Editor: Prof Charles Kelly

Journal Pre-proof

Multicenter inter-laboratory analysis of routine susceptibility testing with a challenge panel of resistant strains

Corentin Deckers , Reza Soleimani , Olivier Denis , Pierre Bogaerts , Catherine Berhin , Hector Rodríguez-Villalobos , Julie Descy , Marie Hallin , Claire Nonhoff , Stefanie Desmet , Koen Magerman , Anne Marie Vanden Abeele , Bénédicte Lissoir , Veerle Matheeussen , Kris Vernelen , Te-Din Huang , on behalf of the Belgian National Antibiogram Committee

PII: S2213-7165(22)00005-4
DOI: https://doi.org/10.1016/j.jgar.2021.12.020
Reference: JGAR 1741

To appear in: *Journal of Global Antimicrobial Resistance*

Received date: 19 September 2021
Revised date: 8 December 2021
Accepted date: 27 December 2021

Please cite this article as: Corentin Deckers , Reza Soleimani , Olivier Denis , Pierre Bogaerts , Catherine Berhin , Hector Rodríguez-Villalobos , Julie Descy , Marie Hallin , Claire Nonhoff , Stefanie Desmet , Koen Magerman , Anne Marie Vanden Abeele , Bénédicte Lissoir , Veerle Matheeussen , Kris Vernelen , Te-Din Huang , on behalf of the Belgian National Antibiogram Committee, Multicenter inter-laboratory analysis of routine susceptibility testing with a challenge panel of resistant strains, *Journal of Global Antimicrobial Resistance* (2022), doi: https://doi.org/10.1016/j.jgar.2021.12.020

Highlights

- Quality control should include organisms with relevant and emerging resistances
- A challenge panel of 12 multidrug-resistant organisms were tested by 9 laboratories
- Overall categorical agreements were > 90% for all but one strain
- Higher discrepancies were observed for fosfomycin, meropenem, aztreonam and tigecycline

Multicenter inter-laboratory analysis of routine susceptibility testing with a challenge panel of resistant strains

Corentin Deckers[1], Reza Soleimani[1], Olivier Denis[1], Pierre Bogaerts[1], Catherine Berhin[1], Hector Rodríguez-Villalobos[2], Julie Descy[3], Marie Hallin[4], Claire Nonhoff[4], Stefanie Desmet[5], Koen Magerman[6], Anne Marie Van den Abeele[7], Bénédicte Lissoir[8], Veerle Matheeussen[9], Kris Vernelen[10], Te-Din Huang[1] on behalf of the Belgian National Antibiogram Committee

[1]Laboratory of Microbiology, CHU UCL Namur and Antibiotic-Resistant Gram-Negative Bacilli National Reference Center, Yvoir, Belgium.

[2]Department of Microbiology, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

[3]Department of Clinical Microbiology, CHU Sart-Tilman, Liège, Belgium.

[4]Department of Microbiology, Laboratoire Hospitalier Universitaire de Bruxelles (LHUB-ULB) and National Reference Center for *Staphylococcus aureus*, Brussels, Belgium.

[5]Departement of Laboratory Medicine, Gasthuisberg Ziekenhuis, KUL, Leuven, Belgium.

[6]Departement of Microbiology, Jessa Ziekenhuis, Hasselt, Belgium.

[7]Departement of Clinical Microbiology, AZ Sint-Lucas, Ghent, Belgium.

[8]Service of Clinical biology, Grand Hôpital de Charleroi, Charleroi, Belgium.

[9]Departement of Clinical Biology Universitair Ziekenhuis Antwerpen and National Reference Center for Enterococci, Antwerp, Belgium.

[10]Quality of Laboratories, Sciensano, Brussels, Belgium.

Contact : Dr Corentin Deckers
CHU UCL Namur, site Mont-Godinne
Avenue Gaston Therasse, 1
5530, Yvoir
Tel : +32 81 42 32 07
E-mail : corentin.deckers@uclouvain.be

ABSTRACT

Objectives

To elaborate a new national challenge panel of resistant GNB/GPC strains for the validation of routine antimicrobial susceptibility testing (AST) methods, an interlaboratory evaluation was organized.

