent cause of diarrhoea in our patients, accounted for 10% of toxigenic CD isolated at our institution during 2007. Epidemic strains of ribotype 027 were detected in only 2 patients. All the bin+ CD isolates had deletions in the tcdC gene.

P1719 Molecular toxin genotyping of *Clostridium perfringens* animal isolates by multiplex PCR

M.C. Ossiprandi, L. Zerbini (Parma, IT)*

Objectives: *Clostridium perfringens* is common in the environment and in the intestinal tracts of humans and domestic and wild animals. This microorganism is perhaps one of the most widespread pathogenic bacteria and it is undeniably the most important cause of clostridial disease in animals. The species is classified into five types (A to E), based on the possession of one or more of four major toxin genes (α, β, ε, and ι). However, *C. perfringens* can produce up to 15 toxins in various combinations, including lethal toxins such as perfringolysin O, enterotoxin, and β2 toxin. In this study we evaluated the molecular toxin gene profile of *C. perfringens* animal strains using a multiplex PCR.
Methods: Seventy-eight *C. perfringens* strains were isolated from biological samples of different animal origin (32 rabbits, 20 calves, 14 dogs, 10 pigs, and 2 broilers). All animals (except for 8 healthy dogs) had presented gastrointestinal tract disease.
Presumptive identification of *C. perfringens* was made based on colony morphology (circular-to stellate and smooth), the presence of a distinctive double zone of haemolysis and by Gram staining (Gram-positive short, plump rods with blunt ends, “box cars”). The species identification was confirmed through the Rapid ID32A assay

(bioMérieux). All *C. perfringens* isolates were multiplex PCR-screened for the detection of alfa, β, β2, ε, ι and enterotoxin (CPA, CPB, CPB2, ETX, IAP, and CPE) toxin-encoding genes, as previously described by Baums et al. (2004).
Results: Seventy-two *C. perfringens* isolates (72/78: 92.3%) were type A (CPA gene-positive). Seventeen type A strains (17/72: 23.6%) also possessed the CPB2 toxin-encoding gene. Three strains (3.85%) were type C (CPA and CPB genes-positive) and all possessed CPB2 gene. Finally, 3 isolates (3.85%) were type D (CPA and ETX genes-positive): 2 also possessed CPB2 gene, and 1 CPE gene. Of all the 78 *C. perfringens* isolates, 22 (28.2%) were PCR positive for CPB2 gene.
Conclusion: The results of this study highlight that the majority of our *C. perfringens* isolates were type A and a relatively high percentage of strains carried the CPB2 gene, that varies with the genotype, but that was detected especially in type A in our tested samples.
We can conclude that multiplex PCR may provide a useful and reliable tool for *C. perfringens* genotyping in routine veterinary diagnostics.

P1720 Genomic identification of pathogenic and non-pathogenic Leptospires by multilocus variable-number tandem-repeat analysis

P. Khaki, M. Abdollahpour Alittapeh, S. Moradi Bidhendi, M. Esmaelizad, K. Tadayon (Karaj, IR)*

Background: Leptospirosis is an emerging zoonotic diseases which caused by pathogenic Leptospires. Detection and identification of Leptospires has conventionally been performed by cultural and serological methods. These methods are tedious, time consuming, and potentially biohazardous. Recently several PCR based techniques such as VNTR have been demonstrated to provide useful tools in detection and identification of leptospiral serovars but most these techniques have drawbacks. VNTR can also provide information relating to both the evolutionary and functional areas of bacterial diversity.
Objective: The present study was carried out to set up of VNTR technique for the genotyping of pathogenic and nonpathogenic Leptospiral serovars.
Methods: The pathogenic and non-pathogenic reference serovars of *Leptospira* spp. were obtained from the Microbial Culture Collection at the National Reference Laboratory for *Leptospira* at the Microbiology Department of Razi Vaccine & Serum Research Institute, karaj, Iran. Leptospiral strains were subcultured into the liquid EMJH medium and incubated at 28 for 7 days.
Genomic DNA of Leptospiral serovars were extracted using the phenol-chloroform method. PCR was performed with the three selected VNTR loci (VNTR4, 10, 36). The amplified products were analyzed by agarose gel electrophoresis. The sizes of the amplified products were estimated by comparison with a 100-bp ladder.
Result: All loci successfully amplified in all pathogenic leptospiral serovars with the primers. Although VNTR-4 was unable to discriminate between Grippotyphosa and Hardjo, Icterohemoragaea and Pomona, this locus was thoroughly differentiated between pathogenic and non-pathogenic. VNTR-10 had an appropriate discrimination to differentiate among these strains but was unable to differentiate between pomana and semaranga. VNTR-36 successfully amplified in all of the strains. Although This locus aptly separated serovar pomona from other strains, but could not separated Grippotyphosa and semaranga.
Conclusion: The sizes of the amplified products displayed a wide range of polymorphisms, suggesting variation tandem-repeat copy numbers in the VNTR loci. The combination of the VNTR-4, VNTR-10, and VNTR-36 loci was useful for typing *L. interrogans*. Although VNTR-4 was poorly discriminatory, was the only marker for separation between pathogenic and non-pathogenic. The VNTR method provides rapid typing as well as a highly discriminant assay to identify *L. interrogans* serovars.

P1721 Molecular epidemiology of *Brucella melitensis* strains isolates from human by MLVA-16 (Multiple locus VNTR analysis-16) in Spain

S. González-Cabrero, F. Menegotto, A. Cubero, M.P. Gutiérrez Rodríguez, A. Dueñas, C. Garcia Loygorri, M.A. Bratos, A. Orduña (Valladolid, ES)*

Introduction: Brucellosis remains the commonest anthropozoonosis worldwide and it was a very important health problem in Spain during the past century. Even now the brucellosis still persists in some regions of Spain with a human incidence higher as 3/100.000, where *B. melitensis* causes more than 99% of the human cases. In these areas, molecular epidemiology could be a very useful tool to know the origin and evolution of *Brucella* epidemic.
Objective: The aim of this study is to know the genetic variability of 172 *Brucella melitensis* strains isolates from humans from 1975 till 2008 and its importance in the evolution of the epidemic outbreaks.
Material and Methods: The genomic profile of 172 strains of *Brucella melitensis* was determined by Multiple Locus VNTR Analysis-16 (MLVA-16). The repeated regions with a variable number of 8 minisatellite markers (more preserved), or 8 microsatellite markers (with more discriminative power) were amplified by following the protocol previously by Al-Dahouk et al. The analysis of gels was performed by InfoQuestFP software (BioRad).
Every *Brucella* isolates was identified using biochemistry tests, specific antiserum anti-A or anti-M and a multiplex-PCR.
Results: The 48.2% of studied strains belonged to *B. melitensis* biovar 1, 46% to *B. melitensis* biovar 2 and the 5.8% to *B. melitensis* biovar 3. Using the microsatellite MLVA analysis we found 8 different genotypes, and the locus bruce11 was the most homogeneous. Using the complete MLVA-16 analysis (mini and microsatellite), the 172 human isolates clustered in 140 different genotypes, and the locus bruce04 was the most heterogeneous.
The 172 *Brucella* strains corresponding to the period 1976–2008 are clustered in 8 different genotypes by the microsatellite analysis, and two of these (genotypes 42 and 60) were the most prevalent with a homology of 50% among them. The genotype 42 is represented by *B. melitensis* biovar 2 and the genotype 60 is represented by *B. melitensis* biovar 1. We may conclude that during this period appeared two major epidemic outbreaks, one of them caused by genotype 42 and the second by genotype 60. On the other hand, the genotypes 36 and 55 were represented by a single strain, and we consider that both cases were sporadic. In the present work we discuss the geographical distribution of the *Brucella* genotypes and the evolution of the *Brucella* epidemic in Spain during 1976–2008.

P1722 Dissemination of limited imipenem-resistant *Acinetobacter baumannii* clones in Asian countries

K.S. Ko, H.W. Kim, J.Y. Choi, J.H. Song on behalf of ANSORP*

Objectives: Current ANSORP (Asian Network for Surviellance of Resistant Pathogens) study on hospital-acquired pneumonia (HAP) shows that *Acinetobacter* spp. is the most frequently isolated pathogens in Asian countries. Moreover, it is quite problematic due to high imipenem resistance rate (69.9%) of *Acinetobacter* spp. isolates in the ANSORP study. We investigated the genetic relatedness of imipenem-resistant *A. baumannii* isolates, which caused HAP in adult patients in 8 Asian countries from 2008 to 2009.
Methods: Species identification was performed for 189 *Acinetobacter* spp. isolates by rpoB gene sequence analysis. Genetic relatedness among 123 imipenem-resistant *A. baumannii* isolates was investigated by method of multilocus sequence typing (MLST). Genes encoding OXA carbapenemases and the other antibiotic resistance genes such as blaPER, blaIMP, and blaVIM were detected by PCR.
Results: As a result of species identification using rpoB gene sequences, *A. baumannii* was the most common species (85.4%), followed by

Acinetobacter genomospecies 13TU (6.9%). All but one imipenem-resistant isolates belonged to *A. baumannii*. MLST showed that CC (clonal complex) 22, which includes six sequence types (STs), was the most prevailing (59.0%) and disseminates in several Asian countries such as Korea, Malaysia, Hong Kong, India, Taiwan, and Thailand. The second most common was CC43 (18.0%), which was found in two Asian countries such as India and Malaysia. Most of imipenem-resistant *A. baumannii* isolates belonging to CC22 contained both oxacillinase genes such as blaOXA-23-like and blaOXA-51-like genes and blaPER-1.
Conclusion: Only a few closely related clones of imipenem-resistant *A. baumannii* disseminate in Asian countries. Especially, more detailed investigation on CC22, a clone distributing worldwide, is required.

P1723 Multilocus variable-number tandem-repeat analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium in the Netherlands

M.E. Heck, M. van Luit, K. van der Zwaluw (Bilthoven, NL)*

Objective: *Salmonella enterica* subsp. *enterica* serovar Typhimurium (STm) is a pathogen frequently responsible for outbreaks of gastoenteritis. To elucidate outbreaks an epidemiological subdivision of this serovar is indispensable. Phage-typing and pulsed field gel electrophoresis (PFGE) are among the methods most frequently applied. Both have been used successfully but have the disadvantage that reading the typing results can be subjective and difficult to standardize. Reliable typing results are critical to both detecting and determining the source of an outbreak. Multilocus Variable-Number Tandem-Repeats Analysis (MLVA) is a PCR method based on the amplification and fragment analysis of five repeat loci. Advantages of MLVA are: it is fast, easy to perform, less labour intensive and yield unambiguous typing data. In this publication we want to demonstrate that MLVA is an alternative to type STm.
Methods: For this study we typed 2800 S.Tm isolates which were sent to our lab for serotyping from, 1st Jan. 2006 up to 1st Oct. 2009. All strains were characterised by phage typing and MLVA typing. To test technical skills our lab participated in a MLVA ring trial. MLVA data were stored in a Bionumerics data base. Simpson's diversity indices (DI) were calculated to determine the discriminatory power of both methods. An overall comparison was made between the two typing methods.
Results: All isolates were typeble with MLVA whereas phage typing was unable to type 6% of the strains and yielded 15% atypical strains. The DI's of MLVA- and phage typing were 0.87 and 0.98 respectively.
We found that dubious phage types, which account for a substantial portion of the strains, could be sub-divided by MLVA and MLVA consequently provided a significant understanding of these phage types. The concordance between the methods was good in known outbreaks but as anticipated did not establish a 100% match. MLVA revealed that some outbreaks have not been detected in the past.
Conclusions: MLVA turned out to have a discriminatory power that surpasses phage typing and yields clearly defined data that can be stored and exchanged easily. MLVA can sub-divide 'dubious and meaningless' phagetypes significantly.
Because MLVA is fast, easy to perform and less labour intensive we strongly believe that MLVA typing is an alternative and consequently a step forward in typing *Salmonella enterica* subsp. *enterica* serovar Typhimurium.
A future target is to establish international accessible data base.

P1724 Typing of plasmids of multi-resistant *E. coli* producing or not extended-spectrum β-lactamases using two different assays: replicon typing and relaxase detection

B. Ruiz del Castillo, A. Alvarado García, M. Garcillán-Barcia, F. de la Cruz, L. Martínez-Martínez (Santander, ES)*

Background: Classification of epidemiologically-relevant plasmids is currently based in the detection of plasmid replication and partition regions, leading to their grouping in incompatibility (Inc) groups. Plasmids conferring antibiotic-resistance are mainly transmissible. They have been recently classified in six MOB families according to the evolutionary relationships or their relaxase, a key protein in the transfer process. Here we have accomplished the identification of plasmids in a multiresistant *E. coli* collection from clinical isolates. Two classification approaches have been used: the classical PCR-based replicon typing and MOB identification. Results allowed us to compare the efficiency and specificity of both methods and to depict the nature of the plasmids involved in the multiresistant phenotype of the isolates.
Methods and Results: Transconjugants (Tc) were obtained from 19/20 ESBL and 13/20 non-ESBL isolates. Plasmid DNA from donor and Tc were extracted and characterized by replicon typing and MOB identification using a set of degenerate primers specific for each relaxase family. In the multiresistant, non-ESBL collection, the most prevalent group was IncF (12/13), followed by IncI1 (7/13). Members of the groups IncB/O (3/13), K (1/13), A/C (3/13), and Pa (1/13) were also present. Relaxase detection confirmed these results: MOBF12 (12/13), MOBP12 (8/12), MOBH12 (3/12), MOBP11 (1/13). Cryptic mobilizable MOBQ plasmids without Inc group assigned and IncX plasmids, both missed by the replicon typing method, were also found by MOB amplification. In the ESBL collection, MOBP12 plasmids were the most abundant, being present in all isolates. They cluster members of IncI1 (7/19), IncK (14/19) and IncB/O (12/19) groups. MOBF12 plasmids were the second most prevalent (17/19), while 18/19 were found to belong to the F group (FIA, FIB, Frep) by replicon-typing. Also MOBP11 (IncPa plasmids), MOBQ and MOBC12 (both omitted in the replicon typing) were observed in 2/19, 3/19 and 3/19 isolates, respectively.
Conclusions: Our collection contains transmissible plasmids of different incompatibility groups. MOB detection and replicon typing are complementary methods for classifying plasmids in clinical isolates of *E. coli*. The first one is especially useful for classifying plasmids that harbour multiple replicons and those without Inc group assigned. The use of combined methods provides higher accuracy and more information about the plasmid backbone.

P1725 Serotypes, virulence genes, and PFGE patterns of shiga toxin-producing *Escherichia coli* isolated from Peruvian children

C. Contreras, J. Ruiz, F. Rivera, Y. Sáenz, N. Zavaleta, C. Lanata, R. Maves, C. Torres, T. Cleary, T. Ochoa (Lima, PE; Barcelona, ES; Logroño, ES; Houston, US)*

Objectives: The objectives of this study were to determine the distribution of virulence genes in Shiga toxin-producing *Escherichia coli* (STEC) strains and investigated the genetic relationships of these strains from humans by pulsed-field gel electrophoresis (PFGE).
Methods: We analyzed 18 STEC strains isolated from stool samples of children with and without diarrhoea from four previous cohort studies in Peruvian children. All studies were in the community setting, 485 children (0–36m) were followed in 1987 in Huaraz-Ancash; 313 (6–18m) in 2004 in Villa El Salvador-Lima; 1025 (0–12m) in 2007 in Chorrillos-Lima and 1250 (0–24m) in 2008 in Lima-north. The strains were analysed by PCR to identify the presence of stx1, stx2, eae, ehxA, and chuA genes and the genetic diversity study was carried out by PFGE. The serotypes were determined using polyvalent and/or monovalent antibodies.
Results: We have 18 STEC strains (1/485 strain-Huaraz, 4/313 strains-Villa El Salvador, 10/1025 strains-Chorrillos and 3/1250 strains-Lima-north). Among the isolated STEC strains, stx1 was the most common toxin-gene than stx2 (16/18 89% vs. 2/18 11%), and eae was present in 14/18 (78%). All eae genes were present only in stx1 STEC samples. The other virulence genes, chuA was found 75% (6/8) in DS, 43%(3/7) in controls and 33%(1/3) in ND; and exhA in 63%(5/8), 57%(4/7) and 100%(3/3) respectively. 60%(9/15) of the strains has a typeable serotype, the most frequent is O26:H11 (40%), others serotypes found were O1:H7, O125:H21, O6:H- y O-:H2. PFGE revealed 17 distinct restriction patterns, based on a difference of a single band as a basis for discrimination between isolates. In the dendrogram produced by the UPGMA algorithm, the isolates were clustered in 9 groups (I–IX;

1–5 strains/group) of 85% similarity according to the Dice similarity index. Isolates of the O26:H11, stx1, eae were grouped together in the dendrogram. No relation between the ehxA, chuA genes and characteristics as date of isolated and locality of isolated were found with the distribution in the different PFGE clusters.
Conclusion: The majority of the STEC strains in the community setting were stx1+ and eae+. The absence of haemolytic uremic syndrome may relate to the low frequency of stx2+ strains. The distribution of virulence genes exhA and chuA among diarrhea and control is similar. The STEC strains isolated in different decade share a genetic relationship indicating that the same clones are circulating in Peruvian population in the last 20 years.

P1726 The Diversilab system versus pulsed-field gel electrophoresis: characterization of extended-spectrum β-lactamase producing *Klebsiella pneumoniae*

A. Brolund, S. Haeggman, P.J. Edquist, L. Gezelius, B. Olsson-Liljequist, K. Tegmark Wisell, C.G. Giske (Stockholm, SE)*

Objectives: Nosocomial and community associated spread of ESBL-producing *Klebsiella pneumoniae* is of great concern. Fast and reliable epidemiological typing methods for rapid identification of outbreaks and epidemic strains are of value. For these purposes pulsed-field gel electrophoresis (PFGE) is considered the gold standard method. The main limitations of PFGE are that it is time-consuming and requires rigorous standardization and experienced personnel in order to achieve reproducible results. Hence, faster, simpler and more easily standardized tools are needed. The DiversiLab system (DL) has been proposed for these purposes. We here, to our knowledge, show the first systematic comparison of DL and PFGE on *K. pneumoniae*.
Methods: We compared DL to PFGE on a national collection of 48 *K. pneumoniae* producing extended spectrum β-lactamases, as defined by clavulanic acid-reversible resistance to oxyiminocephalosporins collected in 26 clinical laboratories in Sweden during February – April 2007. Different cut offs for the Dice coefficients were evaluated for PFGE; 90, 85 and 80%. The cut-off for DL was set at 97% identity in addition to electropherogram overlay analysis, where no peak differences were accepted for isolates to be considered identical.
Results: Simpson's index of diversity was higher for DL (88.6%) than for PFGE (85.6 and 83.9% for Dice 90/85 and 80, respectively). The directional information about the partition relations by Wallace coefficients demonstrated that the probability of two strains being classified as the same type by PFGE having the same DL type was 79.6% (95% CI: 57.6–100) to 70.3% (95% CI: 46.9–94.8) (Dice 90/85 and 80). Conversely the probability was 100% regardless of cut-off. Only four of 48 isolates had discordant results with the two methods.
Conclusion: Although the number of isolates investigated in this study was relatively low and the results therefore should be interpreted with some caution, DL and PFGE showed a high degree of concordance. However, if one considers PFGE the gold standard method for probable close epidemiological relatedness in outbreak investigations the apparent higher discriminatory power of DL could represent a challenge for laboratories using this method for primary screening of clonal relatedness.

P1727 Phenotypic and genotypic characterization of *Haemophilus influenzae* strains isolated from children with cystic fibrosis

R. Cardines, M. Giufrè, E. Fiscarelli, M. Accogli, M. Cerquetti (Rome, IT)*

Objectives: Patients suffering for the cystic fibrosis (CF) disease are prone to recurrent respiratory infections caused by a variety of bacterial pathogens including *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex. *H. influenzae* commonly infects the respiratory tract of CF patients early in childhood. The objective of the study was to characterise *H. influenzae* isolates from CF patients included in a 5-year follow-up study (2004–2009).
Methods: Fifty-eight *H. influenzae* strains were recovered from the respiratory secretions of 45 CF patients attending the CF center of the "Bambino Gesù" paediatric hospital in Rome. Eleven of the 45 patients contributed two or more *H. influenzae* isolates over time. The median age of the patients was 5 years (range 0.25–27 years). The capsular serotype was identified by PCR. MICs were determined by E-test. Production of β-lactamase was detected by the cefinase disk test. Genetic relatedness among isolates was assessed by PFGE. The presence of the hia and hmw adhesin encoding genes was detected by PCR and colony blotting techniques, respectively.
Results: Of the 58 strains, 52 (89.7%) were nonencapsulated or nontypeable (NTHi), 4 type e and 2 type a. The rate of β-lactamase producers was 13.8%; 3 strains showed the β-lactamase negative ampicillin-resistant phenotype. Heterogeneous resistance to imipenem was identified in 11 strains (18.9%). No strains were found resistant to cephalosporins and amoxicillin–clavulanic acid. Resistance to azithromycin and chloramphenenicol was 13.8% and 5.2%, respectively. No increase in antibiotic resistance was observed in strains serially isolated from the same patient. By PFGE, the 52 NTHi isolates yielded 47 different profiles but 11 minor clusters were identified, each including from 2 to 6 strains. Of 11 patients with sequential isolates, 4 harboured strains exhibiting identical PFGE profiles, while 7 patients possessed strains without close genetic relationships. Some NTHi isolates from different patients shared related genotypes, but 16 isolates were totally unrelated to each other. A minority of the NTHi isolates harboured the hia and hmw genes, the former being the most frequent (16/58, 27.6%).
Conclusions: Resistance to imipenem in *H. influenzae* has emerged in Italy. Persistence of an identical clone was observed in 36% of patients. No apparent association between antimicrobial resistance and persistence was found.

P1728 Multilocus sequence analysis of *Stenotrophomonas* spp. and delineation of *S. maltophilia*

Z. Vasileuskaya-Schulz, S. Kaiser, D. Jonas (Freiburg, DE)*

Objectives: During the last years, the genus *Stenotrophomonas* has been the focus of applied microbiology and biotechnology. Nevertheless, the genetic and phenotypic heterogeneity within the genus complicates the choice of representative strains for investigation, e.g. for comparative genomics of clinical versus environmental isolates. MLST of 70 *S. maltophilia* strains previously revealed genogroups with DNA-similarities below 95%, which exclusively comprised strains of environmental origin. To unravel the uneven distribution of environmental isolates within the MLST genogroups and to reveal genetic relationships within the genus we extended multilocus sequence typing to analysis of a further 21 *S. maltophilia*-isolates of environmental origin, *Stenotrophomonas* spp. and related genera.
Methods: In this research, 12 *Stenotrophomonas maltophilia* strains, *S. rhizophila, S. terrae, S. humi, S. nitritireducens, S. acidaminiphila, S. chelatiphaga, S. koreensis, Stenotrophomonas* sp. (DSM2408), *Pseudomonas* beteli and Pseudoxanthomonas dokdonensis were characterized by multilocus sequence analysis (MLSA) of seven housekeeping genes (atpD, gapA, guaA, mutM, ppsA and recA). The data from previous multilocus sequence typing of 70 *Stenotrophomonas* spp. were integrated into a larger MLSA.
Results: Our recent data demonstrate that the seven-gene MLSA provides good discrimination of all the investigated species on the intra- and interspecies levels. Based on this, some isolates, previously described as *S. maltophilia,* need to be re-examined and presumably reclassified as *S. rhizophila.* Moreover, the data provide further genetic evidence that *P. beteli* belongs to the genus *Stenotrophomonas* and Px. dokdonensis, described initially as *Stenotrophomonas* dokdonensis is a representative strain of a different genus only distantly related to stenotrophomonads.
Conclusions: Multilocus sequence analysis is a practical and robust approach for distinguishing *Stenotrophomonas* spp. at both the genus and species levels. This approach provides a reliable means for selection of isolates for comparative genomic investigations.

P1729 The prevalence of magA-positive *Klebsiella pneumoniae* strain in a Japanese medical centre

Y. Kohayagawa, A. Nakashima, H. Fukushima (Izumo, Matsue, JP)*

Objectives: *Klebsiella pneumoniae* is a Gram-negative pathogen that causes many infectious diseases, especially urinary tract infections and pneumonia. Primary pyogenic liver abscess caused by *K. pneumoniae*, sometimes complicated by endophthalmitis or central nervous system infection, has recently become an emerging disease in the world. MagA (mucoviscosity-associated gene A)-positive *K. pneumoniae* has been reported to be extremely virulent. We evaluated the prevalence of magA-positive *K. pneumoniae* strains collected in a 679-bed medical centre in Japan.
Methods: Culture, identification, and susceptibility testing of isolates were performed according to standard microbiological methods. We collected clinical *K. pneumoniae* strains from outpatients and hospitalized patients. Isolates were obtained by culture from all types of samples (blood, pus, bile, urine, sputum, etc.). *K. pneumoniae* strains were collected for 1 year (2008 to 2009) and stored at −30ºC before use. We performed bacterial capsular polysaccharide synthesis (cps) genotyping by polymerase chain reaction to detect magA. The medical records of all patients from whom we collected magA strains were scrutinized meticulously.
Results: Sixty-two strains of *K. pneumoniae* from 61 patients were collected. Of the 62 strains, 10 strains were magA positive. We judged that 6 strains from 5 patients (4 patients with pyogenic liver abscess and 1 patient with meningitis) were causal microbes. One patient of pyogenic liver abscess died and the meningitis patient experienced higher brain dysfunction. The other 4 strains were only colonization. Tests for the strains isolated from our patients indicated good susceptibility to antimicrobial drugs.
Conclusion: We found the prevalence of magA-positive *K. pneumoniae* at 16% was not rare. While magA-positive *K. pneumoniae* is extremely virulent, the existence of patients with colonization means there is the possibility of immunotherapy, i.e. vaccine or monoclonal antibody. MagA-positive *K. pneumoniae* is an important microbe which causes pyogenic liver abscess and/or central nervous system infection. Despite advances in intensive care medicine, pyogenic liver abscess and central nervous system infection are still catastrophic illnesses. Many cases of magA-positive *K. pneumoniae* infection have been reported in Taiwan, but few cases have been reported in the rest of Asia, North America or Europe. Further epidemiological investigation of this virulent organism is needed.

P1730 Clonal diversity of high-level gentamicin-resistant *Enterococcus faecalis* isolated from clinical samples in Tehran, Iran

M. Saifi, M.R. Pourshafie, M.M. Soltan Dallal, M. Talebi (Tehran, IR)*

Objectives: Following the worldwide increased of high-level gentamicin resistace (HLGR); we report the clonal relatedness of HLGR *Enterococcus faecalis* HLGR-EF) from clinical samples in Tehran, Iran.
Methods: From September 2006 to 2007, 266 enterococcal isolates were collected from medical centers in Tehran. All strains were identified to species level by conventional and PCR methods. CLSI recommendations were used for antimicrobial susceptibility and gentamicin MICs of HLGR-EF isolates. Analysis of gentamicin resistant genes was done by PCR method. Filter mating experiment was used for conjugation test among HLGR-EF isolates. Boichemical fingerprint typing (PhP typing) was applied for primary typing of enterococcal population. Analysis of the SmaI restriction digest of genomic DNA by pulsed field gel electrophoresis was performed for genotyping of the HLGR-EF isolates.
Results: A total of 190 (71.5%) of the isolates were identified as *Enterococcus faecalis* (*E. faecalis*) and 58(30.5%) of them were HLGR-EF (MIC$\geqslant$1024 μg/ml). All HLGR-EF isolates contained aac(6′)-Ie-aph(2”)-Ia gene. Among 10 mating experiments, only one transconjugant were obtained with the high frequency of 6×10^{-9} for aac(6′)-Ie-aph(2″)-Ia gene.
PhP typing of HLGR-EF isolates included one dominating cluster, 10 smaller clusters and 14 unique patterns. PFGE resulted in 36 different patterns among the 58 isolates. Only 12% (7 isolates from different medical centers) of the HLGR-EF isolates belonged to one PFGE cluster. The other common types consisted of 45% (26 isolates) were limited only to 1–3 medical centers and the rest (43%) were single types.
Conclusion: PFGE showed more discriminatory power than PhP typing. High diversity observed indicating that the isolates belonged to several clonal groups which could be mostly endogenous origin. However one dominating cluster which observed among studied *E. faecalis* population, revealed the clonal dissemination among the HLGR-EF isolates from different medical centers. We detected a strong association between HLGR phenotype and the aac(6′)-Ie-aph(2”)-Ia gene. This finding can be explained by horizontal transmission of this gentamicin resistance gene.

P1731 Genotypic analysis of *Helicobacter pylori* strains circulating in St. Petersburg, Russia at present

R. Ferman, A. Zhebrun, Y. Alyapkina, A. Svarval (St. Petersburg, Moscow, RU)*

A strain-specific *H. pylori* gene, cagA, has been recognized as a marker for strains that confer increased risk for peptic ulcer disease and gastric cancer. VacA of *H. pylori* is a highmolecular weight multimeric pore-forming protein that causes massive vacuolation in epithelial cell lines. As with cagA, no close homologs of vacA exist in other *Helicobacter* species or in other bacteria, which suggests its importance in the specific relationship of *H. pylori* with the human stomach. However, unlike cagA, vacA is conserved among all *H. pylori* strains, although significant polymorphism exists. vacA alleles possess one of two types of signal region, s1 or s2, and one of two mid-regions, m1 or m2, occurring in all possible combinations. *H. pylori* strains with different forms of vacA exhibit varied phenotypes and have particular associations with gastro-duodenal diseases. vacA s2 strains are rarely isolated from patients with peptic ulcers or gastric adenocarcinoma. s1/m2 forms of VacA bind to and vacuolate a narrower range of cells than s1/m1 forms and induce less damage. vacA s1/m1 strains are most closely associated with gastric carcinoma. In our study we analysed peculiarity of *H. pylori* strains that are circulating in Sainkt-Petersburg at present by means of PCR method.
Materials and Methods: We have studied 30 *H. pylori* strains isolated from patients with chronic gastritis, gastroduodenitis, ulcer disease and gastric cancer. The patients were aged from 13 to 65. The presence of 16SrRNA, CagA, vacA was determined. Genotyping of *H. pylori* vacA was carry out for 14 strains.
Results: All studied strains of *H. pylori* revealed 16SrRNA. Estimated that 76.67% of them were cagA-positive. By genotyping vacA it was found out that s1m1 genotype was prevailing (in 57.14% of strains). It was associating with cagA gene in 87.5% cases. The s1m2 vacA genotype was estimated in 21.43% of strains and the s2m2 vacA genotype in 14.29% of strains. There was also one strain of *H. pylori* separated from the patient with ulcer disease that possessed the genotype cagA+/s1m1m2. The strain isolated from the patient with gastric cancer possessed the genotype cagA+/s2m2 vacA of *H. pylori*.
Conclusions: The fulfilled analysis showed that in Saint-Petersburg area *H. pylori* strains with cagA+/s1m1vacA genotype are prevailing. It is known that such strains are associated with more serious pathology therefore dynamic observation of patients with such strains is necessary.

P1732 Molecular-epidemiological diversity of *V. cholerae* strains isolated in Siberian and the far eastern regions of the Russian Federation

A. Kozhevnikova, S. Balakhonov, E. Kulikalova, L. Urbanovich, N. Breneva, M. Afanas'ev (Irkutsk, RU)*

Objectives: Cholera is a severe gastrointestinal disease that due to Gram-negative bacterium *Vibrio cholerae*. Threat of infection's carries from Africa and South-Eastern Asia demand to effective molecular-typing based surveillance measures. The purpose of this study was to evaluate

molecular-epidemiological diversity of *V. cholerae* strains isolated in Siberian and the Far Eastern regions of Russia.
Methods: *V. cholerae* strains (n = 32) have been selected during epidemiological monitoring of Siberia and Far East from 1994 to 2005. Sample set include toxigenic strains (n = 14) from patient and environmental samples, isolated during outbreaks and sporadic cases of cholera in 1994, 1997 and 1999 years and non-toxigenic strains (n = 18) from environment. Strains' isolation and identification was performed according to WHO protocols. Detection of virulence's determinants (ctxAB, tcpA, toxR, attrS, zot, rstC, nanH, ompU), fragments of pandemic islands (VSP-I and VSP-II) and determinants of environmental persistence (hapA1, mshQ and A, mbaA1 and A2, vpsR) was performed by previously described PCR systems. Standard procedure of VNTR typing, that based on amplification of five loci – VcA, VcB, VcC, VcD and VcG, was carried out as described previously.
Results: Twenty nine strains (90.6%) belonged to the O1 serotype, all were identify as El-Tor biovar. Three strains belonged to the O 139 serotype. Main virulence's determinants (ctxAB and tcpA) were detected in 14 (14/32; 43.8%) strains and 11 (11/14; 78.6%) of them had fragments of VSP-I and VSP-II. Significant association between presence of environmental persistence's and virulence's determinants did not observe. Nineteen different VNTR profiles were identified, including 13 (68.4%) unique and 19 strains grouped into six clusters (from 2 to 6 strains in each). Seven main VNTR genotypes formed large subgroup that included strains isolated from patients and environmental samples during cholera outbreaks. Among strains belonged to these VNTR-genotypes we observed complete set of virulence's determinants and fragments of pandemic islands.
Conclusion: Seven VNTR-genotypes, which associated with worsening of cholera's situation in Siberia and Far East, were revealed. VNTR typing demonstrates sufficient discrimination power and, in combination with virulence's and pathogen's determinants PCR detection, may be useful for *V. cholerae* typing in Siberian and Far Eastern regions of Russia.

P1733 Characterization of *Shigella sonnei* isolated from hospitalized children in Tehran, Iran

R. Ranjbar, M.R. Pourshafie, M.M. Soltan Dallal, M. Izadi, N. Jonaidi (Tehran, IR)*

Objective: Shigellosis is one of the main causes of morbidity in children with diarrhea in Iran. The present study was conducted to examine the prevalence of *Shigella* spp., antibiotic susceptibility and the genetic characterization of *S. sonnei* strains recovered from hospitalized children (<12 years old) with acute diarrhea in Tehran, Iran in 2003.
Methods: Fecal specimens and rectal swab collected from patients were cultured and identified as *Shigella* by the conventional methods. The isolates of *S. sonnei,* as predominant species, were further examined. Antimicrobial susceptibility, plasmid profiling, and ribotyping were used to study the relatedness between the *S. sonnei* isolates.
Results: Out of 3050 patients with acute diarrhea, 302 cases were diagnosed as having shigellosis on the basis of clinical presentations and laboratory findings. *S. sonnei* as the predominant *Shigella* spp. (58.9%) was further studied. Majority of *S. sonnei* isolates (94%) were resistant to co-trimoaxozle. On the other hand, they were relatively or completely sensitive to 15 commonly used antibiotics. The extracted plasmids showed 12 different profiles with two closely related patterns constituting 70% of the total isolates. Ribotyping using PvuII, HindIII or SalI restriction enzymes generated a single pattern for all *S. sonnei* isolates.
Conclusion: The results showed that *S. sonnei* has replaced *S. flexneri* as the predominant serogroup in children with less than 12 years of age in the hospitals in Tehran. Single ribotype dominance was also supported by the data that 70% of the isolates harbored closely related plasmid patterns. The results further confirm that continuous monitoring is needed for over a prolonged period in Tehran to detect the changes in the serotype distribution and the antimicrobial resistance pattern of *Shigella*.

P1734 Genotypic and phenotypic profiles of *Streptococcus mutans* in children with and without dental caries

F. Gamboa, M. Chaves, C. Valdivieso (Bogota, CO)*

Objective: *Streptococcus mutans*, an acidogenic and aciduric microorganism that colonizes the oral cavity is recognized as the main causal agent of dental caries. Epidemiological studies have shown a strong correlation between the number of *S. mutans* in the oral cavity and prevalence and incidence of caries. At present different genotypic and phenotypic methods are known to determine the profiles of settling and epidemiological distribution of *S. mutans.* The aim of this study was to investigate the profiles of *S. mutans* isolated from children with and without dental caries by using the AP-PCR (arbitrarily primed polymerase chain reaction) and api-Zym methods.
Methods: In the AP-PCR method, random DNA segments of the target bacterium are amplified with single primers of arbitrary sequence. The api-Zym system (bioMérieux, Marcy-létoile, France) is a phenotypic micro-method that allows simultaneous detection of 19 enzymatic activities from bacterial inoculum. A transversal observational study was conducted, which finally included 120 3- to 5-year-old children (75 with and 45 without dental caries), who attended a preschool institution in Bogota (Colombia).
Results: *S. mutans* was isolated in 15 of the 45 children without-dental caries (33.3%) and in 31 of the 75 children with caries (41.33%). In the 15 healthy patients with *S. mutans,* 24 strains were isolated, while in the 31 caries patients 45 strains were isolated. With api-Zym system were detected 36 different biotypes, 22 for the caries group and 15 of the healthy group. The biotype XX was present in the two groups. With AP-PCR method identified 27 different fingerprinting profiles; both groups of patients shared four of these genomic profiling.
Conclusion: The information shows a great diversity in *S. mutans* genotypes and phenotypes in the studied population.

P1735 *Neoscytalidium* spp. superficial infections in Madrid

R.B. Mohedano, M.S. Cuétara, A. Aguilar, S. Vazquez, S. Rey-Cao, M. Gallego, I. Wilhelmi (Madrid, ES)*

Objective: *Neoscytalidium dimidiatum (N. dimidiatum)* (synamorph *Nattrassia mangiferae*, formerly known as *Hendersonula toruloidea*) is a dematiaceous plant pathogen that can cause endemic (geographical restricted to tropical and subtropical areas) superficial skin and nail infections, clinically indistinguible from Trichophyton (rubrum and interdigitale). In temperate zones, infections have been noted in immigrants from endemic areas. In areas with important immigration such as most European countries, misdiagnosis of this infection is possible if cycloheximide is not withdrawn from the usual culture medium. The objective of this work was to show the epidemiology of *N. dimidiatum* species in a temperate European capital (Madrid).
Methods: Retrospective study (from 2003 to 2009). After a dermatological suspicion, scrapings from nails and skin (soles, palms, toe and finger webs) and in one case a subcutaneous nodule were planted on Sabouraud agar slants and dishes supplemented with cloramphenicol and cycloheximide and in media without cycloheximide. Media were incubated at 25ºC for up to 30 days. Samples were examined after treatment with treatment with potassium hydroxide. *Neoscytalidium* species were identified according to usual macroscopic and microscopic.
Results: *Neoscytalidium* spp. superficial infections were diagnosed in 8 immigrants from endemic areas; a subcutaneous infection (plantar granuloma) was diagnosed in a Spanish patient with no history of travels, but who had close contact (at work) with immigrants.
The table shows the infected sites, species responsible of the infection and geographical origin. In our population direct examination showed sinuous and irregular hyphae in bright field illumination.
No dermatophytes were either visualized or cultured. The response to treatment (oral/topical) was unsatisfactory.
Conclusions: In Spain *Neoscytalidium* spp. is a uncommon agent of dermatomycoses, and misdiagnosis could explain that although

immigration is high, this species is only occasionally identified. This is the first autochtonous *N. dimidiatum* infection in Madrid probably due to close contact of the patient with immigrants with clinical infection due to this agent. Another important and widely documented issue is the very poor response to treatment. In European areas such as London and Paris these infections show an ascending incidence linked to expertise of clinical mycologists and clinical suspicion of dermatologists.

Age/Sex	Origin	Soles	Toe-webs	Toenails/ Fingernails	Subcutaneous nodule	TTO
43/M	Spain	+	–	–/–	+	yes
65/F	Guinea	+	+	+/–	–	yes
24/F	Colombia	–	–	+/+	–	yes
37/F	Cameroon	+	+	+/–	–	yes
40/F	Brazil	+	+	+/–	–	yes
39/F	Guinea	–	–	+/–	–	no
54/F	Rep. Dom	–	–	+/–	–	no
35/F	Guinea	–	+	+/–	–	yes
31/F	Cameroon	–	–	+/–	–	no

Sex: M = male; F = female; TTO: topical and oral treatment.

P1736 Identification of clinical isolates using the RiboPrinter® system

C. Bégot, F. Wegerhoff (Meyrin, CH; Indianapolis, US)*

Objectives: To assess the use of the RiboPrinter® Microbial Characterization System to identify bacterial strains isolated from clinical specimens.
Methods: The RiboPrinter® is an automated ribotyping system based on genomic restriction fragment length polymorphism (RFLP) analysis, and it generates patterns based on the portion of DNA that encodes for ribosomal RNA. These patterns are used to characterize a bacterial sample, and identify the bacterium by comparing it with static composite identification patterns in the DuPont, or Custom ID Libraries. A total of 1870 strains (Gram-negative and Gram-positive) isolated from sputum, urine, skin and skin structure infections were tested.
Results: About 67% of strains were automatically identified with the RiboPrinter® system with "matches" above the similarity threshold (SI $\geqslant$0.85). A further 7% were identified after manual assignment from a list of nearest neighbors generated by the system (SI < 0.85), whereas 26% of strains were not identified by the system. Of the automatic and manual identifications, the most frequent organisms included 64 different genus and species, such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Enterococcus faecalis*.
The strains not identified by the RiboPrinter® system were identified using phenotypic methods such as GNI (Vitek®), API strips and additional biochemicals tests. These included particular strains of *Sphingomonas paucimobilis*, *Providencia rettgeri*, *Proteus vulgaris*, *Citrobacter freundii* and *Enterobacter* species. RiboPrint patterns from bacterial identifications that were obtained with a high confidence level by phenotypic systems were added to the Custom ID Library. The RiboPrinter® system will therefore have an increased chance of recognizing these patterns and identifying the strains in future testing of patient isolates.
Conclusion: The RiboPrinter® offers a dynamic system for accurate automated identification of clinical isolates by simultaneous use of the manufacturer's ID library (6950 bacterial species, 40496 patterns) as well as the custom ID library, thereby increasing the chances for definitive bacterial identification.

Bacterial identification following a molecular approach

P1737 Usefulness of polymerase chain reaction and DNA sequencing in routine diagnostics of bacterial infections

K.H. Jensen, R. Dargis, J.J. Christensen, M. Kemp (Copenhagen, DK)*

Objectives: Sequencing of 16S rRNA genes (rDNA) has been widely used for taxonomic classification of bacteria and has proven to be a useful tool in clinical laboratories for bacterial identification as well. Since 2003 the Clinical Microbiology Laboratory at Statens Serum Institut has offered this analysis as a routine diagnostic procedure on culture negative samples. After six years and more than 2600 analyses we felt it important to evaluate the usefulness of PCR and DNA sequencing as a routine diagnostic tool. The bacteria identified and type of sample material examined may indicate in which type of infections the method may be of particular value.
Methods: The records of 16S rDNA analyses from June 2003 to October 2009 were used to obtain information about the type of sample material, number of samples in which bacterial DNA were found and what bacteria that were identified.
Results: In a six year period from June 2003 to October 2009, 2628 clinical samples have been submitted for detection and identification of bacterial DNA. In 695 of the samples, bacterial DNA was detected and PCR products for sequencing were obtained (Table 1). The bacterial species or clinical relevant genus/group was identified in 556 of the sequenced samples. 421 (76%) and 135 (24%) bacteria were identified at species and genus level, respectively. In 103 (74%) of the 139 cases where no identification could be obtained, the sample was judged as multibacterial by viewing the chromatogram. The main group of bacteria encountered was facultative anaerobic and aerobic strains and the majority of these were Gram-positive strains. The most frequently encountered bacteria in this group were *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Streptococcus intermedius*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Of the anaerobic strains found, more than half were *Propionibacterium acnes* and other Propionibacterium species.
Conclusion: The finding of bacterial DNA in one fourth of culture negative samples demonstrates that PCR and sequence-based detection and identification of bacteria are good supplements to conventional diagnostics.

Table 1: Results of PCR and 16S rRNA sequencing of samples submitted for analysis during a 6-year period

	No. of samples	Bacterial DNA found (% total samples)	Strain identified[a] (% sequenced PCR products)
2003	33	17 (52)	9 (53)
2004	99	25 (25)	24 (96)
2005	201	58 (29)	39 (67)
2006	305	151 (50)	96 (64)
2007	441	112 (25)	88 (79)
2008	694	126 (18)	117 (93)
2009	855	206 (24)	183 (89)
Total	2628	695 (26)	556 (80)

[a]Identification obtained at either genus or species level.

P1738 Bacterial detection with a microfluidic reactor for polymerase chain reaction

J.R. Peham, H. Steiner, W. Grienauer, R. Heer, M.J. Vellekoop, C. Noehammer, H. Wiesinger-Mayr (Seibersdorf, Vienna, AT)*

Objectives: A novel microfluidic reactor, applicable for infectious disease diagnostics was developed. To enable rapid and sensitive bacterial detection in a continuous operation mode, genomic DNA amplification of the 16S rRNA gene was realized by polymerase chain reaction (PCR)

in a flow-through and miniaturised device. A detailed comparison of this microfluidic approach to conventional PCR methods is presented.
Methods: DNA was extracted from pure bacterial cultures by thermal lysis and purified. PCR reagents were added and the complete mix was directly injected into the new device. The cyclic flow PCR device was designed with 40 turns of polytetrafluoroethylene (PTFE) tubing (400 micrometer inner diameter), representing 40 PCR cycles, coiled around a prismatic heating device, operated by Peltier elements. The aqueous 10 microliter PCR mix is driven by a silicone oil of low viscosity through three equidistant temperature zones (95º C, 55º C, 72º C) per turn. Flow rates of 137 nl/s, 274 nl/s and 548 nl/s have been applied to achieve 60, 30 and 15 s retention time per temperature zone respectively.
Results: Target PCR amplicons of 153bp, 418 bp and 720 bp have successfully been amplified with the developed device. The standard laboratory processing time of 3 h could be decreased to 30 min with the microfluidic reactor without loosing PCR efficiency. Using the current design, this will allow analysis of 60 samples per hour in continuous operation.
Conclusion: It has been shown that the presented system can rapidly amplify the 16S rRNA gene and thereby allows a fast detection of bacteria, which is crucial for clinical microbiology. The performance can be increased with smaller sample volumes and parallel tubing, leading to a high-throughput device. Integrating an optical fluorescence detector for dsDNA measurement would evolve this device into a micro total analysis system for infectious diseases.

P1739 Comparison of four real-time PCRs for *Listeria monocytogenes*

D.L. Hess, P. Savelkoul, E. De Boer, A. Heuvelink, L. Spanjaard, C.W. Ang (Amsterdam, Zutphen, NL)*

Objectives: Real-time PCR is a widely used method for detection of microbial nucleic acid sequences. In the past years, several real-time PCR's have been developed for the detection of *Listeria monocytogenes*. The aim of this study was to assess the sensitivity of four real-time PCR's for *L. monocytogenes* strains from food and human origin.
Methods: We selected two primer-probe combinations from literature, based on the number of investigated strains, sensitivity, specificity and Genbank nucleotide BLAST results. The primer-probe combinations are published by Rodríguez-Lázaro et al. and Oravcová et al., targeting the hly gene and actA gene, respectively. These primer-probe combinations were compared with a primer-probe set used by the Food and Consumer Product Safety Authority (VWA, unpublished), targeting the iap gene, and a primer-probe set developed by Huijsdens et al., targeting the hly gene. All PCR's used FAM-labelled probes. 168 human clinical isolates from the Netherlands Reference Laboratory for Bacterial Meningitis (RBM), 111 food isolates from the Food and Consumer Product Safety Authority (VWA), 3 complete genome sequenced strains (ATCC BAA-679 (=EGDe), F2365, HCC23) and *L. monocytogenes* type strain ATCC 15313 were used to determine the sensitivity of the four real-time PCR's. Specificity was assessed using a total of 40 non-*L. monocytogenes* strains, including all other *Listeria* species.
Results: The Huijsdens PCR detected 76 out of a total of 182 *L. monocytogenes* isolates (41.8%). These results were in line with nucleotide BLAST results. The complete set of isolates was analyzed with the remaining three PCRs. The Rodríguez PCR detected 277 out of 279 (99.3%) of all isolates, the VWA PCR and Oravcová PCR both accurately identified 278 out of 279 (99.6%) of all isolates. The undetected isolates originated from four different human clinical cases, all phenotypically as well as AFLP and PFGE typed as *L. monocytogenes.* Preliminary results suggest that detection limits of the PCRs are comparable to previously published results (<100 CFU/ml in the VWA PCR). Specificity of all PCRs is 100%.
Conclusion: The Huijsdens PCR identifies less than half of all isolates tested, whereas the other real-time PCRs identify almost all isolates accurately. The considerable variation in sensitivity of real-time PCRs should be taken into account when selecting real-time *L. monocytogenes* PCR for diagnostic use.

P1740 Multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3

P.G. Higgins, M. Lehmann, H. Seifert (Cologne, DE)*

Objectives: *Acinetobacter calcoaceticus* is a non-pathogenic environmental organism that can colonise hospitalised patients. In contrast, the closely related *Acinetobacter* genomic species 3 (GS3) is a known nosocomial pathogen. In routine diagnostics both these organisms, in addition to *A. baumannii* and *Acinetobacter* genomic species 13TU (GS13TU), are grouped together as the *Acinetobacter calcoaceticus*-baumannii complex (Acb complex). We have previously established a gyrB multiplex PCR to differentiate between *A. baumannii* and GS13TU (Higgins PG et al. (2007) Clin. Microbiol. Infect.; 13: 1199–201). The aim of this study was to develop a simple and rapid PCR-based method to differentiate between *Acinetobacter calcoaceticus* and GS3 that could be used alongside the established multiplex PCR to differentiate within the Acb complex.
Methods: The gyrB genes from 20 non-identical *A. calcoaceticus* and 21 GS3 were amplified by PCR, sequenced and aligned. Species-specific annealing sites were identified. Primers were designed to work under the same cycling conditions as the established multiplex PCR and to amplify products that are easily distinguishable from each other. Primers were tested against 24 *A. calcoaceticus*, 22 *A. baumannii*, 37 GS3 and 29 GS13TU.
Results: All *A. calcoaceticus* tested amplified a 428bp PCR product. GS3 isolates amplified a PCR product of 182bp. Addition of gyrB primers specific for *A. baumannii* and GS13TU unambiguously correctly identified each of the 4 species. No PCR products were amplified when tested against other species of the *Acinetobacter* genus (haemolyticus, junii, johnsonnii, lwoffii, berezinae, guillouiae, radioresistens, beijerinckii, venetianus, ursingii, schindleri, and genomic species 6, 9, 14 and 15TU).
Conclusions: This multiplex PCR can be used to identify and differentiate between *A. calcoaceticus* and GS3. Addition of the primers from the previously established multiplex PCR now allows for the rapid identification of the various species belonging to the Acb complex.

P1741 Isolation of *Ralstonia insidiosa* from cystic fibrosis patients in a Turkish university hospital

O. Dogan, Ö. Köseoglu Eser, P. Vandamme, B. Sener (Ankara, TR; Gent, BE)*

Objectives: The identification of non-fermentative Gram-negative bacilli (NFGNB) isolated from the sputum of cystic fibrosis (CF) patients is still a challenge for microbiology laboratories. Although the clinical importance of such bacteria is debated, they are increasingly recovered in CF patients. The aim of this study was to evaluate phenotypic and genotypic identification and clinical impact of a series of non-fermentative isolates from CF patients.
Methods: A series of 13 NFGNB were recovered from CF patients during September-November 2008 period. All these oxidase positive isolates were either identified as *Ralstonia pickettii* or *Burkholderia cepacia* by BD Phoenix automated system. The isolates were then examined by 16S rRNA gene sequencing for identification. Antibiotic susceptibilities were tested by disk diffusion and the genetic relatedness of them were evaluated by arbitrarily primed-PCR (AP-PCR).
Results: Gene sequencing revealed that 11 of the isolates were *Ralstonia insidiosa*, one was *Pandoraea pulmonicola* and one was *Stenotrophomonas maltophilia*. All the *R. insidiosa* isolates except one and the *S. maltophilia* strain had the similar antibiotic susceptibility pattern as imipenem, amikacin, tobramycin, colistin resistant; ceftazidime, cefepime, meropenem, levofloxacin and ciprofloxacin susceptible. The patients had neither serious clinical symptoms nor acute exacerbation findings. *R. insidiosa* had never been recovered again during their ongoing controls. None of the patients were socially related. AP-PCR analysis of *R. insidiosa* isolates revealed four different genotypes. Seven isolates were observed to be genetically related, while two other isolates

revealed another identical pattern. The remaining was genotypically unique.
Conclusion: Automated bacterial identification systems do not fulfill the demand of adequate identification of NFGNB in CF isolates and should be replaced by molecular methods. Although *R. insidiosa* isolation was regarded as an intermittent colonisation according to these findings, its clinical impact on CF pathogenesis is not yet clear. Since these bacteria are mostly of environmental origin, these results emphasized the need for more cautious infection control measures for CF clinics.

P1742 Polymorphism of pertussis toxin gene in circulating *Bordetella pertussis* strains in Serbia

G. Dakic, T. Pljesa, T. Kallonen, A. Elomaa, M. Svabic-Vlahovic, Q. He (Belgrade, RS; Turku, FI)*

Objectives: Despite the routine use of pertussis vaccines, pertussis remains an important cause of disease among young infants, adolescents and adults. Pertussis toxin (Ptx) is one of the major virulence factors of *Bordetella pertussis* and is included in all of the acellular pertussis vaccines. Ptx consists of five subunits (S1-S5, or PtxA-E). The S1 subunit (PtxA) contains the catalytically active site and has been shown to be immunodominant. So far four ptxA alleles (ptxA1-ptxA4) have been reported. In many countries, the allele ptxA2 and/or ptxA3 are present in most vaccine strains and predominated in the isolates circulating in pre-vaccine era. However, the "vaccine type" strains were gradually replaced by "non-vaccine" type" ptxA1 after the vaccination was introduced. Today, almost all of circulating strains have been ptxA1. However, little is known of polymorphism of ptxA gene in *B. pertussis* isolates in Serbia where whole cell vaccine has been in use since 1957. The aim of this study was to analyze and compare ptxA genotypes in the Serbian isolates collected since 1953.
Methods: The analysis included genotyping of ptxA by sequencing and LightCycler PCR. A total of 70 clinical isolates together with four vaccine strains were tested. The vaccine strains 2047/57 and 1772/57 were isolated in 1957, and the strains 23/81 and 8/84 were isolated in 1981 and 1984. Current composition of the Serbian vaccine contains the four strains and has been used since 1985. The clinical isolates were from 1953 to 1956 (n = 10), from 1957 to 1984 (n = 43) and from 1985 to 2000 (n = 17).
Results: The vaccine strains 2047/57 and 1772/57 represented ptxA2 genotypes, whereas the vaccine strains 23/81 and 8/84 harbored ptxA1 genotypes. Shift from ptxA2 to ptxA1 has been observed in isolates since the late of 1960s. During the period of 1980–1984, all isolates represented ptxA1 genotype. Reappearance of the isolates containing ptxA2 was noticed after the two strains harboring ptxA1 were added into the vaccine in 1985. From 1985 to 2000 35% of isolates tested were ptxA2.
Conclusions: The Serbian vaccine strains showed difference in ptxA. The frequency of modern *B. pertussis* isolates with ptxA2 was high in Serbia compared to that found in most of countries with long vaccination history.

P1743 Detection of anaerobic bacteria within 15 minutes

P.J. Winklehner, C. Mittermayr, F. Winner (Freistadt, AT)*

Objectives: A fast and easy detection system for bacteria is the goal of this project. The use of a DNA-microarray enables reliable and simultaneous detection and differentiation of many microorganisms. The short timeframe requires a PCR-free method. Ease of use is guaranteed by a microfluidic lateral flow device. Proof of principle will be demonstrated by the detection of anaerobic bacteria that are difficult to culture.
Methods: A disposable single use polymer microfluidic chip has been developed. The chip has the same dimensions as a standard microscope slide. It comprises an inlet which can accept solutions from pipets or dropper bottles, a reaction channel and a fluid reservoir that controls the flow in the channel. The flow is driven by capillary forces without the need for external control mechanisms or pumps. The combination of this microfluidic chip with colorimetric detection and visual readout allows the analysis of biological samples without any expensive laboratory equipment.
The platform was applied to a 16S rRNA based analysis procedure. Differentiation was done by a DNA-microarray which was printed into the reaction channel. All reaction steps were conducted at room temperature.
Results: Identification of bacteria by their 16s rRNA is accomplished within 15 minutes. Analysis is done by dropping lysed bacteria and three solutions for washing and colour reaction onto the platform using disposable pipets and dropper bottles. The limit of detection was 5×10^4–1×10^5 bacteria/analysis-volume (=20 µl).
Conclusions: The advantages of this system are low cost mass production by mold injection technology, the possibility for chair-side analysis of infectious diseases and the elimination of complex liquid handling systems or other laboratory equipment. The advantages of microfluidic in the context of microarray analysis are the handling of minute volumes and the reduction of dilutions in order to improve the limit of detection. The marriage of microarray and microfluidics promises the advent of a powerful platform for bioanalytical applications, like the diagnosis of diseases, establishing genetic predispositions or high through put screening for drug discovery.

P1744 Evaluation of polymerase chain reaction as rapid diagnostic tool compared to culture in respiratory tract infections

A. Makri, G. Tzanakaki, S. Kalimeratzi, H. Iliadou, A. Xiroyianni, J. Kremastinou, A. Voyatzi (Athens, GR)*

Introduction: In spite of the availability of effective antimicrobial therapy both otitis media with effusion (OME) and chronic obstructive pulmonary disease (COPD) are still important infections for children, leading to serious health problems.
Objectives: To investigate the diagnostic value of polymerase chain reaction (PCR) for the detection of bacterial DNA in MEE and broncho-alveolar lavage (BAL) specimens and to compare the conventional culture methods accepted as the "gold standard" to the modecular techniques.
Material and Methods: A total of 102 samples collected from hospitalized children (30 BAL and 72 middle ear effusion – MEE) were analyzed. Multiplex PCR protocol was applied for the detection of *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus* spp including *Streptococcus pyogenes* (GAS). Two stepdown multiplex-PCR assays were used for the simultaneous detection of nine main serotypes of *S. pneumoniae* directly in clinical samples.
Results: The frequency of the tested bacteria by the conventional cultures methods was *H. influenzae* 42.1%, St. aureus 21.5%, Gas 14%, *Ps. aeruginosa* 10.7% and *S. pneumoniae* 8.8%. The sensitivity of PCR assay compared to cultures was 92% for *H. influenzae*, *S. pneumoniae* and GAS while the method indicated 100% total specificity. In comparison to the cultures PCR assay was more sensitive for *S. pneumoniae* (20.55 vs 8.85) and GAS (23% vs 14%). The most prevalent serotypes of *S. pneumoniae* were 19F, 6, 14, 19A, 3 and a high proportion were of not typable (not included in the 7-valent conjugate vaccine).
Conclusions: PCR method could be considered as a rapid, reliable and feasible method for the detection of the most common fastidious bacteria that lead to OME and COPD.

Diagnostics: miscellaneous

P1745 Comparison of cell cultures and three conventional PCR for detecting *Chlamydophila pneumoniae* in adults pharyngotonsillitis

C. Bonaccorso, B. Bisignano, R. Timpanaro, A. Stivala, A. Garozzo, G. Tempera (Catania, IT)*

Objectives: Chamydophila pneumoniae is a common cause of acute respiratory tract infections although it is considered like a non conventional pathogen for pharyngotonsillitis. The incidence among children has been well documented whereas there is a lack of epidemiological data about the spreading of this intracellular pathogens in adults. The aim of the study was to investigate the frequency of *C. pneumoniae* detection in clinical samples of adult patients with pharyngotonsillitis through the use of different methods.
Methods: This study investigated 94 throat swabs collected from patients with clinical diagnosis of acute pharyngotonsillitis. After three serial passages in cell cultures the presence of chlamydial inclusions was evaluated by immunofluorescence test (IFA) using FITC-conjugated anti-*C. pneumoniae* MAb. The obtained data were compared with a conventional PCR targeting the 16S rRNA gene and two nested PCRs, targeting the 16S rRNA gene and the ompA gene respectively.
Results: The presence of chlamydial inclusion in cell cultures was observed in 11/94 samples (11.70%) by IFA. *C. pneumoniae* DNA was detected in 12/94 (12.76%) specimens by the 16S rRNA gene nested PCR, 4/94 (4.26%) by ompA gene nested PCR and in 2/94 (2.13%) by 16S rRNA single step PCR.
Conclusion: Our results indicate that there is a diffusion of *C. pneumoniae* in acute pharingotonsillitis in adults. Isolation of *C. pneumoniae* by cell cultures is essential to document the viability of the pathogen even if it is complicated and time-consuming; therefore tests that target pathogen DNA, such as PCR, have become widely used. Our data show poor agreement between the three applied DNA-amplification methods; in fact, 16S rRNA gene nested PCR confirmed all positive results obtained by IFA, while the other two PCR assays had a lower sensitivity.

P1746 Use of ESwab for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* from genital specimens

S. Castriciano, D. Piccoli, C. Mazzi, R. Sforza, M. Valbusa, A. Fenzi, S. Gaino, M. Caputo (Brescia, Bussolengo, IT)*

Objectives: *Mycoplasma hominis* (Mh) and *Ureaplasma urealyticum* (Uu) are common inhabitants of the urogenital tract of women. ESwab (Copan Italia), a Liquid Based Microbiology device, for collection and transportation of clinical specimens, preserves microorganisms at room temperature. This study validates the use of the ESwab kit for the collection and storage of clinical specimens for the detection of *M. hominis* and *U. urealyticum* with the MYCOFAST Evolution 3 test.
Methods: ATCC strains of Mh and Uu and clinical specimens were used for this study. Mock specimens were prepared by adding 100 ul of Mh and Uu culture to Rayon swabs and to the flocked swabs of the Eswab kit. The rayon swabs were added to the UMMt broth, while the flocked swabs were added to the1 ml Amies medium tubes, then different volumes were added to the UMMt broth. Genital specimens (102) for the diagnosis of genital infections, collected with the flocked swab and transported in the Amies medium, were tested with the MYCOFAST Evolution 3 (Elitech, distributed by DID in Italy). Clinical specimens were tested the same day of collection and after 24 hours storage at room temperature (RT) following the method for liquid specimen. Each specimen was vortexed and 300 microliters were inoculated into an UMMt broth vial as per the kit manufacturer procedure. Vials and trays were incubated at 37ºC and read after 48 hours incubation.
Results: In the mock specimens, 300 ul of the ESwab specimens gave the same results as the Rayon swabs after 36 to 48 hours incubation. In the 102 genital specimens 32(31.37%) were positive, 6 Mh and 26 Uu and 70 negative for the same day testing while 34(33.33%) were positive, 9 Mh and 25 Uu and 68 negative when tested after 24 hours at room temperature. There was 100% antibiotic resistance correlation in 6 Mh and 15 Uu at both testing times. The resistance of the other positive was variable with both inoculation times.
Conclusions: ESwab didn't interfere with the performance of the MYCOFAST Evolution 3 for the detection of Mh, Uu, and antibiotic resistance with mock and clinical specimens. Genital specimens, collected with the ESwab kits, can be stored for 24 hours at RT when unable to same day test and can be tested for other STDs from the same specimen.

P1747 Usefulness of galactomannan testing in broncoalveolar lavage fluid for diagnosis of invasive aspergillosis in HSCT recipients

E. Furfaro, M. Mikulska, A. Raiola, F. Gualandi, M. Van Lint, A. Dominietto, V. Del Bono, F. Miletich, A. Bacigalupo, C. Viscoli (Genoa, IT)*

Introduction: The diagnosis of invasive aspergillosis (IA) remains difficult and non invasive diagnostic tests are of increasing interest in clinical settings. In particular the galactomannan assay (GM) has been widely studied in serum, while fewer studies have focused on GM in bronchoalveolar lavage (BAL).
We investigated the role of GM in BAL fluid as a tool for diagnosis of IA in HSCT recipients.
Methods: Recipients of allogeneic HSCT between January 2007 and October 2009 were included in this study. Data from CT scans and GM testing, both in serum and BAL, were evaluated. GM positivity was defined as $\geqslant 0.5$ in a single sample of BAL and IN two consecutive serum samples. Patients were classified as proven, probable, possible and no IA according to 2008 EORTC/MSG criteria.
Results: A total of 61 patients had GM testing performed both in serum and BAL because of clinical signs compatible with invasive fungal infection (IFI) (table 1). Of them, 19 (31%) had typical signs on CT scan that fulfilled the clinical criteria for possible IA according to EORTC/MSG (dense, well-circumscribed lesions, air-crescent sign or cavity). In 6 of them (32%) serum GM was positive, thus upgrading these cases to probable disease. BAL GM was positive in all but one patients with positive serum (5/6) and in additional 5 patients with negative serum GM. Therefore the proportion of patients with possible IA upgraded to probable disease increased from 6/19 (32%) with serum GM positive to 11/19 (58%) with either BAL or serum GM positive. The median GM value in 10 patients with probable IA and positive GM was 1.5 (range 0.52–4.8) and 7 (70%) patients had GM > 1.0. Using GM cut-off for BAL of 1.0, 9 patients could be upgraded to probable IA (32% > 47%). Among 42 patients with no typical pulmonary infiltrates, 9 had positive serum GM and negative BAL GM, 6 had negative serum GM and positive BAL GM, and 27 had both serum and BAL GM negative. Median BAL GM value was 1.9 (range; 0.7–3.7) and in 5 BAL GM was >1.0 (83%).
Conclusion: Our results suggest that BAL GM is useful to upgrade diagnosis of IA from possible to probable (25% increase).
Increasing the cut-off of BAL GM from 0.5 to 1 allows for fewer patients to be upgraded to probable IA.
Many high risk patients with clinical signs of IFI have pulmonary lesions that cannot be classified as typical according to 2008 EORTC criteria and positive GM results are extremely difficult to interpret in this setting.

Table 1. Number of patients with galactomannan testing results from BAL and serum, divided into those with and without typical CT lesions according to 2008 EORTC/MSG

Patients with typical lesion on CT (19)				Patients with no typical lesions on CT (42)			
BAL+		BAL–		BAL+		BAL–	
Serum+	Serum–	Serum+	Serum–	Serum+	Serum–	Serum+	Serum–
5	5	1	8	0	6	9	27

P1748 Study of *Trichomonas vaginalis* polymorphism in conditions of *in vitro* infection

A. Ivanov, N. Razdolskaya, O. Gavrilova, A. Krivoruchko, D. Zaslavsky, I. Timochin (St. Petersburg, RU)*

Objectives: Human urogenital parasite *Trichomonas vaginalis* is considered as poorly cultivated organism, appropriate models of infection are absent. Trichomonas can exist in clinical samples in different morphological forms: pearlike motile, amoeboid, and round immotile. Standard microscopic methods of diagnosis are reliable only for pearlike form.
Methods: Clinical isolates were cultivated on MDCK, L-41, and HEP-2 cell lines. Monolayer (10^6 cell/ml) were inoculated by pearlike forms in concentrations 10^2–10^8 cell/ml. Morphology of parasite was investigated by direct observations and May-Grunvald-Gimsa staining.
Results: Culture L-41 appeared the most suitable. Monolayer was destroyed within 24 h, when infection dose was 10^4–10^6 cell/ml, and within 1 h, when it was 10^8 cell/ml. Differences between *T. vaginalis* strains were not significant. Morphological changes were induced immediately after contact with monolayer, within 1 hour all pearlike forms were transformed into amoeboid. In course of transformation cells increased in size, free flagella lost motility, filose pseudopodium were formed, acsostyle reduced, vacuoles appeared in cytoplasm. Staining revealed that 25% of cells was with 2 and more nuclei. In pearlike forms population part of dividing cells consisted only 6%, and multinuclear cells never were detected. Multinuclear cells revealed correspondent kit of free flagella and inner flagella roots. Amoeboid cells can be considered as stage of proliferation with postponed cytokines. After 6 h pseudo plasmodium of amoeboid forms was detected on place of already destroyed monolayer, and round immotile cells appeared in suspension. Round forms were similar with amoeboid ones: immotile free flagella, reduction of aksostyle, vacuoles in cytoplasm, big variety in size, significant percentage of dividing cells. That round forms appeared when amoeboid cells have been lost contact with monolayer. After full destruction of monolayer amoeboid cells were transformed in typical pearlike motile.
Conclusion: Round form of *T. vaginalis* basing on normal morphological structure, ability to reproduce, to induce destruction of monolayer may be considered as virulent, but not degenerative form. Appearance of round forms in clinical samples is a singe of active infection.

P1749 PCR-based diagnosis of human dirofilariosis achieved on samples embedded in paraffin

S. Gabrielli, S. Tasic, M. Magi, G. Cancrini (Rome, IT; Nis, RS; Pisa, IT)*

Objectives: Aim of this paper is to report the successful application of molecular diagnostics to identify filarial DNA extracted from 5 paraffin-embedded nodules.
Methods: Surgically removed nodules were been processed for histological analysis. Then, the haematoxylin-eosin coloured sections and part of the worms embedded in the paraffin blocks were submitted to DNA extraction, following a by us modified DNA isolation protocol, and to PCR assays using primers previously designed.
Results: Morphological features of the sections of the worms, in optimal state of preservation, were indicative of the presence *D. repens;* PCR assays performed on the paraffin blocks had a successful application and confirmed the identifications.
Conclusions: For many years the diagnosis of human dirofilariosis has been achieved by the histological examination of surgically removed nodules, and the identification of the worms was based on the features of the cuticle of adult specimens. However, identification was impossible when the cuticle was altered by regressive phenomena or in the case of infection by larval stages, and it risked of turning out unreliable in areas where many filarial species are present in animal population. Therefore, molecular diagnostics then developed have to be considered the gold method for the undisputable diagnosis of zoonotic filariae in humans. The employment of this technique and of different DNA extraction protocols on histological sections, to date hampered by the effect of the formalin on the DNA quality, could permit the re-examination of archival formal-fixed cases, and the evaluation of the real importance of each zoonotic filarial species in human infections.

P1750 Evaluation of Liaison toxoplasma IgG and IgM: comparison with Vidia and Architect assays

S. Roisin, C. Liesnard, M-L. Delforge (Brussels, BE)*

Objective: Comparison of three automated diagnostic systems, Liaison (Diasorin), Vidia (bioMérieux) and Architect (Abbott) for the determination of *Toxoplasma gondii*-specific immunoglobulin G and M.
Methods: According to Vidia results, 172 serums were classified in 6 groups: negative, past toxoplasmosis, doubtful immunity, IgM persistence, seroconversions, recently acquired toxoplasmosis, potential cross-reactions. *Toxoplasma* IgG and IgM were tested by two automated methods: Liaison and Vidia. Immunofluorescent assay (IFA) was used as a reference technique in case of discordant results. In a second time, 61 of these sera were tested by a third automated method, Architect. IgG avidity index was determined by Liaison and Architect on 31 sera.
Results: IgG: of 152 specimens tested for IgG, a total of 44 samples were classified as non reactive by Vidia, including 43 specimens that were concordantly non reactive on both Vidia and Liaison and one discordant specimen categorized as true non-reactive by IFA. The relative specificity was 97.7% for the Liaison assay.
Of these 152 specimens, 95 were classified as reactive by Vidia. 92 were concordantly reactive on both Vidia and Liaison. Three samples were discordant, IFA classified 2 samples as non reactive and one as doubtful. The relative sensitivity for Liaison assay was 100%.
Of the 13 samples classified as doubtful by Vidia, 8 were negative, 3 positive and 2 doubtful with Liaison.
IgM: for the117 non reactive sera, using a cut-off of 10 AU/mL, the agreement between the two methods was 100%.
Of 13 sera from 5 seroconvertions and 18 sera of recently acquired toxoplasmosis, 2 were negative on Liaison. IFA was positive for these 2 samples.
Of the 17 sera with persistent IgM on Vidia, 14 samples were discordant if the cut-off of 10 AU/mL was used. All these discrepant samples were negative by IFA.
Of the 20 potentially cross-reacting samples, all were negative by both methods.
Of the 61 sera tested with Architect, the agreement between this assay and Liaison was 100% for IgG. Concerning IgM, Architect showed more positive results in the persistent IgM group.
31 samples were tested for IgG avidity index on Architect and Liaison. Three were discordant. For these 3, the results of Liaison were more concordant with the clinical history.
Conclusions: The fully automated Liaison Toxo IgG and IgM assay provided sensitive and specific measurements and showed a good agreement with Vidia and Architect assays.

P1751 Evaluation of the Architect Toxo IgG, IgM and IgG avidity assays

G. Maine, A. Francisco, R. Stricker, R. Stricker (Abbott Park, US; Cham, Geneva, CH)*

Objective: Evaluate the performance of the ARCHITECT Toxo IgG, IgM, and IgG avidity assays in samples from women with documented seroconversion for toxoplasmosis during gestation and in samples from the *Toxoplasma* Serology Laboratory in Palo Alto, CA.
Methods: Archived patient samples (n = 200) selected from pregnant women (n = 47) with documented recent seroconversion for toxoplasmosis were tested and the results compared to the Abbott AxSYM Toxo IgG and IgM assays. Archived patient samples (n = 400) from the *Toxoplasma*

Serology Laboratory were tested and the results compared to the Sabin-Feldman Dye test, the differential agglutination test (AC/HS), and the Vidas Toxo IgG avidity assay.
Results: In samples from pregnant women undergoing seroconversion for toxoplasmosis the ARCHITECT and AxSYM Toxo IgG and IgM assays detected the same first bleed as Toxo IgG and IgM positive or equivocal in 42/47 and 45/47 patients, respectively. In the remaining patients either the ARCHITECT or AxSYM assay detected the patient as positive for Toxo IgG or IgM one bleed earlier. Detection of *Toxoplasma*-specific IgM before IgG occurred in 5/47 patients (11%). The clinical sensitivity of the ARCHITECT Toxo IgG avidity assay using a cutoff of 4 months post-seronegative bleed was (111/112) = 99.1% (95% CI = 95.1–100%). In samples from the *Toxoplasma* Serology Laboratory the agreement between the ARCHITECT Toxo IgG test and the Toxo IgG + IgM tests with the Dye test was (383/392) = 97.7% (95% CI= 95.7–98.9%) and (389/392) = 99.2% (95% CI = 97.8–99.8%), respectively. Agreement between the ARCHITECT and Vidas IgG avidity assays was (229/234) = 97.9% (95% CI = 95.1–99.3%). The agreement between the ARCHITECT and Vidas Toxo IgG avidity assays with the AC/HS test was (231/241) = 95.9% (95% CI = 92.5–98.0%) and (247/258) = 95.7% (95% CI = 92.5–97.9%), respectively.
Conclusion: The performance of the three fully automated Abbott ARCHITECT Toxo immunoassays was equivalent to the reference assays and easier for a laboratory to perform than the Dye test and AC/HS assay.

P1752 Association of plasma levels of serum amyloid in kidney transplant patients with cytomegalovirus infection

C. Steininger, W. Höpler, H. Kerschner, T. Popow-Kraupp, M. Kundi, J. Kletzmayr, S. Schmaldienst, W. Graninger (Vienna, AT)*

Objectives: Cost-effective pre-emptive therapy of cytomegalovirus (CMV) infection in solid-organ transplantation requires frequent screening of patients for active CMV infection with the use of inexpensive rapid diagnostic assays. Serum amyloid A (SAA) is an acute phase protein that was used successfully to identify in paediatric patients acute respiratory virus infections or in HIV-infected patients CMV infections. We evaluated the usefulness of measurement of SAA and high-sensitivity C-reactive protein (hs-CRP) levels in plasma for the identification of kidney transplant patients with CMV infection.
Methods: Plasma samples (n = 132) were collected from 23 CMV-seronegative patients who either received an organ from a CMV-seropositive (D+/R–, n = 16) or a CMV-seronegative donor (D–/R–, n = 7). All samples were screened for the presence of CMV-DNA and increased levels of SAA or hs-CRP.
Results: Primary CMV infection was diagnosed in 16 (100%) of the D+/R– patients and in one (14%) of the D–/R– patients. CMV-DNA was detected in 70 of the 132 (53%) samples and increased SAA levels were detected in 96% of CMV-DNA-positive samples. Levels of SAA correlated well with those of hs-CRP (P = 0.001). However, levels of SAA were increased in almost all available samples (125/132, 95%) and overall, increased levels of SAA were not associated with presence of CMV-DNA (P = 0.430). Some samples negative for CMV-DNA but positive for SAA were collected concomitantly with manifestations of other acute inflammatory disorders including acute kidney rejection (n = 7), or respiratory, gastrointestinal, or urinary tract infections (n = 8).

P1753 High throughput sequencing and proteomics to identify immunogenic proteins of a new pathogen: the dirty genome approach

G. Greub, C. Kebbi, C. Bertelli, F. Collyn, B. Riederer, C. Yersin, A. Croxatto, D. Raoult (Lausanne, CH; Marseille, FR)*

Objectives: With the availability of new generation sequencing technologies, bacterial genome projects have undergone a major boost. Still, chromosome completion needs a costly time-consuming gap closure. However, incomplete genome data may be sufficiently informative to derive the pursued information. For emerging pathogens such as Parachlamydia, lack of release of genome data during gap closure is medically counterproductive. We thus investigated the feasibility of a dirty genome approach, i.e. the use of unfinished genome sequences to develop an ELISA.
Methods: Genomic DNA of *Parachlamydia acanthamoebae* was sequenced using 454 (GS20) and Solexa technologies. Sequences were assembled using Edena and Newbler. Then, an ORFing was performed with Glimmer. In parallel, two-dimensional immunoblots of bacterial proteins were performed with sera of humans and rabbits positive for Parachlamydia. Spots corresponding to immunogenic proteins were selected by computer-assisted matching of the Coomassie blue-stained gel and immunoblots, and further analyzed by mass spectrometry. The identified immunogenic proteins were then expressed and used to elaborate first steps of an ELISA.
Results: Solexa technology produced 1'655'941 short reads of 36bp, which could be assembled in 8'616 contigs. The latter were assembled with the 566'453 GS20 reads in 95 contigs covering more than 97% of the genome.
The protein database derived from the genome sequences enabled to identify 18 different immunogenic proteins. Some of them, such as chaperonin GroEL (Hsp60) and DnaK (Hsp70) were already known to be antigenic. Five proteins represented good candidates for the development of an ELISA since exhibiting significant reactivity to sera taken from humans infected by Parachlamydia and from immunized rabbits but no cross-reactivity to sera from humans infected with *C. pneumoniae, C. psittaci* and negative controls. The potential of two of these five proteins to develop a diagnostic tool was suggested by preliminary ELISA tests.
Conclusions: This work constitutes the proof of principle for a dirty genome approach, i.e. the use of unfinished genome sequences of pathogenic bacteria, coupled with proteomics to rapidly identify new immunogenic proteins useful to develop specific diagnostic tests such as ELISA, immunohistochemistry and direct antigen detection. These diagnostic tools will allow further evaluations of the pathogenic potential of this *Chlamydia*-related obligate intracellular bacterium.

P1754 Diagnostic significance of α-defensins (HNP 1–3) in recognition of chronic periodontitis in adults

A.K. Szkaradkiewicz, T.M. Karpinski, J. Hofman (Poznañ, PL)*

Objectives: Chronic periodontitis represents a complex, multicausal pathology, in which anaerobs of oral cavity microflora (most frequently *P. gingivalis, T. forsythia* and *T. denticola)* play a principal role. However, laboratory diagnosis of periodontitis is difficult since it is based on detection of the above mentioned periodontopathogens using, mostly, molecular methods. Salivary antimicrobial peptides and, in particular, α-defensins (HNP 1–3), seem to play a very important role in the local immune reaction. Considering the above, the studies aimed at analysis of diagnostic suitability of HNP 1–3 detection in full saliva and in sera of patients with chronic periodontitis.
Methods: The studies were conducted on 50 adults persons. Clinical criteria allowed to distinguish two research groups. Group 1 included 25 patients, 35 to 49 years of age, with chronic periodontitis, in 14 of whom moderate (group 1A) and in the remaining 11 severe periodontitis (group 1B) was diagnosed. Group 2 included 25 individuals, 20 to 42 years of age, with healthy parodontium (the control group). The investigated material involved supernatants of centrifuged full saliva and sera. Levels of HNP 1–3 defensins were estimated using Elisa test (Hycult Biotechnology). In statistical analysis the non-parametric Mann–Whitney test was used ($p < 0.05$).
Results: Levels of HNP 1–3 in saliva supernatants in the patients with moderate or severe chronic periodontitis (groups 1A and 1B) amounted to, respectively, 628±378 pg/ml and 784±640 pg/ml while in the control group (group 2) the level was 246±148 pg/ml. The levels obtained in either group 1A or 1B were significantly different than those in group 2 (control group). In turn, sera of patients with moderate (group 1A) and severe chronic periodontitis (group 1B) contained mean concentrations (±SD) of HNP 1–3 defensins of 89±64 pg/ml and 91±41 pg/ml,

respectively. The results did not significantly differ from those in sera of the control group (109±60 pg/ml).
Conclusion: In the course of chronic periodontitis, independently of its advancement, saliva contains elevated levels of α-defensins (HNP 1–3), which seems to reflect local immune response. Therefore, elevated level of HNP 1–3 in saliva may represent a novel diagnostic marker of chronic periodontitis in adults.

P1755 Evaluation of diagnostic cut-off values of immunoglobulin G antibodies against pertussis toxin

S.C. de Greeff, D.W. Notermans, B. Elvers, H.E. de Melker, P. Teunis, J.F. Schellekens (Bilthoven, Groningen, NL)*

Objectives: Measuring IgG antibodies against pertussis toxin (IgG-PT) with ELISA can be used to diagnose pertussis infection; however, cut-off points have not unanimously been determined. We applied binary mixture models to interprete IgG-PT antibodies in diagnostics samples obtained from patients with suspected pertussis using single serum samples and the increase in paired sera, to determine optimal cut-offs.
Methods: IgG-PT levels were used from clinical diagnostic samples between 1st October, 2003 and 31st December, 2007 and assayed with an in-house IgG-PT ELISA that was calibrated with the international FDA lot 3 IgG-PT reference serum. The validity of cut-off levels in a single serum sample was assessed by selecting patients sampled within 100 days after onset of symptoms (n = 11655). Increase in antibody titre was studied in patients with two serum samples where the second sample was taken within 10–28 days after the first sample (n = 2205).
Children eligible for the acellular booster vaccination at four years of age or vaccination with an acellular in infancy were excluded, to avoid potential interference of a vaccine induced IgG-PT rise.
Binary distribution mixtures were fitted to the age stratified data, using the two fitted components to calculate ROC curves and determine optimum cut-off levels for positives in single samples and increase (seroconversion) in paired samples.
Results: Given the distributions of antibody levels, an IgG-PT titre of 79 U/ml yielded the highest sensitivity (96.5%) and specificity (94.4%) as diagnostic cut-off level. Mixtures fitted to separate age categories produced similar results: for the age-groups 1–9, 10–19, 20–39, 40–59 and >60 years a cut-off for IgG-PT titre of 66 U/ml, 92 U/ml, 81 U/ml, 78 U/ml and 107 U/ml respectively yielded the highest sensitivity (97.2, 95.3, 97.2, 96.8, 97.3%) and specificity (95.8, 91.5, 96.1, 96.0, 95.9%). In addition, a 3.4 fold increase in IgG-PT titre in paired serum samples yielded a high diagnostic sensitivity (99.4%) and specificity (99.7%) for all ages.
Conclusion: We show that diagnostic samples for pertussis can be classified into two subpopulations: a highly reactive population (persons with infection) and a baseline population (persons without infection); from these bimodal distributions, cut-offs for absolute values and dynamics of IgG-PT can be derived that are associated with very high diagnostic sensitivity and specificity.

P1756 Cerebrospinal fluid D-lactate concentration in patients with bacterial and viral meningitis

S. Ziankova, V. Semenov, I. Veramei (Vitebsk, BY)*

Goal: The aim of our study was to investigate the possibility of cerebrospinal fluid D-lactate concentration usage for differential diagnosis of bacterial and viral meningitis.
Methods: D-lactate concentration has been defined in 40 patients with bacterial meningitis. Control group has been composed of 69 patients with viral meningitis, 21 patients with acute respiratory tract infections and meningism symptom and 14 patients with noninfectious origin of CNS lesions. Etiology of meningitis was estimated by bacteriological method of examination, LAT and PCR. CSF D-lactate concentration was determined by the spectrophotometric technique in the beginning of disease and later.
Results: CSF D-lactate concentration in patients with bacterial meningitis was significantly higher than in control group ($p < 0.00001$). CSF D-lactate concentration median in patients with bacterial meningitis in the beginning of the disease was 10.5 mg/l (7.24–13.18 mg/l); in patients with viral meningitis – 1.58 mg/l (0.31–3.88 mg/l). Differential diagnostic level of CSF D-lactate is 6.265 mg/l (corresponding sensitivity 83.33% and specificity 93.48%, area under the ROC curve = 0.932; standard error = 0.042; 95% CI = 0.868–0.971; P = 0.0001). In addition, CSF D-lactate concentration of 8.62 mg/l corresponds to 100% specificity. We didn't reveal significant differences in CSF D-lactate concentration in patients with bacterial meningitis of a various aetiology ($p = 0.83$), but CSF D-lactate concentration in patients with the malignant disease course (focal neurologic symptoms and etc.) was significantly higher than in patients without concomitant encephalitis ($p = 0.036$). CSF D-lactate concentration have been reduced for the treatment of acute bacterial meningitis to a 1.28–4.20 times within 2–4 days provided effective antibacterial therapy and raised in 4.62–9.22 times at negative dynamics.
Conclusions: Thus, CSF D-lactate concentration more than 6.265 mg/l may be used as additional criterion of bacterial meningitis diagnostics. A growth of CSF D-lactate concentration for the process of treatment can be an indicator of inefficacy of antibacterial therapy.

P1757 Antibodies to *Bordetella pertussis* antigens measured in saliva

M. Riffelmann, M. Littmann, C. Hülße, C. Wirsing von Koenig (Krefeld, Rostock, DE)*

Objectives: IgG-anti-pertussis toxin (PT) antibodies used in pertussis serology are usually measured in serum samples. Measuring antibodies in saliva samples could offer an alternative.
Methods: 206 adolescent and adult patients with longer lasting coughs were recruited by 19 sentinel physicians (10 general practitioners, 9 paediatricians) in the Rostock (Germany) region between January 2008 and April 2009. Clinical symptoms were recorded. From every patient, a nasopharyngeal swab, a serum sample and a saliva sample was taken. Saliva samples were collected in a standardized manner by a commercially available device (OraSure®). *Bordetella*-DNA *(B. pertussis* and *B. parapertussis)* was detected by real-time PCR in nasopharyngeal swabs. The levels of serum IgG and IgA, and the levels of saliva IgG and IgA were measured by rate nephelometry. IgG- and IgA-antibodies to PT and filamentous hemagglutinin (FHA) were quantified by a standardized ELISA in serum and saliva samples. In serum samples, IgG-anti-PT levels of $\geqslant$100 IU/ml were regarded as indicative of recent contact, and IgG-anti-PT levels of $\leqslant$40 IU/ml were interpretede as not indicative of a recent contact.
Results: In a total of 45 patients (18%) a recent contact to *Bordetella* was substantiated either by serum antibodies alone or also by PCR (n = 12). PCR was more often positive in adolescent patients. Antibodies in saliva could be measured by the standardized ELISA procedure used for serum samples. The antibodies measured in saliva correlated well with the serum concentration, when the total IgG / IgA content of the saliva samples was taken into account. Similar to serum, IgG anti-PT was found to be more often indicative of recent contact than IgA-anti-PT.
Conclusions: Measuring IgG-and IgA-anti-PT antibodies in saliva samples taken with a standardized device and correlated to the total IgG/IgA content of the saliva could offer an alternative to measuring anti-pertussis antibodies in serum samples.

P1758 Peripheral blood RNA gene expression profiling in the patients with community-acquired bacterial meningitis

M. Lill, S. Kõks, U. Soomets, L.C. Schalkwyk, C. Fernandes, I. Lutsar, P. Taba (Tartu, EE; London, UK)*

Objectives: We aimed to describe the genetic pathways activated during the community acquired bacterial meningitis (BM) and healthy controls by using genome-wide RNA expression profiling combined with functional annotation of transcritpional changes.

Study was performed in 21 patients with BM and their data were compared with eighteen age and sex matched healthy controls.
Methods: We included 21 patients (median age 56.1 years) with culture proven BM hospitalised between the 1st of January and 31st of December 2008. The control group consisted of 18 age and sex matched subjects (median age 55.3 years). The blood samples were collected via venepuncture on admission. The RNA was extracted from whole blood, α and β globin mRNA was depleted and gene expression profiling was performed with GeneChip Human Gene 1.0 ST Arrays (Affymetrix, Santa Clara, USA) enabling the analysis of 28,869 genes.
To verify the genechip results, we chose ten genes (CD177, IL1R2, IL18R1, IL18RAP, OLFM4, TLR5, CPA3, FCER1A, IL5RA, IL7R) from the gene expression profiling data and performed further analyse with real-time (RT) PCR. Quantitative RT-PCR successfully verified previously found differences. Gene expression profile data was analysed by Bayesian modelling. To define changed genetic networks functional annotation of enriched gene sets was used.
Results: BM was mainly caused by *Streptococcus* pneumonia (14) followed by *Neisseria meningitidis* (2) and *Streptococcus agalactiae* (2). Comparing the controls with the patients, we identified the significant changes at p values of <0.05 in 8569 genes, after False Discovery Rate (FDR) correction total of 5500 genes remained significant at p value of <0.01. Functional annotation and network analysis indicated that most of the genes were related to activation of inflammatory processes. Next common of the upregulated genes were responsible for allergic reactions and anaphylaxia. Those changes were seen in our study both among the adults and the children.
Conclusion: This study demonstrates a strong functional evidence of the activated immune response. This may indicate that the protective reactions caused by severe and active infection are even too strong.

P1759 Saccharose-stabilized intravenous immune globulin as a cause of false-positive *Aspergillus* galactomannan assay in patients

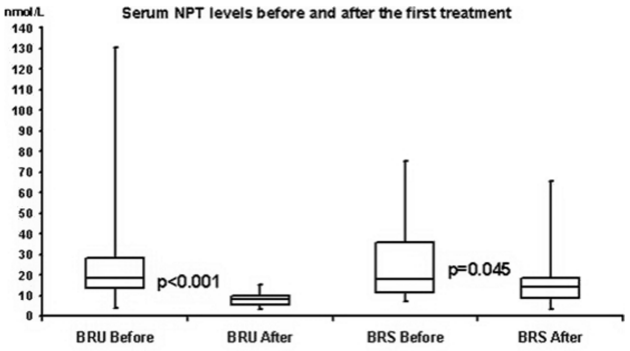
M.E. Bougnoux, H. Coignard, A. Alanio, F. Lanternier, C. Charlier, F. Suarez, E. Catherinot, P. Frange, M. Debré, R. Clement, M. Ben Soltana, M. Lecuit, P. Bourget, O. Lortholary (Paris, FR)*

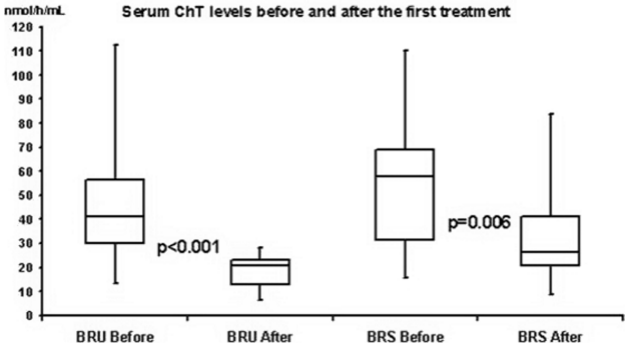
Objective: The detection of galactomannan (galactofuran) antigen (GMA) in serum by the Platelia® *Aspergillus* kit is extensively used for diagnosis of invasive aspergillosis (IA). However, the GMA assay yields a number of false positive results due to cross reactivity. Cross reactivity has already been described with some antibiotics or parenteral nutrition preparations. We observed a recent increase in the frequency of positive tests in patients receiving intravenous immune globulin (IVIG), with no evidence of IA at the time of sampling or during follow up. In this study, we prospectively investigated whether IVIG administration could induce false positive GMA test in 15 patients.
Methods: The sera from 9 adult patients who received saccharose-containing IVIG products (Sa-IVIG) and of 6 pts who received maltose-containing IVIG products (Ma-IVIG) were assayed for the presence of GMA. We also tested for GM (i) batches of IVIG solutions given to each of these patients, (ii) samples of 5 other commercially available IVIG (4 non-sugar- and 1 glucose-containing products), and (iii) each sugar alone at the concentration used to stabilize the IVIG preparations.
Results: GM was detected in sera from 6/9 patients treated by Sa-IVIG (index: 0.7–2), while all sera from patients receiving Ma-IVIG were negative. All Sa-IVIG batches tested yielded positive results (index: 2–7) while the other IGIV solutions were all negative. All saccharose solutions were positive (index: 2–3) while glucose and maltose solutions were negative.
Conclusions: These results demonstrated a strong cross reactivity between the Platelia® kit and Sa-IVIG. These false positive results could be due, in part, to a cross reactivity with saccharose which has a biochemical structure closely related to that of galactofuran which is detected by the assay. Clinicians should be aware of this problem which might lead to inappropriate suspicion of IA.

P1760 Evaluating the validity for using serum neopterin and chitotriosidase levels in follow-up brucellosis patients

Ö. Coskun, S. Öter, H. Yaman, S. Kiliç, C.P. Eyigün (Ankara, TR)*

Due to its high morbidity rates, the systemic inflammatory disease Brucellosis is still an important health problem, particularly in Mediterranean regions. One third of the patients are characterized with musculoskeletal involvement. Principally in chronic cases, there are difficulties in follow-up the success of the treatment. Radiological imaging methods are used in musculoskeletal Brucellosis in addition to standard serological tests. Two macrophage products, namely neopterin (NPT) and chitotriosidase (ChT), are used as novel markers in order to reflect the status of inflammatory diseases. In this study, we aimed to test the validity of these markers in follow-up of Brucellosis patients. A total of 40 Brucellosis cases were included in the study and 27 healthy individuals were used as controls. Twenty of the Brucellosis patients presented involvement of the sacroiliac joint. A 6-week treatment of doxycycline combined with rifampicin or streptomycin was used to treat Brucellosis. Clinical observations and serological outcome was used to decide whether treatment was successful or not. All of the 20 Brucellosis patients without musculoskeletal involvement healed with the first cure of treatment, but all of the *Brucella*-sacroiliitis patients had to be retreated. In addition to routine testing, serum NPT and ChT levels were evaluated after each treatment. The results presented a clear fall in both NPT and ChT levels in parallel to the serological data of the patients. In conclusion, NPT as well as ChT seem to be useful markers in the follow-up of Brucellosis patients and for evaluating the success of therapy.





P1761 Evaluation of cerebrospinal fluid viscosity as a novel diagnostic measure for acute meningitis

F. Yetkin, U. Kayabas, Y. Ersoy, Y. Bayindir, S. Altunisik Toplu, I. Tek (Malatya, Ankara, TR)*

Objectives: In this study we aimed to evaluate the role of CSF viscosity as complemantary measure for diagnosis of meningitis in suspected patients.
Methods: Forty one consecutive patients who undergone lumbar puncture to rule out meningitis were included in the study. The cases

were further categorized into two subgroups: (i) the bacterial meningitis group, whose CSF culture yielded one or more known causative bacteria; and (ii) the aseptic meningitis group, comprising those with negative CSF and blood cultures and recovery without more than 4 days of antibiotic treatment. In the remaining 14 patients diagnosis of meningitis was ruled out. A blood or CSF sample was also placed into a tube containing ethylenediaminetetra-acetic acid (EDTA) anticoagulant for viscosity analysis. Measurements of CSF and plasma viscosity were performed using Brook-field DV-II viscometer (Brookfield, Stoughton, MA, USA).

Results: Among the 41 patients included in the study, 27 patients were diagnosed as meningitis. Of whom 13 patients were aseptic meningitis, 14 patients were bacterial meningitis. Causative agents in bacterial meningitis were found to be *Streptococcus pneumoniae* in 10 (71%) patients, *Neisseria meningitidis* in 2 (14%) patients, *Streptococcus pyogenes* in 1 (7%) patients and *Acinetobacter* spp in 1 (7%) patient. Meningitis was ruled out in 14 patients. CSF protein and CSF viscosity were significantly higher in patients with meningitis compared to non meningitis(Table 1). ROC analysis revealed that CSF viscosity was highly sensitive (100%) and specific (93%) measure for the diagnosis of meningitis in the study population and was comparable to those of CSF protein. Although specificity of plasma viscosity is comparable with CSF protein and viscosity, sensitivity of plasma viscosity was significantly lower than those of CSF protein and viscosity. Additionally patients with meningitis were also divided into two subgroups as having bacterial and aseptic meningitis. CSF viscosity also significantly differed between bacterial and aseptic meningitis.

Conclusion: Although the sensitivity and specificity of the CSF viscosity is comparable to those of CSF protein, CSF viscosity which is a simple and easy method can be used as an adjunctive measure for the diagnosis of meningitis. With the support of further and larger clinical studies CSF viscosity may have a role in the discrimination of bacterial versus aseptic meningitis.

Table 1. Baseline characteristics of patients and cerebrospinal fluid analysis of patients

	Patients without meningitis	Patients with meningitis	P value
Age (year)	49±19	42±15	0.19
Gender (female)	54%	32%	0.19
White blood cell/mm^3	9520±4431	14190±7352	0.058
Erythrocyte sedimentation rate (mm/h)	27±27	55±34	0.006
C-reactive protein (mg/dL)	29±32	120±102	0.005
Plasma viscosity (mpa·s)	1.23±0.06	1.33±0.10	0.002
Cerebrospinal fluid			
Protein (mg/dL)	33.3±10	218±177	<0.001
Glucose (mg/dL)	86±37	50±40	0.001
CSF viscosity (mpa·s)	0.72±0.018	0.84±0.08	<0.001

CSF: Cerebrospinal Fluid.

Automation in the laboratory

P1762 Changing traditional diagnostic medical bacteriology into a lean-automated workflow

M. van Straten, J.J. de Vries, F. Keuren, B.M. Diederen, G. Mulder, D. Veenendaal (Haarlem, Drachten, NL)*

Objective: Most medical microbiological Laboratories (MML) perform bacteriology in which technicians process specimens from start to finish. Some MML improve the work process by lean methodology;efficiency with less costs and labor, conserving high quality. Due to a high difference of specimens and procedures, lean methodology in medical bacteriology is more difficult to implement compared to industrial bacteriology. For this raison we implemented lean-automated bacteriology, supported with a management efficiency tool.

Methods: In September 2008 an automated transportation, inoculation and reading system for bacteriological cultures was implemented in the Public Health laboratory in Haarlem, The Netherlands. This MML services a 550,000 community with 3 teaching hospitals, nursing homes and general practitioners. Per day 500–600 specimens are processed. Culture media are digitally recorded, the digitalized pictures are scored on a computer screen and significant colonies are selected by a software tool for determination and sensitivity testing. The work flow changed from traditional single person to a multi-person multistep controllable workflow. Using lean methodology a scoring tool was developed for efficiency measurement.

Results: Analysis of the traditional workflow showed 4 primary decision and 25 different handling steps. Implementing the automated sample transportation, inoculation and viewing system the number of handling steps reduced to 17 (34% decrease). By spreading decision and handling steps over the day the overall bacteriology laboratory productivity index (i.e. the number of specimens per technicians) increased 25%. Additional overtime by technicians decreased with 33% from 592 in 2007 tot 396 hours in 2009 on a yearly basis. Implementation of automation and changes according lean methodology, further improved the mean variation time of sample throughput.

Conclusions: Implementing automated medical diagnostic bacteriology substantially changes the bacteriological workflow from a difficult to control traditional single work spot to a multistep controllable workflow. A business management software tool is needed to analyze the effectiveness of sample processing including variables as the number of different specimens, culture media, decision and handling steps. Implementing automated bacteriology and changing the workflow according to lean methodology will lead to a controlled continuous improvement of the medical bacteriological workflow.

P1763 Validation of the MicroScan WalkAway-96 for identification of Enterobacteriaceae and detection of β-lactam resistance mechanisms

P. Bogaerts, L. Patteet, M. Ieven, Y. Glupczynski (Yvoir, Antwerp, BE)*

Objectives: Detection of extended-spectrum β-lactamases (ESBL) is a challenging task for diagnostic laboratories. We aimed to evaluate the performance of the MicroScan WalkAway-96 (MScan) instrument (Siemens) for identification and detection of ESBL-producing Enterobacteriaceae (EB) (ESBLE).

Methods: A collection of 180 EB (145 ESBL-, 32 non-ESBL- and 3 carbapenemases-producing isolates) fully identified in the scope of a national Belgian survey in 2008 was used. All isolates were identified by VITEK2 and API20E. Their ESBL status was confirmed by double disk test according to CLSI guidelines. For AmpC producers, boronic acid was used in combination disc synergy tests to confirm the ESBL status. Resistance genes were identified by PCR-sequencing targeting ESBL, carbapenemases, plasmidic and chromosomal AmpC coding genes. Identification and susceptibility testing on MScan were performed with Negative BP Combo 40 panel with integrated ESBL confirmation according to the manufacturer's recommendations. The screening of ESBL-producing microorganisms was based on rules contained within the LabPro Expert System adapted to the current international guidelines.

Results: Of 180 isolates (76 *E. coli*, 36 *K. pneumoniae*, 36 *E. aerogenes,* 16 *E. cloacae*, 7 *K. oxytoca*, 4 *C. freundii* and 5 others) 177 (98%) were correctly identified by MScan.

Regarding the detection of the resistance phenotypes, overall agreement between MScan and reference methods was 81.7%. Among 145 ESBLE, 124 (86%) were detected as ESBL-producers but 4 needed additional testing. Seventeen of 21 (81%) isolates not correctly identified as ESBLs were AmpC producing strains. Among the 32 non ESBL strains, 23 (72%) were correctly assigned as non ESBL isolates including 14 AmpC producers: these required additional testing according to the "possible ESBL" additional remarks by MScan. Nine out of the 32 (28%) non ESBL isolates, (including 5 AmpC producing *E. coli*, 2 K1 *K. oxytoca*, 2 OXA-30 *E. coli*) were however misidentified as ESBLE. For carbapenemases, the 2 MBL and KPC-producing isolates were referred as possible KPC carbapenemase while the OXA-48 expressing isolate was not detected.

Conclusion: Species identification of Enterobacteriaceae by MScan is excellent. Concerning ESBL detection, the system seems more adapted to non-AmpC expressing strains. Differentiation between AmpC and ESBL producing strains remains challenging especially among AmpC producing strains which are often misidentified as ESBLEs.

P1764 Comparative evaluation for automated plate streaking on stool samples using Previ Isola®

S. Zimmermann, M. Trampe (Heidelberg, DE)*

Objectives: We performed this study on automated plate streaking of stool samples using the PREVI Isola® to evaluate the system in terms of sensitivity, quality of the results and time saving compared to the manual method used in our laboratory before.
Methods: A total of 2811 stool samples were analysed within six months. 1358 faecal specimen were plated manually in 3 month (May-July 2009) and 1453 specimen were inoculated by an automated plate streaker (August-October 2009).
For manual streaking the stool was directly plated using a loop, for automated streaking 1g of faeces was diluted in 1.5 ml saline solution (0.45%). The instrument selects the media to inoculate, applies a radical inoculum onto the agar plate and performs a circular streaking (345º) with a disposable applicator. The time measurement for manual and automated performance was recorded for all steps of the procedure.
The suitability of the automated streaking was judged by the technicians as higher, equivalent or lower to the manual streaking (56 samples). A sensitivity test was performed by serial dilutions of the main gastrointestinal pathogens (*Salmonella*, *Shigella*, *Campylobacter* and *Yersinia*) in stool inoculated with both methods.
Over a 3 month period the number of detected bacterial pathogens were analysed in retrospect.
Results: The PREVI Isola® allowed a faster processing of the faeces than the manual method. The suitability of the automated streaking was rated as higher in 48% and as equivalent in 48% of the samples, only 4% of the plates were ranked as lower quality. Serial dilutions of *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* strains in inhibitor free stool of healthy adults resulted in an at least comparable plating efficiency of the automated stool inoculation. Within 1358 stool samples plated manually 12 *Salmonella* species were detected (0.9%) and 3 *Campylobacter* strains (0.2%) between May and July 2009. In 1453 faecal samples processed on the PREVI Isola® (August–October 2009) 24 *Salmonella* species (1.7%) and 6 *Campylobacter* species (0.4%) were found; additionally 2 *Shigella* strains, one Plesiomonas shigelloides and one *Listeria monocytogenes* were isolated.
Conclusion: The PREVI Isola® system seems to produce a higher suitability in streaking of faecal samples. The time saving procedure has a comparable sensitivity for the detection of gastrointestinal bacterial pathogens. A higher detection rate in a retrospective analysis has to be confirmed in further evaluations.

P1765 A new automated test on the Vidas instrument to detect *Escherichia coli* O157 in human stool without enrichment

F. Heskia, P. Mariani-Kurkdjian, S. Martin, J.F. Rey, S. Ait-Ifrane, B. Inçaurgarat (Marcy l'Etoile, Paris, FR)*

Objectives: VIDAS E coli O157 Clinic is an automated ELISA test based on Phage Technology for the qualitative detection of the O157 serogroup (including H7 serotype) directly from human stool. The performance obtained with the VIDAS E coli O157 kit using human stool, without enrichment was evaluated.
Methods: Antibody-based assays can exhibit cross-reactions and non-specific binding resulting in false-positive results. To improve assay specificity, we used bacteriophage recognition elements on the solid phase (VIDAS SPR) responsible for bacterial capture during the ELISA: These recognition elements utilized by bacteriophages to specifically bind to their bacterial hosts are extremely specific as they are essential for phage propagation and survival. To process samples, stools were simply diluted in buffered peptone water. No enrichment was performed. Detection limit and precision studies were performed on artificially spiked human stools. An interference study was performed with 18 of the more common bacteria strains responsible for diarrhoea, including other STEC serogroups (O26, O103, O111, O145). Sensitivity and specificity studies were performed on 149 natural samples routinely collected from *E. coli* National Reference Center in Robert Debré Hospital, Paris, France.
Results: VIDAS E coli O157 displayed a very good detection limit on human stools, i.e 4.5×10^3 CFU/mL. The precision study was performed according to the CLSI document EP5-A2. The total % CV observed was 11.3% for a sample around the cut-off. No interference was observed with bacteria responsible for diarrhoea tested nor with other STEC serogroups. 149 naturally positive and negative human stool samples were tested with the VIDAS E coli O157 assay. Their clinical status for E coli O157 was established following the Robert Debré laboratory algorithm. The VIDAS E coli O157 assay showed a sensitivity of 96.97% and a specificity of 97.41% in comparison to this status.
Conclusion: VIDAS E coli O157 Clinic has several advantages in comparison with classical detection of E coli O157 on SMAC culture. These advantages are processing simplicity (sample dilution, VIDAS reagents and instrument ease of use), quickness (results obtained in 1.5 hour from sample processing to final result), test sensitivity compared to culture, and ease of interpretation (automated result).
The VIDAS E coli O157 Clinic shows good sensitivity with a rapid time to result.

P1766 The yield of pathogens and isolated colonies by automated streaking using the InoqulA®

P.D. Sturm, P.J. Cuppen, C.J. Siebes, S. Kuipers (Nijmegen, NL)*

Objective: Automated streaking by the InoqulA (Kiestra) was evaluated for the yield of (potential) pathogens and isolated colonies.
Methods: Specimens received in the laboratory for routine diagnostics were investigated by standard culture methods with manual streaking (MS). Subsequently, suspensions were prepared from swabs and purulent parts of sputum, in 1 ml saline and 0.5 ml sputolysin, respectively. For automated streaking (AS), 25 microlitre of the suspensions and 10 microlitre of urine was used. Identical culture procedures were followed with respect to culture media, and incubation. Enumeration of growth on urine culture after AS was done by using the extent of growth of isolated colonies on $^1/_4$, $^1/_2$, $^3/_4$, and $^4/_4$ of the agar surface.
Results: In total, 67 swabs, 36 sputum, and 83 urine specimens were evaluated using 283 culture media. Using MS and AS, 171 and 172 potential pathogens were detected, of which 137 and 135 were present as isolated colonies, respectively (Table 1). Of the 11 swab cultures with *S. aureus*, 3 of 22 media showed no growth by AS while 1, 3, and 9 colonies were present by MS. Streptococci and *Pseudomonas* were detected in all cases by both methods. Other Gram negative rods (GNR) were cultured from 22 swabs on 42 media: 8 media showed growth of 1 additional GNR morphology by AS. By MS, 4 and 1 media showed additional growth of 1 and 2 morphologies, respectively. Sputum was cultured on 79 media. *H. influenzae*, *S. pneumoniae*, *Moraxella catarrhalis*, and *S. aureus* were detected on 15 media from 10 sputa by both methods. GNR were detected on 9 media from 5 sputa. With MS, 10 morphologies were detected and 11 with AS. Enumeration of growth from urine after AS correlated well with MS. Of the 51 specimens reported as negative by routine culture, 38 showed no growth with AS, and 12 showed scanty growth of single colonies only on $^1/_4$ of the surface, and 1 on $^1/_2$ of the surface. 32 specimens were reported positive of which 2 contained 10^4–10^5 cfu/ml after MS, with growth on 2/4 and 4/4 of the surface after AS, and 30 contained $>10^5$ cfu/ml with growth after AS on 2/4 in 2 specimens and on 4/4 in 28. In the 32 positive urine specimens, 46 uropathogen morphologies were detected by both methods, and 1 addidtional GNR was detected by AS. With both MS and AS a similar number of pathogens were present as isolated colonies.
Conclusion: AS resulted in similar detection of pathogens as with routine MS. Isolated colonies were obtained equally by both methods.

	Pathogens detected		Isolated colonies	
	MS	AS	MS	AS
Swabs, n = 67; 121 media				
S. aureus	22	19	19	18
Streptococci	7	7	7	7
Pseudomonas	9	9	9	9
GNR	62	64	40	37
Sputum, n = 36; 79 media				
S. pneumoniae	2	2	2	2
H. influenzae	5	5	5	4
M. catarrhalis	3	3	3	3
S. aureus	5	5	4	3
GNR	10	11	7	7
Urine, n = 13; 83 media				
uropathogens	46	47	41	45
Total	171	172	137	135

P1767 Setting a new improved standard for quantization of bacterial growth using the InoqulA

C. Visser, H. Knol, B. de Wever (Amsterdam, NL)*

Objectives: In routine bacteriological diagnostics semi-quantitative cultures are used. The quantization of urine cultures is even more exact since a standard volume is used for inoculation. Recently several automated inoculation systems have been introduced in routine diagnostics. One of these systems is the InoqulA (KIESTRA Lab Automation, the Netherlands). This system differs from the other systems and manual inoculation in using a magnetic bead instead of an inoculating loop or similar device. To determine the quantity of bacterial growth when using this novel inoculation system a new standard had to be set to be able to interpret the amount of growth.
Methods: An 8-fold dilution series of an *Escherichia coli* strain was prepared. 10 microliter of each dilution was inoculated on Orientation agars (Becton Dickinson) and incubated over night at 37ºC O2. Per dilution 4 plates were inoculated; 3 with different patterns on the InoqulA and 1 manually.
Results: See table 1.
Conclusion: A clear difference in amount of growth corresponding to the inoculated numbers of bacteria could be observed. This difference was more distinct on the plates which were inoculated automatically with the InoqulA than on the manually inoculated plates. Quantization is very well possible when using this novel inoculation system. Because the system allows a wide variety of inoculation patterns this standard has to bet set for each different pattern.

Concentration in cfu/ml *Escherichia coli*	Absolute number of bacteria/agar	Result
7×10^{8}	7×10^{6}	Single colonies on last 1/3 of the agar (depending on the pattern). First 2/3 of the agar shows confluent growth
7×10^{7}	7×10^{5}	Idem
7×10^{6}	7×10^{4}	Single colonies on second half of the agar. First half shows confluent growth
7×10^{5}	7×10^{3}	Single colonies up to 2/3 of the agar
7×10^{4}	7×10^{2}	Single colonies on first half of the agar
7×10^{3}	7×10^{1}	Single colonies up to max. 1/3 of the agar
7×10^{8}	7×10^{0}	Only growth on site of inoculation
7×10^{8}	7×10^{-1}	No/scant growth on site of inoculation

P1768 *In vitro* comparison of two blood culture systems for the detection of polymicrobial sepsis

V. Özenci, S. Muhammed, L. Klingspor (Stockholm, SE)*

Objectives: To compare the performance of Bactec aerobic (AE), anaerobic (AN) and mycosis and Bact/ALERT aerobic (FA) and anaerobic (FN) blood culture vials in detection of polymicrobial sepsis caused by yeasts and bacteria.
Methods: A total of 6 reference and 28 clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Candida glabrata* were included in the study. Different polymicrobial models of *C. albicans* + *S. aureus*, *C. albicans* + *E. coli*, *C. glabrata* + *S. aureus*, *C. glabrata* + *E. coli*, *C. albicans* + *C. glabrata* were prepared. Each combination was inoculated in five different blood culture vials. Then the two systems were compared for culture positivity and time to detection (TDT).
Results: Twenty-four mixed cultures with a yeast and a bacteria were prepared and tested in 120 blood culture vials. Mycosis vials from Bactec could detect yeasts in all 24 cultures (100%). The aerobic vials from both Bactec and BacT/Alert could detect both yeasts and bacteria in 22/24 cultures (91.66%). In two mixed cultures Bactec AE vials could detect only yeasts whereas BacT/Alert FA vials could detect only yeasts in one and only bacteria in the other mixed culture. Bactec AN vials could detect both microorganisms in 19/24 (79.16%) and only bacteria in 5/24 (20.83%) mixed cultures. The BacT/Alert FN vials could detect both microorganisms in only 7/24 (29.16%) and only bacteria in 14/24 (58.33%). Seven polymicrobial sepsis models with one *C. albicans* and one *C. glabrata* isolates were also tested. Mycosis vials could detect both yeasts in 7/7 (100%) mixed cultures. The aerobic vials from Bactec and BacT/Alert could detect both yeasts in 3/7 and 2/7 mixed cultures, respectively. The Bactec AN vials could detect both yeasts in 6/7 mixed cultures whereas BacT/Alert FN vials could not detect any yeast growth in 7 mixed cultures tested. The mean + STdev för TDT were significantly shorter in mycosis vials (17.8+1.6 h) compared to all other vials (29.03+4.36 h) in the study.
Conclusion: The present study shows that Bactec and BacT/ALERT have different characteristics in detection of polymicrobial sepsis with yeasts and bacteria. Only Bactec mycosis vials could detect growth of all yeasts in polymicrobial sepsis models tested. The aerobic vials from Bactec and Bact/Alert performed similarly whereas the BacT/Alert FN vials significantly inferior in detection of yeasts compared to Bactec AN vials.

P1769 Molecular versus conventional methods and Vitek2 NH card for identification of fastidious Gram-negative rods in the clinical microbiology laboratory

R. Zbinden, A. Zbinden, J. Schönenberger, G.V. Bloemberg, E.C. Böttger (Zurich, CH)*

Objectives: The use of 16S rRNA gene sequence analysis in the clinical laboratory as a means of identifying fastidious Gram-negative rods (GNR) was evaluated.
Methods: A total of 130 isolates, subdivided into two groups, were analyzed by comparing biochemical identification with that of 16S rRNA gene sequencing; (i) a control group of 84 well-identified clinical isolates, analyzed retrospectively, and (ii) a study group of 46 clinical isolates, collected and analyzed prospectively.
Results: Conventional identification correctly identified 55 of the 130 isolates (42%) to species level, in particular Aggregatibacter aphrophilus, *Cardiobacterium hominis*, *Eikenella corrodens*, Pasteurella multocida, and 22 isolates (17%) correctly to genus level, notably *Capnocytophaga* sp.; 53 of the isolates (41%) were not identified or misidentified. Additional analysis of a subgroup of 81 of the 130 isolates by the colorimetric VITEK 2 NH card (Bio-Mérieux, Marcy-l'Etoile, France) revealed that this system supports the identification of fastidious GNR similar to that of conventional biochemical reactions (39.5% correct identification to species or genus level; 36% incorrect assignment to species or genus level; 24.5% no identification). In comparison, 16S

rRNA gene sequencing correctly identified 117 (90%) of the isolates to species level; of the remaining 13 isolates 12 (9%) were correctly identified to genus level and 1 (1%) to family level.
Conclusion: The 16S rRNA sequencing is an effective means for identification of fastidious GNR in the clinical microbiology laboratory, which are difficult to identify by conventional methods.

