European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-020-04059-4

ORIGINAL ARTICLE

Multicenter study of automated systems for colistin susceptibility testing

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Received: 12 August 2020 / Accepted: 30 September 2020

Abstract

Purpose Broth microdilution (BMD) stays as the reference testing method for determination of antimicrobial susceptibility testing (AST) to colistin and is considered essential for patient management and for monitoring of colistin resistance. This multicenter study aimed to evaluate the performance of automated systems for colistin AST among Enterobacterales as an alternative for BMD since the majority of laboratories use automated systems as first-line method.

Methods Twenty colistin resistant (COL-R) including 10 MCR producers and 10 colistin-susceptible (COL-S) Enterobacterales isolates were blindly tested for colistin susceptibility with the routine automated AST systems used by 8 laboratories (3 with BD Phoenix, 3 with Vitek2 and 2 with MicroScan). Additionally, 3 reference strains (*E. coli* ATCC 25922, *E. coli* NCTC 13846, and one COL-R mcr-negative *K. pneumoniae* M/14750) were tested in triplicate by each laboratory.

Results and conclusion Results were compared with BMD performed at the reference laboratory. BD Phoenix and MicroScan automated AST systems provide accurate and reproducible categorical results for the testing of colistin in Enterobacterales. However, Vitek2 system showed poor performance for the detection of COL-R isolates especially those with MICs close to the susceptibility breakpoint (categorical agreement of 88% and precision categorical agreement of 81%).

Keywords Colistin · Enterobacterales · Antimicrobial susceptibility testing · Automated systems · MicroScan · BD Phoenix · Vitek2

Introduction

The multidrug antimicrobial resistance of Gram-negative has become a major public health issue impacting negatively on the clinical outcome of infected patients. Colistin is an old antibiotic that regained popularity as a last resort drug to treat infections caused by these multidrug-resistant organisms (MDROs). The emerging resistance to colistin (COL-R), whether arising by chromosomal mutations or by plasmid-mediated (MCR) mechanisms (which in all cases lead to modifications of the lipopolysaccharides of the outer membrane in gram-negative bacteria), is now recognized in animals, food animal products, and human samples, and it represents a new threat for global public health [1, 2]. A reliable and

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Published online: 06 October 2020

reproducible antimicrobial susceptibility testing (AST) method is required for patient management and for monitoring of colistin resistance. Broth microdilution (BMD) is the recommended method for MIC determination of colistin both by CLSI and EUCAST since 2016. However, routine clinical laboratories rarely perform reference BMD, which requires freshly prepared or frozen antibiotic solutions. Other susceptibility testing methods, such as agar dilution, disk diffusion, and gradient diffusion, are currently not recommended [3–5]. A number of more user-friendly commercial automated AST systems derived from BMD method have become available. This multicenter study aimed to evaluate the performance of routine automated systems used in Belgium for colistin AST among Enterobacterales.

Material and methods

A panel of twenty colistin resistant (COL-R) including 10 MCR producers and of 10 colistin-susceptible (COL-S) non-duplicated human clinical isolates belonging to different species among Enterobacterales (Table 1) was blindly tested for colistin susceptibility with the routine automated AST systems used by 8 laboratories (3 with BD Phoenix NMIC-408 panel [Becton-Dickinson, Sparks, USA], 3 with Vitek2 AST-N366 cards [bioMérieux, Marcy l'Etoile, France], and 2 with MicroScan Neg MIC EN52 panel [Beckman Coulter, CA, USA]).

The clinical isolates were characterized for *mcr* genes at the national reference center using an in-house multiplex PCR as described by the European Centre for Disease Prevention and Control [6, 7]. The two *E. coli* strains carrying the *mcr-1.5* or *mcr-3.2* gene were provided by the French National Reference Center for Antibiotic Resistance [8, 9]. Species identification was verified at participating laboratories using local identification method.