Methods

Results of 12 well-characterized MDR strains tested by 9 laboratories using local disk diffusion (DD) and automated AST (AUST) methods were compared to the reference broth microdilution.

Results

Overall categorical agreements (CA) ranged from 70% to 100% for both DD and AUST and were > 90% for all but one strain for all antibiotics.

Conclusions

Our multicenter AST study showed good reproducibility and the panel can be used as national resistant reference strains for routine AST validation.

Keywords: interlaboratory assay, quality control, multidrug-resistant organisms, automated susceptibility testing, disk diffusion

Introduction

Reliable antimicrobial susceptibility testing (AST) results among multidrug-resistant (MDR) Gram-negative bacilli (GNB) and Gram-positive cocci (GPC) are critical to avoid the use of inactive antibiotics and to define active drugs for the treatment of causal infections [1, 2]. Appropriate quality control GNB/GPC strains with specific resistance mechanisms to challenge AST methods additional to the recommended ATCC susceptible strains are often lacking. In 2015, the Belgian National Antimicrobial susceptibility testing Committee (NAC) initiated the development of a first national collection of GNB/GPC strains that could serve as a validation panel (NACP1) for new AST systems and for the implementation of European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [3]. However, some resistance determinants of relevance were not covered. Here we aimed to elaborate a second national challenge panel (NACP2) of GNB/GPC strains including relevant resistance traits not covered in the NACP1, based on the agreement results of a multicenter evaluation of routine AST methods.

Materials and Methods

Nine gram-negative bacilli (GNB) and 3 Gram-positive cocci (GPC) clinical strains were selected based on their specific resistant determinants that were previously characterized by three national reference centers (NRC) for multidrug-resistant organisms (Table 1). Strains were subcultured and provided to 9 proficient clinical microbiology laboratories selected based on their geographical distribution, a broad coverage of various routine AST methods used and their experience in performing AST studies (see acknowledgements section).

Isolates were tested by the 9 laboratories in 2020 using their routine AST methods including disk diffusion (DD) from 3 different disk manufacturers (Bio-Rad (Hercules, USA) (n=4), Becton-Dickinson (BD, Franklin Lakes, USA) (n=2), ROSCO (Taastrup, Denmark) (n=2) and by two different automated AST (AUST) systems : Vitek 2 (Biomerieux, Marcy l'Etoile, France) (n=4: AST-N366 (n=3), AST-N367 (n=1), AST-N353 (n=1), AST-P652 (n=3), AST-P655 (n=2), and AST-P650 (n=1)), and BD Phoenix Automated Microbiology System (BD) (n=3: NMIC-417 (n=2), NMIC408 (n=1) and NMIC-502 (n=1), PMIC-90 (n=2) and PMIC-96 (n=1)) according to each manufacturers' instructions following EUCAST methodology. Recorded raw results were interpreted according to the EUCAST 2021 clinical breakpoints (except for tigecycline interpreted using PK/PD breakpoints) [4]. The reference results were obtained by broth microdilution method (BMD) using Sensititre (ThermoFisher Scientific, Waltham, USA) customized panels (BEGN5A for GNB, B0101B for *S. aureus* and BENRC2 for *E. faecium*) at the NRCs. Categorical agreement (CA; agreement of category results), very major errors (VME; susceptible by the evaluated routine method and resistant by the reference method), major errors (ME; resistant by evaluated routine method and susceptible by the reference method) and minor errors (minE; susceptible or resistant by the evaluated routine method

versus intermediate by the reference method or vice-versa) rates comparing the results of DD/AUSTs and the reference BMD were calculated [5]. We set at 90.0% agreement as threshold to accept/reject strains [5].

Results

Altogether, 2117 (1817 [GNB] + 300 [GPC]) organism-drug results were obtained. The resistance rates per antibiotic tested against GNB attained between 44% to 100% and against GPC from 33% to 100%. All results of agreement and error rates are detailed in Tables 1 and 2.