P1770 Rapid identification and antimicrobial susceptibility testing of bacterial strains from positive blood cultures with combination system Bactec/SST/Phoenix®

B. Kowalska-Krochmal, I. Dolna, A. Szkarlat, E. Wrzyszcz, S. Balicka (Wroclaw, Cracow, PL)*

Objectives: Rapid microbial diagnostic may accelerate intoduction of accurate antibacterial treatment. The aim of our study was estimation of agreement direct diagnosis of positive blood culture bottles in to Phoenix® automated system (BD) with standard methods.
Methods: 64 monobacterial positive Bactec® (BD) blood cultures bottles (n = 64) were estimated. Positive culture – 8.5 ml was aspirated to vacutainer tube – SST (BD) and centrifugated in a swinging bucket rotor at 2,000g for10 min. After centrifugation supernatant was discarded, bacterial pellet over the gel was resuspensed and inoculated in Phoenix® ID broth to obtain 0.5 MC Farland. According to Gram staining, adequate the Phoenix® ID/AST panels were choosen and performed with agreement of manufacturer's instruction. Results of direct identification and direct susceptibility tests were compared to the standard methods (Phoenix, disk diffusion method and E-test®) with used pure overnight bacterial cultures on the plates. Discrepaciens in the identification were resolved by API® or ID Crystal®.
Results: Among 64 blood cultures 44 were Gram-negative rods and 20 Gram-positive cocci. In 3 cases of Gram-negative rods, both direct and standard identification, was impossible. Direct identification was correct in 87% – 53/61 (among Gram-negative rods – 90% and Gram-positive cocci – 70%). For Gram-negative rods: in direct and standard AST Phoenix® were shown total agreement results for amikacin, cefepime, ceftazidime, imipienem and meropenem, 2 major errors one for gentamicin and one for piperacillin/tazobactam,; in direct method vs disk diffusion test was observed total agreement only for imipenem and meropenem, 1 very major error – cefepime and 5 major errors (gentamicin – 1, cefepime – 1, ceftazidime – 1, piperacillin/tazobactam – 2); in direct method vs E-test respectively total agreement – ceftazidime, imipenem, meropenem; 1 very major error – cefepime. For Gram-positive cocci: direct method was in total agreement with standards methods for vancomycin and teicoplanin; 1 very major error was confirmed for oxacillin.
Conclusions: (1) Direct identification and susceptibility test performed with used combination system (Bactec/ SST/Phoenix® test) was shown as accurate method for Gram-negative rods. (2) Direct method seems to be not acceptable in identification of Gram-positive cocci especially *Staphylococcus* species.

P1771 Comparative evaluation of a new commercial method Vitek 2 vs. CLSI M38-A2 reference method for susceptibility testing of antifungal agents against filamentous fungi

S. Vega, M.T. Gonzalez, A. Romero, A. Martos, C. Castro, A. Gonzalez, E. Martin-Mazuelos (Salamanca, Seville, ES)*

Objectives: The incidence of invasive and opportunistic fungal infections has increased significantly over the past two decades. Emerging fungal pathogens include species of *Aspergillus* and other filamentous fungi as *Fusarium* spp and *Scedosporium* spp. The variable susceptibility to established antifungal agents has created a need for *in vitro* test to determine the susceptibility of clinical isolates of filamentous fungi. Our objective was to compare the automated commercial system VITEK2 (bioMérieux, SA) was compared to CLSI broth microdilution method (M38-A2) for determining the *in vitro* susceptibility to amphotericin B, flucytosine, voriconazole and fluconazole against filamentous fungi.
Methods: A total of 57 clinical isolates were evaluated: 14 *A. fumigatus*, 14 *A. terreus*, 12 *A. flavus*, 11 *Scedosporium* spp and 6 *Fusarium* spp. Quality control was ensured by testing the strains: *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019. A flavus ATCC 204304 and *A. fumigatus* ATCC 204305 were used as reference strains in each set of experiments. The AST-YS01 VITEK 2 system was realized according to manufacturer instructions. Broth microdilution test was performed in accordance with CLSI guidelines (document M38-A2) and read at 48 hours of incubation. The results obtained were expressed as MIC90 (mg/L) and essential agreement within±2 dilutions.
Results: The MIC90 results obtained by the VITEK 2 system for all species and antifungal agents tested were two or three dilutions higher than those obtained by BMD, with the exception of fluconazole.
The overall agreement between both methods against *Aspergillus* spp was 67% for amphotericin B, 60% for flucytosine, 57% for voriconazole and 53% for fluconazole. For *Fusarium* spp, the best agreement was obtained with flucytosine and voriconazole (86%), and the worst results were obtained with amphotericin B (57%) and fluconazole (29%). *Scedosporium* spp showed the best agreement with flucytosine (83%) and voriconazole and fluconazole (70%) but lower concordance was obtained with amphotericin B.
Conclusions:
1. The VITEK 2 system reliably detected fluconazole resistance, as all strains tested showed MIC90 ⩾ 64 mg/L.
2. Good concordance was obtained with *Fusarium* spp and flucytosine and voriconazole, *Aspergillus* spp and amphotericin B and *Scedosporium* spp with flucytosine.
3. More studies with resistant strains are necessary to comprobe the ability of VITEK 2 system to detect antifungal resistance in filamentous fungi.

P1772 A comparative study to determine the recovery rate of micro-organisms of bloodstream infections: two versus three blood culture specimens

T. Shanthachol, G. Suwanpimolkul, C. Suankratay (Bangkok, TH)*

Background: There has been the development of automated and continuous-monitoring blood culture system which is more sensitive for the detection of microorganisms. Whether two or three blood cultures obtained during a 24-hour period using this automated system achieving a higher recovery rate of microorganism remains to be determined. Our study was aimed to compare the recovery rate of microorganism of blood-stream infections using two and three blood culture specimens.
Methods: A prospectively investigator-blinded study was conducted in patients who needed to have blood cultures in Medicine wards and intensive care units as well as emergency room of King Chulalongkorn Memorial Hospital, Bangkok, Thailand between September 2008 and January 2009. Three blood culture specimens were obtained from each patient during a 24-hour period. Each specimen was inoculated into an aerobic bottle of blood culture broth (Trek Diagnostic Systems, Cleveland, OH, USA), and then was incubated at 37OC for 7 days.
Results: Of 210 patients, there were 48 (22.85%) unimicrobial episodes with 3 blood cultures obtained during a 24-hour period. There were 30 (14.28%) and 18 (8.5%) episodes of true pathogen and contaminant, respectively. Nineteen (63.3%), 21 (70%), and 30 (100%) were detected with the first one, two, and three blood culture specimens, respectively ($P < 0.05$ between the recovery rate of the first two and three blood culture specimens). There were 21 (43.75%) and 30 (62.5%) episodes of true pathogen detected with the first two and three blood culture specimens, respectively. There were 33 (68.75%) and 15 (31.25%) isolates of Gram-positive and Gram-negative bacteria. Among 33 Gram-positive bacteria, *Streptococcus pneumoniae* was the most common isolate (5, 15.2%), followed by coagulase-negative *Staphylococcus* (4, 12.1%) and *Enterococcus faecalis* (3, 9.1%). Among 15 Gram-negative bacteria, *Escherichia coli* was the most common isolate (5, 33.3%), followed by *Pseudomonas aeruginosa* (4, 26.7%) and *Klebsiella pneumoniae* (3, 20%).

Conclusions: To the best of our knowledge, our study is the first in Asia to determine the recovery rate of microorganisms of blood-stream infections using the automated blood culture system. Three blood culture specimens are required to achieve the recovery rate of more than 99%.

MALDI-TOF

P1773 Detection of highly pathogenic bacteria by MALDI-TOF MS

M. Blaschitz, L. Meidlinger, U. Sagel, G. Wewalka, F. Allerberger, A. Indra (Vienna, AT)*

Objectives: Highly pathogenic bacteria both acquired by natural infection or after deliberate dissemination cause severe diseases in humans, thus their fast identification is of paramount importance. Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI TOF MS) represents a rapid tool for the species specific identification of microorganisms due to ribosomal protein profiles by mass spectrometry.
Methods: *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella* spp., *Burkholderia mallei* and *B. pseudomallei* were cultivated under Biosafety Level (BSL) 3 conditions. Colonies were picked, inactivated and subsequently tested for survival. Ribosomal proteins were extracted from killed cells and measured by the microflex™ LT MALDI-TOF system. Data were analysed using the Bruker Daltonics Maldi Biotyper 2.0 and Flex Analysis 3.0 software and the reference library 3.0.
Results: The reference library 3.0 had the following entries available: *Bacillus*: 109 species, *Yersinia*: 11 species, *Francisella*: 0, *Brucella*: 0, *Burkholderia*: 25 species. Of 14 samples measured, all except for the Francisella- and *Brucella* samples were correctly identified at the genus level by the microflex™ LT system. *B. anthracis* was identified as probable *B. cereus, Y. pestis* was identified as probable *Y. pseudotuberculosis* and *B. mallei* and pseudomallei were both identified as probable *B. thailandensis* species.
Conclusion: For an accurate identification of highly pathogenic bacteria by MALDI TOF the MALDI Biotyper Security Library would have been an optimal choice as it comprises reference mass spectra of respective bacteria. However, the present study demonstrates that highly pathogenic bacteria are definitely not identified as any other not highly pathogenic microorganism by the microflex™ LT MALDI-TOF system. Since sample cultivation is required prior to a MALDI TOF measurement, prompt DNA isolation from sample material and subsequent real-time PCR currently represent the fastest and most reliable methods for the identification of highly pathogenic bacteria.

P1774 Development and evaluation of automated sample preparation for bacterial identification with MALDI-TOF MS

C. Lang, O. Dubuis, E.H. Viollier (Basel, CH)*

Objectives: Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) allows for identification of bacteria and yeasts within minutes. The weakness of the systems for high throughput is the manual inoculation of the samples on 3 mm spots on the target plates. We aimed at developing a protocol for automated sample application on the target with a pipetting workstation and to compare it to the manual approach that was already used in our routine microbiology laboratory.
Methods: For automated sample preparation bacterial isolates were suspended in distilled H20 to obtain turbidity at McFarland standards between 2 and 4. A Freedom EVO Clinical liquid handling and robotics platform (Tecan, Männedorf, CH) was adapted to apply the samples and the matrix onto the MALDI-TOF target plate. For the conventional method bacterial isolates were directly smeared onto the target plate and matrix was distributed by manual pipetting. Identification was performed with a Microflex LT MALDI-TOF System / Biotyper 2.0 SR1 software (Bruker Daltonics, D-Bremen). Automated pipetting processes as well as parameters such as sample and matrix volume and drying times were optimised in collaboration with Tecan.
Results: By comparing the identification score of manual and automated sample preparation, we found that in 43% of the tested bacteria isolates (n = 127) automated sample preparation resulted in superior identification. Particularly identification of Enterobacteriaceae and other Gram-negative rods rendered higher yields with this method. On the other hand, in 19% of the isolates identification by manual sample preparation lead to better results. Mainly Streptococci were easier to identify by manual sample preparation.
Conclusion: For high sample throughput automated liquid sample preparation is suited for MALDI-TOF MS. The quality of the identification results is comparable to that of the traditional manual method used in our laboratory. The main advantages are standardisation, improved traceability and reduced hands on time. Due to the long drying time (approximately 25 min) of the bacterial suspension there is no significant reduction in sample preparation time. Furthermore, this method is less flexible than the manual approach and not applicable if conventional and extraction (for yeasts and fastidious bacteria) preparation methods are routinely coapplied. This issue could be resolved if the extraction procedure were applied to all samples.

Quality of results manual versus automated sample preparation

	No. of isolates	Better score		Identification only	
		manual	automated	manual	automated
Enterobacteriaceae	64	4	42	2	6
Gram-negative rods (other than Enterobacteriaceae)	17	1	7	1	2
Staphylococci	23	7	2	1	0
Streptococci	12	9	0	7	0
Other bacteria (anaerobes, Gram-positive rods, *Neisseria*)	11	3	3	2	2
Total	127	24	54	13	10

P1775 MALDI Biotyper, experience in routine clinical bacteriology in a university hospital

E. Bessede, M. Angla-Gré, Y. Delagarde, S. Sep Hieng, A. Menard, F. Megraud (Bordeaux, FR)*

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) appears to have the potential to replace conventional techniques in a routine bacteriology laboratory. Its performance still needs to be evaluated on a large scale. Our aim was to compare MALDI-Biotyper identification of bacteria to the conventional phenotypic identification in a large University Hospital Bacteriology laboratory.
Methods: A prospective study was carried out from April to May 2009. Bacterial isolates from clinical samples were identified by conventional phenotypic bacteriological methods (Phoenix, Beckton Dickinson & Api strips, bioMérieux) and in parallel with a mass spectrometer (Ultraflex III TOF/TOF, Bruker Daltonics, Germany). In case of discrepancy between these results at the genus level, a 16S rRNA and /or rpoB gene sequencing was performed.
Results: Of the 1,013 bacteria tested, 98% were correctly identified at the genus level by the MALDI Biotyper vs. 97% with the conventional methods.
The majority of bacteria could be identified without the step of extraction and gave a score >2. It was even possible to correctly identify bacteria with a score slightly lower that 2 when there was matching with the same species profile several times.
The limits of MALDI Biotyper concerned some *Acinetobacter* species (4 times), rare species not yet in the data basis (6 times) and at this stage the subspeciation for *Salmonella enterica*.
Conclusion: The MALDI Biotyper allowed an accurate identification of bacteria, much quicker than conventional phenotypic methods. The integration of this identification method with the antimicrobial testing remains the challenge for the future.

P1776 Two-year experience with MALDI-TOF MS in a routine microbiology department of a laboratory in Germany

C. Boogen, M. Kostrzewa, U. Weller (Cologne, Bremen, DE)*

Objectives: Introduction of MALDI-TOF mass spectrometry is changing the general workflow in a clinical microbiology laboratory, with the expectation of increased speed and accuracy of microbiological results, thereby enabling improved therapy. We report about our experiences during two years after introduction of the MALDI Biotyper (MBT) system in our routine laboratory.
Methods: As a first and fundamental change, after validation of the MBT system, we cancelled all biochemical identifications except for mycoplasma which are not yet in the MBT database. Basic tests such, as oxidase, catalase and Staphyloccous coagulase were disposed as not needed prior to mass spectrometry. Coagulation tests for subtyping of *Shigella*, *Salmonella*, *Yersinia*, *Haemophilus influenzae*, pneumococci and meningococci were continued. As first step to identification always MALDI-TOF profiling is used. We also stopped to rely on colour change of chromogenic media, as this is generally only due to one biochemichal reaction and known as not always reliable.
Results: Introduction of MALDI-TOF MS for microbial identification significantly reduced the usage of consumables in our laboratory. The extensive reference database gives far better results especially for rare microbes, where the biochemical approaches are limited. For instance, *Helicobacter pullorum* now readily can be distinguished from *Campylobacter* sp..
Workload has been considerably reduced for *Salmonella*. The Kligler test prior to sero-subtyping is only needed after an isolate has been identified as *Salmonella* sp. by the MBT. This frequently eliminates the need for subculture. Identification of anaerobic bacteria is speeded up for several days, since from appearance of colonies on the agar plate the bacterium now readily can be identified. Success rate here is at 95%, a 50% increase over our previous biochemichal methods. Yeasts are quickly and reliably identified by the MBT after extracting the cells prior to mass spectrometry with a success rate of >95%.
Conclusion: Introduction of MALDI-TOF mass spectrometry for identification of microorganisms has led to considerable positive changes to our lab since introduced. Not only that it considerably changed our workflow, it also shorted our turn around time, significantly, and improved quality of results. In addition, workload has been reduced and consumption of consumables, too.

P1777 Rapid identification using MALDI-TOF MS for routine bacterial identification

S. Bocher, R. Abdul-Redha (Copenhagen, DK)*

Objectives: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is increasingly used as a rapid method for identification of bacteria in the clinical microbiology laboratories. The objective of the present study was to evaluate bacterial identification using MALDI-TOF-MS, Bruker, Germany testing both bacterial isolates and positive blood culture specimens.
Materials and Methods: 90 bacterial isolates from positive routine blood cultures and 97 positive blood culture specimens were used in the evaluation.
Methods: All bacterial isolates and bacteria/fungi from blood cultures were tested by routine laboratory identification methods, prior to testing in by MALDI-TOF MS. Bacterial/fungal isolates: Matrix preparation was performed as recommended by the manufacturer (Bruker Daltonics, www.bdal.com). A small portion of a freshly grown colony was smeared onto the MALDI steel target, whereafter 1μL matrix was added and MALDI-TOF mass spectra were generated using Bruker microflex LT instrument. Positive blood culture specimens: 4 mL blood culture was transferred to gel containing vacuette tube and centrifuged at 2500 rpm for 10 min, hereby separating blood cells and bacteria. Following discard of supernatant, the sediment was washed in 1 mL deionised water. After further centrifugation proteins were extracted from the bacterial cells using formic acid /acetonitrile. 1 μL of the extract was spotted onto the MALDI steel target and MALDI-TOF mass spectra were generated. Bacteria were identified using the Maldi Biotyper 2.0 software, taking into consideration the score, number of identical identifications, distance to next best taxon match, next best taxon match and the automatic evaluation of the obtained identification.
Results: Considering culture and phenotypic characterization the gold standard, 34 genera and 77 different species were represented among 90 isolates and 97 blood culture specimens. Concordant results, different genus and inconclusive identifications are given in the table. CoNS and streptococci accounted for 31 of 55 inconclusive identifications.
Discussion and Conclusion: This study showed a high concordance between routine bacterial identification and identification by MALDI-TOF, especially the species frequently causing bacteraemia. The speed and price seems promising for future routine examination of as well bacterial isolates as clinical specimens, ensuring fast and correct treatment of patients.

Table 1: MALDI-TOF-MS results from blood specimens and isolates

Genera	No. of strains/positive blood culture specimens examined / genus (species) level	
	Isolates	Positive blood culture specimens
Bacteroides	3/3 (2)	
Candida	1/1 (1)	
Citrobacter	4/4 (3)	
Enterobacter	1/1 (1)	
Enterococcus	4/4 (3)	4/4 (3)
Escherichia	5/5 (5)	5/3 (3)
Haemophilus	2/2 (2)	1/1
Klebsiella	4/4 (4)	
Moraxella	5/5 (5)	
Proteus	2/2 (2)	
Pseudomonas	3/3 (3)	5/5 (5)
Salmonella	1/1	1/1 (1)
Staphylococcus	9/9 (9)	11/11 (11)
Streptococcus	15/15 (9)	15/13 (2)
Other genera	32/17 (11)	5/5 (5)
Total nr./genus (species)	91/76 (60)	73/67 (54)

[a]In brackets: no. of identical identification(s) using MALDI-TOF-MS and phenotype characterization, respectively, on genus/species level.

P1778 Performance of MALDI-TOF MS for the identification of routine and difficult to identify bacterial strains isolated in a clinical microbiology laboratory

A. Bizzini, K. Jaton-Ogay, C. Durussel, J. Bille, G. Greub, G. Prod'hom (Lausanne, CH)*

Objectives: The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) has recently been introduced in diagnostic microbiology laboratories for the identification of isolates from clinical samples. In this study, we prospectively compared MALDI-TOF to conventional phenotypic identification on routine isolates, and retrospectively assessed the ability of MALDI-TOF to replace 16S rRNA sequencing on isolates which were difficult to identify by conventional methods.
Methods: A) Routine isolates: for a 4 week period, all bacteria and yeast isolates retrieved from clinical samples and identified in our laboratory at the species level using conventional methods (Vitek2 or API systems, bioMérieux, France) were tested in parallel using MALDI-TOF (Microflex LT instrument with the BioTyper automation 2.0 software, Bruker Daltonics, Germany). Colonies were analyzed by MALDI-TOF either by direct deposition on the target plate or after a formic acid:acetonitrile extraction step if no valid result was initially obtained. B) Difficult to identify isolates: 500 bacterial samples representing >100 different species which had been sent for 16S rRNA sequencing were selected from a culture collection.
Results: A) Among 1371 isolates identified by conventional methods, 1278 (93.2%) were putatively identified at the species level by MALDI-TOF, 61 (4.4%) at the genus level, and no reliable identification

was obtained in 32 (2.3%). Among the 1278 isolates identified at the species level by MALDI-TOF, 63 (4.9%) discordant results were initially identified. Most discordant results (42/63) were due to systematic database-related taxonomical differences, 14 were explained by poor discrimination of MALDI-TOF spectra obtained and 7 were due to errors in the initial conventional identification. An extraction step was required to obtain a valid MALDI-TOF identification for 25.6% of the 1278 valid isolates. B) Out of 500 samples which had been previously impossible to identify by conventional phenotypic method, ca. 60% were accurately identified at the species level by MALDI-TOF MS.
Conclusions: MALDI-TOF MS is a fast and reliable technique, which has the potential to replace conventional phenotypic identification for most bacterial strains routinely isolated in clinical microbiology laboratories.

P1779 Reassessment of conventional identification of clinical non-fermenting isolates excluding *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* from cystic fibrosis patients using the MALDI-TOF system

A. Fernandez-Olmos, M. García-Castillo, M.I. Morosini, A. Lamas, L. Máiz, R. Cantón on behalf of the CIBERESP*

Different bacterial species recovered from respiratory samples of cystic fibrosis (CF) patients display distinct degrees of pathogenicity thus requiring different clinical management. Correct identification of these bacteria by conventional microbiology methods is often limited due to low biochemical reactivity. Recently, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) that produces specific mass spectral fingerprints for different organisms has been introduced in clinical microbiology laboratories.
Objective: We reassessed historical routine identification results obtained by WIDER (Fco. Soria Melguizo, Spain) and by API 20 NE (bioMérieux, France) systems using MALDI-TOF (MALDI BioTyper 2.0, Bruker Daltonik, Germany).
Methods: 194 isolates identified by conventional methods (65 *Achromobacter* spp., 56 *Burkholderia* spp., 16 *Acinetobacter* spp., 10 non-*aeruginosa Pseudomonas*, 8 *Chryseobacterium* spp., 7 *Bordetella* spp., 4 *Ralstonia* spp., 3 *Ochrobactrum anthropi*, and 25 other non-fermenting Gram-negative bacilli) recovered from routine sputum cultures from CF patients (1994–2009) and stored at –80ºC were re-identified using MALDI-TOF (direct colony material). Discrepancies were resolved with molecular techniques (PCR of 16S rDNA or other specific genes and sequencing).
Results: 83% of isolates displayed identical identification at genus level when using conventional and MALDI-TOF methods although this figure was only of 57% at species level. Lack of identification (6.1% of isolates) with MALDI-TOF was ascribed to insufficient database entries and was resolved by the molecular approach. Moreover, pathogens such as *A. xylosoxidans* (4) and *B. cepacia* complex (6) that were misclassified by routine methods were identified by MALDI-TOF, being also able to differentiate members of the *B. cepacia* complex. Additionally, other bacteria such as *Burkholderia gladioli* (7) and *Pandoraea* spp. (6) that have never been previously identified by conventional methods in our laboratory were correctly identified by MALDI-TOF.
Conclusions: MALDI-TOF is a quite versatile tool that improves rapid identification of bacteria including non-fermenting isolates that usually have limited biochemical reactivity and different morphotypes that lead to misidentification by classical phenotypic means. However, despite its accuracy, MALDI-TOF database needs to be enlarged mainly with infrequent or even rare species recovered from CF patients.

P1780 The experience of a 2-year application of MALDI Biotyper technique in a routine setting

A. Borovskaya, E. Ilina, S. Sidorenko, A. Kruglov, D. Mudrak, T. Savinova, T. Maier, M. Kostrzewa, V. Govorun (Moscow, RU; Bremen, DE)*

Objectives: The burden falls to clinical microbiology laboratories to provide accurate and rapid identification of the pathogens. Many laboratories rely upon automated microbial identification system. Though, there are some examples in the literature of the misidentification by commercial systems because of unusual strains which may lack key phenotypic characters required for their reliable identification. We demonstrated the prolonged using of MALDI Biotyper technique as a rapid, accurate and cheap method for bacterial species identification in routine practice.
Methods: Species identification in the course of two years of routine bacteriological practice for the broad clinical specimen was done in parallel by both traditional microbiological methods and by MALDI Biotyper technique (Bruker Daltonics, Germany). In addition, 16S rRNA gene sequencing for discordant results was performed.
Results: Wide clinical specimens of 820 patients were investigated. 2780 bacterial colonies from 2003 plates (785 primaries, 549 selective plates and 669 plates for susceptibility testing) were tested by MALDI Biotyper technique. The Biotyper ID did succeed for 2764 (99.4%) colonies, in 16 cases (0.6%) the measuring procedure failed because no mass spectra could be acquired. The efficacy of identification was slightly different in different groups (table), and in 126 (4.5%) cases there was no reliable identification (log score <1.7).
For 98 from 110 cases of discordance the correctness of MALDI Biotyper ID was confirmed by 16S rRNA gene sequencing.
Conclusion: The MALDI Biotyper technique demonstrates as suitable for accurate species identification of quantum amount of species prevalence in routine clinical setting with the diagnostic sensitivity 99.4%.

Table. Distribution of MALDI BiotyperTM ID in microbial groups

Group of microorganisms	Log score $\geqslant 2$ species ID	$1.7 \leqslant$ Log score $\leqslant 2$ genus ID
Non-fermenting bacteria	152	54
Coryneform bacteria	73	45
Anaerobic microorganism	2	3
Enterobacteriaceae	623	132
Genus *Staphylococcus*	585	102
Genus *Streptococcus*	249	127
Genus *Enterococcus*	203	27
Other Gram-positive bacteria	59	49
Other Gram-negative bacteria	76	28
Total	2061 (74%)	576 (21%)

P1781 Bacterial identification by Axima Saramis SirWeb MALDI-TOF MS: application in a clinical routine laboratory

O. Dauwalder, H. Meugnier, A.M. Freydiere, N. Baida, Y. Benito, M. Badoz, M. Chomarat, S. Boisset, P. Girardo, M.E. Reverdy, J. Etienne, G. Lina, F. Vandenesch (Bron, FR)*

Objectives: Evaluation of a bacterial identification (BI) by AXIMA SARAMIS SIRWEB-MALDITOF mass spectrometry (MS) in real clinical setting in comparison with a conventional identification by biochemical test systems (CI) and molecular methods (MM) in case of discrepancy.
Materials and Methods: 323 strains prospectively isolated in the laboratory requiring a bacterial identification were included in the study. These strains provided from blood culture (16%), urine sample (29%), sputum (20%) or cutaneous samples (12%), others (23%) and were isolated on

various media (blood agar, chromogenic media) after 24 to 72 hours of incubation. Strains were identified simultaneously by biochemical panels (BP) using Phoenix (Becton Dickinson) or API systems (bioMérieux) and by SM AXIMA (Shimadzu) coupled with the SARAMIS spectral database (Anagnostec) and the SIRWEB-MALDITOF software (I2A) using α-cyano-4-hydroxy-cinnamique matrix. We did a single test for with each method. In case of non-identification by MS, strains were retested with or without formic acid extraction. Discrepancies were resolved by 16S rDNA-sequencing (MM) [PCR/sequencing, le BIBI (www.pbil.univ-lyon1.fr/bibi)].
Results: Of the 323 strains tested, 305 (94.4%) yielded a concordant BI to the species level between MS and CI. For 87.6% of these strains, the BI was obtained within one minute. A second test was required for 23.5% of strains. For 4 strains (1.2%), the MS provided no BI even after formic acid extraction. For 14 strains (4.3%) a discrepant BI was obtained between MS and CI requiring MM methods. Out of these 14 strains, 6 were identified correctly by the MS either to the genus or to the species level while 5 were identified by the CI to the genus or to the species. For the 3 last strains, the BI obtained with CI and MS were different from that obtained by MM.
Conclusion: MALDI-TOF MS method performed equally well with the CI for bacterial identification in our clinical routine laboratory with the advantage to be a low cost and fast method.

P1782 Comparing conventional identification of bacteria to identification with MALDI-TOF in a routine clinical setting

Y. Han, D. Radjenovic, U. Nydegger, M. Wydler, L. Risch, M. Risch (Pjongjang, KP; Liebefeld, CH)*

Background: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF) bacterial identification is in the process of completing, if not replacing bacterial identification by morphological and biochemical techniques (conventional identification, CI).
Methods: In order to assess comparability of the results obtained with MALDI-TOF to CI, we have analysed 204 clinical samples with the following origins: 66 samples were urine specimens, 17 positive blood cultures, 27 genital specimens, 27 wound swabs, 23 fecal specimens and the remainder were 6 sputum and various other samples taken under precaution to avoid commensal contamination. MALDI-TOF was performed with a Bruker microflex system, whereas CI comprised the VITEK II and API system, both bioMérieux.
Results: Of the 204 isolates identified, 72 were Gram-positive and 130 were Gram-negative strains and 2 were yeasts (candida). In 27 samples (13.2%), a disparity of the species and/or genus name could be seen. The disparity appeared with 16 Gram-negative (12.3%) and with 11 Gram-positive germs (15.3%; n.s.). In the latter 6 samples showed the same disagreement with *Streptococcus pneumoniae* (MALDI) and *Streptococcus mitis*/oralis (CI) constellation. Among Gram-negative samples, most instances exhibited a difference on the species level only, e.g. *Enterobacter cloacae* versus Enterbacter kobei. According to genus- and species-differences, we classified the disparities into two disparity levels (1+ and 2+ differences). Twenty-two differences were 1+. In 5 instances, the differences related to genus disparities: *Enterobacter*/*Raoultella*, *Streptococcus*/*Gemella*, *Pseudomonas*/*Burkholderia*, *Microbacter*/*Sphingomonas* and *Candida*/*Cryptococcus*. The most pertinent difference was *Microbacterium arborescens* (MALDI TOF) and *Sphingomonas paucimobilis* (CI).
Conclusion: We conclude, that there is a good comparability between MALDI TOF analysis and conventional identification procedures (86.8%). Differences are mainly due to disparities at the species level. With respect to clinical decision making, our observations direct special attention to streptococcal species.

P1783 Automated detection of mixed cultures of micro-organisms using MALDI-TOF MS

T. Wenzel, S. Klepel, T. Maier, S. Stumpf, B. Wegemann, M. Kostrzewa (Bremen, Leipzig, DE)*

Objectives: MALDI-TOF mass spectrometry fingerprint analysis for identification of microorganisms recently has emerged as a powerful tool in clinical microbiology diagnostics. Starting with a single colony characteristic profile spectra are acquired for an unknown microbe and identification is performed by bioinformatic comparison with a dedicated database. One current drawback of the technology is that in the case of mixed cultures, e.g. in case of contaminations, generally only one of the microorganisms is detected in automated analyses. Sometimes even any identification is hampered by the mix of profiles in the mass spectra. On the other hand, careful evaluation of the mass spectra derived from two or even three different bacteria in a mixture can be unravelled after inspection by eye, frequently. We present a bioinformatic approach to check automatically spectra for the probable existence of such contaminations.
Methods: Mixtures of different bacterial cultures were prepared in different ratios. Further, several blood cultures spiked with different bacteria were prepared. The blood cultures were incubated for several hours at 35 deg C. Next, they were harvested under standard conditions, purified and prepared for MALDI-TOF MS analysis according to a protocol developed in our lab. Bacteria were analysed after extraction using a microflex benchtop mass spectrometer according to standard procedures. Resulting spectra were automatically analysed using the MALDI Biotyper 2.0 software package and, in parallel, with a novel algorithm which is calculating the possibility of a mixed-culture spectrum.
Results: Several two compound-mixed cultures, from artificial mixtures as well as from spiked blood cultures were unambiguously recognised as mixed-cultures using the novel algorithm. For some mixes, in particular from blood culture experiments, only one species was detected or the analysis failed at all. No false mixture alarm appeared from blood cultures, also no wrong identification was observed. Two compound mixed cultures spiking experiments using other body fluids (e.g. human urine) are under current investigation.
Conclusion: The principle applicability of automated mixed culture identification, even for positively flagged blood cultures, could be demonstrated. The novel algorithm enables a rapid spectra check of real-life samples for probable existence of mixed cultures. Potential of further improvement of sensitivity of the method and algorithm is being investigated.

P1784 Rapid and accurate identification of clinical *Campylobacter jejuni* and *Campylobacter coli* isolates with MALDI-TOF MS

E. Leitner, M. Keimel, G. Feierl, A.J. Grisold, L. Masoud, J. Posch, U. Wagner-Eibel, G. Zarfel, E. Marth (Graz, AT)*

Objectives: Within the genus *Campylobacter*, *C. jejuni* and *C. coli* are the main causes of campylobacter enteritis in humans. In routine diagnostic laboratories the differentiation of *Campylobacter* spp. is still a challenge due to their poor biochemical activity. Basically, the hippurate hydrolysis test and indoxyl acetate hydrolysis test are employed for differentiation. The objective of this study was to show the easy, rapid and accurate identification of *Campylobacter* spp out of clinical samples with the Matrix Laser Desorption/Ionisation – Time of Flight (MALDI-TOF)-based method. Furthermore we wanted to verify the uncommon high portion of 12.5% (56 out of 447) of *C. coli* in 2005.
Methods: At least 54 identified *C. coli* isolates; 54 *C. jejuni* isolates and one *C. fetus* isolate collected from the routine diagnostic laboratory in 2005 were re-cultured on blood and modified charcoal cefoperazone desoxycholat agar plates. Additionally, six different *Campylobacter* spp. reference strains were tested. MALDI-TOF target plates were inoculated by applying a small amount of a single colony directly on the target plate in a thin smear. The microbioal film was overlaid with 1µl of matrix solution and air dried. Mass-spectra were collected with MALDI-TOF spectrometer (Bruker Daltonics) and for analysis the MALDI Biotyper

software was employed. For discordant results the hippurate hydrolysis and the indoxyl acetate hydrolysis test were repeated. Additionally, polymerase chain reaction (PCR) was used for verification.
Results: The results showed that the MALDI-TOF MS was correct in 100% of the identifications, including all reference strains and the 109 test strains. Results were independently from the used agar plate types. In 2005, two *C. jejuni* isolates were misidentified with the traditional methods. These MALDI-TOF results were proven with PCR.
Conclusion: High specificity and fast performance make the MALDI-TOF method a progress for identification of *Campylobacter* spp. strains. We were able to identify two hippurate negative *C. jejuni* strains which represent a minority. Furthermore, we confirmed the high portion of *C. coli* isolates at our laboratory in 2005.

P1785 The performance of MALDI-TOF MS in the identification of Enterobacteriaceae and *Pseudomonas aeruginosa* clinical isolates

G.H. Genzel, R. Schaumann, N. Knoop, W. Schellenberger, A.C. Rodloff, K. Eschrich (Leipzig, DE)*

Objectives: We investigated the ability of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify Enterobacteriaceae and *Pseudomonas aeruginosa* as compared to the identification with biochemical reactions. Moreover, we attempted to separate out strains with Extendend Spectrum β-Lactamase (ESBL) and Metallo β-Lactamase (MBL) production.
Methods: To create a reference mass spectra database, 20 strains of *Escherichia coli*, including 11 ESBL-positive strains, 20 *Klebsiella pneumoniae* including 12 ESBL-positive strains and 40 *P. aeruginosa* strains including 15 MBL-positive strains were analyzed. The strains were identified previously by biochemical reactions. MICs were determined by broth microdilution test. ESBL and MBL were detected with appropriate Etest. Subsequently, all strains were analyzed by MALDI-TOF-MS. Peak lists derived from the mass spectra were analyzed by different methods of multivariate statistical analysis. For this classification, support vector machine algorithms turned out to be most powerful. In an alternative approach, for the Identification of ESBL-positive and MBL-positive strains the whole mass spectra were analyzed instead of peak lists.
Results: A reliable reference spectra database was established with 20 *E. coli*, 20 *K. pneumoniae,* and 40 *P. aeruginosa* strains, respectively. ESBL-producing *E. coli* and *K. pneumoniae* strains and MBL-producing *P. aeruginosa* could not be identified unequivocally.
Conclusion: MALDI-TOF-MS has an excellent ability to identify rapidly *E. coli*, *K. pneumoniae* and *P. aeruginosa*. It can therefore serve as a valuable tool for laboratory diagnosis of these pathogens. On the other hand, ESBL and MBL-producing strains could not be distinguished unequivocally.

P1786 New genotypic and phenotypic analyses of clinically-relevant Gram-negative, non-fermenting bacteria: MALDI-TOF MS as a rapid, high-resolution method for identifying and typing micro-organisms

L.A. Svensson, M. Gomila, S.A. Mihaylova, M. Erhard, E. Moore (Göteborg, SE; Palma, ES; Pleven, BG; Potsdam, DE)*

Objectives: Identification of Gram-negative, non-fermenting bacilli, using phenotypic characterization is problematic. Many of the species of this group are frequent nosocomial infectious agents and are ubiquitous in the environment. The aims of this study were to assess the resolving capacities of "house-keeping" gene sequences, including 16S rRNA, atpD, gyrB, recA, rpoB and rpoD, and to compare a multi-locus sequence analysis (MLSA) with matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry analyses for identifying and typing strains of *Achromobacter*, *Bordetella*, *Burkholderia*, *Pseudomonas*, and *Stenotrophomonas* species.
Methods: Genotypic analyses. Type strains of the focus genera and species-complexes, other well-characterised reference strains and selected clinically-relevant strains representing a range of phenotypic and genotypic similarities were included in this study. Partial genes, 16S rRNA, atpD, recA, gyrB and rpoD recA were amplified by PCR and sequenced. DNA-DNA hybridisation analyses were carried out on selected strains for confirmation of species designations.
MALDI-TOF analysis. Bacterial biomass were prepared from cultures on agar medium and analysed by MALDI-TOF MS, in the positive mode, using the SARAMIS software for analysis [1].
Results: MLSA, using the respective house keeping genes were able to differentiate and identify the most closely related species of the analysed taxa and cluster analyses showed similarities of branching order between species that correlated well between different genes. However, different genes were not equally effective in differentiating species of the different genera.
The MALDI-TOF analyses were effective in differentiating the most closely related species of the respective genera. Good correlation was observed between the results of MALDI-TOF MS and MLSA data.
Conclusion: In most cases, clinically-relevant isolates and strains of Gram negative, non-fermenting bacilli exhibited good agreement between the methods of this study. In some cases, strains previously defined as given species were observed to be genotypically more similar to other species, as well as some strains with highly aberrant phenotypes were almost genotypically identical to the type strain. MALDI-TOF identification was very well correlated to the MLSA results, and is a much less expensive and effectively able to reduce identification times by 24–48 hours.
Reference(s)
[1] Vanlaere E et al. J. Microbiol. Meth. 75: 279–286 (2008).

P1787 Preliminary identification of *Salmonella* serovar Enteritidis by MALDI-TOF MS

U. Sagel, C. Kornschober, A. Indra, M. Blaschitz, B. Springer, F. Allerberger (Vienna, AT)*

Objectives: Human infections by *Salmonella enterica* subsp. *enterica* serovar Enteritidis (S. Enteritidis) are often due to the consumption of contaminated eggs or egg-products. Preliminary identification of this serovar may provide public health officers with a rapid indication of the source of infection. As many routine laboratories send their *Salmonella* isolates to reference laboratories for serotyping, definitive reports about the serovar may be delayed. We studied the applicability of matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI TOF MS) as a tool for rapid identification of S. Enteritidis.
Methods: From the most common serovars identified by the Austrian Reference Centre for *Salmonella* we tested 51 isolates (S. Enteritidis: 10, S. Typhimurium: 10, S. Infantis: 10, S. Hadar: 10, S. Saintpaul: 6, S. Agona: 5) using the mass spectrometer microflex™ LT MALDI-TOF (Bruker Daltonics, Bremen, Germany). Isolates were prepared for testing both by using a specific extraction method and by using the more convenient smear method. Using the MALDI Biotyper 2.0 software we analysed the mass spectra for peak differences between serovars and exported the peak list to STATA 10.1 (STATA Corp, College Station, TX, USA) for further statistical analysis. All m/z-values were rounded to±5, m/z-values of <600 were set as 600.
Results: All 51 isolates were correctly identified as *Salmonella* spp. using the MALDI Biotyper Automation Wizard with both preparation methods. S. Enteriditis often revealed peaks at about m/z 3020 and 6040 while other serovars often showed peaks at m/z 3005 and 6010. Thereof we derived the index = intensity at m/z 3020/intensity at m/z 3005 + intensity at m/z 6040/intensity at m/z 6010 Index values >2.0 point at S. Enteritidis, values ⩽2.0 point to other serovars. Setting the threshold to 2.5, sensitivity and specificity were 90% (9/10) and 100% (41/41) if isolates were prepared according to the extraction method, and 70% (7/10) and 100% (41/41) for the smear method, respectively.
Conclusion: Preliminary testing for S. Enteritidis using MALDI TOF MS appears to be feasible for routine laboratories. Our diagnostical

approach needs to be evaluated using more reference strains before application on a regular basis.

P1788 Rapid identification of coagulase negative staphylococci by MALDI-TOF MS in a clinical lab

P. Rosseel, I. Wybo, K. Vandoorslaer, E. Roebben, I. Van Cauwenbergh, A. De Bel, S. Lauwers (Brussels, BE)*

Objective: Coagulase negative Staphylococci (CNS) are important pathogens especially in device-related bloodstream infections. Correct identification is important but neither the standard biochemical identification nor commercial identification systems are able to correctly differentiate CNS at the species level. We evaluated a MALDI-TOF Mass Spectrometry (MALDI-TOF MS) system for the identification of a set of selected clinical strains and reference strains.
Methods: Strains: 69 clinical isolates (26 recent blood isolates, 14 stock strains with atypical biochemical tests, 30 biochemically good typified stock strains already used in an earlier interlaboraty evaluation) and 4 reference strains, representing 10 different species. The included species were *Staphylococcus capitis*, *S. cohnii, S. epidermidis*, *S. haemolyticus, S. hominis*, *S. lugdunensis, S. saprophyticus, S. schleiferi, S. warneri* and *S. saccharolyticus.* MALDI-TOF-MS was performed by using the direct smear method on the Microflex LT® (Bruker®, Germany).
The resulting mass spectra were automatically analyzed by the Biotyper 2.0 Software® and Bruker library® 3.0.1.0. Thresholds for species and genus identification were respectively a score value of $\geqslant$2.0 and $\geqslant$1.7.
All discrepant results were reanalysed by standard biochemical tests, a second MALDI-TOF-MS analysis and/or 16S rRNA sequencing for definitive species assignment.
Results: 78% of the selected strains could be identified up to species level by MALDI-TOF MS compared to 53% with biochemical testing only. Correct genus identification was reached for 96% of the strains.
Almost all included strains of *S. capitis, S. epidermidis*, *S. hominis*, *S. warneri*, *S. haemolyticus* and *S. saprophyticus* were correctly identified up to species level. If we take into account the prevalence of these species during the last 7 years (total number of CNS isolates: 8561), MALDI-TOF MS can identify 91% of our CNS isolates up to species level.
Conclusions: MALDI-TOF MS is a very fast and accurate identification method for CNS that can lead to a significant improvement in speed and accuracy of CNS identifications in a routine lab.

P1789 Identification of *Campylobacter* isolates by MALDI-TOF MS

I. Wybo, A. De Bel, K. Vandoorslaer, J. Breynaert, P. Rosseel, D. Piérard, S. Lauwers (Brussels, BE)*

Objectives: *C. jejuni* en *C. coli* are the most frequent causes of bacterial enteritis and represent 86.2% of *Campylobacter* isolates in our laboratory. Several other *Campylobacter* species are occasionally associated with diarrhoea or bacteraemia in patients with immune deficiency. MALDI-TOF MS is a new tool for fast and reliable identification of bacteria in the routine microbiology setting. In this study, we evaluated the performance of MALDI-TOF MS in species identification of *Campylobacter* isolates.
Methods: Fifty-seven well characterised isolates comprising 12 species were included. For MALDI-TOF MS sample preparation small amounts of intact cells were directly applied onto the target plate and covered by alfa-cyano-4-hydroxycinnamic acid matrix. Identification was performed with Microflex LT mass spectrometer in combination with the MALDI-Biotyper 2.0 software and Reference Library 3.0.1.0 (Bruker Daltonik GmbH, Bremen, Germany). Results were compared to conventional phenotypic tests and whole-cell fatty acid analysis with the Microbial identification System (MIS-Microbial ID, Newark, Del). The MALDI Biotyper output is a log(score) in the range 0–3.0. Thresholds for species and genus identification were respectively a log(score) of $\geqslant$2.0 and $\geqslant$1.7.
Results: Overall 32/57 (56.1%) of isolates were identified at species level: *C. jejuni* (6/6), *C. coli* (8/8), *C. fetus* (4/4), *C. hyointestinalis* (3/3), *C. gracilis* (1/1) *C. lari* (5/6), *C. jejuni* doyli (3/5), *C. upsaliensis* (1/4) and *C. curvus* (1/8). When the MALDI-TOF results for these 32 isolates were compared with our routine identification, no discrepant results were found. Additionally, 11 isolates (19.2%) were identified to genus level: 6 *C. curvus,* 2 *C. jejuni* doyli and 3 *C. upsaliensis* isolates. Fourteen isolates were not identified including 1 *C. lari,* 1 *C. curvus,* 6 *C. consisus,* 4 *C. mucosalis,* 1 *C. rectus* and 1 *C. sputorum* (1). The latter 4 species are not included in the database.
Conclusion: In our study no *Campylobacter* isolates were misidentified by MALDI-TOF MS. Although some isolates belonging to less common species could not be identified, our results suggest that MALDI-TOF MS is an accurate and rapid method for routine identification of *Campylobacter* isolates. Extension of the Reference Library will improve the performance of the system.

P1790 Use of MALDI-TOF MS for species identification of *Bacteroides* and parabacteroides clinical isolates

I. Wybo, A. De Bel, I. Van Cauwenbergh, K. Vandoorslaer, P. Rosseel, D. Piérard, S. Lauwers (Brussels, BE)*

Objectives: *B. fragilis* and related species may cause severe infections of different origin. Phenotypic identification methods require long incubation and it is not always possible to distinguish between closely related species.
The aim of this study was to evaluate the use and value of MALDI-TOF MS in the rapid species identification of *Bacteroides* and Parabacteroides clinical isolates.
Methods: Two reference strains and sixty-four clinical isolates were selected from previous studies. Twenty different species were included. Routine species identification was obtained by classical biochemical tests. For MALDI-TOF MS one colony was smeared directly on the target plate and overlaid by alfa-cyano-4-hydroxycinnamic acid matrix. Identification was performed with Microflex LT mass spectrometer in combination with the MALDI-Biotyper 2.0 software and Reference Library 3.0.1.0 (Bruker Daltonik GmbH, Bremen, Germany). The MALDI Biotyper output is a log(score) in the range 0–3.0. Thresholds for species and genus identification were respectively a log(score)of $\geqslant$2.0 and $\geqslant$1.7.
Results: Overall 43/66 (65%) isolates were identified at species level. MALDI results were identical to routine species ID for 34 isolates. Nine isolates were originally misidentified: repeating of phenotypic testing (n = 6) or 16S rRNA gene sequencing (n = 3) confirmed MALDI ID. All *B. fragilis* (n = 6), *B. thetaiotaomicron* (n = 9) and *P. distasomis* (n = 6) included were identified at species level. These species represent more than 60% of *Bacteroides* and Parabacteroides isolates in our laboratory. Ten (15.2%) isolates were only identified to genus level. Eight strains (12.1%) were not identified (not included in the database or included with <3 reference spectra). In 5 strains (7.6%) no distinction was possible between closely related species *(B. nordii/salyersiae* and *B. vulgatus*/*B. dorei*).
Conclusion: MALDI-TOF MS is a fast and more accurate method for species identification compared to routine biochemical identification. We estimate that 75% of *Bacteroides* and Parabacteroides isolates wil be identified to species level by MALDI-TOF in our laboratory. Further extension of the database will be needed to improve the performance of this methodology.

P1791 Rapid identification of bacteria from positive blood culture bottles by MALDI-TOF MS fingerprinting

M. Christner, H. Rohde, M. Wolters, I. Sobottka, K. Wegscheider, M. Aepfelbacher (Hamburg, DE)*

Objective: To establish and evaluate a method for rapid identification of bacteria from positive blood culture bottles using MALDI-TOF mass spectrometry.
Methods: Bacteria were isolated from positive BACTEC blood culture bottles by differential centrifugation and prepared for mass spectrometry fingerprinting by formic acid extraction. Spectrum acquisition and

identification was performed with a microflex LT mass spectrometer and the MALDI Biotyper 2.0 software from Bruker. Reference identification was established from agar subcultures using well-proven biochemical and molecular typing methods.
Results: During the study period a total of 304 positive aerobic and anaerobic blood culture bottles from our clinical microbiology laboratory were investigated. Samples that grew yeast (8) or showed no sign of microbial growth by conventional processing (3) were excluded from further analysis. Isolates from the remaining study samples constituted a representative collection of bloodstream pathogens. Results from direct mass spectrometry fingerprinting matched reference identification for 262 of 277 monobacterial and 13 of 16 polymicrobial samples. A second isolate was correctly recognized in 4 of 16 mixed cultures (Gram stain: 2 of 16). The method worked equally well for aerobic and anaerobic culture bottles and was only slightly influenced by culture age. Misidentifications mostly resulted from insufficient bacterial numbers after sample preparation and were almost exclusively limited to Gram-positive isolates. Incorrect direct identification results could be reliably rejected by identification score cut-offs or tests for the bacterial origin of matched signals.
Conclusions: Combined with an efficient procedure for the preparation of bacteria from positive culture bottles, mass spectrometry fingerprinting is a reliable tool for the rapid identification of bloodstream pathogens. Compared to conventional identification from solid media subcultures, time to result is reduced by at least one workday. Providing species-level identification not later than one day after laboratory entry for 80% of our samples, the approach could facilitate targeted treatment optimizations within the critical phase of septic illness.

P1792 High-speed blood culture diagnostic with MALDI-TOF MS

C. Litfin, A. Sohns, A. Koch (Karlsruhe, DE)*

Objectives: Rapid identification of bacteria from blood cultures is essential for an early appropriate therapy in patients with bacteremia. Usually, time consuming subcultivation is needed prior to identification based on biochemical reactions. Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) is an approved method for rapid identification of microorganisms [1]. We evaluated a sample preparation method to obtain identification within 4 hours after detection of bacterial growth directly from blood culture bottles.
Methods: From 646 positive blood cultures (BacT/ALERT®, bioMérieux), containing Gram-positive (N = 364) or Gram-negative (N = 282) bacteria, an aliquot is enriched in brain-heart-infusion. 1 ml of the supernatant is spinned down and the pellet is resuspended in 70% ethanol before the next centrifugation step. The resulting pellet is then treated with formic acid and sonificated. For the last centrifugation step acetonitrile is added. 1 µl of this supernatant is applied on the MALDI-TOF target and HCCA (α-Cyano-4-hydroxycinnamic acid) matrix is added to perform MALDI-TOF MS with a Microflex LT (Bruker Daltonics). Results are compared to those obtained on basis of biochemical and agglutination reactions (MicroScan® WalkAway®, Siemens and API® strips, bioMérieux).
Results: 273 Gram-positive and 270 Gram-negative bacteria provide a result within 4 hours after detection of bacterial growth. 90.8% of the Gram-positives and 98.5% of the Gram-negatives are identified correctly on species level. The positive predictive value is 100% for all tested coagulase negative staphylococci, *Staphylococcus aureus*, enterococci, group A and B streptococci and nearly all Gram-negative rods. Other streptococci are identified correctly on genus level, but cannot be distinguished reliably and require additional identification steps.
Conclusion: The described method provides fast same day identification of bacteria directly from positive blood cultures. This identification procedure allows earlier species specific treatment, resulting in a better patient outcome and cost savings because of early deescalation therapy.
Reference(s)
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P1793 Use of tubes equipped of separating gels for MALDI-TOF assisted bacterial identification in blood cultures

G. Prévost, W. Moussaoui, B. Jaulhac, A. Hoffmann, B. Ludes, M. Kostrzevra, P. Riegel (Strasbourg, Bremen, FR)*

Introduction: Matrix-Assisted Laser Desorption/Ionization – Time Of Flight or MALDI-TOF now represents an attractive challenge for bacterial identification [1, 2], because of the technological and medical interests to provide rapid and cost-effective bacterial identification. The application to blood cultures would constitute an advantage for care of patients and sometimes for therapy.
Materials and Methods: Positive blood cultures were harvested over three months at the Strasbourg University Hospital. Were positive, samples of 1.5 mL of the medium was transferred into tubes containing 1 mL of separating gel. After centrifugation, bacteria present at the gel surface were suspended and recovered, before to be pelleted again and further treated for whole proteins extraction. Mass spectra of most abundant proteins were established onto a BiFlexII – Compass 1.2, Bruker Daltonics, Bremen, GE) as recommanded [1, 2]. Bacterial identifications were then obtained by using BioTyper 1.2 software (Bruker Daltonics).
Results: By including 24 negative controls, 526 samples were considered. Eleven of these samples contained yeast, in 29 cases where genus were only obtained while 555 bacteria could be identified. Thus, compared to the routine, including 21 polymicrobial samples leading to 50 bacteria, MALDI identification was effective for 499 bacteria (89.72%) distributed in 378 with good scores (1.9–3.0), and 72 with middle-ranged scores. For other good identifications, at least the first three identification proposals were identical. Concerning polymicrobial samples, MALDI-assisted identification allowed 22 bacteria to be characterized, according to presence of species ratio. All together, a better identification of Gram-negative bacteria (95% vs 86%) was obtained versus Gram-positive bacteria.
Conclusion: MALDI-TOF-assisted bacterial identification can be applied to blood cultures, and may bring species diagnosis in >90% of the samples, before bacterial culture on agar media. This results can increase the analytical information given to physicians.
Reference(s)
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[2] Mellmann A, et al. (2009) High inter-laboratory reproducibility of MALDI-TOF mass spectrmetry-based species identification of nonfermenting bacteria. J Clin Micorbiol, 47: 3732–4.

P1794 Urinary tract pathogens direct identification from urine samples by MALDI-TOF MS

L. Ferreira, F. Sánchez Juanes, M. González Ávila, D. Cembrero Fuciños, A. Herrero Hernández, J.M. González-Buitrago Arriero, J.L. Muñoz Bellido (Salamanca, ES)*

Objectives: MALDI-TOF mass spectrometry (MS) has been suggested as a fast and reliable method for bacterial identification from culture. Direct analysis of clinical samples may increase the usefulness of this method, since can shorten the time for microorganism identification. We compared conventional methods for UTI diagnosis and urinary tract pathogens identification (automated screening and plate culture) and a fast method based on conventional screening plus MALDI-TOF MS.
Methods: 260 urine samples from outpatients and inpatients with symptoms of UTI, reported as positive by an automated screening device based on flow cytometry (UF-1000i) were processed. Positives samples were studied by culture and conventional identification, and by MALDI-TOF MS.
Results: The screening device reported as positive 20 samples that were negative in culture. All these cases were also negative in MALDI-TOF. Five samples positive in the screening were reported as contaminated in the culture. In these samples, MALDI-TOF reported a non reliable

identification in 2 cases, but reported microorganism identification in 3 cases. 235 samples reported as positive by the screening device, led to significant bacterial growth in culture. 220 of them (93.6%) had bacterial counts (BC) >100,000 CFU/mL, 7 (3.0%) had BC between 50.000 and 100.000 CFU/mL, and 8 (3.4%) had BC <50,000 CFU/mL. In whole, MALDI-TOF MS identified correctly the microorganism involved in the UTI, directly from the urine sample, in 91.8% of cases at the species level, and at 92.7% at the genus level. Failures were observed in only 6.9% of samples. The most frequent microorganism isolated was *E. coli* (173 isolates). MALDI-TOF ME identified the microorganism in 163 cases (94.2%) (97.6%. when BC was >100,000 CFU/mL).
Conclusion: MALDI-TOF MS gives a realiable identification of UTI pathogens, previously to culture, in >90% of positive samples. MALDI-TOF performance is optimal when there is a high BCs and Gram negatives are involved.

P1795 MALDI-TOF ICMS: capability, potentiality and limits in the fast identification of *Trichophyton rubrum* from clinical cases occurrence in Portuguese health centres

L. Pereira, N. Dias, C. Santos, N. Lima (Braga, Gandra, PT)*

Objective: *Trichophyton rubrum* is presently the most common worldwide pathogen causing dermatophytoses such as tinea corporis, tinea capitis, tinea pedis, and onychomycosis [1]. The main aim of the present work was assess MALDI-TOF ICMS as a fast and reliable technique in the identification of *T. rubrum* from clinical cases occurrence in the Portuguese health centres, and evaluates the potentialities and limits of this new microbial identification technique on the taxonomy of these infectious dermatophytes.
Methods: Fungi were grown for 10 days in solid medium (SDA, Sabouraud Dextrose Agar) and then the mycelia were direct transferred from the SDA plate to the MALDI stainless steel template and mixed with 1 ml MALDI matrix solution (75 mg/ml 2,5-dihydroxybenzoic acid in ethanol/water/acetonitrile [1:1:1] with 0.03% trifluoroacetic acid). The sample mixtures were air dried at room temperature. The analyses were performed in our laboratory on an Axima LNR system (Kratos Analytical, Shimadzu, Manchester, UK) equipped with a nitrogen laser (337 nm). The mass range from m/z = 2,000 to 20,000 Da was recorded. *Escherichia coli* strain DH5α with known mass values of ribosomal proteins was used for external calibration. The fungi classification was performed on the SARAMIS software (AnagnosTec mbH, Potsdam-Golm, Germany). Molecular biology was used when appropriated with PCR based-technology. The presence of a 203-bp PCR product confirmed *T. rubrum* identification.
Results: All strains were accurately and consistently identified as *T. rubrum* by MALDI-TOF ICMS combined to SARAMIS database analysis. Spectral mass analysis proven to be a rapid method since the analysis took only a few minutes to perform with the benefit of any laborious sample preparation procedures or any expensive chemical reagent was needed.
Conclusions: The fungal spectral analysis by MALDI-TOF ICMS was as good as molecular biology in order to identify *T. rubrum* but much faster and cheaper.
Reference(s)
[1] Degreef, H. (2008) Clinical Forms of Dermatophytosis (Ringworm Infection) Mycopathologia 166: 257–265.

P1796 A MALDI-TOF assay for the rapid identification of *Aspergillus* and *Candida* sp. in clinical samples

L. Putignani, L. Mancinelli, F. Del Chierico, M. Onori, L. Coltella, P. Bernaschi, E. Fiscarelli, M. Argentieri, L. Pansani, S. Ranno, B. Lucignano, L. Dimiziani, C. Russo, D. Menichella (Rome, Macerata, IT)*

Objectives: Invasive mould infections are becoming worldwide increasing, resulting in significant morbidity and mortality especially in paediatric populations, immunocompromised and transplanted patients. Particularly the phyla Ascomycota, and Basidiomycota are involved in severe infections in children. The Ascomycota includes yeasts of the genera Saccharomyces, Pichia, and *Candida* and many filamentous fungi of the genera *Aspergillus*, Penicillium and *Fusarium*. The Basidiomycota *Cryptococcus neoformans* is an emerging opportunistic human pathogen. Identification of the increasing diversity of fungal pathogens by conventional methods (biochemical methods for yeast and phenotypic methods for mould) is often difficult, time-consuming and frequently, for unusual fungi, inconclusive.
In our study, we exploited the MALDI-ToF MS (Matrix-assisted laser description ionization-time of flight-mass spectrometry) Biotyper to for rapid and specific identification of fungal species from clinical specimens.
Methods: 270 yeast and 80 mould fungi strains were collected from diversified clinical samples in our microbiology unit and grown on Sabouraud solid medium. Protein profiles were provided by using MALDI-ToF MS Biotyper for both clinical and reference strains, the latest purchased from the Centraalbureau voor Schimmelcultures (CBS) culture collection. Generated spectra were acquired and processed to produce appropriate species identification.
The results were compared to conventional biochemical and phenotyping identifications in order to produce concordance data. When appropriate, MALDI-TOF-based results were compared to sequencing-based identification analysis (D2 LSU 28S rDNA).
Results: MALDI-Tof MS provided the correct yeast identification with a sensitivity of 95% and specificity of 94% compared to conventional methods. For mould identification the MALDI-ToF MS provided a 80% of sensitivity, due to the absence of appropriate reference species in the database. The MALDI-ToF-MS identifications provided 100% of concordance with the 28S rDNA D2-LSU-based sequencing analysis for all the analysed isolates.
Conclusions: This study provides a fast and reliable method, based on MALDI-ToF MS detection, for the identification of fungal species in clinical samples. This procedure might overcome current diagnostic tests and may represent a new frontier for the rapid and specific management of fungal infections in paediatric patients.

P1797 Identification of clinical fungi by MALDI-TOF MS: how to deal with growth-dependent variability in peak patterns

M. Erhard, U. Hipler, F. Seyfarth, Y. Gräser, M. Welker (Potsdam, Jena, Berlin, DE)*

The identification of microorganisms by MALDI-TOF MS is about to replace biochemical identification procedures for routine diagnostics. While the mass spectral identification of most bacteria is straightforward, the identification of fungi with whole cell MALDI-TOF MS is more challenging for several reasons. Most importantly, the peak pattern of an individual isolate can change dramatically in dependence of incubation time and medium composition. Especially the transition from non-sporulating to sporulating mycelium generally results in marked differences in mass fingerprints.
One option to overcome this difficulty is to strictly standardize the cultivation conditions of reference and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, particular medium demands, and handling requirements of individual isolates. Another strategy is to obtain reference data from well characterized isolates for different growth conditions. The latter strategy is followed for the Spectral Archive and Microbial Identification System (SARAMIS) by the acquisition of whole cell mass spectra of reference isolates grown on a variety of solid media and at different incubation times. Generally, reference isolates are incubated on three different media and mycelium samples are taken after three different incubation times. By this 3 x 3 approach the variability of mass fingerprints of individual isolates is largely captured and the mass spectra are deposited in the reference database. When multiple isolates of a species are contained in the database, the corresponding data were used to compute SuperSpectra for fully automated identification.

SARAMIS allows the rapid, automated identification of most clinically relevant fungi by direct on-target smear preparation of fresh mycelium taken from agar plates. Examples will be presented for the identification of dermatophytes, yeasts, *Aspergillus* sp., and *Fusarium* sp.

P1798 Comparison of 4 different commercial identification methods and antibiotic susceptibility testing on clinical relevant coryneform bacteria

I. Geerts, A. Smismans, R. Cartuyvels, H. De Beenhouwer, J. Verhaegen, E. Verhoye, J. Frans (Bonheiden, Hasselt, Aalst, Leuven, BE)*

Objectives: Identification of coryneforms is hampered by poorly discriminative biochemical reactions. The aim of this study is to compare 4 commercially available identification methods with 16S rRNA gene sequencing as gold standard. *In vitro* susceptibility was assessed by both MIC determinations (CLSI, M45-P) and disk diffusion for 9 clinically relevant antibiotics.
Methods: A total of 53 clinical isolates of *Corynebacterium* spp. and related genera (22 different species) from clinically significant infections was collected in two Belgian hospitals from november 2008 to may 2009. The strains were tested with API® Coryne strip (bioMérieux), BBL Crystal™ GP system (BD), Vitek 2 ANC card (bioMérieux) and MALDI-TOF (Bruker). Genotyping was performed by sequencing of the 16S ribosomal region.
Antimicrobial susceptibility was performed on Mueller-Hinton agar supplemented with 5% horse blood with both disk diffusion (Rosco Diagnostica) and MIC determination (E-test, bioMérieux). The plates were inoculated with a 0.5 McFarland suspension and incubated in 5% CO_2 at 36ºC for 24–48 h.
Results: Forty-six strains were successfully sequenced. The remaining 7 strains were not retained for the evaluation of the identification methods. MALDI-TOF accurately identified 43 out of the 46 strains (93.5%) to the genus and 38 strains (82.6%) to the species level. API® Coryne, BBL Crystal™ GP system and Vitek 2 ANC card were able to identify respectively 37 (80.4%), 29 (63%), 36 (78.3%) strains to the genus and 18 (39%), 3 (6.5%), 20 (43.5%) strains to the species level.
All isolates (n = 53) were sensitive to vancomycin, linezolid and doxycycline. Sixty-eight % appeared resistant to clindamycin and erythromycin, 62.3% to ciprofloxacin, 47.2% to levofloxacin, 32% to trimetoprim-sulphamethoxazole (TMP-SXT) and 15% to penicillin. Comparison between disk diffusion and MIC determination revealed 15 minor, 6 major and 7 very major errors. The very major errors were related to erythromycin (n = 2), ciprofloxacin (n = 1), levofloxacin (n = 3) and TMP-SXT (n = 1).
Conclusions: Coryneform bacteria can be reliable identified by the MALDI-TOF. API® Coryne, BBL Crystal™ GP system and Vitek 2 ANC yielded erroneous results in 20–30% of the isolates. Antibiotic susceptibility testing is inaccurate by disk diffusion method and should rely on MIC determinations.

Table 1: Correctly identified coryneforms by the different identification methods

Strain (No.)	MALDI-TOF	API Coryne strip	BBL Crystal GP	Vitek 2 ANC
C. amycolatum (11)	11	6	0	10
C. glocuronolyticum (5)	4	5	0	0
C. urealyticum (3)	3	3	0	3
C. ulcerans (2)	2	1	1	1
C. propinquum (2)	2	0	1	0
C. aurimucosum (2)	1	0	0	0
C. xerosis (2)	0	0	0	0
C. coyleae (2)	2	0	0	0
Other (17)	13	3	1	6

P1799 Rapid species identification and differentiation of *Arcobacter*, *Helicobacter* and *Campylobacter* by MALDI-TOF MS analysis and its clinical application

M. Alispahic, K. Hummel, D. Jandreski-Cvetkovic, K. Nöbauer, E. Razzazi-Fazeli, M. Hess, C. Hess (Vienna, AT)*

Objectives: Identification and differentiation of species belonging to the *Arcobacter*, *Helicobacter* and *Campylobacter* genera has become increasingly important, since many of them are recognised as human and/or animal pathogens. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was shown to be a rapid and sensitive method for charcterisation of microorganisms. In this study, we established a reference database of selected *Arcobacter*, *Helicobacter* and *Campylobacter* species. The second objective was to use the reference database to identify 144 clinical isolates and compare the results to molecular method. The third objective was to evaluate the reproducibility using different growth media and age of bacteria, which are relevant in a diagnostic lab.
Methods: Besides the species with significance as food-borne pathogens – *Arcobacter* butzleri, *Helicobacter pullorum*, *Campylobacter jejuni* and *Campylobacter coli* – several other members of these genera were included in the reference library, grown on Columbia agar. In parallel to MALDI Biotyper 1.1 software package (Bruker Daltonik) identification, all strains were analysed by PCR-RFLP.
Results: Strains that made up for the reference database library yielded reproducible and unique mass spectra profiles, which were compared with the Bruker Biotyper database, version 2. It was found that correct identification could be obtained even if the bacteria are stored at room temperature or at +4ºC up to 9 days until being tested. In addition, bacteria were correctly identified when grown on Campylosel-agar, however not when grown on modified charcoal cefoperazone deoxycholate agar. All field isolates gave sufficient spectra for species identification. In total, MALDI-TOF MS identified all 144 clinical isolates at species level.
Conclusion: Altogether, these data show that MALDI-TOF MS fingerprinting is a fast and reliable method for the identification of *Arcobacter* and *Helicobacter* species and their distinction from phenotypically similar *Campylobacter* species with applications in clinical diagnostics. As a result, considering the speed with which reliable identification can be obtained, this technique is well suited for large-scale research and diagnostic analyses.

Molecular tools to diagnose meningitis, sepsis, respiratory tract and bone and joint infections

P1800 Molecular diagnosis of meningococcal meningitis; factors influencing genomic *Neisseria meningitidis* bacterial load within cerebrospinal fluid

T. Darton, M. Guiver, S. Naylor, D. Jack, E. Kaczmarski, R. Borrow, R. Read (Sheffield, Manchester, UK)*

Objective: Polymerase chain reaction (PCR) detection of *Neisseria meningitidis* (Nm) in patients with presumed meningococcal disease has greatly increased diagnostic yield and allows accurate quantification of bacterial genome copies within invasive sites. We sought to identify factors associated with a positive Nm PCR within the cerebrospinal fluid (CSF) of patients with meningococcal disease and the factors that influence the detectable copy number.
Methods: Nm DNA quantification by Taqman PCR was conducted on CSF samples from patients presenting with meningococcal meningitis to hospitals throughout England and Wales during the period 1999–2004, and correlated using multivariate regression to patient, clinical and microbiological variables. Bacterial load (BL) data are log transformed.
Results: The National Reference Laboratory received 383 CSF samples over 5-years from patients with subsequently confirmed meningococcal disease. Of the samples submitted 132 (35%) were Nm PCR positive. Individuals with a positive CSF PCR were at no greater risk of death compared with those with a negative PCR (3.8% vs. 2.4%, p=NS), but those who had a CSF sample sent were at significantly less risk of death when compared to all individuals presenting with MD (n = 1486) (2.9% vs. 10.6%; $p < 0.0001$, chi-square).
The median time from admission to hospital until sample receipt by the laboratory was <24 hours (range 0–25 days). Time delay had no effect on likelihood of PCR positivity or on BL measured. Factors associated with a positive PCR by multivariate regression included serogroup B infection

(B = 1.71, p < 0.0001), older age (B = 0.022, p = 0.002) and antibiotic receipt prior to hospital admission (B = 1.205, p = 0.043).
The median CSF BL was 5.97 copies/ml (IQR, 4.99–7.26). The respective plasma BL for this population was 3.40 (IQR, 2.70–5.63). The CSF BL was significantly greater in individuals with serogroup B than with serogroup C infection (median BL 6.01 vs. 5.09; p = 0.02, Mann–Whitney). There was no correlation between the CSF and plasma BL in individuals with serogroup B infection (p=NS, Pearson's correlation). In serogroup C infection there was a tendency towards a direct positive correlation (r = 0.241; p = 0.074, Pearson's correlation).
Conclusions: These data suggest that in meningococcal meningitis, there is strain to strain variation in penetration of or replication within CSF, and that translocation of bacteria from blood to CSF does not follow a concentration gradient.

P1801 Severe *Mycoplasma pneumoniae* respiratory tract infections in children, suspected on clinical ground, are confirmed by laboratory tests

A. Marangoni, F. Delucca, R. Bergamaschi, D. Zama, F. Specchia, S. Accardo, A. Moroni, R. Cevenini (Bologna, IT)*

Objectives: About 15%-20% of cases of community-acquired pneumonias are estimated to be due to *Mycoplasma pneumoniae*, whereas 1% to 5% of *M. pneumoniae* pneumonias may require hospitalization for respiratory distress. In these cases, a specific diagnosis is essential for the choose of the antimicrobial treatment since β-lactam antibiotics, often delivered to young outpatients with pneumonias, are ineffective against *M. pneumoniae.*
In this study, children hospitalized for suspected pneumonia not-responder to the treatment with β-lactam antibiotics, were studied in order to etiologically diagnose *M. pneumoniae* infection, both by polymerase chain reaction (PCR) and serology.
Methods: Study group. From January 2009 to October 2009 two paired samples (a nasopharingeal aspirate specimen and a serum specimen) were taken on hospital admission from children hospitalized for suspected pneumonia diagnosed on clinical ground and confirmed by radiology. Specimens were obtained from a total of 61 children (aged between 1–14, mean age 5.5) hospitalized at S. Orsola Hospital, Bologna, Italy, during the study period.
Molecular diagnosis of *M. pneumoniae* infection. Automated DNA extraction was performed by using Nuclisens® easyMag system, bioMérieux. Real Time PCR was performed by using MYCOPLASMA pn. Q - PCR Alert AmpliMIX, Nanogen Advanced Diagnostics. System standardization was carried out on Applied Biosystems ABI PRISMTM 7300 instrument. *M. pneumoniae* serology. We used *Mycoplasma pneumoniae* IgG e IgM Novagnost™, Siemens Healthcare Diagnostics, for the serological diagnosis of *M. pneumoniae.*
Results: *M. pneumoniae* was detected by PCR in aspirate specimens of 11 of 61 (18%) patients with pneumonia. All the PCR-positive patients but one had also IgM positive results on serum samples.
In 10/61 aspirate specimens the first result of the amplification reaction was undetermined for the DNA of the human β-globin gene.
Conclusion: The concordance of *M. pneumoniae* PCR and IgM serology in 10 out of 11 (90.9%) young patients hospitalized with pneumonia, confirmed that the timing of pathological samples collection for laboratory investigations in *M. pneumoniae* pneumonias is crucial for optimizing the results. In addition, the rapid acquisition of the laboratory results allowed the clinicians to modify the antimicrobial therapy without delay, by replacing β-lactam antibiotics with macrolides.

P1802 Relevance of PCR-based pathogen detection assay in critically ill patients

S. Sachse, F. Bloos, A. Kortgen, K.H. Schmidt, M. Lehmann, M. Bauer, K. Reinhart, E. Straube (Jena, DE)*

Objectives: Traditional culture based methods for detection of sepsis pathogens take too long to guide empiric antimicrobial therapy in sepsis. Furthermore, blood cultures often yield negative results despite presence of sepsis. PCR based methods may help to overcome this problem since these methods accelerate pathogen identification. We report the performance of PCR based pathogen detection compared to blood culture in ICU patients with suspected infection.
Methods: Patients treated on an interdisciplinary ICU were included into this observational study if a blood culture (BC) was drawn on discretion of the treating physician. Blood cultures and EDTA-blood were taken by sterile venous puncture. The EDTA-blood was processed with a PCR-based assay (VYOO(R), SIRS-Lab GmbH, Jena), which detects a panel of 34 bacterial and 6 fungal pathogens as well as five antibiotic resistances. Data are given as median and interquartile range.
Results: 64 patients were included into this study. Median age was 68.0 (55.5; 74.0) years. 92 pairs of BCs and PCRs have been investigated. 7 (8%) BCs and 29 (32%) PCRs were positive. 42.1% patients had at least one positive PCR and 12.3% patients had at least one positive BC. At study inclusion, 5% of the BCs and 35% of the PCRs were positive. 3 out of 9 pathogens detected in the BC were also reported by the PCR. However, 4 BCs were contaminated with *S. epidermidis* which was not detected by the corresponding PCR. Fungi were detected in 10 PCR specimens compared to 1 *Candida* spp. detection in BC. 50% of the patients with positive PCR amplicon for fungi died. Likewise, 50% of patients (2 out of 4) with more than one positive PCR result died, as well. None of the differences in mortality rates reached statistical significance.
Conclusions: The PCR based assay resulted in considerably higher amount of positive results than the blood culture in this group of high risk patients. Furthermore, the data suggest that PCR can detect a high risk group of patients with positive results for fungi. The fact that repetitive positive PCR results were associated with a high mortality rate further suggests the clinical relevance of microbial DNAemia.

P1803 Development and the evaluation of a real-time PCR assay for molecular diagnosis of polymicrobial sepsis

A. Fouad, A. Kalkanci, A. Seyer, M. Dizbay, O. Tunccan, K. Hizel, K. Caglar, S. Kustimur (Ankara, TR)*

Objectives: The current microbiological diagnosis of sepsis is based on blood culture. However, blood culture systems suffer from several limitations such as lack of rapidity and, in cases such as fungal infections and polymicrobial infections, low sensitivity. In the present report, a real-time PCR assay using a LightCycler instrument, is described for the detection of bacterial and fungal DNA in simulated and clinical samples. The detection of *Aspergillus fumigatus*, *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* were aimed.
Methods: During the proces we have evaluated a range of different primers and probes, targeting different gene regions and including a variety of amplicon detection methods. As a result of this proces, a single-reaction TaqMan PCR assay targets selected in the ITS regions of *Candida* and *Aspergillus* and 16S rRNA gene region of bacteria were selected. Bacterial primers were F-CAACGCGAAGAACCTTACC, and R-ACGTCATCCCCACCTTCC, Gram-positive probe was FAM-ACGACAACCATGCACCACCTG-TAMRA, Gram-negative probe was FAM-ACGACAGCCATGCAGCACCT-TAMRA, *Aspergillus* primers were F-CTGTCCGAGCGTCATTG, ITS-TCCTCCGCTTATTGATAT, probe was FAM-AGCCGACACCCAACTTTATTT-TAMRA, *Candida* primers F-CCTGTTTGAGCGTCRTTT, ITS-TCCTCCGCTTATTGATAT, probe CATTGCTTGCGGCGGTA. Bacterial and fungal amplification was performed using the 50 cycles of 15 s at 95 C and 60 s at 60 C for target amplification. DNA derived from *A. fumigatus*, *C. albicans*, *S. aureus* and *E. coli* as positive control samples.
Results: The analytical sensitivity using pure cultures and EDTA-anticoagulated blood samples spiked with each microorganism cell suspensions was shown to be at least 1 CFU per PCR, corresponding to 5 to 10 CFU/ml blood. To assess the clinical applicability, blood samples of sepsis cases were retrospectively tested and real-time PCR results were compared with results from culture, histology, or a galactomannan enzyme-linked immunosorbent assay (ELISA) results. Sepsis cases were diagnosed correctly. Spiked blood samples had a 100 percent rate of

positive testing for all four types of microorganism (10/10 replicates) at each time point.

Conclusions: Our data suggest that the PCR assays may be appropriate for use in clinical laboratories as simple and rapid screening tests and might become an important tool in the early diagnosis of sepsis in the future.

P1804 Rapid same-day identification of 69% of bacteria and yeast from positive blood culture bottles using five PNA FISH™ fluorescence in situ hybridization probes

D.S. Hansen, L. Leerbeck, H. Kofod, B. Bruun (Hillerød, DK)*

Objectives: Early diagnosis of bloodstream infections is essential for rational antibiotic therapy and minimizing morbidity and mortality. Routine identification must await subculture and are thus unavailable on a same day basis, subjecting patients to broad spectrum empiric antibiotic therapy until identification. Fluorescence in situ hybridization (FISH) testing provides results within 1.5 h. The purpose of this study was to evaluate a five PNA FISH probe approach with regard to feasibility and coverage of the spectrum of positive blood cultures.

Methods: The assay is performed directly on smears from positive blood culture bottles (BCB) with fluorescence labeled peptide nucleic acid (PNA) probes targeting rRNA of the micro organisms. During 8 weeks in August – October 2009 all positive blood cultures detected daytime Monday to Friday were included. Each BactAlert blood culture set (BCS) consisted of two BCB. On all positive BCB wet mount and Gram stain microscopy were done. Based on this data fluorescence microscopy of blood smears stained with the relevant set(s) of PNA FISH™ (AdvanDx) probes were done as follows:

- Gram negative rods – *E. coli*/*P. aeruginosa* and EK (*E. coli*-*K. pneumoniae*)/*P. aeruginosa*
- Gram positive cocci (GPC) in clusters – *S. aureus*/CNS
- GPC in pairs and chains – *E. faecalis*/other enterococci (OE)
- yeast – *C. albicans*/*C. glabrata*.

If more than one BCB from a patient were positive, but had same sample date and Gram microscopy, only one PNA FISH microscopy was done. Conventional identification was performed in parallel.

Results: In the study period 2,631 BCS from 1,026 patients were cultured. Of these, 298 BCS from 169 patients were positive. PNA FISH was done on 135 (45%) of the BCS and on 116 (69%) of the patients. PNA FISH resulted in identification in 93 (69%) BCS tested; 80 (69%) of patients tested. Also a negative PNA FISH result has diagnostic implication, e.g. GPC in pairs and a negative *E. faecalis*/other enterococci PNA FISH doubles the propability for pneumococci.

All PNA FISH results were correct except for a non-hemolytic streptococcus which was falsely judged positive in the OE assay (orange, not red colour).

Conclusion: PNA FISH using five probe sets is a rapid and accurate method for identification of micro organisms directly from positive BCB and provides important information for appropriate antibiotic therapy. The method is reliable, easy to perform, and saves time for technologists, clinical microbiologists and clinicians.

P1805 Use of a commercially available multiplex real-time PCR assay (SeptiFast) to detect bacterial and fungal pathogens in septic patients

J. Sóki, E. Szabó, E. Hajdú, A. Lázár, E. Nagy (Szeged, HU)*

Objectives: The aim of this study was to evaluate the usefulness of the SeptiFast PCR system for rapid diagnosis of 21 pathogens in septic patients with or without antibiotic therapy and compare the results with blood culture tests.

Methods: Blood samples were taken from patients displaying symptoms of sepsis for blood culture (Bactec, Becton-Dickinson) and EDTA-blood sample was collected for the multiplex real-time PCR (SeptiFast, Roche) tests. Patients were from different ICUs and the haematology department of our University Hospital.

Results: 162 parallel samples were tested: blood culture sets (1 aerobic and 1 anaerobic bottle) were cultured and SeptiFast PCR tests were preformed. The two diagnostic methods were compared in two groups of patients: (1) patients laying in ICUs with several types of disease background other than haematological disorders and (2) patients with haematological malignancies with fever. While in the case of the first group of patients (n = 36) the results were concordant with some superiority of the SeptiFast system (11 both positive with concordance in the pathogens, 1 only blood culture positive 4 only PCR positive and 21 both negative). Out of the four cases where SeptiFast tests were positive and the blood cultures were negative in two patients *Aspergillus fumigatus* was detected by SeptiFast and in two other patients, already receiving antibiotics, *E. coli* and *Enterobacter* cloaceae/aerogenes were detected. In the case of haematology patients (n = 126) the two types of tests gave somewhat divergent results (10 both positive with concordance in the pathogens, 17 only blood culture positive, 14 only PCR positive and 85 both negative). Among the only blood culture positive samples (altogether 19 isolates) 2 patients were found positive with pathogens (*Clostridium* sp, *Pseudomonas putida*), which are not included into the SeptiFast kit, and 12 different coagulase-negative staphylococci were considered as contaminants. In all cases where the SeptiFast was positive and the blood cultures were negative antibiotic therapy was already introduced before taking samples.

Conclusion: The SeptiFast system cannot as yet replace blood cultures in the initial diagnostics of septic patients with or without haematological disorders, but may provide helpful information in episodes with persistent fever during antibiotic therapy and detect fungal infections difficult to prove by blood culture method.

P1806 Evaluation of molecular detection of bloodstream pathogens in 144 patients arriving in the emergency room with clinical signs of sepsis

M. Avolio, P. Diamante, S. Zamparo, M.L. Modolo, S. Grosso, P. Zigante, N. Tosoni, R. De Rosa, P. Stano, A. Camporese (Pordenone, IT)*

Objectives: In this study we wished to compare traditional Blood Culture (BC) with multiplex Real-time PCR, LightCycler SeptiFast Test M Grade (Roche Molecular System), in 144 patients with suspected bloodstream infections arriving at emergency room (ER) in order to know the agreement degree about those two methods applied in this category of patients.

Methods: 144 adult patients admitted to ER of our hospital with at least two criteria of the systemic inflammatory response syndrome were included in the study. During a febrile episode a single venipuncture was used to draw samples for 2 x 3 bottles of Bact/Alert (bioMérieux, Marcy l'Etoile, France). Immediately after the blood was drawn for the BC (8–10 mL/bottle), a 1.5 mL of whole blood was collected in sterile EDTA-KE tubes (Sarstedt, Nümbrecht, Germany) for the molecular method.

Results: SeptiFast results were compared with the results obtained with all culture bottles taken on different time points during a 24-h period and arrived simultaneously in our laboratory, showing a sensitivity of 92.5%, a specificity of 91% with a PPV and NPV of 78.7% and 97.1% rescpectively (values obtained by excluding CoNS and DNA not detectable by SeptiFast).

A median of 36 hours was necessary to obtain Gram staining and at least 72 hours, calculated from the arrival of the sample to the laboratory, for final bacterial species identification of pathogens using blood culture and biochemical identification. A median time of 15 hours (range 6–30 hours) was sufficient to obtain a molecular analytical result.

Conclusion: Several studies have underlined the limitations of both molecular and BC methods in diagnosis of BSI (Westh et al. Clin Microbiol Infect 15(6), 2009; Bouza et al. Clin Infect Dis 39, 2004). Our study confirms that SeptiFast is limited by its test menu, the specificity of its primers and probes, and the genetic variability of the target site, but on the other hand, an inadequate sample volume, antimicrobial therapy before drawn or the number of repeated blood draws affected

the sensitivity of BC. On the basis of our results, either in terms of concordance of results, either in terms of TAT, despite its limitations, SeptiFast could be useful as an adjunct to traditional culture methods to facilitate detection of BSI (Andrade et al. Shock 30(1), 2008), especially in cases where BC is negative, but BSI is strongly suggested. For these clinical conditions we wish to further investigate the use of SeptiFast.

P1807 Real-time PCR seems to be not superior to blood cultures during the treatment course of infective endocarditis in anticipating valves sterilization

P. Fanourgiakis, E. Kraniotaki, E. Platsouka, E. Perivolioti, M. Katsimpoulas, E. Gikas, A. Tsarbopoulos, A. Skoutelis, O. Paniara (Athens, GR)*

Objectives: To find out whether real-time PCR in blood could be a sensitive tool during the antibiotic treatment of infective endocarditis that could monitor the bacterial load in blood and probably in vegetations. If this hypothesis proved to be true, it should have serious implications in the management of IE with regard to duration of treatment, earlier recognition of complications or treatment failures and perhaps to animal models of IE.
Methods: We conducted our study during our experiments regarding the efficacy of daptomycin in rabbit enterococcal endocarditis. After left catheterization (day 0) 10^8 CFUs of a clinical strain *Enterococcus faecium* were inoculated on day 2. On day 4 daptomycin was initiated (days 4–8) and on day 9, animals were sacrificed, vegetations excised and quantitatively cultivated. Before inoculation, before treatment initiation and before sacrifice, blood samples were drawn for routine culture and PCR. Hemoglobin was removed, and DNA was extracted using the High Pure PCR Template Preparation Kit (Roche). The extract was eluted in 100 μl of elution buffer. LightCycler *Enterococcus* Kit MGRADE (Roche) was employed. 5 μl of the extracted DNA were used in a total of 20 μl reaction mixture. A standard curve of *E. faecium* DNA consisting of seven 10-fold dilutions of a stock solution of 10^8 CFU (10^7 CFU equivalents/PCR) was included in each run for automatic calculation of the bacterial DNA load. Analytical sensitivity was determined to be less than 10 CFU equivalents/PCR. No false positive results were observed.
Results: All blood cultures on day 4 were positive whilst on days 2 and 9 were sterile. None excised vegetation was sterile. Mean bacterial load on vegetations (mean log10 numbers per gram±SD) was 5.75±0.89. Results of real-time PCR were positive during proved bacteremia but negative when BCs had turned sterile No correlation seems to exist between bacterial DNA load (BDL) on day four and final bacterial load on vegetations.
Conclusions: Our results propose that real-time PCR is not sensitive enough in the case of animal enterococcal endocarditis to detect bacteremia when blood cultures turned negative. Thus its utility in predicting valves sterilization is improbable.

Table 1: Results of real-time PCR on day 4 and of quantitative cultures of excised vegetations

Rabbits	Blood-PCR day 4 Log_{10} copies/reaction	Vegetations Log_{10} CFU/g
1	4.511	6.47
2	3.382	6.51
3	5.014	6.44
4	2.548	5.8
5	1.604	4.05
6	2.959	6.25
7	4.707	5.87
8	1.988	5.95
9	4.504	5.97
10	3.817	4.23
Mean±SD	3.503±1.2	5.75±0.89

P1808 Usefulness of a microarray method for the diagnosis of bone and joint infections directly on clinical samples: a new application of Prove-it™ sepsis

E. Allais, S. Boisset, A.M. Freydière, T. Ferry, S. Lustig, J. Etienne, S. Tigaud, F. Vandenesch, F. Laurent (Bron, Lyon, FR)*

Objectives: We evaluate the performance of Prove-it™ Sepsis assay (PISA) (Mobidiag®) for rapid detection and identification of bacteria directly from bone and joint infections (BJIs) clinical samples, compared to conventional culture and universal 16S PCR. This microarray assay, targeted gyrB, parE and mecA genes, was initially developed to identify bacterial species (n > 50) in positive blood cultures in less than 3 hours.
Methods/Results: We selected 114 clinical BJI samples, for which microbiological diagnosis had been previously established either by culture (C+) or by PCR16S-sequencing (C–/PCR16S+). All these positive samples were chosen within the spectrum of bacteria included on the array. Sixteen negative clinical samples (C–/PCR16S–) were also included.

- C–/PCR16S– (n = 16): 15 samples were negative; 1 sample was positive with *S. epidermidis* which has been confirmed by the tuf gene amplification and sequencing
- C+/PCR16S+ (n = 32): 23 samples (71.9%) were positive with concordant identification, 3 samples were positive and concordant but revealed the presence of a second species; 6 samples remained negative
- C+/PCR16S– (n = 20): 5 samples (25%) were positive and concordant; 15 samples remained negative as PCR16S
- C–/PCR16S+ (n = 62): 36 samples (58%) were positive and concordant, 4 samples were positive with a mix of 2 to 4 species; 22 samples remained negative.

A new generation of PISA (StripArray) including specific probes for *Kingella kingae* and *Propionibacterium acnes* was also tested on culture-negative synovial fluids from children (n = 159):

- C–/PCRKingella+ (n = 45): 35 were positive for *K. kingae* (77.8%). Ten samples remained negative, but seven revealed positive-Kingella probes in insufficient number to meet the strict positive identification criteria, deserving complementary studies.
- C–/PCR16S– (n = 114): 110 samples were negative and 4 samples were found positive *(K. kingae, P. acnes, S. aureus*, *S. pneumoniae*).

Conclusions: The preliminary results of this rapid and cost-effective method are promising. Its advantages include: no sequencing step, detection of a large panel of bacterial species with a single multiplex PCR assay, detection of polymicrobial BJIs, detection of mecA. Currently, the new version of PISA is further evaluated with prospective BJI clinical samples as well as optimization of DNA extraction/amplification protocols is underway to improve the sensitivity of the test. The new version of PISA could eventually replace conventional 16S PCR for the diagnosis of BJIs.

P1809 Detection of uncultured organisms in paediatric bone and joint infections by a Multiplex real-time PCR panel

N. Ahmad, P. Marsh, A. Monk, A. Pallett, K. Saeed, N.M.P. Clarke, S.N. Faust (Southampton, UK)*

Objectives: The clinical features of osteoarticular infection (OAI) can vary considerably in children of different ages. Treatment is often started and continued empirically due to the low sensitivity of conventional blood and tissue culture in these patients. Better and more rapid microbiological diagnostic techniques are required to ensure more accurate therapy in order to reduce morbidity and overall (patient and financial) costs of treatment.
In a review of microbiology data at Southampton University Hospitals NHS Trust (SUHT), a defined pathogen was identified in only 8% of the 1200 samples received for culture and microscopy. Therefore our objectives were to develop a sensitive, specific and rapid multiplex PCR protocol for the detection of uncultured pathogens in paediatric OAI, and to investigate the level of infections left undetected by culture-based detection.

Method: A new real-time PCR diagnostic protocol was developed to identify the presence of the most common organisms known to cause bone and joint infections including the emergent paediatric pathogen *Kingella kingae* (identified from both the literature and basic local alignment search tool (BLAST) in samples taken from children with OAI at SUHT. A real-time PCR panel comprising of two triplexes was developed using three different reporter dyes (one for each target).
DNA extractions were carried out using an automated system, MagNA pure LC by Roche® using the MagNA Pure LC DNA isolation kit III (bacteria and fungi) Roche®..Real-time PCR was carried out using a Rotor-gene 6000 (Corbett).
Results: 95 paediatric bone and joint samples from SUHT were assayed from 56 patients (age range 270 days to 192 months) by PCR. Pathogenic bacteria were detected in 22 out of 56 patients where culture-based detection failed (including 6 K. Kingae, 7 *Staphylococcus aureus* and 3 *Streptococcus pneumoniae*) and 6/56 where culture confirmed the diagnosis.
Conclusions: We have developed a new, rapid (same day) method for detection of uncultured pathogens direct from paediatric bone and joint samples. Important OAI pathogens detected by PCR were missed by culture-based detection. Work is ongoing to establish the clinical value and cost-effectiveness of widespread introduction of this technique into routine clinical practice.

P1810 Comparative evaluation of four serological methods and a quantitative real-time PCR assay in asymptomatic subjects with a previous history of brucellosis

M.J. Castaño Aroca, E. Navarro, M. Andicoberry, S. Lorente, J. Solera (Albacete, ES)*

Objectives: *Brucella* spp. antibodies, despite falling to low levels, can remain measurable after recovery from acute brucellosis. This makes it difficult to distinguish between cured patients, chronic brucellosis patients and those healthy individuals living in areas in which brucellosis is endemic. This is the basis for the controversy in the definition of the chronicity of the disease. The presence of *Brucella* spp. DNA in clinical samples in this setting remains unknown.
The aim of the present study was to evaluate both serological tests and a quantitative real time PCR (QPCR) assay for the presence of *Brucella melitensis* DNA in a cohort of asymptomatic subjects with a well-documented history of brucellosis.
Methods: Twenty asymptomatic subjects that had been diagnosed with brucellosis between three and 12 years prior to the study were included. Subjects were followed ranging between three to 34 months (mean 15 months). Between three and 6 blood and serum samples (172 samples in total) were screened by QPCR, classical serological tests (Rose Bengal, standard agglutination test (SAT) and Coombs test) and the commercial Brucellacapt® test. The methodology used for detecting *B. melitensis* DNA have been previously described by our group(a).
Results: All subjects had negative Rose Bengal test results. The SAT test showed antibody titres in three (15%) subjects (range, 1/20–1/80). The Coombs test showed antibody titres in four (20%) subjects (range, 1/160–1/640). The Brucellacapt® showed antibody titres in 10 (50%) subjects (range, 1/40–1/160). Seropositive samples by SAT or Coombs test were also positive by Brucellacapt®.
Brucella melitensis DNA was found in five (25%) subjects with a mean bacterial DNA load of 218 copies/ml in blood samples. All of them had seropositive samples by the Brucellacapt® (range, 1/40–1/160). However, only five (33.3%) out of the remaining 15 subjects with negative QPCR samples had seropositive samples by Brucellacapt® (range, 1/40–1/80) ($p = 0.03$; two tails Fisher test).
Conclusion: The individuals harbouring *B. melitensis* DNA in their blood are more likely to show a seropositive sample than the remaining participants. These findings suggest that the presence of bacterial DNA at low levels derived from latent bacteria are capable of producing a week immune response in the host.
Reference(s)
(a) Castano MJ et al, J Clin Microbiol 2009 Jul 1; 47(7):2084–9.

P1811 Quantitative real-time PCR may be the only diagnostic tool for chronic brucellosis patients

E. Navarro, M.J. Castaño Aroca, E. Pescador, M.D. Muñoz, J. Solera (Albacete, ES)*

Objective: A minority of chronic brucellosis patients shows low or negative antibody titres (1) that make diagnosis difficult. In the present study we evaluated the usefulness of a quantitative real time PCR (QrtPCR) assay in the diagnosis of this group of patients.
Methods: Seventeen symptomatic patients with a well-documented history of brucellosis that had been diagnosed between 3 and 34 years previously were followed for more than one year. At the entry of the study, six (16%) patients were seronegative by all the three conventional serological methods. Three of them had a focal complication (one multiphocal motor neuropathy of upper limbs, one brucellar orchitis and one spondylitis). The remaining three had unspecific symptoms such as asthenia, arthralgias and/or myalgias. From these patients we analyzed a total of 167 samples including 83 blood samples, 83 serum samples and one sample of synovial fluid. Samples were screened by QrtPCR for the presence of *Brucella melitensis* DNA and conventional serological tests (Bengal Rose (BR), standard agglutination test (SAT) and Coombs test). Collected samples were analyzed randomly in a single blinded way. The methodology used for detecting and quantifying the bacterial DNA have been previously described by our group (2).
Results: Samples from the six patients were seronegative by the three conventional serological methods (BR, SAT and Coombs test). Regarding the QrtPCR assay, *B. melitensis* DNA was found in the six patients, quantifying 98 ± 221 copies/ml ($n = 7$), 642 ± 1106 copies/ml ($n = 13$) and 149 copies/ml ($n = 1$) in blood, serum and synovial fluid samples, respectively.
Conclusion: QrtPCR may be the only method for diagnosis of chronic brucellosis patients when conventional serological tests show negative results.
Reference(s)
[1] Imboden JBl. Arch Intern Med. 1959;103:406–14.
[2] Navarro E. Clin Infect Dis 2006;42:1266–73.

P1812 Preliminary evaluation of a rapid oligochromatographic assay for the detection of *Bordetella* sp. in respiratory samples

J.A. Carrillo Avila, I. Pedrosa Corral, M. Pérez Ruiz, J. Mendoza Montero, A. Rojas González, D. González Fernández, J.M. Navarro Marí (Santa Fe, Granada, ES)*

Objectives: To evaluate a rapid and simple new commercial assay, SpeedOligo® Bordetella (Vircell). The assay is based on a multiplex PCR coupled to the visualization of PCR products by means of a dipstick device and is intended for the detection of *Bordetella* species in respiratory samples. Results are available in less than one hour.
Methods: DNAs from 57 respiratory samples (nasopharyngeal exudates or aspirates) previously tested for the presence of *Bordetella pertussis* at Hospital Universitario Virgen de las Nieves (Granada) were used for the evaluation. DNA was extracted using the automated bioMérieux NucliSENS® easyMAG® platform (bioMérieux). The reference method was an "in house" real-time PCR that amplified a 179-bp fragment of the IS481 element of *B. pertussis.* SpeedOligo® Bordetella amplifies two specific *Bordetella* genes: a 114-bp fragment of the IS481 element present in *B. pertussis, B. holmesii* and *B. bronchiseptica,* and a 119-bp fragment of the ptxA promoter gene of *B. pertussis.* The human β-globin gene was used as internal control. Following amplification, the procedure was completed by oligochromatography. Hybridization of PCR products was carried out on a dipstick using probe pairs (for *Bordetella* and internal control detection), one conjugated with colloidal gold and the other immobilized onto nitrocellulose. The results were interpreted by visual reading of 3 lines: two for *Bordetella* gene fragments (IS481 and ptxA) and the third one corresponding to the internal control.
Results: The reference method yielded 26 positive results for *B. pertussis.* SpeedOligo® *Bordetella* was positive in 25 of these. B

pertussis was confirmed in 22 samples. Reaction with only the IS481 element test line was observed for the remaining 3 samples, indicating the presence of either *B. pertussis, B. holmesii* or *B. bronchiseptica.* No false positive or invalid results were observed. A 96.1% sensitivity and 100% specificity was calculated for the new assay.
Conclusion: SpeedOligo® Bordetella is a rapid, sensitive and specific assay for the detection of *Bordetella* infection. The amplification of two different *Bordetella* gene targets may increase sensitivity and specificity of the assay.

P1813 Evaluation of the Septifast molecular test applied to bronchoalveolar lavage samples for the microbiological documentation of severe pneumonia in the intensive care unit

J. Tankovic, J-L. Baudel, R. Dahoumane, A. Galbois, H. Ait-Oufella, J. Lemant, E. Maury, J.C. Petit, B. Guidet, G. Offenstadt (Paris, FR)*

Objectives: An important determinant of outcome in severe community-acquired or nosocomial pneumonia is prompt and appropriate initiation of antimicrobial chemotherapy. To achieve that, rapid and accurate microbiological documentation is of critical importance. We compared the results obtained by microscopic examination and quantitative culture of bronchoalveolar lavage (BAL) samples with those obtained with the rapid molecular test Septifast (SF) applied on those same samples.
Methods: Microscopic examination and culture were performed according to standard protocols. The SF test has been designed for the rapid detection (6 hours) in a 1.5-ml blood sample of the 25 bacteria and fungi representing 90% of the cases of bacteremia. In our study, the protocol for blood was used without modification. Positive results for streptococci, enterococci, CNS and *Candida* spp. were not taken into account, because these microorganismes were considered as commensals. We determined the sensitivities (SE), specificities (SP), positive and negative predictive values (PPV and NPV) of the three techniques.
Results: 34 consecutive patients with suspicion of infectious pneumonia (19 men, mean age 64±15, immunosuppression 50%, IGS II: 43.5 ±18.3, septic shock 29%, mechanical ventilation 41%) were included between April 10 and July 29 2009. One week after the realization of BAL, all the patient files were reevaluated and the diagnosis of infectious pneumonia was confirmed only for 23 of the 34 patients included (community-acquired: 10; nosocomial: 13). The diagnostic accuracies of the three techniques are shown below. The sensitivity of SF for patients with ongoing antimicrobial treatment was significantly greater than those from the conventional techniques.
Conclusion: For patients with severe pneumonia, analysis of BAL samples with the Septifast® molecular test permits a fastest microbiological documentation than culture and is more sensitive than culture, at least for patients with ongoing antimicrobial treatment at the time of realization of BAL.

	Gram stain	Culture	Septifast	p
All infected patients (n = 23)				
SE	0.35	0.48	0.74	NS
SP	0.73	0.82	0.55	NS
PPV	0.73	0.85	0.77	NS
NPV	0.35	0.43	0.5	NS
Infected patients with ongoing antimicrobial treatment (n = 15)				
SE	0.14	0.29	0.79	0.02
SP	0.67	0.78	0.67	NS
PPV	0.4	0.67	0.79	NS
NPV	0.33	0.41	0.67	NS

P1814 Comparison of DNA extraction and real-time PCR methods for the detection of *Coxiella burnetii*

J.J. Tilburg, E.J. van Hannen, M. Hermans, A. Horrevorts, W.J. Melchers, M.H. Nabuurs-Franssen, A.M. Pettersson, J.W. Rossen, M.C. de Vries, C. Klaassen (Nijmegen, Nieuwegein, 's-Hertogenbosch, Amsterdam, Tilburg, Bilthoven, NL)*

Objectives: In the Netherlands there is an unprecedented and ongoing Q fever outbreak with thousands of affected individuals. Routinely diagnosis of Q fever using serological methods are of limited use in the acute phase of the disease and therefore are not sufficient for accurate diagnosis. Real-time PCR however, can be useful in establishing an early diagnosis of acute and chronic Q fever.
Methods: We compared 7 different DNA extraction and real-time PCR methods implemented by 7 medical diagnostic laboratories. Three-fold serial dilutions of DNA extracted from the Nine Mile strain (RS 493) were send to all laboratories to compare the sensitivity of the different real-time PCR methods. In addition, positive clinical serum samples with relative high and low DNA loads were used.
Results: A minimum difference in sensitivity was observed between the real-time PCR methods using three-fold serial dilutions of DNA extracted from the Nine Mile strain (RSA 493). More differences were obtained between several combinations of DNA extraction and real-time PCR methods using actual clinical positive serum samples. Differences in sensitivity of the real-time PCR could partially be explained in the design of the primers and probes. Although the results of the real-time PCR method obtained by 2 out of 7 laboratories yielded the highest percentage overall of positives, the combination of DNA extraction method and real-time PCR of each laboratory individually however, resulted in comparable number of positives for 6 out of 7 laboratories.
Conclusion: This study show that the DNA extraction and real-time PCR method implemented by 6 out of 7 medical diagnostic laboratories can be used for a reliable screening of Q fever suspected patients and can substantially contribute to the early diagnosis of acute Q fever during the ongoing Q fever outbreak in the Netherlands.

P1815 Broad-range survey of tick-borne pathogens from southern Germany

M. Eshoo, O. Nolte, C. Crowder, H. Matthews, F. Li, R. Sampath, D. Ecker (Carlsbad, US; Konstanz, DE)*

Objectives: Early treatment for tick-borne human infections such as Lyme Borreliosis, Babesiosis and Anaplasmosis is critical for a positive prognosis. We developed and validated an Ibis T5000 assay (Ibis Biosciences, Inc., Carlsbad, CA) that can detect and identify a wide range of tick-borne pathogens from ticks and clinical samples / specimens.
Methods: A multi-locus assay was used that employs 8 broad-range PCR reactions with primers targeting all known tick-borne bacterial and protozoan pathogen families. Electrospray ionization mass spectrometry of the PCR amplicons was used to determine their base composition. These base composition signatures were subsequently used to identify the organisms found in the samples. The assay was developed using field collected ticks and a wide range of clinical sample types and has been shown to be sensitive to the stochastic limits of PCR.
Results: Extracts from 178 field collected ticks and ticks removed from patients from Southern Germany were screened using the Ibis broad-range tick-borne pathogen detection assay. 39 of the specimens tested positive for one or more *Borrelia* species *(B. afzelii, B. burgdorferi* s.s., *B. garinii, B. lusitaniae,* B.spp. LB-2001, *B. valaisiana* and *B. spielmanii)*. In addition 4 specimens were positive for *Anaplasma phagocytophilum* and 8 (4.5%) were positive for *Babesia microti* including 3 *Babesia*/*Borrelia* co-infected ticks.
Conclusions: We demonstrate broad-range detection of tick-borne pathogens in a single assay using ticks from Southern Germany. The Ibis assay detected a diverse range of *Borrelia* species and surprising high frequency of *Babesia microti*. The Ibis T5000 system and the next generation PLEXID systems can be completed within five hours from specimen processing to result reporting, provides rapid and accurate

detection and identification of a broad range of pathogens causing tick-borne human infections.

P1816 Improving performance of bacterial identification DNA arrays by ROC analysis guided probe design

H. Peter, T.T. Bachmann (Edinburgh, UK)*

For the development of DNA microarrays for bacterial species detection it is crucial to design oligonucleotide probes with a high degree of specificity and sensitivity. However, even if probes were very specific in silico, their experimental performance on the array is often less optimal, thus reducing sensitivity and specificity of the system. The definition of an experimentally guided probe assessment and threshold definition should therefore improve the array characteristics in terms of limit of detection and detection specificity. In this study we used ROC-Analysis (Receiver Operating Characteristic) for genotyping DNA microarrays to optimise and streamline probe validation, resulting in advanced sensitivity and specificity.

We present a ROC based approach on the example of the development of a DNA microarray for the detection of toxic cyanobacteria (170 specific probes). The study is based on 120 reference hybridizations of 30 reference strains. For data analysis we developed a mathematical MS Excel based tool, which automatically carries out ROC-Analyses for all probes. The tool is designed to specifically suit the needs of genotyping arrays. For every probe the mathematical sensitivity and specificity was calculated for a wide range of cut-offs and processed into one graph, to receive the ROC-curve. The area under the curve (AUC) was used as a quality measure to either keep or abandon an individual probe. Only the best probes with an AUC value >0.9 should be used for an optimal array platform. In addition, individual cut-offs were calculated for each probe, to increase the sensitivity, maintaining maximum specificity. The sensitivity could be adjusted by modifying the probes' individual cut-off and the associated, accepted false positive rate. In the presented example, we set a false positive rate of 2%. To confirm the approach, a test set of 50 additional hybridizations with target DNA from reference strains was successfully performed.

In conclusion and after further examples on other genotyping DNA array modules we consider the ROC approach on probe validation as a highly useful and versatile tool to achieve significantly improved assay performance which is in particular required for successful translation into diagnostic applications.

Molecular detecting of bacteria and their resistance genes

P1817 Molecular diagnosis of septicaemia: SeptiFast vs. blood culture

M. Kotnik, C. Luger, A. Huber-Wechselberger, S. Stumvoll, A. Bocksrucker, B. Sammer, S. Metz-Gercek, P. Apfalter, H. Mittermayer (Linz, AT)*

Objective: Rapid diagnosis of bacteraemia is of prime importance in the management of febrile patients. The SeptiFast (SF) blood assay detects 19 bacterial and 6 fungal pathogens in blood samples by nucleic acid amplification. Molecular biology methods provide faster results than conventional culture methods, hence SF allows a rapid diagnosis of septic episodes. We evaluated SF in comparison to blood culture in patients with suspected septicaemia.

Methods: Blood samples for SF were processed according to the manufacturer's specification. In short whole blood sample undergoes mechanical lysis with ceramic beads. In further purification steps DNA is extracted. PCR is carried out in three parallel real time reactions. The amplicons are detected by fluorescence emission. Blood cultures were processed according to the SOP's of the respective laboratories. Over a period of two and a half years we tested 440 patients and compared SeptiFast (SF) results to BC.

Results: For 384 out of 440 samples complete data were available. 82 (21.4%) of samples were positive in BC, 122 (31.8%) in SF. Of 122 detected pathogens by SF, 65 were isolated from BC. Differing results between the two methods were obtained in 45 instances, BC was only positive in 8 (9 pathogens) and SF in 36 cases, respectively. In BC only the following pathogens were detected: *S. epidermidis*; β-haemolytic Streptococci (1); *S. aureus* (2); *E. coli* (1);*E. coli* and *C. albicans* (1); *C. albicans* (1); *C. glabrata* (1); *C. parapsilosis* (1); and BC-negative and SF-positive samples: *S. epidermidis*; β-haemolytic Streptococci (3); *S. pneumoniae*; S. spp. (6); *S. aureus* (3); *E. faecalis* (1); *E. faecium* (1); *E. coli* (6); *K. pneumoniae*/oxytoca (5); *P. aeruginosa* (5); *C. albicans* (4); *C. tropicalis* (1); *A. fumigatus* (1).

Conclusions: SF detected considerably more microorganisms than BC. The most striking differences were observed in *S. pneumoniae*, *E. coli*, *Klebsiella*, *P. aeruginosa* and *C. albicans*. In nine cases, however, BC yielded pathogens, which were not recovered by SF. In our study SF has been proven as a valuable tool for rapid diagnosis of septicaemia with a higher recovery rate than BC.

P1818 Biochemical and molecular characterization of *Escherichia albertii* isolated from clinical samples

L. Nimri, N. Strockbine, C. Tarr, E. Sowers (Irbid, JO; Atlanta, US)*

Objectives: *Escherichia albertii* sp. nov. is a newly recognized species whose initial members were isolated from Bangladeshi children with diarrhea. Identification of these organisms is problematic for clinical laboratories due to limited phenotypic and genotypic data upon which to base diagnostic algorithms. The study aimed at the characterization of additional isolates is needed to define the boundaries of phenotypic and genotypic variation for developing reliable identification methods to support studies to address their role in disease, and epidemiology.

Methods: Selected coliform isolates from the CDC reference collection were screened by PCR for allelic variation in two housekeeping genes (lysP and mdh) specific for *E. albertii.* Twenty strains were identified as members of the *E. albertii* lineage and characterized phenotypically with a set of 45 traditional biochemical tests and panels of *E. coli* and *Shigella* typing antisera. Isolates were also tested by PCR for 14 virulence markers [eae, cdtB, phoE, stx1, stx2, stx2f, ehx, bfpA, ipaH, LT, ST, phoE, EAgg and EAF].

Results: Findings from biochemical tests revealed that all *E. albertii* strains are non-motile, methyl red positive and Voges-Proskauer negative. All produced acid from the fermentation of D-glucose (with gas), D-mannitol, D-mannose and failed to ferment lactose, sucrose, dulcitol, L-rhamnose, and melibiose within 48 hr at 35 C. Variable reactions (11%-89% positivity) were noted for the following tests: indole, lysine decarboxylase, ornithine decarboxylase, D-sorbitol, maltose, trehalose, glycerol, mucate, acetate and β-galactosidase. Most isolates were rough or failed to agglutinate with *E. coli* and *Shigella* antisera. Agglutination of 4 strains was observed with *E. coli* antisera against the following O antigens: O63 (2), O101 (1) and O180 (1). All isolates were positive for the eae gene, 17/20 (85%) were positive for the cdtB gene, 2/20 (10%) were positive for ehx, 18/20 (90%) were positive for phoE, and 1/20 (5%) was positive for stx2f. None were positive for the remaining virulence markers.

Conclusion: These findings illustrate the variability extent within this lineage and highlight the importance of including information on *E. albertii* strains within commercial databases to improve the identification of these bacteria.

P1819 Extended-spectrum β-lactamases and their detection applying on-chip PCR

I. Barisic, S. Schoenthaler, C. Noehammer, H. Wiesinger-Mayr (Seibersdorf, AT)*

Objectives: The gold standard for pathogen identification and characterization in clinical routine are cultivation-based methods. However, these methods are laborious and generally last from blood withdrawal to antibiotic resistance determination two to three days, sometimes even

weeks. Hence we develop a DNA-based high throughput method that can characterize pathogens from blood within a few hours.
Methods: First steps included the download of all available extended spectrum β-lactamase (ESBL) DNA sequences from NCBI and the construction of a database with the sequence management tool ARB. Phylogenetic trees of the ESBL genes using the Neighbour-Joining method were calculated and primers targeting DNA encoding those enzymes were designed. The primers were covalently attached to a solid support by printing them onto a specifically modified glass slide using a microarrayer. A PCR mix was then applied onto the slide surface and the slide was put into a thermo cycler. The main difference to conventional PCR is the covalent attachment of the two primers to the microarray surface. In an additional reaction Cy3 labelled CTPs were incorporated into the amplified and attached DNA and signals were analyzed using a microarray scanner.
Results: Phylogenetic analyses based on amino acid alignment revealed two distantly related groups of ESBLs. The OXA β-lactamases form a very diverse group showing low homology to other known ESBLs. The second group includes the remaining ESBLs like TEM, SHV, CTX-M, TLA, PER, etc. but is still less diverse then the OXA group.
DNA amplification with on-chip PCR could be achieved. Preliminary results indicate adequate sensitivity which has been evaluated by real time PCR. Specificity will be validated by DNA sequencing.
Conclusion: An important precondition in medical diagnostics is a low number of working steps thus single tube reactions like multiplex-PCRs are preferred. Since multiplex PCRs are however well-known to cause various difficulties, our method of choice to detect the high number of different β-lactamases is based on on-chip PCR circumventing the primer dimer formation in complex primer mixtures.

P1820 MALDI-TOF MS analysis and molecular typing by pulsed-field gel electrophoresis of environmental Vibrio isolates

R. Eddabra, R. Mimouni, O. Meunier, L. Moussaoui, G. Prevost, J.M. Scheftel (Strasbourg, FR; Agadir, MA)*

Objectives: Pulsed field gel electrophoresis (PFGE) is widely used as an epidemiological tool for the typing and identification of bacterial strains. In contrast, Matrix-Assisted Laser Desorption Ionisation/Time-of-Flight Mass Spectrometry (MALDI-TOF MS) offers a new and promising approach to rapid identification and differentiation of bacterial strains. This study compares a novel Matrix Assisted Laser Desorption/Ionisation–Time of Flight (MALDI-TOF)-based method to PFGE patterns for Vibrio differentiation.
Methods: 30 Vibrio environmental isolates (14 *V. cholerae* non-O1, 10 *V. alginolyticus,* 5 *V. fluvialis* and one strain of *V. metschnikovii)* identified by Vitek 2 system (bioMérieux). Differentiation of Vibrio isolates was done using PFGE of NotI-digested genomic DNA. Computer-assisted analysis of the PFGE banding patterns was performed using Fingerprinting IITM software (Bio-Rad Laboratories). In addition MALDI-TOF-MS was performed. Small amounts of intact cells of a single colony were directly applied onto the target plate, mixed with matrix solution and air dried. Mass spectra were acquired using Ultraflex II MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) and analyzed by BioTyper™ 1.1 software.
Results: Out of the 30 strains of Vibrio examined in this study, 6 isolates could not be typed by PFGE and consistently appeared as a smear on the gel. In general, high genetic biodiversity among the isolates was found regardless to the isolation source. The results of MALDI TOF analysis show that the spectral profile, consisting of about 20 to 25 prominent peptide peaks masses ranging between 2 and 12 kDa, have indeed taxonomic significance and appears to be a rapid and inexpensive method to taxonomically cluster environmental isolates. The main spectra of Vibrio isolates have been included in the BioTyper library.
Conclusion: This study proves that the MALDI BioTyper system is an easy to use and highly suitable tool for the Vibrio species differentiation. MALDI-TOF MS could cluster the isolates from each species into the same pattern, whereas PFGE had lower discriminatory ability between species.

P1821 Prognostic value of chromogranin A at admission in critically ill patients

M.H. Metz-Boutigue, F. Schneider, T. Lavaux, D. Zhang, J.E. Herbrecht, C. Bach, H. Chung, P. Garnero, D. Aunis (Strasbourg, FR)*

Objectives: Critically ill patients are usually admitted with clinical and biochemical signs of systemic inflammation as a consequence of their disease. A recent study was conducted to evaluate the significance of Chromogranin A (CGA), a stress marker released by chromaffin cells and neutrophils, for prognosis of critically ill patients with systemic inflammatory response syndrome (SIRS) or sepsis [Zhang D and al., Clin Chem, 2009]. This study is now completed by a comparative proteomic analysis of the CGA-derived peptides present in serum from patients and healthy controls.
Methods: We measured in 53 patients and 14 healthy controls the serum concentration of CGA, procalcitonin and C-reactive protein. We also assessed the Simplified Acute Physiological Score (SAPS) in the patients. The proteomic analysis of the CGA-derived peptides present in serum was performed by using reverse phase HPLC and Western blot.
Results: Serum CGA concentrations were significantly increased in SIRS patients when compared to healthy controls, with a median value of 115 μg/L. When infection was associated with SIRS, patients had the highest increase in CGA with a median value of 138.5 μg/L. CGA concentrations positively correlated with inflammation markers (procalcitonin, C-reactive protein) and the Simplified Acute Physiological Score (SAPS II). Receiver Operating Characteristic (ROC) analysis showed that CGA is equivalent to SAPS as an indicator for 28-days mortality. We reported also the comparative expression of complete CGA and its natural derived fragments in serum of critically ill patients and healthy controls. These data are related to the processing machinery acting during sepsis.
Conclusion: Patients with CGA concentration superior to 71 μg/L have a significantly shorter survival. A Cox model confirmed that CGA and SAPS were independent predictors of outcome.