The same manufacturing lot of AST cards/panels was tested at different laboratories for each of the three systems according to the manufacturers' instructions. Results were compared with BMD Sensititre [Customized Sensititre panels (BEGN4A/4B); Thermo Fisher Scientific, MA, USA] considered the reference method and categorized according to EUCAST interpretative guidelines [10] (susceptible ≤ 2 mg/L and resistant > 2 mg/L). The range of tested concentrations for each method was as follows: for BMD Sensititre ≤ 0.25 to > 64 mg/L; for BD Phoenix ≤ 1 to > 4 mg/L; for Vitek2 ≤ 0.5 to > 16 mg/L; and for MicroScan ≤ 2 to > 4 mg/L. Isolates with an invalid result for colistin would be retested with the same method with the same lot of cards. The accuracy of each automated system compared with BMD was calculated with the following parameters: categorical agreement (CA, agreement in the categorical interpretation of the MICs), very major errors (VME; false susceptibility), and major errors (ME; false resistance). A method was considered acceptable for categorical reporting only if all the following criteria were fulfilled: CA ≥ 90% and VME < 1.5% and ME < 3% [11, 12].

Additionally, colistin susceptibility of three reference strains (*E. coli* ATCC 25922 (MIC 0.25–2 mg/L), *E. coli* NCTC 13846 (MIC 4–8 mg/L) [5], and *K. pneumoniae M/14750* (MIC 16–32 mg/L), a COL-R *mcr*-negative (chromosomal mutation in the regulator gene *mgrB*) provided by the National External Quality Assessment Program), was tested in triplicate at different days by each laboratory to assess the intra- and inter-laboratory reproducibility of the testing methods. The reproducibility was estimated by calculating the precision categorical agreement (PCA) defined as the agreement with the interpretative results (susceptible/resistant) of the precision test isolate using EUCAST interpretive criteria. A method would be considered reproducible for category reporting if PCA was ≥ 95% [12].

Results

Colistin reference MICs ranged from 0.5 to 2 mg/L for COL-S and from 4 to 32 mg/L for COL-R strains. A total of 90, 90, and 60 colistin results for the 30 collection strains were obtained by Vitek2, BD Phoenix, and MicroScan, respectively (Table 1). No invalid results were observed in this study. While a CA of 100% was observed for BD Phoenix and for MicroScan, Vitek2 failed 11 times (VME of 12%) to detect 9 COL-R isolates giving a CA of 88% [CI 95%: 79.4% - 93.0%]. The majority of COL-R errors were observed with *E. coli* (7/9) including six *mcr*-expressing strains having MICs that straddle the breakpoint. All these *E. coli* isolates had MICs of 4 mg/L by BMD Sensitre, while one MIC of ≤ 0.5 mg/L and six MICs of 2 mg/L were obtained with Vitek2. Among the 11 VME by Vitek2, 9 were observed from the same center Hospital VK_3. Two isolates, *E. aerogenes* S17 and *E. cloacae* S19, showing MICs of 8 and 32 mg/L by BMD Sensitre, respectively, yielded 2 VME each with Vitek2 from two different laboratories (Hospital VK_1 and Hospital VK_2).

A total of 27, 27, and 18 results for the 3 reference strains were obtained by Vitek2, BD Phoenix, and MicroScan, respectively. The PCA was 100% for BD Phoenix and for MicroScan, while Vitek2 yielded 5 incorrect false susceptible results for the *E. coli* NCTC 13846 strain (two errors from Hospital VK_2 and three errors from Hospital VK_3) resulting in a PCA of 81% [CI 95%: 63.3–91.8%] (Table 2).

Discussion

Colistin is one among the few last therapeutic options for infections with multidrug-resistant Gram-negative bacteria.

Table 1 Characteristics and colistin susceptibility results of the 30 collection strains by the 3 automated systems