Regarding GNB strains, all except *E. cloacae* NAC2-2 had CA ranging from 88.3% to 98,6%. *E. cloacae* NAC2-2 yielded the lowest CA of 73.3% with high number of ME for amikacin and tigecycline, and of minE for aztreonam, meropenem and ciprofloxacin/levofloxacin. Considering antibiotics individually, the highest CA was observed for piperacillin-tazobactam and extended-spectrum cephalosporins (<2% error rates). Fosfomycin showed the highest unacceptable major discrepancies rates among all antibiotics for DD/AUST methods with VME of 0/21% and ME of 13/6.4% respectively. Meropenem showed the highest minE rate at 17.1/18.3% (DD/AUST). Aztreonam and ciprofloxacin also had high minE at 8.2/13.5% (DD/AUST) and 8.6/10.4% (DD/AUST), but all observed with *E. cloacae* NAC2-2 strain.

Among MDR GPC strains, the CA for both methods were more than 90%. For *S. aureus* NAC2-1, excellent agreement was observed for all antibiotics tested except one VME for linezolid using ROSCO tablet, one ME for rifampicin by Vitek 2 and 8 minE discrepancies for sulfamethoxazole-trimethoprim using DD methods. For the two MDR *E. faecium* strains, one false-susceptible ampicillin result was obtained by ROSCO tablet with NAC2-4 strain and one false-resistant tigecycline result was given by Vitek 2 with NAC2-5 strain (Table 2).

Discussion

In 2016, a first study supported by the Belgian NAC developed a EUCAST challenge panel for AST based on the susceptibility results of a collection of strains evaluated in 20 laboratories. The pilot-testing study resulted in a selection of 28 GPC and GNB strains that can be used for both automated AST testing and DD testing. The use of that panel aimed to facilitate the implementation of new AST methods and the switch to EUCAST breakpoints in clinical laboratories [3]. While this first panel covered a wide spectrum of susceptibility profiles, several emerging resistance mechanisms were not included.[6, 7]. Therefore, we compiled a second challenge panel of 12 MDR strains reflecting resistance mechanisms among GNB and GPC recently documented in Belgium such as acquired colistin resistance (including a plasmid-mediated *mcr-1*-positive), OXA-48 carbapenemase-

producing *Enterobacterales*, OXA-23 carbapenemase-producing *A. baumannii* and linezolid resistance (including a *cfr*-positive methicillin-resistant *S. aureus* and *optrA*-positive *vancomycin-resistant E.faecium*) to test clinical laboratory routine methods.

Our multicentric study in 9 proficient clinical laboratories showed reproducible routine AST results (CA>90% for 10/12 strains) between laboratories and methods despite expected variation in inhibition zone reading by DD and difference in AUST systems and cards used.

Our data obtained from GNB susceptibility testing showed that fosfomycin, tigecycline, meropenem and aztreonam were more prone for discrepancies. For fosfomycin, we observed numerous discrepant results which could not be considered as true errors since we did not perform agar dilution as the reference method [8-9]. Therefore, the reliability of our challenge panel against fosfomycin could not be certified based on our observations. When fosfomycin was excluded from the analysis, the overall CA increased above the 90% acceptance cut-off for all strains except NAC2-2 (Table 1). High ME observed for tigecycline by AUST could be in part explained by the MIC values that were close to the EUCAST PK/PD breakpoints for three of these strains (NAC2-2, NAC2-6 & NAC2-7). Interestingly, all tigecycline-R strains were correctly identified. These data were in line with other studies showing the trends of AUST to overcall tigecycline resistance especially in species other than *E. coli* and we would suggest to confirm AUST tigecycline-R results by BMD for MDR GNB strains[OBJ][OBJ]. As several GNB strains were included for their resistance to carbapenems (Table 1), we observed discrepancies for meropenem between AST methods that were mainly minE (17.1/18.3% for DD/AUST), while VME remained low (1.4%) for both routine methodologies. The disagreements were detected mostly for strains showing low levels of meropenem resistance including NAC2-2, NAC2-3, NAC2-9 and NAC2-6, an OXA-48 carbapenemase-producing *E. coli* known to be frequently meropenem susceptible[OBJ][OBJ]. The variability of meropenem AST results in GNB MDR strains was previously reported[OBJ][OBJ] and our data highly support verification of meropenem susceptibility by determination of MIC using BMD especially when it is considered as therapeutical option for the infection by these MDR organisms. More specifically regarding DD method, we observed a higher number of errors for ROSCO tablets compared to paper disks, mainly for meropenem (data not shown), similar to other previous study[OBJ][OBJ].Interestingly, we did not observe any discrepancy for piperacillin-tazobactam, ceftazidime, and cefepime which have been highlighted in previous studies[OBJ][OBJ]. For temocillin, the CA (>96%) was excellent as the majority of the tested strains were highly resistant. The two colistin-resistant strains (NAC2-6 and NAC2-7) were correctly interpreted by all methods used. NAC2-2 strain did not attain the acceptance criterion (CA <90%) and was not withheld in the final panel. This strain yielded a high number of discrepancies for amikacin, tigecycline, meropenem, ciprofloxacin and aztreonam (Table 1) potentially explained by the reference results within the 'susceptible increased exposure' category for most of these antibiotics.