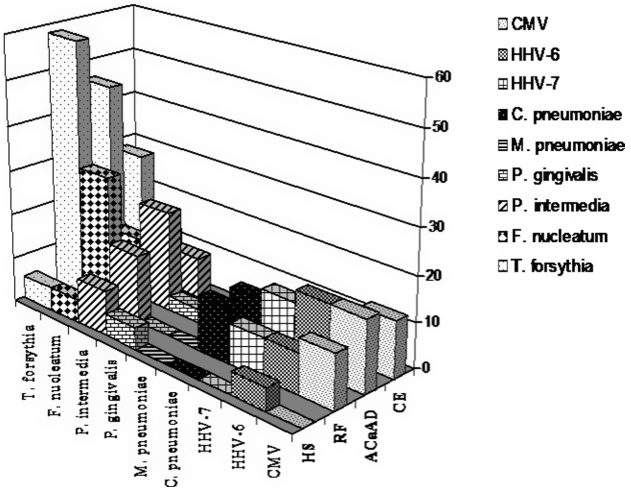
P1822 Presence of infectious agents in plasma samples from patients with atherosclerotic disease

R. Musumeci, B. Sibra, A. Giuliano, A. Cialdella, A. Careddu, G. Parati, C. Cocuzza (Monza, IT)*

Background: During the last decades, numerous clinical and epidemiologic studies have explored the possible direct or indirect role of infectious agents in the pathogenesis of atherosclerosis. The infectious agents that have in recent years gained considerable interest as having a potential role in atherosclerosis include: *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, Cytomegalovirus, HHV-6, HHV-7, and more recently, periodontal pathogens (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum*). In previous studies, the high prevalence of infection by some of these pathogens in the human population has represented a major drawback in demonstrating an association between microbial agents and atherosclerosis by means of serological methods. More recently newer diagnostic assays, such as quantitative Real-Time PCR, have been developed for the direct detection of infectious agents from atherosclerotic plaques; however few studies have applied these methods to analyse the presence of a wide panel of pathogens in plasma of atherosclerotic patients.
Objective: To evaluate the presence of β-herpesviruses (CMV, HHV-6 and HHV-7), periodontal and atypical bacteria in patients with atherosclerosis as compared to patients with only risk factors for the disease.
Methods: Detection by qRT-PCR assays of microbial DNA in plasma samples in patients with previous cardiovascular events (CE, n = 32), demonstrated atherosclerotic carotid artery disease but no events (ACaAD, n = 37), with only risk factors but no evidence of disease (RF, n = 33) and healthy subjects (HS, n = 20).
Results: The prevalence of microbial pathogens as demonstrated by DNA detection in patients plasma is shown in the figure.

The median value (mean±SD) of the total pathogen DNA load/ml was found to be 26 (32±24) in HS, 999 (5173±11706) in RF, 843 (2617±5244) in ACaAD and 2258 (52154±179205) in CE.

Conclusions: Both patients with only risk factors and with known cardiovascular disease showed a relatively high prevalence of microbial pathogen DNA present in plasma samples. However the total microbial DNA load or "pathogen burden" was found to be markedly higher in patients with previous documented cardiovascular events.



Prevalence of microbial pathogens in patients' plasma samples.

P1823 Improvement of *Legionella pneumophila* early diagnostics in severe community-acquired pneumonia patients admitted to an intensive care unit

N. Poblet-Mas, M. DelaTorre, J.M. Calvera, J. Garcia-Gil (Girona, ES)*

Objectives: Due to its difficult diagnostic, *Legionella pneumophila* (LP) incidence might be underrated. An early diagnostic is crucial to apply an appropriate antibiotic therapy. The main goal of this work was to assess the accuracy of a newly developed real time quantitative PCR method (RTi-PCR) specific for all known serogroups of LP as a routine analysis. Our secondary aim was to evaluate the aetiology of severe community-acquired pneumonia (sCAP) and the incidence of polymicrobial infections.

Methods: During a 24-month period, 345 specimens (blood, bronchoalveolar lavage and urine) from 135 patients (95 affected of sCAP and 40 controls) admitted in the intensive care unit (ICU) of a hospital in Girona (Spain) with sCAP were included prospectively. Etiological agents were analysed by culturing, enzimoimmunoassay (EIA) techniques and RTi-PCR for LP, using the fis gene as target. An internal amplification control (IAC) was included in each PCR reaction to minimise contamination and to reduce the chance of false negative results.

Results: Among the 95 sCAP affected patients included in this study, LP was identified by RTi-PCR in 41cases (33 LP-positives in BAL, 25 in urine and 23 in serum samples), whereas 17/95 cases were LP-positive by EIA and 27/95 by culture. LP-positives by RTi-PCR were correlated with EIA and culture ($P < 0.001$).

Streptococcus pneumoniae (SP) was present in 33/95 patients, *S. aureus*, *H. influenzae*, *E. coli*, and *Pseudomonas aeruginosa* in 5/95, 4/95, 4/95, 2/95 of the patients, respectively. No bacterial aetiology was found in 27% of sCAP patients.

In a 22.1% of the LP infected patients, antibiotic therapy was initiated before specimens were obtained. In a 45% of these patients the antibiotics were changed after diagnosing. Polymicrobial infections accounted for 32% of the cases with sCAP. Significant differences between sCAP and control patients ($P < 0.05$) in mortality rate, shock and ICU length of stay were only observed when the infection was polymicrobial.

Conclusions: The RTi-PCR method for is more accurate than culture or EIA, and allows a rapid diagnostic of LP infections. It is especially important in patients in whom antibiotic therapy has been initiated. We propose its use as a complementarily routine analysis in hospitals.

LP and SP are the leading causes of all sCAP cases admitted in the ICU, and polymicrobial infections are important since they present a worse prognosis and a more aggressive clinical evolution.

P1824 Optimizing the workflow in a diagnostic laboratory by combining an internally controlled parasitical real-time PCR with an internally controlled enteric real-time PCR for simultaneous detection of both parasitic and bacterial pathogens in faecal samples

E. van Zanten, S. Hofs, A.M. Kooistra-Smid (Groningen, NL)*

Objectives: Routine enteric diagnostics on fecal samples in our laboratory consists of two real-time PCR assays targeting 4 bacterial enteric pathogens and *Giardia lamblia*. When specific parasitic diagnosis, other than *G. lamblia,* is requested, microscopy is used. The Triple Faeces Test (TFT) is employed for the detection of cysts and Chlorazol-Black staining for the detection of trophozoites. Microscopy is time consuming and relies heavily on well trained personnel. Moreover, results obtained abundantly yield information on non-pathogenic parasites. This study describes a real-time PCR assay targeting *Cryptosporidium*, *Entamoeba histolytica* and *Dientamoeba fragilis* to complement our existing multiplex PCR assays, reducing hands on time and creating a single work flow for both bacterial and parasitic diagnosis.

Methods: A total of 533 faecal samples were analyzed. For 228 samples, specifically requested for parasitic diagnostics, both microscopy and parasitic real-time PCR was performed and for 305 samples, for which only routine diagnostics was requested, additional parasitic real-time PCR was performed. Microscopy consisted of a TFT test which was assessed by two lab technicians for the presence of cysts and trophozoites. Chlorazol black staining was performed by the technicians on samples flagged suspicious for trophozoites. DNA extraction for TFT samples was performed on the unpreserved faeces. After easyMag DNA extraction real-time PCR was performed using the ABI7500 system.

Results: Parasitical microscopy on the 228 samples yielded: 12 *D. fragilis,* 33 *Blastocystis hominis*, 8 *Endolimax nana*, 5 *Entamoeba coli*, 1 Entamoeba hartmanni and 1 Iodamoeba bütschlii. Real time PCR on these samples resulted in: 45 *D. fragilis* and 2 Crytosporidia. The 305 samples additionally screened with PCR yielded 37 *D. fragilis* and 2 *Cryptosporidium* positive samples.

Conclusion: The incorporation of *D. fragilis, E. histolytica* and *Cryptosporidium* in our enteric real-time PCR assays improves detection rates of pathogenic parasites, other than *G. lamblia,* from 5% to 16%. Moreover, incorporating a parasitic real-time PCR in the enteric PCR workflow resulted in detecting a considerable number of pathogenic parasites in samples originally not suspected of parasitic infection. Furthermore, a patient is no longer asked to collect faeces over a three days period, alleviating patient effort and speeding up time to result.

P1825 Vocma, Variation-tolerant Capture Multiplex analysis – a new multiplex method for detecting virus, bacteria and fungi

C. Öhrmalm, R. Eriksson, M. Jobs, B. Herrmann, Å. Melhus, J. Blomberg (Uppsala, SE)*

Objectives: We have developed a new multiplex method for simultaneous detection of nucleic acid from virus, bacteria and fungi, called VOCMA, Variation-tolerant Capture Multiplex Analysis. Today the panels of VOCMA Gastroenterits and VOCMA Sepsis are 7-plex and 22-plex resp.

The method is variation tolerant i.e. it can detect nucleic acid from viruses like calici virus (winter vomiting disease) or influenza virus, which are highly disposed to genomic variation with the aim of avoiding

the immune defense of the host. The variation tolerance is also allowing VOCMA to cover whole groups of similar genes like e.g antibiotic resistant genes as ctxm group I and ctxm group IV.
Methods: VOCMA is based on well established steps as nucleic acid extraction, followed by amplification and labelling of the target sequences of the pathogen. Target specific probes are attached to the different xMAP microspheres and a mixture of beads, consisting of the panel of interest, are allowed to hybridize to the sample. With a Luminex® 200 flow cytometer the amplified and labeled nucleic acid of the target genes are identified and quantified.
Result: The VOCMA method is a highly multiplex (e.g. 22-plex VOCMA sepsis) and provides tolerance to variation in the nucleic acid sequence. Even though it is variation tolerant the VOCMA is still highly specific for the intended target. The method, which is being further optimized, has shown an analytical sensitivity of 10–10000 targets/reaction for both the VOCMA Gastroenterits and VOCMA Sepsis.
Conclusion: VOCMA is a multiplex and specific method for detection of nucleic acid of different microorganism. By utilizing xMAP technology and Luminex® 200 the composition of the multiplex panels can easily be changed, combined and expanded, which creates a large flexibility of the assay. A third panel which contains virus, bacteria and fungi causing respiratory infectious diseases is also under development. A multiplex method like VOCMA both saves time, reduces the cost per microbe analysed, as well as the consumption of sample, and increases the possibility of discovering co-infections.

P1826 Improved accuracy of DNA quantitation using a chemistry model-based method for estimating nucleic acid quantities in PCR amplified samples

B. Archer, M. Dobbs, R. Hazelo, M. Exner (Cypress, US)*

Objectives: Quantitative interpretation of a PCR result requires a mathematical algorithm or method to determine the amount of template in a sample. The method most commonly used depends on a specified threshold and a calibration plot of the fractional cycle at threshold crossing vs. the logarithm of the DNA amount in the sample. We have developed a new method based upon physical models in which parameters represent attributes of the PCR chemistry. This study sought to examine the potential of this method for determining concentrations of CMV, BKV, and EBV viruses in clinical specimens, and to compare the results with those obtained using threshold based methods. In addition, the ability of the chemistry method to assess the efficiency of individual PCR reactions was evaluated.
Methods: Analytical studies were performed to evaluate linearity, reproducibility, and accuracy of the chemistry based method in comparison with a threshold based method. For these studies, the 3M integrated cycler was used to run serial dilutions of viral DNA standards for each target being tested, and observed quantity values were compared to expected quantity values. In addition, suboptimal PCR reactions (containing inhibitory substances) were analyzed, and accuracy of quantitation in these specimens was monitored.
Results: The chemistry model-based method was found to be both accurate and precise over a very wide dynamic range of template concentrations, with a linear range beyond what was observed for the threshold based model. The chemistry method also was able to provide a more accurate quantification of DNA in samples for which PCR conditions were not optimal and replication efficiency was reduced.
Conclusions: By eliminating the assumptions required by threshold methods of quantitation and automatically accounting for replication efficiency in each reaction, the chemistry model-based algorithm provides more accurate quantity determinations from clinical specimens. Using this method will improve performance of diagnostic tests. The estimation of parameters that represent attributes of the chemistry and an estimate of efficiency for each reaction based on these parameters will be helpful in new test development.

Molecular diagnostics for sepsis

P1827 Novel quantitative point-of-care molecular diagnostic system

M. Schamesberger, B. Hierschläger, C. del Rio, C. Wechselberger, M. Sonnleitner (Freistadt, AT)*

Introduction: Molecular diagnostics at the Point-Of-Care (POC) is mostly carried out using simple strip tests. Unfortunately, such tests only give yes/no answers and often have to be reconfirmed by quantitative tests in the lab, which are still time-consuming and have to be performed by expert operators using complex systems. This typical process flow in medical diagnostics often causes significant delay in appropriate medication and facilitates the spread of infectious diseases.
To solve this problem we developed a quantitative POC molecular diagnostics system that is able to simultaneously quantify more than 8 different target bacteria down to concentrations of ~10^4 cfu/ml within 15 minutes, without the need for an expert operator.
Objectives: We addressed following POC platform requirements,
– minimum need for operator intervention in the different steps of the assay
– a simple and reliable but still sensitive detection methodology
– a device that fits typical Point of Care settings like physician offices
Methods: We developed a microfluidic test cartridge which uses transparent surface coated substrates onto which different capture oligonucleotides are deposited. The substrates are then laminated to a proprietary microfluidic channel system.
During the assay procedure, DNA/RNA is extracted and labeled. The remaining assay-steps like hybridization of target molecules, washing, and the final chemiluminescence (CL) reaction are controlled via capillary forces in the microfluidic channel system. CL-signals from different locations on the chip are detected in a compact readout device.
Results and Conclusion: We developed a generic platform for rapid multi-parameter quantitative molecular diagnostics at the POC.
The platform combines a disposable microfluidic test cartridge with a compact device for CL based optical detection. While the use of capillary-flow based microfluidics avoids the need for complex micropumping schemes, CL enables sensitive and robust detection in a very compact optical architecture.
Our system is able to simultaneously quantify more than 8 different target bacteria down to concentrations of ~10^4 cfu/ml within 15 minutes, without the need for an expert operator.
The modular architecture of the platform allows easy adaptation to any type of diagnostic test.

P1828 Dissection of polybacterial clinical samples using group specific broad range primers and DNA sequencing followed by RipSeq analysis

Ø. Kommedal (Bergen, NO)*

Objectives: Detection and identification of bacteria directly from clinical samples by broad range PCR targeting the 16S rRNA gene and DNA sequencing (Direct 16S sequencing) is a valuable tool in clinical microbiology. RipSeq (iSentio, Norway) is a web based application for the analysis of mixed DNA chromatograms that enables the use of this approach on typical polybacterial specimens such as abscesses and empyema. However, the use of a single universal first PCR reaction limits the potential of the method because all bacteria in a sample will be competing for the same reagents, leaving those present at the lowest concentrations at risk for remaining undetected. Also, the RipSeq algorithm has only been validated for chromatograms containing up to three different species. The aim of this study was to reduce the impact of these factors by replacing the universal PCR with three separate group specific broad range PCRs. The group specific broad range primer pairs (A, B and C) all amplified 500 bp of the 16S rRNA gene and were designed to match approximately one third of the human bacterial pathogens each.
Methods: Twenty polybacterial clinical samples were included and investigated using group specific real-time SYBR-green PCRs followed

by DNA sequencing. A positive PCR was defined if fluorescence threshold value (Ct) was reached ⩾3 cycles before the negative control. All samples had previously been analyzed using culture based identification and standard direct 16S sequencing with a single universal primer pair followed by RipSeq analysis.
Results: The results for the group specific direct 16S sequencing are given in Table 1. In nine samples no additional species were found when compared to standard direct 16S sequencing. In the remaining 11 samples a total of 23 additional bacteria were found ranging from 1 to 4 species per sample. Only 27 out of the 74 bacteria identified by group specific direct sequencing grew by culture. Ninety percent of the samples were affected by antibiotics administrated prior to specimen collection.
Conclusion: Group specific direct 16S sequencing and RipSeq analysis identified a high number of pathogens undetected by culture and standard direct 16S sequencing. The method allows for sequence based identification of up to nine species in a sample and can be established in any laboratory with experience in standard direct 16S sequencing.

Table 1. Detailed results for group specific amplification and DNA sequencing

	Sample type	Primer pair A	Primer pair B	Primer pair C
1	Abscess brain*	*G. morbillorum* *S. intermedius*	–	*F. nucleatum*
2	Abscess brain*	*S. intermedius*	–	*F. nucleatum*
3	Abscess brain	*P. micra* *S. intermedius*	*A. aphrophilus* *H. parainfluenzae*	*F. nucleatum*
4	Abscess brain	*A. meyeri* *S. intermedius*	*C. gracilis*	*F. nucleatum* *P. pleuritidis*
5	Abscess brain	*A. meyeri* *P. micra*	*C. gracilis* *Eikenella* sp. *N. elongata*	*F. nucleatum*
6	Abscess liver	*E. faecium*	*E. coli* *K. pneumoniae*	*L. rhamnosus* *S. haemolyticus*
7	Abscess liver	*G. morbillorum* *S. parasanguinis* *S. pyogenes*	*H. parainfluenzae*	*P. melaninogenica*
8	Abscess liver*		*E. coli*	*C. perfringens*
9	Abscess pancreas*	*E. faecium*	–	*B. fragilis*
10	Abscess pancreas	*A. meyeri*	*C. concisus*	*P. melaninogenica* *S. epidermidis*
11	Abscess para-aortic*	*C. tuberculostearicum*	–	*S. haemolyticus*
12	Abscess subcutaneous*	*E. faecium* *F. magna*	–	–
13	Bile*	–	*E. coli*	*F. nucleatum*
14	Pacemaker pouch*	*P. acnes*	–	*S. epidermidis*
15	Empyema	*P. micra* *S. anginosus*	–	*P. nigrescens* *P. stomatis*
16	Empyema*	*S. intermedius*	*C. gracilis*	*Fusobacterium* sp.
17	Empyema	*P. micra* *S. constellatus* *S. mutans*	*C. gracilis*	*F. nucleatum*
18	Empyema	*S. constellatus* *P. micra*	*C. gracilis* *H. parainfluenzae*	*F. nucleatum* *P. melaninogenica*
19	Empyema	*P. micra* *S. intermedius*	*C. gracilis* *Eikenella* sp.	*P. pleuritidis*
20	Empyema	*D. invisus* *P. micra*	–	*P. nigrescens*

* Samples with identical results for standard direct sequencing and group specific direct sequencing.

P1829 A multicentre study of bacteraemia using a new commercial universal 16S rDNA PCR test

S.G. Sakka, M. Lustig (Cologne, Bremen, DE)*

Bloodstream infection is a life-threatening condition with a high mortality rate, especially in intensive care and neutropenic patients. Standard diagnostics is based on blood culturing (BC). However, limitations of BC include relatively low sensitivities and a long time-to-result for the identification of the pathogen, generally over two days and more. On the grounds of data from a multicentre study using a universal 16S rRNA gene PCR assay, SepsiTest™, molecular diagnosis is discussed as a rapid and sensitive tool for the detection and identification of pathogens supportive of BC.
Objectives: A new commercial PCR test, SepsiTest™, for direct detection of bacteria in whole blood was compared to BC in terms of sensitivity, specificity, predictive values and time to positivity (TTP) of bacterial infections of the blood stream of critically ill patients.
Methods: The test, SepsiTest™ (Molzym, Bremen), comprises the extraction and 16S rRNA gene PCR detection of bacterial DNA in whole blood samples. Bacteria in positive samples were identified by sequence analysis of the amplicon. In a prospective multicentre study 342 blood samples from 187 patients with systemic inflammatory response syndrome (SIRS), sepsis, or neutropenic fever were included.
Results: Compared to BC, the diagnostic sensitivity and specificity of PCR/sequencing was 87.0% and 85.8%, respectively. The positivity rate of PCR/sequencing (25.7%) was higher than BC (15.8%). Of 31 PCR/sequencing-positive, BC-negative patients, most of whom received antibiotics, the PCR results of 25 were judged as true or possible to bacteraemia. Using a routine testing workflow, time to positivity of the PCR-test was on average decreased by 40 hours for anaerobe/fastidious infections and by 54 hours for yeast infections.
Conclusions: The PCR approach enables the detection and identification of bacteraemia in blood samples within a few hours. Despite the indispensability of BC diagnostics, the rapid detection of bacteria by SepsiTest™ appears to be a valuable tool, allowing earlier pathogen-adapted antimicrobial therapy in critically ill patients.

P1830 Value of multiplex-PCR (SeptiFast) for the diagnosis of bacterial and fungal pathogens in newborns and children with suspected sepsis

P. Bernaschi, S. Ranno, B. Lucignano, B. Pizzorno, O. Liesenfeld, D. Menichella (Rome, Monza, IT; Pleasanton, US)*

Objectives: Sepsis continues to be a major health problem in neonates and children. Early diagnosis and provision of appropriate antimicrobial therapy strongly correlates with positive outcomes of sepsis. Multiplex PCR has the potential to rapidly identify blood stream infections and fill this diagnostic gap in sepsis patients.
Methods: Between May 2007 and May 2009, multiplex PCR with the ability to detect 25 different bacterial and fungal pathogens (SeptiFAST) was compared to standard blood culture in 1673 paired blood samples obtained from 803 neonates and children with suspected sepsis in a large Italian pediatric hospital.
Results: SeptiFAST identified an additional 97 microorganisms and 90 blood samples that blood culture failed to detect. Coagulase-negative staphylococci (CNS) (29.6%), *Pseudomonas aeruginosa* (16.5%), and *Staphylococcus aureus* (11.6%) were the most frequent pathogens detected by SeptiFAST. Blood culture detected 27 additional microorganisms in 24 SeptiFAST-negative blood samples; 8 of these 27 pathogens were not included in the SeptiFAST menu, 8 additional pathogens were CNS. Contaminants were detected significantly more frequent in blood culture than in SeptiFAST (n = 97 vs. 26, respectively). Excluding contaminants, sensitivity and specificity of SeptiFAST compared to blood culture were 85.0 and 93.5%.
Conclusion: Real-time multiplex PCR served as a highly valuable adjunct to conventional blood culture in neonates and children, adding diagnostic yield, decreasing the number of contaminants, and shortening the time to pathogen identification.

P1831 Evaluation of a commercial multiplex PCR (SeptiFast) in the aetiological diagnosis of community-acquired bloodstream infections

P. Josefson, K. Strålin, A. Ohlin, T. Ennefors, B. Dragsten, L. Andersson, H. Fredlund, P. Mölling, P. Olcén (Örebro, SE)*

Objectives: The SeptiFast test is a commercial PCR test which detects 25 bacterial and fungal pathogens in whole blood samples within 6 hours. We aimed to evaluate the SeptiFast test on consecutive adult patients subjected to blood culturing during one full year at an infectious diseases clinic, predominantly on hospital admission.
Methods: In a prospective study we included for evaluation the first blood culture (BC)/PCR set (two BC bottles and one PCR tube) providing a positive culture and/or PCR result if any positive result was noted. The PCR results were compared with the results of BC and other microbiological data. In addition, positive PCR results without microbiological support were related to the clinical presentation.
Results: Among 1092 included patients BC was positive in 138 patients and PCR was positive in 107 patients. The positive PCR results

were supported by BC in the same BC/PCR set in 50 cases, by other cultures in another 10 cases, and by the clinical presentation in additionally 9 cases. No patient with positive BC was PCR positive for another pathogen. Compared with BC, PCR showed specificities and neg predictive values of >97% for all detectable pathogens. The following sensitivities and positive predictive values (PPV) were noted: *Staphylococcus aureus*, 67% and 43%; *Streptococcus* species, 43% and 77%; *Streptococcus pneumoniae*, 12% and 67%; *Escherichia coli*, 53% and 56%; and *Klebsiella* species 43% and 23%. If other cultures and clinical presentation were included in the reference standard, the PPV for detection of these bacteria rose to 57%, 92%, 100%, 75% and 62%, respectively.

Conclusion: The SeptiFast test showed high specificities for detection of community-acquired blood stream infections. However, the low sensitivities and suboptimal PPV of the test are essential shortcomings that prohibit a routine use of the test in its present form.

Interferon-gamma release assays

P1832 Evaluation of Quantiferon-gold (tuberculin skin test) for the identification of latent tuberculosis infection in would-be transplant recipient patients referring to an Iranian transplant clinic from September 2007 to December 2008

Z. Ahmadinejad, M. Razaghi, A. Jafarian (Tehran, IR)*

Introduction and Objective: Two billion people carry the Tuberculosis (TB) bacteria. The rate of tuberculosis in solid organ transplant recipients has been estimated to be 50-fold higher than in the general population. Diagnosis & treatment of Latent Tuberculosis Infection (LTBI) are the most important strategies to control TB in transplant candidate patients. In this study we aimed to compare Tuberculin Skin Test (TST) with QuantiFERON-TB Gold In-Tube (QFT) in the detection of LTBI in those patients.

Patients and Methods: Patients at the transplant clinic at the Imam Khomeini Hospital, Tehran, Iran between 2008 and 2009 were eligible for inclusion in the study if they were on the waiting list of transplantation. Patients were screened for LTBI with both QFT & TST. Concordance between test results & variables associated with test discordance were assessed.

Results: A total of 61 patients were enrolled in the study. In 10 patients the TST was not read. So, complete results were available for 51 patients. Average age was 40.2±13.2 years. Thirty patients (58.8%) were male. Four patients (7.8%) had positive TST & 7 patients (13.7%) had positive QFT. Two patients (5.9%) had indeterminate QFT. Overall agreement between tests was 82.4% ($k = 0.091$, $p = 0.495$). Discordant test results were seen in 3 TST positive/QFT negative patients & in 6 TST negative/QFT positive patients.

Conclusion: Overall agreement between test results was high, but its percentage was not significant based on p-value ($p > 0.05$) & k value ($k < 0.4$) represented poor agreement. Thus, neither of the two tests is recommended to the patients on waiting list as a more preferable test to detect LTBI. The decision to select QFT or TST will depend on the purpose of testing & resource availability.

P1833 Increasing the yield of interferon-gamma release assay for *M. tuberculosis*

S. Chandrasekharan, F. DeVilliers (Greenock, UK)*

The Interferon-gamma Release Assay (IGRA) is a useful aid to diagnose infection with M tuberculosis (MTB). In our institution the IGRA is performed once weekly using the T-spot®.TB test, a commercial assay by Oxford Immunotec. This *in vitro* test has an acclaimed specificity of 95% towards MTB and is not influenced by previous BCG administration or by infection with common non-tubercle bacilli. There are no patient exclusions (e.g. age, HIV) from the test. The test was introduced in 2006, but suffered a failure rate of 37.3% during that year.

Objectives:
1. To identify the reasons for early failures.
2. To evaluate the impact of guidelines on failure rates and cost per test.

Methods: A retrospective audit of all IGRA tests performed between 2006 and April 2009. All IGRA requests are routinely recorded and resulted in the laboratory information system (LIS); for failed tests, the reasons are recorded and reported. The LIS was mined for the annual total number of tests performed, total number of valid results obtained, total number of failures and reasons for failures. The impact of specific sampling guidelines (issued in 2007) was evaluated.

Results: 484 samples were processed for the IGRA by the T-spot®.TB test; 71 tests failed. The annual failure rates are shown in table1.

During 2006, 84% of failures were due to insufficient viable cells for testing. Introduction of sampling guidelines reduced this to 18% by 2008, paralleled by an overall decrease in failures. The relative contribution of laboratory processes to failure increased from 4% in 2006, to 25% in 2007, and to 55% by 2008.

Conclusion: There has been a marked improvement in the number of reportable IGRA after introduction of specific sampling guidelines. Adherence to an 8 ml minimum specimen volume, or 16 ml in the elderly/immunocompromised maximised the likelihood of extracting adequate effector T-cells. This intervention had the greatest positive impact. As specimen volume and quality improved, the contribution of laboratory techniques towards failures increased. The IGRA test became most cost-effective during 2008. This audit highlighted that laboratory procedure has now become the most important reason for failure. Finally, the audit sounds a warning that the specimen quality issues might be recurring at present (2009).

	2006	2007	2008	2009 (Jan–April)
Failed tests	25	28	11	7
Total tests	67	173	172	72
Failure rate	37%	16%	6%	10%

P1834 Comparison of QuantiFERON® TB Gold with tuberculin skin test for the diagnosis of tuberculosis infection in risk groups

E. Perez-Escolano, J. Gutierrez-Rodriguez, E.M. Menor, J.C. Alados, M.I. López, S. De Tena, M.D. López-Prieto (Jerez de la Frontera, ES)*

Objective: To evaluate the agreement of the QuantiFERON® TB Gold In Tube test (QFT) and the tuberculin skin test (TST) for the diagnosis of tuberculosis infection (TBI) in risk groups (RG), and to establish the utility of QFT as a tool for the indication of TBI treatment.

Methods: We studied 409 immunocompetent persons who came for screening of tuberculosis infection; average age was 41 years (SD: 14.8), 79% were men and 263 (65.4%) had been vaccinated with BCG. All were screened with chest X-ray, TST, QFT (Cellestis, Australia) and risk factors were registered in a questionnaire. TST was performed by Mantoux method and a positive test was defined as an induration $\geqslant$5 mm in non-vaccinated and $\geqslant$15 mm in vaccinated people. QTF was made according to the manufacturer specifications. We considered as vaccinated persons those presenting with a suggestive scar. CDC recommendations were followed for the interpretation of the QFT and the treatment of the TBI. Agreement between TST and QFT was assessed by the Cohen kappa coefficient.

Results: Agreement between the TST and the QFT was 78.7% (Kappa 0.51, CI (0.36–0.65)) among non-vaccinated population. In the vaccinated group, agreement was smaller (69.6%, Kappa 0.38, CI (0.27–0.49). QTF(−)/TST(+) was the most frequently detected discordant result for the non-vaccinated group. Whereas, for vaccinated people, rate of QTF(+)/TST(−) results and QTF(−)/TST(+) results were similar. TST was positive in 107/136 non-vaccinated people, from these 26 were negative for QTF. In the vaccinated group, 120/263 were TST positive, from these 46 were negative for QTF. The indication of TBI treatment made by TST and risk situation was modified in 42% of cases according

to QFT test. We prescribed treatment of TBI by QFT in 25% of the patients that did not have indication according to the TST.
Conclusions: Agreement between TST and QFT was low in vaccinated people, even thought TST was considered positive for 15 mm. Whereas, in non-vaccinated people the agreement was moderate. The use of QFT allows for a better selection of infected individuals and to reduce the number of unnecessary treatment of TBI.

P1835 Whole-blood interferon-gamma release assay for diagnosis of tuberculous lymphadenitis

Y.K. Kim, H.Y. Kim, Y. Uh, M. Cho, C.O. Kim (Wonju, KR)*

Background: Tuberculous (TB) lymphadenitis is not uncommon in Korea. The invasive procedure is needed for the diagnosis of TB lymphadenitis and it is sometimes difficult. We evaluated the usefullness of whole-blood inferferon-γ release assay (QuantiFERON-TB Gold[QTF-G], Cellestis, Carnegie, Australia) for diagnosis of TB lymphadenitis.
Materials and Methods: From July 2007 to August 2009, 67 patients had lymph node biopsy and underwent the QTF-G assay concurrently in Wonju Christial Hospital, Yonsei University Wonju College of Medicine. Sixty-three patients were reviewed retrospectively with exclusion of 4 patients whose QTF-G results were indeterminate. Among them, 16 patients were pathologically confirmed as TB lymphadenitis and 47 patients were diagnosed as other than TB lymphadenitis. The diagnostic performances of the QFT-G assay were evaluated.
Results: Mean age was 35.2±15.9 year old and 18 (28.6%) patients were male. The positive result of QFT-G assay was found in all 16 patients with TB lymphadenitis, but false positive result was found in 6 patients without TB lymphadenitis. The sensitivity of QTF-G was 100% (95% CI, 79.4–100) and the specificity was 87.2% (95% CI, 74.3–95.1). The positive predictive value was 72.7% (95% CI, 49.8–89.3) and the negative predictive value was 100% (91.4–100). In the patients with positive QTF-G results, the value of interferon-gamma was much higher in the patient with TB lymphadenitis than in the patients without TB lymphadenitis (45.53±55.54 IU/mL versus 1.20±1.05 IU/mL, $p < 0.001$).
Conclusion: The value of interferon-gamma must be considered for the interpretation of QTF-G result. The QTF-G would be very helpful for the diagnosis of TB lymphadenitis.

P1836 Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in patients with rheumatic diseases

M. Losi, M. Meacci, D. Ferrari, R. D'Amico, D. Giuggioli, F. Luppi, B. Meccugni, C. Manzini, C. Ferri, P. Roversi, L. Richeldi, F. Rumpianesi (Modena, IT)*

Objectives: Treatment with tumor necrosis factor α (TNF-alfa) antagonists or immunosuppressive drugs in patients with rheumatic diseases is related to an increased risk of tuberculosis (TB), due to reactivation of latent tuberculosis infection (LTBI). Treatment of subjects latently infected with *M. tuberculosis* reduces the risk of developing active TB. The tuberculin skin test (TST) performs sub-optimally in immunocompromised individuals: the new interferon-gamma release assays (IGRA) might improve LTBI diagnosis in this vulnerable population.
Aim of the study was to evaluate in a prospective study the performances of QuantiFERON-TB Gold In-Tube (QFT-IT) and T-SPOT.TB (TS.TB) assays in patients with rheumatic diseases in an area with low prevalence of TB.
Methods: Both IGRA and TST were simultaneously performed in 39 patients (90% Italians, 74% females, mean age 53±14.6 years, 8% BCG vaccinated) affected by rheumatoid arthritis (44%), psoriatic arthritis (26%), ankylosing spondylitis (10%), undifferentiated spondyloarthropathy (5%) and other rheumatic disorders (15%). Twenty-nine patients were on immunosuppressive treatment at the time of testing: 12 (41%) were treated with disease modifying anti-rheumatic drugs (DMARDs), 10 (34%) with steroids and DMARDs and 7 (24%) with steroids only. Most (87%) patients were screened before treatment with TNF-alfa antagonists.
Results: TST was positive (with the 5 mm cut-off) in 9, QFT-IT in 7 and TS.TB in 13 patients (TST vs QFT-IT $p = 0.41$, TST vs TS.TB $p = 0.10$, QFT-IT vs TS.TB $p = 0.0143$). Overall agreement between QFT-IT and TS.TB was substantial (88.5%, $k = 0.71$). Three of the 6 TST-negative/IGRA-positive patients were on immunosuppressive treatment. Indeterminate IGRA results due to low positive control values were more frequent with QFT-IT ($n = 4$, 10%) than with TS.TB (0%, $p = 0.045$).
Conclusions: The results of this study indicate that among patients with rheumatic diseases, TS.TB identifies as infected more subjects, as compared to QFT-IT. Furthermore, indeterminate results are more frequent with QFT-IT than with TS.TB.

P1837 Role of the interferon-gamma assay in the diagnosis of active tuberculosis

M. Losi, A. Fabio, M. Meacci, F. Luppi, R. D'Amico, C. Del Giovane, P. Roversi, M. Apice, B. Meccugni, L.M. Fabbri, L. Richeldi, F. Rumpianesi (Modena, IT)*

Objectives: Diagnosis of pulmonary (pTB) and extra-pulmonary (eTB) active tuberculosis might be difficult when standard microbiological methods are negative, in particular in extra-pulmonary cases. Data are not completely consistent across different studies: the new interferon-gamma release assays (IGRA) might improve the diagnosis of active TB.
Methods: In a multicenter study, we retrospectively evaluated 87 patients with culture-confirmed active TB (77.0% pTB, 23.0% eTB) tested with QuantiFERON-TB Gold In-Tube (QFT-IT) (Cellestis Ltd, Carnegie, Australia). Most patients were male (62.1%), mean age was 40.7±19.0 years: 65 (74.7%) subjects were foreign-born. All QFT-IT assay were performed before initiation of anti-TB treatment.
Results: Indeterminate QFT-IT were 11.9% in pTB and 5.0% in eTB: the test was positive in 71.6% of pulmonary and 80.0% of extra-pulmonary patients (p=ns). IFN-γ levels were similar in the two groups (4.6 pTB vs 7.0 eTB UI/ml, p=ns). Interestingly, 14 patients (16.1%) had determinate negative QFT-IT: 11 (78.5%) had pulmonary disease.
Conclusions: These preliminary data indicate that QFT-IT might be a reliable tool in diagnosing active TB, both in pulmonary and extra-pulmonary cases, in routine clinical practice.

P1838 Use of interferon-gamma release assay in the United Kingdom: compliance with national guidance

C. Ijeh, M.M. Raza, M. Bhattacharya, R. Mukherjee (Milton Keynes, UK)*

Objectives: The National Institute of Healthcare and Clinical Excellence (NICE) in the United Kingdom (UK) has published its guidance on tuberculosis (TB) in March 2006. This includes recommendations for diagnosis of active and latent tuberculosis in various settings including use of interferon-gamma release assay (IGRA). We undertook an audit of the use of an IGRA (ELISpot) in our hospital since July 2006 to establish if this was in compliance with NICE guidance.
Methods: This audit was done in a UK district general hospital. The microbiology laboratory system was used to identify all patients who had an ELISpot test requested between July 2006 and December 2008. Case notes of these patients were then reviewed to establish the indication for the test, whether a prior Mantoux test was performed and compliance with NICE guidelines.
Results: 54 adult patients had the test done during the period of the study. Approximately half (48%) of patients were between 16–35 years of age with an equal sex distribution. 75% of the ELISpot tests were requested by a member of Respiratory or Infectious Diseases team. In 29 patients the test was done for diagnosis of suspected latent TB but of these only 13 requests were compliant with NICE guidance in this area. In the other 16 cases the non-compliance was due to age being greater than

35 (7), absence of prior Mantoux testing (3), both age and no Mantoux (4) and in 2 cases the Mantoux reading was not high enough to justify the ELIspot testing. In the remaining 25 patients the test was done for suspected active TB in various presentations including lymphadenopathy, pleural effusions, cavitating lung disease, discitis, persistent cough. As NICE does not recommend IGRA as a routine test in the diagnosis of active TB therefore compliance with NICE recommendations for use of IGRA was seen in 13/54 cases (24%).

Conclusion: In our hospital use of IGRA for the diagnosis of TB is in compliance with national guidance in only a minority of cases (24%). There are clear recommendations for its use in the diagnosis of latent tuberculosis but still its use in this area was outside the guidance in 16/29 cases of suspected latent TB. Use of IGRA is not recommended in the diagnosis of active TB but may have a role in certain cases. Therefore we recommend that use of the test is restricted by requiring requesting or prior approval by a Respiratory/infectious Diseases Physician.

P1839 Performance evaluation of an in-house interferon-gamma release assay in the diagnosis and follow-up of active tuberculosis disease

L. Alagna, C. Fortis, L. Galli, P. Scarpellini (Milan, IT)*

Objectives: To evaluate the performance of the interferon-gamma release assays (IGRA) for the diagnosis of the active tuberculosis disease (TD) and to describe the changes of the IGRA during the follow-up.

Methods: 238 patients (pts)[M = 140, F = 90] have been tested with the IGRA because suspected of active TD.187 out of 238 were tested with the tubercoline skin test (TST). Pts were classified according to the American Thoracic Society (ATS) classification. The home-made IGRA detects the number of specific γ interferon producing T cells by means of an enzyme-linked immunospot assay, using a restricted pool of synthetic highly immunogenic peptides derived from ESAT-6 and CFP-10 proteins. For the evaluation of the IGRA follow-up in pts with active TD (15 pts), we considered this timeline: T0=mean of the IGRA values up to one week after the start of the therapy, T1=second and third months after the start of the therapy and T2=end of the therapy. The TST and the clinical classification were taken as reference when compared to IGRA. McNemar test and k statistics were calculated. Linear regression was applied.

Results: Among pts with active TD (ATS3) no agreement was detected between the IGRA and TST (k = 0.0767, 95% CI [0.126–0.280]); sensibility (SS)=84%, specificity (SP)=24%, Negative Predictive Value (NPV)=50% and Positive Predictive Value (PPV)=61%. There was a high number of pts with positive IGRA and negative TST [23/30 (77%), p = 0.0013]. We analysed the performance of both tests in relation to the clinical classification (ATS3 vs non ATS3): TST, SS=59%, SP=70%, PPV=55% and NPV=72% and no agreement was found (k = 0.278, 95% CI [0.139–0.418]). IGRA, SS=82%, SP=62%, PPV=54% and NPV=86%; agreement was fair (k = 0.4015 [0.293–0.509]). During follow up, significant decrease of IGRA quantitative values was observed [median slope = –2.98 spot forming cells per million lymphocytes per week, p = 0.0215 (SFCML)]. 13 out of 15 pts show a decrease of values (median –4.23 SFCML, p = 0.021).

Conclusions: Our results show a different performance of TST and the IGRA in pts with active TD. In particular, given the high SS, IGRA has a better performance than TST. Regarding the follow up, the decline in the IGRA values during the therapy is significant; further studies can help to evaluate if this decline could predict a favourable outcome.

P1840 Assessment of the use of the Quantiferon-TB gold in-tube assay for the diagnosis of TB infection in Lothian, Scotland

A-L. Seagar, C. Doig, C. McSparron, A. Hill, I. Laurenson (Edinburgh, UK)*

Objectives: The Quantiferon-TB Gold In-tube assay (QTBG; Cellestis) detects IFN-γ which is released from patients T-cells in response to *M. tuberculosis* specific antigens. Unlike the TST, results are unaffected by BCG vaccination and most atypical mycobacteria. We assessed appropriateness of assay use from Lothian area patient data from September 2007 to July 2009.

Methods: Scottish and NICE guidance recommends use of interferon-gamma release assays following a positive or unreliable TST. We do not recommend routine use in diagnosis of active TB infection (ATBI). Clinical information sheets completed at the time of sampling were used to assess whether the patient had ATBI or latent TB infection (LTBI). ATBI was determined if TB symptoms were indicated (e.g. weight loss and fever). LTBI was determined in patients with a positive TST result, travel history or CXR changes. If ATBI or LTBI could not be determined from the sheets then an "unknown" verdict was recorded.

Results: Samples were collected from 104 patients. Samples from 7 patients were unsuitable for QTBG testing. 18 (17.3%) QTBG positive, 68 (65.4%) negative and 11 (10.6%) indeterminate results were obtained. 10% of all specimens received were from patients ⩽5 years of age. 76 of 97 patients (78.3%) tested by QTBG had clinical details recorded on the data sheets. ATBI, LTBI or an "unknown" diagnosis was identified in 35 (36.1%), 46 (47.4%) and 23 (23.7%) of patients respectively. 10 (21.7%) of LTBI cases produced a QTBG positive result and 4 (11.4%) of ATBI cases produced a positive result. 14 of 83 (16.9%) of patients assessed to be at high risk of TB produced a QTBG positive result. 54 (65.1%) of these patients had a positive TST result (9 [16.7%] were QTBG positive). 31 patients had a TB contact (3 [9.7%] QTBG positive). 8 of 15 patients who had an abnormal CXR and 12 of 14 patients with a history of travel or living abroad were investigated for LTBI (3 [37.5%] and 4 [33.3%] were QTBG positive respectively). 2 of 6 immunocompromised patients produced QTBG indeterminate results.

Conclusions: 36.1% of QTBG tests were used in assessment of ATBI generally considered inappropriate as false negatives are well documented. In Lothian, QTBG is not currently being used to support the diagnosis of LTBI for healthcare workers and new entrants. To allow the correct interpretation, advice and audit sufficient clinical information is required with requests.

Clinical details*	Total	ATBI	LTBI	Unknown	QTBG-positive (%)	QTBG-negative	QTBG-indeterminate	QTBG Not done#	TST Positive (QTBG-positive)
TB contact	31	14	15	2	3 (9.7)	22	3	3	23 (2)
Travel/lived abroad	14	2	12	0	4 (28.6)	8	2	0	12 (3)
Abnormal CXR	15	7	8	0	4 (26.7)	9	1	1	8 (3)
TB symptoms only	3	3	0	0	2 (66.7)	1	0	0	2 (1)
Pre-BCG	5	0	5	0	1 (20.0)	4	0	0	1 (0)
Anti-TNF	3	0	3	0	0	2	1	0	3 (0)
Other	12	9	3	0	0	9	1	2	5 (0)
No clinical details	16	N/A	N/A	16	4 (25.0)	10	1	1	9 (2)
No data sheet	5	N/A	N/A	5	0	3	2	0	N/A
ALL	104	35	46	23	18 (17.3)	68	11	7	63 (11)

*One detail per patient; #Six overfilled tubes and one incubated incorrectly.

P1841 Diagnostic challenges of tuberculosis peritonitis in patients with and without liver cirrhosis

C. Yang, C.H. Liao, P.R. Hsueh (Taipei City, TW)*

Objectives: Little was known of the diagnostic features of tuberculosis (TB) peritonitis among patients with liver cirrhosis compared with those without liver cirrhosis. We conducted a retrospective study in patients with and without liver cirrhosis from over a period of 8 years to analyze the clinical features for TB peritonitis.

Methods: The medical records of all patients with TB peritonitis who were seen at National Taiwan University Hospital and Far Eastern

Memorial Hospital from 2000 through 2008 were reviewed. The diagnosis of TB peritonitis was based on culture of ascetic fluid and/or peritoneal biopsy specimens.
Results: During the study period, 73 patients (32 with liver cirrhosis and 41 without liver cirrhosis) had TB peritonitis. Acid-fast smears (AFS) of ascites were negative in all patients. The patient with liver cirrhosis had less co-existing pulmonary tuberculosis (9% vs 29.2%, $p < 0.05$) and less ascites total protein level (2.19 g/dL ±1.0 vs 3.90g/dL ±1.7, $p < 0.05$). Although the white cell differential count in ascites was lymphocyte-predominant between the two study groups, almost one-third patients with liver cirrhosis were treated as spontaneous bacterial peritonitis (SBP) because of elevated absolute neutrophil count (⩽250 cell/mm^3) in ascites. More than half patients with liver cirrhosis died before the diagnosis of TB peritonitis was established (59.4% vs 19.5%, $p < 0.05$). In addition, fewer patients with liver cirrhosis could complete the anti-TB treatment than patients without liver cirrhosis (25% vs 60.9%, $p < 0.05$).
Conclusion: TB peritonitis had nonspecific and variable manifestations. High mortality rate and difficulty in the treatment of TB peritonitis were observed in patients with liver cirrhosis. It should be considered if the treatment of SBP had poor clinical response.

P1842 Immunological diagnosis of tuberculous uveitis: first experiences

M. Peracchi, L. Fallico, R. Manganelli, A. Sattin, G. Palù, S. Tognon (Padua, IT)*

Objectives: To determine whether Quantiferon-TB Gold In Tube (QFT-IT) is a useful alternative to TST in the immunological diagnosis of tuberculous uveitis and whether it evolves in response to anti-tuberculous therapy.
Methods: We identified 38 patients, seen at the Clinical of Ophthalmology-University of Padua, Italy, with suspected TB uveitis in a two years period (2006–2008). Their medical records, including chest radiographs, and ocular visit were reviewed. All of them were subjected to QFT-IT pre-therapy, while 8 patients were also screened after treatment. TST was performed in 20 patients. Fifty percent of the patients had been previously diagnosed with tuberculosis resolved after antitubercular treatment (ATT).
Results: all patients resulted positive to QFT-IT before therapy and correlated, when tested, with TST. No indeterminate results occurred. Six patients were found to have culture positive TB pulmonary infections. The QFT-IT mean positive value for the 19 patients with previous TB, was 6.4 (0.39–37.53), while the mean positive value for patients never diagnosed with TB before was 16.1 (0.59–46.83). Four patients refused therapy, 28 assumed recommended ATT for at least 6 months and 6 are actually under ATT. After treatment all patients but one had decrease of recurrences. The value of QFT-IT decreased after therapy in five out of eight patient tested so far.
Conclusion: these preliminary results suggest that positive Quantiferon-TB Gold In Tube correlated with a positive TST, although QFT performed better in terms of specificity and had a better compliance by the patients. For this reason QFT-IT should be useful, instead of the TST, to presume a TB uveitis and aid the decision to initiate ATT.

P1843 Clinical usefulness of Elispot assay for diagnosis of tuberculosis in a Korean population

H. Kim, C.H. Kim, M. Hur, M. Park, W. Song, H.J. Kang, K. Lee (Seoul, KR)*

Objectives: Tuberculosis-specific ELISPOT assay (T-SPOT.TB, Oxford Immunotec, UK) is a test that detect interferon-gamma producing T-cells after stimulating patient's lymphocytes with two kinds of tuberculosis-specific antigens (ESAT-6 and CFP-10). We evaluated clinical usefulness of T-SPOT.TB test in Koreans whose incidence of tuberculosis is intermediate and BCG vaccination has been mandatory at birth.
Methods: T-SPOT.TB test was performed in 79 patients and 64 healthy volunteers and these patients and volunteers were classified into four groups: group 1, patients with active tuberculosis (n = 30); group 2, patients with not-active or treated tuberculosis (n = 27); group 3, patients without tuberculosis (n = 24); group 4, healthy volunteers without tuberculosis history (n = 62). Positive rates and average spot counts of T-SPOT.TB test were obtained among these groups.
Results: Positive rates of group 1 (96.4%) and group 2 (92.3%) were higher than those of group 3 (31.6%) and group 4 (27.4%) ($P < 0.0001$). Sensitivity deduced from group 1 and specificity deduced from group 4 of T-SPOT.TB test was 96.4% and 72.6%, respectively. The average spot counts of group 1 and group 2 were higher than those of group 3 and 4 ($P < 0.001$). There was a tendency of increasing positive rate with increasing age. Overall agreement between T-SPOT.TB test and TST (tuberculin skin test) was 63.8% (kappa=0.29).
Conclusions: T-SPOT.TB test seemed to be a very useful screening and supplementary test for diagnosis of tuberculosis due to high sensitivity. However, positive results of T-SPOT.TB should be cautiously interpreted because of high positivity in treated tuberculosis patients and healthy volunteers in Korea.

New insights in *Streptococcus* spp. detection

P1844 Molecular and clinical characteristics of invasive group A, C and G streptococcal disease in western Norway

B. Kittang, S. Skrede, N. Langeland, H. Mylvaganam (Bergen, NO)*

Objectives:
- Determine the incidence and clinical manifestations of invasive disease caused by group A, C and G streptococci (GAS, GCS and GGS) in western Norway during February 2006-March 2009.
- Identify possible correlation between clinical syndromes and virulence gene profiles.

Methods: We included 110 patients with invasive disease caused by GAS, GCS and GGS within an area in western Norway populated by approximately 405 000 inhabitants. All the 107 available bacterial isolates were emm typed and screened for the presence of 11 GAS superantigen genes (SAg genes) and the gene encoding streptococcal phospholipase A2 (SlaA). The 48 GCS/GGS isolates were also subjected to PCR with primers targeting speGdys.
Results: Sixty invasive GAS (iGAS) infections were registered, corresponding to a mean annual incidence rate of 5.0/100 000. Invasive GCS/GGS (iGCS/GGS) accounted for the remaining 50 infections (mean annual incidence rate of 4.1/100 000). The 30 day case-fatality rate was 10% and 2% among patients with iGAS and iGCS/GGS disease, respectively. Non-necrotic skin or soft tissue infections were the most common clinical manifestations of both iGAS (27%) and iGCS/GGS (60%) disease. Necrotising fasciitis (NF) was diagnosed in 14 patients with iGAS infections (23%) and in one patient with iGCS/GGS infection. Streptococcal toxic shock syndrome (STSS) developed in six patients with iGAS disease and in one patient with iGCS/GGS disease. emm1, emm3 and emm28 accounted for 32 (54%) of the GAS isolates. emm3 was associated with three cases of NF that all developed STSS, and emm 1 was associated with three cases of NF and two cases of STSS without NF. SAg gene profiles were conserved within most of the GAS emm types, although five different profiles were obtained within isolates of emm28. Nineteen GAS isolates possessed SlaA, including nine out of 10 isolates of emm3, six out of 13 isolates of emm28 and all four isolates of either emm9 or emm75. stG643 (n = 11) was the dominating GCS/GGS emm type, and this type together with stC74a, stG10, stG485, stG6 and stG652 accounted for 77% of the GCS/GGS isolates. speGdys was detected in 35 out of 48 GCS/GGS isolates and was restricted to certain emm types.
Conclusions:
- The incidence of both iGAS and iGCS/GGS disease was high in our community;
- NF was an unusually frequent manifestation of iGAS disease;
- emm1 and emm3 were associated with severe clinical infections;
- emm28 isolates seemed to have different phage preferences.

P1845 Quality management of *Streptococcus pyogenes* detection by Gen-Probe in a regional hospital

R. Rennie, T. Bennett, S. Juraville (Red Deer, CA)*

Objectives: This study was designed to investigate reasons for large numbers of indeterminate *Streptococcus pyogenes* detection(GASD) results in a large community based hospital when tested by Gen-Probe Group A *Streptococcus* Direct (Gen-Probe Inc., San Diego CA) that then required culture as a gold standard to arbitrate the observations. Solutions were implemented which modified the manufacturer's recommendations and reduced the number of these imprecise results.
Methods: The investigation was divided into two six month study periods (A and B). Throat swabs were received in Copan 140CQ transport medium and were processed in each period according to the manufacturer. All samples were then processed using the Gen-Probe GASD kit and were read on the Gen-Probe Leader 450. Results were recorded as Negative (<4500 RLU). We have established an indeterminate range of 4500–10000 In the first study period, samples were read 5 min. after incubation (manufacturer's instructions – within 1 hr). In the second study period, samples were allowed to cool for a defined 15 min. before reading. Samples from each period that were in the Indeterminate range were re-plated on anaerobic BAP and examined after 24 hr incubation for β-haemolytic streptococci typical of *S. pyogenes*. Suspect colonies were then grouped using antisera to groups A, C, and G.
Results: In periods A and B there were 2558 and 1147 positive GASD results respectively (i.e. >10,000 RLU) that were not further tested. For samples in the indeterminate range, in period A there were 592 that required repeats and culture, and of these 103 were culture positive. In period B, there were 542 In the Indeterminate range, with 39 that were culture positive. In period A, 345 samples were between 4500–5000 RLU; this was reduced to 194 in period B. Positive cultures in the modified grey zone were reduced from 17. 4 to 7.1%.
Conclusion: The manufacturer does not currently have a grey zone. Results are considered positive at >4500 RLU. Increasing the positive cut-off to >10,000 RLU and further investigation of our in-house established grey zone results, the number of tests requiring repeated Gen-Probe and culture was reduced by approx. 50%. Further, extending the cooling period before reading resulted in more consistent observations and reduced back-up cultures by greater than 50%. We conclude that these modifications improve the accuracy of GASD results with better turn-around, and lower cost for the laboratory.

P1846 New insight in *Streptococcus agalactiae* phylogeny provided by multilocus sequence typing in comparison with multilocus enzyme electrophoresis

L. Mereghetti, G. Héry-Arnaud, J.L. Jany, P. Lanotte, S. Gouriou, N. van der Mee-Marquet, A. Rosenau, M.F. Lartigue, R. Quentin (Tours, Brest, FR)*

Objective: Multilocus enzyme electrophoresis (MLEE) was the first technique used to explore the genetic diversity of *Streptococcus agalactiae* population, and it has remained a reference method for many years. The aim of this study was to compare multilocus sequence typing (MLST), a more recent typing method that has never been formally evaluated for *S. agalactiae* species, to MLEE.
Methods: We compared MLST to MLEE by studying a collection of 113 *S. agalactiae* human strains well-characterized by MLEE. We first re-analyzed previous MLEE data using the Sequence Type Analysis and Recombinational Test software. The phylogeny provided by the MLST data was then analyzed with the allelic profiles and with the nucleotide sequences of the seven MLST genes concatenated into a single "supergene". The maximum-likelihood (ML) analyzes were conducted on the supergene obtained from the MLST data.
Results: The congruence between the two typing methods was 87.3%, with a significative statistical association ($P < 2.2 \times 10^{-16}$). The 58 MLEE types, based on the analysis of 12 enzymes, resolved into seven new clonal groups, while MLST was able to distinguish four main genetic divisions (CC12, CC17, CC19 and CC23) and 42 sequence types based on the analysis of nucleotidic sequences of seven housekeeping genes. Discriminatory index was 0.939 for MLEE while it was 0.897 for MLST. However, phylogenetic position of CC23 differs between methods. CC23 was confirmed by ML analysis using the ModelTest software with the MLST data. This is all the more interesting that CC23, unidentified by MLEE, is marked by specific biological features: (i) CC23 strains are the lonely that resist to bacteriophages from hyperinvasive CC17 strains, (ii) CC23 strains express greater variation in molecular weight of putative "species-identifying" biomarker ions than strains of other clonal complexes, and (iii) there is a discrepancy between CC23 strains low adherence to human fibrinogen and the high prevalence of fbsB gene within these strains. Conversely, ML shows a widely distribution of strains that were supposed to cluster into CC19. This was consistent with the high recombination rate observed between strains, as assessed by determining the recombination pairwise homoplasy index ($P = 3.48 \times 10^{-13}$).
Conclusion: Even though discriminatory power of MLEE is higher than MLST, phylogenetic analysis based on MLST data brings considerable information concerning the genetic structure of *S. agalactiae* population.

P1847 The diagnostic utility of lytA real-time PCR in sputum and nasopharyngeal swabs for pneumococcal pneumonia and colonization in HIV-infected adults

W. Albrich, P.V. Adrian, N. van Niekerk, M. Wong, M. Khoosal, P. Zhao, A. Deatly, M. Pride, M. Sidhu, K. Jansen, S.A. Madhi, K.P. Klugman (Aarau, CH; Soweto, ZA; Pearl River, US; Atlanta, US)*

Objective: Pneumococcal aetiology is underestimated in patients with pneumonia as specimens are unavailable from lung tissue. We evaluated the diagnostic accuracy of real-time PCR in HIV-infected South African adults who either had X-ray confirmed pneumonia with pneumococci identified on at least one diagnostic specimen or were asymptomatic outpatients serving as controls.
Methods: lytA real-time PCR was applied on sputum and nasopharyngeal (NP) swabs of HIV-infected patients with X-ray confirmed pneumonia; and on NP swabs in HIV-infected asymptomatic controls. Pneumonia was assumed pneumococcal if *Streptococcus pneumoniae* was identified in blood culture, sputum culture or Gram stain or urinary Binax® Now.
Results: In 91 pneumonia patients with evidence of pneumococcal aetiology, sensitivity of lytA PCR in sputum was 92.3%, and 97.3% in patients who had pneumococci identified by sputum culture. lytA PCR from NP swabs was positive in 90 of 100 (90%) pneumonia patients and in 79/83 (95.2%) of those with a positive NP swab culture; in comparison, it was positive in 57 of 288 (19.8%; RR: 4.55, $p < 0.001$) asymptomatic controls, and in 23/29 (79.3%; RR: 1.20, $p = 0.02$) of controls with a positive NP swab culture. Among those with positive lytA PCR from NP swabs, log10 copies/ml were significantly higher in patients (mean: 6.86; 95% CI: 6.18, 6.52) than controls (mean: 4.02; 95% CI: 3.59, 4.45; $p < 0.001$). There were significant correlations between log10 of quantitative NP colony counts achieved by microbiological culture and of lytA PCR copies/ml (r: 0.7, $p < 0.001$). The AUC-ROC of lytA PCR for pneumococcal aetiology in pneumonia patients was 0.7 for both sputum and NP swab.
Conclusion: lytA real-time PCR from sputum and NP swab is highly sensitive for detection of both pneumococcal colonization and pneumonia in South African HIV-infected adults with a large burden of pneumococcal disease. Higher copy numbers correlate with clinical disease and higher bacterial loads as identified by standard microbiological tests. Quantitative lytA real-time PCR on sputum or NP swab may be a promising tool for diagnosis of pneumococcal pneumonia, both for clinical diagnostic and epidemiologic purposes.

P1848 Evaluation of real-time PCR in whole blood to detect pneumococcal infection in children

J. Chantreuil, S. Cantagrel, Z. Maakaroun, G. Baty, L. Bret, A.L. Bastier, F. Monceaux, E. Saliba, A. Goudeau, P. Lanotte (Tours, Orléans, FR)*

Objective: *Streptococcus pneumoniae* is a major pathogen implicated in invasive diseases in children such as pneumonia with or without pleuritis, meningitis and bacteremia. Actually, the laboratory diagnosis of invasive pneumococcal disease (IPD) relies on culture based-methods. The interest of the rapid urine antigen is controversed in children. The objective of this study was to determine the benefit of specific *S. pneumoniae* real-time PCR on serum samples suspect of IPD.
Methods: Children suspected of IPD between November 2008 and May 2009 were enrolled. The pneumococcal diseases suspected were classified in four clinical categories according to the probability of *S. pneumoniae* with the clinical and biological data. Real-time PCR of ply and/or lytA genes on sera were performed in a second time.
Results: We prospectively enrolled 76 children, aged from seventeen days to twelve years. Five cases were considered as certain based on pneumococcal positive blood cultures: four pleuro-pneumonia, and one lobar pneumonia; 38 infections were likely (24 pleuro-pneumonia, five pneumonia and nine isolated fever), 26 possible, and eight unlikely. The secondary analysis with real-time PCR on serum samples identified 12 pneumococcal infections with a prevalence of 15.8%. The sensibility of PCR was 60% in certain infections (2/5 with positive blood cultures) but 75% in the pleuro-pneumonia versus 44.4% with the culture alone. The global sensibility to detect IPD with the PCR method was 83.3% (10/12) and the specificity 97%. Only one false positive was found with the amplification of the gene ply: the blood culture was positive with group A *Streptococcus*.
Conclusion: Our study demonstrates the interest of real-time PCR especially in infections with pleural affect. The benefit can be discussed in septic shock and meningitis. Moreover our study has shown the greatest specificity of the amplification of the lytA gene than ply gene.

P1849 A semi automated real-time duplex PCR assay for rapid detection of *Streptococcus pneumoniae* directly from clinical samples

M. Sidhu, P. Zhao, A. Deatly, S. Huijts, M. Pride, K. Jansen (Pearl River, US; Utrecht, NL)*

Background: Accurate diagnosis of pneumococcal infections is critical in assessing the effectiveness of pneumococcal-conjugate vaccination on colonization in light of both serotype switching and replacement. Sensitive, rapid, and reliable detection is essential for surveillance of nasopharyngeal carriage. We developed and characterized a semi-automated, real-time duplex PCR assay to detect *S. pneumoniae* (SPn) directly from clinical samples.
Methods: Targeting the lytA and ply genes simultaneously, we tested 91 SPn serotypes in addition to 26 non-related bacterial strains. Assay sensitivity was established by spiking different concentrations of SPn into naïve human blood. The method was then applied to DNAs extracted by automation from 100 μL of 150 blood (chest X-ray confirmed CAP), 22 pleural fluid (PF), and 147 cerebrospinal fluid (CSF) specimens. In positive DNA, the Prevnar 13 vaccine serotypes were identified by capsule specific real-time PCR.
Results: Detection limits for lytA and ply genes were reproducibly established at 160 CFU/mL of blood. Only SPn strains were amplified showing an assay specificity of 100%. Of the 150 blood samples, 13% were detected by blood culture while 14% by PCR. Detection rates in PF and CSF were 91% and 12%, respectively.
Conclusion: Our highly sensitive (1.6 CFU per reaction) and specific PCR assay offers rapid detection of SPn in clinical specimens. Substantially increased detection rates observed in PF swabs indicate the usefulness of this assay on samples obtained from sites with higher bacterial burden. Details of the method and a comparison between PCR and blood culture method will be presented at the meeting.

P1850 Differentiation of *Streptococcus pneumoniae* from *Streptococcus mitis*/*Streptococcus oralis* by recA-gene-based PCR

A. Zbinden, N. Köhler, G.V. Bloemberg (Zurich, CH)*

Objectives: 16S rRNA gene analysis is the gold standard for molecular identification of bacteria. However, the discriminatory power of the 16S rRNA-gene is too low to differentiate *S. pneumoniae* from *S. mitis* and *S. oralis*. We propose the recA-gene as additional, more discriminatory target for proper identification to species level.
Methods: A recA-PCR was developed and investigated with a preliminary number of strains. Type strains of *S. pneumoniae*, *S. mitis* and *S. oralis* as well as 18 strains of *S. pneumoniae* identified by conventional phenotypic methods including colony morphology, susceptibility to optochin and bile solubility, were analysed. With a specific set of recA-primers, a 313-bp fragment of the recA-gene was amplified and sequenced. The recA-sequences were aligned for the search of positions that distinguished those closely related species (molecular signatures). 20 strains formerly identified as belonging to the group *S. pneumoniae*/*mitis*/*oralis* by 16S rRNA-gene sequencing were tested using the recA-PCR for species identification.
Results: Partial recA-gene sequence similarity between the type strains was 95.2% for *S. pneumoniae* and *S. mitis,* 91.4% for *S. pneumoniae* and *S. oralis* and 91.7% for *S. oralis* and *S. mitis.* The recA-sequences of all 18 isolates phenotypically identified as *S. pneumoniae* showed >99.7% sequence homology to recA-sequences of *S. pneumoniae*. Within the amplified recA-fragment of the type strains and the 18 *S. pneumoniae* isolates, we found 7 signature nucleotides specific for *S. pneumoniae*. Those nucleotides were found in all the *S. pneumoniae* strains and differed from those of the type strains of *S. mitis* and *S. oralis* in every single position.
The recA-sequences of the 20 strains grouped to *S. pneumoniae*/*mitis*/*oralis* revealed <95.9% sequence homology to recA-sequences of *S. pneumoniae*. Additionally, differentiation from *S. pneumoniae* according to the 7 specific nucleotide positions was determined for all 20 isolates, the identification of which resulted in *S. mitis* or oralis.
Conclusion: Our investigations present a more discriminatory molecular tool than the 16S rRNA-gene for proper identification of pneumococci. A recA-gene based PCR and analysis of 7 signature positions within the amplified fragment allowed an accurate differentiation of *S. pneumoniae* from *S. mitis*/*S. oralis.*

P1851 Rapid detection of vancomycin-resistant enterococci from rectal swabs by the Cepheid Xpert vanA/vanB assay

N. Bourdon, R. Bérenger, A. Mouet, C. Lesteven, F. Borgey, M. Fines-Guyon, R. Leclercq, V. Cattoir (Caen, FR)*

Objectives: The GeneXpert™ vanA/vanB real-time PCR assay (Cepheid) is a fully automated process that allows detection from rectal swabs of the major vancomycin resistance genes, vanA and vanB, in less than 1 hour. The purpose of this study was to assess the accuracy of this technique in comparison with vancomycin-resistant enterococci (VRE) selective culture after broth enrichment, as a reference technique.
Methods: From April to July 2008, 804 rectal swab specimens were obtained from 794 patients hospitalized at the university hospital of Caen, France. Each swab was suspended in 1 ml of sterile water by vortexing. For culture, a 5-ml BHI broth containing 10 mg/L of aztreonam and 3 mg/L of vancomycin was inoculated with 100 μl of the suspension. Broth cultures were incubated aerobically for 24 h, then subcultured onto chromogenic ChromID VRE™ agar plates (bioMérieux) and incubated aerobically for 24, 48 and 72 h. Identification of enterococci and of vancomycin resistance genes was performed by a multiplex PCR assay. For real-time PCR, the suspension was discharged into a cartridge and automatically processed for extraction, PCR and detection. The rest of the suspension was kept frozen at −80ºC. For samples VRE(−) by culture but vanB(+) [possibly containing resistant anaerobes], Schaedler broths containing aztreonam (10 mg/L) or

aztreonam (10 mg/L) + vancomycin (3 mg/L) were inoculated with 100 µl of the frozen suspension, incubated for 72 h and subcultured.
Results: Eleven samples (1.4%) yielded VRE by culture, including 8 *E. faecium* vanA(+) and 3 *E. faecium* vanB(+) whereas 793 remained negative. Real-time PCR assay was positive for vanA and vanB in 12 (1.5%) and 115 (14.3%) samples, respectively. Eight of the vanA(+) samples and 3 of vanB(+) samples were confirmed as VRE by culture. Sensitivity of the PCR technique was 100% for both vanA and vanB genes whereas specificity was 85.4% and 86%, respectively. PPV was 8.7% and 2.6% for vanA and vanB genes, respectively, whereas NPV was 100% for both genes. Screening for Gram-positive anaerobes harbouring the vanB gene was performed on 15 specimens. No vanB(+) anaerobic bacteria was detected.
Conclusion: This is the first report of the use of the Xpert™ vanA/vanB kit for detecting VRE from rectal specimens. This rapid (<50 min) technique showed a low prevalence (1.3%) of carriage of VRE among patients in our facility. Due to its excellent NPV, this method may reduce laboratory labour allowing to control only positive PCR results.

P1852 Xpert VanA/VanB for managing an outbreak of VRE

L. Cavalié, D. Clavé, M.F. Prère, S. Malavaud, M. Metais, J.M. Conil, N. Kamar, L. Lavayssiere, L. Rostaing, N. Marty (Toulouse, FR)*

Objectives: From late October 2008 to April 2009 the University Hospital of Toulouse was facing an outbreak of vancomycin resistan *E. faecium* (VRE) type B. This led to the introduction of screening by PCR using the new technology Xpert vanA-vanB (Cepheid).
Method: Following the detection of VRE in a patient in the nephrology ICU department all admitted and contacts nephrology patients were screened. Additional contact precautions were set up with three dedicated cohort areas (carriers, contacts and incoming patients). The screening of patients was performed by perianal swabbing and culturing on BEA vancomycin agar or chromID VRE ™ (Biomerieux). The plates were incubated 24–48 H and suspect colonies were identified by Vitek 2 and antibiogram. Carriage of the van A or van B gene was confirmed by molecular biology (Genotype *Enterococcus* ®, Hain Lifescience).
After confirmation of the outbreak, screening was performed using the real-time PCR Xpert VanA/VanB assay (Cepheid) to speed up result availability. Swabs were collected using double-headed swabs (Copan 139C). These were transported in liquid Stuart medium.
Results: We screened 1000 different patients (all techniques combined) over 10 months, some patients were analysed several times. We found 31 *E. faecium* van B (outbreak), 2 *E. faecium* Van A and 1 *E. faecalis* vanA.
182 samples were screened with both Xpert VanA/VanB and culture. 121 were negative in both, 19 positive in both, 38 positive with PCR and negative with culture. One was culture positive but PCR negative. The sensitivity for Xpert VanA/VanB was determined to be 95%; specificity 76.1%; PPV 33.3% and NPV 99.1%. These results led us to consider negative all PCR negatives without the need for culture confirmation. PCR positives were confirmed by culture using the second swab as recommended by Cepheid.
Conclusions: The high NPV of Xpert VanA/VanB makes it very useful for screening. In an outbreak situation, a negative result can be delivered in less than an hour compared to up to 5 days for culture allowing early release from isolation of patients on admission. The cost for the analysis should be weighed against the reduced cost of unnecessary isolation (rooms, equipment, personnel). A positive PCR result needs to be confirmed by culture and the patient kept in isolation until the results are available.

P1853 Development of a high-throughput vanA and vanB gene real-time PCR assay for the screening of vancomycin-resistant enterococci from enrichment broth and clinical isolates using the LightCycler 480 instrument

T.M. Pryce, I.D. Kay, J.P. Morgan, G.W. Coombs (Perth, AU)*

Objectives:
1. To increase the throughput of our existing 32-capillary LightCycler 1.0 (LC1) vanA and vanB gene assay to a 96-well capacity using the LightCycler 480 (LC480) system.
2. Compare PCR sensitivity, cost and turnaround time for each assay.
3. Evaluate an internal control for the LC480.

Methods: Patient rectal swabs cultured in Enterococcosel broth containing 6 mg/L vancomycin (EV broths) which turned visually black (n = 370) were screened for the presence of both vanA and vanB genes using the LC1 and LC480. Twenty clinical isolates were also tested. DNA standards from vanB *Enterococcus faecalis* and vanA *E. faecium* control strains were used to determine the analytical sensitivity of each assay. EV broths were extracted using the MagNAPure robotic instrument. Clinical isolates were extracted by a heat-lysis method. Identical primer-probe sequences and thermal cycling conditions were used for each assay, however for the LC480 detection system the vanA fluorophore was modified from LC Red-705 to LC Red-610. To detect the presence of an internal control, 5µL aliquots of an internal control construct (Bioline Cat. No. AUS-99005) was added to 50 EV broths, then extracted and tested in parallel in a modified LC480 assay containing specific internal control primers and probes.
Results: From the 370 black EV broths, 25 (6.7%) and 42 (11.3%) were vanB positive using the LC1 and LC480 assays respectively. All LC1 positive broths were LC480 positive. All negative LC480 broths were LC1 negative. All clinical isolates tested were concordant. The analytical sensitivity for vanA and vanB was 5pg and 50pg respectively for LC1 and 5pg for vanA and vanB for the LC480. The internal control was amplified for all broths tested and no inhibition was observed. For a routine run of 25 tests the material cost of the LC480 assay was approximately 2AUD per test compared to 4AUD per test for the LC1. Turnaround time was reduced for the LC480 depending on the number of tests performed.
Conclusions: The LC480 demonstrated a 10-fold increase in the analytical sensitivity for the detection of vanB in the *E. faecalis* DNA standard. Detection of vanB in the cultured EV broths from rectal swabs increased 4.6% using the LC480. Significant cost savings were achieved and turnaround times were reduced for the LC480. In addition, incorporation of an internal control may be useful for monitoring test performance and reduce reporting of false-negative results.

P1854 Direct PCR detection and identification of oral streptococci from carious dentin samples

C.S. Stingu, J. Thiel, R. Schaumann, A.C. Rodloff (Leipzig, DE)*

Objectives: *Streptococcus mutans* has been implicated as a major etiological agent of dental caries. On the other hand there are recent studies that indicated that also low pH non mutans oral streptococci have the ability to produce dental caries. Previous research has identified *S. mutans* in carious dentine. Little is know about the prevalence of the other acidogenic streptococci. Our goal was to use molecular methods to detect the most important species of oral streptococci (mutans and the low pH non mutans streptococci) in the carious dentin.
Methods: 27 patients (mean age 41 years) with clinical proof of dental caries were enrolled in this study. None of the carious lesions had signs of pulpitis. All lesions were cavitated with exposure of the dentin. Samples of the carious dentin were collected with a sterilized STOMA excavator 4621.15 taking care not to allow the excavator to touch the adjacent and cervical enamel to prevent sample contamination. The dentin samples were then immersed in 1 ml brain heart infusion broth. The samples were vortexed for 30 s and stored at −20ºC until analyzed. The samples were then brought to the room

temperature and DNA was extracted using Dneasy Blood&Tissue for Gram-positive bacteria from Qiagen GmbH, Germany according to the manufacturer's instructions and prolonging the incubation time with proteinase K up to 2 hours. Genotypic identifications were performed by species-specific PCRs for: *S. mutans, Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus* parasanguinis, *Streptococcus gordonii*, *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus intermedius*.

Results: A number of 10 different species of oral streptococci were identified with a mean of 3.6 strains per dentin sample. The most prevalent species were *S. gordonii, S. oralis* and *S. anginosus* (present in 77.8%, 62.96%, 48.14% of samples, respectively). *S. mutans* was detected in 12 dentin samples (44.44%). Significant numbers of *S. anginosus* were only present in dentin samples were *S. mutans* was also present. Significant differences between male and females and between smokers and non-smokers with respect to prevalence of oral streptococci were not found.

Conclusions: The low pH non mutans streptococci *S. gordonii, S. oralis* and *S. anginosus* dominate the carious dentine flora. They outnumber the mutans streptococci which were present in less of half of carious dentin samples.

P1855 High-level fluorescence labelling of Gram-positive pathogens

S. Aymanns, S. Mauerer, G. van Zandbergen, C. Wolz, B. Spellerberg (Ulm, Tubingen, DE)*

Objectives: Fluorescence labelling of bacterial pathogens has a broad range of interesting applications including the observation of living bacteria within host cells. To facilitate fluorescence labelling of streptococci and related genera we wanted to improve green fluorescent protein (GFP) expression in these species.

Methods: We constructed a novel vector based on the *E. coli* streptococcal shuttle plasmid pAT28 that can propagate in numerous bacterial species from different genera. The plasmid harbours a promoterless copy of the green fluorescent variant gene egfp under the control of the CAMP-factor gene promoter of *Streptococcus agalactiae* and was designated pBSU101.

Results: Upon transfer of the plasmid into streptococci, the bacteria show a distinct and easily detectable fluorescence using a standard fluorescence microscope and quantification by FACS-analysis demonstrated values that were 10–50 times increased over the respective controls. To assess the suitability of the construct for high efficiency fluorescence labelling in different Gram-positive pathogens, numerous species were transformed. We successfully labelled *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* subsp. *equisimilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus mutans*, *S. anginosus* and *Staphylococcus aureus* strains utilizing the EGFP reporter plasmid pBSU101. In all of these species the presence of the cfb promoter construct resulted in high level EGFP expression that could be further increased by growing the streptococcal and enterococcal cultures under high oxygen conditions through continuous aeration.

Conclusion: The plasmid pBSU101 harbouring the egfp gene under the control of the CAMP factor promoter of *S. agalactiae* results in high-level fluorescence in numerous streptococcal species and related genera. It represents a versatile novel vector for *in vitro* and *in vivo* studies of bacterial pathogenesis.

Molecular virology – epidemiology

P1856 Tick-borne infections in natural populations from Altai mountains, Russia

A. Grishechkin, O. Morozova, L. Karan, E. Isaeva, V. Zlobin (Moscow, RU)*

Objectives: Tick-borne encephalitis in Altay region, Russia for 2007–2009 remains high (26 clinical cases per 100,000 men) and exceeds the average disease level in 10 times. The aim of current study was to analyze tick-borne viral and bacterial infection agent prevalence and genetic diversity among ixodid ticks.

Methods: In 2007 404 ticks (mainly Ixodes persulcatus Schulze with a few samples of Hyalomma concinna) have been collected in Altai Mountain near to village Mangerok (85.7N, 51.8 E). Tick-borne encephalitis virus (TBEV) was detected by ELISA, bioassay on permissive PS cells, haemagglutionation test, RT-nested PCR or RT-real time PCR. *Borrelia*, *Ehrlichia*, *Anaplasma*, *Bartonella* DNA were detected using PCR with specific primers.

Results: ELISA allowed us to reveal TBEV in 68 from 404 samples (16.8±1.8%). Molecular typing with TaqMan subtype-specific probes showed the absolute dominance of Siberian genetic subtype. *Borrelia*-specific DNA was detected in 20.8%±1.8% samples, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum* – in 5.2±2.0% and 1.5±1.2% samples analyzed, respectively. Definition of virus loading by a method Real-time RT-PCR has shown a range of threshold cycles Ct 21–33 that corresponds 10,000–1,000,000 genes-equivalents in individual tick. The sequencing 790-bp fragments of gene E followed by phylogenetic analysis using neighbor-joining and bootscanning was performed. Nucleotide sequence data of the PCR products have confirmed the dominance of viruses Sibirian subtype in Altai mountains, however also presence of viruses European subtype was revealed.

Conclusions: The TBEV infection rate among ixodid ticks in Altay Mountain, Russia was high and despite the abundance of Siberian genetic subtype was dangerous for non-immune tourists from non-endemic regions. Beside the TBEV tick-borne bacterial pathogens can cause serious infections among populations.

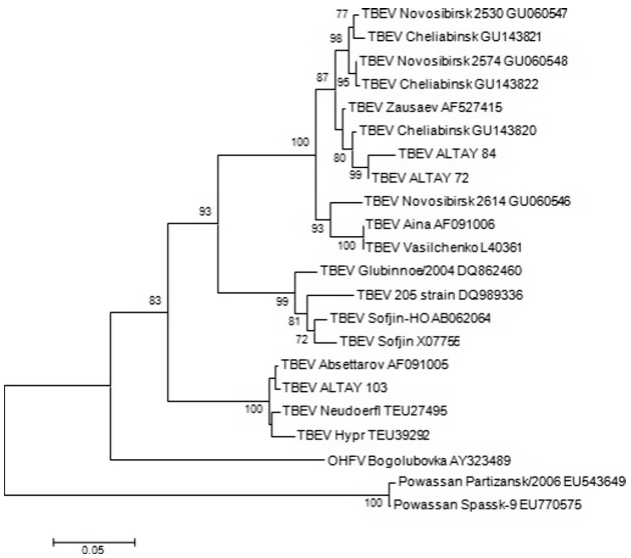


Figure 1. Phylogenetic tree, based on 790-bp fragment gene E sequences of tickborne encephalitis virus (TBEV). Original names of TBEV are presented. The tree was constructed by the neighbor-joining method.

P1857 Molecular epidemiology of norovirus outbreaks in Edinburgh, Scotland

A. Holmes, L. Garcia, F. Cameron, K. Cloy, J. Danial, J. Cepeda, P. Simmonds, K. Templeton (Edinburgh, Basingstoke, UK)*

Objectives: The aim of this study was to determine the genotypes responsible for norovirus (NoV) outbreaks in Edinburgh and to study the temporal occurrence of the genotypes to enable a better understanding of the epidemiology of the outbreaks.

Methods: A total of 161 samples positive for NoV genogroup II (GII) RNA by reverse transcription (RT) real-time PCR during routine diagnostic testing were investigated. Samples were obtained from a representative number of outbreaks (n = 66) of gastroenteritis that

occurred between February 2008 and May 2009 in Edinburgh healthcare facilities. Nested RT-PCR, targeting a 311bp region of the major capsid gene, and sequencing was used to genotype the NoV strains.
Results: 118 of the 161 samples (73%) were successfully amplified and sequenced. A total of 20 NoV strains belonging to six different genotypes (GII.1, GII.2, GII.3, GII.4, GII.6 and GII.12) were detected. The predominant genotype was GII.4, which included 13 variants separated into three genoclusters, 2006a (n = 3), 2006b (n = 6) and GII.4/2007 (n = 4). The majority of outbreaks (65%) were caused by 2006b, and two variants of 2006b, v1 (Hu/GII-4/Kumamoto2/2006/JP) and v4 (Hu/GII.4/VIC4681/2007/AU). These variants circulated at different times; initially 2006b was the predominant strain causing outbreaks at multiple healthcare facilities, and continued to cause outbreaks throughout the study period. 2006b v1 was prevalent during April and May 2008, whilst 2006b v4 predominated from January 2009 until May 2009. Linking this data with the incidence of NoV during the study period showed large increases in NoV activity to coincide with the emergence of these 2006b variants.
Conclusion: In 2006, GII.4 2006a was the predominant genotype detected in Scotland. This study reveals 2006b variants are now the predominant circulating strains, in Edinburgh at least, with other types circulating for shorter periods or sporadically. Importantly, increases in norovirus activity coincided with the introduction of new NoV 2006b variants, highlighting the need for an active surveillance system to allow the rapid identification of new, highly transmissible strains.

P1858 Spread and outbreaks of enterovirus types in Novosibirsk, Russia

A. Demina, V. Ternovoi, S. Netesov (Novosibirsk, RU)*

Non-polio enteroviruses cause a wide variety of clinical diseases frequently associated with the affection of central nervous system. They are recognized now as the most common cause of viral meningitis.
Purpose of this study: Investigation of the clinical, epidemiological features and etiology of enteroviral infections (in particular, one of its wide-spread forms – aseptic meningitis (AM)) in Novosibirsk, Russia.
Materials and Methods: We have analyzed 951 case-records of adult patients which were being treated in Novosibirsk Municipal Infectious Diseases Clinical Hospital No. 1 because of the "aseptic meningitis" diagnosis during the period 1993–2008. We also have analyzed 203 specimens of cerebro-spinal fluid (CSF) and feces from the patients with the same diagnosis in 2008–2009.
Results: There were 3 significant outbreaks of AM: in 1993–74 patients, 1998–97 pat., 2004–194 pat. The morbidity among men was 5–10% higher than among women. Among the patients high portion comprised the persons younger 30 years. The majority of cases occurred during July-October. The main possible sources of infection were: swimming in water reservoirs (30%); contact with the patients with AM or acute respiratory viral infection (12%); recreation in summer camps (6%); usage of raw water (6%) or raw milk (2%). Aseptic meningitis was acute infection, but the majority of cases were fast and mild infections. Fever was noted at all patients; 83% – febrile fever; 15% had two- or three-wave fever. 78% had headache; 60% had sickness and retching. We observed the significant number of specific symptoms which are typical for the enteroviral infection: catarrhal inflammation – 40%; rhinitis – 13%; exanthema – 9%; scleritis – 5%; diarrhea – 5%. The analysis of CSF showed that three-digit cytosis predominated in all years. The nature of pleocytosis was neutrophilic, mixed or lymphocytic in the different years. Diagnosis was established on the basis of clinico-epidemiological information and was confirmed by PCR detection of enterovirus RNA: in 70% – in the CSF, in 9% – in the blood and in 21% – in both CSF and blood. In 2008–2009 we have analyzed 203 specimens of CSF and feces by the RT-PCR and we found the following enteroviruses: ECHO30 – 4 samples; Enteroviruses90 – 6; Coxsackie76 – 1.
Summary: The epidemic ascent of diseases occurs each 5–6 years. Upshot of all aseptic meningitis was favorable. Mainly there were isolated ECHO30 and enterovirus90 in Novosibirsk region.

P1859 Astrovirus in Italy, emergence of a novel 2c lineage

M.A. Platia, V. Rotolo, C. Colomba, A. Cascio, V. Martella, G.M. Giammanco, S. De Grazia (Palermo, Messina, Bari, IT)*

Objectives: Human astroviruses (HAstVs) are enteric viruses associated with gastroenteritis in young children in both developed and developing countries. The pathogenic role of HAstVs is still disputed since they are frequently found in mixed infection. In the present study we investigated on the prevalence of HAstVs in Palermo, Italy, in 2002–2005. Genetic diversity of HAstVs was determined by sequence analysis of ORF2 which allows to predict serotypes (1 to 8).
Methods: 708 faecal samples were obtained from children, less than five years, hospitalised in Palermo from January 2002 to December 2005. The samples were screened for the presence of HAstVs by EIA and positive specimens were analysed by RT-PCR to obtain ORF2 amplicons for genotyping through sequence and phylogenetic analysis. All stool specimens were also tested for presence of rotaviruses and noroviruses by RT-PCR.
Results: HAstVs were found in 28 (3.95%) patients. Mixed infections were found in 50% of the HAstV-positive samples. Coinfections with rotavirus were the most frequent. There were no significant differences in symptoms between patients with HAstV single infection and mixed infections. Fourteen HAstVs were genotyped by sequence analysis and HAstV types-1, -2, and -4 were detected. In particular, HAstVs-1 were identified in 2003–2005, HAstV-2 and -4 circulated only in 2002. In the phylogenetic tree, all Italian HAstVs-1 belonged to lineage 1d and HAstVs-4 to lineage 4b. The two Italian HAstVs-2 segregated in a well defined genetic cluster that differed from the presently described lineages and was designated 2c. Conserved nt polymorphisms allowing to differentiate the novel 2c lineage were observed.
Conclusion: The data obtained in this study confirm the role of HAstVs as agents of acute gastroenteritis in children and indicate HAstV-1d as the predominant type. This lineage, already circulating in Palermo in 1999, re-emerged in 2003 and continued to circulate until 2005. In 2002, HAstV-2c first appeared in Italy after occasional detection in other geographic areas. The emergence and re-emergence of different genotypes and lineages of astrovirus may depend on population immunity pressure. Nation-wide investigations concerted at an international level could be useful to gather more information on the impact of HAstV on enteric disease in children. Knowledge of the viral types circulating worldwide is required to plan prevention and control strategies.

P1860 Molecular epidemiology of enterovirus types causing aseptic meningitis in Greece, 2007–2009

S. Papadopoulou, N. Apostolopoulou, E.T. Piperaki, V. Pitiriga, N. Spanakis, A. Tsakris (Athens, GR)*

Background: Enteroviruses is considered the most common cause of viral meningitis. The detection and identification of the enteroviruses in such cases contributes to the proper use of antibiotics and to the assessment of their epidemiological profile. The aim of the present study was to detect and identify by molecular methods the circulating types of enteroviruses causing aseptic meningitis and encephalitis in Greece for the period 2007–2009.
Methods: One hundred and ten CSF specimens from patients-both children and adults-from 5 tetriary care hospitals in Athens with diagnosed or suspected aseptic meningitis, were examined in order to determine a possible enteroviral etiology.
RNA was extracted from CSF using the QuickGene-810 extraction system. RT-PCR targeting the 5-UTR of viral genome was used for detection of enteroviruses. RT-nested PCR targeting the VP1 gene, followed by sequencing, was then performed in order to determine the enterovirus serotype. The primers were supplied by Macrogen ltd and the RT-PCR reactions were carried out using the QIAGEN One-step RT-PCR kit.
Results: Fifty nine specimens (53.6%) were positive for the presence of enterovirus and 26 (44%) of the positive specimens were successfully typed. coxsackie B5 was determined as the predominant serotype

(34.6%), followed by echovirus 30 (26.92%), echovirus 6, 9, 13 and coxsackie virus B4 (7.7% each), coxsackie A9 and echovirus 11 (3.8% each). Among the 59 patients 37 were male and 22 were female. The vast majority were children up to15 years old (96%) and only 4% were adults (>15 years). All, but one, patients who were found to be enterovirus positive showed abnormal CSF profile, with higher than normal white cell blood count (>5/mm^3) and/or elevated protein level (>45 mg/dl) and all presented the typical clinical symptoms of aseptic meningitis.
Conclusions: Eight co-circulating enterovirus serotypes causing aseptic meningitis were identified. Coxsackie virus B5 was most frequently isolated, followed by echovirus 30. The prevalence of coxsackie virus B5 as well as the presence of echoviruses 6, 13, 11, 30 was also previously reported in Greece. However, echoviruses 15 and 4 that previously reported to cause outbreaks in Greece were not isolated in this study.

P1861 Two prevalent *Pseudomonas aeruginosa* genotypes are unique in Dutch patients with cystic fibrosis and absent in other patient populations

R. van Mansfeld, M. Bonten, R. Willems (Utrecht, NL)*

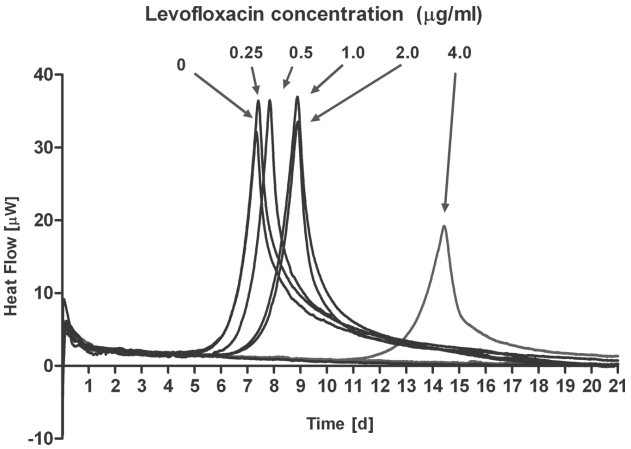
Objectives: We previously demonstrated, based on multi locus sequence typing (MLST), the presence of prevalent clones of *Pseudomonas aeruginosa* in Dutch cystic fibrosis (CF) patients. Sequence types (STs) ST406 and ST497 were found in 15% and 5% of the patients colonized with *P. aeruginosa*, respectively. ST406 and ST497 were not genetically linked to previously described international epidemic clones, which were not found in this CF population. In the study we aimed to determine whether ST406 and ST497 were unique for CF patients.
Methods: 205 *P. aeruginosa* respiratory isolates from intensive care unit (ICU) patients, 100 *P. aeruginosa* respiratory isolates from CF patients and 103 multiple resistant *P. aeruginosa* strains isolated in our hospital between 2006 and 2008 were genotyped with Multiple Locus Variable Number of Tandem Repeats Analysis (MLVA). The prevalence of specific genotypes and genetic diversity, expressed as the Simpsons index of diversity (ID) with 95% confidence interval (CI), were compared between the three clinical settings. Particularly, the prevalence of ST406 and ST497 equivalent MLVA types (MT) in non-CF patients was investigated.
Results: 272 MTs were identified in 408 isolates. The ID of all isolates was 0.995 (CI 0.994–0.997), indicating a highly diverse population, and IDs were not significantly different between the three clinical settings. Only 17 MTs were shared between the different clinical settings and in all three settings more prevalent MTs were found. These were MT80 (5%) and MT265 (15%) in the hospital hygiene collection and MT155 (10%) in the ICU collection. However, these MTs were not unique to one clinical setting, and also obtained from patients in other settings. The CF related clones ST406 and ST497, represented by MT27 (21%) and MT11 (7%) were unique to CF patients.
Conclusion: The population structure of *P. aeruginosa* obtained from different clinical settings is highly diverse and characterized by many unique and some more prevalent genotypes. The two prevalent genotypes previously documented in the Dutch CF population, which are not genetically linked to previously described epidemic clones, appeared unique to the CF related *P. aeruginosa* population and were not found in other clinical settings.

Susceptibility testing bacteria

P1862 Activity of different antibiotics against *Borrelia burgdorferi* determined by bacterial heat production (microcalorimetry)

Y. Achermann, M. Vogt, A. Trampuz (Zurich, Baar, Lausanne, CH)*

Objectives: Antimicrobial susceptibility testing in *Borrelia burgdorferi* sensu lato is not routinely performed due to laborious procedure and lacking correlation with treatment outcome. The MIC and MBC values determined by the colorimetric microdilution assay provide no information on the antimicrobial activity over time. Therefore, we evaluated the activity of 4 antibiotics by measurement of heat production using an ultrasensitive real-time instrument (microcalorimeter, detection limit 025 microwatt).
Methods: We used *B. burgdorferi* sensu stricto (strain 1408/94) isolated from a patient with erythema migrans. Microcalorimetry (detection limit 0.2 μW) was performed in duplicate by adding 0.1 ml of culture containing 10^6 CFU of *B. burgdorferi* in 3.9 ml BSK-H medium containing serial 2-fold dilutions of standard antibiotics (amoxicillin, doxycycline, rifampin, levofloxacin). Heat production was measured at 37ºC under static conditions for up to 21 days. The minimal heat inhibition concentration (MHIC) was defined as the lowest antimicrobial concentration inhibiting heat production within 14 days after heat was detected in cultures without antibiotics.
Results: Heat of *B. burgdorferi* was detected in absence of antibiotics after 2 to 8 days and reached a peak of 30 and 50 microwatt. The peak of heat-flow was proportionally delayed with increasing dose of amoxicillin, whereas the peak was also decreased with doxycycline and levofloxacin (Figure). Growth of *B. burgdorferi* was not affected by rifampin up to 16 μg/ml. The MHIC (μg/ml) was 0.5 (amoxicillin), 1 (doxycycline), 8 (levofloxacin) and >16 (rifampin). At amoxicillin concentrations >0.5 μg/ml, identical heat flow curves were observed even after 14 days delay.
Conclusions: Doxycycline showed the highest activity against *B. burgdorferi* by microcalorimetry, followed by amoxicillin and levofloxacin, whereas rifampin showed no antiborrelial activity. The MHIC values correlate well with the reported MIC values (Hunfeld et al., 2000). Microcalorimetry is a useful tool for rapid and real-time assessment of growth characteristics of and antimicrobial activity against *B. burgdorferi* and potentially other slow-growing microorganisms.



P1863 Chip-calorimetric evaluation of the efficacy of antibiotics and bacteriophages

J. Lerchner, D. Mueller-Hagen, I. Klare, H. Roehr, A. Wolf, F. Mertens, R. Mueller, W. Witte (Freiberg, Viersen, Wernigerode, DE)*

Objectives: Rapid detection of the antibiotic resistance of bacterial strains from clinical material would allow a faster intervention and implementation of infection control treatments and an early selective antibiotic therapy. In the clinical practice, however, conventional antibiotic susceptibility tests often need 24 h until a result is obtained and necessary actions can be taken.
The metabolic heat production of bacteria is an excellent possibility to measure their physiological activity and could therefore be used for a rapid discrimination of resistant and non-resistant bacterial strains. Unfortunately, conventional calorimeters suffer from high sample need, slow operation, and high costs which restrict their application in clinical laboratories. The presented work demonstrates that a new type of calorimeters developed on silicon-chip technology enables the detection of antibiotic resistance on a minute time-scale.
Methods: In the present study, a prototype chip calorimeter was used [1]. Crucial part of the calorimeter is a heat power transducer comprising

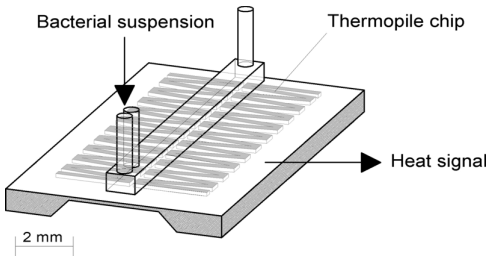
a silicon thermopile chip, a heat sink, and a measurement chamber (see Fig.). At least a volume of 100 µl of a bacterial suspension is necessary to fill the measurement chamber. A few minutes after sample injection, a stable heat signal is available displaying the present metabolic activity of the bacteria. The sensitivity of the calorimeter is 20 nW related to the heat production of about 10^4 bacteria. For a clear discrimination of antibiotic resistance and non-resistance by the presented micro-calorimetric method, about 10^5 bacteria are required.

Results: The antibiotic or bacteriophage susceptibility of different strains of *Staphylococcus aureus* and *Escherichia coli* was studied. For instance, for *S. aureus* strains 1 ml of tryptic soy broth with and without a breakpoint concentration of cefoxitin, respectively, were inoculated with 100 µl of a suspension of a single fresh colony in 220 µl tryptic soy broth in each case. After incubation of the samples at 37ºC for 90 min the calorimetric measurements were performed.

Conclusions: The comparison of the heat production of cultures incubated with bacteriophages and antibiotics to those without these antibacterial preparations enabled a clear discrimination of resistant and non-resistant strains already after totally 120 min.

Reference(s)

[1] J. Lerchner, A. Wolf et al., Thermochim Acta 447 (2008) 48–53.



P1864 Multiplex PCR for rapid detection of genes encoding class A carbapenemases

S.G. Hong, S.K. Hong, J.Y. Huh, M.S. Kang (Gyeonggi-do, Seoul, KR)*

Objectives: Class A carbapenemases (CACs) include the SME, IMI/NMC-A, SFC, KPC, and some type of GES families. The genes for SME, IMI/NMC-A, SFC enzymes are all chromosomally located except IMI-2 and the genes for KPC and GES enzymes are carried on plasmid. KPC producers have induced severe treatment problems in hospitals around New York and have also been reported in Europe, South America and China. Therefore, the confirmation of type of CACs is important to ensure optimal therapy and to prevent their spread. This study was to develop a multiplex PCR assay to detect and differentiate CAC genes in a single reaction.

Methods: The strains comprised 11 CAC-producers (1 SME-producing *Serratia marcescens*, 2 IMI/NMC-producing *Enterobacter* cloaceae, 2 KPC-producing Enterobacteriaceae and 6 GES-producing *Klebsiella pneumoniae*), 8 metallo-β-lactamase (MBL) producers (2 IMP-producing *Pseudomonas aeruginosa*, 3 VIM-producing *P. aeruginosa*, 1 IMP-producing *Acinetobacter baumannii*, 1 SIM-producing *A. baumannii* and 1 VIM-producing Alcaligenes feacalis), and 5 non-carbapenemase-producing Enterobacteriaceae. Four primer pairs were designed to amplify fragments of 4 CAC families (SME, IMI/NMC-A, KPC, and GES). The PCR were done for the detection of CAC genes with above strains.

Results: The multiplex PCR detected all the genes for 4 CAC families that could be differentiated by the fragments size according to the gene types. All non-CAC producers did not show PCR product bands.

Conclusion: This multiplex PCR appears to be a simple and useful approach to detecting and distinguishing CAC genes in carbapenem resistant strains that show negative results to the detecting test for MBL producers. Therefore, this method should be helpful for characterization of CACs and in controlling the spread of pathogens producing these enzymes.

P1865 Detection of point mutations on 23S rRNA of *Helicobacter pylori* and resistance to clarithromycin by PCR-RFLP in Spain

S. Agudo, T. Alarcón, G. Pérez-Pérez, M. López-Brea (Madrid, ES)*

Objective: The identification and characterization of clarithromycin resistant *Helicobacter pylori* strains is a long and expensive process that requires culture and isolation of *H. pylori* from gastric biopsies. The purpose of the present study was to identify *H. pylori* strains resistant to clarithromycin and determine the most common point mutation present in the 23S rRNA gene using a PCR-RFLP technique.

Methods: Gastric biopsies were obtained by endoscopy from paediatric patients with gastric symptoms. *H. pylori* was cultured according to standard microbiological procedures and clarithromycin resistance determined by E-test. DNA extraction was performed by NucliSens platform with the NucliSens magnetic extraction reagents (bioMérieux) according to manufacturers instructions. Clarithromycin resistant and sensitive strains were determined by PCR and DNA sequencing for mutations in 23S rRNA gene. Restriction fragment length polymorphism was performed using BsaI enzyme to detect restriction sites that correspond to the mutation.

Results: We found 42 out of 118 (35.6%) strains resistant to clarithromycin by E-test. E-test results were confirmed for the presence of point mutation in 34 (88.1%) of these strains. There were 8 *H. pylori* strains resistant to clarithormycin by E-test but without any point mutation in the 23 rRNA gene. Mutation A2143G was found in 85.3% of the strains. Restriction enzyme analyses with BsaI was able to detect the presence of that mutation in 100% of the resistant cases.

Conclusion: PCR-RFLP is an usefull method to detect clarithromycin-resistant *H. pylori* infection in countries with a high prevalence as Spain. Only A2143G mutation could be detected with this assay, but it is the most prevalent in Spain as well as in the other countries. It may be useful to apply this methodology before choosing regimens of *H. pylori* eradication.

P1866 Evaluation of the new WalkAway® plus automated microbiology system for identification and antimicrobial susceptibility testing of Gram-negative bacilli in routine practice

L. Armand-Lefèvre, C. Moreau, M. Perrot, A. Andremont, C. Muller-Serieys (Paris, FR)*

The WalkAway® plus (Siemens) is an automated system designed for identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens.

Objectives: We have evaluated performance of the new WalkAway® plus instrument for ID and AST of clinical Enterobacteriaceae (Eb) and non fermentative Gram-negative bacilli (Nfb) isolated in a bacteriology laboratory.

Methods: The MicroScan® Neg Fermenteur Combo Type 47 and Neg Non fermenteur Combo Type 48 panels were tested for Eb and Nfb, respectively. ID results were compared to those of conventional biochemical ID systems: API 20E (Eb) and API 20NE (Nfb) (bioMérieux, La Balme les Grottes). AST category results (S, I, R) were compared to those of the standard disk diffusion method after expertise using SIR software (I2A), and according to CA-SFM guidelines. Discrepant results were retested in duplicate. ID discrepancies were resolved by 16S rRNA sequencing. AST discordant results (I or R instead of S results and vice-versa), were resolved by MIC determination (E-test, AB biodisk).

Results: A total of 331 Eb (144 *E. coli*, 58 *Klebsiella* spp, 45 *Proteus* spp, 44 *Enterobacter* spp, 16 *Citrobacter* spp, 13 *S. marcescens*, 11 others) and 105 Nfb (84 *P. aeruginosa*, 8 *A. baumannii*, 4 *S. maltophilia,* 9 others), recovered from routine cultures, were tested. The percentages of concordant ID at the species levels were 97.6% (323/331) and 96.2% (101/105) for Eb and Nfb, respectively. The rate of categorical complete agreement for AST of Eb ranged from 90.6 to 99.4% for β-lactams, from 95.5 to 97.3% for aminoglycosides, 98.8% for fluoroquinolones and from

97.7 to 99.1% for other antibiotics. The lowest value (90.6%) was for the genus *Proteus* and aminoglycosides. Overexpression of penicillinases in *E. coli* caused a low concordance rate (90.6%) for the combination of amoxicillin and clavulanic acid, using the WalkAway® plus system. Among 18 ESBLs producing isolates, 4 were not detected (all were from the genus *Enterobacter*). Complete agreement for AST of *P. aeruginosa* ranged from 95.2 to 100% for β-lactams, to 95.2% for aminoglycosides and 98.8% for ciprofloxacin.
Conclusion: The new WalkAway® plus instrument is a reliable microbiology automated system for ID and AST of Enterobacteriaceae and non fermentative Gram-negative bacilli commonly isolated in a clinical laboratory.

P1867 Correlation of ciprofloxacin and levofloxacin sesitivity interpretation in Gram-negative bacteria

B. Grabein, M. Hoeck, A. Toniolo, B. Wiedemann for the EPICENTER Network*

Objectives: For infections due to Gram negative pathogens, Ciprofloxacin and Levofloxacin are the most commonly used fluoroquinolones. Both drugs have the same mechanism of action and resistance. Their activities and pharmacokinetics are, however different. Using data from theEPICENTER Network group, we analysed whether it is possible to test only one of the above drugs and if results for the other ca be deduced.
Methods: At present time four laboratories participate in the network using the automated BD PHOENIX-systems measuring MICs. The BD EPICENTER Data-Management-System is used for the evaluation of the data in the laboratory and for the transfer of the data to the concentrator, where the drug susceptibility data are interpreted using appropriate breakpoints. Copy strains are excluded. Quality control assays are routinely performed. Data of two German and one Italian laboratory have been analyzed. Using EUCAST breakpoints MIC results were interpreted for ciprofloxacin ($S \leqslant 0.5$, $R > 1$) and levofloxacin ($S \leqslant 1$, $R > 2$) for Enterobacteriaceae and *P. aeruginosa*.
Results: We analysed 54 137 strains: 35254 *E. coli*, 4319 *E. cloacae,* 7109 *K. pneumoniae,* 6175 *P. mirabilis* and 1280 *P. aeruginosa* strains. Results for ciprofloxacin and levofloxacin were virtually identical for *E. coli*, *E. cloacae,* and *K. pneumoniae* (see table). Major errors were below 1% for all Enterobacteriaceae. In the case of *P. mirabilis* 9.39% minor errors were detected. Including minor errors, an almost perfect correlation was found between results for Enterobacteriaceae with agreement in over 99% of cases. The results with *P. aeruginosa* were less convincing: 3.15% major errors and 14.26% minor errors were detected. Using modified breakpoints for Levofloxacin ($S \leqslant 2$, $R > 4$) correlation levels were increased: perfect agreement in 92.25% of cases, 0.61 major errors, and 6.48% minor errors.
Conclusion: The data show that, for the investigated enterobacterial species either of the drugs can be tested; results can be deduced for the missing one. Using common EUCAST breakpoins, this appears not to be possible for *P. aeruginosa*. Shifting the breakpoints ($S \leqslant 2$, $R > 4$) significantly improved the agreement of MIC interpretation. This correlates with the wild type cut off values for Ciprofloxacin and Levofloxacin of 0.5 and 2 mg/l, respectively.

Table 1. Correlation of ciprofloxacin and levofloxacin sensitivity results

	E. coli	*E. cloacae*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Identical	99.22%	98.80%	97.67%	90.53%	82.51%
Major error	0.06%	0.25%	0.34%	0.18%	3.51%
Minor error	0.23%	0.36%	1.38%	9.39%	14.26%

P1868 Comparison of the agar dilution and the disc diffusion method in 242 *Campylobacter* spp. strains

M. Lehtopolku, P. Huovinen, A. Siitonen, E. Eerola, P. Kotilainen, A. Hakanen (Turku, Helsinki, FI)*

Objectives: Several studies have been published comparing the disk diffusion and agar dilution or broth dilution method in determining susceptibilities in *Campylobacter* spp. In some of the previous studies results have been in line between the disk diffusion and agar dilution method, but there have also been results, where disk diffusion method has not been reliable. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is presently developing a disk diffusion test for routine antimicrobial susceptibility testing. The aim of this study was to compare the agar dilution method with disk diffusion method in *Campylobacter* spp.
Methods: The *in vitro* activities of 10 antimicrobial agents against 242 *Campylobacter* spp. strains collected in Finland between 2003 and 2008 were analyzed by standard agar dilution method and disk diffusion method according to CLSI; Muller-Hinton agar plates with 5% of sheep's blood. In addition, a number of strains were tested using the EUCAST disk diffusion test; MH-F plates containing Muller-Hinton agar with 5% of defibrinated horse's blood and 20 mg/l beeta-nicotinamide adenine dinucleotide. These results were compared to the CLSI method. The evaluated antimicrobials were erythromycin, azithromycin, clarithromycin, spiramycin, telithromycin, clindamycin, ciprofloxacin, nalidixid acid, tetracycline and tigecycline.
Results: According to our results, there was a notable variation in the results between agar dilution and disk diffusion methods regarding all antimicrobials tested. Of the 33 erythromycin-resistant strains ($MIC \geqslant 16$ mg/l), 29 strains did not show any erythromycin inhibition zone (<6 mm) and 4 strains showed inhibition zones between 18–42 mm. In addition, among the 209 erythromycin-susceptible strains ($MIC < 16$ mg/l) tested, erythromycin inhibition zone varied between 10–55 mm. In disk diffusion analyses, repeatability was good only in approximately half of the strains, whereas in the rest of the strains inhibition zones varied significantly although tests were performed according to the same instructions. The disk diffusion results by the EUCAST MH-F plates were similar to those of the CLSI method.
Conclusions: It is a major concern that the disk diffusion method may not be a reliable tool for susceptibility testing of *Campylobacter* spp. Further studies are needed whether all susceptibilities of campylobacters should be done using a MIC based method.

P1869 Efficacy of disinfectants against *Legionella pneumophila* by an inverse biofilm assay

M.E. Portillo, A. Aguinaga, C.A. Alonso, J. Leiva (Pamplona, ES)*

Objectives: *Legionella pneumophila* persists in man-made aquatic installations despite treatments. More information about disinfectants could improve the effectiveness of treatments. This study tests the susceptibility of both the planktonic and biofilm bacteria against 3 disinfectants used in cooling tower treatments.
Methods: We determined the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), biofilm's minimal inhibitory concentration (BMIC) and biofilm's minimal bactericidal concentration (BMBC) of sodium hypochlorite (SH), chloramite T trihydrate (CT) and benzalkonium chloride (BC) against 6 *L. pneumophila* serogoup 1 isolates (5 native isolates and 1 type culture collection ATCC 33152). The MIC and BMIC were determined by the conventional microdilution method in a buffered yeast extract broth. The MBC and BMBC were determined by a variant of the Calgary's method. We determined these values after 3 h and after 48 h of disinfectant exposure. Replicated assays produced identical results for all strains.
Results: The distribution of MIC and MBC values was similar for each disinfectant against all strains after 3 h and 48 h treatment. The MIC and BMIC values were similar too. (Table 1).

Conclusions: The method described for biofilm formation and determination of biofilm susceptibility for *L. pneumophila* is reproducible. Efficacy of disinfectants is similar against planktonic and biofilm bacteria. The more effective disinfectant tests is benzalkonioum chloride. Disinfectants can be applied in shock and continuous treatments of water systems. Our results demonstrate that 3 h treatments are as effective as 48 h treatments for these disinfectants.

Strains	Disinfectants	Treatment time 48 h			Treatment time 3 h		
		MIC (ppm)	BMIC (ppm)	CBI/BMIC	MIC (ppm)	CIB (ppm)	CBI/MIC
CUN1	CT	1024	1024	1	512	2048	4
	SH	128	256	2	128	256	2
	BC	2	4	2	2	4	2
CUN3	CT	1024	1024	1	512	512	1
	SH	256	256	1	128	128	1
	BC	2	2	1	2	2	1
CUN4	CT	1024	1024	1	512	512	1
	SH	256	512	2	128	512	4
	BC	1	1	1	2	2	1
CUN5	CT	1024	2048	2	512	1024	2
	SH	256	512	2	128	256	2
	BC	1	1	1	4	4	1
CUN6	CT	1024	2048	2	512	1024	2
	SH	256	256	1	512	512	1
	BC	1	1	1	2	2	1
ATCC 33152	CT	512	1024	2	64	1024	16
	SH	128	512	4	32	1024	32
	BC	1	4	4	2	8	4

P1870 *In vitro* susceptibility of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients to aztreonam and tobramycin in a biofilm susceptibility test panel

R. Rennie, L. Turnbull, C. Brosnikoff, N. Brown, B. Rawal, M. Olson (Edmonton, CA)*

Objectives: Both aztreonam(AZ) (conditional approval in Europe and Canada as CAYSTON) and tobramycin(TO) (world-wide as TOBI)are available as inhalation modules for treatment of *P. aeruginosa* causing pulmonary infections in cystic fibrosis(CF) patients. These microorganisms often exist as a biofilm and are difficult to treat. We have tested AZ and TO in a biofilm susceptibility test (bioFILM PA™, Innovotech, Inc, Edmonton, Alberta) against 149 strains of *P. aeruginosa* isolated from CF patients to better understand their potential efficacy for treatment in CF patients.
Methods: Isolates were tested for inhibition of planktonic and sessile (biofilm) growth in the bioFILM PA™ module. Isolates were grown on plastic pegs in a 96 well format by placing in an orbital shaker at 110 rpm to generate identical biofilms on the pegs. The pegs were then placed into trays containing Mueller-Hinton broth with antimicrobial agents at varying concentrations, both alone and in combination. After overnight incubation, the pins were washed, and placed in a recovery medium to measure inhibition of the biofilm cells. Isolates were categorized as (S), (I) or (R) according to CLSI systemic breakpoints.
Results: 27 (18%) and 7 (5%) strains did not grow in the control wells in the MIC and biofilm state respectively. These were rough variants that did not form biofilms well. Of the 122 strains that formed biofilms on the bioFILM PA™ pegs, for AZ alone, 73% and 43% were susceptible by MIC and biofilm respectively. For TO, 63% and 34% were susceptible respectively. The remainder were either I or R. When AZ and TO were analysed combined in the same wells, 93% were susceptible by MIC and 64% by biofilm test. Two additional strains (1%) were I in the combination by MIC and 14 strains (9%) were I by biofim test. The remainder of those that formed biofilms were R.
Conclusion: The bioFILM PA™ data are of value in determining the potential effectiveness of these inhaled antimicrobials on strains of *P. aeruginosa* in a biofilm state. These *in vitro* results suggest that there are important differences in the effect of these agents on cells in a biofilm. There is greater resistance in the biofilm to individual agents thought to be effective for therapy of *P. aeruginosa*. Combinations of these agents showed significantly greater efficacy *in vitro*. Such isolates should be tested by a standardized method such as bioFILM PA™ to understand the potential for effective therapy or treatment failure.

P1871 Direct application of E-test on positive blood cultures – more than fast susceptibility testing only?

J.V. Bjørnholt, T. Brastad, L. Kløvfjell, B.O. Johnsen, C.T. Andersen, T.M. Leegaard (Oslo, NO)*

Objective: To validate and implement direct Etest on positive blood cultures in a routine laboratory.
Methods: Samples taken directly from consecutive Bactec blood culture bottles flagging positive and with Gram stain showing bacteria. They were plated onto 140 mm Mueller-Hinton plates with 5% sheep blood and Etest MIC's were determined for: 1) Gram-negative rods: cefotaxime, meropenem, ciprofloxacin, co-trimoxazole, gentamicin and one optional antibiotic. 2) Gram-positive cocci in clusters: vancomycin, gentamicin, ampicillin, clindamycin with adjacent erythromycin disk for MLS-B, cefoxitin, one optional antibiotic. In addition they were plated onto an oxacillin screening plate. 3) Gram-positive cocci in chains: Penicillin G (low), vancomycin, gentamicin (high), ampicillin, clindamycin with adjacent Erythromycin disk for MLS-B and one optional antibiotic. The plates were read as early as possible, after 3–8 hours dependent on growth (Etest I), and reincubated for reading next work day (Etest II). All isolates were identified and susceptibility testing was performed according to routine methods (VITEK 2 or Etest depending on species and additional methods if needed). Time to Etest I and discrepancies between Etest I and II and routine susceptibility were recorded.
Results: 155 positive blood cultures were included, 120 analysed per protocol. (19 mixed cultures and 16 not indicated when Etest was read were excluded). 5 positive cultures did not show any growth on resistance plates (3 anaerobic isolates, 1 *Haemophilus influenzae*, 1 *Actinomyces* spp.). Median time to Etest I 6 hours (range 3–24). There were no major inconsistencies between Etest I and II or between Etest I and routine susceptibility, except from difficulties in interpretation of co-trimoxazole for Gram-negative rods and clindamycin for staphylococcus and enterococcus species, see table 1.
Conclusion: Direct Etest on positive blood cultures gives rapid and reliable results on antibiotic susceptibility although bacteriostatic antibiotics should be interpreted carefully. Maybe even more important than the fast susceptibility testing was the continuous monitoring of growth which resulted in a much faster identification of the isolates. An automated ID or manual ID could be initiated at the same time as the reading of Etest I, meaning that in many cases identification and susceptibility testing was on the clinicians desk 24 hours earlier than previously.

Table 1. Proportion of major groups of isolates with inconsistencies between Etest I and II, and affected antibiotics. Proportion of isolates where Etest I could not be read due to insufficient growth

	Inconsistencies*	No growth	Drugs affected
Enterobacteriaceae	1/30	1/30	co-trimoxazole
Non-fermenters	2/10	1/10	co-trimoxazole
Staphylococcus aureus	3/16	2/16	clindamycin/ampicillin
Coagulase neg. staphylococci	10/29	5/29	clindamycin/ampicillin
Enterococcus species	1/10	3/10	clindamycin
Other Gram-positive cocci	2/9	0	clindamycin/penicillin G
Corynebacterium species	NA	6/6	All

*defined as ⩾2 dilutions difference between readings.