Collection strains				MIC (mg/L) by BD Phoenix ($n = 90$)			MIC (mg/L) by MicroScan ($n = 60$)			MIC (mg/L) by Vitek2 ($n = 90$)				
Strain no	Species	Reference result (*mcr* gene)	Reference MIC (mg/L)	≤ 1	4	> 4	≤ 2	4	> 4	≤0.5	2	4	8	≥16
S01	*E. coli*	R (*mcr*-1)	4		3				2		**1**		2	
S02	*E. coli*	R (*mcr-1)*	4			3			2				1	2
S03	*E. coli*	R (*mcr-1)*	4		3				2	**1**			2	
S04	*E. coli*	R (*mcr-1)*	4		3				2		**1**		2	
S05	*E. coli*	R (*mcr-1*)	4		3				2		**1**	1	1	
S06	*E. coli*	R (*mcr-1*)	4		2	1			2		**1**		2	
S07	*E. coli*	R (*mcr-1.5)*	4		3				2		**1**	2		
S08	*E. coli*	R (*mcr-3.2*)	4		2	1			2			1	2	
S09	*K. pneumoniae*	R (*mcr-1*)	16			3			2					3
S10	*Salmonella sp.*	R (*mcr-1*)	8			3		1	1					3
S11	*E. coli*	R	8			3			2					3
S12	*E. coli*	R	4		2	1		1	1		**1**		2	
S13	*E. coli*	R	4		1	2			2			1	2	
S14	*K. pneumoniae*	R	32			3			2					3
S15	*K. pneumoniae*	R	32			3			2					3
S16	*K. pneumoniae*	R	16		1	2			2				1	2
S17	*E. aerogenes*	R	8		2	1			2		**2**	1		
S18	*E. aerogenes*	R	16			3			2					3
S19	*E. cloacae*	R	32			3		1	1	**2**				1
S20	*E. cloacae*	R	4		2	1		2					3	
S21	*K. pneumoniae*	S	0.5	3			2			2	1			
S22	*K. pneumoniae*	S	0.5	3			2			3				
S23	*K. pneumoniae*	S	0.5	3			2			3				
S24	*E. coli*	S	0.5	3			2			3				
S25	*E. coli*	S	0.5	3			2			3				
S26	*E. coli*	S	0.5	3			2			3				
S27	*E. aerogenes*	S	2	3			2			3				
S28	*C. freundii*	S	1	3			2			3				
S29	*E. cloacae*	S	0.5	3			2			3				
S30	*K. oxytoca**	S	1	3			2			3				

S, susceptible; R, resistant

*One laboratory has identified S30 as *K. pneumoniae* using MicroScan Neg MIC EN52 panel (verified twice on two referrals) while the strain was identified as *K. oxytoca* using MALDI-TOF MS by all other laboratories

Consequently, accurate and reliable testing of colistin susceptibility has now become essential for clinical laboratories worldwide. In this multicenter study, we evaluated the performance testing of three commercial semi-automated systems used in eight laboratories for colistin susceptibility testing. The rationale for evaluating these tests was to validate a method as accurate as BMD that would be more convenient for routine clinical laboratory use. The same lot of AST cards was distributed to these different laboratories for each of the three automated AST systems to minimize between-lots production variability. Automated AST systems were easy to operate with short hands-on time (~ 2–5 min) compared with the more labor-intensive and expensive BMD. BD Phoenix and MicroScan automated AST systems provided accurate and reproducible categorical results for the testing of colistin in Enterobacterales. Vitek2 showed unacceptable rates of false-susceptible results, especially with *E. coli* isolates which had MICs of 4 mg/L by BMD and with the *Enterobacte*r spp. isolates. The high rate of VME exceeded the CLSI recommendation of ≤ 3.0% [11]. Use of this system without confirmation would lead to an underestimation of colistin resistance. Based on our observation of COL-R isolates yielding a MIC

Table 2 Characteristics and colistin susceptibility results of the 3 reference strains by the 3 automated systems

Reference strains		MIC (mg/L) for *E. coli* ATCC 25922 * (target MIC range 0.25–2 mg/L)[a]			MIC (mg/L) for *E. coli* NCTC 13846 * (target MIC range 4–8 mg/L)[b]				MIC (mg/L) for *K. pneumoniae* M/14750 * (target MIC range 16–32 mg/L)[c]	
Automate	**Laboratory**	**≤ 0.5**	**≤ 1**	**≤ 2**	**2**	**4**	**8**	**> 4**	**> 4**	**≥ 16**
BD Phoenix	Hospital BD_1		3			3			3	
	Hospital BD_2		3			3			3	
	Hospital BD_3		3			3			3	
MicroScan	Hospital MS_1			3				3	3	
	Hospital MS_2			3		1		2	3	
Vitek 2	Hospital VK_1	3					3			3
	Hospital VK_2	3			**2**	1				3
	Hospital VK_3	3			**3**					3
Total		**9**	**9**	**6**	**5**	**11**	**3**	**5**	**15**	**9**