Our evaluation on GPC AST shows excellent CA between laboratory/methods (≥ 90%) for most antibiotics. False susceptibility (VME) to ampicillin of the two *E. faecium* strains was observed only in one laboratory using ROSCO tablets for DD. Hence, we suggest confirming ampicillin-S results in *E. faecium* strains by alternative methods [18]. For glycopeptides, no error was detected with both vancomycin resistant *E. faecium* correctly detected. While the *optrA*-positive linezolid-resistant *E. faecium* NAC2-4 was correctly identified by all methods, linezolid resistance in *cfr*-positive *S. aureus* NAC2-1 was missed by one laboratory using ROSCO tablets and another using BD Phoenix. We recommend using other methods (E-test or BMD) to confirm linezolid resistance [19, 20]. For tigecycline, all three GPC strains were correctly categorized as susceptible.

Our study has limitations. First, the number of challenge strains selected to be complementary to the previous 2016 panel was limited. However, the new panel focused on MDR strains covering a large spectrum of emerging or prevalent resistance mechanisms that could be more challenging for the routine AST methods by clinical laboratories. Also, a reproducibility study should be carried out to challenge intra-laboratory conditions. Finally, the observations of our study performed in 9 clinical laboratories in Belgium should be confirmed in a larger number of laboratories with other settings.

Conclusion

Using a panel of MDR strains with a wide spectrum of emerging resistant determinants, our multicenter study showed that routine DD and AST methods are overall reliable for AST and resistance detection. However, the exact determination of resistance mechanisms still requires phenotypic and/or genotypic confirmatory tests. Discrepancies and variability of results for few anti-microbials (especially meropenem, aztreonam, tigecycline and linezolid) raised concerns, thus we highly recommend confirmation by BMD method. In addition, the accuracy of AST for antibiotics that can be used as rescue therapy for the treatment of MDR strains such as tigecycline, fosfomycin and colistin need to be improved Finally, based on the global agreement between methods/laboratories (>= 90%) with exclusion of fosfomycin, all but one (*E. cloacae* NAC2-2) strains could be used as reference resistant strains in a national panel for validation of routine AST methods.

Acknowledgements

We thank the members of the Belgian National Antimicrobial susceptibility testing Committee:
Olivier Denis, Stefanie Desmet, Youri Glupczynski, Marie Hallin, Te-Din Huang, Bénédicte Lissoir. Koen Magerman, Veerle Matheeussen , Pierrette Melin, Karl Mertens, Hector Rodriguez, Anne-Marie Van Den Abeele, Kris Vernelen.

We thank Audric Deckers for his help in compiling the statistics for this article.

Declarations

Funding: The Belgian national reference center is supported in part by the Belgian Ministry of Social Affairs through a fund within the national health insurance system (INAMI-RIZIV).

Competing Interests: The authors declare that they have no conflict of interests.