P1872 Evaluation of different Mueller-Hinton agars for disc diffusion susceptibility testing of tigecycline, doxycycline, minocycline and tetracycline against multi-resistant bacteria

R. Cayô, J. Calvo, C. Fernández-Mazarrasa, L. Martínez-Martínez (Sao Paulo, BR; Santander, ES)*

Objectives: We evaluated the accuracies of different commercially available Mueller-Hinton agars (MH) for susceptibility testing of tigecycline (TGC), doxycycline (DX), minocycline (MN) and tetracycline (TE) against multiresistant bacteria by disc diffusion method (DD).
Methods: A total of 75 clinical isolates (one per patient, clonally unrelated as assessed by REP-PCR and/or PFGE) were evaluated: 15 *A. baumannii* (Ab), 15 *E. faecium* (Ef), 15 ESBL(+) *E. coli* (Ec), 15

ESBL(+) *K. pneumoniae* (Kp) and 15 Methicilin-Resistant *S. aureus* (MRSA). Discs of 15 μg (TG) and 30 μg (DO, MN and TE) from Oxoid® were used. Eleven different freshly prepared MH (<24 h old) were evaluated according to CLSI, from the following manufacturers: Difco®, Merck®, Oxoid®, BioRad®, Remel®, Pronadisa® and Sigma®. Iso-Sensitest (Oxoid) was also tested. Results were compared to broth microdilution according to CLSI, using fresh MH broth (<12 h old) from Difco. CLSI and FDA breakpoints were used for tetracyclines and TG, respectively.

Results: For MRSA, all MH presented an overall category agreement (CA) of 93.3–100% compared with the reference method. High correlation was also observed for: TE for Ef, Ec and Kp (except Merck-MH I and Iso-Sensitest, minor errors-mE=13.3% for both); TGC for Ef (except Merck-MH I, major errors-ME=15.4%) and Ec (except Merck-MH I and Oxoid, mE=53.3% and 13.3%, respectively). Also, the following combinations showed CA > 90%: (Ef/Oxoid), (Kp/Remel and Pronadisa-MH II) and (Ab/Difco-MH I) for DX; (Ef/Remel), (Kp/Pronadisa-MH II and Merck-MH according CLSI), (Ec/all MH except Merck-MH I) for MN; (Kp/Difco-MH I and Iso-Sensitest) for TGC; and (Ab/Remel) for TE. Most VME were detected in Ab, with rates >20% when testing DX (Merck-MH according CLSI, Oxoid, Sigma-MH I and Iso-Sensitest), MN (Difco-MH I, the both MH from Merck, Sigma-MH I and Iso-Sensitest), TE (Difco-MH I) and TGC (Sigma-MH I). ME were detected in the following combinations: (Ef/Merck-MH I), (Kp/Merck-MH I, Remel, Bio-Rad and Pronadisa-MH I), (Ab/Merck-MH I, Remel and Sigma-MH II) for TGC; (Ef/Difco-MH I, Oxoid, Remel, Bio-Red, Pronadisa-MH I and Sigma-MH II) for DX; (Ab/Sigma-MH II) for TE and MN.

Conclusions: Our results enlarge the observation of the effect of differences in the MH agar formulations not only for TGC, but also for tetracyclines susceptibility. Thus, until we have more conclusive results, caution should be exerted when testing these antimicrobial agents against clinical isolates.

P1873 A comparative study of antibiotic gradient devices for meropenem, ceftriaxone and clindamycin against CLSI and BSAC reference methods

S. Reed, D. Crabtree, E. Scopes (Basingstoke, UK)*

Objectives: The aim of this study was to compare the performance of meropenem, ceftriaxone and clindamycin M.I.C.Evaluator™ strips (Oxoid) and Etest™ strips (bioMérieux) against the CLSI and BSAC broth /agar dilution reference methods.

Methods: A range of clinically significant organisms were tested, including anaerobes, staphylococci, streptococci and Enterobacteriaceae. These were grown overnight on Columbia Blood Agar and a 0.5 (1.0 for anaerobes and some streptococci) McFarland suspension of each isolate was used for both the GOLD standard agar/broth dilution and plate inoculation. Both BSAC and CLSI reference methods were followed. All inoculated plates were incubated in appropriate conditions for 24 h or 48 h (anaerobes). Results were read and used to determine essential agreement (EA).

Results: Meropenem, ceftriaxone and clindamycin M.I.C.Evaluator strips achieved an EA of >90% across all groups of organisms when compared with both CLSI and BSAC reference methods. However, with the organisms groups and method tested, the Etest strips demonstrated <90% essential agreement, with 33% of meropenem, 39% ceftriaxone and 17% clindamycin falling below this figure. See Table 1.

Conclusion: Meropenem, ceftriaxone and clindamycin M.I.C.Evaluator strips, both BSAC and CLSI methods achieved an essential agreement of greater than 90%. Etest strips were not able to display the same level of essential agreement with 30% of the results falling below 90% essential agreement.

M.I.C.Evaluator strips performed consistently and significantly better than Etest strips for meropenem (p = 0.029), ceftriaxone (p = 0.002) and clindamycin (p = 0.034).

Gradient diffusion devices are a rapid, easy and reliable alternative to the reference methods for measuring organism susceptibility to meropenem, ceftriaxone and clindamycin.

Table 1. Essential agreement (%) for each antibiotic tested

		Meropenem		Ceftriaxone		Clindamycin	
		CLSI	BSAC	CLSI	BSAC	CLSI	BSAC
Enterococci	M.I.C.E.	**96.97**	**100.00**				
	Etest	**90.91**	**100.00**				
Staphylococci	M.I.C.E.	**93.33**	**100.00**	**93.33**	**96.43**	**100**	**100**
	Etest	**90.00**	**100.00**	86.67	78.57	**94.95**	**98.04**
Streptococci	M.I.C.E.	**95.24**	**95.24**	**100.00**	**100.00**	**99.22**	**96.38**
	Etest	**95.24**	47.62	**90.48**	**95.24**	88.33	**93.36**
Pseudomonas	M.I.C.E.	**90.48**	**95.12**	**97.78**	**90.48**		
	Etest	86.06	**95.12**	**93.33**	**90.48**		
Acinetobacter	M.I.C.E.	**100.00**	**100.00**	**100.00**	**93.75**		
	Etest	**100.00**	**96.77**	**100.00**	**100.00**		
Haemophilus	M.I.C.E.	**90.00**	**90.00**	**100.00**	**94.74**		
	Etest	80.00	85.00	83.33	79.00		
Neisseria	M.I.C.E.			**92.31**	**100.00**		
	Etest			**92.31**	**100.00**		
Moraxella	M.I.C.E.	**100.00**	**100.00**	**100.00**	**90.00**		
	Etest	**100.00**	**90.00**	80.00	**100.00**		
Anaerobes	M.I.C.E.	**93.33**	**95.45**	**90.91**	**100.00**	**96.77**	**95.59**
	Etest	**93.33**	81.82	86.96	86.96	**94.62**	**90.91**
Enterobacteriaceae	M.I.C.E.	**97.51**	**97.50**	**94.26**	**94.53**		
	Etest	**90.76**	84.57	80.09	86.85		

Surveillance

P1874 Antimicrobial activity of doripenem against clinical bacterial pathogens from Europe, 2009

D. Farrell, G. Moet, H. Sader, S. Putnam, R. Jones (North Liberty, US)*

Objectives: To evaluate the *in vitro* antimicrobial activity of Doripenem (DOR) against prevalent Gram-negative and -positive pathogens isolated in Europe (EU) during 2009. DOR is an approved carbapenem in EU for the treatment of nosocomial pneumonia (NP), including ventilator-associated pneumonia (VAP), complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI).

Methods: A total of 5,481 consecutive, non-duplicate isolates from a wide variety of infections were collected from 24 medical centers located in Europe, Turkey, and Israel during 2009. Species identification was confirmed by the central monitoring laboratory and all isolates were susceptibility (S) tested using reference CLSI broth microdilution methods (M7-A8, 2009) against DOR and comparator agents.

Organism (no. of strains)	Cumulative % inhibited at doripenem MIC (mg/L):								
	⩽0.06	0.12	0.25	0.5	1	2	4	8	>8
Enterobacteriaceae (1,480)	83	94	98	99	99	99	99	99	100
Acinetobacter spp. (65)	–	3	14	26	42	58	66	74	100
P. aeruginosa (277)	4	20	34	56	68	75	86	93	100
S. aureus (1,398)	74	77	79	83	86	89	92	96	100
MRSA (318)	1	4	10	26	41	52	65	81	100
MSSA (1,080)	95	99	99	99	100	–	–	–	–
CoNS (454)	23	30	37	45	58	70	76	84	100
MR-CoNS (376)	10	15	24	34	49	64	72	81	100
MS-CoNS (78)	85	100	–	–	–	–	–	–	–
Enterococcus faecalis (357)	–	–	–	–	1	31	89	99	100
beta-haemolytic strep. (212)	100	–	–	–	–	–	–	–	–
S. pneumoniae (485)	81	82	86	97	99	100	–	–	–

Results: Doripenem was very active against Enterobacteriaceae, inhibiting 99% of isolates at 0.5 mg/L (MIC 90, 0.12 mg/L). DOR had good activity against *P. aeruginosa* (MIC50/90, 0.5/8 mg/L) and most *Acinetobacter* spp., inhibiting >70% of isolates at 8 mg/L (MIC50/90 = 2/>8 mg/L). Against Gram-positive pathogens, DOR had very high activity against methicillin-susceptible *S. aureus* (MSSA), MS-coagulase-negative staphylococci (CoNS), β-haemolytic streptococci,

and *S. pneumoniae* with MIC90 values of ⩽0.06, 0.12, ⩽0.06, and 0.5 mg/L, respectively. DOR was less active against MRSA and MR-CoNS with both having an MIC50 of 2 mg/L, as well as *E. faecalis* (MIC50, 4 mg/L). DOR was not active against the vast majority of *E. faecium* (MIC50, >8 mg/L, range 0.25–>8 mg/L).

Conclusions: DOR exhibited a wide spectrum of antimicrobial activity against 5,481 contemporary EU pathogens and excellent activity against most Gram-positive pathogens except for MRSA, MR-CoNS and Enterococci. Against Gram-negative pathogens, DOR exhibited excellent activity against Enterobacteriaceae and good activity against many multidrug resistant *P. aeruginosa* and *Acinetobacter* spp. This data supports the use of DOR as therapy for hospitalized patients, in whom carbapenem therapy would be warranted to treat serious and typically difficult-to-treat infections, such as NP, cIAI, VAP, and cUTI in Europe, Turkey and Israel.

P1875 Ceftobiprole activity when tested against clinical bacterial pathogens from Europe, 2009

D. Farrell, G. Moet, H. Sader, S. Putnam, R. Jones (North Liberty, US)*

Objectives: To evaluate the *in vitro* antimicrobial activity of ceftobiprole (BPR) against prevalent Gram-positive and -negative pathogens isolated in Europe (EU) during 2009. BPR is an investigational drug seeking regulatory approval in EU for the treatment of complicated skin and skin structure (cSSSI) infections due to methicillin-resistant (MR) staphylococci. Ceftobiprole was also evaluated in clinical trials for the treatment of nosocomial pneumonia (NP), including ventilator-associated pneumonia (VAP).

Methods: A total of 5,481 consecutive, non-duplicate isolates from a wide variety of infections were collected from 24 medical centers located in Europe (Belgium, France, Germany, Ireland, Italy, Spain, Sweden, Switzerland, United Kingdom), Turkey, and Israel during 2009. Species identification was confirmed by the central monitoring laboratory and all isolates were susceptibility (S) tested using reference CLSI broth microdilution methods (M7-A8, 2009) against BPR.

Results: Against Gram-positive pathogens, BPR had high activity against methicillin-susceptible (MS) SA, MS-coagulase-negative staphylococci (CoNS), β-haemolytic streptococci, and *S. pneumoniae* with MIC90 values of 0.5, 0.25, ⩽0.06, and 0.5 mg/L, respectively. BPR was active against MRSA and MR-CoNS with both having a MIC90 of 2 mg/L with 99% of isolates inhibited at 2 and 4 mg/L, respectively. BPR was not active against the vast majority of *E. faecium* (MIC50, >8 mg/L, range 0.12–>8 mg/L), but was active against *E. faecalis* (MIC50/90, 0.5/4 mg/L). Against Gram-negative pathogens, BPR was active against the majority of Enterobacteriaceae with >80% inhibited at 0.12 mg/L (MIC90, 4 mg/l), but had limited activity against *Acinetobacter* spp. (MIC90, >8 mg/L) and *P. aeruginosa* (MIC90, >8 mg/L).

Organism (no. of strains)	Cumulative % inhibited at ceftobiprole MIC (mg/L):								
	⩽0.06	0.12	0.25	0.5	1	2	4	8	>8
S. aureus (1,398)	1	1	31	79	94	99	100	–	–
MRSA (318)	–	–	–	11	74	99	100	–	–
MSSA (1,080)	1	1	40	99	100	–	–	–	–
CoNS (454)	3	11	23	40	76	94	99	99	100
MR-CONS (376)	2	4	8	27	71	93	99	99	100
MS-CoNS (78)	8	47	94	100	–	–	–	–	–
E. faecalis (357)	1	12	32	66	73	89	97	99	100
β-haemolytic strep. (212)	99	100	–	–	–	–	–	–	–
S. pneumoniae (485)	78	80	84	98	100	–	–	–	–
Enterobacteriaceae (1,480)	78	83	85	87	88	88	90	90	100
Acinetobacter spp. (65)	2	5	14	19	28	31	35	35	100
P. aeruginosa (277)	–	–	–	3	15	45	64	78	100

Conclusions: BPR exhibited a wide spectrum of antimicrobial activity against 5,481 contemporary EU pathogens and excellent potency against most Gram-positive pathogens including MRSA (MIC90, 2 mg/L) and MR-CoNS (MIC90, 2 mg/L). Against Gram-negative pathogens, BPR exhibited high potency against most Enterobacteriaceae, but was much less active against *P. aeruginosa* and *Acinetobacter* spp. This data showing the potent activity of BPR against a broad range of key cSSSI and NP (including some VAP) pathogens and are consistent with a potential role of BPR in targeted therapy for hospitalized patients with these infections in Europe, Turkey and Israel.

P1876 *In vitro* activity of ceftaroline tested against leading Gram-positive and -negative European bacterial pathogens collected in 2009

M. Castanheira, R. Jones, H. Sader (North Liberty, US)*

Objective: To evaluate the activity of ceftaroline (CPT) against prevalent Gram-positive and -negative pathogens isolated in Europe during 2009. CPT is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. pneumoniae*, as well as against common Gram-negative pathogens.

Methods: A total of 4212 consecutive, nonduplicate isolates from bloodstream, skin and skin structure, and respiratory tract infections were collected from 24 medical centres in Europe, Turkey, and Israel during 2009. Species identification was confirmed by the central monitoring laboratory and all isolates were tested for susceptibility (S) to CPT and comparator agents using reference CLSI broth microdilution methods.

Results: CPT inhibited 99.9% of *S. aureus* strains (22.5% were MRSA) at 2 mg/L. Only one MRSA strain (0.1% of all *S. aureus* strains) from Italy had CPT MIC of 4 mg/L. CPT MIC90 of MRSA strains was 1 mg/L, at least 16-fold lower than both ceftriaxone (CRO) and cefepime (FEP). CPT activity against coagulase-negative staphylococci (CoNS; 83.3% methicillin resistant) was similar to that against *S. aureus* (99.9% of *S. aureus* strains had MIC ⩽2 mg/L). CPT inhibited all tested *S. pneumoniae* at 0.25 mg/L. Against 19 (3.9%) penicillin-resistant pneumococci (MIC ⩾2 mg/L), CPT MIC50 and MIC90 values were 4- to 32-fold lower than values for all other β-lactams. All β-haemolytic streptococci (BHS) and all but one viridans group streptococci (VGS) were inhibited at ⩽0.25 and 0.5 mg/L, respectively. CPT MIC90 values for BHS and VGS (0.03 and 0.06 mg/L, respectively) were lower than those of penicillin, CRO, and FEP against these streptococci groups. CPT activity against Enterobacteriaceae (MIC50 0.12 mg/L) was similar to CRO and FEP (MIC50s ⩽0.25 and ⩽0.12 mg/L, respectively). Extended-spectrum β-lactamase (ESBL) phenotype was observed in 12.2% of *E. coli* and 20.0% of *Klebsiella* spp., and all cephalosporins tested showed limited activity against ESBL-producing strains. *H. influenzae* strains were highly S to CPT (MIC90, 0.015 mg/L).

Conclusions: CPT demonstrated enhanced activity against European staphylococci, including MRSA, different streptococcal groups, and *H. influenzae*. CPT also demonstrated activity against Enterobacteriaceae that is similar to currently available broad-spectrum cephalosporins.

Organism (no. tested)	Cumulative % at CPT MIC (mg/L)					MIC (mg/L)	
	⩽0.25	0.5	1	2	4	50%	90%
S. aureus (1200)	69.0	86.4	97.1	99.9	100.0	0.25	1
MRSA (270)	2.9	40.0	87.4	99.6	100.0	1	2
CoNS (432)	54.6	84.9	92.8	99.0	100.0	0.25	1
S. pneumoniae[a] (485)	100.0	–	–	–	–	⩽0.008	0.12
BHS (195)	100.0	–	–	–	–	⩽0.008	0.03
VGS (80)	97.5	98.7	98.7	98.7	98.7	0.03	0.06
E. faecalis (357)	–	2.2	28.3	65.8	72.5	2	8
E. coli (788)	75.2	81.7	85.6	87.8	89.0	0.12	16
Klebsiella spp. (294)	69.7	75.1	78.6	80.3	81.3	0.12	>32
Enterobacter spp. (146)	52.7	60.3	63.7	68.5	69.9	0.25	>32
H. influenzae (235)	100.0	–	–	–	–	⩽0.008	0.015

[a]Includes 19 (3.9%) penicillin-resistant strains (MIC ⩾ 2 mg/L).

P1877 Activity of ceftaroline against recent (2009) and multidrug-resistant *Streptococcus pneumoniae* isolates from Europe and the United States

D. Farrell, G. Moet, P. Rhomberg, H. Sader, R. Jones (North Liberty, US)*

Objective: To determine the activity of ceftaroline against recent (2009) *S. pneumoniae* (SPN) and multidrug-resistant (MDR) SPN isolated in Europe (EU) and the United States (USA). Antimicrobial resistance in SPN and MDR-SPN are increasing globally and rapidly in some countries, including the USA. Ceftaroline is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive organisms including SPN and MDR-SPN and is currently in late stage clinical development.
Methods: Susceptibility testing for ceftaroline and commonly used antimicrobials was performed by CLSI broth microdilution methods on a total of 987 isolates from the 2009 Ceftaroline Surveillance Program. MDR-SPN status was determined by resistance to 2 or more classes of antimicrobials.
Results: Ceftaroline MIC50, MIC90, and MIC range (all mg/L) against all isolates and resistance phenotypes are listed in the Table. Ceftaroline was very active against all isolates, with MIC50 and MIC90 of ⩽0.008 and 0.12 mg/L. MIC50 and MIC90 were slightly higher in drug-resistant SPN and MDR-SPN than in non-MDR-SPN isolates. The highest ceftaroline MIC found was 0.5 mg/L. MIC90 was 1 log2 dilution higher in USA isolates than in EU isolates due to the higher prevalence of MDR-SPN in the USA (21.5% vs. 14.2% in EU). Ceftaroline was very active against isolates resistant to the commonly used antimicrobials penicillin, ceftriaxone, erythromycin, levofloxacin, trimethoprim/sulphamethoxazole (T/S), and tetracycline.
Conclusions: Antimicrobial resistance in SP and MDR-SPN continues to escalate each year, highlighting the need for new antimicrobials. This study demonstrated the potent *in vitro* activity of ceftaroline against recent (2009) SPN isolates, regardless of MDR status, resistance phenotype, or geographic location (EU or USA). These data suggest that ceftaroline may emerge as an important therapy for infections caused by SPN resistant to β-lactams and other commonly used antimicrobials as well as MDR strains.

	n	MIC_{50}	MIC_{90}	Range
All isolates	987	⩽0.008	0.12	⩽0.008–0.5
All Europe	485	⩽0.008	0.12	⩽0.008–0.5
All USA	502	0.015	0.25	⩽0.008–0.5
MDRSP	177	0.12	0.25	⩽0.008–0.5
Penicillin ⩾4 mg/L	109	0.25	0.25	0.06–0.5
Ceftriaxone ⩾2 mg/L	105	0.25	0.25	0.06–0.5
Erythromycin ⩾2 mg/L	344	0.12	0.25	⩽0.008–0.5
Levofloxacin ⩾4 mg/L	11	0.06	0.25	⩽0.008–0.25
T/S ⩾4 mg/L	218	0.12	0.25	⩽0.008–0.5
Tetracycline ⩾8 mg/L	242	0.12	0.25	⩽0.008–0.5

P1878 Oritavancin activity against Gram-positive pathogens isolated from United States and European medical centres, 2008–2009

R. Mendes, R. Jones, H. Sader (North Liberty, US)*

Objectives: To evaluate the antimicrobial susceptibility (S) of Gram-positive (GP) organisms collected from United States (USA) and European (EU) medical centres. The International Oritavancin (ORI) Surveillance Program was initiated in 2008 as part of the SENTRY Antimicrobial Surveillance Program to monitor the activity of ORI and various comparators in the USA and EU. We report the results obtained by this program during the 2008–2009 period.
Methods: GP isolates (11,853) were consecutively collected from 55 hospitals in the USA (61.0%) and 13 EU member states. Isolates were submitted to a central laboratory where bacterial identifications were confirmed using standard algorithms and Vitek 2. Isolates were tested for S against ORI and comparators by CLSI methods (M07-A8, 2009). CLSI (M100-S19, 2009) and EUCAST (2009) interpretative criteria were applied, when available.
Results: Infection sites included: blood (bacteremia; 43%), skin and skin structure (26%) and lower respiratory tract (19%). ORI (MIC90, 0.06 mg/L) was 8-fold more potent than daptomycin (DAP; MIC90, 0.5 mg/L) and 16 to 32-fold more active than vancomycin (VAN; MIC90, 1–2 mg/L) or linezolid (LZD; MIC90, 1–2 mg/L) against staphylococci. Clindamycin (MIC90, >2 mg/L; 54.0–81.3% S) and levofloxacin (LEV; MIC90, >4 mg/L; 25.8–61.9% S) exhibited limited activity against staphylococci. While tetracycline (⩾90.0% S) and trimethoprim/sulfamethoxazole (⩾98.6% S) showed generally higher S rates against *S. aureus*, sub-optimal activity was noted against coagulase-negative staphylococci (51.9–86.4% S). ORI tested against *E. faecalis* (0.03 mg/L; 4.2% VAN resistant [R]) displayed an MIC90 value 2-fold lower when compared to *E. faecium* (0.06 mg/L; 60.5% VAN R). Among the comparators, only DAP and LZD were active against all enterococci (MIC90, 2 mg/L; ⩾99.3% S), whereas *E. faecalis* were also S to ampicillin (MIC90, 2 mg/L; ⩾99.8% S). ORI (MIC90, ⩽0.008 mg/L) was ⩾128-fold more active than VAN (MIC90, ⩽1 mg/L), LZD (MIC90, 1 mg/L), LEV (MIC90, 1 mg/L) and ceftriaxone (MIC90, 1 mg/L) against *S. pneumoniae*. The activity of ORI (MIC90, 0.12 mg/L) was comparable to that of penicillin (MIC90, 0.06 mg/L; 100.0% S) against β-haemolytic streptococci.
Conclusions: Based on MIC90 values, ORI demonstrated potent *in vitro* activity against this contemporary collection of GP pathogens. In addition, the ORI activity was not adversely affected by resistance to other currently-marketed antimicrobials agents.

	MIC (mg/L)									
	Oritavancin	Vancomycin			Daptomycin			Linezolid		
Organism (No. tested)	90%	90%	%S[a]	%S[b]	90%	%S[a]	%S[b]	90%	%S[a]	%S[b]
S. aureus (6,731)	0.06	1	100.0	100.0	0.5	99.9	99.9	2	>99.9	>99.9
MRSA (3,020)	0.06	1	100.0	100.0	0.5	99.8	99.8	2	99.9	99.9
CoNS (1,082)	0.06	2	100.0	99.3	0.5	99.6	99.6	1	98.9	98.9
MRCoNS (792)	0.06	2	100.0	99.1	0.5	99.7	99.7	1	98.6	98.6
E. faecium (669)	0.06	>16	39.0	39.0	2	99.4	–	2	99.3	99.3
E. faecalis (1,178)	0.03	2	95.6	95.6	2	100.0	–	2	100.0	100.0
SPN (1,198)	⩽0.008	⩽1	100.0	100.0	–	–	–	1	100.0	100.0
BHS (526)	0.12	0.5	100.0	100.0	0.25	100.0	100.0	1	100.0	100.0
VGS (199)	0.03	0.5	100.0	100.0	0.5	99.5	99.5	1	100.0	100.0

[a]CLSI and [b]EUCAST interpretation criteria, if available.
MRSA = methicillin-resistant *S. aureus*; CoNS = coagulase-negative staphylococci; MRCoNS = methicillin-resistant CoNS; SPN = *S. pneumoniae*; BHS = beta-haemolytic streptococci; VGS = viridans group streptococci; – = not available.

P1879 Spectrum of activity of oritavancin and comparison agents tested against contemporary *Staphylococcus aureus* collected in European hospitals

R. Mendes, H. Sader, R. Jones (North Liberty, US)*

Objectives: To assess the spectrum of activity and potency of oritavancin (ORI) and comparators tested against *S. aureus* (SA) recovered from hospitalized patients in Europe (EU) during the 2008–2009 International ORI Surveillance Program, as part of the SENTRY Antimicrobial Surveillance Program. This analysis includes categorization of methicillin-resistant SA (MRSA) based on antimicrobial resistance (R) patterns.
Methods: 3,788 consecutive, non-duplicate SA were collected from medical centers located in 13 EU countries. Isolates were submitted to a central monitoring laboratory and species identification performed by Gram stain, biochemical tests and Vitek 2, when needed. Susceptibility (S) testing was performed using CLSI methods (M07-A8, 2009). Regional interpretive criteria (EUCAST, 2009) were applied, when available. ORI activity was also evaluated according to R patterns. MRSA displaying R phenotypes to at least four classes of drugs were considered as multidrug-resistant (MDR).
Results: The majority of isolates were from bacteremia (39.9%) and skin and skin structure infections (35.8%). The overall MRSA rate was 25.6% and highest in Greece (59.5%), followed by Poland (40.9%),

Belgium (38.5%), Ireland (36.0%) and the United Kingdom (30.6%). MRSA rates in other EU countries were <30.0%. Among MRSA, 30 R patterns were noted and MDR strains comprised 39.3%. SA were very S to vancomycin (VAN; 100.0%), daptomycin (DAP; 100.0%), linezolid (LZD; 100.0%), teicoplanin (99.3%) and trimethoprim/sulfamethoxazole (99.2%). Levofloxacin, erythromycin and clindamycin (71.7–87.9% S) showed suboptimal activity. ORI (MIC50/90, 0.03/0.06 mg/L) was eight-fold more potent than DAP (MIC50/90, 0.25/0.5 mg/L) and 16 to 32-fold more active than VAN (MIC50/90, 1/1 mg/L) and LZD (MIC50/90, 2/2 mg/L). ORI exhibited stable and potent activity (based on MIC50/90 results) against SA, regardless of MRSA categorization, sampling year, country of origin and R patterns, including a MDR phenotype (see Table).

Conclusions: ORI demonstrated consistent and potent *in vitro* activity against this contemporary collection of EU SA strains. The ORI MIC50/90 values did not vary when tested against SA from different countries, sampling years or R phenotype. In addition, ORI was at least eight-fold more potent than comparators. Continued longitudinal surveillance of new and currently marketed agents against this important clinical pathogen is a prudent practice to monitor for emergence of R.

Organism[a] (no. tested)	MIC (mg/L)		Number (cumulative %) inhibited at oritavancin MIC (mg/L) of:					
	50%	90%	≤0.015	0.03	0.06	0.12	0.25	0.5
S. aureus (3,788)	0.03	0.06	972 (25.7)	2019 (79.0)	704 (97.5)	86 (99.8)	6 (>99.9)	1 (100.0)
MSSA (2,816)	0.03	0.06	748 (26.6)	1493 (79.6)	511 (97.7)	59 (99.8)	5 (100.0)	–
MRSA (972)	0.03	0.06	224 (23.1)	526 (77.2)	193 (97.0)	27 (99.8)	1 (99.9)	1 (100.0)
Resistance pattern[b]								
OX, LE, CL, ER (254)	0.03	0.06	54 (21.3)	131 (72.8)	59 (96.1)	9 (99.6)	0 (99.6)	1 (100.0)
OX, LE, ER (236)	0.03	0.06	54 (22.9)	135 (80.1)	44 (98.7)	3 (100.0)	–	–
OX, LE (201)	0.03	0.06	46 (22.9)	109 (77.1)	38 (96.0)	8 (100.0)	–	–
OX, LE, CL, ER, TC (81)	0.03	0.06	26 (32.1)	46 (88.9)	5 (95.1)	3 (98.8)	1 (100.0)	–
OX (51)	0.03	0.06	12 (23.5)	25 (72.5)	13 (98.0)	1 (100.0)	–	–
MDR (382)	0.03	0.06	87 (22.8)	205 (76.4)	76 (96.3)	12 (99.5)	1 (99.7)	1 (100.0)

[a]MSSA = methicillin-susceptible SA; MRSA = methicillin-resistant SA.

[b]Most prevalent R patterns noted among MRSA. Intermediate and R results grouped as R. Criteria for susceptibility were those published by EUCAST (2009). OX = oxacillin, ER = erythromycin, CL = clindamycin, LE = levofloxacin and TC = tetracycline. MDR = R to at least four classes of drugs. Non-S results for vancomycin, daptomycin or linezolid were not observed.

P1880 Antibiotic senstivity of urinary tract isolates from outpatients in the Sirte region, Libya, 2005–2008

S. Abddulsalam Bagar, B. Abdallah, M. Tayar (Sirte, LY)*

Objectives: To document the incidence of resistance against commonly used antimicrobials in uropathogens isolated from outpatients in Sirte, Libya.

Methods: Six thousand nine hundred and thirty-one consecutive urine samples were collected from patients with symptoms suggestive of urinary tract infections at the New Central Polyclinic in Sirte, Libya from 2005 to 2008. Samples were cultured on MacConkey Agar with crystal violet and pure isolates with colony counts of 104 or above were identified and tested for antimicrobial resistance by the Kirby-Bauer method.

Results: One thousand six hundred and ninety-nine cultures were positive. *Escherichia coli* was isolated from 1265 female and 301 male patients, *Klebsiella* spp. was isolated from 81 female and 18 male patients, *Pseudomonas aeruginosa* was isolated from 16 female and 9 male patients and *Proteus* spp. was isolated from 7 female and 2 male patients. In *E. coli*, the highest resistance rates (20 to 70%) were seen against Ampicillin, Co-trimoxazole, Doxycycline, Chloramphenicol, and Nalidixic acid, with little difference across age and sex groups. Resistance rates against 1st generation cephalosporins, Gentamicin and Nitrofurantoin were 7–8% among infants but increased to around 20% with age and there was little difference between the sexes. Resistance against Amoxycillin–clavulanate and Amikacin was absent among infants but rose with age to reach levels of 13% and 14% respectively in men above the age of 50 years. Resistance to Ciprofloxacin was absent among infants and increased with age up to 5%. Only 25% of *E. coli* isolates were simultaneously sensitive to the older antimicrobials (Ampicillin, Co-trimoxazole, Doxycycline, Chloramphenicol, Nalidixic acid, 1st generation cephalosporins, Gentamicin and Nitrofurantoin) but no isolate was simultaneously resistant to all of them.

Conclusion: Given the high rates of resistance to commonly used antimicrobials such as Ampicillin, Co-trimoxazole, Doxycycline, Chloramphenicol, Nalidixic acid, and even 1st generation cephalosporins and Gentamicin, one cannot recommend their use for severely ill patients without a sensitivity test report. Among the orally active antimicrobials, Nitrofurantoin remains a fair choice for lower urinary tract infections in an outpatient setting without sensitivity testing. Ciprofloxacin showed even lower resistance rates but its use should be restricted to preserve the general utility of all fluoroquinolones.

Point of care testing

P1881 Simple and rapid high-throughput test for high-sensitivity antigen detection of swine influenza virus

J.O. Koskinen, T. Vuorinen, A.E. Soini (Turku, FI)*

Objective: Few options are available for prompt high-throughput diagnostic testing in pandemic respiratory infection outbreaks. The use of conventional rapid tests is limited by their testing capacity and compromised sensitivity. The use of centralized testing is limited by the total price of the testing, the long time-to-result, and by the capacity of the specialized laboratories.

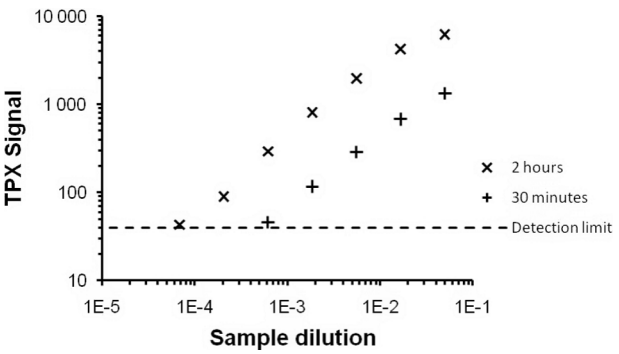
A novel methodology for rapid testing of pandemic influenza viruses is based on separation-free ArcDia TPX assay technique and dried reagents. Nasopharyngeal swab sample is combined with sample buffer, the tube is vortexed, and 20 μl of the sample is dispensed into an assay well. The well is sealed for incubation and automated kinetic measurement by the fluorometer. The test can be operated in batch-mode (up to 1000 tests a day) or in random-access mode.

The objective of this study was to evaluate the performance of the new test for the detection of inactivated standard strains of swine (H1N1) and avian (H5N1) influenza A viruses.

Methods: The new test was compared to a commercial state-of-the-art lateral flow test and to a highly sensitive central laboratory antigen detection test (time-resolved fluorometry, TR-FIA) by analysis of sample dilution series. It is well recognized that the TR-FIA technique is superior in sensitivity to standard colorimetric ELISA. Hemagglutinin titre of the swine influenza virus stock was 32.

Results: The new test returned positive all swine influenza virus samples equal or stronger than the dilution 1:1620 (from the original stock) at the 30 minute time point. At the 2 hours time point, all samples equal or stronger than the dilution 1:14580 returned positive (Figure). The lateral flow test returned positive samples equal or stronger than the dilution 1:540, which still gave visible but very faint line. The TR-FIA test returned positive all samples equal or stronger than the dilution 1:4860.

Conclusions: The analytical sensitivity of the new test for both viruses was 10–20 times better compared to the lateral flow test and 2–3 times better compared to the laboratory immunoassay test. The new test can report strong and medium positive samples in 15–30 minutes. Low positive and negative samples are reported in 2 hours. The new methodology would be well suited for rapid screening of pandemic respiratory infections, both in specialized virological laboratories and in health-care units to enable prompt laboratory testing and high sample through-put.



P1882 Relative lymphopenia is not useful in selecting patients for testing for influenza A virus H1N1

K.J. O'Connell, S. Bergin, B. Hanahoe, C. Faherty, M. Cormican (Galway, IE)*

Objectives: A recent publication by Dr. Cunha and colleagues suggested that relative lymphopenia appears to be a marker to identify those patients likely to have H1N1 and thus to merit selection of patients for RT-PCR testing. To determine if there is an evidence base for this suggestion we retrospectively analysed complete blood count results on all patients presenting to our emergency department who were tested for Influenza A virus (H1N1) using real time reverse transcriptase polymerase chain reaction (rtRT-PCR).
Methods: Between July 1st and 31st October 2009, 263 patients presenting to our hospital emergency department were tested for Influenza A virus by rtRT-PCR. Complete blood count results were available for 205 patients (111/125 adults and 94/138 children).
Results: Patients ranged in age from less than one year to 88 years and comprised 51% males and 49% females. Relative lymphopenia ($\leqslant$21%) was present in 27/33 adults who tested positive for H1N1 and in 60/78 adults who tested negative. (82% vs 78% respectively). In children, relative lymphopenia was observed in 20/34 (59%) of those who tested positive compared with 36/60 (60%) who tested negative.
Conclusions: Our data indicates that a recent suggestion that relative lymphopenia can be used to prioritise patients for testing for H1N1 is not supported by evidence. Relative lymphopenia may be a non-specific feature associated with acute viral respiratory tract infection and should not be used in prioritizing patients for testing for influenza virus infection.

P1883 Identification and characterization of blood-derived pathogens within a few hours enabled by DNA microarray assay automation and integration

H. Wiesinger-Mayr, S. Schoenthaler, J.R. Peham, I. Barisic, C. Noehammer (Seibersdorf, AT)*

Objectives: We present a rapid method for identification and characterization of pathogens from different infectious diseases, by combining PCR amplification with microarray detection both integrated in one analytical platform.
Methods: For identification microbial DNA amplification and labelling is carried out using universal primers targeting 16S and ITS rRNA. The microbial panel for oligonucleotide microarray detection, applying 20- to 30mer probes, was extended for the identification of 70 pathogens relevant for different infectious diseases with pathogen occurrence in blood. Antibiotic resistance patterns of Gram positive and Gram negative species are determined by multiplex PCR and labelling prior to chip hybridisation. Result readout is related to the method used in identification testing.
Results: As expected from sequence similarity of the 16S and ITS target genes, members of the Enterobacteriaceae family are too conserved for accurate species identification based on single species specific DNA probes. However, typical hybridisation patterns of each species could be successfully employed for accurate discrimination. The combination of rank normalisation and nearest-centroid method enabled a correct determination of 100% of the bacteria at genus level and 98.7% at species level. After thorough assay optimization (DNA isolation, PCR amplification, PCR target labelling) we succeeded to identify 10 *E. coli* cells per mL whole blood. Further probes for the detection of fastidious and intracellular pathogens have been introduced. Validation of microarray-based antibiotic resistance testing using clinical isolates showed good agreements with conventional identification methods. For Gram positive species twelve different antibiotic resistance genes are analysed using the conventional multiplex-PCR and microarray hybridization setup. For the simultaneous detection of β-lactamases in Gram negative species and identification of different ESBL types an on-chip PCR approach has been chosen.
Conclusion: Relying on our promising results from pathogen microarrays we currently develop a Lab-on-a-Chip (LOC) device aiming to miniaturize and accelerate the diagnostic assay further. Thus different approaches have been tested for efficient DNA amplification followed by DNA labelling and detection. A software for automated pathogen classification including above mentioned statistical analysis has been developed and successfully applied.

P1884 A simple blood culture bacterial pellet preparation for accurate direct bacterial identification and antibiotic susceptibility testing with the Vitek 2 system

G. Prod'hom, C. Durussel, J. Bille, G. Greub (Lausanne, CH)*

Objectives: Blood cultures are the best approach to establish the etiology of bloodstream infections. Rapid identification and antimicrobial susceptibility tests (AST) are pivotal to guide antibiotic treatment. This may be accurately done using the Vitek 2 system inoculated with subcultures. However, direct testing of bacterial pellets from positive blood cultures do not provide consistent results. Thus, we developed a specific procedure to prepare pellets from positive blood culture that may be directly used to identify both Gram-positive and Gram-negative bacteria with Vitek cards.
Methods: Pellets from positive blood culture vials (Bactec 9240) were treated with ammonium chloride lysing solution and centrifuged. GN and GP Vitek cards were used for the identification of Gram-positive cocci and Gram-negative rods, respectively. The AST-P586 and AST-GN26 Vitek cards were used for AST of staphylococci and Gram-negative rods, respectively. Identification and AST were repeated using subcultures (gold standard). Identification was classified as correct, low-level of discrimination, misidentification, and non-identification. For AST, discordance results were classified as: very major error when the result of direct method was susceptible and that of the reference method was resistant; a major error was the opposite (resistant vs susceptible). All other errors were minor errors.
Results: Among a total of 122 blood cultures, 90 (74%) gave correct identification by Vitek direct inoculation. Among 38 Gram-negative rods, 36 (95%) were correct and 2 (5%) gave low discrimination. Among 84 Gram-positive cocci, 54 (64%) gave a correct identification. The number of low discrimination, misidentification and non-identification were 6 (7.1%), 19 (22.6%)(12/19 *S. intermedius)*, and 5 (5.9%), respectively.
AST results were analysed for 86 isolates with congruent identification. For 34 Gram-negative rods, the overall categorical agreement was 96.9%. The number of very major, major, minor was 1 (0.15%), 5 (0.7%), 15 (2.2%), respectively. 8/21 errors occurred with one isolate (*K. pneumoniae*). For 52 staphylococci, the overall categorical agreement was 97%. The number of very major, major, minor was 0 (0%), 2 (0.2%), 27 (2.7%), respectively.
Conclusion: Bacterial pellets from positive blood cultures prepared with an ammonium chloride-driven hemolysis allow direct inoculation of Vitek cards used for identification and antimicrobial susceptibility testing with relatively good accuracy.

P1885 Evaluation of the *Legionella* V-Test for detection of *Legionella pneumophila* antigen in urine samples

J.P. Bruin, M.F. Peeters, E. Ijzerman, B.M. Diederen (Haarlem, Tilburg, NL)*

Introduction: We evaluated a new immunochromatographic assay (*Legionella* V-TesT, Coris BioConcept, Gembloux, Belgium) for its ability to detect *Legionella pneumophila* serogroup 1 antigen in urine. Test devices were read at various time points to determine the optimum incubation time in relation to performance. The results were compared with those obtained by the BinaxNOW urinary antigen test (Binax, INC., Scarborough, USA).
Materials and Methods: The *Legionella* V-TesT was evaluated against the BinaxNOW urinary antigen test using frozen urine samples. Urine samples were collected by the Laboratory for Medical Microbiology and

Immunology, St. Elisabeth Hospital, Tilburg and the Regional Laboratory of Public Health, Haarlem, The Netherlands between 1999 and 2009 and stored at −70ºC until processing was performed. A case of confirmed *Legionella* pneumonia was defined according to the European Working Group on *Legionella* Infections (EWGLI) criteria. Urine samples from patients with respiratory tract infections other than *Legionella* infections were tested in a similar manner to test the specificity of the assays. Only one sample per patient was included. All *Legionella* positive and negative urine samples were read at 15, 30, 45 and 60 minutes.

Results: A total of 189 samples were tested. Four samples yielded nonvalid results in the *Legionella* V-TesT; these samples were not included in the calculations. Sensitivity and specificity were estimated as, respectively, 82% (97/118; 95% confidence interval [CI], 74 to 88%) and 99% (1/71; 95% CI, 91 to 100%) for the *Legionella* V-TesT test and 84% (99/118; 95% CI, 76 to 90%) and 100% (0/71; 95% CI, 94 to 100%) for the BinaxNOW urinary antigen test after 15 min of incubation (table 1). The sensitivities of the *Legionella* V-TesT and BinaxNOW tests increased to 91% (108/118; 95% CI, 85 to 95%) and 91% (108/118; 95% CI, 85 to 95%), respectively, when the tests were examined after 60 min of incubation.

Conclusion: The *Legionella* V-TesT has a performance comparable to that of the BinaxNOW test and could be a good alternative for the detection of *L. pneumophila* antigen in urine from patients suspected of having Legionnaires' disease.

Table 1. Results of BinaxNOW and Legionella V-TesT after 15 min and 1 h of incubation

Test and incubation time (min)	% Sensitivity	% Specificity
V-TesT		
15	82 (97/118)[#]	99 (1/71)
60	91 (108/118)	99 (1/71)
BinaxNOW		
15	84 (99/118)	100 (0/71)
60	91 (108/118)	99 (1/(71)

[#]The values in parentheses are the number of samples positive/total.

P1886 Alarmingly poor performance in *Chlamydia trachomatis* point-of-care testing

L. van Dommelen, F.H. van Tiel, S. Ouburg, E.E. Brouwers, P.H. Terporten, P. Savelkoul, S.A. Morré, C.A. Bruggeman, C.J. Hoebe (Maastricht, Amsterdam, Geleen, NL)*

Objectives: Infection by *Chlamydia trachomatis* (CT) is the most prevalent sexually transmitted disease (STD) worldwide. The most frequently used diagnostic test for CT is a nucleic acid amplification test (NAAT), which is highly sensitive and specific. To further shorten time delay until diagnosis has been made, in order to prevent CT spread, the use of point of care (POC) tests could be the way forward. Three POC tests, Handilab-C, Biorapid CHLAMYDIA Ag test and QuickVue *Chlamydia* test, were evaluated regarding diagnostic performance in comparison with NAAT.

Methods: All women, above the age of 16 years old, consulting at an STD clinic between September 2007 and April 2008, were asked to participate. Women were asked to complete a short questionnaire and to collect 6 self-taken vaginal swabs (SVS). SVS 2 was used for NAAT and SVS 3 to 5 were randomized for the different POC tests. SVS 1 and 6 were used for determining quantitative CT load to validate the use of successively SVS. All POC tests were performed without knowledge of NAAT results. NAAT was used as the 'gold standard' for sensitivity. During the consultation, demographic data were collected and, if indicated, samples were collected for other STD diagnostics. A questionnaire was handed out to add more specific questions concerning CT testing methods.

Results: 772 women were included. The median age of first sexual contact was 16 (range 6–36 years). The median lifetime number of sexual partners was 9 and almost half of these contacts were considered as unsafe sexual contact. During the last six months, the median number of newly acquired sexual partners was 3. CT prevalence was 11% in our population. Sensitivities of the Biorapid CHLAMYDIA Ag test, QuickVue *Chlamydia* and Handilab-C test were 17%, 27% and 12% respectively.

Conclusions: Our results show poorer laboratory performance of the different POC tests than has previously been described and underline the need for good quality assurance of POC tests, especially in view of the Internet era. Although excellent guidelines on CT POC test evaluation exist, these guidelines are regularly ignored, and thus tighter regulations are urgently needed to prevent unrestrained marketing. In our opinion, the CT POC tests evaluated in our study are not ready for widespread use.

P1887 Evaluation of the Immunocard Stat!® Campy for detection of *Campylobacter*: a routine laboratory perspective

A. Dediste, P. Retore, L. Vlaes, O. Vandenberg (Brussels, BE)*

Objectives: To evaluate the use of the new commercialised Immunochromatographic Rapid Test IMMUNOCARD STAT!® CAMPY (Meridian BioScience, USA) allowing a 20 minutes qualitative detection of both *Campylobacter* antigen directly in human stool specimens.

Methods: During four months, all stool samples specimens, from non-hospitalized children and HIV-infected patients (adults and children) submitted to our laboratory were evaluated with IMMUNOCARD STAT!® CAMPY. Results were compared with those obtained by routine culture methods using both a specific medium and a filtration method for the recovery of *Campylobacter* species.

Results: Of the 500 stool specimens cultured, 35 were found positive for either *C. jejuni* or *C. coli,* giving an overall recovery rate of 7.0%. 24 samples were positive by both culture and Meridian Assay and 11 were positive by culture only, giving a sensitivity of 68.6%. In addition, of 465 samples negative by culture, 7 were initially positive by IMMUNOCARD STAT!® CAMPY. We found no cross-reaction with other bacterial enteropathogens isolated in stool specimens. These results confirm thus a high specificity (98.5%) for both *C. jejuni* or *C. coli.* The positive and negative predictive values found were 77.4% and 97.7% respectively.

Conclusion: These data suggest that IMMUNOCARD STAT!® CAMPY is a rapid and easy-to-use test for the detection of both *C. jejuni* and *C. coli* in stool specimens. It could be used for patients for which early antibiotic therapy is needed or for epidemiological studies.

P1888 Usefulness of procalcitonin for early diagnosis and therapeutic monitoring of non-bacterial sepsis, bacterial sepsis, and severe sepsis or septic shock compared with other inflammatory markers

S.Y. Lee, J. Lee, Y.J. Park, K. Han, S.H. Oh, S.P. Choi (Seoul, KR)*

Objectives: Procalcitonin is useful to asscess the severity of bacterial infection. In this study, we evaluated the diagnostic value of procalcitonin in nonbacterial sepsis, bacterial sepsis, and severe sepsis or septic shock, compared with C-reactive protein (CRP), erythrocyte sediment rate (ESR), white blood cell (WBC), and neutrophil. The usefulness of procalcitonin for antibiotic therapeutic guideline and monitoring was also evaluated.

Methods: 146 Patients (50 clinical nonbacterial sepsis, 80 identified (44) and clinical (36) bacterial sepsis and 16 severe sepsis or septic shock) admitted to the emergency room of St. Mary's hospital from May 2009 to August 2009, were recruited into the study. Procalcitonin (Elecsys® BRAHMS PCT), CRP, ESR, WBC, and neutrophil levels were estimated prior to commencement on antibiotics. For 29 patients of them, procalcitonin, interleukin (IL)-6 (Roche Diagnostics), CRP, WBC, neutrophil, and absolute count/% of immature granulocytes (IGs) were monitored at least three times on days 0, 0.5, 1, 2, 3, 4.

Results: Initial serum procalcitonin level was significantly higher in patients with bacterial sepsis (median 0.213 ng/mL) including severe

sepsis and septic shock (median 7.120 ng/mL) than nonbacterial sepsis (median 0.094 ng/mL) (respectively, P = 0.0405 and 0.0201). Of other inflammatory markers, CRP, WBC and neutrophil count showed significant difference between nonbacterial sepsis and bacterial sepsis and CRP also did between nonbacterial sepsis and severe sepsis or septic shock (respectively, <0.05). The areas under the curve of procalcitonin for differentiating bacterial infection and severe sepsis or septic shock were 0.70 and 0.93 (sensitivity 64.2%, specificity 67.9%, optimum cutoff 0.159 ng/mL and sensitivity 81.3%, specificity 86.3%, optimum cutoff 0.921 ng/mL, respectively). It revealed the best performance for differentiating severe sepsis or septic shock and was only comparable to neutrophil count for diagnosing bacterial infection. The procalcitonin was best inflammatory marker for antibiotic therapeutic monitoring and should be monitored after 3 days from admitted day in this study.
Conclusion: Procalcitonin was the most useful marker for severity asscessment of bacterial infection and antibiotic therapy may be applied to patients when their prcocalcitonin concentration is higher than 0.159 ng/mL.In addition, serial procalcitonin measurement offered the information about bacterial infection control.

Internet and electronic resources

P1889 EpiSouth: a new voice in communicable disease epidemiology in the Mediterranean and Balkans

F. Riccardo, V. Alfonsi, M. Fabiani, M.G. Dente, S. Declich for the EpiSouth Network*

Objective: A country's response to a major outbreak and its capacity to limit the spread of disease across international borders are dependant on the international exchange of information and expertise. Although countries bordering the Mediterranean Sea share concerns in the field of communicable disease epidemiology (CDE), they spread across different continents, 3 WHO regions, and are only partially covered by European networks for infectious disease surveillance and control. Local political tensions and conflicts further limit cooperation in the field of public health (PH). The EpiSouth project aims to create a solid information sharing network among these countries.
Methods: The EpiSouth project started among 5 EU countries: Italy, Spain, France, Greece and Bulgaria. Today it includes 26 countries embracing Southern Europe, the Balkans, North Africa and the Middle-East, and a roster of international organizations (EU, ECDC, and WHO) (Figure 1). In the past three years, through its dedicated web-based platform, this peer-to-peer network of epidemiologists studied several aspects of CDE, including vaccine-preventable disease and emerging zoonosis, developed a cross-border epidemic intelligence platform, directories of PH institutions and veterinary/human PH officers, and established a CDE training programme. All the project outputs are freely available on the web (www.episouth.org). EpiSouth produces two regular bulletins and thematic notes on regionally relevant CD issues including Influenza A H1N1.



Results: As of the 3rd of November 2009, recipients from 59 different countries (Europe 64%, Balkans 20%, North Africa 4%, Middle East 7% and Other 5%) had subscribed to the EpiSouth Bulletin, with a 41% increase in number since the first issue, released in September 2007. 13% of recipients come from countries not included in the EpiSouth region, denoting a growing interest in the network also from other countries. Access to the public website increased dramatically during the past three years, with 15,664 different users counted in September 2009.
Conclusions: With its extended geographical reach, highly committed participants and it's accessibility, Episouth has become a leading voice on the epidemiology of CD in the Balkans and Mediterranean Basin. It created cohesion and concrete collaboration among 26 countries with common PH problems and is now an internet resource providing information to hundreds of users inside and outside the EpiSouth region.

P1890 Experiences with a new computer-based system for automated monitoring and surveillance of healthcare-acquired infections in intensive care

W. Koller, A. Blacky, H. Mandl, K.P. Adlassnig (Vienna, AT)*

Objectives: To compare infection alerts and healthcare-acquired infection (HCAI) surveillance results generated by the computer-based system MONI/Surveillance-ICU with the ones generated in parallel by trained infection control staff. MONI/Surveillance-ICU is a new release of an already established knowledge-based expert system used for monitoring of HCAIs in the intensive care units at the Vienna General Hospital.
Method: Direct comparison of surveillance results generated automatically by MONI with the ones generated in parallel by trained surveillance staff and attending clinical specialists who reviewed patient charts and used other on-site information.
Results: In 50 admissions covering 382 patient days, both systems gave identical results in 40 cases (33 without and 7 with infection). In 6 cases MONI detected HCAIs (2 LRT and 4 CVK-related) which had been missed by the attending clinical specialists. In 4 admissions MONI missed HCAIs (3 LRT and 1 CVK-related) which had been reported by human experts. Though MONI in all those cases reported general indicators for infection, specific information on the actual infection site (e.g., radiology and/or microbiology lab reports indicative for LRT-infection) had not been imported into the surveillance database of MONI and was therefore not available for the automated inference process, which on its own was impeccable. MONI made gradual emergence and fading of HCAIs visible during a patient stay.
Conclusion: MONI/Surveillance-ICU proved to be reliable, quick and even better than human observers in sensing and surveilling HCAI in intensive care, provided all relevant information held in the ICU patient data management system and microbiology lab IT was correctly matched and imported into the MONI database. In our present setting, MONI serves excellently its intended purpose of "infection radar" in intensive care. For a fully-automated surveillance reporting system not all relevant criteria are accessible yet and improvements are planned especially in the IT interfaces to radiology and microbiology.

P1891 The potential of Twitter for early warning and outbreak detection

P. Kostkova, E. de Quincey, G. Jawaheer (London, UK)*

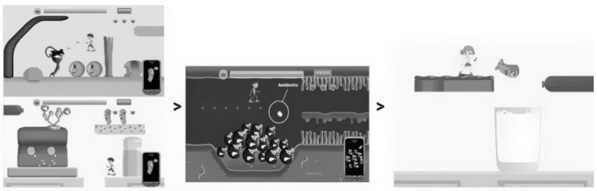
Aims and Objectives: The use of user-generated content in Web 2.0 tools for predicting outbreaks has been seen as a great potential, however, the recent swine flu outbreak in April-May 2009 truly demonstrated the potential of these media for early warning systems. Web 2.0 has generated a great interest recently as a possible media for early warning system for outbreak detection and epidemic intelligence (EI). Traditional systems such as GPHIN, Medisys are well established and used by ECDC and WHO on a daily bases, however, there has been recent interest in the ability to estimate flu activity via aggregating online search queries for keywords relating to flu and its symptoms by commercial companies like Google. However, the search data remain proprietary and therefore not useful for research. The increase in user generated content on the web via social networking services such as Facebook and Twitter, however, provides researchers with a highly accessible view into people's online and offline activity.

Methods (Twitter use on swine flue outbreak): Twitter, a microblogging service that allows people to post and read other users' 140 character messages currently has over 15 million unique users per month. Twitter allow third parties to search user messages using an open source API and return the text along with information from the poster's profile, such as their location, in a format that can be easily stored and analysed.
We searched and collected over 1 million tweets in the period from May until August 2009 and carry on collecting them on a minute bases to understand public concerns, keywords used and the profile of users who discuss these topics on the web.
Results: We found over 1 million tweets reporting flu related illnesses and symptoms via Twitter in this period. Most popular words in tweets were these (frequency in brackets): flu (138, 260), Swine (99, 179), Have (13, 534), Cases (13, 300), H1N1 (9, 134), Has (8, 010) etc. The actual sentence "I have swine flu" appeared 2, 888 times and "I have flu" 1,530 times. Further evaluation of the collected tweets, semantic relationship of keywords, geolocation of posters is underway and will be presented at the conference.
Conclusion: The potential of Web 2.0 system for early warning systems and for better understanding public concerns about their health is enormous, however, further research is required to reveal the underlying principles and implement adequate integration with existing healthcare services.

P1892 Can Web games teach children hygiene and antibiotic resistance? Evaluation of the e-Bug junior games

D. Farrell, P. Kostkova, J. Weinberg, D. Lecky, C. McNulty (London, Gloucester, UK)*

Introduction: e-Bug is a EC funded antibiotic and hygiene teaching resource aiming to reinforces an awareness of microbes, hand and respiratory hygiene and prudent antibiotics use among junior and senior school children across Europe. e-Bug web games were developed for 2 age groups (junior and senior children) teaching the key learning outcomes (LOs) of the e-Bug project and complementing physical packs distributed to schools across Europe.
The e-Bug junior game: The junior platform games has a number of "levels" teaching the given set of LOs. Player, shrunken inside human body, interacts with good and bad cartoon microbes (Fig 1) as well as antibiotics and viruses. Teaching the LOs is implemented through the way the player interacts with microbes (e.g. turning milk into yogurt (Fig 3), finishing course of antibiotics (Fig 2). Children knowledge is tested seamlessly before and after each level in a Game Show style similar to the popular TV game "Do you want to be a Millionaire?"



Evaluation results: Evaluation was primarily conducted in the UK and online demonstrating statistically significant knowledge gain of the learning outcomes. This was complemented by qualitative studies (focus groups and observational studies). 29 pupils took part in the focus groups (and fully completed the pre and post questionnaire) from three schools. Before playing the game, only 4 pupils "agreed" that fungi were microbes while after playing 18. Smaller improvements were seen in other questions including: "We use microbes to make things like bread and yogurt" (11 correct before, 23 correct after) and "Soap can be used to wash away bad bugs" (20 before vs 24 after).
The main evaluation took place in the period of May – August 2009. Each of the completed level was evaluated for statistical significance of knowledge change of the LOs. As many questions were correct before and after the game, the statistically significant improved responses were measured (using McNemar's test), for the following questions: "if you cannot see a microbe it is not there", "most coughs and colds get better without medicine" and in particular "we use good microbes to make things like bread and yogurt". There was a trend towards improved knowledge however in other questions did not reach statistic significance.
Conclusion: The study demonstrated that computer games can teach children about hygiene and antibiotics in an enjoyable way. Further study is needed to evaluate an impact on behaviour change.

P1893 Bibliometric analysis of European publications in infectious diseases and clinical microbiology areas

O. Sipahi, H. Sipahi, M. Tasbakan, H. Pullukcu, B. Arda, T. Yamazhan, S. Ulusoy (Izmir, TR)*

Objectives: In this study it was aimed to make the bibliometric analysis of 1996–2007 and 2007 European publications related to infectious diseases and clinical microbiology areas.
Methods: Bibliomeric data related to 1996–2007 and 2007 were retrieved from SCImago journal and country rank web site (www.scimagojr.org). Data related to infectious diseases and clinical microbiology were accessed by using the infectious diseases subcategory and clinical microbiology subcategory of the database. Top 10 European countries according to total publication number was also detailed with data of citable publications (articles+reviews), total citations, citation per paper and H index.

Table: Top ten European countries both in 1997–2007 and 2007 time periods

Country	Total publications	Citable publications	Total citations	Citation per paper	Hirsch (H) index
Infectious diseases area (1997–2007)					
United Kingdom	5,130	4,220	57,276	12.36	79
France	4,056	3,640	24,138	6.33	53
Germany	1,973	1,728	20,869	12.88	56
Netherlands	1,654	1,513	14,746	11.55	49
Italy	1,644	1,336	10,844	7 89	38
Spain	1,392	1,127	9,390	9.08	37
Switzerland	971	884	14,328	16.7	53
Belgium	903	814	8,864	11.89	41
Turkey	773	518	3,467	5.7	23
Sweden	504	478	5,699	13.12	33
Infectious diseases area (2007)					
United Kingdom	565	440	1,441	2.55	79
France	410	363	679	1.66	53
Germany	245	200	498	2.03	56
Spain	235	173	448	1.91	37
Italy	220	177	430	1.95	38
Netherlands	181	163	400	2.21	49
Switzerland	122	106	391	3.2	53
Belgium	109	100	288	2.64	41
Turkey	94	59	140	1.49	23
Greece	76	55	201	2.64	24
Medical microbiology area (1997–2007)					
Spain	3,634	2,877	15,224	4.31	43
Germany	2,811	2,624	29,711	11.35	60
Italy	2,428	2,241	10,945	4.99	40
United Kingdom	2,191	2,005	24,379	12.3	60
France	1,759	1,556	19,414	11.76	53
Russian Federation	1,356	1,347	2,657	2.08	21
Turkey	1,067	984	2,679	2.9	19
Netherlands	972	916	12,824	14.87	50
Sweden	897	859	9,291	10.63	39
Denmark	826	798	9,739	11.79	42
Medical microbiology area (2007)					
Spain	273	198	492	1.8	43
Germany	268	247	953	3.56	60
United Kingdom	267	235	863	3.23	60
France	199	171	615	3.09	53
Italy	160	141	266	1.66	40
Netherlands	132	120	414	3.14	50
Turkey	116	107	74	0.64	19
Russian Federation	102	98	69	0.68	21
Sweden	90	76	155	1.72	39

Results: When evaluated according to total number of publications, United Kingdom, France and Germany are the top three countries in the infectious diseases area both in 1997–2007 and 2007 periods. In the medical microbiology area in 1997–2007 period top three countries are Spain, Germany and Italy whereas in 2007 top three are Spain, Germany and United Kingdom (See table). United Kingdom which is European number one in infectous diseases, is globally the second after United States in both 1997–2007 and 2007 periods. Spain which is the European number one in medical microbiology, is the third in 1997–2007 period (after United States and Japan) and the fifth in 2007 (after United States, China, Japan and Brazil) (data not shown).
Conclusion: Although at least some of the European countries are quite competitive both in infectious diseases and medical microbiology areas, further progress is needed.

Biofilm

P1894 Response of *Staphylococcus epidermidis* biofilms cells to the effect of farnesol

B. Leite, F. Gomes, P. Teixeira, C. Souza, E. Pizzolitto, R. Oliveira (Braga, PT; São Carlos, Araraquara, BR)*

Objective: *Staphylococcus epidermidis* is a leading cause of medical-device-related infections, especially in immunocompromised patients. The treatment of these infections is further complicated by the emergence of multiresistant strains. The ability of *S. epidermidis* to form biofilms on biotic and abiotic surfaces is believed to contribute significantly to the pathogenesis of these infections. Biofilms are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy. Recently, farnesol has been described as having antimicrobial properties, and therefore a possible action on the prevention of *S. epidermidis* related infections. In previous studies it was shown that 300 microM farnesol was effective against *S. epidermidis* planktonic cells but having only a slight effect on biofilm cells. So, the goal of this study was to assess the antimicrobial activity of higher farnesol concentrations (1 and 100 mM) against biofilm cells of *S. epidermidis*.
Methods: Two *S. epidermidis* strains biofilm-producing (9142 and 1457) were used in this study. Farnesol (0, 1 mM, 100 mM) was added to 24 h biofilm cells. Biofilm formation was assessed through crystal violet (CV) staining that measure total biomass of biofilm and cellular viability through XTT and colony-forming units (CFU/ml).
Results: The results didn't show a significant effect of both farnesol concentrations on biofilm biomass and activity. In fact, biofilm cell reduction was less than 2 Log, similarly to most antibiotics (e.g. tetracycline and vancomycin).
Conclusion: Although the reduction promoted by farnesol was less than 3 Log as requested for an antibiotic agent, its efficacy is similar to vancomycin. On account of that we are now testing the combined effect of farnesol with agents that disrupt the biofilm matrix.

P1895 The influence of oleanolic and ursolic acids on biofilm formation and hydrophobicity of bacteria

A. Kurek, J. Ufnalewska, K.I. Wolska (Warsaw, PL)*

Objectives: Frequent infections caused by *Staphylococcus aureus*, *S. epidermidis* or *Pseudomonas aeruginosa*, are related to their ability to form biofilm. Chemotherapy of such infections is seldom fully effective, due to high biofilm resistance. Pentacyclic triterpenoids, including oleanolic acid (OA) and ursolic acid (UA) can be considered as potential antibiofilm agents. OA and UA are present at the significant amount in many plants of commercial and medical importance. Our previous results showed that OA and UA inhibit growth and survival of several bacterial species, mainly Gram-positive and also influence cell morphology, autolysis, peptidoglycan turnover in planktonic cultures. The aim of this study is to evaluate the ability of OA and UA to impair biofilm formation by pathogenic bacteria and their influence on cell surface hydrophobicity.
Materials and Methods: MICs of OA and UA for planktonic bacteria were determined according to the standards of NCCLS. *S. aureus* ATCC29213, *S. epidermidis* ATCC12228 *P. aeruginosa* ATCC10145 and *E. coli* ATCC23546 were used as planktonic cultures or 24-hour-old biofilm built in the wells of polistyren microplate in the presence of the subinhibitory concentrations: 0.15, 0.25 and 0.5 MIC of OA or UA. BIC and BEC (Biofilm Inhibitory Concentration and Biofilm Eradicating Concentration) were estimated by the crystal violet staining and spectrophotometric measurement and by the BacTiter Glo assay which measures active metabolism of bacteria that survived in the presence of OA or UA. Cell surface hydrophobicity of planktonic bacteria in the presence of the OA or UA was estimated by SAT (Salt Aggregation Test).
Results: It was demonstrated that OA and UA showed antibacterial effect. Both compounds inhibit biofilm formation by bacterial species sudied. The decrease of biofilm formation, especially by two Gram-positive species, 8% and 20%, were observed in the presence of 0.25 and 0.5 MIC of OA/UA, respectively. The lowest concentration of ammonium sulfate giving visible aggregation of bacteria, SAT value, was two-fold and three-fold decreased in the presence of 0.5 MIC OA/UA, for Gram-negative and Gram-positive bacteria, respectively.
Conclusion: Biofilm formation could be inhibited by treatment with OA and UA. Hydrophobicity of the bacterial cell surface are also changed in the presence of the subinhibitory concentration of OA and UA.
This study was supported by Grant No N N302 027937 (MNiSW).

P1896 Biofilm formation is important for *Propionibacterium acnes* pathogenesis and antibiotic susceptibility

A. Holmberg, R. Lood, M. Mörgelin, P. Nordenfelt, B. Söderquist, E. Holst, B. Christensson, M. Rasmussen (Lund, Örebro, SE)*

Objectives: *Propionibacterium acnes* is a common and probably underestimated cause of delayed joint prosthesis infection. Bacterial biofilm formation is important for bacterial pathogenesis and antibiotic tolerance in infections related to foreign material, and *P. acnes* forms biofilm both *in vitro* and *in vivo*. We hypothesed that invasive *P. acnes* isolates form more biofilm *in vitro* than non-invasive isolates. Moreover, we aimed to study the three-dimensional structure of a *P. acnes* biofilm. We also wanted to compare antibiotic susceptibility between *P. acnes* in planctonic and biofilm growth phase.
Methods: Biofilm formation *in vitro* on a plastic surface was analyzed for 93 *P. acnes* isolates either from invasive infections related to foreign material ($n = 45$) or from the skin of healthy controls ($n = 48$). The sequence of recA was used for typing. The biofilm was visualized by scanning and transmission electron microscopy (SEM and TEM) and by fluorescence microscopy (FM). The three-dimensional structure was studied using a wide-field microscope equipped with a motorized stand and deconvolution software. The antibiotic susceptibility was tested using a modified Calgary Biofilm Device.
Results: The majority of isolates from deep infections formed biofilm on the plastic surface, whereas the skin isolates were poor biofilm producers ($p < 0.001$ for a difference). The presence of human plasma inhibited biofilm formation. There were no differences in the distribution of serotypes between the invasive and skin isolates. The biofilm was visualized by different modalities and the multilayered architecture of the *P. acnes* biofilm could clearly be demonstrated by FM. Moreover, an extracellular material within the biofilm was demonstrated by both TEM and SEM. Biofilm formation on beads of bone cement could also be seen with SEM. For selected isolates we have determined the minimal inhibitory concentration (MIC) and minimal biofilm inhibitory concentration (MBIC) for relevant antibiotics. The MIC values obtained were as expected very low, but interestingly MBIC is for most antibiotics much higher.
Conclusions: Our findings demonstrate for the first time that *P. acnes* can form a multilayered biofilm and that biofilm formation is important for *P. acnes* virulence. Moreover, our data suggest that biofilm formation should be considered in the choice of antibiotic treatment for invasive infections with this organism.

P1897 Different biofilm types contribute to resistance of *Staphylococcus epidermidis* against uptake by macrophages

H. Rohde, N. Schommer, M. Hentschke, K. Ruckdeschel, M. Aepfelbacher (Hamburg, DE)*

Objectives: Formation of adherent multi cellular aggregates is the key mechanism involved in *Staphylococcus epidermidis* virulence in device associated infections. Aside from polysaccharide intercellular adhesin PIA, accumulation associated protein Aap and extracellular matrix binding protein Embp act as intercellular adhesins, mediating *S. epidermidis* biofilm accumulation. The aim of this study was to characterize structural features of PIA-, Aap-, and Embp-mediated *S. epidermidis* biofilms in more detail and to evaluate, by using well characterized *S. epidermidis* strain pairs, their specific contribution to biofilm-related *S. epidermidis* immune escape.
Results: PIA-dependent biofilms were characterized by densely packed bacteria, embedded in a PIA containing extracellular matrix. In sharp contrast, neither Aap- nor Embp-mediated biofilms featured a significant extracellular matrix. Despite this marked difference, PIA-, Aap-, and Embp-mediated biofilms protected *S. epidermidis* with similar efficiency against up-take by J774.A1 macrophages. The reduced phagocytic up-take was likewise associated with a significantly reduced NF-kappaB and AP-1 activation and Il-1β production compared to macrophage responses to biofilm-negative *S. epidermidis* strains not expressing the respective intercellular adhesins. Thus, interference of *S. epidermidis* biofilm formation with phagocytosis is due to over-all reduced phagocyte activation. More-over, contact with biofilm forming *S. epidermidis* also induced a reduction in macrophage's LPS responsiveness, suggesting biofilm-related induction of anti-inflammatory host cell events.
Conclusions: Our results not only demonstrate that independent mechanisms of biofilm formation are similar effective in protecting *S. epidermidis* from phagocytic up-take, but point towards specific events during *S. epidermidis* – macrophage interactions which interfere with phagocyte activation. As these result in failure to take up and eradicate *S. epidermidis* a detailed elucidation of the involved mechanisms could open new directions for development of therapeutic strategies to combat *S. epidermidis* infections.

P1898 Unpredictable effects of rifampicin as adjunctive agent in elimination of rifampicin-suseptible and -resistant *Staphylococcus aureus* grown in biofilm

S. Croes, P.S. Beisser, C. Neef, C.A. Bruggeman, E. Stobberingh (Maastricht, NL)*

The use of rifampicin as adjunct in biofilm-associated infections is based on (i) the ability of this antibiotic to penetrate in biofilms and (ii) a presumed activity against dormant bacteria. Yet, its efficacy remains poorly defined and rifampicin-resistant strains frequently emerge during therapy. Therefore, the efficacy against rifampicin-susceptible and isogenic rifampicin-resistant methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated was evaluated. Biofilms were induced in MSSA cultures with various genetic backgrounds. Oxacillin + rifampicin at various concentrations, or oxacillin alone were subsequently added and, after 24 hours, biomass and viable cell counts were determined. Reduction in biomass and viable cell count upon rifampicin treatment varied dramatically and was independent of clonal lineage. Surprisingly, in some cases, biomass was dramatically increased upon treatment with either rifampicin, oxacilin, or both. Overall, subinhibitory concentrations of added rifampicin were effective for biofilm disruption in approximately 20% of the rifampicin-susceptible isolates. Additionally, rifampicin had no additional value in disruption of biofilms of isogenic rifampicin-resistant isolates. Although reductions in were observed in some of these isolates, others exerted a significant increase in viable count. In conclusion, the effect of rifampicin in the reduction of biofilm is unpredictable and the use of rifampicin against biofilm containing rifampicin-resistant variants is unwarranted.

P1899 A model system for genetic analysis of biofilm formation during catheter-associated urinary tract infections

A. Reisner, A. Wöran, M. Holzer, K. Schilcher, E. Stabentheiner, E.L. Zechner (Graz, AT)*

Objectives: Catheter-associated urinary tract infections (CAUTI) are the most frequent nosocomial infections in hospitals and other health care facilities. Catheter insertion in the bladder creates a surface subject to extensive biofilm formation that in turn provides a niche for bacterial growth with inherently increased antibiotic resistance. Despite extensive research using simple biofilm model systems, little is known about the genetic determinants promoting colonization of catheter polymers. We aimed to establish a dynamic model system that allows analysis of molecular factors affecting catheter colonization under conditions mimicking a catheter-associated urinary tract infection.
Methods: Wildtype strains of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* as well as various mutant derivatives that were previously shown to give attenuated biofilm formation in simple laboratory biofilm models were inoculated in a slightly modified model system for a catheterized bladder previously used for studying catheter encrustation (Stickler, et al. (1999) Methods Enzymol 310: 494–501). Models with an inserted all-silicone Foley-catheters (Ch14) were incubated for 1 to 5 days using a constant artificial urine flow of 30 ml/h. Several methods were tested to monitor bladder and catheter colonization including harvest of bacteria followed by determination of colony counts, confocal laser-scanning and electron microscopy, and enzymatic assays revealing microbial activities on the catheters.
Results: All strains formed biofilms on the catheters. Using standardized protocols, bladder and catheter colonization was monitored reproducible over time. We found that the most suitable inoculation strategy to identify mutants attenuated in biofilm formation is to co-inoculate wild-type and mutant strains in an initial ratio of 1:1. Decreased fitness of mutant strains is monitored by shift in the bladder and catheter-colonizing population. The best representation of the biofilm structure formed on the catheter surface was obtained using electron microscopy. Not all mutations that have been shown to give attenuated biofilm formation in simple laboratory biofilm models were also attenuated for catheter colonization in this model mimicking a catheter-associated urinary tract infection.
Conclusion: The presented model system for a catheter-associated urinary tract infection is suitable for application of molecular analysis of catheter colonization.

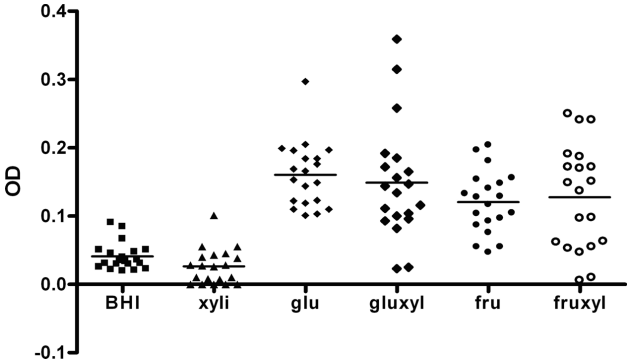
P1900 Effect of xylitol and other carbon sources on *Streptococcus pneumoniae* biofilm formation and gene expression *in vitro*

P. Kurola, T. Tapiainen, J. Sevander, T. Kaijalainen, M. Leinonen, M. Uhari, A. Saukkoriipi (Oulu, FI)*

Objectives: Xylitol inhibits the growth and adherence of *Streptococcus pneumoniae*. In clinical trials, xylitol decreases the occurrence of acute otitis media but does not affect nasopharyngeal carriage of pneumococci. We hypothesized that xylitol inhibits biofilm formation of *S. pneumoniae* and measured pneumococcal biofilm formation and gene expression levels of the capsule gene cpsB and two other genes, autolysin encoding gene lytA and competence gene comA in different growth media *in vitro*.
Methods: Twenty pneumococcal isolates were grown on polystyrene plates for 18 hours in test media containing 0.5% xylitol, 0.5% glucose, 0.5% xylitol and 0.5% glucose, 0.5% fructose, 0.5% xylitol and 0.5% fructose or BHI medium supplemented with 10% horse serum. The polystyrene plates were made in duplicate; one set for RNA extraction and another set for crystal violet staining.
Results: The means of the OD values were 0.04, 0.03, 0.16, 0.15, 0.12 and 0.13 for BHI, xylitol, glucose, glucose supplemented with xylitol, fructose and fructose medium supplemented with xylitol, respectively (Figure). The results differed significantly between the test media ($P < 0.001$). The OD values were significantly lower in xylitol compared

with BHI medium with a mean difference of –0.015 [95% confidence interval (CI) –0.028 to –0.002, P = 0.03]. Xylitol also decreased the expression levels of the cpsB and lytA genes. When the medium was supplemented with glucose or fructose, biofilm formation was enhanced and the inhibitory effect of xylitol on biofilm formation was not observed.

Conclusion: We found that xylitol inhibits the biofilm formation of *S. pneumoniae* whereas other extra carbon sources such as fructose and glucose enhance biofilm formation. The decrease in biofilm formation may further explain the efficacy of xylitol in preventing acute otitis media in previous clinical trials.



P1901 Visualization of *in vivo* biofilm developing on silicone implants and the interaction with the cellular response

M. van Gennip, L. Christensen, K. Qvortrup, M. Alhede, P. Jensen, N. Høiby, M. Givskov, T. Bjarnsholt (Copenhagen, DK)*

Objective: *Pseudomonas aeruginosa* is associated with infections of medical implants in humans, where they form biofilms. The biofilm mode of growth contributes to the tolerance against treatment with antibiotics as well as to the host response. Polymorphonuclear leukocytes (PMNs), which are part of the innate immunity and some of the first cell types to be recruited to the site of infection, most often fail to eradicate the biofilm infection. *P. aeruginosa* produces rhamnolipids which causes the PMNs to undergo a necrotic death. We have previously shown that a *P. aeruginosa* mutant unable to produce rhamnolipids was cleared from flat implants inserted into the peritoneal cavity of mice. By modifying the *in vivo* foreign-body infection model established by Christensen et al. in 2007 using 4 mm silicone tubes (id 4 mm, od 6 mm) instead of flat, square implants, we followed the development of *P. aeruginosa* biofilms as well as the pathogen-host interaction by means of quantitative bacteriology and scanning electron microscopy (SEM).

Methods: Wild-type (WT) *P. aeruginosa* (PAO1) and its corresponding rhlA mutant were harvested by centrifugation from an overnight culture and resuspended in 0.9% NaCl to an OD600nm of 0.1. The implant tubes were colonized for 20-hours with shaking at 110 rpm and inserted in the peritoneal cavity of BALB/c mice at day 0. Tubes removed from euthanized mice were either prepared for SEM (fixed in 2% glutaraldehyde, post-fixed in 1% OsO4, critical point dried using CO2 and sputter coated with gold according to standard procedures) or evaluated for quantitative bacteriology (CFU per implant). Specimens for SEM were investigated with a FEI XL Feg30 SEM microscope.

Results: Quantitative bacteriology showed that the mice were able to clear the rhlA mutant by day 3, but the WT was still cultivated on day 7 post-insertion. The SEM pictures revealed that by day 1 a biofilm had formed, and subsequently a continuously increase in bacterial biomass. As for the host response it was evident that by day 1 an abundant number of PMNs had been attracted and seemed to enter the tubes from the tube openings. Even more PMNs were seen on day 2, however, most of them seemed to be damaged and embedded in the biofilm matrix.

Conclusion: Quantitative bacteriology confirmed our previous findings that a rhlA mutant is cleared faster than the WT. The SEM visualization confirmed this finding and clearly emphasised the WT enabled destruction of the PMNs.

P1902 Oritavancin activity against *in vitro* biofilms derived from clinical isolates of *Staphylococcus aureus* from a phase 2 study

A. Belley, T. McClure, I. Sarmiento, T. Parr Jr., G. Moeck (St. Laurent, CA)*

Objective: Oritavancin (ORI) is an investigational lipoglycopeptide that exhibits activity against *in vitro* biofilms, a bacterial growth state that exhibits remarkable tolerance to antimicrobial agents and consequently poses a serious clinical challenge. We assessed ORI activity against *in vitro* biofilms derived from *Staphylococcus aureus* clinical isolates and tested for relationships between its anti-biofilm activity and both clinical outcome and methicillin-susceptibility status of the isolates.

Methods: *S. aureus* isolates (n = 87; 37 MSSA, 50 MRSA) from the clinically-evaluable population of patients enrolled in a Phase 2, multi-center, double-blind, randomized study of ORI treatment of complicated skin and skin-structure infections were used to form *in vitro* biofilms. All isolates were susceptible to vancomycin. Seventy-two hour biofilms were established in MBEC Physiology & Genetics plates (Innovotech, Edmonton, Canada) at 37ºC with rotation (150 rpm) in tryptic soy broth containing 1% glucose (with daily replacement of media). The minimal biofilm eradication concentration (MBEC), the concentration of drug needed to sterilize the biofilms after 24 hours of exposure, was determined for ORI and vancomycin after a 72-hour recovery period to detect surviving bacteria that tolerated exposure to the antibacterial agents. MBEC distributions were analysed using Fisher's exact test. Results presented are modal MBECs from 4 independent experiments.

Results: Robust biofilms were formed by all 87 clinical isolates of *S. aureus* in MBEC plates after 72 hours incubation as evidenced by recovery of adherent bacteria on the MBEC plate pegs (mean cell density of 6.7±0.9 log cfu/peg). All biofilms exhibited vancomycin MBECs >128 mg/l. In contrast, ORI retained activity against the biofilms (MBEC mode, 8 mg/l; MBEC90, 32 mg/l). The distribution of ORI MBECs for isolates from the cure and failure outcome groups were not statistically different (P = 0.34). However, ORI had significantly lower MBEC values for the MRSA biofilms compared to the MSSA biofilms (P = 0.03).

Conclusion: Against robust *in vitro* biofilms of *S. aureus* for which vancomycin was inactive, ORI demonstrated notable activity at pharmacologically-relevant concentrations of drug. No association between ORI MBEC and clinical outcome in the phase 2 study was evident. This study revealed that biofilms derived from clinical isolates of MRSA were sterilized by ORI at least as readily as biofilms of MSSA.

Oritavancin MBEC (mg/1)	Number of isolates			
	Cure (%)	Failure (%)	MSSA (%)	MRSA (%)
⩽4	4 (9.3)	9 (20.5)	1 (2.7)	12 (24.0)
8	16 (37.2)	13 (29.5)	14 (37.8)	15 (30.0)
16	9 (20.9)	13 (29.5)	10 (27.0)	12 (24.0)
32	10 (23.3)	5 (11.4)	6 (16.2)	9 (18.0)
>32	4 (9.3)	4 (9.1)	6 (16.2)	2 (4.0)
Total	43 (100)	44 (100)	37 (100)	50 (100)

P1903 Effect of weak organic acids on biofilms as observed by scanning electronmicroscopy

Z. Nack, J. Stenos, H. Dunstan, T. Roberts, S. Graves (Geelong, Newcastle, AU)*

Objective: Scanning electron microscopy (SEM) was used to monitor the effect of selected weak organic acids (WOAs) (ascorbic. citric, lactic and malic) and their combinations on biofilm structure. Biofilms were formed by methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus faecium*, multiresistant *Acinetobacter baumannii* and extended spectrum β-lactamase positive *Klebsiella pneumoniae*.

Methods: A 200 µl bacterial suspension (grown in nutrient broth overnight) was added to 10 ml RPMI-1640 and supplemented with 1% ethanol, 1% sodium chloride and 5% foetal calf serum. Using 24 well microtiter plates, SEM aluminium stubs were immersed into 2 ml of the solution above and incubated for 72 hours in a 30ºC incubator.

Following incubation the bacterial suspension was removed and the stubs exposed to 70% ethanol for 30 minutes (positive control) and to 10% WOAs (diluted in sterile distilled water) for 10, 15, 30 and 60 minutes. Following the exposure the stubs were vacuum dried for1 hour and gold coated for 120 seconds for SEM.
Results: Untreated biofilms, formed by any of the multiresistant bacteria showed normal bacterial structure with smooth cell morphology and a well developed extracellular polymeric substances (EPS). Biofilms treated with 70% ethanol (15, 30 and 60 minutes) showed normal EPS structure but bacterial morphologies were affected, including cell deformity, cell collapse and leakage of cytoplasmic material as observed by SEM. Bacterial biofilms treated with selected WOAs and their combination (longer than 15 minutes) showed dramatic changes in their biofilm structure. EPS was completely destroyed and in most cases completely eliminated. Remaining bacterial cells showed major deformity, due to the loss of their cytoplasm ('ghost cells').
Conclusion: Our finding in SEM showed a good correlation with our earlier studies on biofilms exposed to WOAs. Short-term WOAs treatment of biofilms (10 min or less) showed no significant effect, but treatments of 15 minutes or more displayed irreversible damaged bacterial cells and EPS structures. This study strongly indicates that WOAs are effective agents against not just planktonic bacteria but also against biofilm complexes.

P1904 Activity of daptomycin, vancomycin and moxifloxacin alone or in combination with clarithromycin or rifampicin in a novel *in vitro* model of *Staphylococcus aureus* biofilm

J. Parra-Ruiz, C. Vidaillac, W. Rose, M. Rybak (Detroit, Madison, US)*

Objectives: Biofilm is a common virulence factor that protects bacteria from host responses and antibacterial agents. The study aims were to assess the *in vitro* activity of several antimicrobials alone or in combination against 2 *S. aureus* isolates using a novel pharmacokinetic/pharmacodynamic (PK/PD) model of biofilm.
Methods: 1 methicillin-susceptible *S. aureus* (MSSA SH1000) and 1 methicillin-resistant *S. aureus* (MRSA NK315) were evaluated in a CDC biofilm reactor with polystyrene coupons (Biosurfaces Technology Corp., MT, USA). A 36 h biofilm conditioning phase was performed prior to antimicrobials administration. Regimens simulated free peak concentrations of daptomycin (DAP) 10 mg/Kg/24 h, vancomycin (VAN) 1g/12 h, and moxifloxacin (MOX) 400 mg/24 h alone or in combination with rifampicin (RIF) 600 mg/24 h or clarithromycin (CLA) 250 mg/12 h. Media and coupon samples were aseptically removed at 0, 4, 8, 24, 48 and 72 h to assess for the presence of viable planktonic (P) and embedded biofilm (EB) cells. Cidal activity and synergy were defined as $\geqslant$3 log10 decrease in the viable CFU/mL and $\geqslant$2 log10 reduction compared to the most efficient agent alone, respectively. Biofilm recovered on coupons was evaluated by scanning electron microscopy (SEM) at 0, 24 and 72 h.
Results: Starting planktonic and biofilm inocula were about 7.4 and 8.8 log10CFU/mL for SH1000, and about 7.3 and 6.8 log10CFU/mL for NK315, respectively. For SH1000, DAP and MOX were not cidal against P or EB cells. DAP+CLA and MOX+CLA were both cidal against P and EB cells at 24 h and 32 h, respectively. Only DAP+CLA was synergistic and reduced biofilm cells to the limit of detection at 72 h.
For NK315, DAP was bactericidal at 24 h against P cells but bacterial regrowth, uncorrelated to the emergence of resistance was observed after 48 h. In contrast, sustained cidal activity was observed with VAN+RIF from 32 h against P cells. Both regimens reduced biofilm cells only 1.8–2 $\log_{10}$ CFU/mL. The synergistic combination of DAP+RIF was cidal at 8 h against P and EB cells, and decreased the bacterial density to the limit of detection at 72 h. SEM studies confirmed changes observed in biofilm cells CFU/mL.
Conclusion: We developed a novel PK/PD model to assess the *in vitro* activity of antimicrobials against bacterial biofilm. DAP and MOX combinations were the most effective regimens and represent promising alternatives to treat persistent infections caused by staphylococci embedded in biofilm.

P1905 Investigation of daptomycin as a locking agent for line colonization with Gram-positive organisms

F.M. Awadel-Kariem, R.N. Manzoor, H.A. Jeraj, K. Farrington (Stevenage, UK)*

Background: Most line colonization, leading to bacteraemia and/or line removal, is caused by Gram-positive organisms. Line removal can have serious implication for patient management as patients may require the continuous presence on intra-vascular access (e.g. for total parental nutrition, haemodialysis and chemotherapy). Attempts to rescue precious lines especially in patients running out of sites to place central venous catheters have been an important part of line management. This is achieved by "locking" an antibiotic (to which the organism is sensitive) in the colonized lumen(s) of the central lines while the lumen is not in use. Antibiotics used for this purpose include aminoglycosides and glycopeptides. Daptomycin is a new and powerful anti-Gram-positive antibiotic that can be effective against organisms that are resistant to the other agents.
Objective: To assess the stability of daptomycin as a locking agent with heparin and anti-Gram-negative agents, and to evaluate its ability to sterilize simulated line infection with biofilm forming reference strains.
Methods: Daptomycin was prepared as recommended by the manufacturer and mixed with different anti-Gram-negative specific drugs (gentamicin and amikacin) and heparin. Various concentrations were assessed for stability. Once the ideal concentrations for all agents were identified they were employed on an *in vitro* line infection model against biofilm-forming reference strain of MRSA, MSSA, MRSE and VRE. Similar preparations, with Vancomycin, teicoplanin or linezolid substituted for daptomycin, were used as controls. All experiments were carried out in triplicates.
Results: The optimal concentration for daptomycin as a locking agent was identified at 5 mg/ml. The optimal concentration for heparin was 5000 U/ml, and for both gentamicin and amikacin was 5 mg/ml. At these concentrations the three agents could be combined in a stable locking solution. When this solution was tested in the *in vitro* line infection model sterility was achieved with all Gram-positive strains tested. However, sterility was not achieved with the other agents against all the Gram-positive reference strains.
Conclusions: This study confirms that daptomycin can be combined with different anti-Gram-negative agent and heparin in a stable manner that makes it ideal for line locking. Our line infection model has demonstrated that daptomycin is at least as effective as current anti-Gram-positive agents as a locking agent.

P1906 Effectiveness of antimicrobial agents in the prevention of biofilm forming *Staphylococcus epidermidis* growth on the surface of PMMA cement

S.K. Sandiford, J. Tagg, P. Kay, M. Upton (Manchester, UK; Dunedin, NZ; Wigan, UK)*

Objective: *Staphylococcus epidermidis* is one of the major causative agents of biofilm infections associated with indwelling medical devices. The ever increasing resistance of biofilm forming isolates to conventional antimicrobial therapy enforces the requirement for new therapies to prevent initial adhesion of bacteria to biomaterial surfaces.
The aim of the current study was to assess the ability of antimicrobial peptide gallidermin (2164 Da) and a novel inhibitor (NI01) to prevent growth on polymethylmethacrylate (PMMA) bone cement.
Methods: Lantibiotic gallidermin (MIC 62.5μg/ml, MBC 125μg/ml) with an inhibitory activity of >5120 AU/ml and NI01 (MIC 2.18 mg/ml, MBC 4.37 mg/ml) with an inhibitory activity of 2560 AU/ml were incorporated into PMMA bone cement. Columns of bone cement with a diameter of 4 mm and height of 7 mm were attached to the lid of a 96 well microtitre plate. The cement columns were then incubated with 106 cfu/ml clinical biofilm forming *Staphylococcus epidermidis* strain 156 for 1 hour. The columns were then rinsed in phosphate buffered saline (PBS) and immersed in PBS containing 0.25% glucose, (NH4)2

SO4 and 1% TSB. Growth of adhered bacteria was then monitored in real time using a kinetic plate reader over 48 hours, producing a time proliferation curve for each well.
Results: Gallidermin (125μg/ml) and NI01 (4.37 mg/ml) incorporated into columns of PMMA cement resulted in a significant decrease in the growth of clinical *Staphylococcus epidermidis* isolate 156 ($p = 0.001$).
Conclusion: Antimicrobial agents, gallidermin and NI01 were able to inhibit growth of clinical biofilm forming isolate 156. The ability of these inhibitors to prevent cement colonization indicates their potential use to prevent bacterial growth and therefore subsequent biofilm formation.

P1907 Foodborne enterococci in oral biofilm: an *in vivo* human study

A. Al-Ahmad, B. Spitzmüller, J. Maier, A. Wittmer, M. Follo, E. Hellwig, D. Jonas (Freiburg, DE)*

Objectives: Persistence of microorganisms or reinfection are the main reasons for failure of root canal therapy. Enterococci, particularly *Enterococcus faecalis*, have frequently been isolated from root-filled teeth associated with periradicular lesions. Up to now enterococci are considered as transient and not belonging to the normal oral flora. The aim of this study was to examine the ability of food born enterococci to integrate in mature dental oral biofilm.
Methods: Six healthy volunteers with an age range of 25–26 wore dental splints loaded with equal sized pieces of BSE-free bovine enamel. After 3 days, the volunteers consumed cheese which included *E. faecalis*, *Enterococcus faecium*, *Enterococcus avium* and *Enterococcus durans*. The supragingival biofilm was analysed by culture technique. *E. faecalis* was also analysed using fluorescent in situ hybridisation (FISH). Isolated members of the different enterococci found in cheese and biofilm were also confirmed by 16S rRNA gene sequencing. All strains were characterised genotypically by macrorestriction analysis using the restriction endonuclease SmaI and pulsed-field gel electrophoresis. Molecular masses of all bacterial DNA fingerprints were determined using the control strain *Staphylococcus aureus* NCTC 8325. Similarities were presented as dendrograms.
Results: *E. faecalis*, *E. faecium*, *E. avium* and *E. durans* were isolated from the initial biofilm after 2 h, as well as from the mature dental plaque biofilm which developed after 5 days. Similarities between *E. faecalis*, *E. faecium* and *E. avium* isolated from cheese, as well as strains of these species isolated from the initial biofilm and from the 5 day-old biofilm, were revealed by macrorestriction analysis. *E. faecium* and *E. avium* integrated into an already existing 3 day-old biofilm. *E. durans* could be isolated from both the initial and 5 day-old oral biofilm, yet no similarities to the *E. durans* strains isolated from cheese were detected. *E. faecalis* was also detected in dental plaque biofilm using fluorescent in situ hybridisation (FISH). Individual differences among the tested volunteers were shown.
Conclusions: *E. faecalis*, *E. avium* and *E. faecium* could be resident in the dental oral biofilm and not only transient. Fermented food is one source of these enterococci in the oral cavity. The genotypic characterisation of enterococci strains is essential in order to study their origins within the oral cavity.

P1908 Micro-morphological features for the characterization of *in vitro* microbial biofilm development on central venous catheters

M. Lorenzato, F. Tessarolo, I. Caola, F. Piccoli, G. Nollo, P. Caciagli (Trento, IT)*

Objectives: To define new microscopic features for characterizing the *in vitro* development of bacterial biofilm on the surface of central venous catheters (CVCs) by scanning electron microscopy (SEM).
Methods: Three wild strains of *S. epidermidis* (strong, intermediate, and weak biofilm producer according to the Microtiter-plate test) were selected from cultures of infected CVCs. Nine catheter segments were obtained from four types of commercial CVCs: uncoated (STD), benzalkonium chloride coated (BC), silver impregnated (S), and chlorhexidine-silver sulfadiazine coated (CSS) devices. Segments were longitudinally sectioned and glued to cover glasses to obtain thirty-six testing samples (TS) exposing both lumenal and external catheter surfaces as well as the glass area. Each TS was introduced in a screw-cap tube with 3 ml of bacterial suspension (0.5 McF in tryptone soy broth + 1% glucose) and incubated in a oscillating waterbath at 37ºC. After 1, 3 or 7 days, TS were extracted, washed in HEPES, fixed in 2.5% glutaraldehyde in HEPES, dehydrated, dried, and gold sputtered for SEM. For each TS, 16 representative fields were collected at magnifications from 50 to 4000 times. Collected images were reviewed to score TS according to the presence (+) or not (–) of the following micro-morphological features: (a) biofilm demarcation line at the air–liquid interface, (b) bacterial cells with viable morphology, (c) extracellular matrix, (d) three-dimensional architectures (Figure 1). TS scores were grouped according to catheter type, bacterial strain, incubation time and surface of interest. Percentages of "+" scores were finally computed.
Results: Percentages of "+" scores according to catheter type were 62%, 52%, 21%, 11% for STD, BC, S, and CSS respectively. Percentages according to bacterial strain were 38%, 35%, 37% for strong, intermediate, and weak biofilm producer. Percentages according to incubation times were 26%, 40%, and 44% for 1, 3, and 7 days. Percentages according to surface of interest were 21%, 45%, 44% for lumenal, external and glass surface.
Conclusion: Four new morphological features for characterizing the *in vitro* growth of microbial biofilm on CVCs were defined. These features were able to evidence different bacterial colonization and biofilm accretions on antimicrobial CVCs and uncoated catheters.
The micromorphological features (b), (c) and (d) here presented could also have potentialities in assessing microbial biofilm on CVCs recovered from patients.

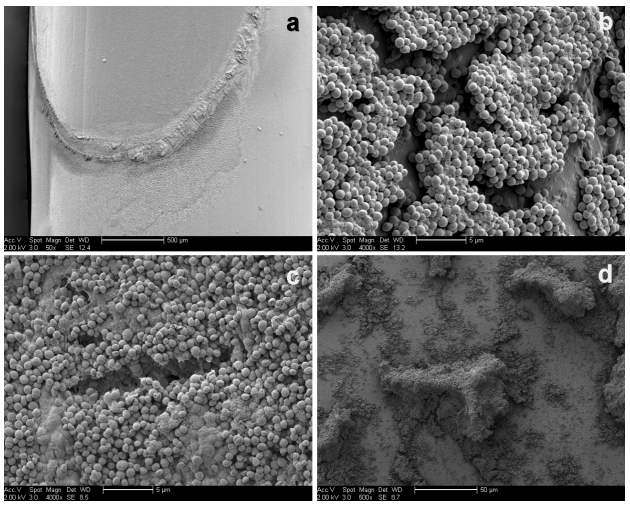


Figure 1. Micro-morphological features for the characterization of microbial film. Representative images for the presence of: (a) biofilm demarcation line at the air–liquid interface; (b) bacterial cells with viable morphology; (c) extracellular matrix; (d) three-dimensional architectures.

P1909 treC contributes to biofilm formation and *in vivo* colonization of *Klebsiella pneumoniae* strains causing pyogenic liver abscess

M.C. Wu, T.L. Lin, P.F. Hsieh, H.C. Yang, J.T. Wang (Taipei City, TW)*

Objectives: Community-acquired pyogenic liver abscess (PLA) caused by *Klebsiella pneumoniae* is an emerging infectious disease. The formation of biofilms is important for bacterial pathogenicity in many tissue infections. Therefore, we sought to identify genes related to biofilm

formation in *K. pneumoniae* and to determine their association with colonization in animals.
Methods: A microtiter plate assay was used to determine the levels of biofilm formed by *K. pneumoniae* clinical isolates and to screen biofilm-altered mutants from a transposon mutant library of a *K. pneumoniae* PLA-associated strain. Deletion and complementation of the candidate gene were performed to investigate its role in biofilm formation by *K. pneumoniae*. Slide culture was used to test the biofilm-forming ability of the isogenic mutants on glass surface. DNA microarray and intragastrical infection in mice were used to compare gene expression and the ability of colonization between the parental strain and the biofilm-deficient mutant.
Results: In a polystyrene microtiter plate assay, higher levels of biofilm formation were demonstrated by *K. pneumoniae* strains associated with PLA. Screening and genetic analysis of a transposon-insertion library revealed a trehalose-6-phosphate hydrolase (treC) mutant that produced decreased levels of biofilm. Biofilm formation was reduced in reknock-out treC mutant and was restored to normal levels by chromosomal complementation. Glass-slide culture confirmed the role of treC in biofilm formation. Using a microarray analysis, differences in a cluster of genes associated with glycerol transportation and metabolism between the parental strains and treC mutant were found. *In vitro* and *in vivo* competition revealed that the treC mutant was attenuated in competiveness with regard to biofilm formation and colonization during infection.
Conclusions: treC contributes to the formation of biofilm and *in vivo* colonization by *K. pneumoniae* PLA strains in intragastrically infected mice.

P1910 Conjugation-mediated protein translocation as a novel strategy to combat biofilm-associated infections

K. Schilcher, A. Reisner, S. Lang, E.L. Zechner (Graz, AT)*

Objectives: Biofilm-associated infections pose a significant challenge in clinical settings because most antibiotics fail to kill cells protected in a biofilm. We aim to evaluate a novel strategy to combat biofilm-associated infections that is based on horizontal gene transfer (HGT)-mediated delivery of toxic proteins to cells located in a biofilm.
Methods: Translocation efficiencies of various Cre-TraI fusion proteins from a conjugation-proficient *E. coli* donor into suspended *E. coli* recipient cells were quantified based on Cre recombinase activity that is associated with acquisition of a permanent chloramphenicol resistance phenotype in the recipient cells. Translocation of the Cre-TraI fusion carrying the best translocation signal was further evaluated in an *in vitro* model for a catheterized bladder (CAUTI model). Bactericidal effects upon addition of a conjugation-proficient *E. coli* strain expressing the Cre-TraI-CcdB fusion protein to an established *E. coli* biofilm were monitored in different model systems using selective plating, electron microscopy and a fluorescein diacetate hydrolysis assay.
Results: Among the tested Cre-TraI fusions, the fusion carrying the TraI region from aa993 to aa1756 was the shortest fusion that still gave translocation levels comparable to the full length protein. In the CAUTI model, Cre-TraI (993–1756) fusion protein was efficiently translocated leading to detectable Cre activities in 17% (CAUTI Model) of the transconjugant population. The ccdB gene encoding a potent inhibitor of bacterial replication was inserted into the vector carrying this Cre-TraI fusion to create a Cre-TraI-CcdB fusion protein. The functionality of the Cre-TraI-CcdB fusion protein was confirmed by lethal effects following expression in *E. coli* hosts. Co-expression of antidote complemented the phenotype. The fusion protein was used to detect bactericidal effects upon translocation to sessile *E. coli* populations in different model systems including an *in vitro* model for a catheterized bladder.
Conclusion: HGT-mediated delivery of toxic proteins may provide a useful strategy to combat biofilm-associated infections. Our initial results prove that protein translocation to recipient cells is detectable at significant levels under conditions mirroring the situation of a catheter-associated infection. Ongoing efforts aim to reveal the efficiency of toxin translocation to combat biofilm-associated infections.

P1911 Biofilm formation by *Acinetobacter* spp. in the air–liquid and solid–liquid interphases

S. Marti, J. Rodriguez-Baño, P. Espinal, M. Catel-Ferreira, J. Vila, H. Seifert, T. Jouenne, E. Dé (Mont-Saint-Aignan, FR; Seville, Barcelona, ES; Cologne, DE)*

Objectives: Biofilm formation by *Acinetobacter baumannii* could be responsible for the high level resistance to desiccation and disinfection. This biofilm can be formed at the air–liquid (ALI) and solid–liquid (SLI) interphases. The aim of this study was to determine the prevalence of these types of biofilm in different members of the genus *Acinetobacter*.
Methods: The study was performed on the clinically relevant members of the *A. baumannii-A. calcoaceticus*(ABC) complex (*A. baumannii*, *Acinetobacter* G3 and G13TU), together with other species less important in the clinical environment *(A. johnsonii, A. lwoffii* and *A. radioresistens)*. The cultures were started at OD600=0.01 in MH broth in polypropylene tubes for the ALI biofilm and in 96-well-plates for the SLI biofilm. This study was performed by duplicate at 25ºC and 37ºC. The results for the ALI biofilm were determined by visual identification while the SLI biofilm was stained with 0.5% crystal violet and solubilised with 95% ethanol to be quantified at 550 nm.
Results: The results for the ALI biofilm would represent the total biofilm formation because the crystal violet stains both structures. Biofilm formation was generally more important at 25ºC than at 37ºC which suggests that this mechanism could be used to persist in the hospital environment. The biofilm formation by the members of the ABC-complex was at least 4 times higher than the other species (80–91% versus 5–24%). In addition, only the isolates belonging to this complex were able to form ALI biofilm; all the other genomic species analysed showed a lack in the formation of this structure. Finally, within the ABC-complex, there was no difference in the amount of SLI biofilm produced; however, the formation of ALI biofilm was almost 4 times higher for *A. baumannii* and *Acinetobacter* G13TU than for *Acinetobacter* G3 (34%, 27% & 9% respectively).
Conclusion: Our results suggest that the members of the ABC-complex had a higher ability to form biofilm than the other genomic species. This feature could be related to the higher presence of these microorganisms (mainly *A. baumannii*) in the hospital environment and therefore increase the risk of infection. In addition, they were also able to form ALI biofilm, an ability that has not been observed yet in the other genomic species.

P1912 Combined effect of copper and silver versus multidrug-resistant clones of *Acinetobacter baumannii*

N. Gordon, J. Turton, D. Wareham (London, UK)*

Objectives: *Acinetobacter baumannii* is a non fermentative Gram-negative cocco-bacillus which has become an increasing problem as a multi-drug resistant nosocomial pathogen, particularly in intensive therapy units (ITU). Its ability to form biofilms enables it to evade routine cleaning and allows colonisation of abiotic surfaces, resulting in numerous outbreaks. The dearth of effective antimicrobials means there is an urgent need for the development of novel management strategies. The antimicrobial effects of copper and silver have been known for centuries and in recent years there has been increasing interest in their use as topical agents, antimicrobial coatings and cleaning agents. In this study we evaluated the synergistic effect of these elements on *A. baumannii* and their effect on biofilm formation, to investigate their potential use against this formidable pathogen.
Methods: Seven MDR *A. baumannii* isolates belonging to the UK South East, OXA-23–1, OXA-23–2, and Burn clones were identified by examination of pulsed field gel electrophoresis profiles. *A. baumannii* ATCC 19606 was used as an antibiotic sensitive type strain. MICs to copper and silver nitrate were determined by broth microtitre dilution with synergy assessed in checkerboard assays interpreted using FICI criteria. The effect of copper and silver on MDRAB biofilm formation at 1/4 MIC was investigated by crystal violet staining of 24 hr cultures grown in polystyrene microtitre plates.

Results: Versus all strains the MIC of Cu(NO)3 was 1024 mg/L and the MIC of Ag(NO)3 was 16 mg/L. MICs were reduced when Cu(NO)3 and Ag(NO)3 were used in combination with marked synergy (FICI $\leqslant$ 0.5) observed versus 3 isolates. In the majority of strains, biofilm formation was unaffected or slightly increased following exposure to Ag(NO)3 or Cu(NO)3 alone. However, when both agents were used in combination a reduction was seen for all strains (range 7–64%), which was statistically significant in four.
Conclusion: The combination of Cu(NO)3 and Ag(NO)3 showed marked synergy against MDR *A. baumannii*, and was associated with reduction in biofilm formation by some strains. The potential use of this combination in cleaning materials, topical formulations and medical device coatings warrants further evaluation.

P1913 Biological cost of antibiotic pressure in *Pseudomonas aeruginosa* biofilm phenotype

I. Machado, H. Lopes, S. Lopes, D. Alves, M. Pereira (Braga, PT)*

Objectives: *P. aeruginosa* (PA) has become recognized as an opportunistic pathogen of clinical relevance mainly when encased in biofilms. Bacteria facing adverse conditions (e.g. in the course of antibiotic chemotherapy) can survive, multiply and form biofilms as a defensive tactic. This work aimed to evaluate the role of antibiotic pressure in biofilm phenotype and in the selection of persisters.
Methods: *P. aeruginosa* from collections (PAO, ATCC, CGCT) and isolated from an endoscope (PAI) were used to form biofilms in the presence of several doses of rifampicin (RIF) and ciprofloxacin (CIP). Biofilm phenotype was evaluated by biomass (CV), respiratory activity (XTT) and number of biofilm-grown cells. MIC and MBC of RIF and CIP against all bacterial strains were assessed.
Results: For all strains, RIF MIC was 32 μg/ml and MBC 64 μg/ml. MIC and MBC of CIP were strain dependent, varying from, respectively, 0.1–0.4 μg/ml and 0.8–3 μg/ml. However, *P. aeruginosa* strains did not loose the ability to form biofilms even when facing extreme doses of CIP and RIF. Biofilm mass was only altered at MIC value, being noticed a big reduction but not total inability of all strains to form biofilms. PAI and PAO were the strains more able to form biofilms even at excessive doses of RIF (64 μg/ml) and CIP (6 μg/ml). Concerning activity, biofilms developed by bacteria that survive in the presence of both antibiotics still able to colonize surfaces, giving rise to metabolically active biofilms also at MBC and 2×MBC. PAI, ATCC and CGCT were the strains that give rise to more active biofilms in CIP and PAI, ATCC and CGCT in RIF. Despite having a good reduction in activity and a reasonable reduction in biofilm mass, results are still worrisome as the number of viable biofilm-cells was very high. There was only a 2-log reduction at MIC and a 3-log reduction at MBC for both antibiotics, being the final cell number around 1E4 CFU/cm^2.
Conclusions: The presence of antibiotics during biofilm formation seems to enforce a selective pressure on bacteria members, eliminating planktonic cells, slowing down biofilm formation and interfering with biofilm phenotype. But cells that survived to antibiotic attack by surface adhesion were the ones among the communities that were more resistant to antimicrobials. These cells may give rise to phenotypically altered biofilms with increasing virulence factors and be the cause of persistent biofilm infections.

P1914 Virulence factors of *Pseudomonas aeruginosa* strains isolated from catheterized patients

K. Olejnickova, V. Hola, F. Ruzicka (Brno, CZ)*

Objectives: Infections caused by *Pseudomonas aeruginosa* are serious problem for patients with cystic fibrosis, catheterized and immunocompromised persons. *P. aeruginosa* is opportunistic pathogen and the imbalance between host's immune system and virulence factors of the bacterium plays important role in the origin of infection. We focused on several virulence factors in *P. aeruginosa*-– the motility (swimming and twitching), the biofilm formation, production of air–liquid (A–L) interface biofilm and resistance to selected antibiotics. Swimming motility allows the bacterium to move in water and low-density environment. Moving on the solid surface, enabled by type IV pili, is called twitching motility. Cells capable of forming biofilm are more resistant to antimicrobial agents and host's immune system. They are often observed in persistent infections.
Methods: We tested 119 strains of *P. aeruginosa* from patients of St. Anne's University Hospital with urinary or bloodstream catheterization. The biofilm formation was tested according to Stepanović et al. (2007). The A–L interface biofilm was tested by growing the biofilm in polystyrene tubes and dividing submerged and A–L interface biofilm. The biofilm formation was quantified by A590 nm. The swimming motility was tested according to Rashid and Kornberg (2000); briefly, we inoculated bacteria with a toothpick and incubated plates for 24 h at 37ºC. Twitching motility was tested by two different methods, which were compared as for reproducibility of results. We used method of Rashid and Kornberg (2000); we inoculated bacterial suspension and incubated plates for 24 h at 37ºC; and method of Zolfaghar et al. (2003); the bacteria were inoculated with a toothpick and incubated for 16 h at 37ºC and subsequently 72 h at room temperature. All methods were assessed by measuring the diameter of the turbid zone.
Results and Conclusion: Both types of motility, antibiotic resistance and production of submerged and A-L interface biofilm respectively differed in particular strains. There is no significant correlation between motility and production of biofilm but we proved correlation between both types of motility ($p < 0.05$; correlation coefficient 0.37 and 0.434 resp.). The resistance profiles showed different patterns in several groups of tested strains. Relationships between virulence factors of *P. aeruginosa* are complex and they will be also subject of following studies.
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P1915 The biocidal activity of silver nanoparticles toward *Pseudomonas aeruginosa* plankton and biofilm

A. Kalicinska, J. Stefanska, K. Szczepanowicz, S. Tyski (Warsaw, Cracow, PL)*

Objectives: The knowledge about silver possessing some antimicrobial activity has been commonly available for many years. Silver or silver coated surfaces are unfavourable environment for microbial colonization and growth. Recently, the rapid development of nanotechnology is observed. It seems interesting to investigate the antimicrobial activity of silver nanoparticles toward cells of clinical *Pseudomonas aeruginosa* strains.
Methods: The silver nanoparticles were synthesized chemically. The average size of nanoparticles as measured by dynamic light scattering (DLS) and scanning electron microscopy (SEM) techniques was below 20 nm. The range of nanoparticles concentrations used in this study was 160–2.5 ug/ml.
Nineteen *P. aeruginosa* isolates (10 from sputum of cystic fibrosis patients and 9 from urine) were under investigation. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of nanoparticles were estimated using standardised plankton cells suspensions of strains. Parallel, 26 h biofilms of analysed strains growing on polystyrene microplate surface were treated with silver nanoparticles concentrations for a 6–48 h contact time. Staining with 3-(4,5-dimethyl-2-thiazolyl)-2,5 diphenyl-2H-tetrazolium bromide (MTT) and measuring the absorbance at A = 554 nm, were applied to estimate the level of viable bacterial cells. The 50% of absorbance reduction after silver nanoparticles treatment was calculated as significant level of antibacterial activity.
Results: Analyzing all strains and different silver nanoparticles concentrations, the following values were calculated: MIC50=20 ug/ml, MIC90=80 ug/ml, MBC50=80 ug/ml, MBC90=160 ug/ml. The MBC/MIC ratio in case of 4 strains was 8, in case of 9 strains was 4 and in case of 2 strains the ratio value was 1. The MIC and MBC values for sputum strains were generally lower then such values for urine isolates. The 6 h contact time of biofilm with silver nanoparticles was ineffective. However, after 24 h, 30 h and 48 h contact times in case of about half

of the strains regardless of isolation site, the biofilm populations were reduced at least in 50%. Silver nanoparticles concentration 160 ug/ml proved to be less effective against biofilm structure, than concentrations range 20–80 ug/ml.
Conclusion: Analysed silver nanoparticles showed antibacterial activity against tested *P. aeruginosa* clinical strains cells, both in plankton form and in biofilm structure.

P1916 Proteomic analysis of *Helicobacter pylori* ATCC 43504 biofilms

P. Pattiyathanee, R. Vilaichone, N. Chaichanawongsaroj (Bangkok, TH)*

The biofilms formation by *Helicobacter pylori* plays an important role not only for environmental survival but also for colonization *in vivo*. However the underlying factors involving *H. pylori* biofilms formation have not been characterized. In the present study, a proteomic analysis was performed in order to compare the difference in protein expression profiles between biofilms-grown cells and planktonic counterparts of *H. pylori*. Three proteins involving in chemotaxis and motility (FlgE, FliD, FlaA) exhibited higher level of expression in biofilms stage. Additional proteins were found only in the biofilms but not in planktonic cells included oxidative stress response (KatA, TrxB, TsaA, electron transport proteins (PorB, HPSH_01160, FldA), elongation factors (EF-TU, EF-P, Tig), heat shock proteins (Hsp60 and GrpE), proteins involved in nitrogen (UreA and UreB) and carbohydrate (AcnB and GltA) metabolism. Proteomic technologies could enhance the understanding of precise mechanisms related to biofilms formation in *H. pylori* which facilitate treatment and prevention of *H. pylori* infection.

P1917 Simvastatin inhibits the biofilm formation of *Candida albicans*

M. Kadowaki, Y. Eriguchi, Y. Uchida, Y. Nagasaki, Y. Harada, K. Sumida, N. Shimono (Fukuoka, JP)*

Objectives: *Candida albicans* forms biofilms on indwelling devices and it is difficult to treat device related infections caused by *C. albicans*. Because of difficulty to eradicate biofilms from devices, it is desirable to prevent making biofilms. Statins inhibit HMG-CoA reductase and are mainly used as the cholesterol lowering agents, however, they showed some additional effects. As to infectious diseases, they are reported to decrease the risk of pneumonia, to improve the survival of patients with sepsis, and so on. In this study, we investigated the potential efficacy of simvastatin (SVS) on the prevention of biofilms formed by *C. albicans*.
Methods: SVS was hydrolyzed in ethanolic sodium hydroxide at 60ºC for 2 h and kept as active open acid form. Three clinical strains of *C. albicans* isolated from blood culture were used throughout this study. Susceptibility testing, the minimal inhibitory concentration (MIC) of SVS were determined based on NCCLS M27A.
Biofilms of *C. albicans* were grown on the discs in the 96 well microplates at 37ºC under several concentrations of sub MIC of SVS for different incubation times. Morphological features of biofilms were observed under the light microscope and the confocal scanning laser microscope (CSLM) used with ConA. Dry weight of biofilms was also measured. The total RNA was extracted from biofilms using RNeasy mini kit. First strand cDNA was made from total RNA using high capacity cDNA reverse transcription kit. Reverse transcription-polymerase chain reaction (RT-PCR) of genes related to biofilm formation was investigated.
Results: The MIC of *C. albicans* strains used in this study against SVS was 16 microg/ml. The formation of biofilms formed by *C. albicans* (CA biofilms) was inhibited microscopically, even if it is incubated under the sub MIC of SVS. The maximum thickness of CA biofilms without SVS was approximately 200 microm. The thickness of CA biofilms under 1/4 MIC was reduced up to 30%. The dry weight of CA biofilms was also decreased dose dependently and showed 50% reduction compared to control. The expression of biofilm related genes, including adherence, hyphal formation, was investigated by RT-PCR. The genes related to hyphal formation were poorly expressed in CA biofilms under SVS exposure.
Conclusion: Our data suggested that simvastatin inhibited *C. albicans* biofilm formation *in vitro*. The administration of SVS will be expected to prevent forming *C. albicans* biofilms on indwelling devices.