*MIC values obtained by BMD Sensitre customized panels (as reference method in this study): 0.5 mg/L for *E. coli* ATCC 25922, 4 mg/L for *E. coli* NCTC 13846 and 16 mg/L for *K. pneumoniae* M/14750

[a] Acceptable MIC values obtained by the automated systems for *E. coli* ATCC 25922: ≤ 1 mg/L (BD Phoenix); ≤ 2 mg/L (MicroScan); ≤ 0.5 mg/L and 2 mg/L (Vitek 2)

[b] Acceptable MIC values obtained by the automated systems for *E. coli* NCTC 13846: 4 and > 4 (BD Phoenix and MicroScan); 4 mg/L and 8 mg/L (Vitek 2)

[c] Acceptable MIC values obtained by the automated systems for *K. pneumoniae* M/14750 > 4 (BD Phoenix and MicroScan); ≥ 16 mg/L (Vitek 2)

of 2 mg/L obtained 8 times by the Vitek2, we would recommend that a colistin result with MIC of 2 mg/L by Vitek2 should be confirmed by an alternative BMD method until revised cards with validated technical improvements by the manufacturer are available. However, no false-resistant results were detected in our study, implying that resistant results could be considered valid for the three systems. These results were in line with two other recent studies which also highlighted poor performances of Vitek2 with VME rates ranging from 29.2 to 36.0% for colistin susceptibility testing [13, 14].

Controversial findings have been reported about the reliability of Vitek2 and BD Phoenix for colistin susceptibility testing. It should be emphasized that performance results could greatly differ depending on the resistance characteristics of the selected organisms (MIC values close or not from the breakpoint value, diversity of the species and genera included). Indeed, studies in which favorable results were obtained for Vitek2 did notoriously only include a very limited number of MCR-producing COL-R *E. coli* isolates with MIC value (4 mg/L) close to the current EUCAST susceptibility breakpoint (≤ 2 mg/L) [15, 16]. On the other hand, our results regarding BD Phoenix contrast in comparison with those by Pfennigwerth et al. reporting a CA of 92.0% and high rate of VMEs with isolates of the *E. cloacae* complex [14], known for the expression of hetero-resistance to colistin [17]. One major drawback is the low representation of *E. cloacae* complex in our collection strains which would challenge the performance of the automated AST systems for colistin susceptibility testing.

Of note, 9 of 11 very major errors of Vitek2 were observed in one particular laboratory (Hospital VK_3). We cannot explain this discrepancy, since the same lot of AST cards and the same strains were tested by skilled staff. Moreover, regular AST internal quality control using recommended QC strains (including *E. coli* ATCC 25922), calibration of the device measuring the bacterial suspension density, and instrument maintenance were performed by the laboratories as part of their continuous quality assurance program. However, this study strongly supports that in addition to a colistin-susceptible principal QC strain (*E. coli* ATCC 25922), testing of a colistin-resistant strain with MIC close to the breakpoint such as *E. coli* NCTC 13846 (*mcr-1* positive; MIC 4–8 mg/L) should be mandatory as internal quality control for colistin AST as per by EUCAST and CLSI recommendations [3].

In summary, a collection of Enterobacterales isolates with well-characterized colistin susceptibility was challenged against three semi-automated AST systems using a standardized study protocol in a multicenter setting. Our study showed relevant differences in performance between the instruments and, moreover, testing by Vitek2 generated unacceptable errors. Therefore, we suggest confirming colistin susceptibility result by a broth microdilution method whenever clinical use is considered. Prospective evaluation of these systems on consecutive clinical isolates in different settings including areas with higher COL-R prevalence should be performed in future studies.

Acknowledgments Members of the National Antibiogram Committee: Olivier Denis, Stefanie Desmet, Youri Glupczynski, Marie Hallin, Te-

Din Huang, Bénédicte Lissoir. Koen Maegerman, Pierrette Melin, Hector Rodriguez, Anne-Marie Van Den Abeele, Kris Vernelen.

We thank Laurent Dortet (CNR de la Resistance aux antibiotiques – France) for providing part of the mcr-positive isolates.

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). This study was also supported by a special funding in the setting of emerging infectious diseases of public health concern through the Medico-Technical Advisory (MTAB) of the Belgian Public Health Institute (Sciensano).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent Not applicable. Given that this study was performed without accessing patient information, approval of the ethics committee was not required.

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