Ethical Approval: Not applicable

Authors' contributions

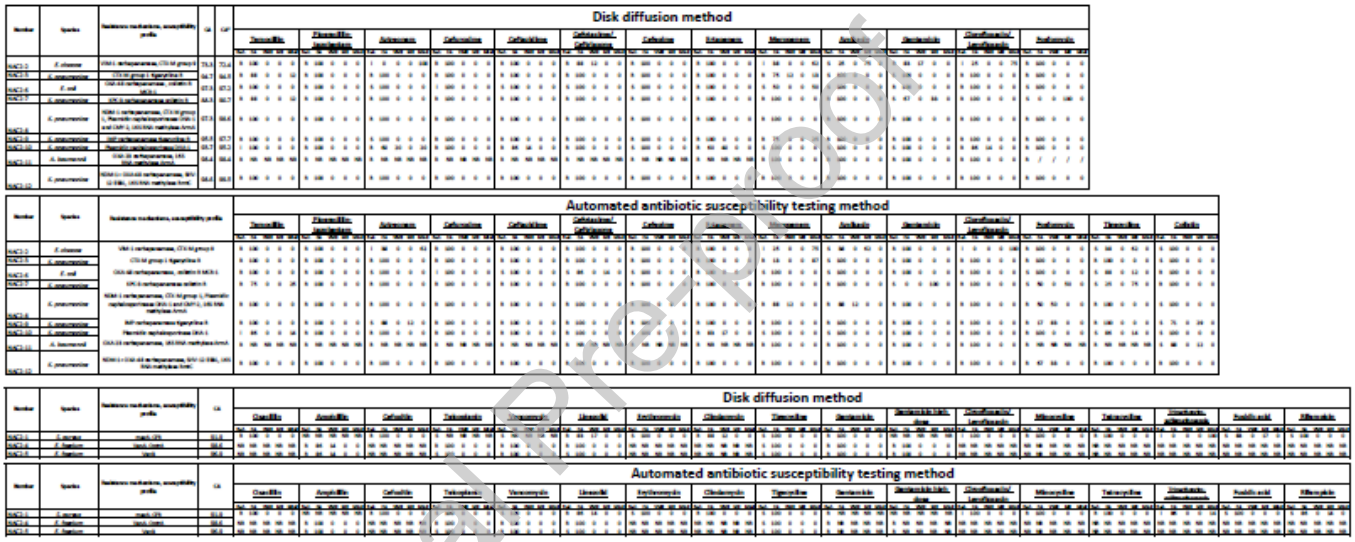
CD, RS and TDH wrote and revised the manuscript. CD collected and analyzed the data. OD and PB revised the manuscript. HRV, JD, HN, SD, CN, KM, AMVdA, BL, VM, OD participated to the study. TDH supervised the whole project. CN, CB, PB characterized the strains at the NRCs. KV organized the storage and the distribution of strains to each laboratory.

References

1. Rolain, J.M., et al., *Do we need new antibiotics?* Clin Microbiol Infect, 2016. **22**(5): p. 408-15.
2. Tamma, P.D., et al., *Infectious Diseases Society of America Guidance on the Treatment of Extended-Spectrum beta-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa).* Clin Infect Dis, 2021. **72**(7): p. 1109-1116.
3. Desmet, S., et al., *Development of a national EUCAST challenge panel for antimicrobial susceptibility testing.* Clin Microbiol Infect, 2016. **22**(8): p. 704-10.
4. *The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021.* http://www.eucast.org.
5. Clark, R.B., M. A. Lewinski, M. J. Loeffelholz, and R. J. Tibbetts, 2009. Cumitech 31A, Verification and Vali- dation of Procedures in the Clinical Microbiology Laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.
6. Huang, T.D., et al., *Increasing proportion of carbapenemase-producing Enterobacteriaceae and emergence of a MCR-1 producer through a multicentric study among hospital-based and private laboratories in Belgium from September to November 2015.* Euro Surveill, 2017. **22**(19).
7. Paridaens, H., et al., *Clinical case of cfr-positive MRSA CC398 in Belgium.* Eur J Clin Microbiol Infect Dis, 2017. **36**(8): p. 1527-1529.
8. Mojica, M.F., et al., *Performance of disk diffusion and broth microdilution for fosfomycin susceptibility testing of multidrug-resistant clinical isolates of Enterobacterales and Pseudomonas aeruginosa.* J Glob Antimicrob Resist, 2020. **21**: p. 391-395.
9. Kaase, M., et al., *Fosfomycin susceptibility in carbapenem-resistant Enterobacteriaceae from Germany.* J Clin Microbiol, 2014. **52**(6): p. 1893-7.
10. Huang, T.D., et al., *In vitro susceptibility of multidrug-resistant Enterobacteriaceae clinical isolates to tigecycline.* J Antimicrob Chemother, 2012. **67**(11): p. 2696-9.
11. Zarkotou, O., et al., *Comparative evaluation of tigecycline susceptibility testing methods for expanded-spectrum cephalosporin- and carbapenem-resistant gram-negative pathogens.* J Clin Microbiol, 2012. **50**(11): p. 3747-50.
12. Bulik, C.C., et al., *Comparison of meropenem MICs and susceptibilities for carbapenemase-producing Klebsiella pneumoniae isolates by various testing methods.* J Clin Microbiol, 2010. **48**(7): p. 2402-6.
13. Tenover, F.C., et al., *Carbapenem resistance in Klebsiella pneumoniae not detected by automated susceptibility testing.* Emerg Infect Dis, 2006. **12**(8): p. 1209-13.