P1918 Drug susceptibility and virulence factors in *Candida glabrata* mutants deficient in proteins involved in histone modification

S. Borecka, T. Schwarzmüller, S. Novohradska, K. Kuchler, J. Subik (Bratislava, SK; Vienna, AT)*

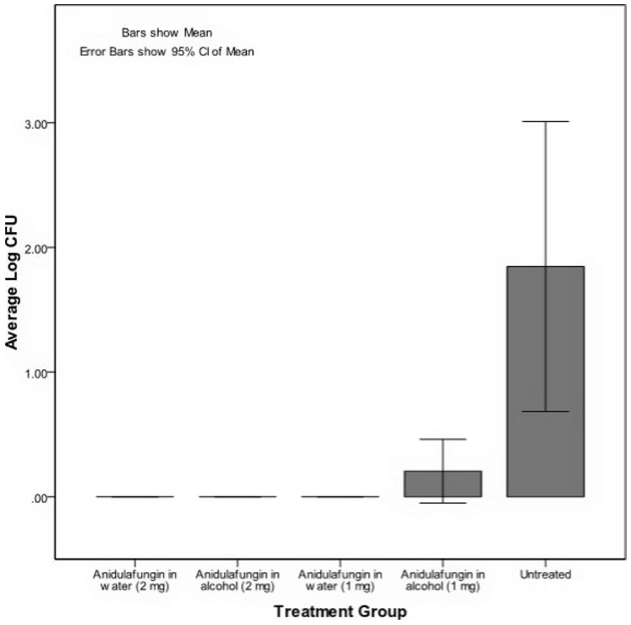
Objectives: The aim of this study was to determine the sensitivity to chemical, osmotic and oxidative stress of *C. glabrata* mutant strains deficient in histone deacetylases and histone methylases, and to assess their factors of virulence.
Methods: Drug susceptibilities were determined by broth microdilution method in 96-well plates according to the CLSI (formerly NCCLS) M27-A3 standard guidelines and by zone inhibition assay. To test for heat shock sensitivity, the strains were incubated at 55ºC and in minute intervals plated out on glucose-rich medium. Cell surface hydrophobicity was measured by the water-octane two-phase assay. Biofilm formation was quantified by staining with crystal violet dye and biochemically using the XTT reduction assay. The quantitative real time PCR was carried out with the Maxima SYBR Green qPCR Master Mix (Fermentas, Vilnius, Lithuania) using the LightCycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany).
Results: The lack of histone modifiers studied in this work only moderately affected the drug susceptibility and stress responses in *C. glabrata* mutant strains. However, the heat shock sensitivity of the strains with disrupted RPD3 and PHO23 genes was increased. Moreover, virulence factors assessed at both 30ºC and 37ºC were significantly altered in some studied strains. Extremely high biofilm formation was observed at 30ºC in strains deleted in the SIR2 and SIR3 genes. On the other hand, lower biofilm formation ability was coupled with deletion of RPD3 and PHO23 genes. The altered biofilm formation in mutant strains was accompanied by changes in expression of the EPA genes encoding major adhesins of *C. glabrata*.
Conclusion: We identified histone deacetylases (encoded by the RPD3, PHO23, SIR2 and SIR3 genes) regulating the biofilm formation in *C. glabrata*. Histone deacetylases Rpd3p and Pho23p may be the targets for novel drugs reducing biofilm formation and enhancing the activity of other antimycotics in combination therapy.

P1919 *In vivo* efficacy of anidulafungin, with and without alcohol, lock therapy against *Candida albicans* catheter-related infection

L. Long, A. Cirino, A. Miller, M. Ghannoum (Cleveland, US)*

Objectives: *C. albicans* (CA) is the most common fungal pathogen associated with colonization and biofilm formation on the surfaces of indwelling medical devices, such as IV catheters. The discovery of antifungals that are able to sterilize prosthetic devices infected with CA biofilms should reduce the morbidity and mortality associated with nosocomial candidiasis. Antifungal lock therapy (AFLT) provides a means for delivering high concentrations of drug to the resistant biofilms formed on catheter surfaces. In this study, we compared the anti-biofilm efficacies of anidulafungin (AND), with (w) and without (w/o) alcohol, in the treatment of CA in a rabbit catheter-associated biofilm model.
Methods: Silicone catheters were surgically placed in New Zealand White rabbits. In 4 experiments, implanted catheters were infected with CA biofilm. Daily flushes with heparinized saline (hep/sal) were performed for 3 days after which blood cultures were drawn to confirm the presence of CA and treatment began. Next, animals were randomized into groups; AND 2 mg w alcohol (n = 6), AND 2 mg w/o alcohol (n = 2), AND 1 mg w alcohol (n = 6), AND 1 mg w/o alcohol (n = 2), and untreated control (UC) (n = 8). Treatment was locked in the catheter

lumen for 8 h/day for 7 days. Animals were sacrificed, and catheters removed for quantitative culture and scanning electron microscopy.
Results: Quantitative culture of catheters treated with AND 2 mg w and w/o alcohol, and 1 mg w/o alcohol yielded zero colony forming units. AND 1 mg w alcohol and the UC yielded 0.21±0.32 and 1.85±1.68, respectively. AND 2 mg w alcohol demonstrated significant efficacy compared to the UC (P = 0.036). There was a trend towards significance with all other AND treated groups. Additionally, there was no significant difference between AND treated groups w and w/o alcohol.
Conclusions: AND 2 mg w alcohol showed significant efficacy when compared to the UC. There was no significant difference between AND w alcohol, and w/o alcohol-treated groups. AFLT with AND 2 mg w and w/o alcohol were able to sterilize intravenous catheters infected with CA biofilms. These findings suggest that future studies examining the ability of AND lock therapy to salvage catheters infected with CA may be warranted.



P1920 Comparison of biofilm production by *Candida albicans* isolates from blood and oral cavity

E. Eraso, I. Miranda-Zapico, G. Quindos, C. Marcos-Arias (Leioa, ES)*

Candida albicans is a major cause of superficial and invasive mycoses. Biofilm production is considered a major virulence factor in candidiasis associated to catheters, prosthesis and dentures.
Objective: To compare biofilm production by *C. albicans* isolates from blood, oral mucosa and dentures.
Methods: One hundred and fifty one oral (51 from denture and 50 from oral mucosa) and 46 blood *C. albicans* isolates were tested for biofilm production. Biofilms were developed in a 100 well polystyrene microtitre plate and incubating during 24 and 48 h at 37ºC. Biofilm production was calculated by a tetrazolium salt (2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide, XTT) metabolic test. *C. albicans* NCPF 3153 and the hypha-deficient mutant *C. albicans* Ca2 were used as reference strains.
Results: All the *C. albicans* isolates studied formed biofilms but showed important differences depending on their origin: 48% of oral mucosa isolates and 35.3% of denture isolates were highly biofilm producers at 24 h. 35.3% of oral mucosa isolates and 52% of denture isolates were intermediate biofilm producers. Conversely, only 2.2% of bloodstream isolates were highly producer and 47.8% of the isolates were found to be intermediate producers. Oral *C. albicans* isolates, both from the denture and the oral mucosa, were found to be more producers of biofilm than bloodstream *C. albicans* isolates, being this difference statistically significant ($p < 0.05$). However, there was not significant difference ($p = 0.18$) when biofilm production was compared between denture oral mucosa isolates, being more producers those isolates from denture and from the oral mucosa.
Conclusion: Biofilm production in *C. albicans* is strongly related to the clinical origin of the isolates: Oral isolates are richer biofilm producers than blood isolates.
Funding: Projects GIC07 123-IT-222–07 (Departamento de Educacion, Universidades e Investigacion, Gobierno Vasco), S-PE08UN35 (Saiotek 2008, Departamento de Industria, Comercio y Turismo, Gobierno Vasco) and PI061895/2006 (Fondo de Investigacion Sanitaria del Ministerio de Sanidad y Consumo de España).

P1921 Morphogene BolA: its role in biofilm formation and respiration of *E. coli* K-12 MG1655

M. Adnan, M. Pereira, I. Machado, G. Morton, S. Hadi (Preston, UK; Braga, PT)*

Objectives: One important and clinically relevant example of *E. coli* adaptation through systematized gene expression is their ability to grow in biofilms in different environmental conditions. Stress response genes are induced whenever a cell needs to survive under adverse growth conditions. One of the possible gene having an influence on this process is morphogene BolA. Therefore, our work aims to understand the stress tolerance response of *E. coli* in relation with BolA gene under different environmental conditions.
Methods: *RNA Extraction*: Bacterial cells were harvested and resuspended into RNAprotect® Bacteria Reagent (Qiagen, UK) and RNA was extracted using Qiagen RNeasy® kit. RNA quality was assessed by agarose gel electrophoresis.
Real time RT-PCR: Real time RT-PCR was used to examine the expression level of BolA gene in biofilms and planktonic cells. Total RNA was extracted, converted to cDNA that was processed using real time PCR (ABI PRISM 7500, Applied Biosystems). Reactions were performed in a 25 μl reaction volume. BolA specific primers were developed in house for this purpose.
Metabolic activity-respiratory activity: The respiratory activity of the several biological (biofilm suspensions or suspended cultures) samples was evaluated by measuring oxygen uptake rates in a biological oxygen monitor (BOM) in short-term assays. The assays were performed in a Yellow Springs Instrument BOM (Model 53).
Results: The expression level of BolA gene was found to be higher in wild type strain in biofilm mode when compared with planktonic phase. This was quantified during both planktonic growth and in biofilms by real time RT-PCR during different stress conditions. This is the first study involving respirometric analysis which showed that BolA gene might have a major role in respiration of *E. coli* in both biofilm and planktonic phases.
Conclusion: It has been shown that the growth rate of *E. coli* remained the same without BolA gene in planktonic cells and also that BolA mutant cannot grow as cell aggregates like wild type *E. coli* does which suggests the contribution of BolA gene in biofilm formation. A comparison between wild type and BolA mutant strains also clearly established the ability of BolA gene to respond and adapt to several stress conditions. The involvement of BolA gene in *E. coli* respiration was observed by checking its glucose metabolism and oxygen uptake under various environmental stress conditions in both wild type and mutant strains.

P1922 The effect of silver and gold nanoparticles on several bacterial species

K.I. Wolska, A. Kraczkiewicz-Dowjat, W. Klapczynska, A.M. Grudniak (Warsaw, PL)*

Objectives: Antibacterial activity of silver and gold ions has been well proved. Recently there is a growing interest connected with silver and gold colloidal nanoparticles (Ag NPs and Au NPs, respectively) which

also show an antimicrobial activity. Until now the number of papers on this subjects is very scarce. Ag NPs and Au NPs can attach to bacterial membranes and penetrate inside cells. The aim of this study was to evaluate the antimicrobial activity of Ag NPs and Au NPs against nine bacterial species, including pathogens, by the MICs values measurement and the estimation of nanoparticles effect on bacterial growth and survival. The influence of Ag NPs on cell morphology and biofilm formation was also investigated.
Methods: Ag NPs and Au NPs were provided by Daunlop, Poland, their diameter varied from 30 to 50 nm. MICs value were measured by two-fold dilution method. The effect of 0.75MIC of Ag NPs and Au NPs on bacterial growth was studied by measuring the absorbance at 600 nm of exponentially growing cultures. The effect on cell survival was estimated by counting of cfu, after plating appropriately diluted samples on solid media. The influence of Ag NPs on cell morphology was examined using scanning electron microscopy (LEO 1430VP, Carl Zeiss) at 2500 x magnification. To study biofilm formation the classic method based on the absorbance of crystal violet by cells adhered to the wells of microtitre dishes was applied.
Results: MICs of Ag NPs of all bacterial cultures varied from 1 to 12.5 micrograms mL^{-1} and MICs of Au NPs, from 5 to 12.5 micrograms mL^{-1}. Both compounds inhibited bacterial growth and survival. Ag NPs showed the stronger inhibition of bacterial growth and survival than Au NPs, the inhibitory effect depended on the bacterial species tested. Ag NPs affected bacterial morphology and impaired formation of biofilms.
Conclusions: Ag NPs and Au NPs are the potent antibacterial agents inhibiting bacterial growth and survival. Ag NPs influence cell morphology and biofilm formation.

Host defence and pathogenesis

P1923 The role of cathepsin X in the immune response to infection with *Helicobacter pylori*

M. Skvarc, A.N. Kopitar, S. Jeverica, B. Tepes, A. Ihan (Ljubljana, Rogaska Slatina, SI)*

Objectives: Cathepsin X has been shown to regulate immune response. Its active role has been demonstrated previously in chronic inflammation of gastric mucosa and tumorgenesis of gastric cancer. In this study we demonstrate its role in presenting the antigens to immune cells and in the immune response to *H. pylori* infection.
Methods: We used the monoclonal antibody against cathepsin X and flow cytometry to determine the level of cathepsin X in THP-1 cells primed with *H. pylori* antigens isolated from subjects suffering from gastritis, who either successfully eradicated the *H. pylori* (seven patients 158/189) or not (seven patients 108/152) after the antibiotic therapy. We also compared the expression of cathepsin X on the THP-1 cells after priming the cells with *H. pylori* antigens and polymixin B, that binds to lipid A, in the same two groups of patients. We also measured the level of IL-6 and, IL-12/23 using flow cytometry. We used Mann–Whitney test to analyze the data.
Results: The group Hp108/152 expressed statistically significant less cathepsin X on the THP-1 cells than the group Hp158/189. We discovered that adding polymixin B to *H. pylori* antigens effects the expression of cathepsin X. Expression was higher in the group 158/189 than in the group 108/152. The difference was statistically significant. The trend was the same as when we tested the expression of cathepsin X with *H. pylori* antigens only. We measured the level of IL-6 and IL-12/23 in the supernatant from the THP-1 cells. The level of IL-12/23 was higher when we used strains 158/189. The difference was statistically significant in comparison with the strains 108/152. LPS does not have such a strong influence on the expression of cathepsin X and on the level of cytokines as we speculated when we added polymixin B. We got the opposite results as we expected.
Conclusion: In our previous study we compared the level of IFN-γ and discovered that strains 108/152 produced weaker immune response than the strains 158/189.
In this study we have proven that different strains of *Helicobacter pylori* have different cathepsin X profils and that the level of cytokines is different when we add *H. pylori* antigens, that were gathered from the two group of patients. The patients 108/152 did not eradicate the *H. pylori* because the antigens from these strains of *H. pylori* are less immunogenic than the antigens from strains 158/189. Apparently cathepsin X plays a vital role in this process.

P1924 Purification and characterization of an anti-pneumococcal capsular polysaccharide IgG antibody preparation

E. Lock, P. Worthington, L. Foyle, L. Garner, J. McGough, R. Barber, P.D. Stubbs, F. Alcock, R. Hughes, G.L. Wallis, A.R. Parker (Birmingham, UK)*

Objectives: *Streptococcus pneumoniae* is a major human pathogen causing pneumonia, sepsis, meningitis and otitis media. The major virulence factor of *S. pneumoniae* is the capsular polysaccharide (PS), of which there are 90 different types each inducing their own serotype specific antibodies. Since anti-PS antibodies are highly protective, efforts have focused on using the PS as immunogens for the development of pneumococcal vaccines. Standardisation of quantitative PS antibody measurement is of utmost importance between different laboratories. The objective of the study was to purify specific anti-PS IgG antibodies against the 23-valent PNEUMOVAX™ vaccine (Merck and Co. Inc).
Methods: Pooled plasma from multiple donors (6 to 10) were clotted. Three independent pools were prepared. Total IgG was purified using ammonium sulphate precipitation and anion-exchange chromatography. Following adsorption of contaminating non-specific PS, pneumococcal capsular polysaccharide(PCP)-specific antibodies were obtained by affinity purification using PNEUMOVAX™ covalently linked to Sepharose. The purified PCP specific IgG was characterised for purity, serotype composition, function, and then quantified.
Results: SDS-PAGE analysis suggested that the purified PCP specific IgG was at least 95% pure. Functionally, the preparation contained no contaminating *Haemophilus influenzae* type b, diphtheria toxoid or tetanus toxoid or contaminating non-specific PS antibodies. The major IgG subclasses in the preparation were IgG2 > IgG1 with negligible levels of IgG3 and IgG4. Immunoglobulin classes IgA and IgM were absent. Serotype analysis indicated that this preparation reacted with all 23 serotypes present in the PNEUMOVAX™ vaccine.
Conclusion: Determination of accurate antibody titre is essential for serodiagnosis, epidemiological investigation and evaluation of vaccine response. We show that it is possible to purify a sample containing 23 serotype specific antibodies produced in response to PNEUMOVAX™ vaccination which could be used to aid standardisation of different technologies to measure antibodies to PCP.

P1925 CD36 is a phagocytic receptor for *Streptococcus pneumoniae* and involved in bacterial clearance during pneumococcal pneumonia *in vivo*

O. Sharif, U. Matt, K. Stich, B. Doninger, S. Knapp (Vienna, AT)*

Objective: CD36 is a class B scavenger receptor that is expressed on several cell types including monocytes, macrophages and platelets and exhibits pleiotropic functions including: adhesion to thrombospondin, inhibition of angiogenesis, transport of long chain fatty acids and clearance of apoptotic cells. Additionally, CD36 has been implicated in the host immune response since it has been shown to act as a co-receptor for Toll like receptor 2 (TLR2) and to play a role in malaria and *Staphylococcus aureus* infection. However, its role in other Gram positive bacterial infections is unclear. Here, using mice mutant in CD36 we sought to examine the role of CD36 in pneumococcal pneumonia, a major cause of morbidity and mortality worldwide.
Methods: WT and CD36 mutant mice were intranasally inoculated with *Streptococcus pneumoniae*. Survival, bacterial counts, neutrophil influx and chemokine/cytokine responses were evaluated *in vivo* in a time dependent manner. *In vitro*, WT and CD36 mutant alveolar and bone

marrow derived macrophages were utilized to study the response to *S. pneumoniae* treatment.
Results: We show that early in infection although CD36 mutant mice have an exaggerated inflammatory response, *S. pneumoniae* bacterial loads are similar in CD36 mutant compared to wild type (WT) mice. However, at later time points, although CD36-mutant mice exhibit impaired bacterial clearance, they surprisingly do not show decreased survival compared to their WT counterparts. *In vitro* studies utilizing CD36 mutant primary cells confirm enhanced early inflammation in response to *S. pneumoniae*. Furthermore, phagocytosis assays show that CD36 mutant alveolar macrophages exhibit impaired phagocytosis in response to *S. pneumoniae*.
Conclusion: These data show that CD36 plays an indispensable role in the lung in response to *S. pneumoniae* infection by virtue of its ability to act as a phagocytic receptor, but suggest mortality is not different because this defect is compensated for by enhanced inflammation.

P1926 Inhibition of macrophage ingested MDR *M. tuberculosis* growth

J. Gvozdeva, L. Kovalchuk, T. Smirnova, L. Gankovskaya, L. Chernousova (Moscow, RU)*

A natural cytokines complex (CNC) and antimicrobial peptides (AMP) is a standardized complex of cytokines produced by pig's activated peripheral blood leukocytes, for which activity of interleukins 1, 2, 6, tumor necrosis factor, migration inhibitory factor, and transforming growth factor have been determined. Besides low-polymeric fraction cytokines, peptides similar to protegrin with direct antiviral and antimicrobial effects were identified.
Aim:to study CNCplusAMP effect on growth of MDR *M. tuberculosis* ingested by macrophages. We used an *ex vivo* model exposed to CNCplusAMP on peritoneal macrophages of C57Bl/6 mice infected by MDR *M. tuberculosis.* Macrophages were preliminarily incubated with CNCplusAMP, 5:1, on 96-well plates using RPMI1640 medium with 5mM HEPES, 2mM L-glutamin, 5%FCS at 5%CO_2. *M. tuberculosis* growth was estimated on day 7 using quantitative PCR, real-time *(M. tuberculosis*-M.bovis DNA test-system). *M. tuberculosis* cytopathogenic effects on macrophages were estimated by LDH release from injured macrophages into media according to the "Promega" instruction. The studies demonstrated that on day 7 *M. tuberculosis* growth rate increased from 5.6±0.03 to 6.0±0.07 lgCFU. Macrophages inhibited mycobacteria growth (5.8±0.03 lgCFU). Preliminary incubation of infected macrophages with CNCplusAMP in the concentration of 5mkg/ml revealed the trend towards complementary inhibition of mycobacteria growth (5.7±0.03 lgCFU). CNCplusAMP in the concentrations of 1mkg/ml and 0.1mkg/ml did not produce reliable influence on mycobacteria growth. CNCplusAMP influence on infected macrophages represented a reliable decrease of cytotoxic effect of *M. tuberculosis* on macrophages: specific macrophage lysis reached 23.8±1.16%($p = 0.002$) at 5mkg/ml of CNCplusAMP and 24.0±0.84%($p = 0.001$) at 1mkg/ml CNCplusAMP as compared to *M. tuberculosis* infected macrophage lysis (30.6±1.23%).
CNCplusAMP activated macrophages, which led to increased bactericidal effect of macrophages on MDR *M. tuberculosis.* The degree of growth inhibition depended on CNCplusAMP doses; the maximal effect was observed at the concentration of 5mkg/ml.

P1927 *Coxiella burnetii* antigen-stimulated dendritic cells mediate protection against *C. burnetii* challenge in Balb/c mice

Y. Wei, X. Wang, B. Wen (Beijing, CN)*

Objectives: *Coxiella burnetii*, an obligate intracellular bacterium, is the etiological agent of human Q fever. To investigate the role played by dendritic cells (DCs) in protective immunity to the pathogen, *C. burnetii* antigens were applied to stimulate DCs and their responses were analyzed.
Methods: The immature DCs derived from bone marrow cells of Balb/c mice were stimulated with *C. burnetii* whole cell antigens (phase I Ag and phase II Ag) and recombinant protein antigens (rP)124, rP105, and rP54, respectively, and expression of MHC-II and cytokines of antigen-pulsed DCs were evaluated by flow cytometry. The antigen-pulsed DCs were transferred to Balb/c mice and the mice were challenged with virulent *C. burnetii* organisms 24 h later. *Coxiella* loads in spleens of mice were measured by quantitative polymerase chain reaction 6 days postinfection.
Results: Compared with mice receiving rP54-pulsed DCs, mice receiving DCs pulsed with phase II Ag, phase I Ag, rP124, or rP105 exhibited significantly lower levels in coxiella loads of spleens, and however there was no significant difference between mice receiving rP54-pulsed DCs and naïve mice. After 24 h of coculturing with homologous antigen-pulsed DCs, expression of CD69, IFN-g, and IL-17 on CD4 T cells from mice receiving phase I Ag-, phase II Ag-, rP124-, or rP105-pulsed DCs were significantly higher than that of mice receiving rP54-pulsed DCs. However, the percentage of CD4+ and IL-17 double-positive cells in mice receiving DCs pulsed with phase I Ag, phase II Ag, rP124, or rP105 were higher than that of mice receiving rP54-pulsed DCs. In addition, higher level of IL-6 and IL-12 and low lever of IL-10 were detected in supernatants of splenocytes from mice receiving DCs pulsed with phase I Ag, phase II Ag, rP124, or rP105, and opponent results were detected in that of mice receiving rP54-pulsed DCs after 24 h of coculturing with homologous antigens.
Conclusion: The data suggest that a vigorous proinflammatory response in DCs is associated with protective immunity against *C. burnetii.* Besides whole cells phase II Ag and phase I Ag, surface protein antigens rP124 and rP105 have the ability to activate DCs to mediate protection against *C. burnetii* infection.

P1928 Increased serum concentration of high-mobility group Box-1 (HMGB-1) in tuberculous lymphadenitis: a pilot study

W.S. Choi, Y.K. Youn, Y.M. Jo, J.Y. Kim, J.Y. Song, J.W. Sohn, H.J. Cheong, W.J. Kim, M.J. Kim, D.W. Park (Seoul, KR)*

Objectives: HMGB-1 is a DNA-binding protein that is passively released by necrotic and damaged cells. Extracellular HMGB-1 is considered as a necrotic marker. Tuberculous lymphadenitis is a chronic granulomatous inflammation with caseation necrosis of the lymph node. The necrotic feature of lymph node usually improves with anti-tuberculous chemotherapy. Sometimes it is difficult to assess the treatment response of tuberculous lymphadenitis. This study is a pilot study to determine the possibility of HMGB-1 as a biomarker for assessing the treatment response of tuberculous lymphadenitis.
Methods: Serum HMGB-1 level was measured quantitatively using a kit (HMGB1 ELISA Kit II, Shino-Test Corporation, Sagamihara-shi, Japan). Sera samples were collected from 14 patients with confirmed tuberculous lymphadenitis before initiating anti-tuberculous chemotherapy and 10 persons (controls) with no evidence of *M. tuberculosis* infection. Tuberculous lymphadenitis was confirmed by pathology, culture or polymerase chain reaction (PCR).
Results: The mean age of 14 patients was 41.86±11.92 years and 12 (85.7%) were female. The mean age of 10 controls was 37.80±8.07 years and 5 (50.0%) were female. There were no significant differences in age ($P = 0.361$) and sex ($P = 0.085$). Among 14 patients, 7 (50.0%) were confirmed by PCR, 6 (42.9%) by pathology and PCR, and 1 (7.1%) by culture and PCR. Serum HMGB-1 level was significantly ($P = 0.002$) elevated in patients (10.94±2.69 ng/ml) before initiating anti-tuberculous chemotherapy compared with controls (7.93±2.52 ng/ml).
Conclusion: The current study revealed that serum HMGB-1 level is elevated significantly in patient with tuberculous lymphadenitis before initiating anti-tuberculous chemotherapy. The result suggested that HMGB-1 could be a biomarker for treatment response. Further studies will be followed to determine the serum HMGB-1 level in the middle and in the end of the treatment.

P1929 Role of leukotrienes in resistance and susceptibility to infection by *Histoplasma capsulatum*

A. Secatto, E. Soares, A. Medeiros, L. Faccioli (Ribeirão Preto, Araraquara, BR)*

Introduction and Objectives: *Histoplasma capsulatum* (*H. capsulatum*) is a dimorphic pathogenic fungus that causes a wide spectrum of diseases. Macrophages are an important phagocytic cells in host defense against fungi. In order to enhance host defense, these resident cells secrete chemotactic substances such as leukotrienes (LTs) and cytokines that recruit effector cells to the focus of infection. LTs are potent lipid mediators of inflammation and host defense, derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid (AA) metabolism. We have been shown that the absence of leukotrienes in genetically modified mice (5-LO–/–) or by treating WT animals with pharmacological inhibitor MK886, have increased susceptibility to infection when they are infected with *H. capsulatum.* Recent studies show that susceptibility or resistance of different strains to certain infections, such as *Leishmania* amazonensis, is associated with differential production of LTs. In the present study, we evaluated the production of LTB4 by peritoneal macrophages (PM) from susceptible and resistant mice after challenge with *H. capsulatum* and the effect of LTs in phagocytosis by macrophages of both strains.
Methods and Results: Macrophages from C57BL/6 (susceptible) and sv129 (resistant) mice were infected for 48 h at a ratio of 1:5 (*H. capsulatum*:macrophage). Supernatants were collected and the production of LTB4 was evaluated by ELISA. The phagocytosis was assessed by fluorescence using unopsonized or IgG-opsonized FITC-labeled *H. capsulatum* and MK886, a LTs inhibitor, was added to the cells previously to the infection. Interestingly, macrophages from resistant mice produced higher levels of LTB4 upon *H. capsulatum* challenge than did those from susceptible mice. As expected, PMs from sv129 phagocytosed 1.9 fold-increased IgG-opsonized-*H. capsulatum* than PMs from C57BL/6. However, phagocytosis of IgG-opsonized-*H. capsulatum* by PMs from C57BL/6 and sv129 are both dependent on endogenous LTs, since when the LTs synthesis is pharmacologically inhibited, the phagocytosis was decreased 10 and 20-fold respectively.
Conclusion: LTs are important mediators involved in the mechanisms of host defense by participating in the patterns of resistance/susceptibility to infection of *H. capsulatum.*

P1930 Identification of new epitopes in the *Trypanosoma cruzi* membrane antigen that are recognized by CD8+ T lymphocytes from Chagas patients

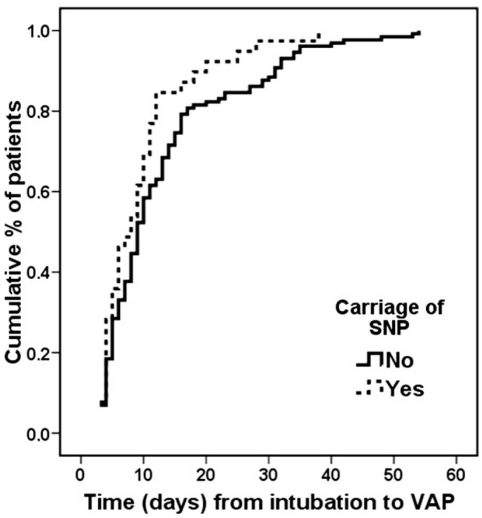
A. Egui Machado, C. Marañon, C. Thomas, M. Segovia, M. López López (Granada, Murcia, ES)*

Objectives: *Trypanosoma cruzi* is the aetiological agent of Chagas disease or American trypanosomiasis, which affects 15 million people in Central and South America, and thus represents an important health problem. CD8+ T cell response has been widely reported as essential for immune-protection against the *T. cruzi* infection. However, only few CD8+ epitopes recognized by chagasic patients have been described, remaining unknown the functionality of specific T cells for most of them. Here we characterized human CD8+ epitopes selected from the deduced amino acid sequence of the *T. cruzi* TcCA-2 gene (TcMe protein) using models of experimental infection in transgenic C57BL/6-A2Kb mice and mononuclear cells from chagasic patients.
Methods: Using SYFPEITHI software, we predicted peptides capable of biding to the human Class I molecule HLA-A*0201 located in the repeated amino-terminus of the TcMe protein. The binding capacity of these peptides to HLA-A*0201 was quantified in T2 cells. We have evaluated the processing and presentation of the peptides during experimental infection throughout the IFN-γ secretion test in splenocytes of infected A2/Kb transgenic mice after *in vitro* stimulation with each peptide. The recognition of epitopes during natural infection was evaluated by measuring IFN-γ and TNF-α secretion in circulating mononuclear cells from chagasic patients and healthy individuals from endemic areas. Finally, we determined the number of TcMe-specific granzyme B-producing cells by an ELISPOT assay in both patients and controls.
Results: We found ten peptides candidates for binding to HLA-A*0201, located in the repeated sequence of the TcMe protein. Five of these peptides showed to be successfully processed and presented in both experimental and natural infection models, producing the expression and secretion of TNF-α and IFN-γ cytokines. We detected granzyme B-producing cells for two of the referred epitopes.
Conclusions: Five new epitopes of the *T. cruzi* TcMe protein restricted to HLA-A*0201 are efficiently processed, presented and recognized by CD8+ T lymphocytes during the natural course of the Chagas disease. Our results suggest that CD8+ T cells specific to these epitopes have both cytotoxic and cytokine secretory capacity in patients with Chagas disease.

P1932 The impact of single nucleotide polymorphisms of the tumour necrosis factor gene for the advent of ventilator-associated pneumonia

A. Kotsaki, A. Savva, F. Baziaka, P. Koutoukas, A. Spyridaki, E.J. Giamarellos-Bourboulis (Athens, GR)*

Objectives: Tumor necrosis factor (TNF)-α is a major pro-inflammatory cytokine produced early during sepsis cascade. Several studies have tried to elucidate the role of single nucleotide polymorphisms (SNPs) within the promoter region of TNFα gene regarding predilection to sepsis or sepsis severity. The results so far have been contradictory with many of the discrepancies grounded to the limited number of patients enrolled. We aimed to define the role of SNPs of the TNFα gene in the development of ventilator-associated neumonia (VAP).
Methods: 214 consecutive patients were followed-up after intubation and admission in three academic intensive care units; 169 developed VAP. Time to development of VAP was estimated. Whole blood was collected in EDTA-coated tubes from those who developed VAP. DNA was extracted by Purigen Blood Core Kit C (Qiagen) standard technique. TNF-α SNPs of the promoter region i.e. –376G/A, –238G/A and 308 G/A were estimated by RFLP and visualization of the digested PCR products after UV photometry on 2.0% agarose gels.



Results: Regarding the –376 SNP 166 (98.2%) patients were carriers of the GG wild-type haplotype and three patients (1.8%) were GA heterozygotes. Regarding the –308 SNP 132 (78.1%) patients were carriers of the GG wild-type haplotype, 35 patients (20.7%) were GA heterozygotes and two patients (1.2%) were homozygotes for the AA mutation. Regarding the –238 SNP 164 (97.0%) patients were carriers of the GG wild-type haplotype and five patients (3.0%) were GA heterozygotes. The allele frequency in the population was in

Hardy-Weinberg equilibrium. Time to advent of VAP was significantly shortened among carriers of at least one SNP than among non-carriers of any SNP (log-rank: 4.08, p: 0.043, Figure).
Conclusions: SNP of the TNFα gene promoter region are associated with a predilection for earlier development of VAP after intubation.

P1933 Prospective analysis of salivary IgA responses in children during the first three months of life against pathogen of oral cavity. Influence of prematurity in this response

R.D. Nogueira, M.C.L. Borges, M.L.T. Sesso, V.P.L. Ferriani (Ribeirao Preto, BR)*

Objectives: The analysis of the mucosal immune system represents an interesting way to understand the microbial colonization in early life, particularly the response of SIgA (Secretory IgA) present in saliva because it represents the first line of defense. Previous study showed in children with 6 months of age, a high complexity of SIgA response to antigens of mutans streptococcal (MS), main pathogen of the dental caries, but little is known about the ontogeny of the mucosal immune system in the first 3 months of life especially in preterm newborn (below 37 weeks of gestation). For this reason, we compared the levels and specificity of SIgA to MS and others species enrolled with initial infection in fullterm (FT) and preterm (PT) early in life.
Methods: Stimulate saliva from 160 children, with 0 day of life (T0) and after 3 months (T3), were enrolled in this study. Salivary IgA and IgM levels were determined by ELISA. Subsets of 24 fullterm (FT) and 24 Preterm (PT) children showing similar salivary IgA levels were paired and matched for gender, racial background, breastfeeding. SIgA antibody reactivity to *Streptococcus mutans* (MS), *Streptococcus sanguinis* (SSA), *Streptococcus mitis* (SMI) and *Streptococcus gordonii* (SGO) Ags was determined in Western blot assays.
Results: Levels of SIgA were statistically different (Whitney test, $p < 0.05$) between groups and in FT were 2.5 times higher than PT children at T0, but not T3 ($p > 0.06$). Significant diversity was observed in IgA antibody response patterns to Ags. The number and intensity of reactive bands was higher in FT than PT children for all antigens tested only T0. In T3 SIgA response was similar in PT and FT. Some antigens were more frequently detected in salivas, such as: 153KDa of SGO, 170 KDa of SSA, 202 KDa of SMI, 185 and 160 KDa of SM. Responses to 153KDa of SGO (glucosyltransferase activity) were unique among those antigens that presented different in their pattern of recognition between FT and PT at T0 (Chi-square:, $p < 0.02$).
Conclusions: The data indicate that salivary IgA responses to Ags can occur in the first day of life and children PT show a diminished response to microorganisms tested that may reflect a lower concentration of IgA, but with 3 months of age PT and FT have the same SIgA response.
CNPq 472928/2007–4, FAPESP 02/07156–1; 04/07425–8.

P1934 Benzodiazepines and cannabinoids lead to enhanced susceptibility and severity of orthopoxvirus infection

H.P. Huemer, C. Lassnig, D. Bernhard, S. Sturm, N. Nowotny, E.U. Irschick, M. Kitchen, M. Pavlic (Innsbruck, Vienna, AT)*

Introduction: Benzodiazepines are widely used as tranquilizers, anticonvulsives and various other indications. The leaves and resin of Cannabis sativa (Indian hemp) are known substances of abuse, but have been also documented since 2000 years as herbal medicine in many cultures. In certain countries, including Austria, its main psychoactive compound THC (d9-tetrahydrocannabinol) is licensed as a medical drug for stimulating appetite in kachectic patients and a means for pain release. There is growing evidence that also other constituents of the resin other than THC may have significant drug effects which may be usable in medicine.
Methods: Balb/c mice were treated with a single dose of diazepam and/or cannabinoids one day before infection with vaccinia virus (VACV) and cowpox virus (CPXV). Infected animals were monitored and weight and disease index recorded. The anti-CPXV antibody response was determined in an ELISA assay. The different substances were also tested for their ability to interfere with the mitogen-mediated immune stimulation of peripheral blood mononuclear cells (PBMC) as well of human and murine spleen cells in proliferation assays using H3-thymidine labeling. Additionally the late apoptosis of whole spleen cells was tested by propidium iodide staining and FACS analysis.
Results: Diazepam as well as cannabis/THC led to earlier onset of disease, prolonged duration of symptoms, higher weight loss and overall disease index in mice infected with VACV. In CPXV infected mice the typical poxviral skin lesions developed after administration of diazepam only and also a significant decrease in the anti-CPXV specific antibody response was observed in the drug treated animals. Cannabinoids, diazepam and to a certain extent also alprazolam inhibited the proliferative response of human PBMC or human and murine spleen cells *in vitro* but did not show noteworthy apoptotic effects. The cannabis resin was fractionated also by HPLC and inhibitory fractions could be also extracted from cigarette smoke extracts.
Conclusions: Together with our recent findings of a human case of unusual severe CPXV infection in a young drug taker these data indicate a certain risk of "soft drugs" for suszeptibility of orthopoxvirus infection. As VACV is still used as an outdated life vaccine against smallpox infection, these data provide a first evidence of a risk of certain drugs for more severe side effects and/or possible interference with the success of vaccination.

P1935 Enhanced anti-influenza IgA production in airway mucosa by clarithromycin and its restoration effects on mucosal immunity in patients treated with oseltamivir

H. Kido, S. Suzuki, K. Iwase (Tokushima, Nagoya, JP)*

Objectives: The antiviral neuraminidase inhibitor oseltamivir (OSV) suppresses influenza viral RNA replication and viral antigenic production, resulting in a limited immune response against virus, particularly airway IgA response. The macrolide clarithromycin (CAM) is used to treat bacterial infections and has immunomodulatory activities. This retrospective study investigated the immunomodulatory effects of CAM in influenza patients who were treated with or without OSV.
Methods: The study recruited 40 children with acute influenza, and grouped them according to the treatment received: five days treatment with OSV (n = 14), CAM (n = 8), OSV+CAM (n = 12) and untreated (n = 6). The Before and after treatment comparisons were made of the level of secretory IgA (sIgA) against influenza A virus (H3N2) and (H1N1), total sIgA, viral RNA copy numbers in nasopharyngeal aspirates and disease symptoms.
Results: Infection induced anti-viral mucosal sIgA in the nasopharyngeal aspirates of most patients of all treatment groups. Particularly prominent increases in the levels were found in the CAM and OSV+CAM groups. Low induction of anti-viral sIgA was observed in the OSV group, but the addition of CAM to OSV augmented sIgA production and restored local mucosal sIgA levels. The frequency of residual cough in the OSV+CAM group was significantly lower than in the other groups including the group treated with OSV.
Conclusion: CAM boosted the nasopharyngeal mucosal immune response in children presenting with influenza A, even in those treated with OSV who had low production of mucosal anti-viral sIgA, and alleviated the symptoms of influenza.

Pathogenesis – impact of antimicrobials

P1936 Quantitative evaluation of *Clostridium difficile* and intestinal lactobacilli in patients with antibiotic associated diarrhoea

I. Smidt, E. Sepp, M. Rätsep, S. Kõljalg, K. Lõivukene, I.H. Löhr, O. Natås, R. Mändar, P. Naaber (Tartu, EE; Stavanger, NO)*

Objectives: (1) To evaluate the correlation between presence of *Clostridium difficile* (CD) toxins and CD population level in the intestine we compared CD counts in direct toxin test positive and negative

(*C. difficile* infection) CDI patients. (2) To evaluate the role of lactobacilli (LB) in colonization resistance against *C. difficile* we compared the counts and diversity of intestinal LB in CDI and non-CDI patients with antibiotic associated diarrhoea (AAD).
Material and Methods: Faecal samples of AAD patients were collected from Norway (n = 42) and Estonia (n = 36). Direct toxin A+B EIA test was performed and quantitative cultures from serial dilutions were made on LAB 160 agar for CD and on MRS and Rogosa agar for LB.
Results: From 43 faecal samples that were culture positive for toxigenic CD strain (CDI cases), 28 were toxin positive and 15 negative by EIA test. In CDI patients CD counts varied from 5 to 7.3 log CFU/g (median 6.3). Although CD counts were somewhat higher in toxin positive CDI cases than in negative cases (medians 6.9 vs 6.0) the difference was not statistically significant. Comparing CDI and non-CDI AAD cases no significant differences were found in total counts of intestinal LB (medians 2.7 and 2.9 log CFU/g), in their prevalence (60 vs 54%) and numbers of different LB strains (medians 1 vs 1). Comparing Estonian and Norwegian AAD patients we found more different LB strains (medians 2 vs 0) and higher LB counts (medians 4.14 vs 0 log CFU/g; p = 0.02) in Norwegian patients. Similar trends were present if comparing CDI and non-CDI samples separately.
Conclusion: In our study positive direct toxin test did not predict higher population level of CD in the intestinal tract of CDI patients. Correlation between intestinal CD counts and severity of disease should be evaluated in future studies. We did not find any correlation between presence of cultivable LB or their count in intestine and presence of CD as cause of AAD. Thus our study does not support the role of LB in maintenance of colonization resistance against CD. Higher counts of intestinal LB in Norwegian AAD patients probably reflect common usage of probiotics containing yogurts in the hospital and in the community rather than differences in antibiotic treatment. Further studies are needed to determine the causes of higher colonization of Norwegians with LB and its possible effect on AAD and CDI incidences.

P1937 Resistance to colistin associated with mutations in pmrB in *Acinetobacter baumannii* and its effect on virulence and bacterial fitness

R. López-Rojas, J. Domínguez-Herrera, M.J. McConnell, F. Docobo-Pérez, Y. Smani, J. Pachón (Sevilla, ES)*

Objectives: *Acinetobacter baumannii* has clinical relevance due to the high number of nosocomial infections that it causes, and its ability to develop resistance to all antimicrobials, including colistin (COL). Recently, mutations in the regulatory system PmrAB were associated with COL resistance in *A. baumannii* strains (Antimicrob Agents Chemother. 2009; 53:3628–34). The aim of this study is to evaluate the effect of COL resistance caused by mutations in the PmrAB system on the virulence and fitness of *A. baumannii*.
Methods: The strains used were *A. baumannii* ATCC 19606 (ATCC), and its COL resistant mutant (RC64) obtained by selective pressure (COL MIC=64 μg/mL). Virulence was assessed in a murine peritoneal sepsis model by inoculating groups of 10 C57BL/6 mice with 8 log CFU/mL and diminishing the inoculum by a factor of 10 until 0% mortality was reached (mortality and time to death were measured). For *in vitro* growth and competition index (CI) experiments, growth curves in Mueller-Hinton broth were performed for both strains separately and in combination, and bacterial concentrations at 2, 4, 8 and 24 h were determined. For *in vivo* growth and CI experiments, groups of 12 mice were infected with both strains separately, and with a 50% mixture of both. In each group 3 animals were sacrificed at 2, 4, 8 and 24 h, and the bacterial concentration in the spleen was determined. In addition, for the *in vivo* CI, 9 animals were inoculated with the mixture and sacrificed at 24 h. In order to characterize pmrA and pmrB mutations, genomic DNA from ATCC and RC64 strains was extracted, amplified with specific primers, and sequenced.
Results: ATCC was more virulent than RC64 in terms of mortality (see table) and time to death (20.0 h vs. 38.4 h respectively, p < 0.001, T-student test). There were no *in vitro* growth differences between strains, separately or when grown together, nor in CI (CI was 1.08). During *in vivo* growth, ATCC reached a maximal concentration in the spleen of 10 log CFU/g, whereas RC64 reached a maximal concentration of 9 log CFU/g. Growing in competition, ATCC remained at 10 log CFU/g, while RC64 decreased to 8 log CFU/g. The *in vivo* CI was 0.016. There were no mutations in pmrA, whereas in pmrB two mutations were identified: arg134cys and ala227val.
Conclusion: The acquisition of COL resistance in *A. baumannii*, related to PmrB mutations (arg134cys and ala227val), leads to an *in vivo* fitness loss and a decrease in virulence.

Mortality	$10^{8.5}$ CFU/mL	$10^{7.5}$ CFU/mL	$10^{6.5}$ CFU/mL	$10^{5.5}$ CFU/mL
ATCC 19606	100%	80%	20%	0%
RC64	100%	0%	0%	0%

P1938 Antibiotic-associated haemorrhagic colitis caused by *Klebsiella oxytoca*

D. Orth, A. Naim, S. Ehrlenbach, K. Grif, R. Würzner (Innsbruck, AT)*

Objectives: *Klebsiella oxytoca* has been isolated from stool samples of patients with *Clostridium difficile*-negative antibiotic-associated hemorrhagic colitis. However, the pathogenic role of this organism has not been fully elucidated yet.
The aim of our study was to investigate the presence of toxin producing *Klebsiella oxytoca* in patients with antibiotic-associated hemorrhagic colitis.
Methods: 2500 stools specimens from patients with the clinical diagnosis antibiotic-associated hemorrhagic colitis and 85 stool specimens of a healthy control group were examined in the period from June to November 2007 in the Tyrol (Austria).
The specimens were cultured on MacConkey agar and investigated for the presence of *K. oxytoca* by standard microbial procedures. The cytotoxic effect of *K. oxytoca* strains was tested on Hep-2 cells and Vero cells, the amount of cytotoxicity was determined by Lactat-Dehydrogenase release. The *Klebsiella* strains were characterized phenotypically by antibiotic susceptibility testing and were typed by Pulsed-Field Gel-Electrophoresis. In addition, all stool samples were investigated for *Clostridium difficile* toxin by ELISA.
Results: 119 of 2500 stool specimens (4.8%) of patients with antibiotic-associated hemorrhagic colitis yielded *K. oxytoca*. In the control group only 2 of 85 specimens (2.4%) were positive for *K. oxytoca*. The rate of cytotoxic strains among *K. oxytoca* was 46% in patients with antibiotic-associated hemorrhagic colitis compared with none in the healthy control group. In addition we could demonstrate that Vero cells are superior to Hep-2 cells for investigating *K. oxytoca* strains for toxin production.
Eleven percent of the stool specimens were positive for *Clostridium difficile* toxin.
Conclusions: Our study demonstrates that beside *Clostridium difficile* cytotoxigenic *K. oxytoca* are a causative agent of antibiotic-associated hemorrhagic colitis. Thus, cytotoxigenic *K. oxytoca* should be included in routine microbiologic diagnostic of stool specimens. Furthermore we recommend Vero cells for determination of the cytotoxic effect of *K. oxytoca* strains.

P1939 Virulence factors and pathogenicity islands in extended-spectrum β-lactamase-producing *Escherichia coli* bloodstream isolates

C. Caneiras, A. Narciso, M. José Salgado, L. Lito, J. Melo-Cristino, A. Duarte (Lisbon, PT)*

Objectives: The aim of this study was to evaluate the relationship between phylogenetic groups, virulence determinants and ESBL production in bloodstream *E. coli* isolates recovered at the Hospital de Santa Maria in Lisboa.
Methods: This study included 45 *E. coli* clinical isolates, identified from blood cultures of patients hospitalized between 2001 and 2007 at Hospital de Santa Maria. It was included representatives of the

major endemic *E. coli* isolates producing CTX-M-15 enzyme (n = 39), as well as others ESBL (n = 6). The isolates were classified according to phylogenetic group and screened by PCR amplification for gene markers of eight pathogenicity islands (PAI) and nine virulence factors genes.
Results: Twenty seven *E. coli* isolates belonged to the virulent extra-intestinal phylogenetic group B2, ten to group D, two to group B1 and six to group A. The genes associated with pathogenicity islands were detected for five different PAIs: PAI IV536 (87%), PAI ICFT073 (51%), PAI IICFT073 (44%), PAI IJ96 (31%) and PAI II536 (16%). Gene markers for PAI I536, IIj96 and PAI III536 were not detected. The most frequent virulence genes were fimH (91%), iucC (62%), and EcpA (91%). The haemolysin hylCA was presented in one isolate (2%) while no papC, sfa, afa and cnf were detected. 55% of the bloodstream isolates showed the virulence pattern fimH, ecpA and iucC genes, followed by fimH and ecpA genes (31%). Only one isolate (2%) presents four of the nine virulence factors studied, namely fimH, ecpA, iucC and hylCA. The prevalence of the virulence factors detected was comparable among all phylogenetic groups, isolates with endemic CTX-M15 enzyme, and among those with non-CTX-M ESBLs.
Conclusions: The same virulence pattern has been observed in pathogenic and commensal isolates what suggested that the combination of virulence factors could not be predictive different pathotypes. The role of virulence factors in the etiology of *E. coli* bacteremia remains unclear, although fimH and ecpA were significantly more prevalent among the endemic CTX-M15 isolates. Our results are generally in accordance with recent reports and indicate a distribution of bloodstream infection isolates in low virulent and highly resistant group, regardless the *E. coli* strain background.

P1940 Correlation between azole resistance and virulence attributes displayed by *C. parapsilosis*

A. Silva, A. Silva-Dias, I. Miranda, C. Pina-Vaz, A.G. Rodrigues (Porto, PT)*

Objectives: *C. parapsilosis* is common yeast, particularly isolated from invasive infections. There is a growing concern about the rise of antifungal drug resistance, although short knowledge is yet available regarding phenotypic characterization of drug-resistant fungal strains. In order to study the correlation between drug resistance and pathogenicity attributes, we induced resistance in one susceptible *C. parapsilosis* clinical strain by sub-culturing it in the presence of increasing concentrations of fluconazole, voriconazole and posaconazole. The set of strains obtained displayed variable degrees of resistance and was characterized regarding their virulence attributes.
Methods: Growth Kinetic. Strains were incubated at 30ºC under agitation for 48 h using an initial inoculum density of 0.1 in liquid YPD medium; growth was assessed by measuring O.D. along time. Adherence Assay. From a overnight culture, a PBS yeast suspension (106 blastoconidia/mL); a stock solution of 105 microspheres/mL in PBS was prepared; equal volumes of both suspension were mixed; the percentage of yeast cells attached to microspheres was assessed by flow cytometry. Biofilm Formation. A initial 5 x 107 blastoconidia/mL suspension was incubated at 37ºC in YPD medium in 12 wells polystyrene plates; after 24 and 48 hours biofilm mass was determined by crystal violet method. Phenotypic Switching. Blastoconidia were plated onto YPD and incubated at 30ºC for 96 h; the presence of distinct colony phenotypes was scored and the frequency of switching was determined. Pseudohyphal Development. Blastoconidia were incubated in RPMI 1640 medium with 10% fetal calf serum for 24 h at 37ºC; pseudohyphal development was checked microscopically.
Results: Overall, specific growth rates and adherence ability displayed by resistant strains were higher compared to the susceptible strain; however, resistant strains were less capable to form biofilm. phenotypic switching and pseudohyphal formation occurred extensively among resistant strains.
Conclusion: Our results demonstrate that *in vitro* induced resistance to azoles alters growth rate, adherence, phenotypic switching and pseudohyphal development of *C. parapsilosis*, considered important attributes favouring invasive fungal infection.

P1941 Quinolones induce a decrease in kidney colonization of uropathogenic *Escherichia coli* associated with a decrease in P-fimbria production

S. Soto, N. Frimodt-Møller, J. Vila (Barcelona, ES; Copenhagen, DK)*

Objective: Urinary tract infections (UTIs) develop in an ascending manner beginning with peri-urethral colonisation, followed by that of the urethra into the bladder causing cystitis, and in some cases, if left untreated, ascension of the ureters into the kidneys establishing acute pyelonephritis. In more than 80% of all cases of uncomplicated acute pyelonephritis, the etiologic agent is uropathogenic *Escherichia coli* (UPEC). The aim of this study was to determine the effect of the partial loss of a PAI induced by quinolones in bladder and kidney colonisation.
Methods: The UPEC HC14366 strain and its non-haemolytical derivative with partial loss of a PAI were selected. An ascendant urinary tract infection animal model was used to evaluate the infectious capacity of these two strains. Six mice were inoculated with these strains, and urine samples from bladder and kidney were collected. RT-PCR was used to determine the expression of the papA and lrp genes. The whole genome of both strains was sequenced using the SOLID procedure.
Results: Both strains were inoculated in mice and the number of bacteria found in the urine, bladder and kidney was determined. The HC14366/PAI– strain presented the same capacity to colonise the bladder as the wild-type strain but lost the capacity to colonise the kidney. The number of CFU/ml or CFU/g of urine in both cases was 10^7 in urine and 5×10^5 in bladder. On the other hand, the number of CFU/g found in the kidney was 103 and 100 in the wild-type strain and in its derivative PAI–, respectively. The expression of P-fimbriae, related to this function, in both strains was analysed by RT-PCR. Differences in gene expression were found between and the wild-type strain and its derivative. The expression of papA was lower in the non-haemolytical strain than in the wild-type strain. The lrp gene was cloned and introduced into HC14366/PAI–. It The lrp and papA genes were found decrease their expression in the HC14366/PAI– strain. However, when this strain is complemented with the lrp gene, the expression became normal.
Conclusion: The partial loss of a PAI induced by quinolones in UPEC causes a decrease in kidney colonisation due to a reduction in pap gene expression mediated by the Lrp protein.

Pathogenesis – host response

P1942 The role of IFN-γ receptor in the control and clearance of *Legionella micdadei* from its experimental murine host

I. Gobin, T. Grubesic, D. Turkovic, D. Rebic, M. Susa, M. Doric (Rijeka, HR; Ulm, DE)*

Objectives: *Legionella micdadei* (Pittsburgh pneumonia agent) is commonly identified as a cause of nosocomial infection. Illness due to *L. micdadei* occurs in heart and kidney transplant patients, HIV-infected patients and other immunocompromised patients. Since the infections by *L. micdadei* occurs predominantly in immunocompromised hosts, we examined the virulence of *L. micdadei* in immunodeficient IFN-γ receptor knockout mice. We explored the kinetics of bacterial clearance and patohistological changes in IFN-gR –/– and control C57Bl/6 mice.
Methods: Pathogen-free 61- to 10-weeks-old IFN-gR –/– and C57Bl/6 mice were infected by intratracheal inoculation with *L. micdadei* (clinical isolate) using a dose of 10^7 CFU. We determined the mortality rate and bacterial clearance from the lung, liver and spleen of infected mice at different time points post infection. We also followed the patohistological changes in these organs during 168 hours of infection.
Results: Our results showed a higher mortality rate of IFN-gR –/– mice in comparison to control C57Bl/6 mice. After intratracheal inoculation of 10^7 CFU of *L. micdadei* all IFN-gR –/– mice died within 12 days, while control mice survived the infection. The numbers of *L. micdadei* recovered from the lungs of IFN-gR –/– mice were significantly higher compared to the control C57Bl/6 mice. At day 7 post infection *L. micdadei* was not detected in the spleens and livers of C57Bl/6 mice,

whereas, IFN-gR –/– mice showed signs of systemic bacterial infection. In comparison to C57Bl/6 mice, IFN-gR –/– showed significantly higher infiltration of inflammatory cells on the 3rd day of infection with progression of disease on 7th day. IFN-gR –/– mice developed severe bronchopneumonia with infiltration of interstitial tissue, while control mice showed only mild peribronchial inflammatory cell infiltration. Nods of inflammatory cells were present in the liver of IFN-gR –/– mice from day 3 after infection in contrast to control mice which cleared the bacteria.
Conclusion: In this study we demonstrate a critical role of IFN-gR in the control and clearance of *L. micdadei* from mice. IFN-gR–/– mice failed to control *L. micdadei* and eventually succumbed to an overwhelming bacterial burden.

P1943 Characterization of the cerebrospinal fluid inflammation in patients with tick-borne encephalitis

D. Grandgirard, H. Schudel, S. Bigler, S.L. Leib (Berne, CH)*

Objective: Tick-borne encephalitis (TBE) is a potentially fatal neurological infection. In most cases, patients recover spontaneously within 2 weeks after onset of symptoms but TBE may cause long-term neurological/neuropsychiatric sequelae in affected patients. Different forms of brain damage including neuronal and glial destruction, spongiform focal necrosis, inflammation and perivascular infiltration have been documented in autopsy cases. Since the virus is rarely detectable in the cerebrospinal fluid (CSF) during the encephalitic stage of disease the main pathogenic mechanism of brain destruction may be the inflammatory host reaction. The aim of this study was to investigate the pathogenesis of TBE by characterizing the pattern of inflammatory mediators in the CSF of patients with TBE.
Methods: Ten patients with confirmed TBE, hospitalized between March-July 2008 in western Switzerland were enrolled in this study. CSF samples were taken for diagnostic purpose on the day of hospitalization, centrifuged and stored at −80ºC until analysis. CSF samples from patients with unrelated indications for lumbar puncture served as controls ($n = 9$). Inflammatory parameters in the CSF were determined with commercially available microsphere-based assays. Statistical analyses were performed using GraphPad Prism 5.01. Clinical data were collected retrospectively in the documented patient's history by chart review.
Results: Inflammatory parameters were significantly increased in CSF from patients with confirmed TBE ($n = 10$) vs. controls ($n = 9$) including white blood cells ($p = 0.001$); total protein ($p = 0.01$); MMP-9 ($p = 0.001$); IFN-γ ($p = 0.0001$); IL-6 ($p = 0.04$); IL-10 ($p = 0.04$); and IL-1RA ($p = 0.0001$). There was a statistically significant positive correlation between defined cytokines e.g. (IL-6/IL-10, $r = 0.91$, $p = 0.002$; IFN-γ/IL-1RA, $r = 0.84$, $p = 0.02$). Importantly, TGF-α was found to be significantly downregulated in TBE patients vs. controls ($p = 0.006$).
Conclusion: Our results support the notion that the inflammatory host reaction is critically involved in the pathogenesis of TBE. In TBE, CSF levels of pro-inflammatory mediators including IL-6, IFN-γ and MMP-9 were significantly increased which was accompanied by an increase in levels of IL-10 and IL-1RA (regulatory proteins of the inflammation). Deciphering the inflammatory host reaction to TBE within the brain compartment will lead to a better understanding of its pathogenesis and in consequence more efficient therapies.

P1944 Concentration of sVCAM-1 and CXCL10, CXCL11, CXCL12, CXCL13 chemokines in patients with tick-borne encephalitis

A. Muszynska-Mazur, J. Zajkowska, S. Pancewicz, M. Kondrusik, S. Grygorczuk, P. Czupryna, A. Moniuszko (Bialystok, PL)*

Objective: The objective of a study was to measure concentration of soluble form of adhesion molecule sVCAM-1 and chemokins: CXCL10, CXCL11, CXCL12, CXCL13 in serum and cerebrospinal fluid (csf) in patients with TBE before and after treatment. We also analyzed usefulness of measurement of concentration of these molecules in diagnostic and monitoring of inflammatory process during TBE.
Methods: Samples of serum and csf were taken from 23 patients hospitalized in The Department of Infectious Diseases and Neuroinfections of Medical University in Bialystok. Patients were divided into 2 groups:
i. patients with confirmed TBE(by detection antibodies against TBE in serum and csf)
ii. control group: patients with excluded TBE by csf evaluation.
In group I serum and csf were evaluated twice: at diagnostic evaluation and after resolving symptoms with control tests. In group II serum and csf – only once. Concentrations of analyzed molecules were measured by ELISA kits (Bender MedSystem, R&D).
Results: We observed increased concentration of soluble form of sVCAM-1, CXCL10, CXCL11, CXCL12, CXCL13 in serum and csf in group of patients with TBE. Concentration of CXCL10, CXCL11, CXCL12, CXCL13 in csf during convalescence decreased, but still was higher in comparison with controls. Higher concentrations of CXCL10, CXCL12, CXCL13 in csf than in serum were observed. Correlation between pleocytosis and concentration of CXCL10 in csf was noticed.
Conclusions:
1. Increased concentration of soluble form of sVCAM-1, CXCL10, CXCL11, CXCL12, CXCL13 in serum and csf, indicates participation this molecules in immunopathogenesis of TBE.
2. Decrease of concentration of CXCL10, CXCL11, CXCL12, CXCL13 in csf during convalescence proves delay in fading of inflammation, despite clinical recovery.
3. Significant increase of concentration of CXCL13 in csf in an acute period of disease in comparison with controls may indicate usefulness of this chemokine as a biomarker of inflammatory process in TBE.
4. Higher concentration of CXCL10, CXCL12, CXCL13 in csf than in serum may be a proof of its local production in central nervous system (CNS) or compartmentalization of inflammation process in neurological phase of disease.
5. Correlation between pleocytosis and concentration of CXCL10 in csf confirms role of this chemokine as a chemoattractant for lymphocytes in TBE.
6. Chemokines CXCL10, CXCL12, CXCL13 may be used as biomarkers of inflammatory process in CNS.

P1945 Caspase-3 activation induces cleavage of tau protein in neurons infected with herpes simplex virus type 1

C. Otth, R. Neira, R. Lerchundi, M. Villalba, K. Vio, M. Concha, A. Zambrano (Valdivia, CL)*

Objective: Herpes Simplex Virus Type 1 (HSV-1) is ubiquitous, neurotropic, and the most common pathogenic causes of sporadic acute encephalitis in humans. Herpes simplex encephalitis is associated with a high mortality rate and significant neurological, neuropsychological, and neurobehavioral sequelae, which afflict patients for life. HSV-1 infects limbic system structures in the central nervous system, and has been suggested as an environmental risk factor for Alzheimer's disease. In a previous study we demonstrated that HSV-1 triggers hyperphosphorylation of tau epitopes serine 202/threonine 205 and serine 396/serine 404 in neuronal cultures, resembling what occurs in neurodegenerative diseases. Therefore, the aim of the present study was to further evaluate at a cellular level other possible mechanisms associated with neurodegeneration triggered by HSV-1 infection in primary neuronal cultures, such as caspase-3 induced cleavage of tau.
Methods: Western blot and immunofluorescence techniques were used to study caspase-3 activation and tau protein processing in mice neuronal primary cultures infected with HSV-1.
Results: It is shown that HSV-1 infection induced caspase-3 activation and cleavage of tau protein at aspartic acid 421, generating an epitope that is recognized by TauC3 antibody. In agreement with our previous study on tau hyperphosphorylation, tau cleavage was also observed during the first 4 hours of infection, before neuronal death takes place.
Conclusions: Neuronal HSV-1 infection induces caspase-3 activation triggering the cleavage of tau at its specific site. This tau processing

has been previously demonstrated to increase the kinetics of tau aggregation and has been observed in neurodegenerative pathologies. Funding FONDECYT 11080067, DID-UACH.

P1946 Human metapneumovirus: interferon sensitivity and production in airway epithelial cells

C. Scagnolari, C. Selvaggi, T. Carbone, A. Soldà, L. Spano, P. Di Marco, F. Maggi, E. Riva, A. Pierangeli, G. Antonelli (Rome, Pisa, IT)*

Objectives: Actually very little is known about Human Metapneumovirus (hMPV) pathogenesis and the activation of innate immune response triggered by infection.
The aim of this study was to investigated whether hMPV is sensitive to the antiviral activity of interferon (IFN) β, leukocyte IFN α and several IFN α subtypes. The sensitivity of hMPV was compared with that of the vesicular stomatitis virus (VSV) which is known to be highly sensitive to the action of all types of IFN. In addition we evaluated the transcript expression of IFN α subtypes after hMPV infection.
Methods: Antiviral effect of IFNs against hMPV or VSV replication in human laryngeal carcinoma Hep-2 cells were analysed by yield reduction assay. The transcriptional induction of the IFN α subtypes in Hep-2 infected with hMPV or transfected with PolyIC were investigated by real time reverse transcript-PCR.
Results: Results indicated that IFNs showed substantially different capacities for inhibiting the hMPV yield in Hep-2 cells. In particular in Hep-2 cells pre-treated with IFNs and infected with hMPV for 24 hours, leukocyte IFN α were less potent than IFN β. Among IFN α subtypes investigated, IFN5, IFN6, IFN8 and IFN10 were the most active while IFN17 and IFN21 were the least potent. IFN1, IFN2b, IFN7, IFN14 had comparable intermediate activity. In addition results indicate that the IC50 values of the different type I IFNs preparations against VSV were significantly lower compared to those against hMPV. Furthermore we found that, over a timecourse of 48 hours of infection, lower levels of IFN α subtypes were transcribed in Hep-2 cells infected with hMPV in contrast with those induced by Poly IC.
Conclusion: This study provides evidence that hMPV are partially resistant to the antiviral activity of IFN type I, although some IFN α subtypes appear to be more active against hMPV than others. Furthermore hMPV appears to be a weak inducers of IFN type I too. The implications of these observations in terms of hMPV pathogenesis is now under study.

P1947 Immune profile in lymphoma patients with febrile neutropenia secondary to high-dose chemotherapy with autologous stem cell support

D. Torfoss, A. Høiby, H. Holte, J. Bjerner, T. Bjøro, G. Gaudernack, G. Kvalheim, T. Mollnes, S. Kvaløy (Oslo, NO)*

Objective: To study the immune profile during episodes of febrile neutropenia.
Methods: During the clinical study "Tobramycin once versus three times daily, given with penicillin G, to febrile neutropenic cancer patients in Norway: a prospective, randomized, multicentre trial" (J. Antimicrob. Chemother. 2007; 59: 711–7) blood samples were taken from lymphoma patients undergoing high-dose chemotherapy with autologous stem cell support when they developed febrile neutropenia and one to two days later. The samples were tested for procalcitonin, a 17 cytokine profile and mannan-binding lectin (MBL).
Results: Sixty-one patients constituted a homologous population with a mild and benign course of febrile deep neutropenia for more than a week and then recovering as expected. Four patients had a positive blood culture (Gram-positives only). There was a mild, but significant, increase in procalcitonin and the following cytokines: IL-1β, IL-4, IL-6, IL-7, IL-8, G-CSF, GM-CSF, INF-γ and TNF-α. There was a significant decrease in IL-5 whilst no change was observed in IL-2, IL-10, IL-12, IL-13, IL-17, MCP-1 and MIP-1β. Six patients were MBL deficient (<100 ng/ml) and another six patients had decreased MBL values, all without a significant different clinical course.
Conclusions: The profiles of procalcitonin, 17 cytokines and MBL suggest a mild proinflammatory response in lymphoma patients with fever of unknown origin and a benign course of febrile neutropenia after high-dose chemotherapy with autologous stem cell support.

P1948 sCD30: a novel prognostic factor for chronic brucellosis?

A. Rafiei, M. Hasanjani Roushan, Z. Hoseini-Khah (Sari, Babol, IR)*

Background: It is suggested that CD26 and CD30 are surface molecules expressed on activated Th1 and Th2 cells, respectively. The aim of the present study was the determination of the levels of soluble (s) CD26 and CD30 co-stimulatory molecules in sera of brucella-infected individuals. The correlations of sCD26 and sCD30 levels with clinical presentation of the disease were assessed.
Methods: The study included 90 brucellosis patients (56 acute disease and 34 chronic form) and 70 healthy controls. The levels of sCD26 and sCD30 were determined by a sandwich enzyme-linked immunosorbent assay in sera of study population.
Results: The serum level of sCD26 and sCD30 were differed in patients with brucellosis. Stratification of patients according to disease status showed significant higher levels of sCD26 in the acute brucellosis compared to chronic disease and controls ($P < 0.0001$). The highest significant levels of sCD30 were shown in patients with chronic brucellosis ($P < 0.0001$).
Conclusion: These findings indicate that sCD30 is more relevant to disease activity than sCD26 in patients with brucellosis. Therefore, sCD30 is a prognostic valuable marker for chronic brucellosis.

P1949 NK cells activity and IL-10 in gastric carcinoma patients with or without *Helicobacter pylori* infection

T.M. Karpinski, A. Szkaradkiewicz, M. Borejsza-Wysocki, M. Drews, P. Majewski (Poznañ, PL)*

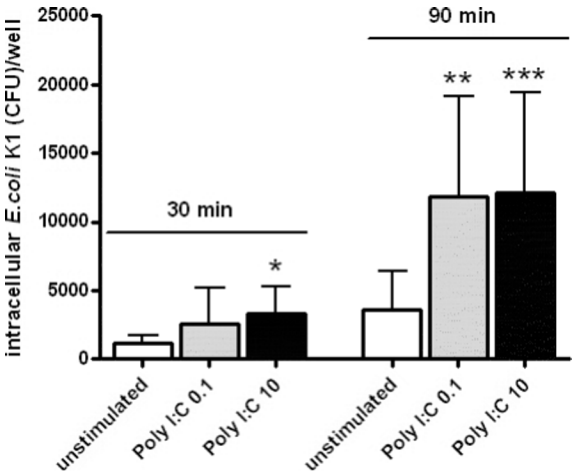
Objectives: The study aimed at analysis of NK cell cytotoxic activity and serum levels of IL-10 in patients with gastric carcinoma, as related to presence of infection with *H. pylori* and tumour advancement.
Methods: The study was conducted on 42 adult patients with gastric adenocarcinoma. Depending on parallel presence of *H. pylori* infection, was distinguished group1 of 15 patients with presence of *H. pylori* and group 2 of 27 patients without *H. pylori* infection. Histological analysis of the gastric tumours disclosed 5 patients with stages I-II and 10 with stages III-IV in group 1 and 9 patients with stages I-II plus 18 with stages III-IV in group 2. The investigated material included intra-operative samples of gastric carcinoma and peripheral blood of the patients. Presence of *H. pylori* in the samples was confirmed using cultures and biochemical tests. Estimation of serum IL-10 levels was conducted by ELISA, using Quantikine HS Human IL-10 Immunoassay kit (R&D System). NK cells were isolated using EasySep Human NK Cell Enrichment Kit (StemCell Technologies). Cytotoxic activity of NK cells toward gastric adenocarcinoma cells AGS (ATCC CRL-1739) was estimated using CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega).
Results: In group 1 cytotoxic activity of NK cells averaged at 36.5±15.2% and it did not significantly differ from that in the group 2. In parallel, mean cytotoxic activity of NK cells in group 1 patients with stages I-II amounted to 42.2±17.1%, and in those with stages III-IV to 31.1±11.6%, while in group 2 it was 38.3±11.3% in patients with stages I-II and 31.1±11.6% in patients with stages III-IV. Mean serum levels of IL-10 were 4.27±3.44 pg/ml in group 1, averaging at 2.38±3.47 pg/ml in the subgroup with stages I-II and at 5.21±3.18 pg/ml in the subgroup with stages III-IV. In group 2 mean levels of IL-10 amounted to 4.05±2.81 pg/ml, averaging at 2.93±2.58 pg/ml in the subgroup with stages I-II and at 4.61±2.82 pg/ml in the subgroup with stages III-IV. The analysis demonstrated no relationship between presence of *H. pylori*

infection in patients with gastric carcinoma on one hand and cytotoxic activity of NK cells or serum IL-10 levels on the other.
Conclusion: The result indicated that development of gastric carcinoma is linked to a significantly decreased cytotoxicity of NK cells and elevated serum levels of IL-10. However, the reduced cytotoxic activity of NK cells and elevated levels of IL-10 do not depend directly on *H. pylori* infection.

P1950 The viral TLR3 agonist poly(I:C) stimulates phagocytosis and intracellular killing of *Escherichia coli* by microglial cells

N. Adam, S. Ribes, S. Ebert, T. Regen, S. Bunkowski, U-K. Hanisch, R. Nau (Gottingen, DE)*

Objectives: *Escherichia coli* K1 meningitis is associated with a high rate of mortality and long term sequelae despite antimicrobial therapy, especially in pediatric patients. Microglial cells, the resident phagocytes in the central nervous system, express Toll-like receptors (TLR) that mediate the innate immune response upon recognition of invading pathogens. Polyinosine–polycytidylic acid [poly(I:C)] is a TLR3 agonist structurally similar to viral double-stranded RNA. Here, we show that a viral TLR3 agonist can stimulate microglial cells and increase their phagocytic activity and intracellular killing of *E. coli* K1, a pathogenic encapsulated bacterial strain.
Methods: Primary cultures of murine microglia were exposed to poly(I:C) at 0.1 or 10 mg/l for 24 h. A control group of unstimulated cells was included in all experiments. After stimulation, supernatants were collected and stored at −80ºC until measurement of cyto-/chemokine levels. Microglial cultures were co-incubated with live *E. coli* K1 for phagocytosis and intracellular killing assays at a ratio of 100 bacteria per cell. Phagocytosis was left to proceed for 30 and 90 min at 37ºC. For phagocytosis inhibition studies, cytochalasin D (CD) was used at 10 microM. For intracellular killing assays, cells were incubated with bacteria for 90 min. After bacterial exposure, microglial cultures were washed and cultured in medium containing gentamicin (200 mg/l). At various time points, cells were washed and lysed with distilled water. Viable intracellular bacteria were enumerated by quantitative plating of serial 10-fold dilutions. ANOVA (followed by Bonferroni's multiple comparisons test) was performed to analyse differences between groups ($n \geqslant 11$); $p < 0.05$ was considered statistically significant.
Results: The supernatants of unstimulated cells were devoid of measurable amounts of cyto-/chemokines. Unstimulated microglia ingested bacteria at a low rate. Poly(I:C) stimulated murine microglial cultures in a dose-dependent manner to secrete TNF-α and CXCL1 and increase their ability to phagocytose ($p < 0.05$ after 30 min, $p < 0.01$ after 90 min) and kill intracellular *E. coli* K1. CD blocked the bacterial uptake by ⩾90%.



Conclusions: Stimulation of microglia with a viral agonist of the TLR system such as poly(I:C) could increase the resistance of the brain to bacterial infections. This may be a promising approach to protect the brain of septicemic patients from meningitis, cerebritis and brain abscess.

P1951 *Yersinia* V-antigen binds to recombinant CD14 and TLR2

D. Reithmeier-Rost, T. Pirch, R. Darveau, K. Jung, J. Heesemann, A. Sing (Munich, DE; Seattle, US; Oberschleißheim, DE)*

Objectives: The virulence antigen LcrV of *Yersinia enterocolitica* O:8 (LcrVO:8) induces IL-10 in macrophages via Toll-like receptor 2 (TLR2) and CD14 thus causing TNF-α-suppression in mice.
Methods: In the present study, using ELISA and Biacore analysis it is shown that this effect is triggered by direct binding of LcrV to both TLR2 and CD14.
Results: The N-terminal, but not the C-terminal part of recombinant (r) LcrVO:8 seems to be responsible for TLR2- as well as CD14-binding, as rLcrVO:81–130 strongly binds to both a TLR2- or CD14-coated sensor chip, whereas rLcrVO:868–334 does not show any interaction. The affinity to TLR2 and CD14 seems to be *Y. enterocolitica* serotype-dependent, since rLcrVO:8 showed stronger affinity to both innate immune receptors when compared to rLcrVO:3. Moreover, the saturation level for rLcrVO:8 was higher than for rLcrVO:3. rLcrVO:8K42Q bearing a point mutation in the TLR2-active region showed rather strong affinity to TLR2. Derived from kinetic studies it could be demonstrated that rLcrVO:8 binding is kinetic-controlled, whereas the interaction of rLcrVK42Q with TLR2 and CD14 is more thermodynamically regulated. rLcrVK42Q even blocks the TLR2 receptor as the dissociation constant is very low. The mutagenesis of CD14 in single aa positions revealed the region responsible for LcrV-binding. Especially aa positions 11 and 37 seem to be important for the interaction indicating that ionic binding is involved. Furthermore, the active regions within CD14 for LPS-, BLP- and LcrV-binding seem to overlap.
Conclusions: LcrV binds to recombinant CD14 and TLR2.

P1952 Screening of the immunoprotective protein from the clinical strain of *Mycobacterium tuberculosis*

S. Suraiya, R. Suppian, J. Ashraful Haq, A.R. Zaidah, M. Musa (Kelantan, MY)*

One third of the world's populations have been exposed to *Mycobacterium tuberculosis*. Approximately 2 million people died each year from the disease. Tuberculosis (TB) was predicted to be the largest single infectious cause of death between 1990 and 2020. In Malaysia, BCG vaccination is mandatory, given to all newborn and will be repeated at 12 years old if no BCG scar observed during routine school children medical examination. The tuberculosis protection by BCG is questionable, but thus far no new TB vaccine available in the market.
The objective of the study was to compare the immune reaction towards the BCG strain as compared to circulating strain of *M. tuberculosis*.
Materials and Methods: The BCG strain and the clinical strain of M tuberculosis were culture in the Middlebrooks supplemented with OADC. The whole protein lysate from both were extracted and the protein profiles were studied by the SDS-PAGE. The serum sample were taken from BCG vaccinated, healthy and The western blotting for detection of IgG were done from pool sera of BCG vaccinated healthy person and pool sera of confirmed TB patients.
Results: The whole protein lysate was successfully extracted from BCG strain and the clinical strain of *M. tuberculosis*. There is higher level of existing immune response against clinical strains of M tuberculosis as compared to immune reaction towards the BCG strains. There is also presence of a specific band around 90KD that only present in the healthy subject but not in the TB patients.
Conclusion: There is a potential protein present in the circulating clinical strain that might have an immunoprotective component. This specific band attracts special interest in the development of DNA based tuberculosis vaccine in the near future.

Pathogenesis – epidemiology/virulence

P1953 Spread of Panton-Valentine leukocidin positive *S. aureus* among patients of a children's clinical university hospital, Riga, Latvia

L. Cupane, N. Pugacova, I. Selga, A. Balode, D. Gardovska, E. Miklasevics (Riga, LV)*

Objectives: Althought *S. aureus* is considered to be an opportunistic pathogen certain clones are more prone to cause invasive disease due to the presence of virulence factors like Panton-Valentine leicocidin (PVL). PVL producing strains can cause severe skin infections and necrotizing pneumonia in previously healthy children and young adults. Aim of this investigation was to detect spread of invasive *S. aureus* among paediatric patients at hospital and presence of PVL.
Methods: Antibacterial susceptibility was determined according to CLSI standards (M2-A9, M100-S16). The luk-PV gene was detected by PCR. Chromatograms of the spa sequences were analysed by Ridom StaphType software (Ridom GmbH).
Results: Investigation of 370 invasive *S. aureus* (21 blood isolate, 349 – from pus) from patients, who were admitted to Children Clinical University Hospital in Riga from November, 2006 to November, 2008 revealed that 241 (65%) isolates carried genes for PVL synthesis. 8 of them were identified as MRSA. Investigation of clonal relationship among the luk-PV positive *S. aureus* showed that majority of the typed strains belongs to the spa type t435, or closely related types. Retrospective analysis of patients medical histories from November 2006 throught March 2007 showed, that majority of patients were hospitalised in surgery department – 64%, others in therapeutical profile departments – 27%. Patients were hospitalised mainly with purulent skin and soft tissues infections like furunculosis, absceses, limfadenitis and mastitis.
Conclusions: the obtained data provides evidence for wide spread of PVL producing spa type-t435 staphylococci in community.

P1954 The prevalence of Panton-Valentine leukocidin toxin in *Staphylococcus aureus* from a Birmingham teaching hospital during three distinct time periods, 2002–2009

K. Hardy, S. Shabir, C. McMurray, M. Oliver, P. Hawkey (Birmingham, UK)*

Objectives: To investigate the prevalence and epidemiology of panton-valentine leukocidin (PVL)toxin carrying *Staphylococcus aureus* over a seven year time period.
Methods: Three *S. aureus* collections from distinct time periods from Birmingham, UK were examined for the presence of PVL toxin using PCR. Collection 1: 115 MRSA and 110 MSSA isolates from nasal screening samples from intensive care unit patients (Feb 2002 to Feb 2004). Collection 2: 793 MRSA isolates from nasal screening samples from surgical patients (Nov 2005 to May 2007). Collection 3: 412 MRSA and 410 MSSA strains from a mixture of clinical and screening samples (Dec 2008 to Apr 2009). All isolates were non-duplicate patient isolates. All PVL– *S. aureus* isolates were epidemiologically typed using staphylococcal interspersed repeat unit (SIRU) typing looking at seven variable number tandem repeats located around the genome, with a subset being analysed using MLST.
Results: Forty four (2.4%) of the 1831 *S. aureus* isolates screened were positive for PVL. The overall prevalence of PVL in MRSA isolates was 1.1% (14/1320), decreasing from 2.6% in the first collection to 0.9% and 1.0% respectively in collections 2 and 3. Six of the PVL-MRSA isolates were highly related (<2 loci different) by SIRU typing and were all ST22. Four of these isolates belonged to collection two. The prevalence of PVL within the MSSA isolates was 5.6% (29/520), increasing from 2.7% in collection 1 to 6.6% in collection 3. Ten of the isolates were closely related (<2 loci different), all belonged to ST22 and were all from collection 3. In addition four isolates were closely clustered (<1 loci different) and all were ST30, the second most predominant type. All of these isolates were from collection 3.
Conclusions: This study demonstrates that the prevalence of PVL amongst MRSA isolates has not increased over the last seven years, with the highest prevalence being observed amongst ITU screening samples from 2002–04. The prevalence of PVL amongst MSSA isolates showed an increase between the ITU screening samples and the clinical samples in the third collection, but this may have been due to the samples being from infected patients rather than screening for carriage. The most predominant MLST type (ST22) was the same for both MRSA and MSSA isolates and is the same as the most common UK hospital MRSA strain.

P1955 Determination of *Staphylococcus aureus* and presence of staphylococcal enterotoxin A by molecular and microbiological methods in meatball meals cooked by two-bedded treatment institutions

F. Uyar, C. Mirza, E. Ruh, B. Sener, A. Alp, Y. Beyhan (Ankara, TR)*

Objective: This study was planned and carried out for rapid determination of *Staphylococcus aureus* and Staphylococcal Enterotoxin A presence in meatball meals cooked in two different bedded treatment institutions (institution A and institution B) by using molecular and classical microbiological methods. Two genes, nucA and entA, were targeted in terms of *S. aureus* and Staphylococcal Enterotoxin A, respectively.
Methods: In this study, a total of 60 samples (30 samples from each institution) were included and the ability of real-time PCR technology for detection of nucA and entA genes was examined. FRET and TaqMan probes were designed for nucA and entA, respectively. Additionally, samples from meatball meals were also inoculated into sheep blood agars and *S. aureus* specific barid-parker media for further identification of *S. aureus*.
Results: By using real-time PCR, nucA and entA positive strains were detected in 23.1% and 16.5% of the samples from institution A and institution B, respectively. By the classical microbiological methods, *S. aureus* and *S. aureus* Enterotoxin A positive strains were detected in 16.5% and 6.6% of the samples collected from institution A and institution B, respectively. The results from molecular procedure were compared with those obtained from classical procedure and no statistical difference was reported ($p > 0.05$).
Conclusion: Our study shows that real-time PCR technology can succesfully be performed for rapid demonstration of *S. aureus* and enterotoxin A producing *S. aureus* strains in the food samples from food poisoning cases.
Our study also underlines the importance of the hygenic rules and sustainable training of personnel which should be ensured to prevent food poisoning cases in institutional food service systems.

P1956 Enterotoxins production, biotyping and genetic fingerprinting in *Staphylococcus aureus* isolated from milk and dairy products

A. Cataleta, E. Crisetti, L. Latorre, C. Nardella, S. Tola, G. La Salandra (Foggia, Sassari, IT)*

Objectives: *Staphylococcus aureus* is an important food-borne pathogen because of its ability to synthesize one or more staphylococcal enterotoxins (SEs). The present study aims to characterize 209 enterotoxigenic strains of *S. aureus* isolated from milk and dairy products during 2008–2009, in order to determine the ecological origin of the strains by biotyping and to obtain genetic fingerprinting, using pulsed field gel electrophoresis (PFGE).
Methods: Genomic DNA was extracted from overnight culture of *S. aureus* by boiling. The detection of the genes encoding for 12 SEs (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEM, SEN, SEO) was carried out using three multiplex PCR assays. All the 209 enterotoxigenic strains were biotyped by detection of some properties of

the strain tested and genotyped by PFGE after SmaI restriction treatment. PFGE banding patterns were identified by computer analyses (Applied Maths). Dendrograms were created using the unweighted pair group method using geometric averages (UPGMA) and Dice's coefficient.
Results: The results obtained indicate a prevalence of classical SEs encoding genes (sea, seb, sec, sed and see), and the high incidence of the "new" SEs encoding genes. Most of the isolated strains carried, alone or in association, the sed gene (42%), followed by sea (35.4%), sej (29.6%), ecg cluster: seg, sei, sem, sen, seo (26.8%), sec (23.2%), seh (5.7%), seb (3.8%) and see (1.9%). Of the 209 analyzed strains, 52.6% belonged to the Non-Host-Specific ecovar followed by the ovine (15.8%), human (13.9%), poultry-like (4.3%) and bovine (1.4%) ecovars. Same strains (12%) were not included in studied ecovars. All the strains were typable by PFGE and showed a great genetic heterogeneity both among the bovine and the ovine food isolates.
Conclusion: The results of this study remark the presence of enterotoxigenic strains of *S. aureus* in milk and dairy products. To correctly evaluate the foodborne risk linked to the consumption of foods contaminated with *S. aureus* it is crucial to monitor them to characterize the isolates and to gain information about their potential ability to synthesize SEs and their traceability; accordingly here we have applied a molecular approach for typing the 209 *S. aureus* strains. Further studies are required to better elucidate the relationship between the pulsotype and the presence of particular type of SEs.
This work was supported by IZS PB 006/08 RC.

P1957 Pneumococcal clones and the role of pili in invasive disease in Iceland, 1995–2008

K. Kristinsson, H. Erlendsdottir, M. Hjalmarsdottir, T. Gunnarsdottir, H. Jensdottir, H.D. Johannsdottir, G. Haraldsson (Reykjavik, IS)*

Objectives: The pneumococcal capsule is recognised as one of the most important virulence factor for invasive disease. Apparently, virulence may vary between certain clones belonging to the same capsular type. The importance of other virulence factors is not as clear. Pili have recently been recognised as potentially important virulence factors. Our aim was to analyse the pneumococcal clones causing invasive disease in Iceland, especially with regard to the presence of pili.
Methods: Invasive pneumococcal disease has been recorded for the whole Icelandic population since 1975. Since 1995 most of the invasive pneumococcal isolates (blood and CSF) have been collected and stored at −80ºC. We subcultured all viable isolates for serotyping and molecular typing using PFGE, after DNA restriction with the SmaI enzyme. MLST typing was performed on representative isolates of the most prevalent PFGE clones. The presence of pili was verified using the slightly modified PCR method of Paterson and Mitchell (2006) and the separation of the pili into clades was determined using the PCR method of Moschioni et al (2008).
Results: Of the 498 pneumococci isolated from invasive infections, 459 were from blood and 39 from CSF. 121 were from children <7 years old, 13 from 7–17 year old, 174 from 18–64 year old and 190 from >64 year old. Of the 491 that were serotyped, the most frequent types were 7F/7 (86), 14 (60), 4 (41), 9V (35), 6B (32), 23F (31), and 19F (19). PFGE typing has been performed on 460 (92%) isolates. The number of clones within serotypes varies significantly. For serotype 7F, all isolates belong to only two clones (ST191 and ST218). The prevalence of the most common clones varies between years, with a decline in the number of ST90 (type 6B) and ST218 (type 7F). Pilus genes could not be detected in the two most prevalent clones (ST191 and ST218). Of the 7 most common clones, pilus genes were detected only in ST162 (type 9V), ST205 (type 4) and ST90 (type 6B), the first two of clade I and the last of clade II.
Conclusion: The clonal diversity within serotypes varies between serotypes. Clonal incidence can change with time, which may not be reflected in serotype incidence. The presence of the pilus genes in only 3 of the 7 most common invasive clones, indicates that it is not important for invasiveness.

P1958 Temporal variations among invasive pneumococcal disease serotypes in children and adults and pneumococcal vaccine coverages in Germany from 1992 to 2008

M. Imöhl, R. Reinert, M. van der Linden (Aachen, DE; Paris, FR)*

Objectives: This study was performed to evaluate the serotype distribution of *S. pneumoniae* among isolates with invasive pneumococcal disease (IPD) that were sent to the German National Reference Center for Streptococci (NRCS) between 1992 and 2008, analyse temporal trends and, supplementary, calculate the vaccine coverages for the 7-valent, 10-valent and 13-valent (in development) pneumococcal conjugate vaccines.
Methods: A population and laboratory based surveillance study of invasive pneumococcal disease was conducted in Germany. Cases from January 1, 1992 to December 31, 2008 were included in this study.
Results: A total of 12,137 isolates from invasive pneumococcal disease was collected between January 1, 1992 and December 31, 2008. Data on serotypes were available for 9,394 isolates. The leading serotypes were serotypes 14 (16.5%), 3 (8.0%), 7F (7.6%), 1 (7.3%) and 23F (6.0%). Concerning the epidemic serotypes 1–3 and 5, a slight increase was noticed during the last years, reaching levels around 20% among children and adults in 2008. Variations in serotype distribution over the years are partly extensive, especially concerning serotype 14 (min 7.4%, max 33.5%). Serotype 14 is considerably more frequent among children (22.5%), than among adults (13.7%). Serotype 1 and serotype 7F have been increasing clearly over the last approximately 10 years, both among children and among adults. Serotype 3 is far more common among adults (10.2%) than among children (3.3%). The increase observed during the last years seems to be slightly higher among adults than among children. Concerning serotype 19A no clear change in frequency can be observed during the period under study. Variations in serotype distribution also affect theoretical vaccine coverages. The overall serotype coverage for the 7-valent conjugate vaccine was 45.4% during 1992–2008. For the 10-valent vaccine and the 13-valent vaccine (in development) the coverages were 60.9% and 76.6%, respectively.
Conclusions: Temporal changes in the incidence of different serotypes have been reported from different countries. However, in particular against the background of the general recommendation of pneumococcal conjugate vaccination for children <2 years in Germany at end of July 2006, ongoing nationwide surveillance is necessary to observe further developments of pneumococcal serotype distribution in Germany.

P1959 Allelic variation in internalin genes of *Listeria monocytogenes* isolated from humans, small rodents and mollusks

E. Zaytseva, S. Ermolaeva, G. Somov (Vladivostok, Moscow, RU)*

L. monocytogenes is facultative intracellular pathogen, which causes an invasive disease with such severe symptoms as septicemia, meningitis, meningoencephalitis and stillbirths in human and animals. The surface and secreted proteins of the internalin family play a main role in *L. monocytogenes* invasion into eukaryotic cells. The structure of internalins includes LRR- (for leucine-rich repeat) domains. Among both eukaryotes and prokaryotes the LRR domains are involved in specific protein-protein interactions.
The purpose of this study is to analyze distribution of various internalin gene alleles among *L. monocytogenes* isolated from different sources and regions.
Methods: 50 and 17 *L. monocytogenes* isolates obtained in Far East and in European parts of Russia, respectively, were investigated. Four internalin genes – inlA, inlB, inlC, inlE, and the housekeeping gene prs, were partly sequenced. The study included three host specific groups of invasive isolates obtained from tissues of aborted fetuses (n = 19), small rodents (n = 13), marine organisms (n = 13). Control groups included isolates obtained from food (n = 22).
All isolates were divided into 32 sequence types (STs) according to results of sequencing. Geographic and host specific allelic variations in *L. monocytogenes* genes encoding invasion factors of the internalin family were revealed. The region specificity of STs to suggests the

polyclonal distribution of *L. monocytogenes* strains in geographically distant regions. Some STs were isolated from particular hosts with high frequency although they were not host specific. There were ST1, ST6 and ST9 among isolates from aborted fetuses, ST1 among rodent isolates and ST17 among marine organism isolates.
The analysis of allelic variations in individual genes revealed a statistically relevant host specific prevalence of particular alleles for the following genes: inlA and inlC among aborted fetus isolates, inlB among rodent isolates, inlA, inlC, inlE among marine isolates. Alleles of all tested genes were distributed uniformly in control group. Alleles of the prs gene were distributed uniformly in all groups. Obtained results confirm with the knowledge on role of internalins in host specificity of *L. monocytogenes.*

P1960 *Helicobacter pylori* cagA subtyping and its role in identification of mixed infections

Z. Karimi, M. Douraghi, Y. Talebkhan, S. Saberi, M. Bababeik, M. Mohammadi (Tehran, IR)*

Objectives: Cytotoxin-associated gene A product (CagA) is one of the most important virulence markers in pathogenesis of *Helicobacter pylori* (Hp) strains. Different investigations show that 60% of Western and approximately all of the East Asian *H. pylori* strains are cagA positive. Variation in the type and number of EPIYA-motif in the 3' end of CagA has provided this protein with varying degrees of risk in cagA+ strains of different geographical regions. The Hp cagA subtype status and the presence of multiple infections among Iranian GI patients were investigated in this study.
Methods: Totally, 202 *H. pylori* infected patients including 38 GC, 34 PUD, 130 NUD were enrolled in this study. cagA diversity was determined in 585 Hp isolates from at least two biopsy specimens from different locations of the stomach. PCR amplification was performed using primers cag2/cag4. SPSS package was used for data analysis.
Results: Based on multiple biopsy sampling, 54 percent of the examined patients suffered from multiple infections (harboring more than one cagA subtypes) while this percentage had been diminished to 12.8% when just one antral biopsy specimen was obtained. Of these 202 patients, 585 isolates were recovered which produced 711 Hp strains of which 659 (92.7%) were cagA-positive distributed in the following nine different categories: 400bp (1.2%), 450bp (4.7%), 500bp (2.7%), 550bp (56.3%), 600bp (2.3%), 650bp (28.5%), 750bp (3.8%), 800bp (0.2%), 850bp (0.3%).
Conclusion: According to this investigation, approximately half of Iranian dyspeptic patients were infected with more than one cagA subtype Hp strains which thereby identifies cagA typing as an accessible marker for detection of mixed infections. Furthermore, these findings indicated that obtaining more than one biopsy sample from each patient and assessing multiple strains from one location are critical for accurate identification of the infecting cagA subtypes.

P1961 *Helicobacter pylori* vacA and cagA gen: changes over a period of 15 years in Spain

S. Agudo, T. Alarcón, P. Urruzuno, G. Pérez-Pérez, M. López-Brea (Madrid, ES)*

Introduction: The presence of cytotoxin-associated gene A (cagA) and expression of vacuolating cytotoxin activity encoded by vacuolating cytotoxin gene A (vacA) are considered the two major virulent markers of *H. pylori*. VacA and CagA of *H. pylori* strains showed genetic variability. The aim of this study was to determine the prevalence of CagA and VacA genotypes among *H. pylori* isolated in Madrid, Spain over two periods: 1994 and 2008.
Methods: We obtained 64 biopsies from symptomatic gastric patients from four different regions (Madrid, Aviles, Almeria and Ibiza) of Spain in 1995 and 90 biopsies from patients with gastric symptoms from Madrid, Spain in 2008. Standard microbiological procedures were used for *H. pylori* culture. DNA extraction was carried out with phenol/chloroform/isoamil alcohol (25:24:1) in 1994 and by the NucliSens easyMAG platform with the NucliSens magnetic extraction reagents (bioMérieux) in 2008. VacA genotypes (s, m) were determined by PCR and agarose gel. CagA status was determined by PCR and those CagA negative, we confirmed the absence of the pathogenicity island by "empty-site" PCR. The identification of the number and type of CagA EPIYA motifs was based on sequencing analyses.
Results: The results of VacA are showed in the table 1. Around 27% biopsies were cagA gene positive in strains from Madrid 1994 and 2008. 69% were cagA gene positive in strains from the rest of regions in 1994. We confirmed the lack of pathogenicity island in the rest of strains that were CagA negative. CagA with three EPIYA motifs (ABC) was observed in 53% and 68% of CagA positive strains in Madrid 1994 and 2008, respectively.
Conclusions: In Madrid, Spain most of *H. pylori* isolates are VacA $s2/m^2$ and CagA negative in both periods of time, different to the rest of the regions in 1994. The prevalence of CagA proteins with three EPIYA motifs (ABC) was the most frequently found.

	n	s1m1/m2		s2m2		s2m1	
		n	%	n	%	n	%
Madrid	22	5	22.7	16	72.7	1	4.5
Almeria	20	14	70.0	5	25	1	4.5
Aviles	6	2	33.3	3	50	1	16.7
Ibiza[1]	16	10	62.5	2	12.5	2	12.5
Madrid 2008[2]	90	23	25.6	64	71.1	2	2.2

[1] 2 strains showed mixed signals; [2] 1 strain showed mixed signals.

P1962 Distribution of putative iron-utilization genes in European clinical strains of *Acinetobacter baumannii*

L.C. Antunes, F. Imperi, K.J. Towner, P. Visca (Rome, IT; Nottingham, UK)*

Objective: Iron is an essential nutrient for bacteria and, as such, bacterial iron uptake mechanisms are important factors for pathogenesis. A gene cluster in *Acinetobacter baumannii* is known to be responsible for synthesis of the siderophore acinetobactin. Genomic analysis of sequenced *A. baumannii* strains enabled us to identify further gene clusters putatively involved in biosynthesis/transport of other siderophores, haem utilisation and ferrous iron uptake. The aim of this study was to investigate the distribution of these genes in a collection of 42 representative *A. baumannii* strains from 17 European countries.
Methods: PCR primers were designed to screen for 16 of the putative iron-utilisation genes identified from the genotypic sequence analysis. These primers were used in standard PCRs to screen a diverse collection of 42 European clinical *A. baumannii* strains of known sequence groups. In addition, the reference strain ATCC 19606 and the sequenced and annotated strains ACICU, AYE, ATCC 17978 and SDF were also investigated. The distribution of iron-utilisation genes among the different strains and sequence groups was analysed.
Results: Two putative siderophore gene clusters, including that coding for acinetobactin, were conserved among the clinical isolates of *A. baumannii*, while a third cluster was present in less than 5% of strains. Furthermore, a putative haem utilisation gene cluster was conserved in all strains, while a second haem utilisation cluster was found in approximately 50% of strains. Lastly, the Feo cluster, putatively coding for genes involved in the uptake of ferrous iron, was conserved in all strains. Notably, no apparent correlation was found between the sequence group (lineage) of the strains and the presence/absence of specific iron-utilisation genes.
Conclusions: The apparent universal distribution of the acinetobactin gene cluster, an additional and previously undescribed siderophore cluster, the Feo cluster for the uptake of ferrous iron, and one of two haem uptake clusters, indicates their potential importance for the survival and/or virulence of *A. baumannii* in the clinical setting. It remains to be determined if a second haem uptake cluster, present in approximately

50% of strains, and/or a third siderophore cluster, present in less than 5% of strains, further enhance the survival and/or virulence properties of specific *A. baumannii* strains.

Pathogenesis – bacteria

P1963 Impact of genes upstream of the RD1 region on virulence of *Mycobacterium tuberculosis* and ESAT-6 secretion/modification

D. Bottai, L. Majlessi, R. Simeone, W. Frigui, C. Laurent, P. Lenormand, M. Huerre, C. Leclerc, S. Cole, R. Brosch (Paris, FR; Lausanne, CH)*

The pathogenesis of *Mycobacterium tuberculosis* largely depends on the secretion of key virulence factors, such as ESAT-6 (EsxA) and CFP-10 (EsxB) via the ESX-1/T7SS system. Several genes located in the region of difference 1 (RD1) have been shown to play a role in this process, but little is known about the genes situated further upstream of RD1. Thus, in an attempt to elucidate the role of genes rv3865 and rv3866, in the secretion of ESAT-6 and virulence of *M. tuberculosis*, we constructed rv3865/66 and large scale RD1 knock-out *M. tuberculosis* strains and tested these mutants in various *in vitro* and *in vivo* systems. This analysis showed that inactivation of either of the two genes has a strong attenuating effect on the virulence of *M. tuberculosis*. However, unlike their orthologs in the faster growing model organisms *Mycobacterium smegmatis* and *Mycobacterium marinum*, rv3865 and rv3866 of *M. tuberculosis* do not impact on the secretion and T cell recognition of ESAT-6, but rather contribute to the pathogenicity via an as yet unknown mechanism. These findings indicate that in spite of many similarities, specific differences of the ESX-1/T7SS system exist between *M. tuberculosis* and other less virulent mycobacteria that may reflect the different adaptation processes undergone by each these species.
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P1964 Intracellular penetration capacity of *Mycobacterium tuberculosis* drug-resistant isolates using a murine macrophage model

E. Rey, G. Tudó, S. Borrell, F. Alcaide, G. Codina, P. Coll, N. Martín, V. Mick, M. Montemayor, M. Salvadó, E. Vicente, J. González (Barcelona, ES; London, UK)*

Objectives: To determine the penetration capacity into macrophages of *Mycobacterium tuberculosis* isolates resistant to different drugs compared with susceptible clinical isolates.
Methods: *Isolates*: Seventeen susceptible isolates were collected from tuberculosis (TB) patients of the Hospital Clinic of Barcelona. Forty-two isolates resistant to isoniazid (H) and/or rifampicin (R) were selected from an isolate collection of TB patients attended in five university hospitals of the Barcelona area according to their molecular mechanism associated with resistance. Of these, 5 isolates showed mutation in the katG gene, 11 mutation in the inhA gene, 5 mutation in the rpoB gene, 11 mutations in the katG gene and rpoB and 10 were wild-type at these positions. Mutations in the katG gene were at position 315.
Measurement of M. tuberculosis penetration in macrophages: Murine macrophages from the cell line J774.2 were used as an *in vitro* infection model. The cell monolayer was infected with *M. tuberculosis* isolates in duplicate using a 1:1 MOI. After 3 hours of incubation, the monolayers were washed to remove extracellular bacteria. The monolayers were then lysed. Cellular lysates and inoculum were plated on Middlebrook 7H11 plates and incubated at 37ºC, being read at the end of 3 and 4 weeks. Penetration (%) was calculated as: total CFU cellular lysates / total CFU inoculum.
Results: Lower penetration percentages were observed in resistant isolates inside the macrophages compared with susceptible isolates ($p < 0.05$). H-resistant isolates with mutation in the katG gene and multidrug-resistant (MDR) isolates with mutation in the rpoB and katG genes had a lower penetration percentage than isolates with other mutations or susceptible isolates ($p < 0.05$). Resistant isolates with mutations in the inhA or rpoB genes or resistant without detected mutations had a similar behaviour to that of susceptible isolates. No significant difference was found between isolates with mutation in the katG gene and isolates with mutations in the katG and rpoB genes.
Conclusions: H-monoresistant and MDR isolates of *M. tuberculosis* with mutation at codon 315 in the katG gene have a lower penetration capacity inside murine macrophages, which could be associated with lower virulence. The lack of a significant difference between isolates with a katG mutation and with katG and rpoB mutations suggests the absence of an accumulative effect.

P1965 The transcriptome of extraintestinal pathogenic *Escherichia coli* in response to human serum

N. Cooke, S. Smith, T. Rogers (Dublin, IE)*

Objectives: *Escherichia coli* infections of the bloodstream often originate from the urinary tract, and are associated with high morbidity, mortality and economic cost. The emergence of multidrug resistant strains highlights the need for alternative antimicrobial strategies. The ability to survive in serum is a key attribute in the pathogenesis of uropathogenic *E. coli* (UPEC) related sepsis. It is unknown how UPEC responds to assault by serum at a gene transcription level. The aim of this study is to characterise the transcriptome of *E. coli* when exposed to human serum.
Methods: The bactericidal activity of serum collected from healthy donors was verified. Next, RNA from virulent UPEC strain CFT073, exposed to normal human serum (NHS) and heat-inactivated NHS (HIS), was extracted. RNA was labelled and hybridised to a CFT073 expression microarray. Transcriptional profiles in NHS and HIS were compared. qRT-PCR was employed to validate expression results against an endogenous control. Highly upregulated genes of interest were assessed for a direct role in serum resistance by knockout mutational studies.
Results: Expression microarray analyses revealed 97 and 58 genes were respectively upregulated and downregulated by $\geqslant$3-fold in CFT073 exposed to NHS. Many genes with the highest expression fold differences clustered within functional operons. Among the highly upregulated genes there was a bias towards carbohydrate metabolism systems (fructose, maltose, trehalose) and also, pyruvate formate lyase and colanic acid biosynthesis. Downregulated genes included those for OmpF, fatty acid transport and, ascorbate and galactosamine metabolism. Results from the microarray were verified by qRT-PCR. Knockout mutational studies revealed that the viability of CFT073 mutants lacking gmd (colanic acid biosynthesis), malE (maltose metabolism), yjbE (exopolysaccharide production) or ybiW (pyruvate formate lyase) in NHS were significantly decreased in comparison to the wild-type ($P = 0.0001$, 0.0001, 0.0022 and 0.0025, respectively).
Conclusions: We have identified four genes, gmd, malE, yjbE and ybiW, to have a direct and significant role in the ability of CFT073 to resist the lytic action of NHS. These genes may represent novel virulence-specific therapeutic targets as alternatives to antimicrobial therapy of *E. coli* sepsis.

P1966 *Escherichia coli* clinical isolates causing febrile urinary tract infection. *In vivo* acquisition of a pathogenicity island

S. Soto, P. Ulleryt, S. Zúñiga, J. Vila (Barcelona, ES; Boras, SE; Valencia, ES)*

Objectives: Uropathogenic *Escherichia coli* (UPEC) strains are the most frequent cause of urinary tract infections (UTIs). The persistence of the same *E. coli* strain in the urinary tract may be the cause of recurrent prostatitis. We analysed *E. coli* clinical isolates causing febrile UTI in men, collected previously and posterior to treatment with ciprofloxacin. The aim of this study was to determine if UTI recurrence is associated with relapse or re-infection and evaluate the possible effect of virulence factors of the isolates causing these infections.
Methods: A total of 30 *E. coli* isolates were collected from 15 patients showing febrile UTI in the Shalgrenska University Hospital, Sweden. The first isolate from each patient was collected before antimicrobial treatment with ciprofloxacin and the second after this treatment. The

isolates were analysed by PFGE, REP-PCR and MLST in order to determine the epidemiological relationship. Phylogenetic group and virulence factors were analysed by PCR using gene specific-primers. The whole genome of the 14A and 14F isolates was sequenced using the SOLID procedure.
Results: The two isolates were completely similar and were considered as relapse in only five patients (33.3%), while the first isolates collected from the other 10 patients (66.6%) were different fromthose collected after treatment, considering these cases as re-infections. On comparing the isolates causing relapse from each patient the virulence profile was similar in all cases, except one. However, in the case of isolates 14A and 14F, the first (14A) lacked hly, cnf1, hra and P-fimbriae with respect to the second (14F), all these factors belonging to a pathogenicity island. The isolates 14A and 14F were analysed by MLST to prove their similarity. The results obtained confirm that they are the same strain. Using SOLID sequencing the 14F isolate presented 92 genes which were not presented by the 14A isolate. Among these genes the pefC fimbria, the perC transcriptional regulator, the operon TetR, the CCT cytotoxin and the heat-resistant agglutinin 1 were found.
Conclusion: The results obtained show that horizontal transfer of virulence factors contained in pathogenicity islands had taken place. This fact allows the bacteria to become more virulent and, therefore, to cause a greater damage or colonise new niches.

P1967 Role of EspFu/Tccp in the establishment of the attaching and effacing lesion caused by atypical enteropathogenic *Escherichia coli* displaying different interactions with epithelial cells *in vitro*

S. Rocha, C. Abe, S. Bando, V. Sperandio, W. Elias (Sao Paulo, BR; Dallas, US)*

Enteropathogenic *Escherichia coli* (EPEC) has been classified as typical and atypical (aEPEC), based on the presence or absence of the EAF plasmid, respectively. EPEC cause the attaching-effacing (A/E) lesion on the intestinal mucosa, which is triggered by proteins encoded by the pathogenicity island named LEE. However, some aEPEC strains are unable to cause A/E *in vitro*, despite the presence of LEE.
Objective: Structural and functional analysis of the LEE region of A/E-negative aEPEC strains.
Methods: Four aEPEC strains were studied: O55:H7 (displaying the localized-like adherence pattern/LAL), O55:H7 (displaying the diffuse adherence pattern/DA), O125ac:H6 (displaying the aggregative adherence/AA) and O88:HNM (nonadherent/NA). Adherence and capacity to cause A/E (FAS assay) were investigated in HeLa, HEp-2, Caco, T84 and HT29 cells, and Tir phosphorylation in HEp-2 cells. The espFu/tccp gene was searched by PCR. Transcription of LEE operons was measured by real time PCR (qRT-PCR). Expression of intimin, Tir, EspA, EspB and EspD was detected by immunoblotting. A EspFu-expressing plasmid (pKC471) was used to transform the A/E-negative aEPEC strains.
Results: The adherence patterns observed in HEp-2 and HeLa cells were maintained in all cell lines of intestinal origin. The capacity to cause A/E, to phosphorylate Tir and the presence of espFu/tccP was detected only in the LAL-expressing strain. Transcriptional profiles of LEE as measured by qRT-PCR were analysed in comparison to the atypical EPEC strain BA320 (LAL/FAS+). LEE 1−5 transcription levels were decreased in the AA, DA and NA strains in both culture conditions (DMEM and HeLa cells), except for LEE 4 (espA) which showed higher transcription level in the DA strain in DMEM. All four aEPEC strains studied expressed intimin, Tir, EspA, EspB and EspD. The DA and NA strains harbouring espFu/tccp were able to cause A/E in HeLa and Hep-2 cells. The presence of espFu/tccp was not efficient to induce the A/E lesion *in vitro* by the AA strain.
Conclusions: Despite the incapacity to cause A/E, the transcription and expression of LEE 1−5 demonstrated that LEE is functional in these strains. The incapacity to cause A/E *in vitro* by the DA and NA aEPEC strains is due to the absence of espFu/tccp. The complete mechanism involved in the establishment of A/E by the AA aEPEC of serotype O125ac:H6 remains unknown.

P1968 The anti-aggregation protein dispersin is not involved in the establishment of the diffuse and non-characteristic adherence patterns of *Escherichia coli* on HEp-2 cells

L. Bevilacqua, F. Vasconcellos, W. Elias (Sao Paulo, BR)*

The anti-aggregation protein dispersin is a 10.2-kDa protein encoded by the aap gene. This protein is an important virulence factor of enteroaggregative *Escherichia coli* (EAEC) and was demonstrated to be immunogenic in studies with human volunteers. Mutants in the aap gene express a very intense autoagglutination, indicating that dispersin acts re-covering the surface of the bacterium, diminishing the autoaggregation and allowing the bacteria to disperse along the intestinal tract. Recent findings of our group demonstrated that the aap gene is not exclusive of EAEC, since it was also detected in diffusely adherent *E. coli* (DAEC) and non-pathogenic *E. coli* displaying a non-characteristic adherence pattern to HEp-2 cells.
Objective: Determine the role of dispersin in the establishment of the diffuse and non-characteristic patterns of adherence to HEp-2 cells expressed by *E. coli* strains that harbour the aap gene.
Methods: Three aap-positive *E. coli* strains isolated from children with diarrhea were selected. These strains display the aggregative adherence (AA) pattern (strain 268), the diffuse adherence (DA) pattern (strain 84) and a non-characteristic (NC) pattern (strain 901) on HEp-2 cells. The aap genes of these strains were mutagenized using the suicide vector pJP5603. An internal fragment of aap was amplified by PCR using the EAEC prototype strain 042 as template and cloned in the suicide vector. Conjugation of *E. coli* DH5α lambda-pir harbouring this construct and the wild type strains led to the aap knockout by homologous recombination. The effect of aap inactivation on the adherence ability of the wild type strains was evaluated on HEp-2 cells (3- and 6-h assays).
Results: The correct inactivation of aap in the transconjugates obtained was confirmed by PCR and Southern-blot analysis. The mutants selected were named 268::pLB2, 84::pLB2 and 901/Nal::pLB2. These mutants were tested in HEp-2 cells adherence assays with incubation periods of 3 and 6 h. The results demonstrate that the mutation in aap abolished the AA pattern of the 268::pLB2 mutant, which did not occur in the other two mutants expressing DA (84::pLB2) and NC (901/Nal::pLB2).
Conclusions: the product of the aap gene (dispersin) is involved in the establishment of the AA phenotype, but not in the DA and NC, which are mediated by uncharacterised adhesins.

P1969 Evaluation of frequency of secreted proteins (EspA, EspB, EspD and EspC) and actin polymerization in enteropathogenic *E. coli* isolated in Peru

C. Contreras, V. Bustamante, T. Ochoa, C. Lanata, T. Cleary, J. Puente (Lima, PE; Cuernavaca, MX; Houston, US)*

Objective: The aims of this study is to describe the diversity and relationship between protein secretion of *E. coli* secreted proteins (EspA, EspB, EspD and EspC) and the ability of adherent strains to cause A/E lesions was evaluated by the FAS (Fluorescent actin stain) test, in enteropathogenic *Escherichia coli* (EPEC) isolated in the population.
Methods: Protein secretion (EspA, EspB, EspD and EspC) was analysed in 57 (42 from diarrhea cases and 15 from controls) representative isolates of 120 EPEC strains isolated from children with and without diarrhea under 1 years of age living in Lima, Perú. FAS test was evaluated in 69 (46 from diarrhea cases and 23 from controls) representative strains. Only isolated from episodes with infections due to a single pathogen were evaluated in all cases. The characterisation among typical EPEC (tEPEC) and atypical EPEC (aEPEC) were determined by presence of bfpA gene evaluated by PCR.
Results: EspB/D was the most frequently protein recovery in both groups diarrhea 55%(23/42) and control 27%(4/15); same similarity was observed in tEPEC 7/10 (70%) and aEPEC 20/47 (43%). The protein EspA was more frequently found in diarrhoea episodes than controls (21/42, 50% vs 0/15, 0%; $P < 0.05$). The frequency of EspA among tEPEC y aEPEC was similar. The EspC protein have similar frequency among diarrhea cases 15/42 (36%) and controls 3/15 (20%). The strains

that secreted all proteins were related whit diarrhoea cases compared with controls (17/42, 19% vs 0/15, 0%; $p < 0.05$). In all strains analyzed for FAS test we observed a statistical relationship among tEPEC and actin polymerization ($p < 0.05$); whereas the frequency of actin polymerization is similar between diarrhoea cases and controls (8/46, 17% and 2/23 9%, respectively). The high percentage of strains do not polymerized actin (38/46, 83% in diarrhea cases and 21/23, 91%).
Conclusions: Our finding indicates that few EPEC strains secreted all proteins evaluated in this study. EspA is an important marker for virulent EPEC in human isolates. The small frequency of actin polymerization among the isolates is due to the small frequency of tEPEC in our population.

P1970 Relation between 20-kDa polysaccharide and polysaccharide intercellular adhesin of *Staphylococcus epidermidis*

A. Spiliopoulou, F. Kolonitsiou, M.I. Krevvata, N.K. Karamanos, G. Dimitracopoulos, L.G. Harris, T.S. Wilkinson, A.P. Davies, D. Mack, E.D. Anastassiou (Patras, GR; Swansea, UK)*

Objectives: Extracellular polysaccharide molecules from *S. epidermidis* have proved notoriously difficult to characterise as numerous original descriptions of unique molecules have ultimately been confirmed to be closely related or identical to Polysaccharide Intercellular Adhesin (PIA). In the present work (ESCMID09 Fellowship) we have investigated the relation of 20-kDa PS and PIA.
Methods: 75 CoNS strains isolated from blood cultures and central venous catheter tips in the Clinical Laboratory of the General University Hospital of Patras, Greece, were identified at the species level and screened for biofilm formation, icaADBC presence, and 20-kDa PS expression. Nine different *S. epidermidis* reference strains were also used in the present study. Parallel screening of reference and selected clinical strains for 20-kDa PS and PIA using specific anti-20-kDa PS and anti-PIA rabbit antisera was performed. Susceptibility of both antigens to certain chemicals and enzymes was examined by treatment of bacterial suspensions with sodium meta-periodate, proteinase K and specific N-acetylglucosaminidase, Dispersin B (DspB). Antigen detection was accomplished by immunofluorescence microscopy and ELISA.
Results: Among the clinical isolates, 20 strains were found to be ica+biofilm+20kDaPS+, 10 ica−biofilm−20kDaPS−, 6 ica+biofilm−20kDaPS+, 6 ica−biofilm−20kDaPS+, 5 ica+biofilm−20kDaPS− and 5 strains ica+biofilm+20kDaPS−. All other CoNS were ica−20kDaPS−. All PIA positive reference strains (1457, 8400, and 9142) were 20-kDa PS positive, whereas, a PIA negative strain (1585) was also 20-kDa PS negative. Expression of 20-kDa PS does not seem to be related to icaADBC activity or biofilm formation as isogenic icaA::Tn917 PIA-negative mutants (1457-M10 and 8400-M10) were still 20-kDa PS positive. 20-kDa PS is resistant to treatment with proteinase K, sodium meta-periodate and DspB, whereas, PIA is resistant to proteinase K but sensitive to sodium meta-periodate treatment and DspB. Fractions of Q-Sepharose containing the PIA-antigenic peak were devoid of 20-kDa PS. Finally, the two molecules have distinct antigenic properties, as shown by cross absorption experiments.
Conclusions: 20-kDa PS is a main antigenic determinant of *S. epidermidis*. Our data show that 20-kDa PS is distinct from PIA. It is therefore of great importance to elucidate its biologic properties and role in pathogenesis of biomaterial-associated infections by constructing isogenic mutant(s) devoid of 20-kDa PS.

P1971 Nasal carriage of *Staphylococcus aureus*: a histological approach

N.J. ten Broeke-Smits, J.A. Kummer, A.C. Fluit, E. Boel (Utrecht, NL)*

Objectives: Nasal carriage of *Staphylococcus aureus* is an endemic risk factor for blood-borne and surgical site infections. Around 20% of the human population is persistent carrier of one type of strain and an additional 60% is intermittent carrier of varying strains. Decolonization with topical antibiotics like mupirocin, is rather effective, however relapses are common within months. Nasal colonization is thought to occur in the vestibulum nasi. It has been hypothesized that intermittent carriers could be mucosal carriers, while persistent carriers could use a niche for *S. aureus* cells. However, the exact niche for *S. aureus* to reside in the nose is currently not known. To determine this niche, we performed a study in which sections of different parts of the nose were stained with antibodies against staphylococcal protein A (SpA).
Methods: Study objects were human bodies donated for scientific purposes. After nasal swabs for culture of *S. aureus* were taken, noses were removed anatomically, vertically dissected, formalin fixated and embedded in paraffin. We used 9 out of 37 culture-positive noses and 8 culture-negative noses for staining. To stain for *S. aureus*, sections were incubated with anti-SpA (1 mg/ml), followed by Powervision Goat anti-Mouse/Rabbit/Rat IgG and stained with 3′,3′-Diaminobenzidine (DAB). Sections were counterstained with haematoxylin and visually inspected with light microscopy.
Results: *S. aureus* was detected in the region of the vestibulum nasi in 8 of the 9 culture-positive noses. The majority of the bacteria were found within the cornified layer of the stratified squamous epithelium and in the associated keratin and mucous debris within the vestibulum. Interestingly, in some cases the bacteria were also detected in the outer portion of the hair follicle shafts. No bacteria were detected in the ciliated mucosa covering the major part of the nose or its associated serous glands. Negatively cultured noses did not show stained bacteria indicating that the primary antibody was specific for *S. aureus*.
Conclusion: This study revealed the presence of *S. aureus* in the cornified layer and associated debris as well as in hair follicles in the nasal cavity. This shows that colonization of the nose is indeed restricted to the vestibulum nasi.

P1972 Correlation of biofilm formation with agr polymorphism and quantification of RNAIII in *Staphylococcus aureus*

E. Bae, M.Y. Lee, M.N. Kim, S.H. Lee (Seoul, KR)*

Objective: The purpose of this study is to measure the effect of activity and polymorphism of accessory gene regulator (agr) on production of biofilm in *Staphylococcus aureus*.
Methods: From November 2003 to April 2004, blood culture isolates of *S. aureus* were collected by one per one patient. Minimum inhibitory concentration of vancomycin was determined by Clinical and Laboratory Standards Institute broth dilution method. agr polymorphism and the presence of ica were determined by PCR. Relative quantity of RNAIII transcript was measured by real time reverse transcription PCR compared to those of 16s rRNA. Delta cycles of threshold (delta CT) were calculated from CT of 16s rRNA minus CT of RNAIII. Under the conditions of tryptic soy broth(TSB) or TSB plus 4% ethanol (TSBE) or 3% NaCl (TSBN) or vancomycin of one fourth of MIC (TSBV), biofilm mass produced on polystyrene microtiter plate was stained by crystal violet and OD490 of biofilm substracted from OD490 of blank were averaged from triplicate assays of each isolates.
Results: Total of 46 isolates included 21 methicillin-susceptible *S. aureus* (MSSA) comprising 7 agr1, 4 agr2, 8 agr3, and 2 agr4 and 25 methicillin-resistant resistant *S. aureus* (MRSA) comprising 11 agr1, 12 agr2, and 2 agr3. All of them except one were ica-positive. There was no significant difference found in biofilm production according to agr polymorphism or conditions of biofilm assay, but OD490 of MSSA agr1 were significantly higher than those of other groups of MSSA; 1.97 ± 1.44 vs 0.27 ± 0.08 ($P < 0.001$) in TSBN and 0.89 ± 0.71 vs 0.33 ± 0.15 in TSBV ($P < 0.05$). MRSA showed higher mean OD490 than MSSA; 0.59 ± 0.43 vs 0.37 ± 0.24 ($P < 0.05$) in TSB and 0.79 ± 0.65 vs 0.45 ± 0.27 in TSBE ($P < 0.05$). Mean delta CT were 4.57 ± 2.24 for MSSA and 7.7 ± 3.45 for MSSA ($P < 0.05$). 10 isolates including 8 MRSA of agr2 and 2 MSSA agr3 showed delta Ct over 10 and higher OD490 than the others; 0.67 ± 0.35 vs 0.44 ± 0.36 in TSB ($P < 0.05$) and 0.99 ± 0.65 vs 0.54 ± 0.46 in TSBE ($P < 0.05$).
Conclusion: Difference of biofilm production was not prominent according to agr polymorphism but certain conditions such as salt or vancomycin enhanced the difference. MRSA of agr2 were predominant in agr-defective strains. MRSA and agr-defective strains were correlated with higher production of biofilm.

P1974 Interconnection in phosphoregulation pathways underlines the complexity of *Staphylococcus aureus* virulence

M.E. Cluzel, J.P. Didier, M. Becchi, I. Zanella-Cleon, V. Molle, A.J. Cozzone, B. Duclos (Lyon, FR)*

Objectives: Reversible phosphorylation of prokaryotic proteins on serine, threonine and tyrosine residues has become a growing field of interest, since it is involved in several virulence pathways. The Stk1/Stp couple, including a serine/threonine kinase and its associated phosphatase, is one of the main actors of the phosphoproteome of *Staphylococcus aureus*. Besides this kinase/phosphatase couple, two-component systems (TCS) are specific prokaryotic systems that involve phosphorelay between both sensor and response elements. Response elements are DNA-binding proteins, leading to the modulation of virulence through genetic regulation. Thus, TCS and Stk1/Stp are implicated at different levels of *Staphylococcus aureus* pathogenesis, with distinct phosphorelays. We have focused on these two parallel systems to point out an interconnection that implicates a further degree of virulence regulation.
Methods: We have specifically analyzed the autolysis regulatory locus response element (ArlR) and the staphylococcal accessory element response element (SaeR) proteins. These proteins contain conserved serine and threonine amino acids in their primary structures, as shown by interspecies alignment. We used radioactive ATP labeling, analysis of specific phosphoamino acids, mass spectrometry and site-directed mutagenesis to evidence the interconnection between these two systems and to identify the phosphorylated positions.
Results: Radioactive experiments have pointed out that both response elements ArlR and SaeR are substrates for Stk1. Phosphorylated amino acids are phosphothreonine for SaeR and both phosphoserine and phosphothreonine for ArlR. Two distinct phosphorylation sites have been identified in the N-terminal and C-terminal regions. Considering that the loop formed to link DNA is located in the C-terminal region, phosphorylation may modify the DNA-binding process and thus contribute to the regulation of bacterial virulence.
Conclusion: As response elements ArlR and SaeR have been identified as substrates for the serine/threonine kinase Stk1, the two-component systems and the Stk1/Stp system are therefore shown to be interconnected, suggesting that new antibiotic drugs against *Staphylococcus aureus* could be elaborated to inhibit this interrelation.

P1975 Comparison of expression of virulence genes among invasive and colonizing methicillin-resistant *Staphylococcus aureus* clinical isolates

A. Abu Othman, E. O'Neill, H. Humphreys, D. Hughes (Dublin, IE)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) can mediate infection by the expression of a wide array of virulence factors, encoded by various virulence genes to facilitate invasion and tissue damage. Some virulence markers have been reported to be associated more with invasive MRSA isolates than with colonising isolates. We compared the expression of virulence genes among 20 colonising and 20 invasive (bloodstream) isolates of MRSA.
Methods: The invasive and colonizing (e.g. nasal carriage) isolates were collected in Beaumont Hospital, Dublin, Ireland between 2001–2006. We determined the expression levels of the virulence genes hlg, cna, sea and the accessory gene regulator agr, in isolates grown in the presence of human serum (to mimic the *in vivo* environment) using reverse transcriptase real-time PCR (RT-PCR).
Results: There was considerable variation in the expression of virulence genes among both groups of isolates. Transcription of hlg was up-regulated greater than 2 fold in 60% of invasive isolates compared to 35% of colonizing isolates (P value = 0.0193), cna transcription was up-regulated in 63% of invasive isolates compared to 100% of colonizing isolates (P value = 0.0110). Sea transcription was down-regulated in 100% of invasive isolates and 85% of colonizing isolates (P value = 0.0074). Transcription of RNAIII, the effecter gene of the agr regulation system was up-regulated greater than 2 fold in 40% of invasive isolates and 25% of colonizing isolates but this was not statistically significant. The transcription of RNAIII was not accompanied by a reduction in transcription of cna or increased transcription of hlg and sea.
Conclusions: The pattern of expression of specific virulence associated genes in invasive versus colonising isolates differs, which may contribute to the pathogenicity of MRSA. However, some virulence genes are preferentially up-regulated in colonizing isolates suggesting that colonising isolates have the capacity to promote host damage in certain settings. Therefore bacterial virulence is only one factor in invasive infection and other factors, e.g. the host immune response also contribute to infection.

P1976 Processing of antimicrobial peptides derived from chromogranin A by *Staphylococcus aureus* virulence factors

M.H. Metz-Boutigue, P. Shooshtarizadeh, J.F. Chich, C. Corazzol, S. Lefèvre, B. Guérold, F. Delalande, F. Jehl, G. Prévost, Y. Haïkel, D. Aunis (Strasbourg, FR)*

Objectives: Recent studies have shown that *S. aureus* strains produce virulence factors that are proteolytic enzymes able to interact with tissue components and the host defence mechanisms. The antimicrobial peptides play a crucial role in innate immunity and avoidance mechanisms deployed by *S. aureus* may include proteolytic degradation. The present study is conducted to evaluate the effects of several *S. aureus* virulence factors against the antimicrobial chromogranin A (CGA)-derived peptides.
Methods: By using a combination of RP-HPLC, Edman sequencing and Maldi-Tof mass spectrometry, we analyzed the processing of Chromofungin (CGA57–66), Catestatin (CGA344–364) and Cateslytin (CGA344–358), three potent antimicrobial CGA-derived peptides, after incubation in presence of two *S. aureus* supernatants, (S1, a MRSA strain and S2 a strain without resistance against tested antibiotics). A similar experimental strategy was applied to determine the effects of the endoprotease Glu-C of *S. aureus* on the degradation of CGA-derived peptides released by chromaffin cells during stress. The structure of the new generated peptides was related with the antimicrobial activities against bacteria, fungi, yeasts and *Plasmodium*.
Results: After incubation with *S. aureus* S1 or S2 for 18 h, we observed the degradation of Catestatin and we determined the sequence of new generated fragments. The degradation profile was different with both strains and for the strain S2 aureolysin might be involved in the proteolytic process. In contrast, Cateslytin and Chromofungin remained mostly intact after incubation with either strain. Furthermore, when the material released by chromaffin cells was treated by the Glu-C protease from *S. aureus*, we observed the degradation of numerous antibacterial peptides to generate new antifungal peptides.
Conclusion: We evidence that *S. aureus* subverts innate immunity to degrade highly conserved antibacterial peptides and to produce new antifungal peptides.

P1977 Genetic organization of pathogenicity island XII of group B streptococci

E. Kuleshevich, A.N. Suvorov (St. Petersburg, RU)*

Objectives: Group B streptococci (GBS) are the major cause of neonatal meningitis, sepsis, and pneumonia leading to newborn mortality. At the beginning of XXI-st century sequencing of the genomes of several GBS strains have been carried out. It allowed to discover a number of previously unknown surface protein genes including SspB1(A. Suvorov, 2006). The gene encoding for this protein is localized on the GBS pathogenicity islands (PAI). PAI are mobile genetic elements which might be apprehended by GBS due to horizontal gene transfer and can differ significantly between the epidemic strains. The aim of the work was to analyze the organization of PAI XII which usually carries SspB1.
Methods: Three collections of GBS strains have been studied: 16 from Russia, 12 from USA and 9 from Czech Republic. All the strains have been serotyped using capsular specific antibodies and specific set of PCR primers (C. Poyart, 2007). Localization of the genes on PAI [1] XII have been studied by the following methods: PCR and DNA hybridization.
Results: Genetic organization of pathogenicity island XII GBS containing sspB1 gene have been studied. Following genes have been

chosen for the investigation: sspB1 (gbs1356), putative zeta toxin (gbs1343), putative Zn-finger protein (gbs 1348), putative helicase (gbs1352), putative transposon Tn5252 (gbs1360), type IV secretory pathway protein (gbs1364). G+C content of the genes under study differs from typical GBS G+C content by 4–7%. Gene encoding for the putative protease (gbs1376) located on the verge of the island and present in all GBS strains was used as positive control. Three collections of clinical GBS strains was analyzed. None of the genes belonging to the pathogenicity island under study were found in strain collections from USA or Chech Republick. Island had been found in eight strains from Russian collection. These strains belonged to different serotypes which excludes clonality. Composition of the genes on PAI XII was different between the strains.
Conclusion: Pathogenicity island XII have been found only in eight from sixteen strains of Russian collection but haven't been found in other strain collections. Results obtained allows speculating that virulent GBS strains containing pathogenicity island XII might have specific geographical distribution.

P1978 Evidence for capsular switching in *Streptococcus agalactiae*

E.R. Martins, J. Melo-Cristino, M. Ramirez (Lisbon, PT)*

Objectives: The polysaccharide capsule is a major antigenic factor in *Streptococcus agalactiae* (GBS). Previous observations suggest that exchange of capsular loci is likely to occur rather frequently in GBS, even though GBS is not known to be naturally transformable. The aim of this study was to evaluate the concordance between serotype and the clusters defined by pulsed-field gel electrophoresis (PFGE) and to further characterize any putative transformants to establish unequivocally that capsular switching occurs in GBS.
Methods: We analyzed a large collection of GBS isolates (n = 463) by serotyping and PFGE in order to evaluate the genetic heterogeneity within bacterial clones and to investigate how often they express different capsular types. Each putative transformant (n = 9) was compared to other isolates in the same PFGE cluster by analysis of multiple genes spread across the GBS genome, including multilocus sequence typing (MLST), and surface protein and pili gene profiling. The capsular locus was also characterized in these isolates by restriction fragment length polymorphism (RFLP), sequencing and southern blot hybridization.
Results: We show that capsular switching by horizontal gene transfer is not as frequent as previously suggested. Serotyping errors may be the main reason behind the overestimation of capsule switching, since phenotypic techniques are prone to errors of interpretation. The identified capsular switching events involved the acquisition of the entire capsular locus and were not restricted to the serotype-specific central genes, the previously suggested main mechanism underlying capsular switching. Since we found no evidence of recombination inside the capsular locus we surmise that the recombination breakpoints are located outside this region and most probably outside the cps operon.
Conclusions: Our data, while questioning the frequency of capsular switching, provide clear and unambiguous evidence for *in vivo* capsular transformation in *S. agalactiae* which may be of critical importance in planning future vaccination strategies against this pathogen.

P1979 Adhesion mechanism of *Streptococcus anginosus* to mucosal epithelial cells

Y. Kodama, M. Sasaki, Y. Shimoyama, S. Tajika, S. Kimura (Morioka, JP)*

Objective: Although *Streptococcus anginosus* is a part of the normal flora found in human dental plaque, recent studies indicate that *S. anginosus* infection in oral mucosa can be associated with oral squamous cell carcinoma. The organism possesses a number of pathogenic properties, however, adhesive mechanism that mediate the initial process of *S. anginosus* infection to oral mucosal epithelial cells remained to be elucidated. In this study, the adhesive abilities of *S. anginosus* to mucosal epithelial cells of a human larynx carcinoma cell line (HEp-2 cells) and a gingival epithelial cell line (GE1 cells) as well as the immobilized fibronectin were investigated.
Methods: *S. anginosus* ATCC 10713 and clinical isolates were used. The adhesive ability of *S. anginosus* was assessed by binding of bacteria to the cultured epithelial cells, and the immobilized extracellular matrix proteins. The fibronectin expression of HEp-2 cells was analyzed by Western blotting and RT-PCR assay.
Results: *S. anginosus* can adhere to both mucosal epithelial cells as other oral streptococci do. The adhesive ability could be ascribable to mainly its fibronectin-mediated adherence to the mucosal epithelial cells. It was also indicated that the adhesive ability to HEp-2 cells of the *S. anginosus* isolates from oral cancer tissues was significantly higher than that of the isolates from the plaque sample of healthy subjects. The cell-surface expression of fibronectin in HEp-2 cells was augmented by the bacterial adhesion itself and the autocrine activation of TGF-b1 induced by the bacterial adhesion. Furthermore, the addition of exogenous fibronectin (10 nM) enhanced the *S. anginosus* adherence to HEp-2 cells. The pretreatments with fibronectin of the bacterial cells as well as HEp-2 cells enhanced the *S. anginosus* adherence, and the enhancements were abrogated by the addition of anti-fibronectin antibodies, suggesting the coexistence of a direct adhesion of *S. anginosus* to the fibronectin of HEp-2 cell surfaces and another fibronectin-mediated adhesion mechanism involving a fibronectin bridge between the *S. anginosus* fibronectin-binding molecule(s) and the integrins of HEp-2 cells.
Conclusion: The present findings suggest that *S. anginosus* could adhere to mucosal epithelial cells via multiple adhesion mechanisms, and the adhesive ability to fibronectin could be involved in the pathogenicity of *S. anginosus,* leading to the onset of oral squamous cell carcinoma.

P1980 Isolation of two genetic loci involved in anti-phagocytosis and pathogenesis in *K. pneumoniae* through a Dictyostelium model

Y.J. Pan, T.L. Lin, J.T. Wang (Taipei City, TW)*

Objectives: Dictyostelium has been used for study the interaction of phagocyte-bacteria because it displays highly similarity to mammalian macrophage. Thus we determined to investigate virulence factors of anti-phagocytosis related genes in a clinical strain of *Klebsiella pneumoniae* causing pyogenic liver abscess which is highly resistant to phagocytosis using a Dictyostelium model.
Methods: Plaque assay was used for screening the mutants susceptible to Dictyostelium phagocytosis. The transposon-disrupted genes were further identified in these selected mutants. Unmark deletion mutant and complementation constructs were generated and confirmed with plaque assay. Bacteria carrying a plasmid encoding green fluorescence protein (GFP) incubated with Dictyostelium or human neutrophils were observed using confocal microscopy and the numbers of phagocytosed bacteria were also determined. Five week-old female BALB/cByl mice were intraperitoneally inoculated with 10^3 CFU bacteria. Four to eight mice were infected in each group and monitored for 30 days. Survival of different groups of mice were compared and analyzed with Kaplan–Meier method.
Results: Thirty of 2500 mutants were permissive for Dictyostelium growth. Among the 30 mutants, capsular genes and two non-capsular genes were identified. One is encoding a subunit of protease (clpX) and the other is encoding a lipopolysaccharide transporter (wzm). The two genes were confirmed by generating their deletion and complementation strains. The results showed that clpX– mutant and wzm– mutant became more susceptible to Dictyostelium phagocytosis, and the complementation strains restored the ability of anti-phagocytosis. Moreover, we have proved that the two mutants not only became more easily to be phagocytosed by Dictyostelium but also by human neutrophils. Also, the mutants were attenuated in virulence in a mouse model, implicating they play important roles in pathogenesis of *K. pneumoniae*.
Conclusion: We have isolated 2 genes, clpX and wzm, which are involved in anti-phagocytosis and also *in vivo* virulence of *K. pneumoniae* using a Dictyostelium model.

P1981 **Development of neonatal sepsis is not dependent on the presence of magA, k2A, rmpA and kfu genes in *Klebsiella pneumoniae***

K. Huik, Ü. Parm, K. Denks, M. Pauskar, R. Avi, T. Metsvaht, M. Ilmoja, I. Lutsar (Tartu, Tallinn, EE)*

Background: *K. pneumoniae* is one of the leading causes of infections in neonatal intensive care units (NICU). In adults its pathogenicity has been associated with virulence genes like magA, k2A, rmpA and kfu. Their role in neonatal infections is less well known.
Objectives: To determine the population dynamics of *K. pneumoniae* in two NICUs in Estonia and evaluate the presence of the above mentioned virulence genes among invasive and colonising strains.
Methods: From Aug 2006 to Dec 2007 rectal and nasopharyngeal (NP) surveillance cultures were collected from all neonates admitted with suspected or confirmed early onset sepsis. Blood cultures were obtained as clinically indicated. Isolates were typed using amplified fragment length polymorphism (AFLP) PCR analysis. In parallel, 5 mucosal and 5 invasive isolates in 5 pts with invasive disease were typed using pulse field gel electrophoresis (PFGE). Hypermucoviscosity test was defined as positive string test. The magA (specific to K1 capsule serotype), k2A (specific to K2 capsule serotype), rmpA (regulator of mucoid phenotype) and kfu (an iron uptake system) were detected by PCR using specific primers.
Results: A total of 47/278 pts had mucosal carriage of *K. pneumoniae* and 6 had BSI including 38 colonised pts in unit A (5 with BSI) and 9 in unit B (1 with BSI). Altogether, 88 rectal, 55 NP, and 5 BSI isolates were analysed. The ALFP analysis identified 6 different clonal groups 2 of which predominated in unit A (type A in 26 pts over 7mo and, type K in 6 pts over 2mo), while in unit B all colonising *K. pneumoniae* strains were different. 5/6 BSI were caused by type A; the invasive and colonising strains had similar genotype in PFGE analysis. Hypermucoviscosity strains were detected only among mucosal isolates; in 16/30 isolates in pts with and in 0/118 without BSI ($P < 0.001$). No magA and rmpA genes were detected. The k2A and kfu genes were rare and present in 7/143 and 4/143 mucosal isolates, respectively but only in pts without BSI. A concordance between AFLP type and presence of k2A and kfu genes was identified – type B was related to k2A and type P to kfu.
Conclusions: AFLP method is an alternative in evaluating the spread of *K. pneumoniae* in NICU and allows to identify potential cross-colonisation clusters. Although none of the tested virulence genes was associated with BSI, the testing of colonising strains for hypermucoviscosity appeared to be a simple method identifying patients at risk for BSI.

P1982 **Evidence of the inhibitory activity of the *Lactobacillus paracasei* probiotic strain soluble fraction on the expression of *Pseudomonas aeruginosa* quorum-sensing genes quantified by real-time qRT-PCR**

M.C. Chifiriuc Balotescu, A. Cotar, C. Bleotu, S. Dinu, D. Pelinescu, V. Lazar (Bucharest, RO)*

Objectives: The purpose of this study was to demonstrate by real-time PCR the inhibitory activity of the *Lactobacillus paracasei* subsp. *paracasei* probiotic culture filtrate on *Pseudomonas* (Ps.) *aeruginosa* quorum-sensing expression.
Methods: The study was performed on 30 *Ps. aeruginosa* strains isolated from different clinical specimens. The characterization of quorum-sensing systems (las and rhl) of *Ps. aeruginosa* analysed strains was performed by PCR using specific primers for detection of genes lasI, lasR, rhlI and rhlR. The *Ps. aeruginosa* strains were grown in the presence of subinhibitory concentrations of sterile supernatant of probiotic culture, that exhibited inhibitory effects on growth and adherence to the inert substrate. Total RNA was extracted from standard bacterial cultures, and from bacterial cultures grown in the presence of probiotic cultures filtrates versus controls was performed by real-time qRT-PCR with SYBR Green I fluorescent dye using the instrument Mx3005 (Stratagene). In addition, the expression of each target gene (lasI, lasR, rhlI and rhlR) in the control versus experimental sample was normalized using the 16S rRNA reference gene.
Results: The results of PCR assays showed that all *Ps. aeruginosa* strains possess all quorum sensing tested genes. Also, the results of the real time qRT-PCR showed that in all *Ps. aeruginosa* strains grown in the presence of probiotic culture filtrate, the level of expression is reduced comparatively with the control cultures. The expression level of lasI and rhlI gene in experimental samples was reduced with 35.6% comparatively with the control samples. The expression level of lasR and rhlR gene in the experimental samples was reduced with 41.6% and respectively 41.5%.
Conclusion: The comparative quantification of quorum sensing genes expression in *Ps. aeruginosa* strains grown in the presence of probiotic culture filtrate proved the inhibitory effect of soluble molecules accumulated in the supernatant on the quorum sensing genes expression level. These results prove that the inhibition of virulence factors regulation mechanisms by soluble molecules secreted by probiotics could represent an interesting way of attenuation of pathogenicity and virulence in *Ps. aeruginosa* nosocomial strains.

P1983 ***Pseudomonas fluorescens* can increase the permeability of Caco2/TC7 and modify the actin cytoskeleton**

A. Madi, P. Svinareff, N. Orange, M.G. Feuilloley, N. Connil (Evreux, FR)*

Context: *Pseudomonas fluorescens* has been proposed as a normal low-level resident of the intestine (Wei et al, 2002). However, its I2 specific antigen has been shown highly prevalent in the serum of patients suffering from crohn's disease (Arnott et al, 2004) suggesting a putative role of this bacterium in the initiation of the pathology.
Objectives: As we have recently demonstrated that *P. fluorescens* MF37 is able to bind and exert cytotoxic effects on undifferentiated Caco-2/TC7 cells (Madi et al, 2008), the aim of the present work was to investigate the interaction of *P. fluorescens* MF37 with fully differentiated Caco-2/TC7 monolayers.
Methods: Caco-2/TC7 cells were seeded onto inserts and cultivated in Dulbecco's modified Eagle's medium containing 10% fetal calf serum at 37ºC in 5% CO2 during 21 days. The differentiated cells were infected with *P. fluorescens* MF37 at a multiplicity of infection (MOI) of 100. Bacterial cytotoxicity was evaluated using the CytoTox 96 assay kit (Promega). Transepithelial resistance (TER) was estimated using a Millicell-ERS apparatus and the paracellular permeability was quantified by fluorimetry using 4 kDa FITC-Dextran beads. The F-actin cytoskeleton was visualized with Alexa fluor® 488 phalloidin.
Results and Conclusions: No cytotoxicity was observed after 24 h-infection of the differentiated Caco-2/TC7 cells with *P. fluorescens* MF37. However, a slight decrease of TER was obtained correlated with an increase of paracellular permeability. This increase of permeability was associated with a profound reorganization of the F-actin cytoskeleton. Further studies will help to understand the signaling pathways involved in these mechanisms and might partially explain the implication of *P. fluorescens* in the etiology of crohn's disease.
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P1984 ***Acinetobacter baumannii* and other genomic species: characteristics of adhesion and biofilm**

E.H. Takagi, J.C. Campos, C.R. Taddei, M.B. Martinez (São Paulo, BR)*

Bacterial species of *Acinetobacter calcoaceticus-A. baumannii* complex are nosocomial pathogens of increasing importance, but the mechanisms involved in their virulence are largely unknown. This study investigated

the potential of different species of this complex to produce biofilm on abiotic surface and adhere to eukaryotic cells: Hep-2, NCI-H292 and MRC-5. A modified PCR-based method was created and used to distinguish between *Acinetobacter baumannii* and *Acinetobacter* genomic sp. 13TU and 3TU. This genomic identification method exploits differences in gyrB gene and 16S-23S rRNA gene spacer region sequences. Thirty non-clonal isolates from intensive unit care patients, 18 were identified as *A. baumannii*, eight as A. 13TU and four A. as 3TU. During this study, 3 clinical strains of A. 3TU, 3 of A. 13TU and 3 of *A. baumannii*, were examined for biofilm formation and adherence assays. Biofilm formation was evaluated by quantitative method through microtitre plate assay by means the crystal-violet staining method. Adherence of clinical isolates to eukaryotic cells was examined by light microscopy. The strains under this study displayed high variability in biofilm formation among isolates from the same species. None isolate adhere to Hep-2 cells. On NCI-H292 cells it was possible to observe differences, such as *A. baumannii* presents dispersed adherence, while A. 13TU shows some cluster cells adherence and A.3TU did not adhere. However, dispersed adherence was observed by *A. baumannii*, A. 3TU and A. 13TU with variability in number of bacterial cells adhered on MRC-5 cells. This study describes a PCR-based method that allows the identification of major clinical species from *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. Differences on assays performed on NCI-H292 cells could be a possible explanation for a minor isolation frequency of A. 3TU and A. 13TU.

P1985 Environmental *Stenotrophomonas maltophilia* strain is less virulent than clinical strain from cystic fibrosis patient

A. Pompilio, V. Crocetta, S. Pomponio, A. Bragonzi, V. Holà, E. Fiscarelli, R. Piccolomini, G. Di Bonaventura (Chieti, Milan, IT; Brno, CZ; Rome, IT)*

Objective: *S. maltophilia* is considered an emergent pathogen in cystic fibrosis (CF). Since *S. maltophilia* is widespread in the environment, it is important to determine the potential virulence of environmental strains for CF patients. For the first time, in this study two *S. maltophilia* strains of environmental and clinical origin were compared for their virulence in a mouse model of airway infection.
Methods: DBA/2 strain mice (n = 10/group) were exposed once to an aerosol with PBS only (control) or PBS containing *S. maltophilia* Sm111 CF strain or C39 environmental (waste water) strain. At day 1 and 3 post-exposure mice were sacrificed, and the lungs were observed in situ for macroscopic damage, then homogenized for quantitative bacteriology and cytokine (IL-6, IFN-g, TNF-a, MIP-2) measurements. In each group, two mice were randomly selected for histology. To evaluate dissemination of infection, spleens were homogenized and cultured.
Results: Clinical strain was able to persist longer than environmental one in the lungs of infected mice (day 1: $4.1\pm2.8\times10^6$ vs $10.9\pm11.1\times10^3$ CFU/g; day 3: $2.5\pm3.2\times10^4$ vs $1.8\pm3.5\times10^3$ CFU/g, respectively; $P < 0.01$). Dissemination of infection was significantly more frequent in mice exposed to clinical strain (day 1: 90 vs 10%, day 3: 40 vs 0%, respectively; $P < 0.01$). Exposure of DBA/2 lungs to environmental strain caused a mortality rate comparable to that observed with clinical strain (10 vs 20%, respectively). Macroscopic lung examination did not reveal significant differences in lung damage although lung histopathology showed a significantly higher polymorphonuclear infiltrate in the alveolar septa of mice infected with Sm111 strain (60 vs 20% of visualized lumens, respectively; $P < 0.05$). At day 1, cytokine levels were significantly higher in lungs infected with clinical strain than environmental one (TNF-a: 498.8 ± 92.4 vs 187.0 ± 56.2 pg/lungs; IFN-g: 198.4 ± 44.6 vs 19.03 ± 22.9 pg/lungs; IL-6: 977.2 ± 297.6 vs 457.5 ± 590.0 pg/lungs; MIP-2: 2703.4 ± 157.3 vs 2124.6 ± 279.8 pg/lungs, respectively; $P < 0.01$). At day 3, cytokine levels were comparable in both groups.
Conclusion: Our results showed that *S. maltophilia* environmental strain is potentially virulent although less than the corresponding CF strain, thus suggesting that environment could be a potential reservoir for the acquisition of infection by CF patients.
This work was supported by the Italian CF Research Foundation; grant FFC7/2007.

P1986 Transfer of a putative hyaluronidase hylEfm and distribution of its putative pathogenicity island in different *Enterococcus faecium* isolates

J. Laverde Gomez, C. Fleige, W. van Schaik, G. Werner (Wernigerode, DE; Utrecht, NL)*

Objectives: The increasingly important nosocomial pathogen *Enterococcus faecium* possesses a putative hyaluronidase gene, hylEfm, which has been a supposed pathogenicity factor coded on a pathogenicity island (PAI)-like structure of ca. 20 Kb (Rice, L. et al 2003). Distribution of this putative virulence factor varies among clinical strains of *E. faecium*. We aimed to investigate the structure and distribution of the putative pathogenicity factor hylEfm and its putative PAI among various clinical isolates and resolve the composition and structure of the hylEfm-plasmid.
Methods: We re-annotated the supposed PAI-like element by comparing available genomic contigs of *E. faecium* strains DO and U0317 (Willems, R. and van Schaik, W. personal communication) using DS Gene 1.5 software. Presence of this structure was investigated by long template PCR, regular PCR and sequencing in 40 *E. faecium* isolates of different clonal types and complexes. We identified the genomic localization of hylEfm in our set of strains by Sma-I and S1-nuclease macrorestriction of genomic DNA resolved by PFGE and analyzed by southern hybridization (Roche®). Transfer of hylEfm among *E. faecium* was tested by *in vitro* filter mating. Sequencing of 2 selected plasmids was done using 454 technology.
Results: The hylEfm putative PAI is a 17.824 bp element similar but not identical to the structure previously described. This cluster is conserved in 40 hylEfm positive strains with slight differences downstream hylEfm. The island was exclusively located on large plasmids (150 to 350 kb). hylEfm-plasmids were horizontally transferred using *in vitro* filter-mating. Sequencing of selected plasmids resolved conjugation and plasmid related determinants, additional to sugar uptake and metabolic genes, heavy metal resistance, several antibiotic resistance clusters and additional pathogenicity factors such as surface-exposed pili genes on the same plasmid with the putative hylEfm PAI.
Conclusions: The putative hylEfm PAI structure is highly conserved and located on transferable plasmids of different sizes. hylEfm conjugative plasmid bears additional putative pathogenicity factors and antibiotic resistance genes. These findings suggest horizontal gene transfer of virulence factors and antibiotic resistance gene clusters by a single genetic event (conjugation) which might be triggered by heavy antibiotic use common in health care units where *E. faecium* is increasingly prevalent.

P1987 A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region

S. Agudo, T. Alarcón, G. Pérez-Pérez, J. Martínez, M. López-Brea (Madrid, ES)*

Introduction: VacA, the vacuolating cytotoxin encoded by the vacA gene is a highly polymorphic. It has two regions: signal (s) and middle (m) that are well characterized markers of virulence. Functionally, s1/m1 strains cause more extensive vacuolation and clinically, are associated with gastric atrophy. Recently, a new polymorphic region of vacA designated intermediate (i) region, was described as a major determinant of vacA toxicity. The aim of this study was to determine the prevalence VacA genotypes and determine the association with VacA intermediate among *H. pylori* isolated in Madrid, Spain.
Methods: We obtained 118 biopsies from patients with gastric symptoms, from June 2008 to January 2009. Standard microbiological procedures were used for *H. pylori* culture. DNA extraction was carried out by the NucliSens easyMAG platform with the NucliSens magnetic extraction reagents (bioMérieux). VacA genotypes (s, m) were determined by PCR and agarose gel. VacA i genotype were determined by PCR and DNA sequencing in all s1m2 and in 10 random s2m2 and s1m1 strains. The criteria used for determining i-type (either i1 or i2) were based on the amino acid sequences.

Results: The results of VacA are showed in the table 1. Two i-region types were identified, i1 and i2. S1/m1 and s2/m2 strains were exclusively i1 and i2, respectively. 15 out of 16 (93.7%) s1m2 strains were i1.
Conclusions: The s2m2 was the most frequent vacA mosaicism, observed in 63% of our patients. This study shows the strong association between vacA i1 genotype and s1 allele of vacA gene. Further studies are needed in other to know if vacA i-region is an important marker of *H. pylori* toxicity.

	Total	
	n	%
s1m1	22	19.3
s1m2	16	14.1
s2m1	4	3.5
s2m2	72	63.2
Mixed strains	4	3.4
m1m2s1	2	
m1m2s2	2	

P1988 Demonstration of the role of heat shock proteins in *Vibrio parahaemolyticus* pathogenesis by *in vitro* and *in vivo* approach

G. Pircalabioru, M.C. Chifiriuc Balotescu, B. Gilea, V. Lazar, L. Dascalu, G. Enache, M. Hirt, A. Dragodan, M. Chivu, C. Bleotu (Bucharest, RO)*

Objective: Different cellular fractions of *V. parahaemolyticus* haemolytic and non-haemolytic strains grown under sub-lethal heat and osmotic stress were analyzed in order to evaluate the survival profiles, the morphological changes, the levels of heat shock proteins (hsp) and their immunogenicity using *in vivo* experimental models.
Methods: Different cellular fractions (supernatants concentrated by ultrafiltration, periplasmic and cellular lysates) were obtained from bacterial cultures submitted to different stress conditions and further used for: i) the assessment of total protein (Bradford) and hsp concentration (Western blot, using monoclonal antibodies against hsp 10, 60 and 70 kDa); ii) *in vivo* immunization of holoxenic mice by repeated i.p. injection, once a week during 10 weeks. After immunization, the hsp antibodies were assessed by ELISA and the mice were challenged with wild *V. parahaemolyticus* strains.
Results: Our studies confirmed the expression of different hsp, i.e. GroES (10kDa), GroEL (60kDa) and DnaK (70kDa). GroEL exhibited the highest expression after heat, osmotic and combined stress and GroES the lowest, being expressed only after heat stress. The haemolytic strain constitutively over-expressed high levels of GroEL, while the non-virulent *V. parahaemolyticus* strain exhibited GroEL in direct relation with the heat shock. No cross-protective effect was observed between different stress factors. The heat shocked cells exhibited a more intensive hemolytic activity on blood agar, proving the role of the heat-stress proteins in the increase of pathogenic potential. The osmotic stress induced important changes in cell morphology, with the occurrence of fusiform, spiraled forms with low affinity for the Gram staining. The immunized batches exhibited good survival rates after challenge with the virulent *V. parahaemolyticus* strain. The supernatant of heat stressed bacterial cultures induced the early occurrence of anti-hsp antibodies (after 4 immunizations), while after 8 immunizations, the anti-hsp antibodies were present in the majority of the tested batches, the most immunogenic fraction proving to be the heat-stressed culture supernatants.
Conclusion: The hsps accumulated in the supernatants and total cellular extracts exhibited a protective role against the infection with *V. parahaemolyticus* virulent strains, the good survival rates being correlated with high titers of anti-hsp antibiodies.

P1989 Locations of proteases in cellular fractions and association with vesicles in *Porphyromonas gingivalis*

S. Oishi, M. Miyashita, A. Kiso, Y. Kikuchi, O. Ueda, K. Hirai, Y. Shibata, S. Fujimura (Shiojiri, JP)*

Objective: Arginine gingipain (RGP) and lysine gingipain (KGP) are major proteases of *P. gingivalis* and are important in the pathogenesis of periodontitis. These proteases are found in the culture supernatant, in the envelope and within the cytoplasm. Two types of proteases were found to exist in the culture supernatant, free from any cellular components and vesicle-bound forms. Since vesicles of Gram-negative bacteria may contribute to the transportation of their virulence factors to the host cells, we undertook to investigate the interaction of proteases and vesicles.
Methods: *P. gingivalis* ATCC 33277, W83 and 381 were cultured anaerobically and the envelope and crude extract of the cells were prepared by a combination of ultrasonication of cells and centrifugation. Vesicles were collected from the culture supernatants by ammonium sulfate precipitation (40% saturation) and centrifugation. Solubilization of the vesicles and the envelope was carried out using a detergent. Protease and peptidase activities were determined using p-nitroanilide derivatives of amino acids and peptides.
Results: Locations of RGP in the cellular fractions in the crude extract, envelope, vesicles and culture supernatants were 48%, 16%, 17% and 31%, respectively, while the corresponding values of KGP were 47%, 10%, 7% and 36%, respectively, indicating that distribution rates of both proteases in the cellular fractions are similar. Although the molecular masses of RGP and KGP in the culture supernatant had previously been determined as 43 kDa and 46 kDa, respectively, their molecular masses of RGP and KGP solubilized from the vesicles were estimated to be over 1,500 kDa, since they eluted in the void volume of the column of Sephacryl S-300. Interestingly, the conversion to the macromolecular size could not observed in other enzymes tested such as mono-, di- and tripeptidyl peptidases as well as alkaline phosphatase. Therefore, this conversion may be restricted to the proteases. When vesicle and culture supernatants containing free RGP and KGP were mixed and incubated, neither RGP nor KGP seemed to bind to vesicles. Both RGP and KGP bound to the vesicles were found to be more stable than the unbound forms in the heat treatment at 60 C.
Conclusion: The cellular locations of proteases of *P. gingivalis* in the culture supernatant, crude extract, vesicle and envelope were determined and molecules of the proteases bound to the vesicle were confirmed to increase the molecular size.

P1990 Rifampin protects human lung epithelial cells against resistant *Acinetobacter baumannii* clinical isolates pathogenesis

Y. Smani, J. Domínguez-Herrera, R. López-Rojas, M.J. McConnell, F. Docobo-Pérez, J. Pachón (Seville, ES)*

Objectives: In recent years, it has become apparent that rifampin, in addition to having antimicrobial activity, can act on eukaryotic cells modulating the production of host mediators. The aim of this study is to evaluate the cytoprotective effect of rifampin on resistant *Acinetobacter baumannii* clinical isolates that induce cell death in human lung epithelial cells.
Methods: Three *A. baumannii* strains: ATCC 19606 (susceptible to rifampin), 1327 and 113–16 (both resistant to rifampin) were used. Standard inoculum 5×10^5 cfu/mL was used for time-kill studies. Cellular viability and apoptosis were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Live/Dead and DAPI after addition of ATCC 19606, 1327 or 113–16 (10^8 cfu/mL) for 24 h in presence or absence of different concentrations of rifampin (0.5×, 1× and 2× MIC of each strain). Host mediators associated with *A. baumannii* pathogenesis like superoxide anion and cytokine (TNF-α and IL-6) were measured. Moreover, bacterial count was performed to evaluate the efficacity of rifampin in A549 cells culture medium in presence of these strains.

Results: At standard inoculum, time-kill curves showed that rifampin was bactericidal (a 3 log reduction in cfu/mL) at 2× MIC against 1327 and 113–16 and at 4× MIC against ATCC 19606 at 24 h. The assessment of A549 cells survival by the MTT assay and Live/Dead showed that rifampin reduced the cell death induced by ATCC 19606, 1327 and 113–16. Nuclear staining of A549 cells with DAPI showed that rifampin reduced the apoptosis induced by these strains. Moreover, we determined that the cytoprotective effect of rifampin was associated with the decrease of superoxide anion, TNF-α and IL-6 concentrations induced by these strains in A549 cells. Surprisingly, the bacterial count performed in A549 cells culture medium pretreated with rifampin showed that rifampin at 0.5×, 1× and 2× MIC of both resistant strains (1327 and 113–16) does not reduce significantly the bacterial concentrations for 24 h.
Conclusion: These data demonstrate for the first time that rifampin is able to attenuate the pathogenesis of resistant *A. baumannii* clinical isolates without being relevantly bactericidal. Indeed, cytoprotective effect of rifampin was observed on the decrease of dead cells induced by *A. baumannii* by reducing oxidative stress and pro-inflammatory cytokines release.

Pathogenesis – case reports

P1991 Condyloma acuminatum caused by HPV13: a case report

E. Blioumi, D. Chatzidimitriou, E. Gavriilaki, H. Kalekou, A. Markopoulos, E. Nikoloussi-Emmanouil, E. Diza, D. Antoniades (Thessaloniki, GR)*

Introduction: Condylomata acuminata are benign proliferative lesions of the stratified squamous epithelium of the genitalia, the perianal region, mouth and larynx, induced by human papillomavirus (HPV). HPV types usually detected are: 2, 6, 11, 16, 18, 31, 33, 51, 53, 54. Herein we present a case of an oral condyloma accuminatum HPV13, consisting in the first description of HPV13 as an etiological factor of condyloma acuminatum.
Material and Methods: We describe an 18-year-old female patient with multiple non-tender oral lesions, reporting condylomas of the perianal region, anorexia and depression in her medical history.
Clinical examination revealed multiple, non-tender, wide-based exophytic nodules, with a normal colour and a size of 0.2–0.8 cm, found on the labial mucosa, the buccal mucosa, the lateral surface of the tongue and on either side of the lingual frenum.
The lesions were surgically excised. Sections of paraffin-embedded tissue were stained by hematoxylin-eosin stain for histopathological examination. DNA extracted from the paraffin-embedded tissue was amplified by polymerase chain reaction (PCR) in standard protocols. Nested PCR, using primers (MY09/MY11 and GP5+/GP6+) targeting the L1 gene, and sequencing were conducted.
Results: From clinical and histopathological examination condylomata acuminata were diagnosed. HPV infection was confirmed by nested PCR. Sequencing revealed HPV type 13.
Conclusions: It should be emphasized that to our knowledge, oral condyloma acuminatum induced by HPV13 has never been reported.

P1992 *Orientia tsutsugamushi* meningitis or meningoencephalitis

D.M. Kim, N. Yun (Gwang-Joo, KR)*

Background: *Orientia tsutsugamushi* spreads via the bloodstream and lymphatics. It induces vasculitis leading to symptoms of systemic organ invasion including meningitis and meningoencephalitis, which are life-threatening manifestations.
Methods: We conducted a prospective case–control study of scrub typhus patients who were admitted to Chosun University Hospital between 2004 and 2008 to investigate the clinical and laboratory features of patients with scrub typhus meningitis or meningoencephalitis, and the therapeutic outcomes, and to determine the predictor factors. Cases were 22 patients with scrub typhus meningitis or meningoencephalitis, and controls were 303 patients without meningitis or meningoencephalitis.
Results: Multivariate analysis demonstrated that the presence or absence of pneumonitis was associated with the occurrence of scrub typhus meningitis and meningoencephalitis (OR, 8.9; $P < 0.001$; CI, 2.9–27.2). Although appropriate antimicrobials such as doxycycline agents were administered at an early stage, mengititis or meningoencephalitis still occurred.
Conclusions: Physicians should be aware that meningitis or meningoencephalitis may develop during appropriate drug therapy such as doxycycline. Close observation and great care are essential for patients with risk factors associated with meningitis or meningoencephalitis, particularly pneumonitis. Increasing the dosage of doxycycline, or administering antimicrobial agents such as rifampin with good penetration to the central nervous system, might be considered in such cases.

P1993 Case of endocarditis due to *Bacillus cereus*

O. Barraud, N. Hidri, P. Manea, K. Ly, F. Garnier, F. Denis, M.C. Ploy (Limoges, FR)*

Objectives: *Bacillus cereus* is an ubiquitous telluric bacteria mainly responsible for food borne infections due to toxins production. Isolation of the bacteria is of particular concern in immunocompromised patient, but can also be related to rare clinical infections (endophtalmitis, fasciitis, meningitis, peritonitis . . .) in non-immunocompromised patients. We describe here a case of endocarditis due to *Bacillus cereus*.
Methods: Eighteen blood cultures were sampled from a 65 years-old patient presenting a 25 mm per 10 mm vegetation contiguous to the right ventricular pacemaker sensor. Bottles were incubated into the BacT/ALERT® system (bioMérieux®). After pacemaker withdrawal, two electrodes were cultivated into thioglycolate broth for 48 hours. Bacteria were identified using molecular biology targeting 16S RNA-encoding DNA and rpoB gene. Susceptibility testing was performed using Mueller-Hinton agar according to the recommendations of the French Society for Microbiology.
Results: Concerning blood cultures, two aerobic and one anaerobic bottles derived from three different samples were detected positive. Gram staining detected presence of long Gram positive rods and culture grew two different morphotypes with different antibiograms. Both electrodes yielded Gram positive rods with three different macroscopic aspects. For all morphotypes obtained, sequencing of the 16S RNA-encoding DNA was unable to discriminate the species among *Bacillus* genus, contrary to the sequencing of rpoB gene which lead to the precise identification of *Bacillus cereus* sensu stricto.
Conclusion: Endocarditis due to *Bacillus cereus* has been exceptionally previously reported in the literature. This study underlines the contribution of molecular biology in bacterial species identification, notably the interest of using rpoB gene to discriminate the *Bacillus* species among the genus.

Staphylococcal bacteraemia

P1994 Molecular variability of methicillin-resistant *Staphylococcus aureus* strains isolated from blood in the Czech Republic, 2000–2005

M. Fridrichova, H. Zemlickova, K. Tyllova, V. Jakubu, P. Urbaskova (Prague, CZ)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually viewed as a major nosocomial pathogen that is associated with hospitals and healthcare facilities. It is also known as a cause of community acquired infections. Prevalence of MRSA infections is still increasing in most of countries but there are also places where decreasing trend of these infections is seen. Prevalence of MRSA infections in the Czech republic increased from 4.3% in 2000 to 12.9% in 2005. The aim of the study was to describe clonality of MRSA blood isolates collected in years 2000–2005.
Methods: Thirty three national microbiology laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS)

submitted to the National Institute of Public Health MRSA isolates collected from individual patients in each hospital from 2000 to 2005. *S. aureus* strains were identified by conventional methods. All MRSA isolates (n = 144) were confirmed by the MRSA Screen latex agglutination test and PCR detection of the mecA gene. Antimicrobial susceptibility testing was based on the method recommended by the Clinical and Laboratory Standards Institute (CLSI). Clonality of MRSA isolates were characterized by SCCmec typing and spa typing.
Results: Spa typing revealed 20 different spa types and 4 SCCmec types. Ten spa types were identified in at least 2 isolates The most prevalent spa types were as follows: t003 (n = 52) with SCCmec type II, t030 (n = 45) with SCCmec type III, and t002 (n = 11) SCCmec type II. Since the first isolation in 2000, spa type t003 has gradually replaced spa type t030 that was highly prevalent in the Czech Republic until 2003. The proportion of spa type t003 increased from 0% in 2000 to 80% in 2005 while that of spa type t030 decreased from 60% to 8.6%.
Conclusion: A significant shift in the prevalence of spa types was observed. Spa type t030 with SCCmec type III (Brazilian clone) has been replaced by spa type t003 (Rhine Hesse clone) with SCCmec type II.
This work was supported by a research grant from the Internal Grant Agency, Ministry of Health (CZ), IGA 9642–4.

P1995 Invasive methicillin-resistant *Staphylococcus aureus* in a Romanian university hospital – do we have the entire picture?

E. Szekely, L. Lorinczi, D.V. Bilca, A. Foldes (Târgu Mures, RO)*

Objectives: Many surveillance studies are based on the evaluation of the frequency of MRSA (methicillin-resistant *S. aureus*) defined as percentage of methicillin resistant strains from the total number of invasive *S. aureus* isolates. Results might be influenced by local sampling habits, as collection of blood samples mainly in severe cases already on antibiotic treatment may lead to overestimation of resistance. On the other hand, expression of frequency as incidence rate seems to be a more precise approach although the results still might be influenced in case of low sampling rate, which leads to underestimation of the incidence. We intended to estimate the local epidemiology of invasive MRSA in a Romanian university hospital with 1200 beds.
Methods: We evaluated the frequency of blood culture MRSA isolates during a 4 year period (2005–2008) expressed both as percentage of total *S. aureus* isolates and as incidence rate. Sampling rate and the percentage of blood samples collected after initiation of antibiotic therapy were also assessed.
Results: The percentages of methicillin-resistance were as follows: 73% in 2005, 71% in 2006, 70 in 2007, 56% in 2008 ($p = 0.22$). Incidence rates varied annually between 5, 5, 7 and 6/100000 bed-days ($p = 0.37$) in the same period. Blood culture sampling rates increased annually during the study period from 2 to 4.2/1000 bed-days ($p < 0.05$). Samples were collected from patients on antibiotic therapy in case of 79% of blood culture sets.
Conclusions: The very high percentage of MRSA did not correlate with the relative low incidence rates found. This discrepancy could have resulted from selective sampling and low sampling rates and suggests that neither methods of assessing frequency are reflecting reality. This was the first study from Romania which analyzed comprehensive epidemiological data regarding MRSA in a large university hospital.

P1996 Incidence and mortality of *Staphylococcus aureus* bacteraemia in Iceland

H. Asgeirsson, O. Gudlaugsson, K. Kristinsson, S. Heiddal, M. Kristjansson (Reykjavik, Akureyri, IS)*

Objectives: To determine the incidence, source and mortality of *Staphylococcus aureus* bacteraemia in Iceland.
Methods: Cases of *S. aureus* bacteraemia occurring between January 1 1995 and December 31 2008 were identified by clinical microbiological laboratories performing blood cultures in Iceland. Patients 18 years or older with a positive blood culture for *S. aureus* were included. Recurrent episodes were classified as re-infections if they occurred more than 90 days apart or if the isolates had a different antibiotic susceptibility profile. If recurrences took place within 90 days they were considered a relapse of the original bacteraemia. From December 1 2003 to November 30 2008 clinical data regarding source was collected from patients' charts (307 out of 308 available, 99.7%). National population statistics and dates of death were retrieved from the National Registry.
Results: 692 individuals from 18 institutions had 721 distinct episodes of *S. aureus* bacteraemia. Mean age at diagnosis was 62.6 years (range 18 to 99) and male to female ratio was 1.43, extending throughout the study period. During 1995 to 2008 age specific incidence of bacteraemia rose from 22.7 to 28.9 per 10^5 per year ($p = 0.016$, trend across years) and 30 day case fatality rate decreased from 27.9% to 8.7% ($p = 0.001$, trend across years). Susceptibility to penicillin increased from 14.3% to 20.7% ($p = 0.13$, trend across years), with no correlation between penicillin sensitivity and case fatality ($p = 0.8$). Four cases of methicillin resistant *S. aureus* bacteraemia were identified (0.6%), all of whom lived. Among 308 episodes in 2003 to 2008, 125 (40.5%) were hospital acquired, 82 (26.6%) health care associated and 101 (32.8%) community acquired. There was no difference in case fatality between groups ($p = 0.36$). The most common sources of bacteraemia were intravascular catheters (20.2%) and skin infections (13.4%) whereas the focus was unknown in 23.5%. The 30 day case fatality was highest among those with pneumonia (29.6%) and unknown source (22.2%), and lowest for those with skin (2.4%) and intravascular catheter related (8.1%) infections ($p = 0.04$).
Conclusion: During 1995 to 2008 a significant increase in the incidence of *S. aureus* bacteraemia in Iceland and a significant reduction in 30 day case fatality were noted. The reason for this change is not clear. The source of bacteraemia could be identified in majority of cases and different foci were associated with a difference in mortality.

P1997 MSSA bloodstream infection (BSI), MRSA's evil twin! A review of the epidemiology, risk factors and outcomes among hospitalized patients with *Staphylococcus aureus* (MSSA & MRSA) bacteraemia

M. Przybylo, J. Chang, M. Lau, M. Qulaghassi, A. Khan, A. Guleri (Blackpool, UK)*

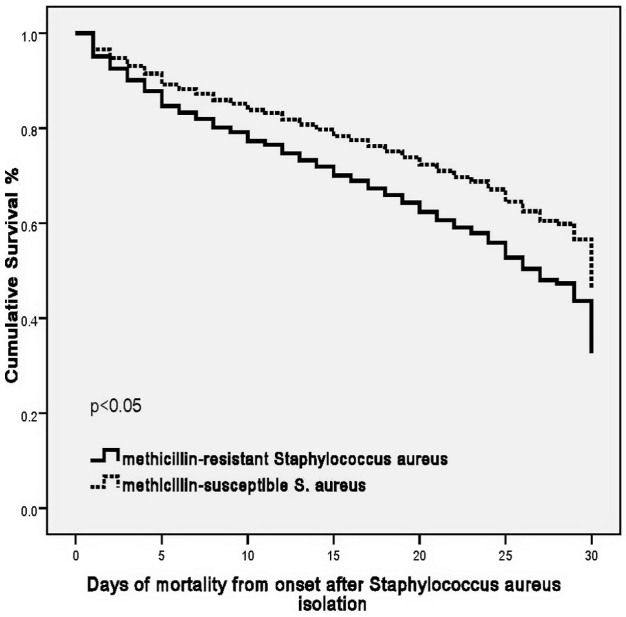
Objective: *Staphylococcus aureus* (meticillin sensitive – MSSA & meticillin resistant – MRSA) blood stream infections(BSI) are associated with morbidity, mortality and associated costs. While MRSA-BSI has received significant attention of the media, department of health, NHS and infection control teams (ICT), rates of MSSA BSI have remained unabated. This study is aimed to determine the epidemiology, risk factors, mortality and morbidity for MSSA & MRSA BSI in a large district hospital and inform the new trust MSSA/MRSA containment programme (MMCP).
Method: Cases with SA-BSI were identified from pathology database. Data was extracted at case note review on patients with BSI with MSSA & MRSA between Nov07 – Aug09. Parameters investigated included indication for blood culture, demographics, apache scores, outcome, timing & appropriateness of antibiotic treatment, length of hospital stay, intensive care admissions and mortality.
Results: Data on 68 cases of SA-BSI (52 MSSA & 16 MRSA) are presented here. Average age was 61years (MSSA) & 72years(MRSA) respectively with a comparable male/female ratio (3:2). 66% (MSSA) and 56%(MRSA) patients recovered to be discharged from hospital. Average length of stay was 23.2d (MSSA) and 26.3d (MRSA). Average Apache II score at the time of blood cultures were 13 (MSSA) & 16(MRSA). Intensive care admissions were 18% (MSSA) and 19%(MRSA) respectively. 20%(MSSA) & 56%(MRSA) had a 'Do not attempt resuscitation' order at the stage of their illness. Mortality was recorded in 34%(MSSA) and 44%(MRSA) SA-BSIs. 71%(MSSA) and 80% (MRSA) had pre-48 h BSIs. 84% (MSSA) and 54%(MRSA) had optimal antibiotics initiated before results of blood cultures were known.

100% of MSSA & MRSA – BSI were initiated on optimal antibiotics after Microbiology consultations.
Discussion: The study revealed significant morbidity, increased length of stay and mortality associated with SA-BSIs. A significant (71% MSSA & 80% MRSA) SA-BSI were pre-48 h. SA bacteraemia is associated with increased length of stay putting a financial burden on the health services. The findings of this study have been used to inform the new trust MSSA/MRSA containment programme. Details to be presented.

P1998 **Risk factors and attributable mortality in patients with nosocomial methicillin-resistant *Staphylococcus aureus* bacteraemia**

Y.Y. Chen, L.C. McDonald, F-D. Wang, C.S. Chen, I.H. Chen (Taipei City, TW; Atlanta, US)*

Objectives: *Staphylococcus aureus* bacteremia in patients is an increasingly prevalent problem. Particularly, methicillin-resistant *S. aureus* (MRSA) is one of the most serious healthcare issues currently influencing morbidity, length of hospitalization, healthcare costs, and mortality. This study was to examine the clinical significance of MRSA and methicillin-susceptible *S. aureus* (MSSA) bacteremia.
Methods: The retrospective cohort study was conducted in a 2,900-bed tertiary refer medical center. All patients admitted to the hospital who had developed nosocomial infections (NIs) from 1999 to 2008 were eligible. The diagnosis of NI was based upon criteria established by the Centers for Disease Control and Prevention. The Cox regression analyses were used to estimate the risk factors of MRSA and prognostic factors of mortality.
Results: A total of 1,230 patients were diagnosed with nosocomial *S. aureus* bacteremia. The proportion rate of MRSA was 78%. The dependent variable for Cox regression was length of ICU stay prior to the onset of the first infection, after controlling potential risk factors for MRSA bacteremia, used of mechanical ventilator (Relative Risk [RR], 1.19; 95% confidence interval [CI] 1.02–1.39) and insertion of central venous catheter (CVC) (RR, 1.42; 95% CI 1.03–1.94), were independent risk factors ($p < 0.05$) for MRSA. The difference between the crude mortality rates of MRSA bacteremia (42.4%) and MSSA bacteremia (24.1%) was 18.3% with statistical significance ($P < 0.001$). The dependent variable for Cox regression was length of ICU stay from after the onset of the first infection to mortality, after potential prognostic factors of mortality were adjusted, MRSA bacteremia (RR 1.46, 95% CI 1.07–1.99), age (RR 1.01, 95% CI 1.01–1.02), used of mechanical ventilator (RR 1.05, 95% CI 1.02–1.09), and insertion of CVC (RR 1.32, 95% CI 1.07–1.632) were independent factors ($p < 0.05$).



Conclusion: We found MRSA bacteremia to have a higher attributable mortality than MSSA bacteremia. In addition, invasive devices were the important risk factors and prognostic factors after adjusting for covariates. This study emphasizes the importance of continuous efforts in prevention of device-associated infections.

P1999 **The clinical feature and mortality of community-associated methicillin-resistance *Staphylococcus aureus*: comparison with healthcare-associated methicillin-resistant and methicillin-susceptible *S. aureus* bacteraemia**

S. Kang, S.M. Choi, H. Jang, K.H. Park, S. Jung (Gwangju, KR)*

Objective: No conclusive data is available about the difference in mortality between in patients with community-associated methicillin-resistant *Staphylococcus aureus*(CA-MRSA) bacteraemia and Health-care associated methicillin-resistant *Staphylococcus aureus*(HA-MRSA) bacteremia. In this study, we compared the outcome of CA-MRSA bacteraemia with that of HA-MRSA bacteraemia and that of methicillin-susceptible *Staphylococcus aureus*(MSSA) bacteraemia.
Methods: 556 adult patients with *S. aureus* bacteremia were analysed, who were admitted to Chonnam National University Hospital and Hwasun Chonnam National University Hospital in Korea from January 2005 to December 2008. The CA-MRSA was defined as *S. aureus* carring SCCmec type IV or IVa by multiplex PCR.
Results: Of the 556 *S. aureus* bacteraemia, 205(36.9%) were by MSSA, 290(52.2%) by HA-MRSA, and 61(11.0%) by CA-MRSA. Among 351 MRSA isolates, 240(68.4%) carried SCCmec type II, 49(14.0%) SCCmec typeIII, 22 (6.3%) SCCmec type IV, and 39(11.1%) SCCmec type IVa. Of the 61 patients with CA-MRSA bacteraemia, 56(91.8%) had risk factor for healthcare exposure, and 52(85.2%) were caused by clindamycin susceptible isolates. Only one CA-MRSA isolate carried Panton-Valentine leukocidin gene(PVL+), and no HA-MRSA isolate carried PVL. The 30-day mortality in patients with CA-MRSA bacteraemia was significantly higher than that in patients with MSSA bacteraemia(34% vs 21%, $P = 0.007$), but was not different with that in patients with HA-MRSA bacteraemia (34% vs 31%$P = 0.649$). In multivariate analysis using Cox-porportional hazard model, the independent risk factors for 30-day mortality were higher APACHEII score and cancer. After adjustment of these factors, the 30-day mortality in patients with CA-MRSA bacteraemia was not significantly higher than that in patients with MSSA bacteraemia(hazard ratio 1.17; 95% CI 0.67–2.04; $P = 0.585$) or that in patients with HA-MRSA bacteraemia(hazard ratio 0.85; 95% CI 0.52–1.37; $P = 0.846$).
Conclusions: The 30-day mortality in patients with CA-MRSA bacteremia was comparable with 30-day mortality in patients with HA-MRSA bacteremia, but was significantly higher than 30-day mortality in patients with MSSA bacteremia. However, after adjustment of other factors, mortality in patient with CA-MRSA bacteraemia without PVL gene was not different with mortalities in patients with MSSA bacteremia and in patients with HA-MRSA bacteremia.

P2000 **Insights in *Staphylococcus aureus* strains from bacteraemia and infective endocarditis in Italy**

F. Campanile, D. Bongiorno, S. Borbone, A. Goglio, A. Raglio, F. Suter, M. Venditti, C. Mancini, C. Venturelli, F. Rumpianesi, S. Stefani (Catania, Bergamo, Rome, Modena, IT)*

Objectives: *Staphylococcus aureus* bacteraemia (SAB) is a serious infection both in hospital and the community, possibly leading to infective endocarditis (IE), with a high risk of mortality despite aggressive therapy. The glycopeptides use has been recently challenged by various forms of low-level resistance. This study evaluated the distribution of MSSA and MRSA isolates from bacteraemia (B) and infective endocarditis (IE) in Italian hospitals, their antibiotic susceptibility features – focusing on the emergence of hVISA strains – and their genotypic relationships.

Methods: 106 *S. aureus* strains from patients with bacteraemia (58) or infective endocarditis (48), were collected from 3 Italian hospitals between 2004 and 2008. MICs for anti-Gram + drugs were determined according to CLSI guidelines; vancomycin heteroresistance was tested by macro Etest, and confirmed by PAP; PFGE and SCCmec (the latter only for MRSA) analyses were also performed.
Results: From 106 *S. aureus* strains, 30 were MRSA (13 MRSA-B and 17 MRSA-IE) and 76 MSSA (45 MSSA-B and 31 MSSA-IE). The hVISA phenotype was particularly diffused among MRSA strains, both from B (4/13) and IE (7/17), but an increase was shown also among MSSA (7 MSSA-B and 6 MSSA-IE). 3 PVL positive strains were detected only among MSSA. All *S. aureus* strains showed similar but not genetically related PFGE profiles. There was a major diffusion of SCCmec IV (subtypes a, c and g), related to a spread of CA-MRSA, both in IE and B patients (47 to 61%); HA-MRSA strains were associated to SCCmec types I/IA in 3 MRSA-B and 8 MRSA-IE strains. There was a correlation among hVISA strains and SCCmec types I/IA (7/11). Concerning antibiotic resistance, almost all the HA-MRSA/hVISA were MDR strains; a low rate of resistance was observed among MSSA, with only one showing the hVISA phenotype.
Conclusion: In this study we analysed the circulation of *S. aureus* strains cause of bacteraemia and infective endocarditis in Italy, their antibiotic and genotypic features, showing the emergence of hVISA phenotype among MSSA and MRSA strains, and the major spread of CA-MRSA.

P2001 Clinical characteristics of *Staphylococcus lugdunensis* bacteraemia

S. Choi, E.H. Song, E.J. Lee, J.B. Jun, J.W. Chung, J.M. Kang, M.S. Lee, S. Choi (Seoul, Gangneung, Ulsan, Pohang, KR)*

Objectives: The clinical significance of *S. lugdunensis* bacteremia has been largely unknown, mainly understood from the fatal cases of infective endocarditis. To evaluate the frequency and severity of *S. lugdunensis* bacteremia, we retrospectively reviewed all the cases with *S. lugdunensis* bacteremia from 7 tertiary care hospitals in Korea.
Methods: Identification of clinical isolates was performed using MicroScan Gram-positive combo panels (Dade Behring, CA, US) or Vitek 2 card for Gram-positive cocci (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibilities were determined by the same automated systems using standard criteria prescribed by the Clinical Laboratory Standards Institute.
Results: There were 61 patients with *S. lugdunensis* bacteremia. The isolation rates of *S. lugdunensis* were ranged from 0.3% to 1.2% and from 0.9% to 3.4% among all blood isolates and CNS blood isolates, respectively. Of the 61 patients, 17 had significant *S. lugdunensis* bacteremia. Majority (82.4%) had underlying diseases, which were mostly nonfatal underlying diseases according to the criteria of McCabe and Jackson (70.6%). Resistance rates to antimicrobials were as follows; penicillin (87.5%), oxacillin (31.2%), gentamicin (12.5%), erythromycin (13.3%), tetracycline (6.3%), ciprofloxacin (6.3%), clindamycin (12.5%) and fusidic acid (9.1%). Multidrug resistant isolates had hospital-associated bacteremia with long hospital stay before bacteremia. The probable sources of bacteremia were unknown source (41.2%), catheter (29.4%) and skin/soft tissue (29.4%). All Patients with catheter-related bacteremia had healthcare associated infection. Infective endocarditis was observed in 4 patients with community-associated infection except one. Severe sepsis (17.6%) and septic shock (5.9%) were rarely observed. Inappropriate antimicrobial therapy was not observed in fatal cases. Bacteremia-related death was not observed.
Conclusion: Significant *S. lugdunensis* bacteremia occurred rarely in the clinical practice. Significant bacteremia developed with mild clinical presentation frequently in patients with nonfatal underlying diseases. Whereas catheter-related bacteremia was frequent in hospital-associated infection, infective endocarditis was frequent in community-associated one. Bacteremia caused by multidrug resistant organisms was found more frequently than that of previous studies, especially in patients with longer hospital stay before bacteremia.

P2002 Delayed removal of catheter deteriorates clinical outcome in patients with catheter-related bloodstream infection due to coagulase-negative staphylococcus

M. Kamimura, Y. Yoshino, K. Tatsuno, T. Kitazawa, Y. Ota (Tokyo, JP)*

Objectives: Coagulase-negative staphylococcus (CNS) is commonly a cause of catheter-related bloodstream infection (CRBSI). Catheters are frequently retained CNS CRBSI. The aim of the study is to evaluate whether the timing of catheter removal influence on outcomes in case of CNS CRBSI.
Methods: From April 2003 to December 2006, all the patients with CNS bacteraemia at the University of Tokyo Hospital were enrolled. Clinical data were retrospectively collected from medical records; age, gender, clinical background, and acute physiology and chronic health evaluation (APACHE) II score, the timing of initiation of appropriate antimicrobial treatment and catheter removal. The endpoints were 7-day mortality and clinical improvement.
Results: 153 cases were enrolled in the study period. Seven-day mortality and the ratio of clinical improvement were 3.9%, 69%, respectively. For 7-day mortality, high score of APACHE II (>18), older age (>60 years old) and delay of appropriate anitimicrobial treatment (>48 hours after onset) were identified as risk factors, by univariate analysis, although high score of APACHE II was the only negative prognostic factor by multivariate analysis (odds ratio (OR) 14.26; $p = 0.011$). For clinical improvement, high score of APACHE II (OR 0.116; $p = 0.005$) and delay of catheter removal (OR 0.404; $p = 0.014$) were identified as negative prognostic factors by multivariate analysis.
Conclusions: In addition to the severity of the illness, the delayed removal of catheter deteriorated clinical response in patients with CNS CRBSI.

P2003 Cotrimoxazole versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteraemia – a retrospective cohort study

E. Goldberg, M. Paul, Z. Samra, O. Talker, M. Raskin, R. Chazzan, L. Leibovici, J. Bishara (Petah Tikva, IL)*

Objectives: To evaluate the efficacy and safety of cotrimoxazole versus that of vancomycin in adults with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia.
Methods: We identified 1006 clinically significant consecutive episodes of *Staphylococcus aureus* bacteremia between 1999 and 2009. Demographic, bacteriologic and clinical data, including the presumptive focus of infection and outcomes were retrieved retrospectively. We identified 38 patients with MRSA bacteremia treated with cotrimoxazole as the main therapeutic agent, defined as at least one week of continuous treatment with cotrimoxazole alone or with another drug other than vancomycin, daptomycin, linezolid or quinupristin/dalfopristin. We matched them with 76 patients treated with vancomycin as the main agent. The groups were matched for age, sex, functional status, endovascular source of infection, appropriateness of empirical antibiotic therapy, presence of a foreign body, sepsis severity and Charlson score. The outcomes collected were 30 day mortality, persistent bacteremia, relapse and adverse events. Persistent bacteremia was defined as positive blood culture (BC) >14 days after the first positive BC. Relapse was defined as recurrence of the same phenotype >30 days after the first positive BC within 12 months.
Results: The baseline characteristics of patients in the two groups are presented in the table. Overall, the groups were well matched with no significant differences in the baseline characteristics. Thirty day mortality was not significantly different between the groups [cotrimoxazole 13/38 (34.2%), vancomycin 31/76 (40.8%); $p = 0.49$]. There was only one case of relapse in the cotrimoxazole group (2.6%) compared with 9 cases in the vancomycin group (11.8%). Incidence of relapse or persistent bacteremia was lower in the cotrimoxazole group (3/38, 7.9%) than in the vancomycin group (13/76, 17.1%), although the difference was not statistically significant ($p = 0.182$). There was no significant difference

between the groups in renal failure as a complication [cotrimoxazole 11/38 (28.9%); vancomycin 21/76 (27.6%)].

Conclusions: Within the limitations of a small retrospective study, cotrimoxazole had a safety and efficacy profile similar to vancomycin and may offer an attractive additional therapeutic option for MRSA bacteremia. A prospective, randomized controlled trial is warranted.

Table 1: patient characteristics

Variable	Cotrimoxazole group (n = 38), N (%)	Vancomycin group (n = 76), N (%)
Age	74.7±15.9*	75.8±13.7*
Female gender	26 (68.4)	51 (67.1)
Bedridden patients	17 (44.7)	34 (44.7)
Diabetes	15 (39.5)	34 (44.7)
McCabe score:		
None	11 (28.9)	18 (23.7)
Ultimately fatal disease	20 (52.6)	40 (52.6)
Proximally fatal disease	5 (13.2)	12 (15.8)
Charlson score	3.53±2.46*	3.21±2.29*
Hospital acquired infection	20 (52.6)	43 (56.6)
Endovascular source of infection	9 (23.7)	18 (23.7)
Skin and soft tissue source of infection	7 (18.4)	19 (25)
Presence of central venous catheter	4 (10.5)	13 (17.1)
Appropriate empirical treatment	12 (31.6)	26 (34.2)
Septic shock	10 (26.3)	27 (35.5)

*Mean±standard deviation.

Endocarditis and cardiovascular implantable electronic device infections

P2004 Continuing changing profile of infective endocarditis – results of a repeat one-year population-based survey in France, 2008

B. Hoen, M. Célard, F. Alla, V. Le Moing, T. Doco-Lecompte, M. Revest, C. Strady, E. Cambau, A. Bouvet, C. Selton-Suty, X. Duval for the AEPEI Study Group on infective endocarditis*

Objectives: Important changes in the characteristics and management of infective endocarditis (IE) were observed over the past decades. For the third time we conducted a one-year, population-based prospective survey to describe the changes in epidemiological, clinical, and microbiological characteristics, surgical management, and outcome of IE, as compared to the results of the 2 similar surveys we had conducted in France in 1991 and 1999.

Methods: We collected data of all adult patients hospitalized for IE in 2008 in the regions that participated in the previous 2 studies and in 2 additional regions, accounting for a total of 20 million inhabitants, i.e. 32% of the whole French population. Cases were notified by the patient's attending clinicians, echocardiographists, surgeons, and/or microbiologists. For each patient, a case report form was filled out by a specially trained research nurse. A validation committee reviewed all forms. Of the 983 cases notified, 487 cases fulfilled the inclusion criteria, were Duke-Li definite and were analysed. Standardised annual incidence rates (SAIR) were calculated and compared between the 3 surveys.

Results: Sex ratio was 3M/1F. Patients' mean age was 62.4 years. 50.7% of patients had no known valve disease and 22.6% had a prosthetic valve. The distribution of causative microorganisms was as follows: *Staphylococcus aureus* 26.9%, oral streptococci 18.5%, group D streptococci 12.5%, enterococci 10.5%, coagulase-negative staph 10.1%, pyogenic streptococci 4.7%, other streptococci 1.8%, and other pathogens 8.0%. More than one and no microorganism was identified in 1.6% and 5.3% of the cases respectively. Blood cultures were negative in 8.4% of the cases. Valve surgery was performed in 48.3% of the patients during the initial hospital stay. Overall in-hospital lethality rate was 21%. Overall SAIR of IE in 1991, 1999, and 2008 were 35.0, 33.4, and 32.4, per 100.000 inhabitants respectively. Specific incidence rates increased for IE in patients with no known valve diseases and IE due to *S. aureus*, decreased for group D streptococcal IE, and remained stable for oral streptococcal IE.

Conclusion: Incidence of IE appears stable in France. This repeat population-based survey confirmed the increasing proportion and incidence rate of *S. aureus* IE as well as the increasing incidence of IE in patients with no known valve disease. Conversely, the incidence of group D streptococcal IE sharply decreased.

P2005 Rising incidence and persistently high mortality of bacteraemia: a 15-year population-based study in Denmark

M. Søgaard, M. Nørgaard, L. Mortensen, H.C. Schønheyder (Aalborg, DK)*

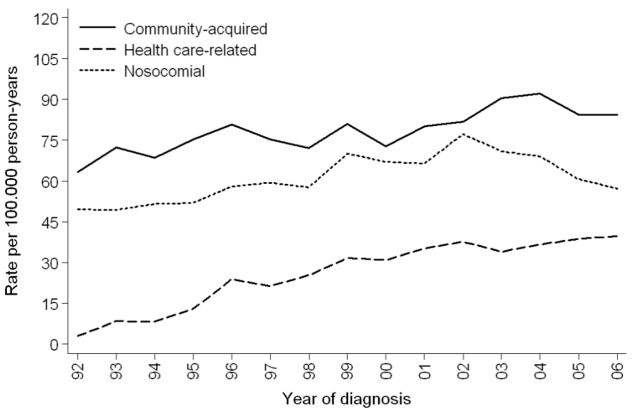
Objective: Bacteraemia incidence has increased during the last decades, but population-based data are sparse. We have examined temporal trends of community-acquired, health care-related, and nosocomial bacteraemia within a defined population.

Methods: We conducted a population-based cohort study to examine changes in incidence and 30-day mortality associated with community-acquired, health care-related, and nosocomial bacteraemia in North Jutland County, Denmark between 1992 and 2006. All patients with a first-time bacteraemia diagnosis were identified in regional databases and followed for all-cause mortality through the Danish Civil Registry System. We determined age- and gender-standardised incidence rates and adjusted mortality rates associated with origin of bacteraemia and calendar period.

Results: We identified 12,535 patients with a first-time bacteraemia episode; 5,941 (47.4%) were community-acquired, 4,619 (36.9%) nosocomial, and 1,975 (15.8%) health care-related. Between 1992 and 2006, the overall incidence increased from 116 to 181 per 100,000 person-years, corresponding to an age and sex adjusted incidence rate ratio of 1.56 (95% CI 1.34–1.80). Age- and sex standardized incidence rates of community-acquired and nosocomial bacteraemia peaked at 92.1/100,000 and 77.2/100,000 in 2004 and 2002, respectively, whereas the incidence of health care-related bacteraemia increased steadily from 2.9/100,000 in 1992 to 39.8/100,000 in 2006 (Figure 1).

The overall 30-day mortality was 16.4% in patients with community-acquired bacteraemia, 22.2% in patients with health care-related bacteraemia, and 28.2% in patients with nosocomial bacteraemia. During the 15-year study period, the 30-day mortality decreased among patients with community-acquired bacteremia (19.0% in 1992–1996; 15.1% in 1997–2001, and 15.4% in 2002–2006), but was virtually unchanged in patients with health care-related (23.4%, 22.1%, and 22.0%, respectively) and nosocomial bacteraemia (27.9%, 29.1%, and 27.7%, respectively). Because of the increasing incidence, the total number of deaths per 5-year period increased from 757 in 1992–1996, to 920 in 1997–2001, and 1036 in 2002–2006.

Conclusion: The incidence of bacteraemia, regardless of origin, increased considerably in Denmark during the last 15 years. The 30-day mortality only improved for community-acquired bacteremia, which is of clinical and public health concern.



P2006 **Transoesophageal echocardiography in *Staphylococcus aureus* bacteraemia – when is it dispensable?**

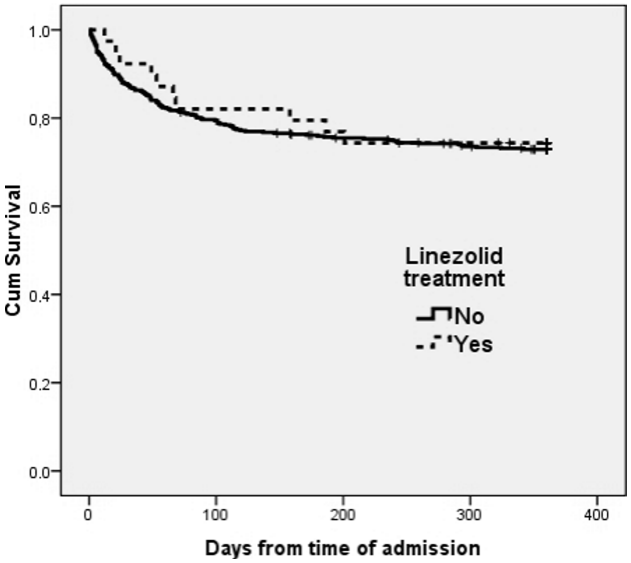
A. Kaasch, S. Rieg, H. Birkholz, S. Neumann, G. Peppinghaus, G. Peyerl-Hoffmann, W. Kern, H. Seifert (Cologne, Freiburg, DE)*

Objectives: Infective endocarditis (IE) is a severe complication in patients with *Staphylococcus aureus* bacteraemia (SAB). Therefore, it has been suggested that transoesophageal echocardiography (TEE) should be performed in every patient with SAB. In some clinical scenarios however, IE is unlikely to occur. We sought to develop and validate criteria for the indication of transoesophageal echocardiography (TEE) in patients with SAB.
Methods: Data from 570 consecutive patients with SAB was assessed. All patients were enrolled in the prospective INSTINCT study from January 2006 to June 2009 at the Cologne and Freiburg University hospitals. Patients with and without IE were analyzed for predisposing factors, clinical features, diagnostic procedures, and outcome. The diagnosis of IE was based on the Duke criteria and patients were followed closely for the development of IE during a 3 months follow-up period. Guided by clinical judgement, criteria for performing TEE were defined as: community-acquired SAB, prosthetic heart valve, vascular implant, hemodialysis, injection drug use, documented or possible (no follow up blood cultures taken in due time) prolonged bacteraemia (>3 days), vertebral and non-vertebral osteomyelitis. The suitability of the criteria set was assessed retrospectively.
Results: In 9.5% (54) of patients with SAB, a diagnosis of IE was made during hospitalization. Further 3 patients (0.5%) developed IE during the follow-up period. The aortic (45%) and mitral valves (38%) were most commonly affected; 19% of cases were prosthetic valve infections.
Of all patients with SAB, 460 (81%) fulfilled one or more criteria for echocardiography, mainly possible or documented prolonged bacteraemia (57%), community acquisition (46%), occurrence of a vascular implant (16%) and hemodialysis (9%). All patients with a final diagnosis of *S. aureus* IE fulfilled at least one criterion for performing TEE (sensitivity: 100%). Among patients not fulfilling any of the criteria for performing TEE, none developed IE during hospitalization and follow-up (negative predictive value: 100%). The positive predictive value of TEE increased from 10% to 14.6% when using the criteria set.
Conclusion: A simple criteria set for patients with *S. aureus* bacteraemia was developed that allows to safely reduce the number of echocardiographic evaluations for infective endocarditis by about 20%.

P2007 **Linezolid in infective endocarditis – it seems to work**

T.K. Lauridsen, L.E. Bruun, R.V. Rasmussen, C. Moser, H.K. Johansen, M. Arpi, C. Hassager, N.E. Bruun (Copenhagen, DK)*

Background: The increasing number of resistant bacterial strains in infective endocarditis (IE) emphasizes the need for a constant development of new antimicrobials. Linezolid is a new antibiotic with effect on Gram-positive cocci. Only few casuistic reports describe its utilization in treatment of IE.
The objective of this study is to report our experience with linezolid from a large contemporary cohort of IE patients.
Methods: In a prospective cohort study data from 521 consecutive patients were collected at two tertiary University Hospitals in Copenhagen, Denmark. Only patients with culture negative IE or IE due to Gram-positive micro organisms were included in the study. Main endpoints were in-hospital and post-discharge mortality in IE patients receiving linezolid for a part of their treatment period vs. non-linezolid treatment.
Results: 521 patients were enrolled in the study, 39 patients received linezolid treatment, and 482 received non-linezolid treatment. Reasons for changing to linezolid were allergic reactions (N = 10), nephrotoxicity (N = 2) and inadequate response to given treatment (N = 17). There were no significant differences in age, gender or co-morbidity such as diabetes, kidney dysfunction, heart failure, cardiac surgery or neurological diseases. There were no differences in the infected valve location or complications to IE such as pseudoaneurysms or intracardiac abscesses. Median duration of linezolid treatment was 18 days (range 2–69 days). No differences in in-hospital mortality (10% vs. 14%, NS) or post-discharge mortality at 12 months follow-up (26% vs. 27%, NS) linezolid vs. no linezolid, respectively were observed. Only few side effects of linezolid were noted. During linezolid therapy >28 days, 4 of 9 patients developed drug related anemia, requiring blood transfusions.
Conclusion: No difference was found between in-hospital and post-discharge mortality in IE patients receiving linezolid for a part of their treatment period vs. patients with conventional treatment. Despite our results, controlled studies are warranted before linezolid more generally can be recommended for treatment in IE.



P2008 **Effect of management interventions on short- and long-term mortality in patients with cardiovascular implantable electronic device infections**

K. Le, M. Sohail, P. Friedman, D. Uslan, S. Cha, D. Hayes, W. Wilson, J. Steckelberg, L. Baddour (Rochester, Los Angeles, US)*

Objective: To evaluate the impact of management interventions on in-hospital and overall mortality among patients with cardiovascular implantable electronic device (CIED) infections.
Background: CIED infections are associated with mortality during both index hospitalization and overall mortality over long-term follow-up. The specific impact of management interventions has been poorly defined.
Methods: We retrospectively reviewed all cases of CIED infection seen at Mayo Clinic Rochester between 1991 and 2008. Mortality was considered from time of presentation to time of death during the index hospitalization, and to time of death during the subsequent follow-up period. The impact of management interventions were identified using Cox proportional hazard regression.
Results: Of the 418 patients who presented with CIED infection, 30 (7.1%) died during the median of 13 days of hospitalization, and 140 (33.5%) died during the median of 0.95 years of subsequent follow-up. Of the 396 patient who underwent device removal, 39 (9.8%) of them developed extraction complications, and seven (1.7%) of them died. In a multivariable analysis that included adjusting for significant clinical predictors of mortality, device extraction complications were associated with elevated in-hospital mortality (HR 9.86 [3.01, 32.34]). Despite the risk of complications related to extraction, our data suggest that overall, patients benefited from device removal. Antibiotic therapy without device removal was associated with increased in-hospital mortality (HR 7.69 [2.88, 20.56]), and over-all mortality (HR 2.18 [1.05, 4.48]) when compared to combined management with antibiotic therapy plus device removal. Additionally, the timing of device removal had an impact on in-hospital mortality. Compared to device removal at initial hospital

presentation, device removal after failure of antibiotic therapy was associated with increased in-hospital mortality (HR 3.22 [1.09, 9.50]).
Conclusions: Our findings indicate that although device removal can result in complications that are associated with increased in-hospital mortality, these complications are rare. Therefore, timely initiation of antibiotic therapy and complete device removal early on are crucial for increasing in-hospital survival.

P2009 Endocarditis: effects of routine implementation of echocardiography in the work-up of high-risk patients with Gram-positive bacteraemia

F.J. Vos, C.P. Bleeker-Rovers, P.D. Sturm, W.J. Oyen, B.J. Kullberg (Nijmegen, NL)*

Objectives: Although the advice from the literature is clear, routine echocardiography is only performed in a minority of patients with *S. aureus* bacteraemia. The aim of the study was to evaluate if routine performance of echocardiography in a high risk patient group with Gram-positive bacteraemia leads to an increased percentage of diagnosed endocarditis and to a better outcome when compared to clinically driven requests for echocardiography.
Methods: In this prospective case control study, a regime of "obligatory" echocardiography within two weeks after admission in the study group was compared to a perfectly matched historical control group in the same hospital in whom echocardiography was performed at the discretion of the attending physician.115 patients with Gram-positive bacteraemia and at least one risk factor for the presence of metastatic infection were prospectively included between 2005 and 2008 and compared to a matched historical control group of 230 patients treated between 2001 and 2004. Endocarditis was diagnosed according to the Duke criteria.
Results: Echocardiography was performed significantly more often in the study group (81.7% vs. 27.4%, $p < 0.001$). The yield of echocardiography was 23% in the study group and 30% in the control group ($p = 0.7$). In the absence of a heart murmur, 70% of patients did have an echocardiogram in the study group against 21% in the control group ($p < 0.001$). Endocarditis was diagnosed significantly more often among study patients, 22/115 (19.1%) vs. 17/230 (7.4%) in the control group ($p = 0.002$). In the control group, endocarditis was first detected by autopsy in an additional two patients. No differences in adherence to AHA guidelines concerning choice and duration of antibiotic treatment were found between both groups in patients with definite endocarditis. Overall mortality was 19% in the total number of study patients compared to 32% in control patients ($p = 0.007$). In the subgroup of endocarditis patients, overall mortality was 23% in study patients and 63% in control patients ($p = 0.012$).
Conclusion: Correctly diagnosing endocarditis in a larger proportion of patients was associated with a significant decrease of mortality rates, probably because endocarditis was recognised only in an advanced stage in all historical controls who were therefore not treated adequately. Therefore, echocardiography should be performed in all patients with Gram positive bacteraemia and at least one risk factor for complicated infection.

Emerging vaccines

P2010 Protective effect of DNA vaccine cocktail including plasmids encoding *Toxoplasma gondii* major surface antigen 1 and complete Rhoptry2 against lethal toxoplasmosis in BALB/c mice

K. Hosseinian Khosroshahy, F. Gaffarifar, S. D'Souza, Z. Sharifi, A. Dalimi Asl (Tehran, IR; Brussels, BE)*

Objectives: *Toxoplasma gondii* is an obligate intracellular protozoa that causes toxoplasmosis in human and animal. In recent years, significant progress has been made in the identification of vaccine candidates which can induce a protective response.
Like other unicellular organisms *Toxoplasma gondii* is composed of various immunogenic antigens. Excreted/Secreted antigens and surface antigens maybe the best forms of antigens for stimulation of the cell mediated immune response and hence they appear to be good candidates for vaccine against toxoplasmosis.
In this study we used complete Rhoptry proteoin2 (ROP2) and major surface antigen 1 (SAG1) genes of *Toxoplasma gondii* as DNA vaccine cocktail.
Methods: Genomic DNA extracted by phenol-chloroform method and amplified by PCR method. The PCR products cloned in to pTZ57R/T plasmid and then confirmed by sequencing.
Result of sequencing indicate a 1686 bp fragment of ROP2 gene of *Toxoplasma gondii* was cloned into pTZ57R/T plasmid. The results of BLAST in NCBI showed that this gene has 99% and 98% homology with RH strain Gene Bank Accession Numbers. Z36906.1 and S 54994.1 respectively. Then this gene subcloned into pc DNA3 and after transfection of Eukaryotic cell (CHO) with this recombinant plasmid (pc-ROP2), expression of this gene was confirmed by SDS-PAGE and western blot.
Afterwards, we investigated the efficacy of pc SAG1 + pc ROP2 (DNA cocktail) with or without adjuvant Alum in female inbred BALB/c mice against toxoplasmosis. Mice were intramuscularly immunized three times at 3 week interval.
Results: DNA cocktail immunization induced a protection against a lethal challenge with the highly virulent *Toxoplasma gondii* RH strain, whereas control groups were not protective.
Anti-*T. gondii* IgG values (OD) increased markedly in the case groups, which were significantly higher than those of control groups ($P < 0.05$). The results of cytokine (IFN-γ, IL-4) assay show that mice immunized with pc SAG1 + pc ROP2 elicited stronger Th1-type cellular immune responses than those immunized with empty plasmid, or phosphate buffer salin (high level of IFN-γ and low level of IL-4).
Conclusion: Our study indicates that the DNA vaccine cocktail (pc ROP2 + pc SAG1) is more powerful and efficient than single vaccines, and the co-delivery of Alum not enhanced the potency of DNA vaccine. These results support further investigations to achieve a multi-gene anti-*T. gondii* DNA vaccine.

P2011 Immnunization of dogs with a recombinant cysteine proteinase from *Leishmania chagasi* in an endemic region (Teresina-PI, Brazil) of visceral leishmaniasis

P.H.C. Pinheiro, S. Katz, J.H.L. Ferreira, C.L. Barbiéri (Teresina, São Paulo, Picos, BR)*

A recombinant protein, rLdccys1, produced by expression of the gene encoding a 30 kDa cysteine proteinase from *Leishmania* (*Leishmania*) chagasi, was used for immunization of dogs and evaluation of its possible protective role against L. (L.) chagasi infection in an endemic region of visceral leishmaniasis (VL), Teresina, Piauí State, Brazil.
Cellular immune responses induced by the recombinant antigen were evaluated after immunization of dogs with rLdccys1 plus *Propionibacterium acnes*. Peripheral blood mononuclear cells isolated from rLdccys1-immunized dogs showed significant stimulation indexes after *in vitro* incubation with either rLdccy1 or L. (L.) chagasi amastigote extracts. Cytokine dosages in the supernatants from lymphocyte cultures showed significant levels of IFN-γ, whereas IL-10 was not detected.
Whereas 3 from 4 dogs immunized with rLdccys1 plus *P. acnes* and challenged by intraperitoneal injection of 1×10^4 L. (L.) chagasi amastigotes survived ten weeks after challenge, control dogs which received either PBS or *P. acnes* died after four and six weeks, respectively. The load of L. (L.) chagasi amastigotes in spleen, liver, and bone marrow from rLdccys1-immunized dogs was significantly reduced in comparison to that of non immunized controls. A significant concentration of IFN-γ and basal levels of IL-10 were detected in sera from dogs immunized with rLdccys1.
All dogs immunized with rLdccys1 plus *P. acnes* and challenged by the bite of L. (L.) chagas infected *Lutzomyia* longipalpis survived until sixteen weeks after challenge, whereas control dogs injected with PBS or *P. acnes* died after seven and nine weeks, respectively. Control dogs showed a significant number of L. (L.) chagasi amastigotes in liver and

spleen, but no parasites were found in rLdccys1-immunized dogs. During immunization with rLdccys1 there was an increase of serum levels of IFN-γ in the immunized dogs that peaked one week after challenge. In contrast, a very low concentration of IL-10 was detected in these animals.
Overall, these findings indicate that L. (L.) chagasi recombinant cysteine proteinase is potentially useful for immunoprophylaxis of canine VL. We believe that results obtained open perspectives for immunization of dogs in the field and evaluation of the impact on the disease incidence.

P2012 Antibody dynamics after tick-borne encephalitis and Measles–Mumps–Rubella vaccination in children post early thymectomy

M. Zlamy, C. Goeggele, J. Schönlaub, R. Würzner, H. Holzmann, L. Zimmerhackl, M. Prelog (Innsbruck, AT)*

Objectives: In a previously published prospective controlled cohort study, thymectomized children, who had thymectomy in early childhood due to open heart surgery, completed a three-dose immunization regimen in order to analyze the clinical impact of immunological alterations in thymectomized patients after exposure to a new antigen (tick-borne encephalitis virus (TBEV) vaccine). In the previous study, thymectomized children showed significantly lower TBEV IgG antibody levels after the second vaccination when compared to healthy age-matched controls, but a normal response after the third vaccination.
Methods: The present study was aimed to analyze the TBEV-specific IgG antibody response 3 years after the third TBE vaccination in 22 thymectomized patients compared to 37 non-thymectomized healthy controls from the previously published cohort, to identify patients with waning antibody titers. Additionally, the serum samples were tested for measles, mumps and rubella IgG antibody concentrations after immunization with live-virus-attenuated vaccine administered post thymectomy. The TBEV-, measles- and rubella-specific IgG antibody avidity was analyzed by an adapted ELISA.
Results: Although there was a great inter-individual variety in the TBEV-specific IgG concentrations, thymectomized patients showed equal levels compared to healthy controls. The humoral immune response to measles, mumps and rubella was normal in thymectomized patients. There was no difference in avidity maturation of TBEV-, measles- and rubella-specific IgG antibodies between patients and healthy controls.
Conclusions: Despite a delayed primary humoral immune response to new vaccine antigens, thymectomized patients are able to produce and maintain an appropriate memory immune response to TBEV and live-virus-attenuated vaccines administered post thymectomy. The follow-up data underline the hypothesis of a normal memory function but a diminished primary humoral immune response to new antigens in thymectomized patients, which may also have an impact in later life with increased risk of morbidity or mortality due to infectious diseases with new pathogens.

P2013 Distribution of pertussis antibodies among Iranian children of different age groups

A. Eslamifar, A. Aghakhani, M. Banifazl, A. Khadem-Sadegh, A. Ramezani (Tehran, IR)*

Objective: Pertussis is a highly communicable, vaccine-preventable respiratory disease characterized by paroxysmal cough often accompanied by inspiratory whoop and posttussive emesis. Although the largest number of reported cases is among young infants, the most dramatic increase in pertussis incidence has been reported among adolescents and young adults. In this study we determined the distribution of antibody levels against pertussis antibodies among children of different age groups in Tehran, Iran.
Methods: Plasma samples of 833 children (459 male and 374 female) between ages of 6–20 years were tested for the presence of pertussis toxin (PT), filamentous hemagglutinin (FHA) and different lipopolysaccharides (LPS) IgG antibodies by Enzyme-linked immunosorbent assay (ELISA). All cases vaccinated five times with DTP vaccine. The pertussis antibodies >24 U/ml was considered positive. The children were separated to three age groups: Group 1 (6.0–10.9 years; n = 361), Group 2 (11.0–15.9 years; n = 284) and Group 3 (16.0–20.9 years; n = 188).
Results: The overall prevalence of pertussis antibodies was 45.5% (95% CI: %42.1-%48.9). The mean antibody titer was 43.3±47.8 U/ml. Pertussis antibodies prevalence rates according to age groups were as follows: Group 1; 39.1%, Group 2; 45.8% and Group 3; 57.4%. Pertussis antibodies positivity in terms of age groups was significantly different from each other ($P < 0.001$) and significant elevation in pertussis antibodies rates was observed with increasing age. For all age groups, there was no statistically significant difference between genders regarding to pertussis antibodies positivity.
Conclusion: Our study showed that *Bordetella pertussis* infection is widespread and on the rise in Iranian adolescents and young adults. Booster vaccination of this age group with acellular pertussis vaccines appears to be the most logical approach to disease prevention in adolescents and control the circulation of the organism.

P2014 Cross-reactivity evaluation of *Neisseria meningitidis* serogroups A and B outer membrane vesicles as a method to induce cross-immunity against both serogroups

A. Sharifat Salmani, S.D. Siadat, M.R. Aghasadeghi, K. Parivar, S.M. Sadat, S. Amini, M. Razavi (Tehran, IR)*

Objective: Outer membrane vesicle (OMV) of *Neisseria meningitidis* is an immunogenic structure and would promote specific and long-lasting serologic responses against the organism. All the serogroups of *N. meningitidis* poses this outer membrane structure which is released from the cell surface during growth of the organism.
Method: OMV of *N. meningitidis* serogroups A and B purified through Classen method. Serogroup B OMV injected intramuscularly to animal model (rabbit). Booster doses injected 2 and 4 weeks after the first immunization. Serum samples of immunized animals collected two weeks after any injection. Two different ELISA kits designed with serogroup A and B OMV as the solid phase antigen to assay total IgG titer against Serogroup A and B OMV. Optimized concentration of OMV determined by checkerboard and coated. The probable cross-reactivity between these two antigens was evaluated by measurement of anti-serogroup A OMV IgG in the animals immunized by the serogroup B OMV.
Results: OMV of *N. meningitidis* serogroup B elicited high titer of specific antibody. The produced antibody against serogroup B OMV was highly cross-reactive with serogroup A OMV. The elicited titer against Serogroup A OMV was close to the titer of anti-serogroup B OMV.
Conclusion: OMV of *N. meningitidis* serogroup B has efficiently promoted the synthesis of anti-OMV IgM and IgG.Sera of immunized animals with serogroup B OMV reacted with serogroup A OMV and high titers of anti-serogroup A OMV detected in the sera of serogroup B OMV-immunized animals. The detected titer of anti-serogroup A OMV in the sera of animals immunized against serogroup B OMV reveals the cross-reactivity between OMV of A and B serogroups. Since OMV consists of different outer membrane proteins, anti-OMV antibodies may be protective against Meningococci. The protective antigen of available vaccine for Meningococcal meningitis is a capsular polysaccharide. Immune responses against polysaccharides mostly lacks memory, isotype switching and affinity maturation of antibodies that may leads to weak opsinization of the microorganism and increase of microorganism chance to survive. Opsonophagocytic activity of anti-OMV antibodies would be studied to determine the cytolytic activity of these antibodies and the probability of the OMV application as the protective antigen in a new meningococcal meningitis candidate vaccine to overcome drawbacks of the available vaccine and induce cross-immunity against both of serogroups A and B.

P2015 Recombinant group B streptococcal peptides as protection against broad range of pathogens

A.N. Suvorov, G.F. Leontieva, M. Grudinin, M. Stukova, A. Shaldzhyan, E. Romanko, K. Grabovskaya, L. Meringova, I. Koroleva, N. Duplik, A. Korjueva, T. Gupalova, A. Totolian, O. Kiselev (St.Petersburg, RU)*

Objectives: Group B streptococcus (GBS) is known as the major cause of newborn mortality and morbidity in the elderly. This pathogen together with other Gram positive bacteria can follow and complicate the viral infections. Several research groups are working on GBS vaccine employing various polysaccharides or peptides. In our previous studies we have selected several surface localized GBS proteins as the antigens for complex GBS vaccine. Some of the peptides under study share immunogenic domains with the proteins from other bacterial species. The goal of the present study was to evaluate the possibility to construct a vaccine protecting not only against GBS, but group A streptococcus (GAS), pneumococcus and influenza A virus.
Materials and Methods: Recombinant streptococcal peptides based on ScaAB, SspB1, ScpB, Clp and Bac immunogenic regions were expressed in pQE cloning system and affinity purified. Equimolar quantity of recombinant HA protein was added to the set of bacterial antigens for viral protection. Immunogenicity in mice model was evaluated by ELISA. Protection *in vitro* was done by opsonophagocytosis. *In vivo* protection was accomplished on either previously immunized mice or after adding the protective IgG together with the microbes. Viral load in the lungs was determined by a TCID50 assay on Vero cells.
Results: Bacterial surface polypeptides under study provided protection against the challenge with the most of GBS and GAS strains in all models tested. In addition ScaAB and SspB1 antibodies were able to opsonize the pneumococci. The mixture of peptides performed better range of protection compared to the single antigens. Introduction the influenza virus antigen in the vaccine composition decreased the viral load without the loss of protectivity against bacterial infection.
Conclusion: Recombinant polypeptide vaccine has a potential for protection not only against the bacterial but viral-bacterial infections.

P2016 The humoral immune response of infants to potential vaccine candidates of *Moraxella catarrhalis*

S.J. Verhaegh, C.P. de Vogel, K. Riesbeck, T.F. Murphy, E.R. Lafontaine, H.A. Moll, A. van Belkum, J.P. Hays (Rotterdam, NL; Malmo, SE; Buffalo, Athens, US)*

Objective: *M. catarrhalis* is a frequently found nasopharyngeal commensal of young children that has the capacity to also cause upper respiratory tract infections. *M. catarrhalis* outer membrane proteins (OMPs) play a significant role in the colonization and infection process, with several potential OMP vaccine candidates having been described. However, relatively little is known about the development of the humoral immune response to *M. catarrhalis* OMP vaccine candidates within the first few years of life.
Methods: Nine different potential *M. catarrhalis* vaccine candidates from laboratories in the USA and Europe were included: OMPs $Hag^{385–863}$, $McaP^{51–333}$, MhaC, $MID^{764–913}$, $MID^{962–1200}$, orf238, orf296, $UspA1^{557–704}$ and $UspA2^{165–318}$. Serum samples were collected from cord blood and venepuncture at 6, 14 and 24 months of age from a cohort of Dutch infants participating in the Rotterdam Generation R Study. Antigen-specific IgG, IgA and IgM levels against the respective *M. catarrhalis* OMPs were measured using the Luminex bead based flow cytometry technique, and a timeline of antibody responses was obtained.
Results: Antigen-specific IgG, IgA and IgM levels showed extensive inter-individual variability over time. The level of antigen-specific IgG in cord blood was significantly higher for Hag, MID^{764}, MID^{962}, UspA1 and UspA2 than at 6 months of age ($P < 0.001$). However, IgG levels against MID^{764}, MID^{962}, UspA1 and UspA2 rose significantly between 6 months to 2 years of age. IgG levels to *M. catarrhalis* OMPs Hag, McaP, MhaC, orf238 and orf296 remained relatively low and did not significantly increase over the 6 month to 2 year time period. IgA and IgM levels to all 9 OMPs were relatively low throughout the study period. However, IgA levels to the OMPs Hag, MID^{764}, MID^{962}, UspA1 and UspA2, and IgM levels to MhaC, MID^{764}, MID^{962}, UspA1 and UspA2 increased significantly ($P < 0.05$) over the 6 month to 2 year time period.
Conclusions: This is the most intensive study of the infant immune response to potential vaccine candidates of *M. catarrhalis* performed to date. Infants develop a range of IgG, IgA and IgM responses to potential *M. catarrhalis* vaccine candidate proteins in a time dependent manner, though IgG responses predominate. Antibody responses to the major OMPs MID/Hag, and UspA1 and UspA2 were most significant. Further studies are required in order to determine whether these immune responses correlate with protection from re-colonization and infection.

P2017 Implications of universal childhood pneumococcal vaccination on invasive pneumococcal disease in HIV population – a review of current infecting serotypes

C. Rock, T. Coulter, K. Rock, N. Conlon, C. Dowling, J. Jackson, C. Feighery, C. Bergin (Dublin, IE)*

Objectives: Invasive pneumococcal disease (IPD) is associated with high morbidity and mortality. HIV is a well described risk factor with annual incidence of up to 1% in the AIDS population. Vaccination with 23-valent pneumococcal polysaccharide (23 PPV) is recommended, with 5 yearly booster. Childhood vaccination schedule in Ireland includes CPV7 since 2008. We review IP isolates in adult patients (HIV positive and negative) for serotypes, risk factors prior to introduction of universal childhood CPV 7 and examine potential benefit of prime boost strategy.
Methods: Laboratory surveillance and medical records were reviewed for sex, age, HIV status, comorbidities of all IP isolates from 2006 to 2008, IP serotype from March 2007.
Results: There were 98 IP isolates, 96 (98%) blood culture and 2 (2%) PCR on cerebrospinal fluid. 30 were HIV positive, 68 HIV negative. HIV positive: 21 (70%) male, average age 38, risk factors for HIV; 25 (83%) IVDU, 4 (13%) African HIV negative; 34 (50%) male, average age 61. IVDU (83%) and malignancy (28%) were strong risk factor for IPD in HIV positive and negative respectively. IP serotype was known for 20/31 (64.5%) HIV positive, 27/67 (40.2%) negative. HIV positive vs negative: 15 (75%) vs 26 (96.3%) in PPV23, 5 (25%) vs 21 (80.7%) in both vaccines, 5 (25%) vs 5 (18.5%) in neither vaccine, 10 (50%) vs 5 (18.5%) in PPV23 but not CPV7. These differences in vaccine coverage are important when considering prime boosting strategy. There was no difference in antibiotic sensitivity of isolate per HIV status. 83% were fully sensitive, 13% intermediate sensitivity to penicillin. Only 7 (21%) HIV had PPV23 prior to IPD, 3 had serotype covered in PPV23, all IVDU with low CD4 count, none required ICU admission.
Conclusion: CPV7 should not replace PPV23 in adult HIV population but prime boosting approach may confer greater immunity. IPD in HIV population as a surrogate marker for lack of access to healthcare in IVDU population. Targeted strategies are required to engage this cohort in vaccination services.

P2018 Changing epidemiology of *Streptococcus pneumoniae* in Morocco, 2006–2008 – implications for national immunization programme

N. El Mdaghri, H. Belabbes, J. Najib, K. Zerouali, M. Benbachir (Casablanca, MA)*

Objectives: To assist decision makers in determining the best choice of pneumococcal conjugate vaccines (PCV) to be introduced soon in national program immunization in Morocco.
Methods: Susceptibility testing and serotyping were performed on *Streptococcus pneumoniae* (Sp) isolates recovered from invasive diseases in children 5 years or less hospitalized in the children university hospital in Casablanca (Morocco) in 2006–2008 and compared with surveillance data from earlier periods.
Results: From 1998–2001 to 2006–2008, the overall prevalence of penicillin non-susceptible pneumococcal isolates increased from 15.3%

(12% with intermediate resistance) to 24.4% (15.7% with intermediate resistance). This increase was particularly of concern among paediatric isolates (21.4% to 43.3% versus 13% to 15% among adult isolates). The prevalence of non susceptible isolates to other antibiotics were for amoxicillin (2.7%), ceftriaxone (0.7%), erythromycin (14.3%), chloramphenicol (8%) and tetracycline (30.5%). Seventeen serotypes were recognized among the 83 isolates recovered from invasive diseases in children ⩽5 years in 2006–2008. Predominant serotypes were 19F (14.5%), 23F (13.25%), 14 (12%), 6B (10.8%), 5 (10.8%), 19A (9.6%) and 1 (8.4%). Pneumococcal isolates with higher rates of penicillin non-susceptibility were serotypes 14 (100%), 6B (89%), 19A (75%), 23F (54.5%) and 19F (41.7%). Serotypes included in the PCV-7, PCV-10 and PCV-13 accounted for 54%, 76% and 88% respectively but the potential coverage of these vaccines regarding penicillin non-susceptible isolates would be 71.4%, 76.2% and 90.5% respectively. The serotype distribution fluctuated significantly with time, showing an increase of PCV-7 and PCV-7 related serotypes among children invasive isolates, from 28% in 1998–2001 to 65% in 2005–2008.

Conclusions: This study documents the increase of penicillin-non-susceptibility among Sp isolates causing invasive diseases in paediatric population in Morocco and the fluctuation of serotype distribution over the time before conjugate vaccine introduction. These changes stress the need for continuous and country wide laboratory surveillance to guide treatment recommendations and conjugate vaccine formula choice.

P2019 Serotype coverage of present and future pneumococcal vaccines

A.W. Rosingh, E. Canton, G.F. Fagundez, F. Gonzalez, M. Gobernado on behalf of the MIVA Network*

Background: Infection with *Streptococcus pneumoniae* can cause invasive pneumococcal disease (IPD). IPD is a cause of high mortality and morbidity worldwide. The currently available vaccine includes 7 serotypes (PCV7) and the two future vaccines include 10 (PCV10) and 13 (PCV13) serotypes. To make decisions about including a pneumococcal vaccine, a description of the serotype distribution of *S. pneumoniae* is essential.

Objective: The aim of this study is:

1. To describe the serotype distribution of isolates of *S. pneumoniae* from IPD in the Comunidad Valenciana (5.1 million inhabitants) during the year 2007, in the whole population and in infants (<15 years of age).
2. To determine the serotype coverage of present and future vaccines.

Materials and Methods: A total of 362 strains of *S. pneumoniae* causing IPD in the Comunidad Valenciana, were collected from 22 participating hospitals during 2007. 72 Of these isolates were from infants. Serotyping was performed by serum slide agglutination (Denka-Seiken, Tokyo, Japan), and if necessary by the Quellung reaction. The Quellung reaction was also used as gold standard.

Results: The serotypes of *S. pneumoniae* found in order of prevalence in the whole population were: 19A (10.8%), 1 (10.8%), 14 (10.2%), 8 (8.6%), 7 (7.7%), 3 (6.9%), 4 (4.4%), 7F (3.6%), 6A(3.9%), 22 (2.8%), 11 (2.5%). The rest of the serotypes were less than 2.5%. The serotypes of *S. pneumoniae* found in order of prevalence in infants were: 7 (26%), 1 (25%), 14 (15%), 19A (12%), 18 (5%), 8 (3%), 6 (3%), 4 (2%), 16 (2%), 19F (2%), 22 (2%), 23A (2%), 23F (2%), and 24 (2%).

The serotype coverage of the pneumococcal vaccine in the whole population is 22.8% by the PCV7, 41.3% by the PCV10, and 62.9% by the PCV13.

The serotype coverage of the pneumococcal vaccine in infants is 29% by PCV7, 80% by PCV10 and 95% by PCV13.

Conclusions:

- The three most prevalent serotypes in the whole population, with percentages >10%, causing IPD in the Comunidad Valenciana during the year 2007 are 19A, 1 and 14.
- The four most prevalent serotypes in infants, with percentages >10%, causing IPD in the Comunidad Valenciana during the year 2007 are 7, 1, 14 and 19A
- Taking into account the high serotype coverage (80% by PCV10 and 95% by PCV13) of the two future vaccines, it might be recommended to include the future PCV10 or PCV13 vaccine in childhood immunization programs.

P2020 Immunization with the 7-valent pneumococcal conjugate vaccine in children leads to herd immunity among adults in Germany

M. van der Linden, R. Reinert, M. Imöhl (Aachen, DE; Paris, FR)*

Objectives: *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. In July 2006, vaccination with pneumococcal conjugate vaccine was generally recommended by the German Health authorities for all children up to the age of 24 months. In fact, the vaccination program started in January 2007 in all federal states, with the exception of Saxony where vaccination of children started 9 months earlier. In this study, we present the indirect effects of routine vaccination of young children with PCV7 on the rates of IPD in the adult population (herd immunity effect).

Methods: The National Reference Center for Streptococci has monitored the epidemiology of invasive pneumococcal disease (IPD) in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system, including 265 laboratories throughout Germany. For three federal states (since 2001 in North Rhine-Westphalia, since 2006 in Saxony and Bavaria) a population-based surveillance has been conducted. In January 2007, a nationwide web-based surveillance system was introduced. The present analyses include IPD cases documented between 2002 and 2009. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.

Results: The number of reported IPD cases considerably increased with enhanced surveillance from 421 in the pneumococcal season 2003–2004 to 1964 in 2008–2009. From 2002 to 2006, the percentage of vaccine (PCV7) type IPD in adults in Germany varied between 42.9% and 48.5%. In 2007–2008, this percentage dropped to 33.4%, in 2008–2009 to 25.2%, and in Saxony even to 14%. While reporting of IPD is not mandatory in Germany and therefore calculation of incidences is not possible, this reduction of the percentage of IPD cases caused by vaccine serotypes indicates an indirect vaccination effect. Of particular interest, the13-valent vaccine (PCV13, in development) reveals a coverage of 68.0% based on the most recent (2008–2009) German data on IPD in adults.

Conclusions: While the burden of disease due to IPD is substantial in Germany, the strong reduction of IPD in children after introduction of the routine childhood immunisation with PCV7 was followed by a considerable reduction in the percentage of IPD cases in adults caused by the PCV7 serotypes.

P2021 Determination of serotypes distribution among *Streptococcus pneumoniae* in North America by multiplex PCR

C. Lascols, M. Hackel, K. Nichol, D. Hoban, S. Bouchillon, B. Johnson, J. Johnson, M. Dowzicky (Schaumburg, US; Winnipeg, CA; Collegeville, US)*

Background: *Streptococcus pneumoniae* is a major causative agent of infections among young children and the aged. The pneumococcal capsule is a major virulence factor in *S. pneumoniae*. To optimize the development of future conjugate vaccines and to evaluate their efficacy, it is essential to monitor the changes in the sero-epidemiology of *S. pneumoniae*. Accurate serotype determination of *S. pneumoniae* is critical as vaccine development presently relies on serotype prevalence data.

Methods: 184 *S. pneumoniae* clinical isolates collected through the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) were tested. Based on published serotype distribution, we devised a simple PCR-based assay of four sequential multiplex reactions to reliably deduce

specific pneumococcal serotypes for a large number of isolates. PCR results were confirmed by the conventional Quellung reaction.
Results: The most common serotype was 19A (16.8%) followed by serogroup 6B/A (9.2%), serotypes 22F (7.6%) and 3 (5.4%).
- The first PCR reaction allowed determining the serotype for 46% of the isolates.
- Overall, this multiplex typing scheme identified the serotype of 92% of the isolates with 100% correlation with the Quellung reaction. 8% of isolates were non-typeable by PCR.
- 33 various serotypes were identified (see table).

Serotypes (%) identified

19A: 16.8%	4, 33F and 35B: 2.7%
22F: 7.6%	15C, 17F, 23F and 9V: 2.2%
3 and 6B: 5.4%	10A, 15B and 16F: 1.6%
7F: 4.4%	1, 8, 31, 33A and 38: 1.1%
11A, 6A and 19F: 3.8%	7A, 9A, 15A, 18B, 20, 34 and 35F: 0.6%
14, 12F and 18C: 3.3%	Non typeable: 8.2%

Conclusions: This study confirms the high prevalence of serotype 19A in the United States and confirms the accuracy and utility of multiplex-PCR for serotyping, which could be of valuable use in microbiology laboratories to monitor sero-epidemiological changes. Additionally, this method appears to be a fast and cost-effective way to analyze large numbers of isolates.