14. Justesen, U.S., et al., *Comparison of Rosco Neo-Sensitabs with Oxoid paper disks in EUCAST disk diffusion antimicrobial susceptibility testing on Mueller-Hinton agar.* Eur J Clin Microbiol Infect Dis, 2013. **32**(5): p. 621-5.
15. Rhodes, N.J., et al., *Unacceptably high error rates in Vitek 2 testing of cefepime susceptibility in extended-spectrum-beta-lactamase-producing Escherichia coli.* Antimicrob Agents Chemother, 2014. **58**(7): p. 3757-61.
16. Jang, W., et al., *Evaluation of MicroScan WalkAway and Vitek 2 for determination of the susceptibility of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates to cefepime, cefotaxime and ceftazidime.* J Antimicrob Chemother, 2013. **68**(10): p. 2282-5.
17. Karlowsky, J.A., et al., *Comparison of four antimicrobial susceptibility testing methods to determine the in vitro activities of piperacillin and piperacillin-tazobactam against clinical isolates of Enterobacteriaceae and Pseudomonas aeruginosa.* J Clin Microbiol, 2003. **41**(7): p. 3339-43.
18. Joste, V., et al., *Enterococcus faecium and Ampicillin Susceptibility Determination: Overestimation of Resistance with Disk Diffusion Method Using 2 Micrograms of Ampicillin?* J Clin Microbiol, 2019. **57**(3).
19. Doern, C.D., et al., *Investigation of Linezolid Resistance in Staphylococci and Enterococci.* J Clin Microbiol, 2016. **54**(5): p. 1289-94.
20. Jones, R.N., G. J. Moet, L. Woosley, H. S. Sader, and T. R. Fritsche. *Critical evaluation of linezolid susceptibility testing using two automatedsystems (Vitek, Vitek2)*. 2007; Available from:https://www.researchgate.net/publication/5461584_Increasing_Incidence_of_Linezolid-Intermediate_or_-Resistant_Vancomycin Resistant_Enterococcus_faecium_Strains_Parallels_Increasing_Linezolid_Consumption.

Table 1. Characteristics and results (rates in %) of interlaboratory testing for the challenge panel NAP2.



Abbreviations: categorical agreement,CA; CA*, categorical agreement excluding fosfomycin; Broth microdilution, BMD; Disk diffusion,DD; extended-spectrum b-lactamase, ESBL; susceptible increased exposure, I; not realized,NR; percentage very major error, VME; percentage major error, ME; percentage minor error, minE; reference result by broth microdilution, Ref; resistant, R; sensitive, S.

Table 2. Categorical susceptibility rates and discrepancies per antibiotic for the challenge panel NACP2.