P2022 Surveillance of invasive pneumococcal disease in adults in Asturias, Spain

A. Rueda, R. Rico, L. Garrido, P. Capón, R. Carballo, I.S. Folgueras, F. Pérez, A. Fleites (Oviedo, ES)*

Objectives: The 7-valent conjugate pneumococcal vaccine (PCV7) was introduced in Asturias, in 2001, with an individualized immunization use. We evaluated the impact of PCV7 on trends in invasive peumococcal disease (IPD) in adult patients, serotype distribution and penicillin resistance.
Methods: Population-based surveillance. We have studied consecutively all invasive isolates of *Streptococcus pneumoniae* at the Hospital Central (Oviedo, Asturias), serving a adult population of 281849–299669, during 2001–2008. Demographic data were obtained from official statistics. 98% of strains were serotyped at the National Center Ref. (Madrid). Penicillin susceptibility was determined by microdilution method and oxacillin disk (CLSI). A Chi-square test was used.
Results: A total of 297 cases were identified: 69.7% pneumonia, 12.5% meningitis, 11.4% sepsis, 4.4% empyema, 2.0% others. The average rates of incidence (cases per 100000/year) were compared in 2001–04 vs. 2005–08. Overall IPD incidence increased from 11.8 (135 cases, range 9.5–13.2) to 13.7 (162 cases, range 9.3–16.9). Age-specific trends in IPD incidence were: among adults $\geqslant$65 yrs. (142 episodes) 25.3 vs. 28.3 and among adults 15–64 yrs. (155 episodes) 7.7 vs. 9.5. The annual incidence of bacteremic pneumonia increased from 8.6 to 9.2 and data for meningitis were 1.7 vs. 1.4. A total of 32 serotypes/groups were identified. Between 2001–04 and 2005–08 the proportion of PCV7 serotypes decreased from 44.4% to 25.8% ($p = 0.001$) corresponding with a significant decrease ($p = 0.004$) in the prevalence of serotype 6B (8.3% to 1.3%) and a decrease (not significant) of serotypes 4, 14, 18C, 23F. Vaccine-related and nonvaccine serotypes increased from 55.6% to 74.2% corresponding with a significant increase in the prevalence of serotypes 6A (0.7% to 6.3%, $p = 0.013$) and 7F (3.8% to 11.3%, $p = 0.017$) and not significant increase in 19A, 22, 23A. During 2005–08, 73.0% of the detected serotypes were included in the 23-valent vaccine. In this comparative period, similar frequency of penicillin non-susceptible *S. pneumoniae* isolates was observed: 33.3% vs. 30.9% ($p = 0.650$) and so was a non significant decrease in penicillin-resistant strains 14.1% vs. 7.4% ($p = 0.074$).
Conclusion: Our results show a slight increase in the incidence of IPD in adults. Changes in the distribution of prevalent serotypes were found. The penicillin non-susceptible rates remain unchanged. Surveillance should be continued.

P2023 Serotype distribution of *Streptococcus pneumoniae* isolated from patients with invasive pneumococcal disease in 2008

A.W. Rosingh, E. Canton, G.F. Fagundez, F. Gonzalez, M. Gobernado on behalf of the MIVA Network*

Objective: To describe the serotype distribution of *S. pneumoniae* associated with invasive pneumococcal disease in patients in the Valencia Community (5.1 million inhabitants), Spain, during the year 2008, after introduction of the 7 valent pneumococcal conjugate vaccine (PCV7).
Methods: We serotyped *S. pneumoniae*, isolated from blood, spinal fluid (CSF) and sterile liquid cultures collected during the year 2008 in patients who were diagnosed with invasive pneumococcal disease in any of the 22 participating hospitals. Serotyping was performed by antiserum agglutination (Denka Seiken, Tokyo Japan). If necessary, more detailed serotyping was done by the Quellung reaction (Staten Serum Institute).
Results: 335 Isolates of *S. pneumoniae* from 335 patients (blood cultures, CSF and sterile body fluids) were serotyped. The serotypes in order of frequency were: 7F (17.6%), 19A (14.9%), 1 (13.1%), 3 (10.4%), 14 (6.3%), 22 (3.9%), 19F (3.3%), 11 (3%), 8A (2.7%). These serotypes represent a total of 77.2% of the total number of isolates that were serotyped. The other serotypes counted for percentages of less than 2.5%.
Conclusions: Of the serotypes isolated in this study, only 14.4% are included in the PCV7-vaccine. Continued surveillance is needed to guide development of future formulations of conjugate vaccines and to monitor the effects of continued vaccine use.

P2024 Invasive *Streptococcus pneumoniae* serotypes associated with adult and paediatric isolates from the United States

M. Hackel, C. Lascols, D. Hoban, S. Bouchillon, J. Johnson, M. Dowzicky (Schaumburg, Collegeville, US)*

Background: *Streptococcus pneumoniae* (SPN) is a major cause of invasive diseases and upper respiratory tract infections. Introduction of the pneumococcal conjugate 7-valent vaccine (PV7) into the US childhood vaccine schedule in 2000 has significantly reduced invasive pneumococcal disease in children and adults, with concurrent reduction in the seven vaccine serotypes. Consistent monitoring of possible replacement serotypes is essential to determine possible antibiotic resistance patterns as certain serotypes are more closely associated with antibiotic resistance, as well as to determine targets for future vaccines. In this study we evaluate the serotypes of invasive SPN isolates from pediatric and adult patients from 2004.
Methods: The capsular serotypes of 275 invasive SPN isolates collected in the US through the Tigecycline Evaluation Surveillance Trial were determined using sequential multiplex PCR and confirmed using the Quellung reaction. Invasive isolates were defined as those from normally sterile sites, such as blood, CSF and other body fluids.
Results: Results are presented in the table.
Conclusions: While PV7 serotypes have declined since 2000, in 2004 approximately 20% of the invasive isolates from this study were from the seven vaccine types (4, 6B, 9V, 14, 18C, 19F, 23F). Pediatric and adult patients were equally likely to carry PV7 serotypes. Serotype 19A was the most common serotype in both groups, which is cause for concern as this serotype is often non-susceptible to penicillin and erythromycin. Continued monitoring of post-vaccine serotype trends is on-going and will be vital to the management of pneumococcal disease.

	Total	PV7 serotypes,	Non-PV7 serotypes, N (%)						
	N	N (%)	19A	6A	3	22F	15	7	Others
Pediatric	46	9 (20)	13 (28)	3 (7)	2 (4)	3 (7)	0 (0)	4 (9)	12 (26)
Adult	230	50 (22)	38 (17)	9 (4)	15 (7)	13 (6)	10 (4)	10 (4)	85 (37)

P2025 **Variable serological response to PPV in HIV-positive patients – a need to review pneumococcal boost-prime strategies?**

A. Brown, C. Rock, C. Ní Bhuachalla, T. Coulter, C. Dowling, C. Bergin (Dublin, IE)*

Objectives: International guidelines recommend 23-polyvalent polysaccharide pneumococcal vaccination (PPV) for HIV-positive patients with CD4 >200 cells/mm^3. Historical data shows suboptimal response to vaccination in HIV-positive patients when measured by pneumococcal-specific IgG and vaccine serotype-specific IgG2. There is little data to support the efficacy of vaccination in consistently producing serological response, or to assess the role of conjugate vaccine in adults.
Methods: In a cross-sectional study, 82 random blood samples were taken from HIV-positive outpatients. The samples were assessed for pneumococcal IgG and IgG2. IgG2 levels >69 microgram/L are considered good serological evidence of response in the absence of baseline titres with which to compare post-vaccination response. Demographic, vaccination and laboratory data was recorded. Results were analysed using Fisher's exact test and two-tailed p values with GraphPad InStat.
Results: Four patients were excluded who had no prior history of pneumococcal vaccination, and two due to incomplete data. The remaining 76 patients (M = 47, F = 29) were aged between 21 and 71 years (mean= 39.1yrs, SD 9.86) at the time of first vaccination. Twenty-six patients (34%) received booster. Sixty-one patients (80%) were taking ART at the time of sampling. Thirteen patients (17.1%) had a vaccine serotype-specific IgG2 titre ⩾69 microg/L at the time of random sampling. Achieving an IgG2 titre ⩾69 microgram/L was not associated with any of the measured variables – age <35 yrs vs >35 yrs at vaccination (p = 0.54); male vs female sex (p = 0.54); CD4 count <200 cells/mm^3 vs ⩾200 cells/mm^3 at first vaccination (p = 0.58); CD4 count <350 cells/mm^3 vs >350 cells/mm^3 at first vaccination (p = 1.00); CD4 count <350 cells/mm^3 vs >350 cells/mm^3 at time of sampling (p = 0.34); ART at sampling time vs no ART (p = 1.00); single vs boosted doses (p = 0.19); <234 weeks (4.5 years) since vaccination vs >234 weeks (p = 0.37).
Conclusions: In the HAART era only a minority of patients show adequate levels of vaccine serotype-specific IgG2 regardless of time since vaccination or booster dosing. Although immunoglobulin measurements are a surrogate marker of immunity, there is no reliable predictor of whether a HIV-positive patient will mount adequate serological response to PPV. Further studies are needed to assess the nature of response to PPV, alternative conjugate prime-boosting strategies, and the cost-effectiveness of mass vaccination programmes.

P2026 **Rabies vaccine: A comparative study on adverse events of four 0.1 ml intradermal booster doses on day 0 with conventional booster vaccination**

T. Tantawichien, M. Benjawongkulchai, T. Tantawichien (Bangkok, TH)*

Booster vaccination of previously immunized persons with potentially exposure to rabies is two doses of cell-culture vaccine (CCV) intramuscularly (IM) or intradermally (ID) on days 0 and 3 as WHO recommendation. We previously demonstrated that four 0.1-mL ID booster doses administered on day 0 induced significantly higher titers of neutralizing antibody (Nab) than did conventional booster doses to previously vaccinated subjects. Since 1998, our institute's guideline has recommended use of either (1) the four 0.1-mL ID booster doses given on day 0, or (2) the conventional two 0.1-mL ID booster doses given on days 0 and 3 for previously vaccinated individuals. During the past 10 years, a total of 5,116 previously vaccinated patients who had exposed to rabies (65% had severe exposure as WHO category III) were given four 0.1-mL ID booster doses of CCV on day 0. None of all patients had any severe adverse reactions and there were no reported rabies deaths. We therefore carried out a prospective study on comparing the 4-site ID booster regimen in terms of safety and tolerance with conventional 2-site ID booster regimen.
Methods: Our patients who received ID booster vaccination were re-cruited after providing informed consent approved by ethical committee. The safety endpoints assessed the frequency and severity of solicited local, systemic symptoms and serious adverse events collected at 30 minute post-vaccination, and during the 7-day period following booster vaccination.
Results: Three hundred patients (251 patients received 4-site ID booster regimen; gr. A and 49 patients received 2-site ID booster regimen; gr. B) were recruited. Ninety patients (87 patients in gr. A and 3 patients in gr. B) who received tetanus toxoids simultaneously were excluded. Among 164 patients in gr. A (mean age 43.0 years) and 46 patients in gr. B (mean age 44.6 years), there were no reports of serious side effects other than minor symptoms. Local pain was more frequent in gr. A (20.1%) than gr. B (6.5%), however, the incidence of itchiness, erythema at site of injection and local lymphadenopathy were similar in both groups. The incidence of mild generalized symptoms such as fever (9.1%), headache (10.9%), dizziness (11.6%), nausea (6.7%) were more often with the gr. A than gr. B (2.2%, 2.2%, 6.5% and 2%).
Conclusion: The four 0.1 mL-site ID booster regimen has been proven to be highly immunogenic and more convenience but has a few disadvantages, such as local pain and mild adverse events.

P2027 **Long-term protection provided by hepatitis B vaccine and need for booster dose: a meta-analysis**

J. Poorolajal (Hamadan, IR)*

The duration of protection provided by hepatitis B vaccine is still unknown but can be estimated through long-term follow-up studies. Electronic databases and conference databases to December 2008 were searched. Reference lists of articles were screened and the studies authors and manufacturers were contacted for additional unpublished references. Randomized clinical trials and prospective cohort studies addressing the long-term protective effect of hepatitis B vaccine were included in this meta-analysis. We assessed 42 separate cohorts involving overall 11,090 subjects; 34 cohorts involving 9356 subjects were included in the final meta-analysis.
Results indicate that the overall cumulative incidence of HBV breakthrough infection 5–20 years post-primary vaccination was 0.007 [95% CI: 0.005 to 0.010] with a variation among studies from 0 to 0.094. Available data do not allow us to exclude an increased risk for infection with time since vaccination.
We conclude that the protection provided by three or four doses of monovalent HB vaccine persists for at least two decades in the great majority of immunocompetent individuals. Additional studies are needed for assessing vaccine efficacy for longer periods of time and the need of booster doses in different subgroups of population.

P2028 **Cellular immune activation and regulatory mechanisms after influenza vaccination**

S. Wang, H. Lei, C.C. Liu (Tainan City, TW)*

Background and Purpose: Influenza virus infections can cause primary influenza viral pneumonia; exacerbate underlying medical conditions. Influenza vaccination is the most effective method for preventing influenza virus infection and its potentially severe complications. Two broad categories of regulatory T cells (Tregs) have been described, CD4+CD25+ Treg subset and transcription factor forkhead box P3 (Foxp3). We conduct this study 1) to explore the role of regulatory T cells and effector T cells immunological mechanisms after receiving influenza vaccination; 2) to characterize the expression of regulatory T cells and antibodies production after influenza vaccination.
Material and Method: Healthy volunteers (n = 35) at the time of blood collection donated whole blood via venous puncture before influenza vaccination and two weeks after the immunization in each year. Peripheral blood mononuclear cells (PBMCs) were isolated from

EDTA whole blood by Ficoll separation. Following activation, fixation and permeabilization was performed using either the fix/perm buffer recommended by eBiosciences for detection of Foxp3 (PCH101).
Results: The mean white blood cell counts and mean absolute lymphocyte counts were similar before and after vaccination. The ratio of expression frequency of CD4+CD25+Foxp3+/isotype and CD127 increased significantly after vaccination, in comparison with that of before vaccination ($p < 0.05$). The expression frequency of CD3 also enhanced after vaccination. There was no difference of immunophenotypes expression frequency on PBMCs of CD4+, CD8+, and CD20+, before and after vaccination.
Conclusions: Use of the CD4+CD25+Foxp3+ and CD127 phenotype to discriminate Tregs may permit better understanding of the role of Treg cells in vaccination. Natural Treg cell participate in the immune response of influenza vaccination. The altered expression of natural Treg cell may be used as a surrogate measurement of regulatory function and may influence the efficacy of protective immunity.

P2029 Biological and immunological characteristics of a new lipopolysaccharide-based conjugate vaccine for brucellosis

S.D. Siadat, M.R. Aghasadeghi, M. Mohammadi, M. Kheirandish, D. Norouzian, J. Izadi Mobarakeh, M. Razavi, M. Zangeneh, S.M. Sadat, A. Sharifat Salmani, A. Moshiri (Tehran, IR)*

Objective: The development of an efficacious vaccine for brucellosis has been a challenge for scientists for many years. At present, there is no licensed vaccine against human brucellosis. To overcome this problem, currently, antigenic determinants of *Brucella* cell wall such as Lipopolysaccharide (LPS) are considered as potential candidates to develop subunit vaccines. Since naturally occurring strains lacking LPS show reduced survival, LPS is considered to be a major virulence factor. Also, the LPS of *B. abortus* is considered one of the most important antigens from the point of view of the primary targets of the innate immunity.
Methods: We have undertaken detoxified of *Brucella abortus* S99 LPS by basic hydrolysis and resultant amine groups were used for their conjugation to *Neisseria meningitidis* serogroup B outer membrane vesicle as carrier protein using carbodiimide and adipic acid-mediated coupling and linking respectively. Groups of ten Balb/c mice were injected intramuscularly with 5 μg of dLPS alone, combine or conjugated on 0, 14 and 28 days. Sera were taken before and 14 days after each injection. The anti-LPS IgM and IgG were measured in serum.
Results: The molar ratio (dLPS/OMV) of the resulting conjugate was 45:1. The dLPS-OMV conjugates was the most immunogenic compound that stimulated following the first injection an increase in IgG titre of about 9.50, 5.80 and 4.53 fold higher than that produced against LPS, LPS in non covalent complex to OMV (LPS+OMV) and LPS with complete Freund's adjuvants, respectively. The highest anti-LPS IgG titer was detected two weeks after the third injection (the day 42) of dLPS–MV conjugates.
Conclusion: Our previous studies demonstrated that LPS+OMV was immunogenic in mice and elicited high level of anti-LPS IgG titers. In this study, conjugation of LPS to OMV was designed and the antigenicity of this conjugated was evaluated by ELISA. The conjugated elicited higher titers of IgG than LPS+OMV, that showed a 120- to 165-fold rise of anti-LPS IgG in mice. These results indicate that the conjugated LPS obtained by us, can be used as a brucellosis vaccine after further investigation.

P2030 How long after the last vaccine dose can pertussis serology not be used?

M. Riffelmann, M. Littmann, C. Wirsing von Koenig, C. Hülße (Krefeld, Rostock, DE)*

Objectives: IgG-anti-pertussis toxin (PT) antibodies used in pertussis serology can be induced either by vaccination with acellular vaccines or by infection with *Bordetella pertussis*. As a consequence, diagnostic serology cannot be correctly interpreted for some time after vaccination. However, it is unclear, how long this interference lasts in clinical practice.
Methods: 206 adolescent and adult patients with longer lasting coughs were recruited by 19 sentinel physicians (10 general practitioners, 9 paediatricians) in the Rostock (Germany) region between January 2008 and April 2009. Clinical symptoms and vaccination history were recorded. *Bordetella*-DNA *(B. pertussis* and *B. parapertussis)* was detected by real-time PCR in nasopharnygeal swabs. IgG- and IgA-antibodies to PT and filamentous hemagglutinin (FHA) were quantified by a standardized ELISA. IgG-anti-PT levels of $\geqslant$100 IU/ml were regarded as indicative of recent contact, and IgG-anti-PT levels of $\leqslant$40 IU/ml were interpretede as not indicative of a recent contact. IgG-anti-PT levels between 40 IU/ml and 100 IU/ml were complemented by measuring IgA-anti-PT and IgG/IgA-anti-FHA.
Results: In a total of 45 patients (18%) a recent contact to *Bordetella* was substantiated either by serology alone or by PCR ($n = 12$). PCR was more often positive in adolescent patients. The share of patients with pertussis in the study population was similar to other rates observed in previous studies. ROC-curve analysis substantiated that a cut-off of IgG-anti-PT between 30 and 40 IU/ml was sufficiently sensitive and specific. A survival time analysis comparing the time to the last vaccine dose between the pertussis group and the non-pertussis group showed that the Kaplan–Meier plots were similar one year after the last vaccine dose.
Conclusions: Our results indicate that diagnostic pertussis serology is difficult to interpret for one year after vaccination with acellular pertussis vaccines.

Molecular diagnosis of TB

P2032 Evaluation of GeneXpert MTB/RIF assay on pulmonary and extra-pulmonary samples in a high-throughput laboratory

S. Naidoo (Johannesburg, ZA)*

Objectives: In South Africa, tuberculosis (TB) and HIV co-infection is wide spread and these patients are more prone to have smear-negative TB or extra-pulmonary TB. Most nucleic acid amplification methods have poor sensitivities with smear-negative samples and culture results from these samples can take weeks, leading to higher mortality rates and further spread of multi-drug resistant TB. The GeneXpert MTB/RIF test integrates and automates sample processing and simultaneous detection of *Mycobacterium tuberculosis* complex and rifampicin resistance, using real-time PCR, within single-use disposable cartridges. The time to result is less than 2 hours and it requires minimal training. We have assessed the performance and feasibility of the Xpert MTB/RIF assay for rapid identification of MTB complex and rifampicin resistance, in both pulmonary (smear negative and smear positive) and extra-pulmonary samples in a routine diagnostic high-throughput TB laboratory in South Africa over a period of two months.
Methods: The results of 1140 sputum samples were compared with validated conventional methods including smear microscopy, liquid culture (MGIT) and culture identification (Accuprobe). In addition, the GeneXpert results from 361 extra-pulmonary samples including bone marrow, cerebrospinal fluid, lymph nodes and urine samples were also compared with conventional testing.
Results: Xpert MTB/RIF results showed great sensitivity (99.8%) and specificity (94.1%) compared to conventional testing in pulmonary samples, resulting in a PPV of 97.6% and NPV of 99.4%. Results also correlated very well with conventional drug susceptibility testing, with sensitivity of 99.4% and specificity of 98.8% (PPV 95.2% and NPV 99.8%). We also showed that TB can be successfully identified in various types of extra-pulmonary samples with the Xpert MTB/RIF test (sensitivity 93.5%, specificity 99%, PPV 98.7% and NPV 94.6%).
Conclusions: The Xpert MTB/RIF assay has proved to be a user-friendly system with remarkably high sensitivity and specificity, in both pulmonary and extra-pulmonary samples, in a high-throughput laboratory in South Africa. The routine implementation of the test has significantly alleviated the workload of the laboratory and lead

to much quicker result reporting and earlier treatment of patients than conventional TB testing.

Pulmonary samples		
	Culture positive	Culture negative
MTB detected	800	20
MTB not detected	2	318
Sensitivity = 99.8%		PPV = 97.6%
Specificity = 94.1%		NPV = 99.4%
	RIF Resistant	RIF Sensitive
RIF Resistant	157	8
RIF Sensitive	1	635
Sensitivity = 99.4%		PPV = 95.2%
Specificity = 98.8%		NPV = 99.8
Extra-pulmonary samples		
	Culture positive	Culture negative
MTB detected	157	2
MTB not detected	11	191
Sensitivity = 93.5%		PPV = 98.7%
Specificity = 98.7%		NPV = 94.6

P2033 Molecular typing of clinical isolates of *Mycobacterium tuberculosis* complex obtained in Bogotá (Colombia) based on Spoligotyping and MIRU-VNTRs

I. Cerezo, Y.J. Jiménez, J. Hernández, T. Zozio, N. Rastogi, M.I. Murcia (Bogotá, CO; Guadeloupe, FR)*

Objectives: The present study focused on molecular typing of *M. tuberculosis* strains isolated from TB patients diagnosed in Bogotá-Colombia between 1995 to 2006. Our primary aim was to have a first insight on the population structure of tubercle bacilli in Bogotá.
Methods: we studied 154 clinical isolates collected from different hospitals in Bogotá. The strains were genotyped using spoligotyping and mycobacterial interspersed repetitive units-variable number of tandem repeats MIRU-VNTRs (classical 12 loci format) in order to determine the percentage of unique and clustered isolates. Spoligotypes in binary format and 12-digit MIRU patterns were entered in the SITVIT2 proprietary database of the Pasteur Institute of Guadeloupe to assign major phylogenetic clades and sub-lineages for *M. tuberculosis* isolates. In this database, SIT (Spoligotype International Type) designates spoligotyping shared by 2 or more patient isolates, whereas MIT (MIRU International Type) designates 12-locus MIRU patterns shared by 2 or more patient isolates, as opposed to "orphan" patterns designating patterns reported for a single isolate. The discriminatory power of the methods used was calculated using the Hunter Gaston Discriminatory Index (HGDI).
Results: Using Spoligotyping as first molecular marker and MIRU-VNTRs as second marker we obtained 100 single patterns and 54 grouped strains into 15 clusters. The lineages found in our sample were in the following order: Latin American & Mediterranean (LAM) 49.25%; Harlem, 25.97%; ill-defined T, 12.33%; S family 1.3%; X lineage, 1.3%; Beijing, 0.65%, and unknown, 7.14%. The MIRU-VNTRs patterns corresponded to 51 MITs for 111 strains and 43 orphan patterns. The most frequent patterns were MIT190 (n = 12), MIT45 (n = 10), and MIT25 (n = 9). The HGDI of both methodologies gave a value of 0.983. In our setting, the HGDI of a five loci subset (MIRU- 26, 40, 10, 31, 23) contributed most to the discriminatory power of the 12-loci format used.
Conclusions: *M. tuberculosis* lineage distribution showed that more than $^3/_4$ of strains in Bogotá are commonly found in Latin-America, Caribbean, and Europe. This observation reflects the common post-Columbus history of Colombia and its Latin-American neighbors as well as the strains brought by 20th century immigrants from Europe. We also show the usefulness of MIRU-VNTRs to detect polyclonal infections, and high stability across time allowing us the detection of chronic infections and endogenous reactivations.

P2034 Analysis of an additional 9-loci MIRU-VNTR confirms a large single clone of *Mycobacterium tuberculosis* in the West Midlands, United Kingdom

A. Gibson, J. Evans, S. Gardiner, G. Smith, P. Hawkey (Birmingham, UK)*

Objective: The Mercian strain of *Mycobacterium tuberculosis* is the single most prevalent strain of tuberculosis in the West Midlands, UK. It was first identified in 2003 after the start of routine DNA fingerprinting and accounted for 171/1899 (9.0%) of all clustered cases between 2004–2008. Archive data suggests Mercian *M. tuberculosis* has been circulating for at least 14 years, however it appears to be geographically restricted as only 6 isolates have been identified outside the West Midlands. Routine 15-loci Mycobacterial Interspersed Repetitive Unit-Variable Number tandem Repeat (MIRU-VNTR) typing identified a common profile unique to Mercian strains. The aim of this study was to determine if the addition of the optimal 9-loci (P Supply et al, 2006. J Clin Microbiol 44:4498–510) to the 15-loci MIRU-VNTR typing panel would sub-divide this highly prevalent clade.
Methods: A total of 182/198 (91.9%) Mercian isolates identified between 2003–2008 were studied. In addition, isolates (n = 19) identified as the Mercian strain through previous epidemiological investigations from 1996–2002 were analysed. All isolates (n = 201) had previously undergone 15-loci MIRU-VNTR typing and were further typed for the additional 9 loci using a WAVE® System.
Results: A large single clone remained after the addition of 9-loci to the MIRU-VNTR typing panel. The most common Mercian strain type (type A) accounted for 170/201 (84.6%) isolates. The remaining 31 isolates were single locus variants (2 clusters of 23 and 5 isolates respectively [type B and C] and 2 unique isolates [type D and E]) along with a double locus variant (1 isolate, type F). Only 3 out of 9 loci were variable. Analysis according to year of isolation showed the most prevalent MIRU-VNTR type (type A or "True Mercian"), was present in isolates from all study years. In 2000 the first variant type was identified (type B), followed by type C in 2002, type D in 2005 and types E and F in 2006, suggesting that the Mercian strain is evolving slowly over time.
Conclusion: The addition of 9-loci confirmed the presence of a highly prevalent conserved clone (True Mercian) containing 170 isolates. To the best of our knowledge this is the largest single *M. tuberculosis* clone to date, based on the optimal 24-loci MIRU-VNTR typing method. True Mercian may have unique genetic properties that enable it to outcompete other more widely encountered strains in the UK.

P2035 Molecular genetic analysis of multidrug-resistant *Mycobacterium tuberculosis* in Bulgaria

V. Valcheva, I. Mokrousov, G. Slavchev, N. Rastogi, N. Markova (Sofia, BG; St.Petersburg, RU; Abymes, FR)*

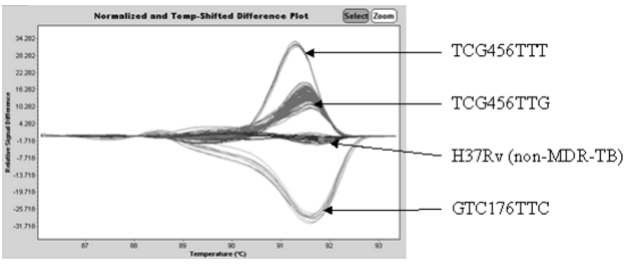
Objectives: Multidrug-resistant tuberculosis (MDR-TB) in Bulgaria has reached alarming proportions (WHO estimation: 10.7% of new TB cases), much higher than in the EU neighbours. Here, we studied the population structure and drug resistance patterns of *Mycobacterium tuberculosis* strains in Bulgaria.
Methods: A total of 133 *M. tuberculosis* clinical isolates from different regions across Bulgaria were analyzed by spoligo-, 24-MIRU-VNTR, IS6110-RFLP typing and rpoB, katG, inhA, embB drug resistance mutations analysis.
Results: The spoligotype-based population structure of *M. tuberculosis* in Bulgaria was sufficiently heterogeneous. The Beijing genotype strains were not found. Novel 15/24-loci MIRU-VNTR format achieved the highest discrimination. Three types of the rpoB mutations were found in 20 of 27 RIF-resistant isolates; rpoB S531L was the most frequent (17 strains). Eleven of 23 INH-resistant isolates had katG S315T mutation.

A mutation in embB306 was found in 7 of 11 EMB-resistant isolates. A monoresistance was found in 15 of 37 drug-resistant isolates.
Conclusion: *M. tuberculosis* population in Bulgaria is dominated by several global and Balkan specific spoligotypes. rpoB and embB306 mutations may serve for rapid genotypic detection of the majority of RIF and EMB-resistant strains in Bulgaria; the results on INH resistance are complex and further investigation of more genes is needed. Transmission of MDR-TB in Bulgaria is not influenced by the global spread of the Beijing genotype which apparently has not yet reached this country. A local circulation of the particular area-specific clones appears to be an important factor to take into consideration in the molecular epidemiological studies of tuberculosis in Bulgaria.
This work was supported by grant DMU 02/01.

P2036 Fast detection of frequent multidrug resistance conferring mutations in *Mycobacterium tuberculosis* isolates using a duplex high-resolution melting curve assay

A. Pietzka, A. Stöger, F. Allerberger, W. Ruppitsch (Vienna, AT)*

Objective: Rapid diagnosis of multidrug-resistant (MDR) tuberculosis has substantial impact on progression and spreading of the disease. A widely used surrogate marker for MDR-TB testing is resistance against rifampicin, which is caused by diverse mutations in the RNA polymerase β-subunit gene (rpoB). The aim of this study was a general improvement in multidrug-resistance testing of *Mycobacterium tuberculosis* isolates by optimisation and simplification of PCR based methods. As a model we used a duplex high-resolution melting (HRM) curve analysis approach to scan for the most frequent mutations in cluster I and outside cluster I of the rpoB.
Methods: Thirty-four MDR-TB and nineteen fully susceptible *Mycobacterium tuberculosis* strains were used to develop a duplex HRM PCR assay to screen simultaneously for the V176F mutation and cluster I codon 456 mutations of the rpoB on a LightCycler480 instrument using LightCycler 480 software 1.5 (Roche Diagnostics, Penzberg, Germany). For analysis melting curves were normalised, temperature-shifted, and a difference plot was generated (Figure 1).
Results: Parallel gene scanning of two distinct regions within the rpoB allowed the correct identification of 34 MDR-TB isolates and all 19 non-MDR-TB isolates in a single closed-tube assay format. HRM curve analysis generated four distinct and highly reproducible curve profiles each specific for the respective SNP. Thus, non MDR-TB isolates were easily distinguishable from MDR-TB isolates independent of the kind of mutation. In addition the method allowed a differentiation between MDR-TB isolates due to the specific SNP conferring rifampicin resistance (Figure 1).
Conclusions: The developed duplex high resolution melting assay represents a diagnostic improvement in terms of speed, simplicity, accuracy and cost-effectiveness. Moreover, since the entire amplification product is scanned for sequence variations any mutation can be detected by HRM due to melting curve profiles that will be unrelated to the non-MDR-TB melting curve profile used as a standard.



P2037 Multiple mutations in the katG encoding catalase proxidase in isoniazid-resistant *Mycobacterium tuberculosis* isolates correlate with high level of resistance in patients with active pulmonary tuberculosis in Iran

Z. Bostanabad, M. Rahimi, P. Adimi, M. Shekarabei, H. Hosseinaei (Parand, Tehran, IR)*

The aim of this study was to investigate the significance of multiple-mutations in the katG gene, predominant nucleotide changes and its correlation with high level of resistance to isoniazid in *Mycobacterium tuberculosis* isolates that were randomly collected from sputa of 42 patients with primary and secondary active pulmonary tuberculosis from different geographic regions of Iran. Drug susceptibility testing was determined using the CDC standard conventional proportional method. DNA extraction, katG gene amplification and DNA sequencing analysis were performed. Thirty four (80%) isolates were found to have multiple-mutations (composed of 2–5 mutations) in the katG gene. Increased number of predominant mutations and nucleotide changes were demonstrated in codons 315 (AGC_ACC), 316 (GGC_AGC), 309 (GGT_GTT) with a higher frequency among patients bearing secondary tuberculosis infection with elevated levels of resistance to isoniazid (MIC μg/ml – 5–10). Furthermore it was demonstrated that the combination of mutations with their predominant nucleotide changes were also observed in codons 315, 316 and 309 indicating higher frequencies of mutations among patients with secondary infection respectively. In this study 62% (n = 21) of multi-mutated isolates found to have combination of mutations with predominant nucleotide changes in codons 315 (AGC_ACC), 316 (GGC_GTT), 309 (GGT_GGT) and also demonstrated to be more frequent in isolates of patients with secondary infections, bearing higher level of resistance to isoniazid (5–10 μg/ml).

P2038 Rapid identification of Beijing *Mycobacterium tuberculosis* strains by high-resolution melting analysis

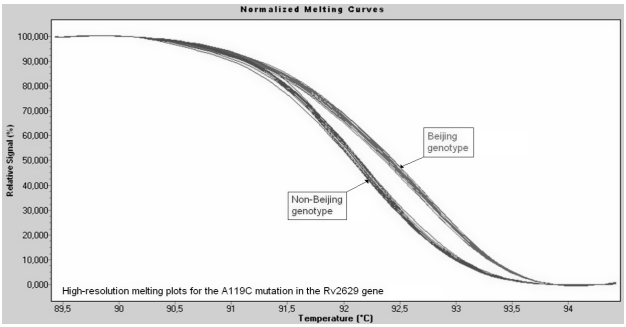
Y. Navarro, M. Alonso, F. Barletta, M. Martinez Lirola, E. Gotuzzo, E. Bouza, D. García de Viedma (Madrid, ES; Lima, PE; Almeria, ES)*

Background and Objective: Genotypic analysis of *Mycobacterium tuberculosis* (MTB) has enabled us to define several lineages. Among these, the Beijing family is considered highly virulent and transmissible, has been associated with resistance in certain settings and involved in severe outbreaks, making it one of the most closely monitored lineages. Therefore, rapid identification of cases infected by Beijing MTB strains could be relevant at a clinical and epidemiological level. Our aim in this study was to develop and evaluate a new rapid procedure to identify Beijing MTB isolates using high-resolution melting (HRM) analysis.
Methods: We designed a real-time polymerase chain reaction followed by HRM analysis based on the identification of the single-nucleotide polymorphism A191C in the Rv2629 gene, which is a marker for the Beijing lineage. We analysed thirty-two MTB strains (18 representatives of the Beijing genotype and 14 representatives of the non-Beijing genotype, according to their spoligotypes) and 44 smear-positive clinical respiratory samples. Finally, 84 MTB strains were selected from a prospective cohort of 145 isolates from 5 areas in Peru between December 2008 and March 2009.
Results: HRM analysis efficiently differentiated Beijing and non-Beijing strains from the 32 reference strains. HRM was able to assign a Beijing/non-Beijing genotype in 100% of respiratory specimens with a high bacterial load and in 73.1% of those with a low/intermediate load. An indirect analysis based on the identification of heteroduplexes when Beijing and non-Beijing DNA were combined in the reaction succeeded in assigning a genotype in 50% of the cases where the direct HRM analysis had failed. Our HRM design identified 10.7% of the Peruvian strains as belonging to the Beijing genotype; this proportion reached 20% in the North Lima area.
Conclusion: Our HRM-based method is a rapid, reliable, and sensitive method for the efficient identification of cases infected by high-risk Beijing MTB strains. A Beijing/non-Beijing genotype was succesfully

assigned in 100% of the MTB isolates and in 90.9% of the respiratory specimens assayed. Its application to a Peruvian sample of MTB isolates revealed high representativity of Beijing strains, especially in the Lima area.

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P2039 Oligonucleotide array-based identification of *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria

Y. Jenh, R. Jou, P. Lu, Y. Yang, W. Huang, H.M. Chen, T. Chang (Tainan, Taipei City, Kaohsiung, TW)*

Mycobacterial infections have been a serious health problem worldwide. Conventional methods for the identification of *Mycobacterium* are time-consuming and identification can be difficult for closely related nontuberculous mycobacteria (NTM). Rapid differentiation of NTM from *Mycobacterium tuberculosis* complex (MTBC) is of clinical importance since antimicrobial agents used to treat infections caused by NTM are different from those caused by MTBC. In addition, accurate identification of members in MTBC may have epidemiological value. The aim of this study was to develop an oligonucleotide array to rapidly differentiate NTM from MTBC, to differentiate members in MTBC, and to identify 19 clinically important species of NTM. Specific oligonucleotide probes (18 to 25-mers), immobilized on nylon membrane, were designed from the 16S-23S rRNA gene intergenic spacer (ITS) region and gyrase B gene (gyrB). The method consisted of PCR amplification of the ITS regions using mycobacteria universal primers, followed by hybridization of the digoxigenin-labeled PCR products to oligonucleotide probes on the array. Three probes were found to useful for differentiating NTM from MTBC, and a combination of multiple probes could effectively differentiate members in MTBC. Moreover, probes were successfully designed to identify 19 species of NTM. The current method provides a rapid and accurate tool to differentiate NTM from MTBC, to identify different species in MTBC, and to identify clinically important species of NTM. The identification procedures can be finished within 24 h, starting from isolated colonies.

P2040 Molecular epidemiology of *Mycobacterium tuberculosis* in an Italian north-eastern area

L. Fallico, M. Peracchi, R. Manganelli, G. Palù (Padua, IT)*

Objectives: The control of tuberculosis (TB) requires methods for rapid detection and tracing sources of infection, so that further transmission can be arrested. Objective of this study was that to characterize the molecular epidemiology of *M. tuberculosis* in an area of northwester Italy surrounding Padua.

Methods: *M. tuberculosis* strains isolated in the Laboratory of Microbiology and Virology of the Hospital of Padua during three years (1 January 2006/ 31 December 2008) from patients with pulmonary and extra pulmonary TB, were genotyped by spoligotyping. The study population (383 isolates) included 101 strains collected during 2006, 139 during 2007, and 143 during 2008. Spoligotypes were analyzed using the International Spoligotype Database (SpolDB4) available at the following link: http://www.pasteur-guadeloupe.fr/tb/bd_myco.html.

Results: The 3-year survey showed that 108 isolates were grouped in two major spoligotypes (ST1378, belonging to the CAS family, 57 isolates; ST1, belonging to the Beijing family, 17 isolates) and 8 minor spoligotypes distributed among the CAS, Bovis, U, EAI families.

Of the remaining isolates, 15 were present as orphan (only one reported worldwide) while 275 were not present in the database. Among these, 110 isolates clustered in 9 major spoligotypes; including 5 (3 clusters), 6 (2 clusters), 8, 21, 25, and 29 isolates respectively; 65 isolates clustered in 27 minor spoligotypes. Finally, 100 isolates were orphan.

Conclusions: Our results demonstrate a characteristic epidemiological pattern of *M. tuberculosis*, represented by two well known major spoligotypes (ST1378 and ST1) and 9 major spoligotypes not yet reported in the SpolDB4 database. The identification of large clusters of isolates belonging to still unreported spoligotypes suggests the possibility of local outbreaks with ongoing transmission. At the best of our knowledge, this is the first report showing the presence of isolates belonging to the Beijing family in northern Italy.

P2041 Routine non-tuberculous mycobacteria detection and molecular identification, Athens, Greece, 2005–2009

S. Nikolaou, D. Papaventsis, S. Karabela, P. Ioannidis, A. Sainti, E. Konstantinidou, I. Marinou, S. Kanavaki (Athens, GR)*

Objective: Infections caused by non-tuberculous mycobacteria (NTM) are diagnosed with increasing frequency. The purpose of this study was to present NTM isolations during a 5-year period, in Athens, Greece.

Methods: From Jan 2005 to Oct 2009, 85540 clinical specimens were tested. Microscopy was performed by the Ziehl–Neelsen stain, culture in both Löwenstein-Jensen (LJ) and in the automated BACTECTM MGIT 960 (Becton Dickinson, USA). Identification at the species level was done by the Genotype *Mycobacterium* CM/AS (Hain Lifesciences, Germany). Susceptibilities to first and second-line anti-tuberculous drugs were determined with the classical proportion method on LJ (Biomerieux, France and Liofilchem, Italy) and the MGIT 960. Tests were performed with the following critical concentrations based on methods recommended by the CLSI for *M. tuberculosis* (MTB) and slow-growing mycobacteria: isoniazid (0.2 ug/ml for LJ), rifambicin (40 ug/ml for LJ), ethambutol (2 ug/ml for LJ), streptomycin (5 ug/ml for LJ), clarithromycin (4, 16, 64 ug/ml for MGIT), and ofloxacin (2 ug/ml for MGIT).

Results: 2540/85540 (2.9%, range 2.7–3.0%) acid-fast bacteria were isolated. 2215/2540 (87%, range 85–90%) acid-fast isolates were identified as MTB, while 325/2540 (12.8%, range 10–15%) were NTM. 277/325 (85%) were successfully identified on species level. In total: 70 isolates (25%) were *M. avium,* 53 (16%) *M. fortuitum,* 35 (11%) *M. intracellulare,* 26 (8%) *M. chelonae,* 23 (7%) *M. gordonae,* 19 (6%) *M. peregrinum,* 11 (3%) *M. abscessus* and 11 (3%) *M. kansasii,* 9 (2.5%) *M. xenopi,* 6 (2%) *M. malmoense,* 6 (2%) *M. lentiflavum,* and 6 (2%) *M. celatum.* Only 2 isolates (0.5%) were identified as *M. marinum.* Susceptibility testing revealed the following resistance rates to clarithromycin and ofloxacin: *M. avium* 20% and 80%, *M. intracellulare* 16% and 100%, *M. fortuitum* 70% and 66%, *M. chelonae* 20% and 80%, respectively. *M. kansasii* was 67% resistant to isoniazid and 22% to rifampicin. All *M. gordonae* isolates were found susceptible to quinolones.

Conclusions: During the study period, NTM detection rate remained constant. The NTM:MTB detection rate was estimated at 1:7 to 1:10. Identification to species level has been substantially improved by molecular techniques. *M. avium* complex (MAC) was the most frequent species. Interpretation of the results of susceptibility testing remains controversial, and determination of NTM clinical significance relies upon good collaboration between clinicians and microbiologists.

P2042 BacTec MGIT 960 system evaluation for susceptibility testing of *Mycobacterium tuberculosis* against second-line anti-tuberculosis drugs

S. Nikolaou, D. Papaventsis, S. Karabela, E. Konstantinidou, I. Marinou, A. Sainti, S. Kanavaki (Athens, GR)*

Objective: The purpose of this study was to evaluate the BACTECTM MGIT 960 system (Becton Dickinson, USA) for drug susceptibility testing (DST) of multi-drug resistant (MDR) *Mycobacterium tuberculosis* (MTB) clinical isolates to second-line anti-tuberculous drugs.
Methods: The accuracy of the MGIT 960 system was compared to susceptibility testing by the proportion method in Löwenstein-Jensen (LJ) slants (Biomerieux, France and Liofilchem, Italy). Forty seven MDR MTB clinical strains isolated from previously untreated patients were included (1 isolate/patient). For MGIT 960, testing concentrations used were: Ofloxacin (OFL) 2 μg/ml, Rifabutin (RIF) 0.5 μg/ml, Ethionamide (ETH) 5 ug/ml, Capreomycin (CM) 2.5 μg/ml. For LJ, drug concentrations were: OFL 5 and 10 μg/ml, RIF 30 and 50 μg/ml, ETH 20 and 30 μg/ml, and CM 20 and 40 μg/ml. Discordant results were repeated by both methods. The ability to detect true resistance (sensitivity), the ability to detect true susceptibility (specificity) for MGIT 960, and agreement between the 2 methods were calculated. The chi-square test was used to compare agreement ratios at a significance level of 95% cases.
Results: The prevalence of resistance to second-line anti-tuberculous drugs of the 47 MDR MTB strains based on the LJ method was: OFL 8.51–12.57%, RIF 12.77–27.66%, ETH 36.17–42.55%, CM 8.51–17.02%. The MGIT 960 system showed sensitivities ranging from 50 to 100%, and specificities from 79.41 to 90.70%, for OFL, RIF, ETH and CM, respectively. Overall agreement between MGIT 960 and the LJ method was 80.61%. The two methods had 87.23% ($k = 0.554$, MODERATE), 82.98% ($k = 0.628$, GOOD), 80.85% ($k = 0.601$, GOOD) and 82.98% ($k = 0.397$, FAIR) agreement at +1 log2 dilutions for MICs of OFL, RIF, ETH and CM, accordingly. At +2 log2 dilutions, agreement remained the same for OFL, decreased for RIF and ETH (to 72.34% ($p < 0.05$) and 78.72% ($p > 0.05$), respectively), and increased to 91.49% ($p < 0.05$) for CM. The median time for obtaining susceptibility results was 7.29 days (range 4.11–11.22 days).
Conclusions: This study shows that nowadays, the BACTECTM MGIT 960 system is useful for rapid and reliable drug susceptibility testing of MDR MTB clinical isolates against second line anti-tuberculous drugs. However, further standardization is needed in order to optimise the clinical relevance of the results obtained.

P2043 Evaluation of the GenoType MTBDRPlus assay for the diagnosis of tuberculosis and rapid detection of rifampin and isoniazid resistance in clinical specimens

C. Cavusoğlu, D. Gursel, H. Bozkurt (Izmir, TR)*

Objectives: *Mycobacterium tuberculosis* remains one of the most significant causes of death from an infectious agent. Several molecular methods have been developed in recent years for the diagnosis of tuberculosis and rapid detection of drug resistance in clinical specimes. The Genotype MTBDR plus assay (Hain Lifescience GmbH, Nehren, Germany) is a novel kit-based method for the detection of the most common mutations in the *M. tuberculosis* rpoB, katG, and inhA genes. The aim of this study was to determine the performance of the Genotype MTBDRplus assay for diagnosis of tuberculosis and rapid detection of rifampin and isoniazid resistance in smear-positive clinical specimens.
Methods: A total of 90 clinical specimens of 57 patients were included in the study. The Genotype MTBDRplus assay was used according to the instructions of the manufacturer. The results obtained by the Genotype MTBDRplus assay were then compared with the results obtained by culture and phenotypic drug susceptibility testing (DST).
Results: A total of 90 clinical specimens which 82 smear-positive and 8 smear negative were included in the study. Overall 80 interpretable results were obtained for 90 specimens (88.9%) with readability rates significantly higher in clincal samples graded 4+, 3+, and 2+ compared to 1+ (Table 1). Among the 80 interpretable clinical specimens obtained from 47 patients, 71 clinical specimes obtained from 43 patients were pansuspcetible, 5 clinical samples obtained from 2 patients had INHr and RIFs pattern, 2 clinical samples obtained from 1 patient had INHr and RIFr pattern, and 2 clinical samples obtained from 1 patient had INHs and RIFr pattern. The overall rate of concardence between the results of the MTBDRplus assay and those of the DST for the assessment of RIF resistance was 97.3%. The overall rate of concordance between two tests for assessment of INH susceptibility was 98.6%.
Conclusion: In conclusion, the test has a short turnaround time and simultaneously provides the RIF and INH susceptibility pattern in 1 working day. Although the MTBDRplus assay could be a useful tool for rapid identification of RIF- and INH-resistant *M. tuberculosis* in smear positive clinical samples, the test results must always be confirmed by drug susceptibility testing.

Table 1. MTBDRplus assay results according to the auramine-rhodamine microscopy staining for the culture positive 90 clinical samples

MTBDR*plus*	Number (%) of specimens					
Test result*	Smear 4+	Smear 3+	Smear 2+	Smear 1+	Smear-negative	Total
MTBDR*plus* +	18 (85.7)	19 (100)	22 (88)	12 (70.6)	6 (75)	7 (85.6)
MTBDR*plus* ±	0 (0)	0 (0)	1 (4)	2 (11.8)	0 (0)	3 (3.3)
MTBDR*plus* –	3 (14.3)	–	2 (8)	3 (17.6)	2 (25)	10 (11.1)
Total	21	19	25	17	8	90

*MTBDR*plus* + interpretable and concordant test results with DST; MTBDR*plus* ± interpretable and discordant test results with culture and DST; MTBDR*plus* – invalid test results.

P2044 Detection of rifampin resistance mutations in *Mycobacterium tuberculosis* by TaqMan probes

V. Slizen, L. Surkova, L. Titov, O. Zalutskaya, S. Zaker (Minsk, BY)*

Steady tendency of increase in tuberculosis incidence is complicated by alarming spread of multidrug resistant strains. Most of rifampin-resistant strains of *M. tuberculosis* possess genetic alterations within an 81-bp fragment of the rpoB gene that encodes the b-subunit of DNA dependent RNA. Many different allelic variations have been detected within this locus, and specific rpoB genotypes are known to be associated with high-level rifampin resistance. Identification of the importance of rpoB and katG in antituberculosis drug resistance has led to the application of a variety of rapid methods to detect mutations in these genes. The aim of this study was to evaluate method for rapid detection of the major mutations causing rifampicin resistance of *M. tuberculosis* using real-time amplification and TaqMan probes.
Methods: Forty four clinical isolates of multi drug resistant *M. tuberculosis* were studied and their mutation profiles in 81-bp region of rpoB gene were characterized. For this purpose 411-bp PCR-products of rpoB gene of *M. tuberculosis* were sequenced. Real-time PCR with TaqMan probes was used to detect mutations in codons 516, 526, 531 of rpoB gene.
Results: It was found as much as twenty types of mutations locating in codons 507, 508, 510, 512, 516, 520, 521, 523, 525, 526, 531 with the prevalence of mutations in triplets 510, 526, 523, 531 comprising 78.9% of all mutations. According to our sequencing data mutations in codons 510, 526, 523, 531 encountered in 47.7, 45.5, 38.6, 29.5% of isolates correspondingly. Mutations in codons 516, 526, 531 were detected TaqMan technology in 9.1, 38.6, 27.2% isolates correspondingly hence the sensitivity of the TaqMan assay was 89%, specificity 95%.
Conclusion: MDR *M. tuberculosis* belongs to "mutator" strains having the universal mechanisms for generating whole-genome mutations; such method as real-time PCR with TaqMan probes for SNPs detection in the most frequently mutated codons allow to determine whether the strain "mutator" or not. Selective pressure of anti-TB drugs induces accumulation of resistance associated mutation and expansion of such clones.

P2045 Mutations in rpoB gene in rifampin-resistant *Mycobacterium tuberculosis* isolates identified in the West-Bohemian region of the Czech Republic

J. Amlerova, T. Bergerova, D. Cervena, J. Hrabak (Plzen, CZ)*

Objectives: Laboratory of Mycobacteriology of the Department of Microbiology of the Faculty of Medicine in Plzen serves the whole West-Bohemian Region of the Czech Republic with ca 567 500 citizens and two centers specialized in tuberculosis treatment. In the last years, many new factories employed thousands foreign workers (usually from the Far East) have been opened in this region. Therefore, there is a permanent migration of the people in some cities. Interestingly, despite these social-geographical characteristics, the rifampin resistance in the *Mycobacterium tuberculosis* isolates seems to be low. The aim of this study was to determine the resistance mechanisms in the rifampin resistance isolates.
Methods: Since the February of 2004, the isolates of rifampin-resistant *M. turberculosis* have been stored. Resistance to rifampin was determined by a proportion method. A 305-bp region covering the 81-bp rifampin resistance determining region of the gene of β-subunit DNA-dependent RNA polymerase (rpoB) was amplified and sequenced.
Results: During the study period, 344 *M. tuberculosis* isolates were identified. Ten isolates were found to be resistant to rifampin (2.91%). Sequencing analysis of the rifampin resistance region revealed the single mutation in codon 531 (Ser – Leu) in all resistant isolates.
Conclusions: Our data indicate that the resistance to rifampin in the isolates collected in the industrial area of the Czech Republic is low. The resistance mechanism was identified as a mutation in codon 531 responsible for the cross-resistance to rifamycin derivatives (rifampin, rifapentine, rifabutin, and rifalazil). The typing based on insertion sequence IS6110 analysis and restriction fragment length polymorphism (RFLP) is ongoing.
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P2046 Assessment of GenoType MTBDRplus assay performance compared to culture using respiratory and non-respiratory samples in Scotland

A-L. Seagar, A. Rayner, P. Claxton, I. Laurenson (Edinburgh, UK)*

Objectives: The GenoType MTBDRplus (MTBDR+) assay (HAIN Lifescience) was evaluated for rapid, direct detection of *Mycobacterium tuberculosis* complex (MTBC) and simultaneous resistance to rifampicin (RIF) and isoniazid (INH) in specimens using line-probe technology. The MTBC detection and drug susceptibility results were compared to those obtained by culture and Bactec MGIT 960.
Methods: 322 specimens (266 respiratory and 56 non-respiratory) that were AFB-positive (n = 293) or had high suspicion of TB were examined from June 2006 to November 2009. MTBDR+ testing was performed once weekly as part of the routine reference laboratory service. Additionally 96 MTBC cultures were extracted for INH and RIF resistance testing only and the results compared to phenotypic susceptibility results.
Results: The MTBDR+ assay was positive for MTBC in 182 specimens. Compared to culture the sensitivity, specificity, positive and negative predictive values for the MTBDR+ assay were 84.9%, 85.4%, 89.6% and 79.3% respectively. 11 of 29 MTBC-culture positive specimens not detected by MTBDR+ contained large numbers of AFB on microscopy. 19 of 154 (12.3%) MTBC culture-positive respiratory specimens produced negative MTBDR+ results. 10 MTBC culture-negative respiratory specimens and 4 non-respiratory specimens that gave positive results using the MTBDR+ assay were from known TB patients. No specimens contained PCR inhibitors. Assay sensitivity for MTBC detection was 87.7% for 266 respiratory specimens and 73.7% for 56 non-respiratory samples.
163 of the 170 (95.9%) specimens with interpretable resistance results and found to contain MTBC were sensitive to both RIF and INH. INH mono-resistance was detected in 6 specimens and both RIF- and INH-resistance was detected in a single specimen. The MTBDR+ resistance results were confirmed in all 122 cases where Bactec MGIT 960 results were available. For the 96 culture extracts, 98.9% of MTBDR+ RIF and 86.7% of INH resistance results concurred with the phenotypic susceptibility results.
Conclusions: The MTBDR+ assay is appropriate for weekly batching of small specimen numbers. Assay sensitivity was 87.7% for respiratory specimens but reduced for non-respiratory specimens. 19 MTBC cases 'missed' using MTBDR+ require further investigation. Overall, the direct RIF resistance results correlated well with BACTEC MGIT 960 but some INH resistant strains may be missed.

MTBDRplus result	Culture result			
	MTBC	NEG	MOTT	Total
MTBC-POS	**163**	19	0	182
MTBC-NEG	29	**39**	**72**	140
Total	192	58	72	322

P2047 Evaluation of XpertMTB/RIF assay and amplified *Mycobacterium tuberculosis* direct test in direct detection of pulmonary *M. tuberculosis* complex

J.S. Lin, C. Lin, R. Hsiao, L. Shih (Changhua, TW)*

Objective: Nucleic acid amplification test on respiratory samples for rapid detection of *Mycobacterium tuberculosis* complex (MTBC) aids to the early diagnosis of pulmonary tuberculosis. Recently, the XpertMTB/RIF (XMTB; Cepheid, SunnyVale, CA) has been introduced as a semi-quantitative nested real-time polymerase chain reaction *in vitro* diagnostic test for rapid identification of MTBC and rifampicin resistance on pulmonary samples. The aim of this study is to compare the detection sensitivity of pulmonary MTBC using MTB assay and the Amplified *Mycobacterium tuberculosis* Direct test (MTD; Gen-Probe, Sandiego, CA) against mycobacterial culture.
Methods: Clinical respiratory specimens submitted for MTD test were enrolled. Only samples with a smear positive (one to two plus) for acid-fast bacilli (AFB) were eligible for further testing. After MTD testing and inoculation onto culture media, the rest of samples were kept frozen at −70ºC until XMTB testing. Both MTD and XMTB assays were tested once on each sample respectively. The mycobacterial culture used both liquid and solid media and was regarded as a gold standard in this study.
Results: A total of 38 unrelated respiratory samples were recruited for comparison. Of them, 27 were culture-positive and 11 were culture-negative. When compared with mycobacterial culture, both MTD and XMTB assays had the same detection sensitivity of MTBC, i.e., 92.6% (25/27). The concordance rate between MTD and MTB assays was 84.2%, i.e., six discrepant samples consisting of MTD–/XMTB+/culture+ (n = 2), MTD+/XMTB–/culture+ (n = 2) and MTD+/XMTB–/culture– (n = 2), respectively.
Conclusions: This preliminary study suggests that XMTB test is comparable with MTD in terms of MTBC detection on AFB-positive respiratory samples and aid to the early diagnosis of pulmonary tuberculosis.

P2048 Diagnosing pulmonary tuberculosis in a low prevalence setting – the Xpert MTB/RIF test

T. Bodmer, A. Ströhle (Berne, Niederwangen, CH)*

Objectives: Rapid detection of *Mycobacterium tuberculosis* (MTB) in respiratory specimens is the key to breaking the chain of transmission and initiating drug therapy. The novel Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) simultaneously detects both MTB and rifampin (RIF) resistance in sputum. We evaluated the assay's ability to detect MTB not only in sputa, but in all types of respiratory specimens.

Methods: Beginning on the 7th September 2009 500 µl aliquots of decontaminated sediments were tested, following clinical request, using the Xpert MTB/RIF assay according to the manufacturer's instructions. All respiratory specimens were decontaminated (NALC); sediments were examined by fluorescence microscopy and cultured (35ºC/8 weeks) on liquid and solid media. Mycobacteria were identified via partial sequencing of the 16S rRNA gene and the GenoType® MTBC assay (Hain Lifescience, Germany). Drug susceptibilities were assessed by the Bactec MGIT 960 method (BD Diagnostics, Switzerland).
Results: To date, 231 specimens from 149 patients (mean, 1.58 specimens per patient) have been tested, sputum (n = 83); bronchial secretions (n = 70); and broncho-alveolar lavage (n = 78). No sample inhibition was observed. Four (1.7%) tests had to be repeated once due to ERROR flags. MTB was isolated from 6 (2.6%) specimens. Of these, 5 (2.2%) were positive by the Xpert MTB/RIF test (sensitivity, 83.3%; C.I. 95%, 43.6–96.9). Four (1.7%) specimens yielded non-tuberculous mycobacteria; none of these were positive by the Xpert test (specificity, 100%; C.I. 95%, 98.3–100). The NPV was 99.5% (C.I. 95%, 97.5–99.9%); the PPV was 100% (C.I. 95%, 56.5–100). The assay was flexible, robust and readily integrated into the laboratory workflow taking 2 hours to perform.
Conclusions: The Xpert MTB/RIF provides rapid and specific detection of MTB in all types of NALC-decontaminated respiratory specimens. The sensitivity of the assay was good. Further testing will allow a more robust assessment of its potential to rule out transmissible TB on the basis of a single test result. The assay's role in the diagnosis of extra-pulmonary TB should be assessed.

P2049 Comparison of polymerase chain reaction and Ziehl–Neelsen staining with histopathologic finding in formalin-fixed, paraffin-embedded tissue specimens for diagnosis of tuberculosis

S. Khazaei, B. Izadi, J. Zandieh, A. Alwandimanesh, S. Vaziri (Kermanshah, IR)*

Objective: Even in the 21th century, Tuberculosis to be a problem infecting about 1/3 of the world population. Rapid correct diagnosis is necessary for Therapy. The aim of this study is to compare PCR to detect Mycobacterial DNA on formalin-fixed, paraffing-embedded tissues with Ziehl–Neelsen Staining and Histopathologic Finding.
Methods: Paraffin blocks of the submitted specimens of the patients clinically suspicious for tuberculosis or containing granuloma were selected. Ziehl–Neelsen Staining & TB-PCR (IS6110 element) was carried on. The results of tests were compared by using McNemar chi-square test. Statistical significance was accepted when the P value was less than 0.05.
Results: 45 specimens were included in the study, 35 had granulomas (19with caseous necrosis). Acid-fast bacilli were identified in 17 (37.8%). TB-PCR was positive in 16 (84%) with caseating granulomas, 11 (68.8%) with non-caseating granulomas & 6 (60%) without granulomas. (P value = 0.59) (Table 1).

Comparison of three tests (PCR, Histopathology & Ziehl–Neelsen staining)

Histopathology	Ziehl–Neelsen staining	PCR		Sum, n (%)
		Negative, n (%)	Positive, n (%)	
Without granulomas	Negative	4 (40%)	4 (40%)	8 (80%)
	Positive	0 (0%)	2 (20%)	2 (20%)
	Sum	4 (40%)	6 (60%)	10 (100%)
Caseating granulomas	Negative	3 (15.8%)	9 (47.4%)	12 (63.2%)
	Positive	0 (0%)	7 (36.8%)	7 (36.8%)
	Sum	3 (15.8%)	16 (84.2%)	19 (100%)
Non-caseating granulomas	Negative	5 (31.1%)	4 (25%)	9 (56.3%)
	Positive	0 (0%)	7 (43.8%)	7 (43.8%)
	Sum	3 (31.3%)	11 (68.8%)	16 (100%)

Conclusions: TB-PCR on paraffin-embedded tissue is a potentially useful approach for early, rapid and sensitive test for diagnosis of tuberculosis. It is especially useful when granuloma is seen in tissue section, while acid-fast stain is negative. There is on significant difference between PCR and histopathology finding. (P value = 0.59) where PCR is not available, histopathology finding can be used for detecting and conforming tuberculosis.

P2050 Clues to enhanced virulence in *Mycobacterium tuberculosis*: mapping of IS6110 in Lisbon strains

J. Perdigão, C. Pinto, J. Moniz-Pereira, I. Portugal (Lisbon, PT)*

Objectives: Tuberculosis (TB) is responsible for about 2 million deaths worldwide. National TB control programs have to cope with the enhanced virulence of some *Mycobacterium tuberculosis* (*M. tuberculosis*) strains. It is the case in Lisbon Health region, where a specific strain family, designated Lisboa family, is responsible for the high number of multidrug and extensively drug resistant TB (MDR-TB and XDR-TB, respectively) cases. Given the high prevalence of such strains one may ask what genomic factors may be triggering such high virulence and/or transmission. Genetic expression plasticity may be conferred by the differential location of several genomic mobile elements, of which the most notorious is insertion sequence IS6110. This study intends to map these insertion sequences in a Lisboa family strain to possibly infer on its phenotypic consequences.
Methods: The genomic position of IS6110 in a representative Lisboa family strain, previously characterized by 24 loci Mycobacterial Interspersed Repetitive Unit – Variable Number of Tandem Repeats (MIRU-VNTR), was analyzed through Ligation-Mediated PCR and Heminested Inverse PCR with restriction endonucleases and specific primers directed at IS6110. Amplified fragments were characterized by DNA sequencing analysis.
Results: In this study, using the above methodology, we were able to map the location of six IS6110. The insertion sites were mapped to positions 483580, 932202, 1998809, 2456838, 3480373 and 4183430 relative to *M. tuberculosis* H37Rv genome. Three of these insertion sites were located at intergenic sites, while the others were inserted in Rv0403c (mmpS1), Rv3113 and Rv3732 open reading frames. The latter encode a hypothetical protein with unknown function, a possible phosphatase and a membrane protein. On the other hand, two of the intergenic IS6110 may influence the expression of the upstream genes.
Conclusion: We have identified the location of six IS6110 in the genome of a Lisboa family strain, of which 5 may influence the strain's metabolism. The identification of more insertion sites will allow a better characterization of the influence of these mobile elements in the metabolism and adaptation of these strains. Further search for other insertion sites is ongoing.

P2051 Rapid simultaneous detection of *Mycobacterium tuberculosis* and Beijing/W genotype directly from clinical respiratory specimens

E.T. Leung, M. Ip, N. Lee, L. Zheng, E.W. Chan, R.C. Chan (Hong Kong, HK)*

Objective: Fighting tuberculosis is a challenge and a major public health concern worldwide. Recent clinical and epidemiological studies have highlighted the virulence of *Mycobacterium tuberculosis* (MTB) Beijing/W strains. Beijing/W strains have evolved unique properties, including the abilities to acquire drug resistance more frequently, to disseminate more efficiently, and to evade the protective effect of BCG vaccination. This study aims to develop a rapid, sensitive, reliable and high throughput protocol which has the potential for routine clinical identification of MTB, with simultaneous differentiation of Beijing/W strain type, by using an optimized DNA extraction method and multi-probe multiplex quantitative PCR.
Methods: Three different sizes/materials of beads (200um glass beads, 900£gm glass beads and 0.1 mm zirconia beads) for mycobacterial cell lysis and four different DNA purification methods (Chelex-100 resin, QIAgen DNA mini kit, QIAgen viral DNA/RNA kit, and Invitrogen MyOne SILANE magnetic particles with in-house formulated buffers) were compared for their sensitivities in capturing minute quantities of MTB DNA from clinical specimens. New primers and Taqman

probes were designed for simultaneous amplification of MTB IS6110 gene, MTB RD105 (Beijing/W identification), and human B-globin gene (internal positive control). Specificities of primers and probes were tested. Quantitative PCR conditions and component concentrations were optimized.
Results: The combination of using 200um glass beads and Invitrogen magnetic particles with in-house formulated buffers gave the highest sensitivity. The primers and probes had no amplification with DNA extracts from non-TB clinical specimens and non-tuberculosis mycobacterium isolates. The detection limit of this protocol is 10 copies of IS6110, which corresponds to approximately one H37Rv bacillus. Addition of RD105 and B-globin primers has no interference on the sensitivity and specificity. The turnaround time for detection of MTB from specimen is approx. 7 hours.
Conclusions: The protocol developed provides a rapid, sensitive and reliable reference to physicians while waiting for the traditional culture results. The method is more affordable in comparison with any TB diagnosis kits currently in the market. This protocol has the potential to be widely adopted in clinical laboratories worldwide.

P2052 Rapid detection of ciprofloxacin resistance in *Mycobacterium tuberculosis* clinical isolates by two colorimetric assays

E. Ruh, H.Y. Öcal, P. Yurdakul, Z. Saribas, A. Alp (Ankara, TR)*

Objective: New diagnostics including rapid drug susceptibility tests are attractive for tuberculosis research. Colorimetric assays have previously been used for rifampin and isoniazid susceptibility testings. In this study, we searched the efficiency of two colorimetric assays using tetrazolium violet (TV) and resazurin (RES), in terms of rapid detection of ciprofloxacin resistance in *M. tuberculosis* clinical isolates.
Methods: Thirty isolates were included in this study. Fifteen isolates were tested for TV assay, and 15 were tested for RES assay. The tests were performed in four sets (day 5, 7, 10 and 12) in order to optimize the incubation period. For each isolate, an inoculum of 2 McFarland turbidity was prepared in Middlebrook 7H9 media. One mililiter from these suspensions was transferred into new sterile tubes. Then, 1 ml from 4 micrograms/ml ciprofloxacin stock solution was added into these tubes in order to achieve a final breakpoint value of 2 micrograms/ml and 1 McFarland of inoculum. In the first assay, 100 microliters of TV were added into the control and the drug containing tubes. After incubation for 4 hours, 50 microliters of 0.1 N HCL were added into the tubes. These mixtures were re-incubated for one hour and any change in colour into purple was recorded as bacterial growth. In the second assay, 100 microliters of RES were added into the control and the drug containing tubes. After an overnight incubation, any change in colour into pink was recorded as bacterial growth. The results were compared with those obtained from agar proportion method.
Results: For both of the two assays, no change in colour occured on day 5. Regarding the bacterial growth, changes in colours in the control tubes were detected on day 7. However, three of the isolates tested for TV and 5 of those tested for RES showed only a little change in colour. These isolates successfully changed the colour of the test media on day 10 and 12. The changes in the colour of drug containing media were recorded as resistance to ciprofloxacin and these results matched with those obtained from agar proportion method.
Conclusion: In this study, we showed that, the 7th day of incubation gives important information on bacterial growth and resistance, but in some cases, this should be supported by a further 10th or 12th day incubation. Our results suggest that TV and RES assays can be used for rapid detection of bacterial growth and ciprofloxacin resistance in tuberculosis.

P2053 Isolation of non-tuberculous mycobacterium in patients with suspected pulmonary tuberculosis in Korea

S.H. Lee, K.H. Song, M.H. Kim, L.S. Park, K.H. Choi (Seoul, KR)*

Objectives: In recent, prevalence of pulmonary nontuberculous mycobacterium (NTM) infection has been increased in Korea. The aim of this study was to assess the prevalence of NTM infection and each type of the NTM species in patients with suspected pulmonary tuberculosis in Korea in which was a high prevalence area for mycobacterium tuberculosis (MTB).
Methods: 44,789 specimens were cultured on Lowentein-Jensen media between January 2007 and Jun 2009. Positive cultures were analyzed using acid fast bacilli (AFB) stain and conventional or real-time PCR methods with Cobas Taqman 48 (Roche Diagnosticsm, Mannheim, Germany) in order to differentiate pulmonary NTM from MTB. Each strain of the NTM species was identified by the Line probe assay (LPA) (GenoType *Mycobacterium* CM/AS kit, Hain-Lifescience GmbH, Nehren, Germany). We evaluated the prevalence of pulmonary NTM disease in all specimens and its incidence rates were compared according to each year, sex and age range. Each strain was analyzed for the NTM species identified from the sputum specimens in 2009.
Results: 4,890 of 44,789 (10.9%) specimens were positive for culture. The NTM species were isolated in 561 (11.5%). Rates of NTM positive cultures were 10.7% (184/1723) in 2007, 13.1% (251/1911) in 2008, and 10.0% (126/1256) in half of 2009. Frequency of NTM disease increased significantly in old age and male groups: highest in the 70–79 year old, 66.0% in male versus 44.0% in female. The strains of the NTM species were as follows: *M. intracellulare* (n = 30, 31.9%), *M. avium* (n = 13, 13.8%), *M. abscessus* (n = 12, 12.8%), *M. gordonae* and *M. peregrinum* (n = 10, 10.6%), *M. fortuitum* (n = 7, 7.4%), *M. kansasii* (n = 6, 6.4%), *M. chelonae* (n = 5, 5.3%), and *M. szulgai* (n = 1, 1.1%).
Conclusion: Culture positive rate is about 10% in patients with suspected pulmonary tuberculosis and the NTM species are isolated above 10% of positive cultures. Therefore, it may be important to differentiate MTB from NTM for proper treatment and management. The LPA is very precise and convenient method for NTM identification.

P2054 Analysis of prevalent *Mycobacterium tuberculosis* strains in the United Kingdom: detection, distribution and expansion of MIRU-VNTR profiles containing high numbers of isolates

J. Evans, S. Gardiner, G. Smith, P. Hawkey (Birmingham, UK)*

Objectives: Universal DNA fingerprinting of *M. tuberculosis* strains has been utilised previously in population-based transmission studies and outbreak detection. This 4 year study describes the distribution and expansion of large clusters defined by MIRU-VNTR (Mycobacterial Interspersed Repetitive Units containing Variable Number Tandem Repeats) typing with the aim of identifying expanding clusters of *M. tuberculosis* earlier and directing public health control efforts and resources.
Methods: All *M. tuberculosis* isolates (n = 4,207) referred to the Health Protection Agency Midlands Regional Centre for Mycobacteriology from 2004–07 were typed by MIRU-VNTR using the 3 ETR and 12 MIRU loci on a WAVE(R) System. BioNumerics v5.1 was used to analyse the distribution and expansion of MIRU-VNTR clusters.
Results: Within the 4,207 isolates, 439 clustered MIRU-VNTR profiles containing 2,575 isolates were identified. Profiles containing paired isolates accounted for 196/439 (45%) of all clusters and 392/4,207 (9%) of all isolates. There were 127 (29%) clusters containing $\geqslant 5$ isolates which contained 1,779 (43%) isolates. Clusters containing 2–17 isolates accounted for 415/439 (95%) of all clustered profiles and 1,1544/4,207 (37%) isolates with the largest 5% of all clusters containing 24 MIRU-VNTR profiles which varied from 19–126 isolates in size and contained 1,027/4,207 (24%) of all isolates. In clusters that reached 5 isolates within 2 years, the first two strains were identified within an average of 4.04 months (95% CI: 3.11–4.97) of each other whereas the first two strains in clusters that did not reach 5 isolates within 2 years were identified within 12.12 months (95% CI: 10.94–13.29) of each other.
Conclusion: *M. tuberculosis* MIRU-VNTR profiles normally occur in small-medium sized clusters with few profiles exhibiting rapid expansion rates to reach very high numbers of isolates. These expanding clusters more often have a second isolate appear more rapidly (4 versus 12 months), thus potentially allowing early identification and control.

P2055 Deletion and single nucleotide polymorphism typing of *Mycobacterium tuberculosis* Lisbon family strains in Portugal

J. Perdigão, C. Silva, I. Portugal (Lisbon, PT)*

Objectives: Multidrug and extensive drug resistant tuberculosis poses a very serious threat for public health. Lisbon Health Region has one of the world's most serious situation regarding this problem. Such, is the result of a continued circulation of an endemic and predominant strains of a particular genetic family – Lisboa family. Little is known regarding the phylogeny, relative virulence and genetic background of these strains. The loss or deletion of specific genomic regions constitutes the most important way by which *Mycobacterium tuberculosis* diverges and adapts. Several deletions, named Regions of Difference (RD), have already been described and associated with phyllogeographic lineages. Alternatively, single nucleotide polymorphism (SNP) analysis allows a more precise positioning in the global *Mycobacterium tuberculosis* phylogeny. The characterization of Lisboa family in this manner may elucidate its origin and perhaps be helpful in explaining its high prevalence.
Methods: Three representative clinical isolates of different genetic clusters of *Mycobacterium tuberculosis* (*M. tuberculosis*) strains circulating in Lisbon Health Region were screened for the presence of 16 distinct RDs. Deletion detection was performed by PCR carried out using primers flanking each RD. Confirmation was performed by sequencing analysis. SNP analysis was performed through the analysis of 9 SNP regions, amplified by PCR and characterized by sequencing analysis.
Results: All three isolates were found to possess four of the tested deletions: TbD1, pks15/1, RD174 and RDRIO. It was not possible to discriminate between the strains using this deletion typing approach. However, it was possible to infer on the phylogeography of these strains. The presence of TbD1 and pks15/1 deletion positions the strains in the modern and Euro-American lineage, respectively. On the other hand, RD174 suggests that the analyzed strains are related to the West-African sub-lineage.
SNP analysis revealed that all strains under analysis belong to SNP Cluster Group (SCG) 5.
Conclusion: The present study points toward the fact that Lisboa strains and others circulating in Lisbon belong to modern lineages of *M. tuberculosis*, specifically a widespread lineage very prevalent in West African countries. Also, the position of Lisbon-circulating strains in the global SNP phylogenetic tree became established. This study therefore reveals more on Lisboa family's origin and genomic distinctiveness.

P2056 Evidences for scanty spread of *Mycobacterium tuberculosis* Beijing genotype in the Balkan Peninsula

S. Panaiotov, E. Bachiiska, V. Levterova, N. Brankova, S. Tafaj, V. Katalinic-Jankovic, M. Zolnir-Dovic, R. Durmaz, T. Kantardjiev (Sofia, BG; Tirana, AL; Zagreb, HR; Golnik, SI; Malatya, TR)*

Objectives: To search evidences for spread of *Mycobacterium tuberculosis* Beijing genotype in the Balkan Peninsula.
Methods: Spoligo- and MIRU-VNTR typing was applied for typing of *M. tuberculosis* Beijing genotype in strains collected across the Balkan Peninsula. Personal research data, spoligo- and MIRU-VNTR data available in SpolDB4 database and publications were the sources for data collection of *M. tuberculosis* genotypes in the Balkan Peninsula.
Results: Strains collected from Albania (100), Bulgaria (329), Croatia (2200) and Slovenia (1250) were typed by spoligotyping and MIRU-VNTR. *M. tuberculosis* Beijing genotype was not identified, except for four strains isolated from three Albanian patients, one Russian immigrant in Croatia and one Chinese woman working in Slovenia. Published data for Greece do not give evidences for existence of Beijing genotype in the country. No Beijing genotype strains are found in Bulgaria, Romania and Serbia. Beijing genotype was found in Istanbul, Turkey where 4069 strains were typed. The prevalence rate was 1.13% and these strains were carried over from countries of the former Soviet Union. On Asian Turkey, the Beijing genotype is similar to that obtained from Istanbul.
Conclusions: Our supposition that Beijing genotype was recently introduced in the Balkan peninsula (Albania, Croatia, Slovenia) is supported by the facts that in the Balkan countries Beijing genotype is not or rarely detected and most often is carried over from recent migrations. In the near past (1960s-80s) China and Albania had tight political, commercial and cultural relations. Albanian isolates with Beijing genotype have intrafamilial transmission between brothers-in-law. They both lived in a city where a steel factory was built by Chinese specialists. There are no evidences for spread of *Mycobacterium tuberculosis* Beijing genotype in Bulgaria, Greece, Croatia (with one immigrant exception), Romania, Serbia and Slovenia (one Chinese immigrant). There are no data for Macedonia.
Natural resistance of the local population to Beijing genotype could be supposed. Environmental and cultural factors preventing adaptation of the Beijing genotype to the population could be supposed too. Although our results do not provide direct evidence for resistance of the Balkan populations to *M. tuberculosis* Beijing genotype, it does suggest that associations between host and parasite populations are sufficiently stable for such resistance/adaptation to evolve.

P2057 Analysis of the role of genes ethA, ethR, ndh, mshA, inhA and the inhA-promoter in resistance to ethionamide, a second-line antituberculosis drug

F. Brossier, N. Veziris, V. Jarlier, W. Sougakoff (Paris, FR)*

Objectives: Ethionamide (ETH) is a prodrug which is activated by an enzyme (EthA) and targets the InhA protein involved in biosynthesis of the mycobacterial cell wall. Resistance to ETH has been previously reported to be linked to mutations in genes ethA and its transcriptional regulator ethR, as well as in inhA and its promoter inhA-pro. More recently, two additional genes have been suggested to be involved in resistance to ETH: ndh encoding a NADH dehydrogenase and mshA encoding an enzyme involved in the biosynthesis of mycothiols. Our goal was to investigate the presence of mutations in these genes in a set of clinical isolates showing different levels of resistance to ETH.
Methods: 87 *M. tuberculosis* clinical strains collected in 2003–2004 at the National Reference Center for Mycobacteria were included, 47 ETH-resistant (ETH-R), 24 ETH-susceptible (ETH-S) and 16 showing an intermediate level of resistance to ETH (ETH-I). The drug susceptibility testing was performed on Lowenstein-Jensen medium with the standard proportions method. The genes ethA, ethR, inhA, inhA-pro, ndh and mshA were sequenced.
Results: Of the 47 ETH-R strains, 23 were mutated in ethA and/or ethR, and 29 in inhA and/or inhA-pro (14 in both). The 9/47 strains without mutation in these genes were shown to have no mutation in ndh and mshA. Regarding the ETH-I strains, 7/16 were mutated in ethA, while wild-type ndh and mshA genes were found in 9/9 and 8/9, respectively, of the ETH-I isolates showing no mutation in ethA/ethR, one strain having a N111S mutation in mshA. Finally, of the 24 ETH-S strains, 23 had no mutation in ethA-ethR and inhA/inhA-pro, one displaying a rare mutation ($-47\ g \rightarrow c$) in inhA-pro.
Conclusions: These results strongly suggest that ndh and mshA are not implicated in resistance to ETH in clinical strains of *Mycobacterium tuberculosis*. In 81% of the ETH-R *M. tuberculosis* clinical isolates analyzed in the present study, resistance to ETH resulted from the combination of mutations (1) in ethA-ethR and inhA-pro and (2) in inhA and inhA-pro. In the ETH-I strains, the intermediate level of resistance to ETH was mainly associated with mutations in ethA (7/16) which were all different from those found in the ETH-R isolates. For 17/63 ETH-R or ETH-I strains, the mechanism of resistance to ETH remained unknown as no mutation was found in ethA, ethR, inhA-pro, inhA, ndh and mshA, highlighting the complexity of the mechanisms of resistance to ETH in *M. tuberculosis*.

P2058 Clinical usefulness of rpoB gene mutation analysis in tuberculous lymphadenitis

K.T. Kwon, D.Y. Song, J.Y. Park, J.H. Kim, R.S. Yeol (Daegu, KR)*

Background: Tuberculous lymphadenitis (TL) is the most common extra-pulmonary tuberculosis and frequently shows paradoxical response (PR) during the treatment. Differential diagnosis between PR and treatment failure is not easy without drug susceptibility test (DST). But, it is rarely performed and delayed due to low culture yield and long process. RpoB gene mutation analysis is rapid detection method for rifampin resistance but has not yet been evaluated in TL. To evaluate clinical usefulness of rpoB gene mutatinon analysis in TL, we performed direct sequencing for rpoB gene with fine-needle aspirates (FNAs) from lymph node (LN).
Materials and Methods: A total of 24 cases which revealed positive results upon the polymerase chain reaction (PCR) for *M. tuberculosis* in FNAs from LN were included. With these cases, direct sequencing for rpoB gene was performed. Also, clinical and microbiologic data were reviewed retrospectively and investigated with rpoB gene mutation results.
Results: A total of 18 evaluable rpoB gene sequences were obtained. Six evaluable rifampin susceptibility data (1 resistance, 5 susceptibilities) were compatible with mutation analysis. Two (11%) of 18 cases showed rpoB gene mutation. One case with D516Y mutation showed rifampin resistance in DST. The other case with S522A mutation needs 2nd line treatment and percutaneous drainage for tuberculous psoas abscess. Among 16 cases without mutation, 15 cases (93.75%) were cured with 1st line anti-tuberculous drug and 9 cases (56.2%) showed PR. Among them, 4 cases (44.4%) were treated with steroid, 2 cases (22.2%) were treated with surgical drainage.
Conclusions: It can be rapid and effective to detect rifampin resistance and to manage the clinical course of TL by using rpoB gene mutation analysis with FNAs from LN.

P2059 Comparison of the BacTec MGIT 320 to the BacTec MGIT 960 for the growth, detection and susceptibility testing of *Mycobacterium tuberculosis*

P. Beaty, E. Carrese, M. Warns (Sparks, US)*

Objective: Critically compare the BACTEC MGIT 320 to the BACTEC MGIT 960. The MGIT 320 is a new lower capacity instrument for the growth and detection of *M. tuberculosis* and antimicrobial susceptibility testing using the BACTEC MGIT reagents.
Method: Growth and detection was evaluated in a paired study that included three strains of *M. tuberculosis*. The study design included both instrument position and microbial detection limit as variables. Three dilutions (highest dilution approximately 0 to 10 CFU) per strain were tested. There were 16 replicates per dilution distributed throughout the MGIT 320 or MGIT 960 drawer. A total of 144 paired cultures were analyzed for recovery and time to detection (Wilcoxon Parametric Analysis). Susceptibility testing was compared using one strain in a MGIT SIRE susceptibility test and two strains in a PZA comparison test. A total of 48 AST sets were distributed evenly in the MGIT 320 or MGIT 960 drawer.
Results: There was no difference in total recovery with a McNemar Pvalue = 1. The recovery rate for the MGIT 320 and the MGIT 960 was 93.7 and 93.0%, respectively. All recovery failures were with the highest dilution (0 to 10 CFU). The mean TTD in the MGIT 320 and the MGIT 960 was 355 and 365 hours, respectively. The median time to detection difference was 10 hours (N = 131 paired cultures) earlier in the MGIT 320. The percent agreement for all SIRE tests was 99.6%. The difference was a borderline streptomycin determination (43 versus 38% of streptomycin tests determined resistant, 320 and 960 respectively). There was a mean difference in time to result of the SIRE sets of 7 hours (earlier in the MGIT 320). There was 100% agreement and no time in protocol differences for the 32 PZA tests between the two systems. There were no observable differences in growth characteristics based on total Growth Unit (GU) accumulation in the test sets.
Conclusion: The data demonstrate that the MGIT 320 and the MGIT 960 systems are functionally equivalent for growth, detection and antimicrobial susceptibility testing of *M. tuberculosis*.

P2060 Comparison of INNO-LiPA Rif. TB, genotype MTBDRplus, PCR-RFLP and DNA sequencing for detecting multidrug-resistant *Mycobacterium tuberculosis* isolates

M.A. Mokaddas, N. Al Mutairi, S. Ahmed (Dasma, KW)*

Objective: Rapid identification of multidrug-resistant (resistant at least to rifampicin, RMP and isoniazid, INH) strains of *Mycobacterium tuberculosis* (MDR-TB) is crucial for early institution of appropriate therapy and to limit further transmission. The performance of two line probe assays (INNO-LiPA Rif. TB and Genotype (g) MTBDRplus), PCR-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing was compared for rapid detection of RMP and INH resistance conferring mutations in hot-spot region of rpoB (HSR-rpoB), katG codon 315 (katG315) and inhA regulatory region (inhA-RR) in MDR-TB strains.
Methods: Eighty-two phenotypically documented MDR-TB and 43 pansusceptible *M. tuberculosis* strains isolated from 99 patients in Kuwait during 2001–2008 were analyzed. Mutations in HSR-rpoB and katG315 and/or inhA-RR were detected by INNO-LiPA, gMTBDRplus, PCR-RFLP and DNA sequencing of regions of interest of *M. tuberculosis* genome. Strain relatedness was determined by PCR-based fingerprinting methods and genetic group analysis.
Results: All 43 pansusceptible strains contained wild-type sequences in HSR-rpoB, katG315 and inhA-RR. The RMP resistance was detected in 94% (77/82) and 95% (78/82) of MDR-TB strains by INNO-LiPA and gMTBDRplus assay, respectively. Two isolates with insertion514TTC were detected as RMP susceptible by INNO-LiPA, 1 isolate with L533P mutation was detected as RMP susceptible by gMTBDRplus while 3 isolates were detected as RMP susceptible by both the assays. Two of the latter 3 isolates contained a novel I572F mutation that is outside the HSR-rpoB. Rate of concordance of INNO-LiPA and gMTBDRplus assays for RMP resistance detection with HSR-rpoB sequencing was 96% (120/125) and 97% (121/125), respectively. INH-resistance was detected in 72% (59/82), 72% (59/82), 27% (22/82) and 93% (76/82) of MDR-TB strains by katG315 PCR-RFLP, katG315 sequencing, inhA-RR sequencing and gMTBDRplus assay, respectively. Majority of MDR-TB isolates in Kuwait were genotypically distinct strains.
Conclusions: The gMTBDRplus assay alone while three other tests combined (INNO-LiPA or HSR-rpoB sequencing, katG315 PCR-RFLP or katG315 sequencing and inhA-RR sequencing) were required to accurately detect MDR status of ~87% (71/82) of MDR-TB strains. The high cost of gMTBDRplus assay is the main limitation of this test for rapid detection of majority of MDR *M. tuberculosis* strains for proper management of the disease when MDR-TB is suspected.

P2061 Cluster association of gyrA mutations in extensively drug-resistant tuberculosis isolates from Lisbon, Portugal

J. Perdigão, R. Macedo, L. Brum, I. Portugal (Lisboa, PT)*

Objectives: Previous reports have shown that a high rate of extensively drug-resistant (XDR) tuberculosis (TB) exists in Lisbon Health Region. One of the factors contributing to this high rate is the high prevalence of specific clusters. Moreover, Portugal has the highest rate of fluoroquinolone prescription rate in the European Union. Such, high usage may be selecting *Mycobacterium tuberculosis* strains resistant to fluoroquinolones in infected patients. In this study we intend to characterize which mutations are conferring fluoroquinolone resistance in Lisbon Health region and eventually correlate them with specific clusters.
Methods: We have analyzed 26 fluoroquinolone-resistant *M. tuberculosis* strains, all of which XDR-TB, isolated in Lisbon Health Region during the year of 2005. An internal fragment of gyrA gene of each isolate was amplified by PCR and characterized by sequencing analysis.

All strains were also genotyped by 12 loci Mycobacterial Interspersed Repetitive Unit – Variable Number of Tandem Repeats (MIRU-VNTR).
Results: We have found three different missense mutations in gyrA gene: S91P, D94A and D94G. The most common mutation was S91P, which was present in 11 isolates. One isolate did not have any mutation. The 26 isolates were divided by two MIRU-VNTR clusters: Lisboa3 and Q1. Both have been previously associated with XDR-TB. Mutations S91P and D94G were associated with Lisboa3 cluster, while mutation D94A was associated with Q1.
Conclusion: We verified that an association exists between the mutations conferring fluoroquinolone resistance and genotype. Such mutations may be helpful in determining a clinical isolate's genotype and possible association with XDR-TB. We also verify that given the high clonality of the analyzed isolates, active XDR-TB transmission is taking place.

P2062 *Mycobacterium tuberculosis* Beijing family strains: analysis of molecular typing and of the epidemiological and clinical factors associated with an emerging lineage in the urban setting of Milan, 1996–2007

F. Zanini, M. Carugati, C. Schiroli, A. Lombardi, E. Mazzola, L. Codecasa, A. Gori, F. Franzetti (Milan, Monza, IT)*

Objectives: Among the most prevalent *Mycobacterium tuberculosis* strains, the Beijing genotype raises major concern because of its global spreading, hyper-virulence and association with multiple drug resistance (MDR). The aim of the study was to evaluate the role of Beijing family in the urban setting of Milan from 1996 to 2007 and to identify epidemiological and clinical factors associated with this genotype strain.
Methods: 2401 culture positive tuberculosis (TB) cases diagnosed in Milan and surrounding area were included. Epidemiological, microbiological and clinical data were assessed. The genotypic profile of the clinical isolates was obtained by spoligotyping method. Mycobacterial interspersed repetitive unit (MIRU) typing was executed on Beijing isolates to assign MIRU type (MT). The χ^2 test and a multivariate logistic regression model were used to identify predictors significantly associated with Beijing strains.

Table 1. Multivariate logistic analysis of epidemiological and clinical factors associated with Beijing related tuberculosis

Epidemiological and clinical features	AOR[§]	(95% CI)[#]
Gender		
Female	1*	
Male	1.4	(0.77–2.54)
Age group		
⩾40 yr	1*	
⩽40 yr	1.24	(0.59–2.58)
Geographic area of patient's born		
Italy	1*	
China	118.0	(43.7–321.0)
Asia (without China)	6.25	(2.11–18.6)
Eastern Europe	4.97	(1.66–14.9)
Southern America	3.45	(1.31–9.09)
Africa	1.48	(0.41–5.31)
Site of tuberculosis		
Pulmonary	1*	
Extra-pulmonary	0.40	(0.05–3.21)
Lymph nodes	1.62	(0.64–4.13)
Disseminated	1.03	(0.43–2.49)
Drug susceptibility of clinical isolates		
Susceptible	1*	
Other resistance	0.83	(0.36–1.89)
MDR	4.07	(1.81–9.15)
HIV co-infection		
Absent	1*	
Present	2.31	(0.92–5.76)

[§]Adjusted odds ratio (AOR), [#]Confidence interval (CI), *Reference category.

Results: Beijing family accounted for 67 isolates (2.8% of the total). From 1996 to 2007 its prevalence raised from 1.8% to 3.2% (p=.104). At the univariate analysis, the Beijing group displayed a lower average age at the diagnosis ($p < 0.0001$) and a higher proportion of foreign patients ($p < 0.0001$) and MDR cases ($p = 0.009$). The multivariate logistic analysis (Table 1) confirmed that foreign origin increased significantly the risk of having a Beijing related TB [AOR = 6.0 (95% CI:2.5–13.4)]: the highest values were observed among Chinese [AOR = 118.0 (95% CI:43.7–321.0)] and Eastern European patients [AOR = 4.9 (95% CI:1.7–14.9)]. MDR [AOR = 4.1 (95% CI:1.8–9.1)] and HIV infection were also independently associated with Beijing genotype but the latter without a significant statistical difference [AOR = 2.3 (95% CI:0.9–5.7)]. Sixty-six isolates were grouped in 28 MIRU types. The predominant MT resulted the founder strains MT11 and MT33, always present during study period and including isolates belonging to a wide range of nationality. One type not previously identified (called S10) was detected exclusively in four Italian patients.
Conclusion: Beijing family was an emerging lineage in metropolitan area of Milan and it displayed to be predominant in young foreign people, causing preferentially lymph-nodal and disseminated TB. Foreign born origin and MDR pattern were significantly associated to Beijing related TB. The Beijing MIRU types founders confirmed to be overriding and related to geographical origin of patients.

P2063 Comparison of rapid drug susceptibility tests for *Mycobacterium tuberculosis* clinical isolates

M. Aranzamendi Zaldumbide, C. Fernandez Mazarrasa, L. Martínez-Martínez, J. Agüero Balbin (Santander, ES)*

Objectives: Drug resistant tuberculosis is a growing concern worldwide. Clinical microbiology laboratories should provide reliable results on susceptibility testing to assess effective treatment schemes and appropriate intervention measures to control the disease. In the present study, the efficiency of Etest and genotypic detection of isoniazid (INH) and rifampin (RAMP) resistance was evaluated by comparing results to those of the multiple proportion method as a reference.
Methods: 100 *Mycobacterium tuberculosis* strains were selected from a stock collection of clinical isolates (January 2000–July 2009). CMI was determined by Etest in 50 strains known to be INH-R (n = 42) or INH+RAMP (n = 8) and in 50 susceptible strains. The genome regions associated with INH-R (including the codon 315 of the katG gene and the fabG1(mabA)-inhA regulatory region) and RAMP-R (81-bp hot spot region of the rpoB gene called RRDR) were amplified in all the strains by PCR and the DNA sequences were studied.

Multiple proportion method

		INH	RAMP
Etest	VME rate	38%	0%
	ME rate	0%	1.08%
	mE rate	19%	1%
	CA rate	81%	99%
Genotypic detection	VME rate	42%	0%
	ME rate	0%	0%
	mE rate	21%	0%
	CA rate	79%	100%

Results: Very Major Error (VME) rates for INH susceptibility testing were 38% for Etest and 42% in genotypic detection with Category Agreement (CA) rates of 81% and 79%. In contrast, CA rates for Etest and genotypic detection were 99% and 100% respectively, for RAMP. Mayor Error(ME) rate for RAMP using Etest was 1.08%. Overall a significant number of isolates harbouring the mutation S315T in the katG and the total of multiresistant isolates showed high-level resistance (INH >256 mg/L, RAMP >32 mg/L). The results are summarized in Table 1.
Conclusions: Our results demonstrated a low sensitivity of these methods to detect INH-R strains, and points to the need of finding out

new phenotypic methods and other mutant regions. On the other side, confirm the feasibility of implementing both methods as fast and accurate alternatives for assessment of RAMP-R, which in turn is a marker for multiresistance.

P2064 Characterization of purified recombinant ESAT6, CFP10 and ESAT6/CFP10 fusion protein of *Mycobacterium tuberculosis* C for detection of human tuberculosis in Iran

M. Hemmati, Z. Mostafavi-pour, A. Seghatoleslam, M. Rasti, S. Ebadat (Shiraz, IR)*

Mycobacterium tuberculosis (M.tb) is major cause of human tuberculosis (TB) which kills millions of people annually. The two secretory proteins of M.tb that recently have been focused on, are Early secretory antigenic target 6 (ESAT6) and Culture filtrate protein 10 (CFP10). These proteins are potent T-cell antigens recognized by over 70% of tuberculosis patients. In addition the two proteins are dominant γ interferon (IFN-g)-induced antigens which has led to their proposed usage as diagnostic reagents for Human tuberculosis via ELISPOT (Enzyme Linked Immuno Spot) assay. To see whether each one of these proteins or the combination can detect M.tb from nontuberculosis mycobacteria with high specificity in biological fluids, we produced the proteins from M.tb C strain that was provided by Razi Institute. esat6, cfp10 and esat6/cfp10 fusion gene were amplified by Polymerase Chain Reaction (PCR). Cloning of the genes into pGEX4T1 plasmid was performed. Recombinant plasmids were sequenced to confirm the insertion. *E. coli* strain BL21 was transformed with the recombinant plasmid. The expressed proteins were purified by affinity chromatography with Glutathione agarose beads in order to produce GST-tagged proteins, and then analyzed by SDS-PAGE and western blotting. Purity and antigenicity of the proteins were confirmed by western blotting. In this study ESAT6, CFP10 and ESAT6/CFP10 fusion protein obtained successfully. In order to compare the proteins alone or their combination in detection of TB, we performed ELISPOT assay by separation of tuberculosis patient WBC and analyzed effect of the proteins ESAT6, CFP10 and ESAT6/CFP10 in production of IFN-g in comparison with PPD. Results indicated high sensitivity and specificity of these proteins in comparison with PPD in detection of tuberculosis.

P2065 A comparison of two specimen preparation methodologies and their effect on the outcome of standard diagnostic protocols used for mycobacteria detection

G. Skenders, L. Broka, I. Prokopovica (Riga, LV)*

Objectives: Sputum and other respiratory specimens are the primary means to diagnose pulmonary tuberculosis and other mycobacteria infection. In order to be useful for diagnostic protocols, these specimens must be liquefied and decontaminated, but the failure to control pH inherent in conventional protocols for specimen preparation can significantly reduce the number of viable mycobacteria in the patient sample and negatively affect analytical protocols. In this study we attempted to examine the effect of two separate specimen preparation methods on the outcome of common diagnostic procedures for AFB infection.
Methods: Pulmonary samples submitted for diagnosis were split evenly and subjected to two separate specimen processing methodologies; the conventional methodology using NALC, 3% NaOH and M/15 Phosphate Buffer, and a new methodology offered by the NAC-PAC™ EA3 [Alpha Tec Systems Inc., Vancouver, WA]. Once prepared for diagnosis each sample was initially screened via Auramine O / Rhodamine B staining, and, if positive, organism concentration was graded based on the WHO/IUATLD evaluation scale. All samples were cultured using Lowenstein-Jensen medium at 36ºCelsius for 8 weeks. Culture positive specimens were graded based upon the number of colony forming units present.
Results: Of the 113 samples submitted for diagnostics 22 were positive for AFB by either microscopy or culture. Of these positive samples all 22 were detected using the Alpha Tec methodology and 20 were detected using conventional specimen preparation methodology. The two samples undetected using conventional methodologies were smear negative but culture positive using the Alpha Tec methodology. One sample that was smear negative but culture positive using the conventional methodology was smear positive using the Alpha Tec methodology. Four smear negative culture positive samples showed non-correlating growth, three samples using the Alpha Tec methodology showed a greater number of AFB colonies, one sample using the conventional methodology showed a greater number of colonies.
Conclusion: The Alpha Tec NAC-PAC™ EA3 AFB Specimen Processing reagent system identified positive smears and cultures otherwise missed by the conventional NALC/NaOH/M/15 Buffer methodology.

Aggregated results

	Smear–/Culture–	Smear+	Smear–/Culture+
ATS	91	14	8 (3 with increased CFU, 2 undetected using NaOH/PBS)
NaOH/NALC	93	13	7 (1 with increased CFU)

TB – clinical aspects

P2066 Tuberculosis in immigrants: clinical findings and epidemiological trends in the last decade in an area of northern Italy

P. Brugnaro, E. Morelli, F. Eseme, M. Fiscon, F. Cavinato, F. Ebo, S. Grandesso, U. Fedeli, C. Ziprani, E. Raise (Venice, Castelfranco Veneto, IT)*

Objectives: The immigration wave occurred in Italy in the last decade is dramatically acting on the epidemiology of tuberculosis (TB) in our region. The aim of our study was to compare the prominent disease features between Italian and immigrant patients with TB who came to our attention in the last ten years.
Methods: From January 2000 through May 2009 all consecutive patients with diagnosis of TB admitted to our hospital were included in this study. Immigrants patients were compared with native residents in relation to the following data: demographics, comorbidities, clinical and radiological findings of TB disease, drug resistance.
Results: Among the 188 consecutive patients with TB diagnosis Italians were 92 (49%) and immigrants were 96 (51%). The frequency of foreign-born patients was significantly higher in the period 2005–2009 (71.9%) than in the period 2000–2004 (28.1%; $p < 0.001$). Among immigrants the median time from arrival in the host country to TB diagnosis was 36 months (range 1–336). Italians patients had a greater mean age (64.3 versus 30.0 years; $p < 0.001$) and a higher frequency of concomitant chronic illnesses (35.9% versus 6.3%; $p < 0.001$), whereas foreigners were more commonly affected by social risk factors such as low-income and homelessness (25% vs 2.2%; $p < 0.001$). Immigrants patients had a higher frequency of sputum positive pulmonary TB on direct microscopy (65.6% vs 42.4%; $p = 0.001$) and radiological signs of lung cavities (52.9% versus 29.3%; $p = 0.007$). The detection of lung cavities on chest-X-ray was strictly associated with smear-positive pulmonary TB ($p < 0.001$). Drug resistance was detected in 30 out of 109 *Mycobacterium tuberculosis* isolates (27.5%): multidrug-resistant *M. tuberculosis* isolates were 3.7%. The distribution of resistances did not show difference between local and immigrant patients (30.8% in immigrants versus 22.7% in local patients for any resistance; $p = 0.356$).
Conclusions: During the last decade the prevalence of foreign-born patients with tuberculosis surpassed the native patients. Immigrant are represented by otherwise healthy younger people, with a recent arrival from countries with high incidence of TB. They develop more commonly smear-positive pulmonary TB with cavitary lung lesions compared to the local patients. Updated surveillance and preventive strategies of screening, diagnosis and treatment of TB are mandatory in the ever-changing scenario of immigration in our country.

P2067 Epidemiological investigation of a tuberculosis cluster including an untraceable infant case in the Tyrol, Austria, 2006–2009

R. Fretz, W.M. Prodinger, P. Much, A. Indra, M. Prem, W. Volkan, J. Schwanninger, A. Luckner-Hornischer, F. Allerberger (Vienna, Innsbruck, Kitzbühel, Kufstein, AT)*

The incidence of tuberculosis (TB) disease in Austria has steadily declined from 1480 TB cases in 1997 (18.6/100 000) to 890 TB cases in 2008 (10.7/100 000). A cluster of isolates from 9 TB cases was identified with identical spoligotype (International Type – SIT276) and indistinguishable 25 loci MIRU-VNTR and IS6110-RFLP genotypes. All TB cases (including a 6m old infant) were diagnosed in the Tyrol in the years 2006–09.

Methods: To enlighten the chains of TB transmission, a 3-stage strategy was applied: The databases of the NRCT and of DHMM were searched from 2002 onwards (year of implementation of general spoligotyping at NRCT) about information on further TB cases with identical spoligotype; the already conducted TB contact investigations, the TB notification reports and hospital discharge letters were reviewed, and a network analysis among the identified contacts of TB cases was carried out. A 2nd round of personal interviews with the Tyrolean TB cases was performed to obtain in-depth information around an individual's exposure to infection prior to diagnosis.

Results: In total, 15 TB-cases with spoligotype SIT276 were identified from 5 Austrian provinces: Carinthia (n = 1), Styria (n = 2), Upper Austria (n = 2), Vorarlberg (n = 1), and the Tyrol (n = 9). Of these, the 9 isolates from the Tyrol had an identical VNTR and/or RFLP genotype. All Non-Tyrolean TB cases were diagnosed earlier than the Tyrolean TB cases. Besides the local and temporal clustering of the Tyrolean TB cases, the age structure differed between Non-Tyrolean and Tyrolean TB cases: the median age was 68y (range: 53–78y), respectively 21y (range: 6m–59y). The epidemiological investigations revealed a probable chain of transmission from a female consultant to her male colleague, and from him to his girl-friend. Furthermore, possible transmission chains from the index case (a waitress with cavitary TB) to the female consultant and to 2 further TB cases were established. However, no apparent epidemiological links to the infant TB case and to the 2 remaining cases were found.

Conclusion: The investigation underlines the role of genotyping to be able to clarify growing TB clusters and links to rare cases, e.g. in infants. This infant TB case's untraceable link to an emerging cluster nevertheless appears as probable, and illustrates the low risk in industrial countries for contracting TB from non-family sources at infant age.

P2068 Extrapulmonary tuberculosis in intermediate-TB endemic area

Y.J. Kim, S.I. Kim, Y.R. Kim, K.W. Hong, M.W. Kang (Seoul, KR)*

Background: Tuberculosis (TB) in Korea is still common, and remains as important cause of mortality. The characteristics of extrapulmonary TB vary from country to country. This study is to evaluate the epidemiology and clinical outcome of extrapulmonary TB in intermediate-TB endemic area.

Methods: From January 2004 and December 2007, we reviewed retrospectively inpatient medical records of the patients who had been with extrapulmonary tuberuculosis(TB).

Results: Among 140,661 inpatients, 168 patients (0.1%; 57 male, 111 female) were diagnosed as extrapulmonary TB, including cervical lymph nodes (131/168, 77.9%), intestine and/or peritoneal (13/168, 7.7%), genitourinary (7/168, 4%), pleural (6/168, 3.6%), meningeal (4/168, 2.4%), bone (2/168, 1.2%), psoas abscess (2/168, 1.2%), laryngeal (1/168, 0.6%), and disseminated TB (2/168, 1.2%) cases. The mean age was 39 years, and 36.3% of cases were <30 years of age. Previous tuberculosis history was seen in 44 cases (26.2%) of patients, HIV infection in 5 cases (2.9%). In 143 (85%) patients, the diagnosis was established on histopathologic methods, in 10 cases on microbiologic methods, and in 6 cases on PCR method. In 10 cases who confirmed by microbiologic methods, one patient was MDR TB case. TB-related mortality showed in one case with HIV-infected patient, who diagnosed with disseminated TB. Five patients (2.9%) experienced recurrence of extrapulmonary TB after the cessation of anti-TB treatment.

Conclusion: Cervical lymph nodes were the most common site of extrapulmonary TB. This study shows a different epidemiologic pattern in that the majority of extrapulmonary TB patients are female with young age. Further study is needed to identify the cause the higher incidence of extrapulmonary TB infection in younger females.

P2069 Tuberculous meningitis in adults – a 6-year survey

E. Miftode, G. Juganariu, D. Leca, A. Vata, O. Dorneanu, D. Teodor, A. Teodor, C. Manciuc, C. Dorobat (Iasi, RO)*

Objective: To assess the present epidemiology, clinical presentation and outcome of adult patients with tuberculous meningitis (TBM) (except HIV-patients).

Method: We conducted a retrospective study that included all adult patients diagnosed with tuberculous meningitis in an Infectious Diseases Hospital.

Results: In the past 6 years 84 patients with TBM were hospitalized in the Hospital of Infectious Diseases Iasi. The patients age ranged from 16 years to 73 years (mean, 31 years). The neurological state at the time of admission was: coma in 22 patients (26%), other consciousness abnormalities (dizziness, changing in personality) in 18 patients (20%), and cranial nerve palsies in 6 cases (7%). A history of contact with tuberculosis was detected in 12% of adults and prior tuberculosis was mentioned in 19% of them. In 23 cases (27%) a pulmonary location of tuberculosis was revealed. Pulmonary lesions had a miliary pattern in 13 cases (15.5%), fibro-cavitary – in 8 cases (9.5%), and pleural effusion in 2 cases (2.3%). Tuberculin skin test elicited a positive reaction only in 21% of patients. The CSF smear for acid-fast bacilli was positive in 7 patients (9%). A positive culture result *M. tuberculosis* was available only in 13 patients (15.5%). The rate of quantiferon test positivity in the blood was 69% (29 cases). The level of albumin in CSF was very high (>3g/l) in 30 patients (35.7%) (much more frequently in adults than in children – p = 0.048) and glucose level, markedly low (<0.3 g/l) in 29 cases (34.5%) – with no differences between adults and children.

Sixteen patients (19%) developed neurological complications during the hospitalization: hydrocephalus (4 cases), cranial nerves paralysis (4 cases), hemiplegia/monoplegia (3 cases), tuberculomas (5 cases), and 8 patients died (9.5%).

Conclusions: We observed a high rate of complications in our study, due to the late presentation of patients to the hospital. The low rate of mycobacterium documentation requires other methods of diagnosis such as the DNA/RNA polymerase chain reaction which has become the method of choice for early diagnosis. Several specimens and large volumes of CSF fluid are important for obtaining higher cultures and smear positivity rates.

P2070 Tuberculous meningitis: evaluation and review of 32 cases

U. Demirpek, U. Dizer (Istanbul, Ankara, TR)*

Objectives: Tuberculous meningitis(TBM) is the most devastating form of meningitis and prompt accurate diagnosis remains the key to its treatment. Conventional microbiology has limited utility and nucleic acid-based methods have not been widely accepted for many reasons. Therefore, evaluation of common clinical and laboratory features of the TBM patients could be contribute to its fast diagnosis, consequently treatment.

Methods: Over a six-year period (1999–2005), clinical and laboratory data of the 32 patients admitted to 1000-bed hospital were reviewed retrospectively.

Results: Male to female ratio was 27:5. The mean age of the patients was 21.7 years; ranged 20–36 years. The criteria evaluated were BCG vaccination previously, tuberculous contact, initial symptoms, CSF (cerebrospinal fluid) analysis, culture for *M. tuberculosis,* TB

PCR(polymerase chain reaction), chest X-ray, MRI of the head. Ten(10) of the patients have had BCG vaccination beforehand. Five(5) of the patients have had TB contacts. The symptom present on admission were fever in 20 patients (62.5%). All of the patients had CSF leucocytes ranged 40–1480 cells/mm^3. Only one patient was diagnosed by acid-fast stain from CSF.Protein quantity ranged between 60–760 mg/ml CSF.*M. tuberculosis* had been cultured 13 patients (41%). PCR analysis of CSF for *M. tuberculosis* were positive for all of the cases (100%). Five of the patients (16%) had abnormalities of the chest X-ray.21 patients (66%) had abnormalities found on MRI.Hydrocephalus found in eight of the patients on MRI (25%). Just four of the patients had tuberculomas on MRI.

Conclusion: TB is a significant health issue in terms of outcome and sequela. TB meningitis is difficult to diagnose due to the nonspecific clinical presentation of patients; the initial differential diagnosis includes many other diseases. Early diagnosis and laboratory confirmation is important to manage the treatment. Positive cultures, ranging 15–75 per cent, have lower rates to confirm diagnosis. Molecular methods and neuroimaging could be used for fast and accurate diagnosis.

P2071 Extra-pulmonary tuberculosis: epidemiology and risk factors

J.F. García-Rodríguez, H. Álvarez-Díaz, M.V. Lorenzo-García, E. Solla-Babio, A.I. Mariño-Callejo, P. Sesma-Sánchez (Ferrol, ES)*

Objective: To describe the epidemiology and risk factors associated with Extra-pulmonary tuberculosis (EPTB).

Method: The new tuberculosis (TB) cases diagnosed from 1991 to 2008 in a sanitary area with 218000 caucasian population were classified as either EPTB or pulmonary tuberculosis (PTB). EPTB encompassed pleural, lymphatic, genitourinary, bone and/or joint, skin, meningeal, peritoneal, gastrointestinal and unclassified cases. We excluded patients with concurrent EPTB-PTB and disseminated TB (miliary). Of all diagnosed cases, 1.233 (63.9%) were prospectively followed in a monographic unit of TB. A standardized protocol was developed for each patient, including demographic characteristics, drug susceptibility test results, and risk factors, considering: female gender, age, BCG vaccination, history of contact with TB patients, gastrectomy, smoking, alcoholism, diabetes mellitus, chronic renal failure (CRF), neoplasia, use of immunosuppressive drugs/steroids, albumin and human immunodeficiency virus (HIV) status. Variables were compared between EPTB and PTB groups.

Results: Among 1,929 cases diagnosed, 1,037 (53.8%) were PTB and 649 (33.6%) were EPTB, including pleural 281, lymphatic 184, genitourinary 44, bone and/or joint 36, skin 31, meningeal 28, peritoneal 18, and other 27 cases. A reduction in the number of cases of EPTB appears to be slower than that showed in cases of PTB: 283 EPTB and 505 PTB cases in the 1991–1996 period versus 136 and 178 respectively in the 2003–2008 period. An increase of EPTB has taken place from 35.9% of the cases of TB in the 1991–1996 period to 43.4% in the last six years, $p = 0.02$. Compared with PTB, EPTB was associated with female gender (56.4% vs 33.7%), age average (43.3±22.5 vs 35.7±17.3 years old), BCG vaccination (10.5% vs 20%), contact with TB patients (24.4% vs 36.4%), gastrectomy (0.4% vs 1.7%), smoking (23.5% vs 52.5%), alcoholism (5.3% vs 20.6%), and CRF (1.8% vs 0.3%), $p < 0.05$. Multivariate logistic regression analysis showed that being female (OR 1.78; 95%IC: 1.35–2.34), age (OR 1.02; 95%IC: 1.01–1.03), alcoholism (OR 0.37; 95%IC: 0.23–0.6), smoking (OR 0.43; 95%IC: 0.32–0.57) and BCG vaccination (OR 0.63; 95%IC: 0.43–0.93) were associated with EPTB.

Conclusions: The proportion of EPTB has increased. Risk factors to EPTB differ from those of PTB. The proportional increase in EPTB could be explained by older population and increase of female gender, and by a decline of smoking and alcohol use habits.

P2072 Long-term efficacy of 6-month therapy with isoniazid and rifampin for pleural tuberculosis

J.F. García-Rodríguez, H. Álvarez-Díaz, E. Solla-Babio, S. Sánchez-Trigo, A.I. Mariño-Callejo, P. Sesma-Sánchez (Ferrol, ES)*

Introduction: Incidence of tuberculosis (TB) is high in our city and primary drug resistance is <4%. Objective: To investigate the long-term efficacy of a 6-month treatment regimen of isoniazid and rifampicin (6HR) for pleural TB in the HIV-negative population.

Methods: HIV-negative patients with pleural TB attended in our hospital from 1991 to 2008 entered a 6HR treatment protocol and were prospectively followed in our TB Unit for 10.6±4.3 years (1–18.1 years). The diagnosis of pleural TB was based on pleural fluid analysis and cultures and/or pleural biopsy pathology or cultures. Patients with empyema and those with associated pulmonary infiltrates were not included. Pleural effusions were deemed slight if they affected less than one fifth of the hemithorax, moderate if they affected between one fifth and one third and extensive if they affected more than one third. Treatment regimen consisted of H 5 mg/Kg and R 10 mg/Kg daily for 6 months. In patients with extensive effusion that caused dyspnea, therapeutic thoracocentesis and respiratory physiotherapy were performed. Demographic and clinical variables (including pleural fluid analysis, BCG, tuberculin skin testing, chest x-ray, acid-fast bacillus smears, Lowenstein culture, assessment of treatment tolerance and illness evolution) were collected in each case. Chest x-ray was performed at the end of the treatment. Then, patients were followed by telephone interview.

Results: 93 patients followed: 51 men and 42 women, mean age 32.3±15.9 years old (14–79). The diagnosis was confirmed by culture or histological examination in 51.6% of cases; in 22.6% of patients the pleural effusion was exudative with lymphocytosis and ADA > 45 UI/l; in 25.8% of the patients the diagnosis was based on a compatible clinical picture and successful treatment. Pleural effusions were: slight 32.3%, moderate 28% and extensive 39.8%. Susceptibility tests were performed in 24 cases: all *M. tuberculosis* were susceptible to first-line agents. All patients completed treatment, 14 (15%) developed drug side effects and 3 (3.2%) toxic hepatitis. In 2 cases it was precise to modify the treatment and to prolong its duration (1 due to H-hepatitis and 1 due to R-fever). An overall success was achieved in all patients; 15% developed residual pleural thickening and none of them required surgery.

Conclusion: 6HR should be adequate for pleural tuberculosis in HIV-negative population if the incidence of primary drug resistance is <4%.

P2073 Clinical features and outcomes of genito-urinary tract infection caused by non-tuberculous mycobacteria at a university hospital in Taiwan, 1996–2008

C. Yang, C.Y. Liu, C.H. Liao, P.R. Hsueh (Taipei City, TW)*

Background: Genitourinary infections caused by nontuberculous mycobacteria (NTM) are rarely reported.

Methods: The medical records of all patients with GU NTM infections treated at National Taiwan University Hospital from 1996 to 2008 were retrospectively reviewed.

Results: Fifteen patients were identified, of whom 10 (67%) were male. More than two-thirds of patients had underlying diseases, and the most common one was chronic renal disease. Only one patient had acquired immunodeficiency syndrome. Acid-fast smears (AFS) of urine were negative in all patients. Eleven of the 15 isolates were stored and available for further confirmed by sequencing of their 16S rRNA gene. *Mycobacterium avium* complex (MAC) was the most common etiology of genitourinary NTM infections and was isolated in five (33%) patients. Rapidly growing mycobacteria (RGM) were isolated in four (27%) patients: two each for *Mycobacterium abscessus* and *Mycobacterium fortuitum*. Of 12 patients receiving anti-NTM treatment, only four received adequate prescribed regimens, and none died of NTM infections. Another two patients died of refractory urosepsis before urinary NTM infections were diagnosed. Comparisons of clinical

characteristics between these 15 patients and 43 patients with GU tuberculosis reported previously were also analyzed. Patients with GU NTM infection were more likely to report constitutional symptoms, seek medical help within one month after onset of symptoms, and develop leukocytosis. However, patients with GU tuberculosis were more like to have ureteral strictures and abnormal chest radiographs associated with active ore inactive tuberculosis.
Conclusion: Although rare, GU NTM infections were significantly and potentially life-threatening, and should be considered in the differential diagnosis of genitourinary infections, especially those unresponsive to conventional antibiotic treatment.