Group of strains	Antibiotic	BMD results				DD method					AUST method					Overall CA (%)
		n	S(%)	I(%)	R(%)	n	VME(%)	ME(%)	minE(%)	CA(%)	n	VME(%)	ME(%)	minE(%)	CA(%)	
Gram-negative bacilli	Temocillin	9	0	11	89	63	0	0	3.2	97.8	63	0	0	4.8	95.2	96.0
	Piperacillin-tazobactam	9	0	0	100	62	0	0	0	100	63	0	0	0	100	100
	Aztreonam	9	22	11	67	52	1.9	0	13.5	84.6	61	0	1.6	8.2	90.2	87.7
	Ceftazidime	9	11	0	89	61	1.6	0	0	98.4	62	0	0	0	100	99.2
	Cefotaxime/Ceftriaxone	9	11	0	89	62	1.6	0	0	98.4	56	0	1.8	0	98.2	98.3
	Cefepime	9	22	0	78	55	0	0	0	100	63	0	0	0	100	100
	Ertapenem	9	0	0	100	40	5	0	0	95	46	2.2	0	0	97.8	96.5
	Meropenem	9	22	11	67	70	1.4	0	17.1	81.5	70	1.4	0	18.3	80.3	80.9
	Ciprofloxacin/Levofloxacin	9	0	11	89	69	1.4	0	8.6	90	71	0	0	10.4	89.6	89.8
	Amikacin	9	44	11	56	70	0	8.6	0	91.4	70	1.4	7.2	0	91.4	91.4
	Gentamicin	9	33	0	67	54	1.8	3.7	0	94.5	69	0	11.1	0	89.9	91.3
	Tigecycline	9	44	0	56	NR	NR	NR	NR	NR	62	0	20.7	0	79.3	79.3
	Fosfomycin	9	22	0	78	23	0	13	0	87	48	21.3	6.4	0	72.3	77.1
	Colistin	9	56	0	44	NR	NR	NR	NR	NR	65	0	4.6	0	95.7	95.4
Gram-positive cocci	Oxacillin	1	100	0	0	3	0	0	0	100	7	0	0	0	100	100
	Ampicillin	2	0	0	100	14	14.3	0	0	85.7	10	0	0	0	100	91.6
	Cefoxitin	1	0	0	100	8	0	0	0	100	4	0	0	0	100	100
	Teicoplanin	3	66	0	33	8	0	0	0	100	19	0	0	0	100	100
	Vancomycin	3	33	0	66	12	0	0	0	100	24	0	4.2	0	95.8	97.2

	Linezolid	3	33	0	66	17	5.6	0	0	94.4	18	5.3	0	0	94.7	97.3
	Erythromycin	1	100	0	0	7	0	0	0	100	7	0	0	0	100	100
	Clindamycin	1	0	0	100	8	12.5	0	0	87.5	7	0	0	0	100	93.3
	Tetracycline	1	0	0	100	5	0	0	0	100	6	0	0	0	100	100
	Minocycline	1	100	0	0	5	0	0	0	100	4	0	0	0	100	100
	Tigecycline	3	100	0	0	10	0	0	0	100	9	0	0	0	100	100
	Gentamicin	3	0	0	100	9	0	0	0	100	0	0	0	0	100	100
	Gentamicin high dose	3	0	0	100	8	0	0	0	100	10	0	0	0	100	100
	Ciprofloxacin/Levofloxacin	1	0	100	0	7	0	0	0	100	4	0	0	0	100	100
	Trimethoprim-sulfamethoxazole	1	0	100	0	8	0	0	100	0	7	0	0	14.3	85.7	40.0
	Rifampicin	1	100	0	0	5	0	0	0	100	7	0	14.3	0	85.7	91.7
	Fusidic acid	1	100	0	0	8	0	14.3	0	85.7	7	0	0	0	100	92.4

Abbreviations: Automated antibiotic susceptibility testing, AUST; categorical agreement,CA; Broth microdilution, BMD; Disk diffusion,DD; susceptible increased exposure, I;Not realized,NR; percentage very major error, VME; percentage major error, ME; percentage minor error, minE; reference broth microdilution, BMD; resistant, R; susceptible, S.