P2074 Intracranial tuberculomas: report of 24 cases

F. Bouattour, B. Hammami, D. Lahiani, I. Maaloul, C. Marrekchi, K. Ben Mahfoudh, M. Ben Jemaa (Sfax, TN)*

Central nervous system involvement in the form of TBC meningo-encephalitis is found in approximately one in five of patients with extra pulmonary tuberculosis.
Among the patients with tuberculosis only 1% develop intracranial tuberculomas.
The aim of this study is to characterize the clinical and radiological manifestations of brain tuberculoma.
Methods: This was a retrospective case survey of 24 patients admitted to infectious disease department over a 17-year period (1993–2009). Criteria used for the diagnosis of a tuberculoma were suggestive findings on a CT scan or MRI, supported by clinical and biological investigations and confirmed by the culture of CSF.
Results: We identified 7 men and 17 women affected by intracranial tuberculomas, without documented immunodepression. The mean age of this group was 28 years (14–60years). The main neurological symptoms and signs were meningeal irritation in 15 patients, focal neurological signs in 14 and alteration of consciousness in 11.
Mycobacterium tuberculosis was isolated in cerebrospinal fluid of 4 patients. Tuberculomas were solitary in 5 cases and multiple in 19 cases, with miliary aspects in 5 cases. They are particularly found in supratentorial compartment. Associated hydrocephalus was detected in 8 patients, infarction in 4 and meningitis in 20. Anti tuberculosis treatment was given to all patients associated with corticosteroid therapy for 17 patients. Paradoxical development or enlargement of tuberculomas was reported in 7 cases. With anti tuberculous chemotherapy for at least 12 months, the course was favorable without sequelae in only 11 patients. Permanent neurological sequelae were found in 4 patients.
Conclusions: The clinical presentation of intra cranial tuberculoma was polymorphous. MRI is useful for diagnosis and for follow-up during treatment. CNS tuberculosis continues to be a condition which carries significant morbidity and mortality.

P2075 *Mycobacterium bovis* infections in a tertiary-care centre in Mexico: a case–control study

R. Franco, M. Bobadilla, A. Ponce de León, A. Galindo, A. Martinez, B. Chavez, L. Rodriguez, A. Hernandez, C. Lee, J. Sifuentes (Mexico City, MX)*

Objective: To determine the clinical and epidemiological characteristics of *M. bovis* (MB) disease in comparison with *M. tuberculosis* (MTB) disease in patients treated in a tertiary-care centre in Mexico.
Methods: We included all MB clinical isolates recovered from patients seen at this centre from 01/2000 to 12/2007. We randomly selected as controls two cases of MTB disease per each MB case. Demographics, clinical manifestations, treatment and outcome were collected. All isolates were properly identified by conventional biochemical tests, confirmed and grouped by RFLP and spoligotyping. Antimicrobial susceptibility testing was done by the radiometric method. For comparison we used X2 or Fisher's exact test accordingly, and a p value <0.05 was considered significant.
Results: From a total of 226 MTB complex isolates recovered during the study period, 52 were MB (23%) and 174 MTB (77%); 40% of MB isolates were recovered in the last two years. Spoligotyping revealed different MB lineages (BOV, 2; BOV-1, 1; BOV-2, 19; BOV-3, 8; no lineage, 14; not done 1). Only 45 MB were recovered for further analysis; these patients were compared with a group of 90 MTB cases. Patients with MB were younger, and showed a trend towards having chronic liver disease. MB disease caused more gastrointestinal symptoms and MTB more respiratory symptoms. Localized disease was seen in 24 (53.3%) and in 62 (68.9%) patients, ($p = 0.07$); pulmonary involvement in 10 (22%) and 34 (37.8%); extra-pulmonary disease in 35 (77.8%) and 56 (62.2%), respectively ($p = 0.06$). The frequency of HIV/AIDS was the same in both groups. We were able to follow-up 36 (89%) MB cases and 79 (88%) MTB cases; no difference in the intensive phase of treatment was noted, however MB patients received longer treatment with more than two drugs during the maintenance phase. The cure rate was high in both groups 87.5% and 95.7%, respectively, and the rate of relapse was low. Pansusceptibility was seen in 75% of MB and 90% MTB ($p = 0.026$) isolates; 11% of the MB isolates were resistant to streptomycin and 9% to isoniazid, without differences between groups.
Conclusion: MB disease seems to be a re-emergent problem in Mexico, although the burden of disease remains unknown. Surprinsingly, we found a higher resistance rate among MB isolates, although the cure rate was very high in both groups.

P2076 Tuberculosis meningoencephalitis with severe neurologic sequelae in an immigrant family's child

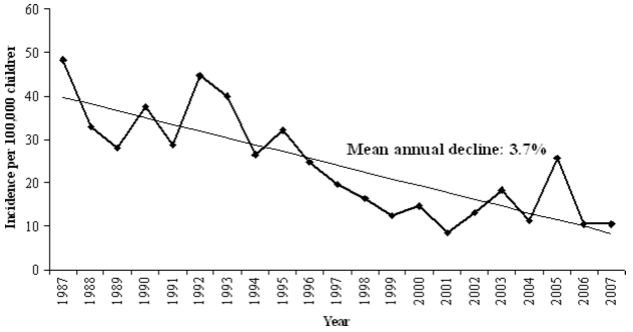
K. Kart Yasar, F. Pehlivanoglu, G. Sengoz, E.R. Ince, S. Sandikci (Istanbul, TR)*

Objective: Tuberculous meningitis (TBM) is the most devastating manifestation of tuberculosis. It is a challenge for clinicians because of the difficulty in making an early diagnosis and the severe consequences of delayed treatment. The aim of this report is to point out relation between migration and tuberculosis based on a 14-year old child of an immigrant family with tuberculous meningoencephalitis.
Case: A 14-year old child of an immigrant family was applied to our clinic with the complaints of fever, headache and vomiting lasted for 10 days. The emergent cranial CT scan showed no pathological findings thereby lumbar puncture was performed to examine cerebrospinal fluid (CSF). The findings were: CSF cell count: 57/mm^3 leucocyte (60% lymphocyte), 18/mm^3 erythrocyte and glucose: 13 mg/dl (simultaneous blood glucose 109), protein 243 mg/dl. Laboratory tests: WBC 12300/mm^3, haemoglobin 11.7 g/dl, CRP 12 mg/dl and Anti HIV antibody negative.
On the same day sudden loss of conscience, bradycardia, hypotension developed and the patient transferred to the intensive care unit (ICU) ceftriaxone and acyclovir therapy was started. The patient's mother had a tuberculosis history and general condition of the patient didn't ameliorate, for these reasons antituberculous therapy was started on the second day and vital findings were improved on seventh day. The patient was transferred to the ward from ICU. The patient was evaluated as stage III and cranial MRI showed multipl tuberculoma, basal meningitis and minimal hidrocephalus, thorax CT showed miliary pattern. CSF PCR for tuberculosis (GeneXpert MTB-RIF, Cepheid®, USA) was positive, *Mycobacterium tuberculosis* was isolated from CSF and Quantiferon TB gold test (Cellestis®, Australia) was positive. During the hospitalization period convulsion, hearing loss, aphasia, motor deficits developed and the patient was discharged from the hospital on the 24th day with mild sequelae (aphasia, minimal motor deficits and hearing loss). Follow examinations has been continuing.
Conclusion: Migration, crowded living conditions and positive family history contribute the severe course of tuberculosis as TBM and miliary TB forms. Tuberculosis control may prevent these severe manifestations of the disease.

P2077 Childhood tuberculosis in a large city: incidence evolution and factors associated to unsuccessful treatment completion during a 21-year period

J.L. Nelson, A. Moreno, A. Orcau, N. Altet, A. Martinez-Roig, J. Cayla, M. Casals, J.P. Millet, F. Moraga and the Childhood Tuberculosis Working Group of Barcelona*

Objectives: To date, few studies have been published on childhood tuberculosis (TB). Furthermore, the global burden cannot be accurately assessed due to lack of epidemiological data and consistently-used definitions across different regions. The objective of our study was to examine the TB incidence evolution and factors associated with an unsuccessful TB treatment outcome of children in Barcelona, Spain during a 21-year period.
Methods: The study utilized an observational longitudinal design and included TB cases less than 15 years of age, residing in Barcelona, who started treatment between 1987 and 2007, and were registered in the TB control program. Number of cases and incidence were calculated for the total study population, according to Barcelona consensus data, and stratified by year of treatment initiation, sex, and age. A logistic regression analysis was performed to analyze the factors associated with unsuccessful treatment completion (defined as treatment outcome other than cured), calculating odds ratio (OR) and corresponding 95% confidence interval (CI).
Results: One thousand children were included in the study: 512 (51.2%) were male, 922 (92.2%) were Spanish, and 466 (46.6%) were under 5 years of age. Regarding treatment outcome, 925 (92.5%) cases were considered cured, 2 (0.2) died due to TB, 4 (0.4%) died due to other causes, 31 (3.1%) transferred out of Barcelona, and 37 (3.7%) were lost to follow-up. Incidence dropped from 48.1 cases per 100,000 children in 1987 to 10.6 cases per 100,000 children in 2007 ($p < 0.001$), with an annual decline of 3.7% (Figure 1). Factors associated with an unsuccessful treatment included age under 5 years (OR = 1.8; CI 1.0–3.3), residence in the inner-city district (OR = 1.8; CI:1.2–3.1), and an unknown radiology result (OR = 7.5; CI:2.6–24).
Conclusions: Childhood TB incidence declined during the study period, probably due to increasing development of the TB control program created in 1987. Children under 5 years generally contributed more cases and represented a higher incidence whereas small differences existed by sex. The proportion of successfully treated patients in our study was high and transfer-out cases had an important presence within unsuccessful treatment outcomes. According to the multivariate analysis results, we found that evaluating successful treatment by comparing cured and all non-cured cases is not conclusive in a setting with a high percentage of cured and transfer-out cases.



Incidence of childhood tuberculosis per 100,000 children by year (n = 1000). Barcelona, 1987–2007.

P2078 Description of tuberculosis cases involving both respiratory and extra-respiratory sites and genotypic characterization of their isolates

Y. Navarro, M. Herranz, M. Ruiz Serrano, M. Martinez Lirola, E. Bouza, D. García de Viedma (Madrid, Almeria, ES)*

Background and Objectives: The application of molecular tools has revealed that infection by *Mycobacterium tuberculosis* (MTB) could be more complex than initially assumed. We analysed tuberculosis cases in which MTB was isolated from both respiratory and extrarespiratory specimens. Our aims were to describe these cases, determine their proportion to the total number of TB cases, and apply systematic molecular analysis to identify cases with clonally complex infections (mixed infections, coexistence of clonal variants, compartmentalized infections) as a first step in the in-depth characterization of these strains.
Methods: We analyzed the TB databases of two hospitals between January 2003 and October 2009. Patients were distributed in 3 groups according to the number (1 to 3) of extrarespiratory specimens from different tissues yielding MTB in addition to the respiratory sample. Genotyping of the MTB isolates from the selected cases was performed by MIRU-VNTR.
Results: In 97 out of 1733 isolates (5.6%), MTB was isolated from at least 1 extrarespiratory specimen in addition to the respiratory sample. The extrarrespiratory specimens most frequently yielding MTB were urine (40 cases), blood (31), pleural fluid (17), stool (8), and CSF (7). In most cases (70, 72%), MTB was isolated from only one extrarespiratory specimen in addition to the respiratory sample (Group 1). Additionally, in 27 cases (28%), MTB was isolated in 2 or more different extrarespiratory specimens: Group 2, 16 cases; and Group 3, 11 cases. Preliminary genotyping results from 21 representatives (10, 6, and 5 cases form Groups 1, 2, and 3, respectively) showed identical VNTR-MIRU-types for all the the isolates in 16 cases and genotypic differences in the isolates from the remaining 5 cases (in 1, 3, 4, 6, and 16 loci).
Conclusion: The isolation of MTB from both respiratory and extrarespiratory specimens was not anecdotal. In certain cases, the extrarespiratory infection involved several different tissues. Preliminary genotyping data allowed us to identify cases infected with isolates showing genotypic differences (mostly subtle) at the respiratory and extrarespiratory sites. Further genotyping could help us determine more precisely the degree of bacterial clonal complexity in these patients.
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P2079 Clinical features and outcomes of disseminated infection caused by non-tuberculous mycobacteria

K. Kao, C. Chou, P.R. Hsueh, H.Y. Chen, C.Y. Chen, C. Huang (Taipei City, Douliu, TW)*

Objectives: This study investigated the clinical characteristics and prognostic factors of patients with disseminated non-tuberculous mycobacteria (NTM) infection in Taiwan.
Methods: This retrospective study included patients who fulfilled the criteria for disseminated NTM infection at a medical center in Taiwan from January 2004 to December 2008. Data on etiology, clinical characteristics, laboratory findings, and outcome were collected from medical records.
Results: Disseminated NTM infection was diagnosed in 40 patients during the study period. The mean age was 46 years (range 22–79), and male gender was predominant (80%). More than half of the patients (n = 22, 55%) had HIV infection and fever was the most common initial presentation (n = 21, 52.5%). There were 13 episodes of co-infection with other bacterial pathogens in 11 patients (30%). Anemia, elevated alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, and C-reactive protein were commonly noted in disseminated NTM

infection. The most common site of NTM isolation from culture was blood (62.5%) followed by respiratory tract (52.5%). *M. avium* complex was the most common isolated species (70%). The overall mortality rate due to disseminated NTM infection was 30%. Univariate survival analysis showed significantly higher mortality rates in female patients, patients without anti-NTM treatment, and patients co-infected with other bacterial pathogens. Multivariate analysis showed that lack of anti-NTM treatment was the only prognostic factor for poor outcome ($p = 0.001$).
Conclusions: Maintaining a high level of suspicion and starting appropriate anti-NTM treatment promptly after diagnosis are crucial to improve outcome of patients with disseminated NTM infection.

P2080 Epidemiological monitoring of pulmonary tuberculosis in a correctional facility population, Athens, Greece, 2005–2009

S. Karabela, D. Papaventsis, S. Georgoulas, S. Nikolaou, P. Ioannidis, E. Konstantinidou, A. Sainti, I. Marinou, S. Kanavaki (Athens, GR)*

Objective: Correctional facilities are extremely high-risk environments for tuberculosis (TB) infection (co-infection with HIV is common), due to overcrowding, poor nutrition, limited access to health care, unsafe drug injecting practices and unprotected sex. However, limited data are available concerning the TB epidemiology in prisons. The purpose of the present study was to investigate the rate of TB in a large prison, in Athens, Greece.
Methods: Korydallos Prison Complex, Athens, is the main correctional facility in Greece, housing maximum security men and women. The majority of the nowadays 2.002 detainees are foreigners (1287, 64%), and 715 (36%) are Greeks. Between January 2005 and October 2009, a case-finding through self-referral survey was carried out in Korydallos Prison Complex Hospital. Prisoners who had symptoms suggestive of pulmonary TB were included in the study (cough of at least 1 week duration or fever or haemoptysis), underwent chest radiography, were offered HIV testing, and three "spot specimens" of sputum were tested for TB by both conventional and modern molecular methods. The sputum specimens were processed at the National Reference Laboratory for Mycobacteria, Athens.
Results: In total, sputum samples from 190 prisoners were tested (most were men <40 years old). Twenty (10.5%) had pulmonary TB: 6 were taking anti-tuberculous treatment (5 from Africa and 1 from Greece) and 14 were undiagnosed at the start of the study; 11 (5.8%) were sputum-smear positive and 9 (4.7%) were sputum-smear negative. The prevalence of active pulmonary TB in the prison's population determined by the number of persons with active TB divided by the total number of prisoners was approximately 250 per 100,000, which is 15.6 times higher than the general population (OR = 15.66, 95% CI: 9.44 to 25.96). Total resistance rates of TB isolates were: 30% for isoniazid, 25% for both rifampicin and ethamboutol, 15% for pyrazinamide, and 25% for streptomycin. 3/20 (15%) of TB isolates were multidrug-resistant strains (MDR), and 2/20 (10%) were extensively drug-resistant strains (XDR).
Conclusions: Pulmonary TB in Korydallos Prison Complex was 15.6 times more common than in the general population in Greece. This ratio was slightly above the mean ratio in prisoners compared to civilians in Europe. Moreover, MDR/XDR TB was prevalent in the study population. Improving living conditions in correctional facilities and adhering to TB control programmes are urgently required.

P2081 Extrapulmonary tuberculosis as an aetiological factor of increasing importance in patients with fever of unknown origin

G. Stevanovic, M. Pelemis, M. Pavlovic, L. Lavadinovic, B. Milosevic, S. Stojakovic, J. Nikolic, N. Mitrovic (Belgrade, RS)*

Objectives: The aims of this study were to determine prevalence of extrapulmonary tuberculosis in patient with fever of unknown origin (FOU) who was HIV negative, to show prevalence of, and types of extrapulmonary tuberculosis (TB) and to determine trends of prevalence among patients with FOU.

Methods and Results: During period 1994–2009, 2842 with FUO were evaluated and treated in the Clinic for infectious and tropical diseases, Belgrade. Extrapulmonary TB were diagnosed in 134 (4.7%) patients. Genitourinary TB in 73 patients (renal – 60, orhiepididymitis – 4, salpingo-oophoritis – 9); TB lymphadenitis in 13; meningitis in 15; TB pericarditis in 8, spondylodiscitis in 6, liver TB in 4 and in one patient small intestine TB. In 14 patients we did not confirm tuberculosis and after pulmonary TB was excluded, they were treated empirically with antituberculous drags, and had a good response. As a diagnostic methods we used: PPD skin test, cerebrospinal fluid (CSF), sputum and urine cultivation, computed tomography, echocardiography, intravenous pyelography, patohistological examination of lymph nodes, intestine and liver, and gynecological laparoscopy. For urine and CSF specimens PCR test was used after year 2001. The sensitivity of conformation test were: CSF culture 100% (PCR 100%), urine culture 45% (PCR 68%), for histopathology lymph nodes 78%, small intestine 100% (single patient) and liver 85%. As a diagnostic criteria, clinical course of the illness, radiological examination, laparoscopy and other endoscopic examinations and response to empiric therapy, were also used. Incidence of extrapulmonary TB was in slight increasing after year 2001. Isoniazid, rifampin, pyrazinamide, ethambutol and streptomycin were used for treatment. Multi drug resistant TB were confirmed in 4 patients and in 7 patients (drug sensitive TB) had relapses of the illness after treatment.
Conclusions: Extrapulmonary TB is increasing cause factor in patients with FOU and should be always considered during evaluations of this patients.

P2082 Incidence of thromboembolic events in hospitalized tuberculosis patients and its risk factors

M. Marjani, P. Baghaei Shiva, P. Tabarsi, D. Mansouri, M. Masjedi (Tehran, IR)*

Background: Pulmonary tuberculosis may be associated with a hypercoagulable state but there are few studies about incidence of deep vein thrombosis (DVT) and pulmonary thrombo-embolism (PTE) in tuberculosis patients. This study was performed to determine risk factors and incidence of DVT and PTE in admitted tuberculosis patients.
Methods: In a cross sectional study we calculated incidence of diagnosed thromboembolic events in all pulmonary and extra pulmonary new cases of tuberculosis patients admitted in our center since August 2007 to August 2009. Prophylactic strategies for prevention of DVT and PTE have been performed for high risk patients.
We randomly selected 100 tuberculosis patients without thromboembolic complications as control group and compared risk factors between case and control groups.

Table 1. Comparison of risk factors between case and control groups

	Case group, N (%)	Control group, N (%)	OR	95% CI	P-value
Male gender	21 (78)	48 (48)	3.79	1.411–10.189	0.006*
Age (yrs), mean	64	50.4			
⩾50 yrs	15 (56)	49 (49)	1.301	0.554–3.057	0.545
Active smoker	10 (37)	24 (24)	1.863	0.853–4.609	0.175
Opium	12 (44)	22 (22)	2.83	1.16–6.93	0.019*
IDUs	4 (15)	12 (12)	1.275	0.376–4.325	0.696
Diabetes mellitus	3 (11)	12 (12)	0.917	0.239–3.512	0.899
HIV	2 (7)	8 (8)	0.920	0.184–4.609	0.919
HTN	8 (30)	13 (13)	2.8	1.24–7.74	0.039*
Total	27	100			

Abbreviations: OR = Odds ratio, CI = Confidence interval, * = Significant, IDU = Intravenous drug user, HIV = Human immunodeficiency virus, HTN = Hypertension.

Results: The incidence of established thromboembolic complications was 27 of 1011 admitted tuberculosis patients (2.7%; 95% confidence interval: 1.8 to 3.9%). It was 1.8% for DVT and 1.0% for PTE. Thromboembolic events and DVT were more common in men than women, 3.8% versus 1.4% (p-value= 0.019) and 3.1% versus 0.4%, (p-value= 0.001) respectively.

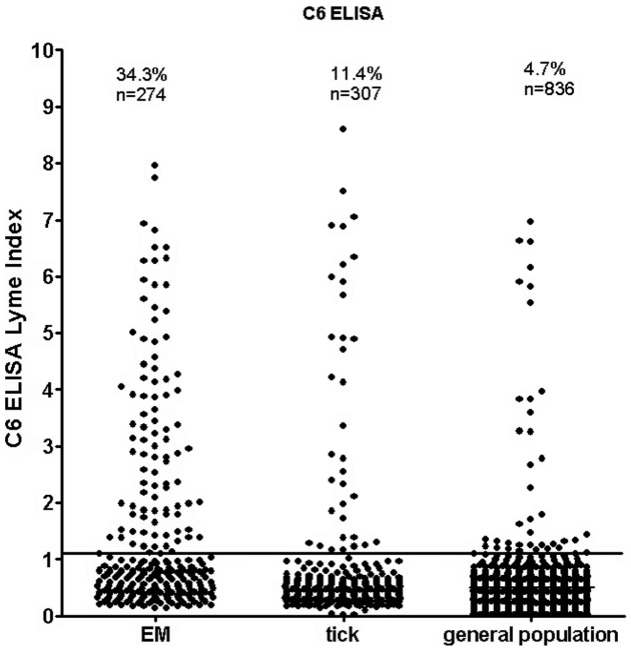
Also, in this study 100 tuberculosis patients without thromboembolic complications were entered randomly as the control group and risk factors between case and control groups were compared. Hypertension was more common among patients with thromboembolic events: 30% versus 13% (p-value= 0.039). Also opium addiction was more common among cases with TEE than control group: 44% versus 22% (p-value=0.019).
Other than sex, history of HTN and opium addiction there was not any relation among thrombo-embolic events and other risk factors such as smoking, diabetes mellitus or Rifampin dose between case and control groups.
Conclusion: It must be considered that this study was performed in a tertiary hospital and this may cause selection bias because many of our cases were complicated but it was enough to evoke special attention to thrombo-embolic events in admitted (and probably non-admitted) tuberculosis patients. Patients should be educated about its symptoms and clinicians should consider prevention, diagnosis and management of these events as soon as possible.

Lyme disease

P2083 Combining C6 ELISA and IgM immunoblot for the detection of antibodies in early Lyme infection

T. Herremans, A. Hofhuis, D.W. Notermans, N. Nozari, C. Nijhuis, L.M. Kortbeek, J. van der Giessen (Bilthoven, NL)*

Objectives: Lyme borreliosis is a zoonotic disease transmitted by ticks and caused by the spirochetes of the *Borrelia burgdorferi* sensu lato group. Diagnosis depends on clinical suspicion, the recognition of characteristic symptoms, and serology. Although frequently applied, serodiagnosis is not indicated in Erythema migrans (EM) patients because clinical signs are specific and serology is usually negative during this phase of Lyme disease. We used a population of EM patients to evaluate which assay or combination of assays provides the best algorithm for identifying early Lyme infections.
Methods: We analyzed 274 EM patients serologically at the start of their symptoms and approximately 12 weeks thereafter. Sera were tested in a commercially available C6 ELISA, an in-house inhibition ELISA based on the flagella antigen, and by an in house IgM and IgG immunoblot. In addition, sera from 307 tick bite patients taken directly after they were seen by their GP and from the general population of the Netherlands (n = 836) were also investigated to determine background levels.



Results: With 34.3% positive EM patients, the C6 ELISA was the most sensitive assay, followed by the IgM immunoblot (19.7%), the inhibition ELISA (10.9%) and the IgG immunoblot (6.9%). An algorithm using both a C6 ELISA and/or a IgM immunoblot positive results gave the highest sensitivity (41.2%). Examination of follow-up serum samples gave similar results in seroprevalence. In tick bite patients, the C6 ELISA showed the highest seroprevalence (11.4%) followed by IgG (7.2%), IgM (5.2%) and the inhibition ELISA (4.6%). Low C6 ELISA results could usually not be confirmed by immunoblot. In the general population, we identified 4.7% C6 positive persons. Increasing the cut-off level to a Lyme Index of 1.70 (the average + 3 x Standard Deviation) improved the specificity of the C6 ELISA from 95.3% to 97.8%, with a sensitivity loss in EM from 34.3% to 26.6%.
Conclusion: Serology in EM is insensitive, and the antibiotic treatment probably prevented further antibody development, making follow-up serology of limited clinical value. For these reasons, the serological investigation of EM patients should not be recommended. C6 ELISA values between 1.1 and 1.7 should be interpreted with caution in the absence of other confirmative serology. However, a combination of the C6 ELISA and the IgM immunoblot proved to be the most sensitive combination for detecting antibodies in early Lyme infections.

P2084 Comparison of eight ELISAs and five immunoblots for detection of anti-*Borrelia* antibodies reveals large differences between test strategies

C.W. Ang, D.W. Notermans, M. Hommes, A.M. Simoons-Smit, M. Herremans (Amsterdam, Bilthoven, NL)*

Objectives: (1) To compare a wide range of methods (ELISA, immunoblot, whole cell, recombinant) for detecting anti-*Borrelia* antibodies (2) To investigate the influence of the choice of assay on the result in a two-tier testing algoritm.
Methods: 92 serum samples from clinically well defined patients were tested in eight ELISA systems. Samples were tested in four assays based on whole cell antigens (Enzygnost, Moran, VIDAS, Virion/Serion), two assays with whole cell antigens supplemented with VlsE (Euroimmun, Virotech) and two assays using recombinant proteins (Immunetics, Mikrogen). A subset of samples was tested in five immunoblots. One whole cell blot (home-made), one whole cell blot supplemented with VlsE (Virablot) and three recombinant blots (Euroimmun, Mikrogen, Virotech).
Results: The number of positive ELISA results in the group of patients suspected for *Borrelia* ranged from 34% (Moran) to 61% (Virotech), depending on the ELISA assay used. The specificity of the ELISA's differed widely. The percentage of positives in samples from syphilis patients and patients with recent M pneumoniae infection ranged from 0% (Immunetics) to 38% (Virotech). Reactivity in healthy controls was low to absent in all assays.
Comparison of the immunoblots yielded large differences in inter-test agreement and showed at best a moderate agreement between tests. Kappa-values for IgM ranged from 0.02 (Virablot vs Euroimmun) to 0.50 (home-made vs Mikrogen). Agreement on the presence of IgG anti-*Borrelia* was better with kappa-values between 0.37 (home-made vs Euroimmun) and 0.81 (home-made vs Mikrogen). There was only very limited agreement on the presence of specific immunoreactive bands. Even between recombinant blots there was substantial disagreement. Some immunoblots gave positive results in samples that had been tested negative by all ELISAs.
The percentage of positive blots, following a positive ELISA result depended heavily on the choice of ELISA-immunoblot combination and ranged from 60–100%. In only 76% of the samples all 40 ELISA-immunoblot combinations yielded the same result (either positive or negative).
Conclusion: ELISA's and immunoblots for detecting anti-*Borrelia* antibodies have widely divergent sensitivity and specificity. Immunoblots for detecting IgM show only very limited agreement. The choice of ELISA-immunoblot combination severely influences the number of

positive results making exchange of test-results between laboratories with different methodologies hazardous.

P2085 Spectrum of clinical forms and seasonal distribution of Lyme borreliosis in children

P. Mikolášek, L. Homola, L. Krbková, J. Pavelka, D. Bartošová (Brno, CZ)*

Objectives: The aim of the study was to identify clinical forms and the course of Lyme borreliosis (LB) with in relation to the season, gender and age of children during the period. of 28 months.
Methods: 453 children were examined for dermatoborreliosis in out patient clinic or they were admitted for neurologic and joint involvement of LB from May 2007 to September 2009. Children with early localized borreliosis were diagnosed clinically. Children with disseminated disease fullfilled EUCALB criteria.
Results: 453 children (55% boys / 45% girls) with LB represent 4.1% of all reported cases during the same period in the Czech Republic, this predomination is opposite to adults in Europe. Cutaneous manifestations were clinically characterized by erythema migrans (EM), erythema migrans multiple (EMM), and borrelial lymphocytoma (BL), all together 76.5%. A prevalence of dermatoborreliosis was detected in the age group seven months to eight years in contrast to Lyme arthritis, which was extremely rare. After the ten years of age skin and joint manifestations were diagnosed equally. Neurologic manifestations (n = 61) were the second largest group 13.5%. The joint involvement (n = 45) was diagnosed in 9.9% of children. There were only four cases of LB in patients of age under one year (EM twice, EMM twice). During 3 following years, the most children 21.4% developed their clinical features in July. In cases with BL two seasonal peaks were seen, from June to August (38%) and from October to December (38%).
Conclusion: 252 children (55.6%) have clinical symptomes of EM. 95 children (21%) suffered from LB disseminated cutaneous stage. 31 children (6.8%) were seen with borrelial lymphocytoma, which is typical for children age.
No suspected diagnoses of acrodermatitis chronica athrophicans was proven during the evaluated period. 16 children have to be admitted for prolonged course of Lyme arthritis, whereas 29 were treated orally. The outcome of neuroborreliosis in all children was excellent. Coinfection with tick-borne encephalitis was confirmed in two children.

P2086 Evaluation of two automated tests on the Vidas instrument to detect anti-Lyme disease antibodies IgG and IgM in human serum, plasma and CSF

G. Bouchard, O. Peter, A. Bonnet-Pierroz, R. Vonlanthen, P. Cosin, N. Ammour, A.M. Brevet, P. Broquedis, N. Dehainault, C. Perret, B. Incaurgarat (Marcy l'Etoile, FR; Sion, CH)*

Objectives: VIDAS Lyme IgG and VIDAS Lyme IgM are two automated ELISA tests for the qualitative detection of IgG or IgM antibodies against *Borrelia burgdorferi* sensu lato *(B. afzelii,* garnii, sensu stricto) in human serum or plasma. VIDAS Lyme IgG can also detect IgG in cerebrospinal fluid. Analytical and clinical performance were assessed.
Methods: Specific recombinant proteins are used on the ELISA solid phase to recognize and capture serum antibodies. The precision study was performed according to the CLSI document EP5-A2 with a sample around the cut-off. Cross reactivities with Spirocheatae bacteria, EBV, SLE, HSV, CMV and non-specific antibodies RF, ANA, HAMA were evaluated.
Results: The total precision % CV observed was <10% for a sample around the cut-off. Cross-reactions observed with Spirocheatae bacteria (*Treponema*, *Leptospira*) and EBV were <4% with VIDAS Lyme IgG test, and <12% with VIDAS Lyme IgM. No significant cross reactivity was observed for FR, ANA, HAMA, SLE, HSV, and CMV.
About 300 human serum samples were evaluated with the VIDAS Lyme IgG and Lyme IgM assays. All sera were from patients with well-established clinical status of early Lyme borreliosis (LB) or early disseminated and late LB. The VIDAS Lyme IgG assay showed a sensitivity of 60% and the VIDAS Lyme IgM assay showed a sensitivity of 72% in early LB (N = 60). When both assays were combined, the sensitivity reached 82% on a selection of sera, including seroconversions. In late LB, 79 out of 81 serum samples were found positive with VIDAS Lyme IgG (2 seronegative Lyme arthritis). Preliminary assays for IgG detection in CSF were performed on 12 samples with intrathecal antibody production (IAP) and 16 without IAP, gave expected results. Specificity for both IgG and IgM assays was assessed using sera from patients with EBV, HAV, syphilis, as well as autoimmune disease, (N = 130). Sera from 6 patients were IgG positive (4.5%) and 20 were IgM positive (15.3%).
Conclusion: The VIDAS Lyme IgG assay, without equivocal status results, showed good sensitivity and specificity and a rapid time to result (27′). The preliminary testing on CSF, performed in the same manner as serum samples, appears promising. The VIDAS Lyme IgM assay, with equivocal status results, showed good sensitivity and moderated specificity. Using specific recombinant proteins, the VIDAS Lyme IgG and IgM assays provide good detection of IgG and IgM allowing precise and accurate assessment of disease status.

P2087 Recommendations for diagnosis and treatment of Lyme borreliosis: a comparison of guidelines and consensus papers from specialist societies and expert groups in Europe and North America

S. O'Connell on behalf of the European Union Concerted Action on Lyme Borreliosis (EUCALB)*

Objective and Background: The European Union Concerted Action on Lyme Borreliosis (EUCALB) initiative promotes Lyme borreliosis (LB) research and evidence-based clinical practice through multi-disciplinary collaborations and a highly-regarded, frequently updated website. Its clinical case definitions for LB were published in 1997 and an updated version is to be published. EUCALB's current programme includes a review of diagnosis and treatment recommendations in Europe, and the evidence on which they are based.
The Infectious Diseases Society of America published updated guidelines for LB in 2006. There has since been considerable public dispute internationally regarding LB diagnostic criteria and treatment duration, particularly for patients with persistent symptoms following standard treatment. Some patient support groups and a minority of physicians in North America and Europe are very active in promoting nonspecific diagnostic criteria and prolonged or multiple repeated antibiotic courses. There is significant evidence that some patients have been seriously harmed by these practices, prompting this EUCALB review.
Method: EUCALB participants collated diagnostic and treatment guidelines prepared independently by European specialist societies and expert groups. Guidelines, including those from the Czech Republic, Denmark, Finland, France, Germany, the Netherlands, Norway, Poland, Slovenia, Sweden and Switzerland were evaluated and compared with respect to diagnostic criteria and treatment recommendations (antibiotic agents, dosages and durations) for erythema migrans, neuroborreliosis, Lyme arthritis and persisting symptoms. They were also compared to those of the IDSA and the American Academy of Neurology.
Results: Recommendations and evidence bases of all of the guidelines will be tabulated to permit easy detailed comparison. There are great similarities in diagnostic criteria and antibiotic choice, with some minor differences in dosing and treatment duration, mainly depending on clinical indication. Doxycycline and amoxicillin are the most frequently recommended oral antibiotics; ceftriaxone is the most commonly recommended parenteral agent. The most common treatment durations range from 10–30 days. Notably, none of these guidelines recommends very prolonged treatment courses for patients with persisting symptoms.
Conclusion: There is overall very good concordance between these independently developed, evidence-based European and American guidelines.

P2088 **Preferential reactivity towards *Borrelia afzelii* in the sera of patients with Lyme borreliosis from the north-east of Poland**

S. Grygorczuk, O. Péter, J. Zajkowska, M. Kondrusik, A. Moniuszko, S. Pancewicz (Bialystok, PL; Sion, CH)*

Objective: Several *Borrelia burgdorferi* sensu lato *(B. burgdorferi* s.l.) genospecies act as causative agents of Lyme borreliosis (LB) in Europe, with apparent differences in their prevalence between countries. Clinical form of a disseminated LB correlates with a genospecies, so the natural course of the disease may vary in different areas. Neuroborreliosis (NB) is more often associated with *Borrelia garinii*, chronic cutaneous lesion (acrodermatitis chronica atrophicans, ACA) with *Borrelia afzelii* and Lyme arthritis (LA) with *Borrelia burgdorferi* sensu stricto *(B. burgdorferi* ss). In the north-east of Poland late Lyme borreliosis presents as NB or ACA. Osteoarticular symptoms are frequent, but usually mild and unspecific, while typical Lyme arthritis is rare. Different genospecies could possibly co-exist in the area, but their prevalence has not been studied so far.
Methods: From the Podlaskie province (north-eastern Poland), a group of 22 patients was selected with a positive serology and symptoms compatible with disseminated Lyme borreliosis lasting for >6 weeks (>6 months in 21), including 7 patients with NB, 10 with osteoarticular symptoms (Lyme arthritis, LA), 3 with ACA and 2 with mixed symptoms (LA + NB and LA + ACA). Of patients classified as LA, 3 had mild arthritis while the others presented with chronic arthralgia and myalgia. Serum samples were serotyped with a previously validated immunoblot assay, able to detect preferential reactivity towards one of the four *B. burgdorferi* s.l. genospecies *(B. afzelii, B. burgdorferi* ss, *B. garinii, B. valaisiana)* in patients with long-lasting active infection.
Results: The preferential reactivity towards *B. afzelii* was found in 19 patients irrespective of the clinical form of LB. The reactivity towards *B. garinii* prevailed in a single patient with LA. No patient had preferential reactivity towards *B. burgdorferi* ss or *B. valaisiana.* In 2 patients with NB no preferential reactivity could be determined.
Conclusions: *B. afzelii* seems to be a main aetiological agent of disseminated Lyme borreliosis in the north-east of Poland. In this area it is responsible for all clinical forms of late Lyme borreliosis, including neuroborreliosis and osteoarticular symptoms. The unspecific clinical course of the osteoarticular LB in the study area may be related to the lack or scarcity of *B. burgdorferi* sensu stricto.

Toxoplasmosis

P2089 **A twelve-month retrospective audit of reactive *Toxoplasma gondii* serology in pregnant women in Ireland**

K. Burns, V. McDonnell, D. Murphy, S. Collum, R. Twohig, J. Connell, W. Hall (Dublin, IE)*

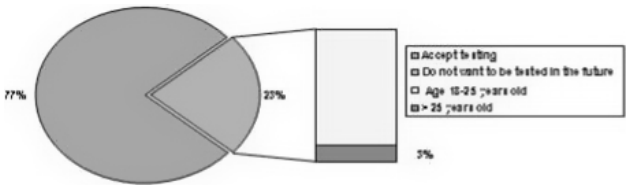
Objectives: Primary maternal infection with *Toxoplasma gondii* may result in transplacental transmission of infection to the fetus. Prompt and accurate recognition of primary maternal toxoplasma infection enables initiation of effective anti-toxoplasma chemotherapy. Interpretation of *Toxoplasma* serology results can prove challenging. Patients may have detectable IgM levels to *T. gondii* which persist for months to years following primary infection and false positive IgM results are not uncommon. Referral to a reference laboratory for further confirmation of reactive *T. gondii* serology is recommended in pregnant women. This audit was carried out to determine the indication for performing *T. gondii* serology on women of childbearing age and to determine the frequency and significance of reactive serology results.
Methods: The results and clinical information of 2,497 samples tested for antibodies to *T. gondii,* received from females aged fourteen to fifty years of age from January 1st to December 31st 2007 were reviewed.
Results: Of 540 women of childbearing age with reactive *T. gondii* serology, 234 (43%) were pregnant or had been recently pregnant at the time of testing. Routine antenatal screening and investigation of intrauterine death were the testing indications for 82% of reactive *T. gondii* specimens.
There was no serological evidence of recent *T. gondii* infection in 218 (93%) of pregnant women with reactive *T. gondii* serology. Twelve pregnant women had a first positive IgM to *T. gondii.* High IgG avidity results were recorded in nine of ten IgM positive specimens, indicating that the *T. gondii* infection was not acquired in the preceding three months.
One sample had low IgG avidity, confirmed at the *Toxoplasma* Reference Unit (TRU) suggestive of a recently acquired *T. gondii* infection. The indication for performing *T. gondii* serology in that case was presence of fetal ventriculomegaly on ultrasound examination.
Conclusions: It was possible to confirm recent acquisition of primary *T. gondii* infection in only one of 234 pregnant women with reactive serology for *T. gondii* in 2007. The results of this audit suggest that testing for *T. gondii* as part of routine antenatal screening in the absence of a clinical indication should be reconsidered owing to the implications of detection of a positive IgM result for a pregnant woman.
Routine testing for *T. gondii* in the investigation of intrauterine death should also be reevaluated owing to the low yield of positive results.

P2090 **Testing for toxoplasmic immunity – are we prepared?**

C. Costache, L.M. Junie, Z. Coroiu on behalf of the ESGP*

The onset of screening programs for toxoplasmic infection in women of fertile age was preceded by studies for the appreciation of contamination level in the area in all countries unrolling this type of follow-up. Lobby among women of fertile age for performing this analysis is carried out in our country by doctors as there is no national program for this.
Methods: Prospective epidemiologic (questionnaire) and serologic study over 280 mothers selected from obstetrics departments, during 2006–2009, (15.896 births). Inclusion criterion was birth of a child with abnormalities clinically possible to be of toxoplasmic origin.
Results: Ig G positive serology in mothers was higher in 21–30 years old interval (34.3%). Relation between positive/negative serology and living area showed statistic significant association ($p < 0.05$) between positive serology for *T. gondii* and rural area. The abortion and still birth rate were 32% in this group. Of all 280 mothers which filled the questionnaire, 77.14%, CI=[66.7%-87.3%] of them express their availability to participate in future testing within a national free screening program, while 22.85%, CI=[12.9%-33.4%] of them do not want to participate, 20%, CI=[11.38%-31.27%] of them being within 18–25 years of age interval. Only 12.85%, IC = [6.1%-23.3%] of mothers have had a test for *T. gondii* infection while 87.14%, IC= [76.7%-93.9%] of them were never checked for this infection. Confirmation of congenital toxoplasmosis diagnostic in 8.6% of neonates was due to Ig. A testing while specific antitoxoplasmic Ig.M was negative.
Conclusions:

1. There is statistic significant association between rural area and contamination with *T. gondii* during fertile age.
2. A small number of women were followed for the appreciation of antitoxoplasmic immunity, a statistically larger percentage did not undergo this procedure ever, even if they had given birth to children with congenital abnormalities or have had pregnancy loss (abortion) in antecedent.
3. A quarter of mothers do not want to be tested, a fifth of this group being very young females (18–25 years of age).
4. Ig.A testing in serologic screening for congenital toxoplasmosis of neonates is important due to Ig.M negative serology.



Percentage of women according to their attitude to future testing.

P2091 Type II *Toxoplasma gondii* strains from human congenital toxoplasmosis: differential phenotypic characteristics *in vitro*

M.P. Brenier-Pinchart, R.L. Bertini, D. Maubon, H. Pelloux (Grenoble, FR)*

Objective: More than 80% of human congenital toxoplasmosis (CT) is attributable to *Toxoplasma* strains belonging to type II lineage in France, while three main lineages have been described in Europe. Our original hypothesis is that type II strains can present specific phenotypic characteristics that differ from one isolate to another which could partially explain the varying degrees of severity observed during toxoplasmosis. The objective was to study the *in vitro* phenotype of type II strains isolated from different clinical forms of CT.
Methods: Four *T. gondii* strains of type II from the French Biological *Toxoplasma* Resource Centre (BRC *Toxoplasma*) originated from different forms of CT and two laboratory reference strains were investigated. Three key steps of the parasite life cycle were compared in human fibroblast model (HFF): i) cell invasion was determined by microscopy 1 h after infection, ii) *Toxoplasma* replication by measuring 3H-uracil uptake 24 h after infection and iii) cyst formation was evaluated by the number of cysts counted after immunofluorescence staining using the cyst-specific BSR4 antibody 7 days after infection (±IFN-γ).
Results: *In vitro*, the invasion rate was significantly higher for one strain isolated from symptomatic CT (retinochoroiditis) than for the reference type II strain (PRU) and for the three other strains. The parasite multiplication level was not significantly different among the four strains compared to this same reference strain. Interestingly, the capacity to form cysts was not equivalent among these strains: the two strains responsible for asymptomatic infection formed significantly fewer cysts than the two strains isolated from symptomatic toxoplasmosis (table 1). The susceptibility of the four strains to IFN-γ did not differ.
Conclusions: The virulence of the three main clonal lineages of *T. gondii* has been described in the mouse model but a close relationship between the genetic structure and the human clinical expression has not yet been demonstrated. In this study, we show for the first time that the phenotypic patterns of type II strains differ in a human *in vitro* model: strains that have led to an asymptomatic infection form fewer cysts than type II strains isolated from symptomatic infection. This result underlines the interest to identify the genetic and/or epigenetic variations responsible for these phenotypic differences and the predictive markers of symptomatic and serious congenital infections.

Table I. Characteristics of the type II *T. gondii* strains

Strain	Genotype	Date of maternal infection[a]	Clinical form of congenital toxoplasmosis (CT)	Number of cysts (Standard Error)
BRC TgH20018A	II	36 WA	Symptomatic CT, retinochoroiditis	25±12.6
BRC TgH20002A	II	34 WA	Symptomatic CT, encephalitis, retinochoroiditis, medical abortion	26.6±18.7
BRC TgH38034A	II	36 WA	Asymptomatic CT	7.4±1.1*
BRC TgH20017A	II	36 WA	Asymptomatic CT	16±10.3*
PRU strain	II	/	Laboratory reference strain	27±12.5
RH strain	I	/	Laboratory reference strain	/

[a] Weeks of amenorrhoea (WA); *$p < 0.05$ from type II PRU based on analysis of variance (ANOVA).

P2092 Congenital toxoplasmosis in France in 2007: first results from a national surveillance system

I. Villena, C. Delmas, T. Ancelle, H. Pelloux on behalf of the National Reference Centre on Toxoplasmosis*

Objectives: Toxoplasmosis is generally asymptomatic when infection is acquired in immunocompetent humans. However, transplacental transmission of *Toxoplasma gondii* may lead to severe congenital infection. The national prevention programme for congenital toxoplasmosis in France has been in place since 1978; however no reliable data was available regarding the annual number of cases or the severity of infection. In 2006, French National Institute of Public Health Surveillance and the National Reference Centre (NRC) for Toxoplasmosis recommended a laboratory-based surveillance system, in order to estimate the perinatal burden of this infection and follow the impact of the national prevention programme. A surveillance system ("Toxosurv" network with laboratories carrying out the diagnosis) was implemented in 2007.
Methods: A case of congenital toxoplasmosis was defined as a foetus, a newborn or a child less than one year in whom was detected the presence of at least one of the following elements: *T. gondii* in body tissues or fluids, synthesis of specific IgM or IgA antibodies, synthesis of specific IgG antibodies within the first 12 months of life, persistently positive IgG until one year of age. Cases diagnosed between the 1st January and the 31st December 2007 in France were declared to the NRC among the process of notification described in 2008.
Results: During 2007, 31 laboratories declared at least one congenital case to the surveillance system with a total of 272 cases notified. Eleven terminations of pregnancy were reported. Among live born infants, 206 were asymptomatic and 28 were symptomatic. Since 818 700 births occurred in 2007, the overall prevalence of congenital toxoplasmosis observed was 3.3 per 10 000 births, the rate of symptomatic congenital toxoplasmosis could be estimated at 0.34 cases per 10000 births.
Conclusion: The surveillance system for congenital toxoplasmosis cases based on the "Toxosurv" network appears to be effective and provides for the first time reliable French data on congenital toxoplasmosis. This surveillance needs to continue to appreciate the overall prevalence of congenital toxoplasmosis. The toxoplasmosis seroprevalence among women of childbearing age is regularly estimated in France through National Perinatal Surveys based on cross-sectional surveys of birth on a national level during a week. With these two indicators it will be possible to perform economical analyses for the development of different strategies of screening.

P2093 Decreased seroprevalence and age-specific risk factors for toxoplasmosis in the Netherlands between 1995–1996 and 2006–2007

L.M. Kortbeek, A. Hofhuis, C. Nijhuis, Y. van Duynhoven, L. Mollema, F. van der Klis, W. van Pelt (Bilthoven, NL)*

Objective: To estimate the toxoplasmosis seroprevalence and determine risk factors for toxoplasmosis in the Netherlands.
Methods: Sera from a nation wide serum bank of the general population of the Netherlands were tested with an in house *Toxoplasma* IgG ELISA, using the same methods as in a previous study performed in 1995/1996 (ref Kortbeek 2004). Additionally, participants returned questionnaires including questions on food habits and contacts with young cats.
Results: Sera and data of 5541 participants were available for analysis. The overall seroprevalence showed a marked decrease to 26.0%, as compared to 40.5% in 1995/1996. Among women of reproductive age, the seroprevalence decreased from 35.2% in 1995/1996 to 18.5% in 2006/2007. In participants aged 20 and older, *Toxoplasma* seropositivity was associated with living in the Northwest, living in urban areas, low educational level, consumption of raw pork, keeping a cat, and not having professional contact with clients or patients. For younger participants, risk factors were keeping sheep or cattle, consumption of raw unwashed vegetables and putting sand in the mouth.
Conclusion: The seroprevalence of toxoplasmosis decreased in the Netherlands. The majority of pregnant women is susceptible to primary infection with *Toxoplasma gondii* and therefore of congenital toxoplasmosis. There is a regional difference in seroprevalence in adults that cannot be explained. Consumption of raw or undercooked pork meat is a risk factor as is contact with cats, but not with young cats.

Reference(s)

[1] Kortbeek LM, De Melker HE, Veldhuijzen IK, Conyn-Van Spaendonck MA. Population-based *Toxoplasma* seroprevalence study in The Netherlands. Epidemiol Infect. 2004; 132(5): 839–45.

P2094 ***In vitro* effects of ivermectin and sulphadiazine on *Toxoplasma gondii***

M. Bilgin, T. Yildirim, M. Hokelek (Samsun, Amasya, TR)*

Objectives: Toxoplasmosis is an infection disease caused by the obligate intracellular parasite *Toxoplasma gondii* occur worldwide. Currently, the most effective treatment for both congenital and acquired toxoplasmosis is the combination of pyrimethamine-sulphadiazine. The aim of this study was to investigate *in vitro* activities of ivermectin and sulphadiazine, against *T. gondii* RH strain on Hep-2 epithelial cells.
Methods: In this study, Hep-2 cells in tissue culture plates were infected with the tachyzoites isolated from peritoneal fluid of mice infected with *T. gondii.* Subsequently, ivermectin and sulphadiazine were added and their inhibitory activities were assessed after 24, 48 and 72 hours incubations using inverted microscopy and ELISA. The toxicities of drugs for Hep-2 cells were also determined with NRUD assay.
Results: Ivermectin was found to have significant inhibitory activity against tachyzoites after 48 h of incubation at the concentration of 5 and 2.5 microgram/ml ($P < 0.01$) and higher concentrations. The 50% inhibitory concentrations (IC50) of ivermectin and sulphadiazine were found 0.27 microgram/ml and 7.3 microgram/ml after 48 h of exposure, respectively. None of the concentrations tested for each drugs demonstrated toxicity to Hep-2 cells after 72 h of incubation.
Conclusion: Our results indicated that ivermectin significantly inhibited replication of the tachyzoites of *T. gondii* RH strain. Further studies are needed to combination of ivermectin with other drugs and animal model for toxoplasmosis treatment.

Sexually-transmitted and other genito-urinary infections

P2095 **Intraprostatic *Trichomonas vaginalis* might be implicated in the inflammatory pathogenesis of benign prostatic hyperplasia**

D. Mitteregger, S. Aberle, A. Makristathis, J. Walochnik, W. Brozek, M. Marberger, G. Kramer (Vienna, AT)*

Objectives: Benign prostatic hyperplasia (BPH) is a progressive disease originating as small nodular proliferations as early as the fourth decade. Forty percent of men in their 70s have developed nodules of large mass producing obstructive symptoms which may progress to acute urinary retention requiring prostatic surgery. Chronic tissue inflammation, invariably present in BPH tissue, has been causally linked to prostate growth and disease progression. The etiology of chronic inflammation is, despite suggestions of autoimmunity, currently unknown. In search of a possible infectious etiology, we investigated the presence of microorganisms in BPH tissue.
Methods: Transurethrally resected samples from 86 patients (mean age 69±8.3 years) were investigated for *Trichomonas vaginalis* by PCR and culture. Proportions of the samples were simultaneously examined for bacteria by culture and broad-spectrum PCR, for fungi by culture, broad-spectrum and multiplex PCR, and for viruses by organism-specific PCR assays. To exclude urinary infection, urine from all patients was investigated for bacteria and fungi by culture. Type and density of leukocytic tissue infiltration was assessed by immunohistochemistry of cryo-sectioned BPH tissue. Several clinical parameters and the characteristics of the infiltrate were compared with microbiological results.
Results: *Trichomonas vaginalis* was detected by PCR in 29/86 (33.7%) tissue samples. Various bacteria were found in 8/55 (14.6%) tissue samples, while fungi were not detected (0/55). Parvovirus B19 and BK virus were identified in 1/17 tissue samples, respectively, and JC virus in 2/17. *Trichomonas vaginalis* in BPH tissue correlated with the presence of a chronic B-lymphocytic infiltrate ($p < 0.01$) and elevated blood monocyte counts ($p < 0.01$).
Conclusions: The bacterial detections in BPH tissue may represent true infections but it remains difficult to interpret these findings since different species were involved. The presence of the identified viral species does not seem to be relevant compared with their general seroprevalence. However, the unexpectedly high detection rate of *Trichomonas vaginalis* in BPH tissue correlating with local and systemic signs of inflammation suggests an implication of that organism in the pathogenesis of BPH. The high prevalence of *Trichomonas vaginalis* in this elderly study population might point towards a yet unrecognized chronic course of male trichomoniasis.
Authors S. Aberle, A. Makristathis and J. Walochnik contributed equally.

P2096 **Congenital syphilis surveillance**

A. Marangoni, A. Moroni, E. Tridapalli, M.G. Capretti, G. Faldella, S. Accardo, R. Cevenini (Bologna, IT)*

Objectives: Congenital syphilis (CS) is mainly a consequence of the lack of antenatal care and control of sexually transmitted infections. The bedrock of the prevention of CS is syphilis diagnosis by serological screening during pregnancy. Current Italian guidelines suggest that all the pregnant women should be tested in the first trimester. Due to the frequently absence of specific signs of infection at birth, Laboratory tests are often the only method for a correct CS diagnosis.
The aim of this study was to evaluate the usefulness of *T. pallidum* IgM Western Blot (WB) and Polymerase Chain Reaction (PCR) on cerebrospinal fluid (CSF) as an aid in the diagnosis of CS during a prospective surveillance study carried out at St. Orsola Hospital in Bologna, Italy, from January 2008 through March 2009.
Methods: All pregnant women during pregnancy and at delivery were screened for syphilis by ARCHITECT® Syphilis TP, Abbott. Positive samples were further analysed by TPHA and RPR tests, Radim. An in-house Western Blot (WB) was also performed.
Infants born to syphilis seropositive mothers were enrolled in a prospective follow up. At birth, tests were performed (including IgM WB). Infants with positive RPR tests at birth born to mothers not adequately treated received also a long bone radiograph as well as a complete CSF analysis, including VDRL (Siemens Healthcare Diagnostics) and PCR testing.
All seroreactive infants received careful follow up examinations and serological testing at 0, 3, 6, 9, 12 months or until the tests became negative.
Results: In this study, positive syphilis serology was noted in 21 pregnant women delivering in our hospital. Four women had never been adequately treated, and two out these four women gave birth to infected newborns. Both the infants had positive IgM WB results on serum samples and also positive PCR and VDRL results on CSF specimens. One baby had characteristic long bone lesions at X-ray examination and bullous eruptions on palms and soles.
Conclusions: These observations confirmed that antenatal syphilis screening facilitates treatment during pregnancy and offsets vertical transmission; moreover, the use of IgM WB and careful CSF examination allowed the identification and treatment of high risk newborns.

P2097 **Large proportion of patients with positive darkfield microscopy samples also have positive syphilis serology**

E. Muldoon, S. Squance, M. Kelleher, G. Courtney, F. Lyons, C. Bergin, F. Mulcahy, B. Crowley (Dublin, IE)*

Background: The diagnosis of primary syphilis can be problematic. Darkfield microscopy requires expertise both in obtaining the specimen for analysis and in making the diagnosis. Sensitivity of darkfield microscopy approaches 80%, however it may not be available in all centres, necessitating the reliance on other methods of diagnosis. There has been a recent increase in syphilis diagnoses in our centre.
Methods: All patients who had a specimen taken for darkfield microscopy from 2007 to March 2009 were identified. Data was collected on the site the specimen was obtained from, the result of the examination by darkfield microscopy and the results of syphilis serology taken on the same day and any subsequent samples sent for syphilis serology. All

statistical data was performed using SPSS 17.0 commercially available software.
Results: 66 samples were collected for darkfield microscopy from patients with clinically suspected primary syphilis. 4 were either incorrectly labelled or unsuitable for analysis and were excluded. Of the remaining 62 samples, 58 (93.5%) were taken from men and the mean age of patients was 36.5 year (range 19–63, median 35.5 years). 14 (22.6%) samples were positive. Of the 14 patients that had a positive darkfield sample, 12 (85.7%) also had a positive EIA on the same day, all of whom had a positive rapid plasma reagin (RPR) titre. All 12 of these patients had a positive syphilis immunoglobulin M (IgM). Of the 2 patients with negative syphilis serology, 1 patient had a positive RPR and IgM 4 weeks after initial presentation and the other patient remained seronegative.
Conclusion: This series demonstrates high degree of correlation between a positive darkfield microscopy examination and syphilis serology. This is in contrast to previous reports that syphilis serology may several weeks to months to become positive after appearance of a primary chancre. This may give solace to physicians who do not have readily available darkfield microscopy facilities and who rely in part on syphilis serology in the diagnosis of primary syphilis.

P2098 Community-acquired *Staphylococcus saprophyticus* urinary tract infections

C. Marne, N. Baez, A. Borque, M. Arias, A. Servera, M.J. Revillo (Zaragoza, ES)*

Objectives: *Staphylococcus saprophyticus* is a coagulase-negative species with urinary tract pathogenic role, causing cistitis mainly in female patients. It has been associated with rectal, vaginal and urethral colonization, hormonal changes during menstruation, sexual activity, use of spermicides and *Candida* infection. Main virulence factors are extracellular slime production, adherence to urothelial cells and urease production. The aim of the study is the update of microbiological and epidemiological characteristics of *Staphylococcus saprophyticus* urinary tract infections.
Methods: Over an eleven year-period (1998–2008), a total of 333524 urine specimens from both hospitalized and outpatients from an area of circa 400000 inhabitants, were cultured by standard methods. Identification and antimicrobial susceptibility were performed by authomated microdilution system.
Results: In our study 255962 (76.7%) urocultures were negative or contaminated and 77562 (23.3%) were positive for bacteria or yeasts. *S. saprophyticus* was isolated from 368 (0.5%) positive urocultures corresponding to 356 patients. Represent 16.4% of all our coagulase-negative staphylococci urine isolates. Sex distribution was 92.4% women (range 8–88 years) and 7.6% men (range 10–89 years). In both sexes 11 patients were under 15 years.
Only one patient had recurrent infection by this species with two episodes.
Autumn was the seasonal period with more cases.
Percentages of susceptibility to antibiotics used for uncomplicated urinary infection treatment in outpatients was: clotrimazole 99%, ciprofloxacin 95%, nitrofurantoin 99%, fosfomycin 38%, cefuroxime 64% and amoxicillin–clavulanic acid 56%.
Conclusions:
1. *S. saprophyticus* was isolated almost exclusively from female patients and half of them were under 30 years.
2. A small percentage were men and and most over 50 years.
3. Infection by this species is exceptional in pedriatric patients.
4. Frequently *S. saprophyticus* clumping factor card test results cannot be interpreted due to aglutination with both positive and negative reagents.
5. Infections can be considered community-acquired.
6. βlactams and fosfomycin should not be used empirically.
7. Quinolones and clotrimazole showed good activity.
8. Due to the high effectiveness of nitrofurantoin against both *S. saprophyticus* and *E. coli*, it must be considered in empirical treatment for uncomplicated urinary tract infections in young women.

P2099 Current state of diagnostics and management of bacterial sexually transmitted diseases in Russia: results of multicentre study

Y.A. Belkova, O.Y. Aleksandrova, B.V. Berezhanskiy, E.N. Bochanova, V.V. Chebotarev, I.L. Chechula, E.P. Cigankova, A. Dekhnich, V.V. Evstafiev, A.V. Grinev, E.V. Eliseeva, E.A. Ortenberg, A. Savicheva, S.V. Sekhin, I.A. Toropova, R. Kozlov (Smolensk, Ekaterinburg, Moscow, Krasnoyarsk, Stavropol, Krasnodar, Vladivostok, Tyumen, St. Petersburg, Yakutsk, RU)*

Objectives: To reveal the main problems in current practice of management of bacterial sexually transmitted diseases (STDs) in Russia.
Methods: A multicenter retrospective study was conducted in 10 cities of Russia. Clinical records of patients treated for bacterial STDs during one year period were collected.
Results: The data on 1250 patients (61% male, 39% female, mean age 28.8±9.2) with early syphilis (n = 341), uncomplicated gonococcal (n = 309), chlamydial (n = 310), mycoplasmic (n = 137) and ureaplasmic (n = 153) infection were analysed. We discovered wide use of outdated low informative methods in diagnostics of STDs which can result in considerable underestimation of its prevalence. Thus the main methods to diagnose syphilis were microprecipitation (81.5%) and complement fixation tests (13.3%). Furthermore in 81% of patients syphilis was diagnosed by microprecipitation alone and in 12.9% by combination of these tests. The most common method to diagnose gonococcal infection was methylene blue stain and/or Gram's stain microscopy (82.5%). For 80.7% of patients it was the only test performed. Culture was done in 18.7% of patients with susceptibility testing only in 2.3% of cases. Polimerase chain reaction and culture test were used to diagnose infections caused by intracellular pathogens only in 37.1% and 14.8% of cases, whereas low informative microscopy, direct immunofluorescence test and ELISA were used unjustifiably often (21.5%, 13.4% and 13.3%). The most common treatment problems were administration of inappropriate antibacterial agents and higher then necessary course doses of antibiotics. Thus in about 30% of patients gonorrhea was treated by inappropriate agents such as tetracyclines (7.2%), aminoglycosides (6.6%), fluoroquinolones (5.7%), macrolids (5.4%) and penicillins (4.2%). Although the majority of syphilis cases were treated by penicillins, 26.9% of patients received ceftriaxone. Chlamydial, mycoplasmic and ureaplasmic infections were treated by macrolids (56.3%), tetracyclines (28.6%) and fluoroquinolones (15.1%), but not all agents were chosen appropriately. Administered therapy went in conflict with national guidelines in 28.2% of patients, international guidelines – in 24.2%. Among correctly chosen agents only 24% and 11%, respectively, were used in recommended course doses (higher dose – 69.1% and 83.7%, lower – 6.9% and 5.3%).
Conclusion: The current practice of STDs diagnostics and management in Russia is in need of revision and correction.

P2100 *Chlamydia trachomatis* proctitis in Italian men who have sex with men: report from an STD unit

R. Beretta, M. Fasolo, F. Mazza, A. Lombardi, G. Orlando (Milan, IT)*

Objective: the burden of sexually transmitted infection (STIs) is increasing all around the world; increasing rates of Syphilis, Gonorrhea, sexually transmitted HAV, HSV and *Chlamydia trachomatis* infections including proctitis (CTP) are observed in high risk patients in our unit. Here we describe a CTP epidemic among men who have sex with men (MSM) attending the STD unit of L Sacco University Hospital and the correlation with other STIs.
Methods: patients attending the STD unit between Oct 07 and Feb 09 with proctitis were included in this analysis. The diagnosis of CTP was achieved through a real time PCR (*Chlamydia* Tr Q PCR

Alert – Nanogen) on anal swabs. All included pts underwent anoscopy, anal swab for bacteria, HSV nucleic acid amplification (HSV2 Q-PCR Alert AmpliMix), TPPA and RPR for syphilis, HIV test and anal HPV genotyping on liquid based cytology (InnoLipa).
Results: 13 pts aged 36.5y (range 26–45) with symptomatic proctitis/proctocolitis and tenesmus, rectal pain, bleeding, discharge and ulceration or anal fissure on ano/colonscopy, have been observed during a 17 months period. Mean time to CTP diagnosis was 23 days (IQR 15–43 days); in 3 pts the diagnostic delay (3–6 months) was due to a misdiagnosis of Crohn's disease on histology. HIV co-infection was observed in 12 pts (92.3%) most of whom (75%) off-HAART; primary syphilis in 4 pts (33.3%), anal *Mycoplasma hominis* in 1 patient (8.33%). Anal HPV infections (10/10 pts tested) were due to High Risk genotypes (HPV-HR). Multiple HPV infections (from 3 to 6 genotypes) in 5 cases (55.5%) were assessed in 9/10 pts. HPV-52 (55.5%), HPV-51 (44.4%), HPV-16, HPV-35 and HPV-66 (33.3% each) were the most frequently observed genotypes. Anal cytology, performed in 8 pts, showed anal LSIL in 7 patients and HSIL in 1 patient. Histology on anoscopic guided biopsies showed AIN-1 in 3 pts and AIN-2 in 1 patient. In 91.67% of the cases multiple infections ($\geqslant$2 pathogens) have been diagnosed in these pts (infections/pt 3.25) (Fig 1).
Anal CT was treated with doxycycline 100 mg BID for 7 days or azitromycine 1 g/d for 3 days with clinical and microbiological resolution.
Conclusions: among patients attending an STD Unit, MSM are at very high risk of sexually transmitted infections. The high rate of multiple infections reported in this work suggest the need for a complete STI evaluation and for specific counseling programs after a diagnosis of an STI in very high risk persons.

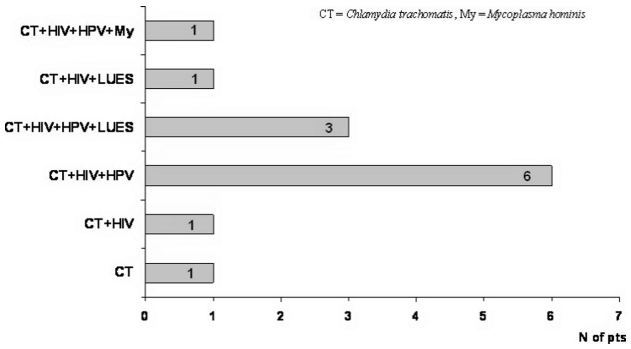


Figure 1. Patients with *Chlamydia trachomatis* proctitis and co-infection with other STIs.

P2101 Prevalence and antibiotic susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in women with vulvovaginitis

S. Baka, I. Tsouma, A. Lykas, A. Panopoulou, I. Patikas, E. Logothetis, E. Kouskouni (Athens, GR)*

Objectives: *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) are members of a unique group of microorganisms that are commonly found in the vaginal flora and have been associated with a plethora of clinical manifestations on the genital system of women. We aimed to study the prevalence and the susceptibility to antibiotics of Uu and Mh isolated from the vaginal secretions in a group of women with vulvovaginitis.
Methods: Vaginal samples from 1902 symptomatic women of reproductive age presenting with signs and symptoms of vulvovaginitis to the Outpatient Clinic of Aretaieion University Hospital from February 2007 to October 2009 were studied. For the isolation and susceptibility testing of both mycoplasmas the commercial kit *Mycoplasma* IST2 (bioMérieux, France) was used. After inoculation onto the respective nutrient medium, the samples were incubated at 36–37ºC for 48 h in aerobic conditions.
Results: Out of the 1902 samples Uu was isolated in 497 (26.1%) samples while Mh in 42 (2.2%), always in association with Uu. Only concentrations of >104 CFU/ml at 48 h were included as positive samples in the study. As for the susceptibility testing to antibiotics, doxycycline and tetracycline displayed the lowest percentages of resistance (0.6% and 1.2%, respectively) as well as intermediate susceptibility (0.2% and 1.4%, respectively) in the isolates studied. Among the macrolides, the same percentages for azithromycin, clarithromycin and erythromycin were 9.6, 11.0 and 12.4, respectively (resistant) and 6.9, 1.8 and 4.4, respectively (intermediate susceptible). In contrast, the genital mycoplasmas tested showed decreased susceptibility to quinolones. Specifically, 33.4% and 6.9% of the mycoplasmas were resistant to ciprofloxacin and ofloxacin, respectively, while the intermediate susceptibility was 50.1 and 43.0%, respectively.
Conclusions: The isolates studied were highly resistant to quinolones, probably due to the increased use in the last years. It is imperative to stop the empirical treatment of genital mycoplasmas and to relay the therapeutic approach on the results of the *in vitro* susceptibility testing since nowadays, with the aid of commercially available systems, it is just a simple laboratory procedure.

P2102 Molecular and serological evidence of the role of *Chlamydia trachomatis* in miscarriage

D. Baud, G. Goy, K. Jaton-Ogay, M. Osterheld, S. Blumer, N. Borel, A. Pospischil, F. Damnon, Y. Vial, G. Greub (Lausanne, Zurich, CH)*

Objectives: Mostly asymptomatic, untreated *Chlamydia trachomatis* infections are responsible for a large proportion of salpingitis, pelvic inflammatory disease, ectopic pregnancy and infertility in women. During pregnancy, *C. trachomatis* is a recognised agent of preterm labour and premature rupture of membranes. However, its role on miscarriage is unclear. We thus analysed patients with or without miscarriage for the presence of *C. trachomatis.*
Methods: Three-hundred heighty six women were prospectively enrolled at the Obstetrical ward of the University Hospital of Lausanne, Switzerland. The "miscarriage" group (n = 125) included women diagnosed with an acute episode of miscarriage. The "control" group (n = 261) included women with an uneventful pregnancy and without any previous history of miscarriage, stillbirth or preterm labour. All sera were tested for IgG and IgA antibodies against *C. trachomatis* with the Ridascreen *Chlamydia* IgG/IgA kit (R-biopharm) according to the manufacturer's instructions. Presence of *C. trachomatis* DNA in vaginal swabs was investigated by real-time PCR targeting the cryptic plasmid, as described previously (Jaton et al., 2006). Placenta were also investigated by histology, and remain to be tested by PCR and immunohistochemistry.
Results: Anti-*C. trachomatis* IgG prevalence was higher in women from the miscarriage group (15.2%) than the controls (7.3%, p = 0.018). This association between miscarriage and anti-*C. trachomatis* IgG remained significant, even after adjustment for age, origin, education and number of sexual partner (odds ratio 2.3, 95% confidence interval 1.1–5.1). Cervico-vginal *C. trachomatis* DNA was more often detected in patients from the miscarriage group (n = 5, 4%) than from the control group (n = 2, 0.7%, p = 0.026). All placentas were analysed for the presence of inflammation. In the chorion, presence of deciduitis was present in 39.5% and 26.2% of patients with and without *C. trachomatis* IgG positive serology (p = 0.081).
Conclusion: In conclusion, our study showed the association between miscarriage and evidence of *C. trachomatis* infection. *C. trachomatis* screening should thus be proposed to all women experiencing a miscarriage, and pre-conceptional screening could be considered to potentially reduce the prevalence of this adverse pregnancy outcome.

P2103 Re-emergence of infectious syphilis among homosexual men and HIV co-infection in Spain, 2003–2008

X. Camino, M. von Wichmann, H. Azkune, M. Goenaga, J. Iribarren, F. Rodriguez-Arrondo, M. Bustinduy, J. Arrizabalaga (San Sebastian, ES)*

Background: An increase in syphilis infections since the mid 1990s has been documented, especially in homosexual men, in different European and North-American cities.
Methods: Retrospectively reviewed all cases of early syphilis diagnosed in a monograph consultation sexually transmitted diseases (STD) between 2003 and 2008. We describe the characteristics of newly diagnosed cases of syphilis.
Results: In that time period, a total of 76 early syphilis: 18 in 2003–2005, 12 in 2006, 22 in 2007 and 24 in 2008. 26 (34.2%) of them agreed with the diagnosis of HIV infection. 70 patients were men and 6 women. The mean age was 36.5 years. About 75% of diagnoses were in homosexual patients and 25% of patients were immigrants. Only 5% of patients were engaged in prostitution. The diagnosis was clinical in 34 patients and all had a score of RPR and FTA-abs/TPHA (+) with quantitative VDRL (+). Predictive factors of HIV coinfection were age >30 years (p: 0.004) and having a HIV positive partner (P: 0.042).
Conclusion: It is seen in the last two years a significant increase in cases of early syphilis, especially in homosexual patients. An important group of them are simultaneously diagnosed early syphilis and HIV. This problem should emphasize prevention campaigns and highlights the need for consultation monograph of STIs.

Brucellosis, Q-fever, tetanus, rickettsiosis

P2104 Sero-epidemiology of Q fever in the Netherlands, 2006–2007, preceding a series of large outbreaks

D.W. Notermans, B. Schimmer, M.G. Harms, J.H. Reimerink, J. Bakker, P.M. Schneeberger, L. Mollema, P. Teunis, W. van Pelt, Y. van Duynhoven (Bilthoven, Den Bosch, NL)*

Objectives: The Netherlands were confronted with a series of large outbreaks of Q fever starting in 2007 and increasing in size with over 2200 reported cases in 2009. Before 2007, Q fever was a rare disease in the country, with on average 17 notified cases annually. Q fever is asymptomatic in the majority of infected persons and might have been missed in symptomatic patients as well before national awareness was aroused by the outbreaks. Seroprevalence of Q fever and risk factors for sero-positivity were investigated in samples taken prior to the outbreaks.
Methods: Serum and a questionnaire were obtained from a representative sample of the Dutch population as part of the 'PIENTER2 project', a population-based study primarily focussed on evaluation of the national vaccination programme, performed in 2006 and early 2007. IgG phase 2 antibodies were measured with an ELISA (Serion). To increase sensitivity, borderline results were considered positive. A subset of ELISA-negative samples was also tested in IFA (Focus Diagnostics). Information on age, ethnic origin and urbanization degree was available for the total study population. Associations of seropositivity with patient characteristics were studied with logistic regression analysis. Seroprevalence was weighted by age and degree of urbanization.
Results: ELISA results were available for 5654 individuals. IgG phase 2 antibodies were detected in 85 samples, of which 47 had borderline levels. In the IFA-tested subset of 504 ELISA-negative samples, 6 (1.2%) had low-level IgG phase 2 titres ranging from 1:32 to 1:128.
Overall weighted seroprevalence based on ELISA results was 1.5% (95% CI 1.2%-1.8%). Seropositivity increased with age from 0.4% (95% CI 0.1–0.7%) in the 0–14 years age group to 2.3% (95% CI 1.4–3.1%) in the 60–79 years age group. Univariately, contact with farm animals and being a Turkish or Moroccan immigrant (1st generation) seem significant risk factors. No significant association was observed for gender, region and degree of urbanisation.
Conclusion: Seroprevalence of Q fever in The Netherlands was very low prior to recent outbreaks. The rate given should be considered a maximum estimate as borderline results were considered positive. Although municipalities included in the study sample were not at the epicentre of the Q fever outbreak, surrounding municipalities did not show a higher seroprevalence, supporting the hypothesis that the Netherlands is confronted with a new emerging Q-fever problem since 2007.

P2105 Decreased health status of Q fever patients one year after the first Dutch outbreak: a case–control study

G.J. Limonard, M.H. Nabuurs-Franssen, J.B. Peters, A. Horrevorts, G. Weers-Pothoff, R. Besselink, J.H. Vercoulen, R. Dekhuijzen, C. Groot (Nijmegen, 's-Hertogenbosch, Herpen, Oss, NL)*

Objectives: Q fever is a zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii*. The two long term complications after primary infection are chronic Q fever in about 1% of patients, and a chronic fatigue syndrome in 10 to 20%. However, the existence of a protracted decreased health status after Q fever remains controversial. The aim of the present study was to determine the health status of the patients of the Q fever outbreak in the Netherlands in 2007, one year after primary infection.
Methods: Health status of the patients from the 2007 Dutch Q fever outbreak was compared to age-, sex- and geographically matched, and Q fever seronegative controls. Health status of both patients and controls was assessed with the Nijmegen Clinical Screening Instrument (NCSI).
Results: A total of 54 Q fever patients from the 2007 Herpen outbreak provided thirty-four age and sex-matched controls from the same neighbourhood. Eleven controls had positive Q fever serology and were excluded. There were no differences in gender (61% vs 42% male), age (53 vs 53 yrs) and comorbidity (41% vs 39%), all $p > 0.05$ between patients and controls. Results on the subdomains of the NCSI are provided in Figure 1. Overall quality of life was decreased in both patients and controls, 59% vs. 39% (ns) respectively. However, Q fever patients had abnormal fatigue scores (eg increased) compared to controls (74% vs 48%, $p < 0.05$). Furthermore, clinically significant fatigue levels were present in 52% of patients vs. 26% in controls, $p < 0.05$. In addition to the primary data analysis, we compared NCSI scores of the seropositive controls who were excluded ($n = 11$) to the scores of seronegative control subjects ($n = 23$). The NCSI scores of seropositive- and seronegative controls were not statistically different in all 8 measured sub-domains of health status ($p > 0.05$ for all sub-domains).
Conclusion: These data strongly support a sustained decrease in health status in Q fever patients in the Netherlands, one year after primary infection. With more than 3000 new Q fever patients in the last 2 years in the setting of the ongoing Dutch Q fever epidemic, these are the first clinical data indicating a major long-term burden of the disease in the years to come. Clinical expression of Q fever infection seems to be necessary for the subsequent development of a long-term decreased health status.

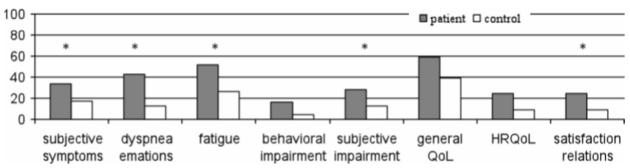


Figure 1. Percentages of clinically relevant scores for each sub-domain of the NCSI for the patient and control group. *$p < 0.05$.

P2106 Evaluation of immune response in brucellosis

N. Aktug Demir, O. Ural, U. Arslan, D. Findik (Konya, TR)*

Objectives: Although all brucellosis patients are treated by the same therapy protocol, while the greater part of patients get over the illness, some become chronically ill and some have rare relapses. The reason for this clinical variation is unknown. Cytokines are important mediators and

there are some investigations into this subject. In the present study we aimed to determine the alterations in cytokine levels during brucellosis and their effects on the diagnosis of relapses.
Methods: Forty-two patients with brucellosis and 43 healhy controls were evaluated in the study. Acute brucellosis was diagnosed according to the results of STA tests and positive blood cultures besides clinical symptoms. Serum samples were collected from the patients on the 1st and 45th days of illness The patients were followed for one year and had controls at three month intervals. If they had relapses serum samples were taken at the time of the ralapse and after the second therapy. If there were no relapses the serum samples were taken at the end of the first year. The cytokine levels (TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, sIL-2R) of the samples were measured by the ELISA method according to the manufacturer's instructions.
Results: The mean serum levels of TNF-α, IFN-γ, IL-2, IL-8, sIL-2R were observed to be significantly higher but the mean IL-4 levels significantly lower in acute brucellosis patients compared to the control group. No significant differences were found between the two groups in terms of IL-6 and IL-10 mean levels. Also there were no significant differences among serum samples taken on the 1st day, on the 45th day and on the 1st year of the illness in terms of TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, sIL-2R mean levels. Relapses were observed in 7 patients during the one year follow up. According to statistical analysis the 1st day IL-2 and IL-6 mean levels were higher in patients with relapses than patients without relapses ($p = 0.027$) but mean levels of sIL-2R were lower. Mean levels of IL-6 on the 45th day increased in patients with relapses.
Conclusion: Our results revealed that 1st day and 45th day IL-6 values are useful tools to detect relapses and also can show the prognoses of the illness. Another important thing is that, on the first day if IL-2 and IL-6 levels are high and sIL-2R is low clinicians need to be careful about relapses.

P2107 Brucellosis: a Tunisian experience

L. Ammari, H. Ben Hamza, H. Tiouiri Benaissa, M. Zribi, B. Kilani, C. Fendri, T. Ben Chaabane (Tunis, TN)*

Brucellosis is a chronic granulomatous infection caused by intracellular bacteria. It is an endemic disease in Mediterranean countries and Tunisia. Brucellosis shows the involvement of many systems and it seems to be responsible for the high incidence of relapse. The aim of this study is to analyze the clinical, laboratory findings and therapeutic features in patients with brucellosis.
Patient and Methods: We conducted a retrospective study of patients who developed brucellosis between January 1986 and December 2008 and admitted in the department of infectious diseases of Rabta Hospital in Tunis. The diagnosis was based on clinical findings compatible with brucellosis, serological tests positive, and/or isolation of *Brucella* species from blood, or other tissues.
Results: 117 cases were included. The mean age was 38 years (13–72 years) and sex ratio was 2.34. An occupational history relevant for *Brucella* exposure was present in 58% of the cases and consumed contaminated animal product was noted in 79% of cases. The mean diagnostic delay was 37 days, much longer in focal brucellosis. Acute brucellosis was predominant, in 66% of cases. The focal brucellosis complications were seen in 31.5%: osteoarticular involvement (73%), nervous system central (21.6%), infective endocarditis (1.7%). Chronic brucellos occurs in 2.5% of cases. Clinical manifestations include non-specific symptoms such as fever (100%), sweats (92%), arthralgia and lower back pain (71%). All the patient had serological titre $\geqslant 1/160$. Overall, 37% of blood culture were positive. 98.5% of the patients were cured by antibiotherapy included Doxycycline and rifampicin. Relapse in follow-up period was observed in 2 patients.
Conclusion: Brucellosis is an infection with multiple presentations. Its early diagnosis was mandatory to avoid severe complications.

P2108 Assessment of prognosis of tetanus and its related factors among Albanian adults

A. Pilaca, A. Ndreu, Z. Delia (Tirana, AL)*

Objective: To assess the associated factors with prognosis of tetanus among the adult population of Albania.
Methods: All reported cases of tetanus ($N = 64$) hospitalized in the regional hospitals of Tirana (the Albanian capital), Shkodra (main district in north Albania) and Korca (one of the main districts in Southeast Albania) for the period 1984–2004. Four patients were excluded from the analysis due to missing information on prognosis. Data on prognosis-related factors included age, sex, incubation period, onset period, as well as location and type of wounds. Multivariable-adjusted binary logistic regression was used to asses the independent association of prognosis (survival vs. death) with covariates.
Results: The overall case-fatality rate was 38.6%. It was higher among older individuals ($P < 0.01$), and in those with a shorter incubation period (<11 days; $P < 0.01$) and with infected wounds ($P = 0.03$). There was no association, however, with location of wounds. Upon simultaneous adjustment for all covariates, age (>50 years vs. <50 years) and a shorter incubation period (<11 days vs. 11–20 days) were positively related to case-fatality (OR = 3.4, 95% CI=1.23–5.13 and OR = 2.41, 95% CI=1.19–4.37, respectively), whereas the association with infected wounds was not statistically significant any further ($P = 0.13$).
Conclusions: Age, incubation period and type of wound are important factors related to prognosis of tetanus in Albanian adults. Health care practitioners should actively seek information on prognostic characteristics of patients with tetanus in order to institute effective and prompt treatment of this disease.

P2109 Prevalence of *Rickettsia helvetica* in *Ixodes ricinus* ticks from a recreational forest in Denmark

B. Kantsø, C. Svendsen, P. Jensen, J. Vennestrøm, K. Krogfelt (Copenhagen, DK)*

Objective: To study the prevalence of *Rickettsia* in specific habitats in a Danish recreational forest.
Methods: 704 ticks of the species *Ixodes ricinus* collected in 3 different habitats were screened for *Rickettsia* DNA by a genus species-specific real-time PCR.
Results: 4.7% (31/662) of the nymphs were positive for rickettsial DNA, The infection rate was highest in May as compared to July, August and October. Ecotone (forest clearing) had elevated prevalence as compared to spruce or beech forests. Sequencing revealed only DNA from *Rickettsia helvetica*
Conclusions: The findings in this study substantiate the previous findings of *R. helvetica* in *I. ricinus* ticks from Denmark, a low-prevalence country of rickettsiosis. Furthermore, it emphasizes that rickettsiosis is a diagnosis to be considered along with that of borreliosis.

P2110 Targets for clinical diagnosis of *Coxiella burnetii* and vaccine development identified by proteomics techniques

G. Flores-Ramirez, R. Toman, L. Skultety (Bratislava, SK)*

Objectives: *Coxiella* burnetti is an intracellular Gram negative bacterium that causes Q fever in humans. Human infections are mainly acquired via the respiratory route by inhalation of aerosols produced by infected farm animals. The aim of the study is to identify candidate proteins for diagnosing of *C. burnetii* using an immunoproteomic approach.
Methods: Whole cell extracts from *C. burnetii* phase II cells were separated by two-dimensional electrophoresis (2-DE) and probed against hyperimmune rabbit serum and sera of three patients with various form of infection. Reactive proteins were matched to a 2-DE gel prepared in parallel, trypsin digested and analyzed by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS). To identify the respective proteins,

the peptides were matched against databases. Bioinformatics tools were applied to predict the possible functions of these proteins.
Results: More than twenty protein spots were identified that reacted with the sera from patients infected with *C. burnetii.* Specific antigens have been detected. The immunoreactive proteins belong to the groups of chaperones/heat shock proteins, ribosomal proteins, metabolic enzymes, transport proteins and hypothetical proteins.
Conclusions: Here we present our first immunoproteomics study of the *C. burnetii* proteins that may allow us to identify possible candidates molecules for serodiagnosis of the infectious agent and vaccine development against Q fever.

Imported infections: tropical and travel medicine

P2111 *Leishmania* mixed infection in great gerbil population of a hyperendemic focus of zoonotic cutaneous leishmaniasis in Iran

A.A. Akhavan, A. Khamesipour, M.R. Yaghoobi-Ershadi, H. Mirhendi, M.H. Alimohammadian, Y. Rassi, N. Shareghi, R. Jafari, M.H. Arandian, H. Abdoli, M. Ghanei, N. Jalali-Zand (Tehran, IR)*

Objectives: Zoonotic cutaneous leishmaniasis, a neglected tropical disease, is a major public health problem in some areas of the old world. *Leishmania major* is widely distributed in various populations of rodents in arid and savannah regions. The disease is endemic in many rural districts of Iran, in 17 out of the 30 provinces. In the current study, natural *Leishmania* infection rate and seasonal variation of the infection in Rhombomys opimus population of a hyperendemic focus of ZCL in Iran was investigated.
Methods: The investigation was conducted over a period of 24 months from October 2006 to October 2008 in Borkhar and Sejzi rural districts, 15–35 km, northeast and east of Esfahan City, Esfahan Province, central Iran, respectively. Regardless of having any obvious lesions, impression smears were prepared from the ear lobes of the animals. Nested PCR assay was used for detection and identification of *Leishmania* species and the results were confirmed using PCR-RFLP.
Results: A total of 58 *R. opimus,* were captured and examined by two diagnostic techniques, direct examination and nested PCR. Fourteen out of 58 specimens (24.1%) were positive by microscopic examination and 34 (58.6%) by the nested PCR. In 29 samples which the amastigote was not seen by through direct examination, the nested PCR showed positive results, and every positive smear was also found positive by nested PCR. Twenty six out of 58 (44.8%) of the gerbils were identified to be infected only with *L. turanica,* and 1 (1.7%) with *L. gerbilli.* A mixed natural infection with *L. major* and *L. turanica* was seen in 12.1% of the rodents. Pure *L. turanica* infection was seen throughout the year whereas mixed infection of *L. major* and *L. turanica* was seen in all seasons except spring. The highest (88.2%) and lowest (22.2%) *Leishmania* infection rate was observed in fall and spring respectively.
Conclusion: It is concluded that *L. major, L. gerbilli* and *L. turanica* circulate in the population of *R. opimus* in central part of Iran. *Leishmania* turanica was the dominant species in the population of great gerbils. Infection with *L. major* alone was not seen in the population of the gerbil. *Leishmania major* infection usually accompanied with *L. turanica* in naturally infected gerbils with the highest rate in fall. It is recommended that the role of *L. turanica* in the epidemiology and transmission of ZCL should be revisited carefully.

P2113 Single *Trypanosoma cruzi* antigens are differentially recognized by Chagas patients after benznidazol treatment

A. Fernández-Villegas, M. Pinazo, C. Marañón, M. Thomas, E. Posada, M. Segovia, J. Gascón, M.C. López (Granada, Barcelona, Murcia, ES)*

Objectives: The objective of this work was to set up a panel of *T. cruzi* recombinant proteins which are differentially recognized by sera from healthy donors and Chagas disease patients at different stages of the disease. We have also explored the dynamics of humoral recognition of these individual antigens after benznidazole treatment, looking for singular recognition patterns, which could be correlated with a peculiar progression of the disease.
Methods: In this study, KMP11, HSP70 PFR2, Tgp63 recombinant proteins and whole soluble proteins of *Trypanosoma cruzi* were used to detect antiparasite antibodies by ELISA. Sera from Chagasic patients living in Spain who were at different stages of the disease (Imported Chagas) were collected pre and post benznidazole treatment together to sera from non-infected subjects coming from endemic areas as a control.
Results: The recognition of all the proteins analyzed in this study was significatively higher by sera from chagasic patients than those from healthy donors. When data were analyzed regarding the stage of the disease, it was observed that indeterminate, cardiac and chronic patients with digestive symptoms significatively recognized the KMP11, HSP70 and PFR2 antigens compared with healthy donors. The recognition level of the recombinant proteins by sera from 6 healthy donors did not show any differences along one year period as an indication of the long term stability of the detected basal antibodies.
The chagasic patients showed a significance decrease of KMP11, HSP70 and PFR2 recognition at 3 and 6 moths after benznidazol treatment. When humoral response in chagasic patients was sorted regarding the stage of the disease, a relevant reduction in the KMP11 antibody level was only observed in patients at the indeterminate stage 6 months post-treatment. Conversely, patients with cardiac symptoms showed a statistically significant decrease of the antibody level specific for PFR2 and HSP70 after 3 and 6 months post-treatment, respectively.
Conclusions: These results let us to the identification of chagasic patients and healthy donors using a panel of recombinant proteins, and to establish specific recognition pattern after benznidazole treatment depending of the stage of the disease.

P2114 Detection of dengue virus in lymphoid tissues of persons with remote dengue infections

V. Nilaratanakul, S. Thaivanich, K. Songcharoen, K. Arunyingmongkol, R. Plongla, M. Sriprapun, W. Kulwichit (Bangkok, TH)*

Objectives: Dengue virus is thought to cause only acute infection and then complete recovery. But some of its peer flaviviruses can persistently reside in hosts and cause diseases. Almost all adults in our country have been infected by dengue viruses, demonstrated by neutralizing antibody assays. Due to its tropism to mononuclear leucocytes, we conduct a study to find evidence of dengue virus persistence in reticuloendothelial tissues from adults without evidence of recent dengue infection.
Methods: We enrolled autopsied cases and patients whose indicated surgery provided tissues (liver, spleen, bone marrow and/or lymph nodes) requiring pathological examination. After completion of each pathological report, a fraction of remaining paraffin-embedded tissue was utilised for reverse transcription-polymerase chain reaction (RT-PCR) by dengue-specific primers. To confirm that there were no acute or recent infections, paired sera were collected just before and 1–4 weeks after surgery for anti-dengue IgM and IgG by standard ELISA. Demographic data and history of recent febrile illnesses were also recorded.
Results: 2 autopsied cases and 57 surgical cases were enrolled. RT-PCR was performed on 51 lymph nodes, 23 livers, 1 bone marrow and 2 spleens. Dengue genome was detected in 2 cases (table). Neither of them had evidence of acute or recent infection by dengue-ELISA using paired sera. On the other hand, there was one case of asymptomatic acute dengue infection in a 81-year-old male patient whose lymph node from extended gastrectomy for cancer at the cardia part of stomach contained no dengue genome. Dengue IgM in his first and second sera, one week apart, was 6 and 94.
Conclusions: Severe dengue infection is believed to be significantly associated with antibody-dependent immune enhancement in secondary infection. In a hyperendemic area like our country, most of clinical dengue infections are secondary; yet, these are mostly either mild or asymptomatic. Persistence of primary dengue virus genome, defective or complete, may play an important interactive role in clinical severity

and dengue pathogenesis when the patient has superimposed infection by another serotype. Our findings may be the first step into a new area of dengue research.

Patients with dengue genome in tissues

No.	Sex	Age	Diagnosis	Tissue	DENV-EISA in paired sera			
					IgM		IgG	
					1st	2nd	1st	2nd
1	M	47	Recurrent colonic cancer with liver metastasis	Liver	12	14	12	11
2	M	70	Lower rectal cancer	Lymph node	1	0	15	6

P2115 Prevalence of *Dientamoeba fragilis* in adults and children in Denmark, tested by routine real-time PCR in patients suspected of enteroparasitic disease

D. Röser, C.R. Stensvold, H.V. Nielsen (Copenhagen, DK)*

Objectives: In recent years, *Dientamoeba fragilis* has become increasingly recognised as an entero-pathogenic parasite associated with various gastrointestinal symptoms. Permanent staining of faeces fixed with e.g. sodium acetate-acetic acid-formalin (SAF) has been the standard diagnostic method in laboratories testing specifically for *D. fragilis.* Diagnosis based on PCR was introduced only recently, and is not widely used as a routine method. The objective of this study was to ascertain the prevalence of *Dientamoeba fragilis* in patients suspected of enteroparasitic disease, assessed by real-time PCR analysis of genomic DNA extracted directly from stool samples.
Methods: Since Aug. 2007, Statens Serum Institut has as the sole laboratory in Denmark offered routine real-time PCR analysis for intestinal protozoa (Taqman assay targeting the SSU rRNA gene) to hospitals and general practitioners. The analysis is performed as either a single request (SR) analysis or as part of a diagnostic package (DP) incl. PCR for *Cryptosporidium*, *Entamoeba dispar*, *Entamoeba histolytica* and *Giardia* duodenalis, on genomic DNA extracted directly from faeces. Sampling period was Aug. 07 – Jun. 09.
Results: A total of 6802 PCR's were performed in the study period, of which 2102 (31%) were positive for *D. fragilis.* The DP-group (n = 4822) showed a lower prevalence of positives (26%) than the SR-group (42%), one suspected reason being a higher degree of treatment control tests in the SR-group. In the DP-group, gender distribution among positives was 55% females and 45% males. In adults (aged >16 years) the prevalence ranged from 12% (50+ years) to 21% (31–50 years). In children (age 0–15) the prevalence was markedly higher, rising from 9% in 0–1 year olds, to 32% in 2–3-year-olds, and to 47% in 4–10-year-olds, hereafter declining to 39% in 11–15-year-olds.
Conclusion: This study showed a higher overall prevalence of *D. fragilis* using real-time PCR (26%), than previously shown using SAF-staining (12%), however dissimilarity between the present and earlier study in terms of age distribution and positive prevalence within age groups have to be considered. Compared to age groups, we found a significantly higher ($p < 0.001$) prevalence in children aged 4–10 years, and a significantly higher ($p < 0.001$) prevalence in adults aged 31–50, with parenthood being the suspected reason for this. In general, real-time PCR proved a reliable and feasible method for detection of *D. fragilis* in stool samples.

P2116 *Rickettsia hoogstraalii*-like organism in the soft tick, *Ornithodoros moubata*

S.J. Cutler, J. Nikitorowicz Buniak (London, UK)*

Objectives: *Ornithodoros moubata* complex (Argasidae) ticks collected from human dwellings in Tanzania carry a partially characterised *Rickettsia*-like organism. Subsequently others have reported a rickettsial organism present in soft ticks feeding on seabirds. As yet we are uncertain as to the phylogenetic position of this organism or its relevance as a potential cause of infection. This is a realistic possibility with the species carried by *O. moubata* as these ticks primarily feed on humans.
Methods: Molecular characterisation of a *Rickettsia*-like organism in 145 pooled *O. moubata* tick samples from Tanzania was undertaken using PCR amplification and sequence analysis. *O. moubata* ticks were imported under license, and total DNA extracted using DNAeasy kits (Qiagen) and QiaCube robot. As preliminary data suggested this *Rickettsia*-like species had greatest homology with *R. akari* and *R. australis,* we screening samples using a real-time PCR's with specificity for OmpB or *R. felis,* and a genus specific assay based upon the citrate synthase gene citrate synthase [CS] (gltA). Samples positive upon screening were further tested using conventional PCR for genes gltA; OmpA; and OmpB. Twenty six rat samples collected from the tick-infested dwellings were similarly tested.
Results: Interestingly, complete concordance between screening assays was not observed for tick samples, with 6 samples only detected by the *R. felis* assay; 8 in only the CS PCR; and 4 in both assays. Only 4 of the rat samples were positive with 4 from tail samples and 2 from each of ear and blood samples. Only limited amplicons were available for sequence analysis as a result of thier significant sequence divergence. Greatest sequence homology was found with *R. hoogstraalii,* a species described in Carios capensis soft ticks recovered from seabirds, USA, and from the hard tick Haemaphysalis sulcata, Croatia.
Conclusions: Significance of this *Rickettsia* for human health is not fully established, but exposure is high as these ticks infest human dwellings with man serving as their principle maintenance host. Intriguingly, *R. felis* has recently been reported from significant numbers of febrile patients in East Africa, possibly relating to the findings reported herein. Even if this species is not significant as a human pathogen, exposure to this soft-tick *Rickettsia* appears significant and thus might confound the application of rickettsial diagnostics in African settings.

P2117 Detection of *Dientamoeba fragilis*: a comparison between real-time polymerase chain reaction and conventional diagnostic assays

A. Calderaro, C. Gorrini, S. Montecchini, F. Gargiulo, N. Manca, G. Dettori, C. Chezzi (Parma, Brescia, IT)*

Objectives: The pathogenic role of *Dientamoeba fragilis* has been controversial for a long time but it was recently reassessed because of a large number of patients with *D. fragilis* infection (without other enteropathogenic agents) reporting gastro-intestinal signs and symptoms solved by a targeted therapy. The laboratory diagnosis of dientamoebiasis is traditionally performed by microscopic examination after permanent staining and/or culture. The fact that conventional diagnosis is time-consuming and requires well-trained personnel led us to evaluate the usefulness of a real-time PCR assay compared to the methods currently used in our laboratory for the diagnosis of infection by *D. fragilis.*
Methods: Conventional diagnostic methods for the detection of intestinal parasites currently used in our laboratory (microscopic examination of fresh and concentrated faecal material and cultivation in Robinson's medium, followed by acridine orange staining when structures resembling *D. fragilis* were observed) were performed on 988 faecal samples belonging to 508 patients with a clinical suspicion of intestinal parasitosis in a period of 41 months. The DNA extracted from the same samples was used in a previously described real-time PCR assay targeting the 5.8S rRNA gene of *D. fragilis* [1].
Results: Conventional methods detected *D. fragilis* in 72 samples of 47 patients while real-time PCR revealed the presence of the protozoan in 189 samples of 107 patients (21% of the analyzed patients). In 60 of these cases dientamoebiasis was diagnosed by real-time PCR alone.
Discussion: In our experience, the evaluated real-time PCR assay showed a higher diagnostic sensitivity than microscopic examination and culture which had underestimated the number of *D. fragilis* infections. This molecular assay could be a rapid and effective tool in our laboratory to be associated with conventional ones for an accurate diagnosis of infection by *D. fragilis* in faecal samples belonging to patients presenting

with signs and symptoms and/or risk factors for intestinal parasitoses, taking into account that dientamoebiasis is suitable to be eradicated by a targeted therapy.

Reference(s)

[1] Verweij et al. Mol Cell Probes 2007; 21: 400–404.

P2118 The diagnosis of giardiasis by a real-time polymerase chain reaction assay as compared to conventional assays

A. Calderaro, C. Gorrini, S. Montecchini, F. Gargiulo, N. Manca, G. Dettori, C. Chezzi (Parma, Brescia, IT)*

Objectives: *Giardia intestinalis* is a world-wide distributed parasitic agent of gastroenteritis with a higher frequency in warm climate. As giardiasis is one of the most frequent parasitoses in our area, the aim of this study was the evaluation of the performance and of the applicability of a real-time PCR assay in comparison with the combination of conventional methods used in our laboratory for the diagnosis of the infection by *G. intestinalis.*

Methods: The DNA from 800 faecal samples collected in a 3-year period and belonging to 403 patients with a clinical suspicion of intestinal parasitosis was used in a real-time PCR assay for the detection of *G. intestinalis* [1]. The same samples were analyzed for the presence of intestinal parasites by conventional methods (microscopic examination from fresh and concentrated faecal material and *G. intestinalis* and *Cryptosporidium* spp. specific antigen detection by immunocromatographic and/or immunofluorescence assays).

Results: *G. intestinalis* was detected in 178 samples of 98 patients by conventional methods, while the real-time PCR assay revealed the DNA of the protozoan in 26 additional samples. In particular in 13 cases the diagnosis was possible only by using the molecular assay evaluated in this study. The detection limit of the real-time PCR assay was 2 cysts per reaction. As concerns the diagnostic performance, the real-time PCR assay showed a sensitivity and a specificity both of 100% as compared to conventional assays for the diagnosis of the infection by *G. intestinalis.*

Discussion: In our experience, the evaluated real-time PCR assay demonstrated to be a specific and sensitive tool for the detection of *G. intestinal* infection. This assay could be successfully associated to the conventional methods for the diagnosis of intestinal parasitoses in our laboratory, in order to perform an accurate diagnosis of giardiasis, especially in groups of selected patients such as subjects presenting with risk factors for faecal-oral infections including immigrants and adopted children from developing countries and travellers returning from the same areas [2].

Reference(s)

[1] Verweij JJ. et al. J Clin Microbiol 2004; 42:1220–1223.
[2] Calderaro A. et al. Diagn Microbiol Infect Dis 2009; In press.

P2119 International travel is a significant risk factor for acquiring zoonotic *Salmonella* bacteraemia

K. Koch, B. Kristensen, H.M. Holt, S. Ethelberg, K. Mølbak, H.C. Schønheyder (Aalborg, Aarhus, Odense, Copenhagen, DK)*

Objectives: To determine the incidence of zoonotic *Salmonella* bacteraemias associated with international travel and to estimate the relative risk according to travel destination.

Methods: We conducted a 10-year population-based cohort study in three Danish regions 1999–2008 (population 1.6 million). Patients with zoonotic *Salmonella* bacteraemias were identified in registries in local departments of clinical microbiology. The patients' travel history was obtained by chart review. Statens Serum Institut is notified of all *Salmonella* isolates in Denmark and information on travel is provided on a voluntary basis; the information is recorded in the national registry of enteric bacterial pathogens. In 2008 Statens Serum Institut undertook a telephone-based survey of the travel history for *Salmonella* patients resident in two of the regions. We computed incidence rates and estimated the relative risk of bacteraemia compared to gastroenteritis in the subset of patients diagnosed 2004–2008. For each geographic region we calculated the odds ratio (OR) adjusted for age and gender by logistic regression.

Results: We identified a total of 311 incident cases of zoonotic *Salmonella* bacteraemia and 6,236 cases of gastrointestinal *Salmonella* infections. Seventy six (24.4%) patients with bacteraemia had a history of international travel within the previous two weeks and no likely source of exposure in Denmark. The overall incidence rate of travel-related bacteraemia was 0.5/100.000 person-years; the incidence rate was highest in the age group 15–24 years (0.8/100.000 person-years). Seasonal peaks differed for travel-related and domestically acquired cases: July-August versus September-October, respectively. Two hundred and twenty five of 1089 (20.7%) patients with gastroenteritis interviewed by telephone had a travel history. Information collected actively and voluntarily was comparable with regard to the distribution of travel destinations. We found the highest risk of bacteraemia for travelling to Sub-Saharan Africa (OR 17.7 [95% CI: 6.4–49.0]) and South East Asia, foremost Thailand (OR 3.7 [2.0–6.9]).

Conclusion: One in four cases of zoonotic *Salmonella* bacteraemia was travel-related. The incidence was highest in the young age group. The relative risk of presenting with bacteraemia was significantly associated with travel to Sub-Saharan Africa and South East Asia. When giving advice to travellers the risk of invasive salmonellosis should be considered.

P2120 Multiplex real-time PCR for the diagnosis of malaria: correlation with microscopy and with clinical presentation

L. Dormond, K. Jaton-Ogay, S. De Valliere, B. Genton, J. Bille, G. Greub (Lausanne, CH)*

Objectives: Malaria is generally diagnosed by microscopy and rapid antigen testing. Molecular methods become more widely used. Here, we investigated the contribution of a quantitative multiplex PCR for malaria diagnosis. We assessed i) the agreement between PCR-based identification and microscopy, ii) the correlation between the parasite load determined by this quantitative PCR and by microscopy and iii) the correlation of parasite load with clinical severity.

Methods: For 83 patients positive by microscopy, the first EDTA-blood sample was tested by PCR to confirm smear-based species identification. Number of parasites/mL of blood was assessed daily using both microscopy and PCR. Clinical criteria of severity were retrieved from medical files and were compared with microscopy and PCR quantification.

Results: Among the 83 patients tested, 1 was positive by microscopy only and 82 were positive by microscopy and PCR. Agreement between microscopy and PCR for the identification at the species level was of 89% (73/82). The 9 discordant results corresponded to co-infections with 2 or 3 species and were attributed to inaccurate phenotypic identification of mixed cases. The number of parasites/mL of blood generally decreased rapidly after treatment start with similar decay curves obtained with both microscopy and PCR. Clinical severity was significantly correlated with high parasite load as determined by microscopy ($p = 0.0163$) and PCR ($p = 0.0264$).

Conclusion: Our PCR proved especially useful to identify mixed infections. The quantification obtained by PCR closely correlated with microscopy-based quantification and with disease severity.

P2121 Haematological toxicity secondary to parenteral sodium stibogluconate in the treatment of New World cutanous leishmaniasis

E. Wise, D. Lockwood (London, UK)*

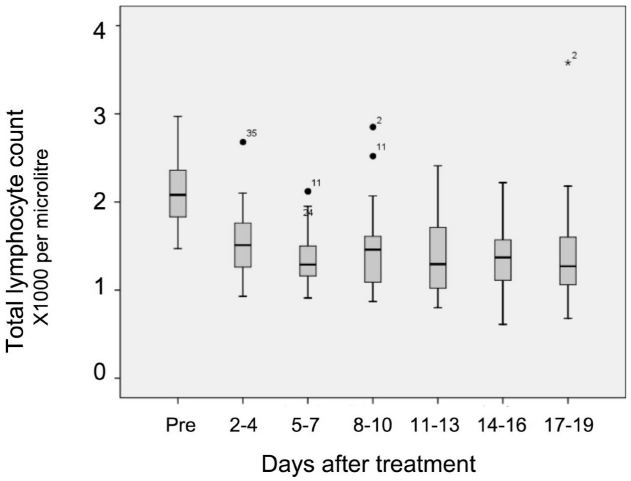
Objectives: Systemic treatment with the pentavalent antimonial sodium stibogluconate is advocated for New World cutaneous leishmaniasis (CL), both to induce healing of the primary lesion and to reduce the risk of dissemination of the parasites to the nasopharyngeal mucosa. Significant toxicity is associated with the parenteral administration of the drug. Despite previous case reports of thrombocytopaenia and

lymphopaenia associated with parenteral use of the drug, this has yet to be adequately investigated or documented. We determined the haematological suppressive effects of intravenous sodium stibogluconate in a case series of patients.

Methods: Since 2003, our institution has treated ~20 returned travellers a year for New World CL. This is done on an outpatient basis, with 21–28 doses of daily sodium stibogluconate infusions. Monitoring of treatment is in accordance with a protocol, using the Hospital for Tropical Diseases' CL 'Integrated Care Pathway'. This stipulates that a full blood count should be performed pre-treatment and on days 1, 4, 7, 10, 13, 17, 19, 21, 24 and 28 after treatment is commenced. We performed a retrospective case analysis of 50 patients, randomly selected, from those with New World CL treated with the ICP between 2003 and 2008. The patients' platelet and lymphocyte counts during treatment were compared to baseline values. The case notes were reviewed for clinical events suggestive of disordered haemostasis or immunocompromise.

Results: There were no documented events of abnormal bleeding or opportunistic infections in our patient group, either during treatment or on follow-up. Repeated blood counts demonstrated a significant fall in both platelet and lymphocte counts after institution of therapy ($P < 0.001$, using repeated measures analysis-of-variance). Inspection of the boxplots showed that the fall in counts was relatively small (~30%). This occurred within a few days of commencing treatment, with no further decline throughout the course of infusions. The Figure shows the boxplot for lymphocyte counts up to 19 days after treatment was started. In one exceptional case, the platelet count fell to <50,000 per μl.

Conclusion: The use of intravenous sodium stibogluconate is unlikely to cause clinically significant suppression of either the platelet or lymphocyte counts. The effect on these blood elements is described by a quadratic function, with an early moderate fall and then no further decline over the course of treatment.



P2122 **Demographic factors associated with serum IgG anti-lipopolysaccharide for *Shigella sonnei* antibodies among 147 Thai volunteers**

S. Chamnanchanunt (Bangkok, TH)*

Shigella sonnei is the most common pathogen of dysentery diarrhea in industrialized countries. The serum IgG anti-lipopolysaccharide (LPS) indicates previous infectious with *S. sonnei.* There were several studies about serum IgG anti-LPS in infected patient, but it had not been reported in asymptomatic individual.

Objectives: The purpose of the study was to explore the association of demographic factors and the titer of serum IgG anti-LPS for *S. sonnei.* In order to identify high-risk group of previous infection for future prevention in un-infected group are necessary.

Methods: This study was performed in Bangkok and its periphery, Thailand, in 2007 to 2008. All Thai volunteers were screened for challenging of shigella vaccine study, and shown the negative result of culture and PCR in stool. Informed consent and complete demographic questionnaires were done at the beginning. Serum samples were tested for serum IgG anti-LPS for *S. sonnei* using ELISA.

Results: Of 147 Thai volunteers aged 20–39 years. The majority were male (63.3%) and 38.8% had high titer (>800) of anti-LPS for *S. sonnei.* The highest titer was in the age group of 31–35 years. The titer for *S. sonnei* was significantly high in female ($p = 0.018$) and farmer groups ($p = 0.01$). Moreover, in 56 (38.1%) volunteers who live outside Bangkok, the titer were statistically higher in older age ($p = 0.048$) and female groups ($p = 0.046$). High titer of anti-LPS for *S. sonnei* was not associated with education level, family income, birth location, and current location in Bangkok, positive of HLA-B 27, anti-HIV, HBsAg, and anti-HCV antibodies. Finally, the logistic regression showed that two independent factors; older age group (31–35 years); OR = 7.2 (95% CI =2.3–22.5) and female group; OR = 2.5 (95% CI= 1.1–5.8) were associated with high titer of anti-LPS for *S. sonnei.*

Conclusions: These demographic data demonstrated that older age (between 31–35 years) and female were associated with high titer for IgG anti-LPS for *S. sonnei* in Thai volunteers. In addition, farmers were high-risk to exposure to *S. sonnei* organism. Therefore, these groups may need vaccine prophylaxis.

P2123 **New anti-*Leishmania* agents: *Cymbopongon citratus* and citral triggers induction on cell-cycle arrest and apoptosis of *L. infantum, L. tropica* and *L. major* promastigotes**

M. Machado, G. Santoro, A. Dinis, M. Santos-Rosa, V. Alves, L. Salgueiro, C. Cavaleiro, M.C. Sousa (Coimbra, PT; Rio de Janeiro, BR)*

Leishmaniasis treatment is limited and considering toxicity, side effects, rate of relapse, cost, length and resistance to available drugs, more attention should be given to the search of new chemotherapeutic options. Extracts prepared by distillation of aromatic plants, the essential oils, are a valuable research option for anti-*Leishmania* leads and drugs as they offer a huge diversity of small hydrophobic molecules, most of them accomplishing theoretical criteria's of druglikeness: easily diffuse across cell membranes and consequently gain advantage in what concerns to interactions with intracellular targets. The present work, study the effects of *Cymbopongon citratus* essential oil and citral on *L. infantum, L. tropica* and *L. major* promastigotes. Essential oil composition were analysed by GC and GC-MS. Antileishmanial activity was performed on promastigotes culture of *L. infantum, L. tropica* and *L. major.* The viability was assessed by tetrazolium-dye colorimetric method and expressed as the concentration that inhibits parasite viability by 50% (IC50). Effects on *L. infantum* promastigotes were analyzed by flow cytometry in order to assess mitochondrial transmembrane electrochemical gradient (JC-1), to analyze phosphatidylserine externalization (annexin V–FITC, propidium iodide) and to evaluate cell cycle (DNase-free RNase, PI). Morphological and ultrastructural alterations were evaluated by light, scanning and transmission electron microscopy. *Cymbopongon citratus* and citral revealed activity against promastigotes growth with IC50 values ranging from 20 to 52 microg/ml. Modifications on normal cell shape, mitochondrial swelling, increase number of autophagosomal structures and cytoplasmatic vacuolization, electrondensity lost and membrane disruption were ultra-structural alterations occurred. Apoptosis/necrosis event, mitochondrial membrane potential and cell cycle were significantly different in treated cells. The leishmanicidal activity was mediated via apoptosis as evidenced by externalization of phosphatidylserine, loss of mitochondrial membrane potential, and cell-cycle arrest at the G(0)/G(1) phase. Taken together, these data indicate that *Cymbopongon citratus* and citral has promising anti-leishmanial activity that is mediated by programmed cell death (PCD). No toxicity was seen on mammalian cell lines. These results provide new perspectives on the development of drugs with activities against *Leishmania*.

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P2124 Development of a novel multiplex real-time PCR method for simultaneous detection and differentiation of *Echinococcus granulosus* and *Echinococcus multilocularis* from cyst samples

A. Caner, T. Inceboz, M. Doskaya, A. Degirmenci, F. Celebi, M. Korkmaz, Y. Guruz (Izmir, Erzurum, TR)*

Objectives: *Echinococcus granulosus* and *Echinococcus multilocularis* cause cystic echinococcosis (CE) and alveolar echinococcosis (AE) in human, respectively. CE most frequently involves liver followed by lungs. CE is endemic in sheep and cattle raising endemic areas in Mediterranean region such as Turkey, South America, Australia, and New Zeland. AE primarily develops in the liver and metastases to other organs. Humans acquire the CE and AE infection by ingesting the eggs present in the dog and fox feces, respectively. To date, eight genotypes (G1–3, G6–10) and two species (G4–5) have been identified within the former species *E. granulosus.* Worldwide, sheep *E. granulosus* strain (G1) is the most frequent genotype detected in humans. Parasitologically sterile cysts or cysts without any protoscoleces and rostellar hooklets may hamper histological differentiation of *E. granulosus* from *E. multilocularis* by pathologists. Currently, diagnostic PCR methods have been developed to differentiate *E. granulosus* and *E. multilocularis* from cyst biopsy and drainage materials. In the present study, a novel multiplex Real Time PCR method has been developed for simultaneous detection and differentiation of *E. granulosus* and *E. multilocularis* in a single reaction tube.
Methods: DNA extraction material obtained from the cysts diagnosed with echinococcosis (8 samples with CE, 12 samples with AE) formerly by serological, histological and radiological tests was used during multiplex real time PCR method. For the simultaneous detection and differentiation of *E. granulosus* and *E. multilocularis* by multiplex real time PCR in a single reaction, specific primers (EchiS, EchiA), hybridization probes (granulosus-640, multilocularis-705) and an anchor probe (Anchor Ech-FL) were designed from the *E. granulosus* genotype 1 mitochondrion and *E. multilocularis* mitochondrion genes. DNA extraction material obtained from the *Taenia* spp., *Trichinella* spp., *Toxocara* spp., and *Hymenolepis nana* were used as negative control.
Results and Conclusion: The analyses of the amplification curves obtained from multiplex Real Time PCR confirmed echinococcosis in 20 cysts samples (sensitivity 100%) and differentiated *E. granulosus* from *E. multilocularis* in approximately 40 minutes. Moreover, different picks obtained from the melting curve analysis differentiated *E. granulosus* from *E. multilocularis* as well. The multiplex Real Time PCR didn't show any amplification curve in the negative samples (specificity 100%).

P2125 Evaluation of a new immunochromatographic assay for the detection of antibodies against *Echinococcus granulosus*

J.M. Delgado, F. Soriano, A. Camacho, J. Rojas, J. Mendoza, A. Rojas (Santa Fe, ES)*

Objectives: To evaluate a new immunochromatographic rapid test for the detection of total antibodies against *E. granulosus* in human serum samples.
Methods: A new immunochromatographic assay (ICA), VIRapid Hydatidosis, has been designed for the qualitative detection of anti-*E. granulosus* in both serum and plasma samples. The new test is based on an HPLC-purified antigen, prepared from a 5/B enriched fraction that was obtained from hydatid fluid. This antigen was used both adsorbed on the gold conjugate and the test line to produce a lateral flow ICA. A control line was included to check the correct performance of the test. Results were visually read out after incubation for 20 minutes at room temperature (see attached figure). The test performance was evaluated with 276 human serum samples: 199 negative sera from Almería and Jaén (Spain) and 77 positive sera from different geographical areas (Spain, Turkey and France). Two CE-marked commercial assays for hydatidosis (an IgG ELISA and an indirect haemagglutination) were used as references to characterize the sera.
Results: 73 out of 77 positive sera showed a distinct red test line in the ICA. 198 out of 199 negative sera showed no reactivity on the test line in the ICA. Sensitivity, specificity, positive predictive value and negative predictive value of the new VIRapid Hydatidosis assay were 94.7%, 99.5%, 98.6% and 98.0%, respectively.
Conclusions: The new test has proved to be able to detect anti-hydatid antibodies in human serum samples with good sensitivity and specificity values. Since serological assays, together with imaging techniques, are the most frequently used techniques for the diagnosis of hydatidosis, this test offers a new diagnostic tool that brings together good performance characteristics with the advantages of rapid tests (easy to run, easy to interpret and easy to store). It suits the demand of both laboratories from developed areas with low prevalence of the disease and those from developing regions, with more limitations in their material equipment.

