Evaluation of three broth microdilution systems to determine colistin susceptibility of Gram-negative bacilli

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Background: The broth microdilution (BMD) method is currently the recommended technique to determine susceptibility to colistin.

Objectives: We evaluated the accuracy of three commercialized BMD panels [Sensititre (ThermoFisher Diagnostics), UMIC (Biocentric) and MicroScan (Beckman Coulter)] to determine colistin susceptibility.

Methods: A collection of 185 isolates of Gram-negative bacilli (133 colistin resistant and 52 colistin susceptible) was tested. Manual BMD according to EUCAST guidelines was used as the reference method, and EUCAST 2017 breakpoints were used for susceptibility categorization.

Results: The UMIC system gave the highest rate of very major errors (11.3%) compared with the Sensititre and MicroScan systems (3% and 0.8%, respectively). A high rate of major errors (26.9%) was found with the MicroScan system due to an overestimation of the MICs for the non-fermenting Gram-negative bacilli, whereas no major errors were found with the Sensititre and UMIC systems.

Conclusions: The UMIC system was easy to use, but failed to detect >10% of colistin-resistant isolates. The MicroScan system showed excellent results for enterobacterial isolates, but non-susceptible results for non-fermenters should be confirmed by another method and the range of MICs tested was narrow. The Sensititre system was the most reliable marketed BMD panel with a categorical agreement of 97.8%.

Introduction

Occurrence of MDR Gram-negative bacilli is a growing concern and has led to a renewed interest in the use of polymyxins (colistin, polymyxin B) as last-resort antibiotics.[1] However, colistin susceptibility testing is currently challenging, with the disc-diffusion method and the Etest systems giving high rates of false-susceptibility results (up to 30%).[2] Since March 2016, the joint CLSI–EUCAST Polymyxin Breakpoints Working Group has recommended the broth microdilution (BMD) method as the reference method to determine susceptibility to colistin (www.eucast.org).[3] However, this method is often not implementable in routine practice due to a laborious manual preparation. Marketed BMD panels such as Sensititre (ThermoFisher Diagnostics, Dardilly, France), UMIC (Biocentric, Bandol, France) and MicroScan (Beckman Coulter, Villepinte, France) systems may be considered as interesting alternatives, but the performances of these systems for detecting colistin resistance have not been carefully evaluated.

To date, only two studies have investigated the performance of the MicroScan system for colistin susceptibility testing. One study included *Acinetobacter* spp. isolates only and showed a categorical agreement (CA) of 87.3%,[4] while the second included *Klebsiella pneumoniae* isolates only and showed a susceptibility of 88.1%.[5] A single study has evaluated the Sensititre method, and a 96% CA with the reference BMD method was found, with no false-susceptibility results reported.[6] The performance of the UMIC system for determining colistin susceptibility has never been assessed.

The objective of this study was therefore to evaluate and compare the performances of the Sensititre system, the UMIC system and the MicroScan system for determining colistin susceptibility using a collection of 185 isolates of Gram-negative bacilli.

Materials and methods

Bacterial isolates

A collection of 185 Gram-negative isolates was tested. Fifty-two isolates were susceptible to colistin, 19 isolates belonged to a genus naturally resistant to colistin (*Proteus*, *Providencia*, *Morganella*, *Serratia* and *Hafnia*) and 114 isolates belonged to various species (*Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Salmonella enterica*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*) and presented acquired resistance to colistin. The colistin-resistant isolates were collected worldwide from clinical samples. Identification was performed using the Microflex bench-top MALDI-TOF mass spectrometer (Bruker, Champs-sur-Marne, France). None of the strains was clonally related.

Molecular genotyping for colistin resistance

Colistin-resistant isolates were screened for the plasmid-mediated colistin resistance genes *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* as described previously.[7–11] Chromosomally encoded mutations in genes potentially involved in colistin resistance (*pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB* and *crrB* genes) were also searched for as described previously.[12–18]

Colistin susceptibility testing

Preparation of the BMD panels

The features of the various commercialized panels used in this study are summarized in Table 1. Each isolate was tested with the four techniques in the same experiment and from the same starting bacterial suspension with a turbidity equivalent to that of a 0.5 McFarland standard. The panels were prepared as recommended by each manufacturer. The BMD reference method was performed according to the EUCAST guidelines (www.eucast.org) in 96-well non-treated polystyrene microplates (ref. 82.1582.001; Sarstedt, Nümbrecht, Germany). Dilutions of colistin sulfate (ref. C4461; Sigma–Aldrich, St Louis, MO, USA) ranging from 0.12 to 128 mg/L were made extemporaneously in CAMHB (ref. YT3462; ThermoFisher Diagnostics), without addition of polysorbate 80 (Tween 80), and a final concentration of 5×10^5 cfu/mL of bacteria was added to each well. All the BMD panels were read visually after 18–20 h of incubation at 35±2°C. The colistin-susceptible ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* strains were included in all the experiments as quality controls.

Analysis of the results

It should be noted that all inspections were visual and not automated. Categorization of the isolates was performed on the basis of the EUCAST susceptible and resistant breakpoints ($\leq$2 and $>$2 mg/L, respectively). For *S. maltophilia* isolates, the same breakpoint of 2 mg/L was arbitrarily chosen, given the lack of EUCAST breakpoints.

Results obtained with the three commercialized BMD panels were compared with those obtained with the BMD reference method. Discrepancies were determined for each method in order to assess how accurately they determined susceptibility to colistin. Isolates for which discrepant susceptibility results were observed were retested twice with the four methods. Unsolved discrepancies were then maintained in the database for performance evaluation. Errors were ranked as follows: very major errors (VMEs), for isolates categorized as susceptible using the marketed panel, but resistant by the BMD reference method (false-susceptibility result); and major errors (MEs), for isolates categorized as resistant using the marketed panel, but susceptible by the BMD reference method (false-resistant result). The number of resistant and susceptible isolates were used as denominators for VME and ME calculations, respectively. CA was defined as the percentage of isolates classified into the same category by the commercialized panel compared with the BMD reference method. Acceptance criteria that provide the requirements and specifications to evaluate performances of antimicrobial susceptibility test devices were those defined by the ISO standards (VMEs and MEs must be $\leq$3% and CA must be $\geq$90%).[19]

Results

The 133 colistin-resistant Gram-negative isolates analysed in this study presented various levels of resistance (MICs ranging from 4 to $>$128 mg/L by using the BMD reference method) (Table 2).

The tested isolates exhibited various genotypes conferring colistin resistance, i.e. related to various chromosomal mutations, and/or acquisition of plasmid-mediated genes (Table 2). Thirty-five *K. pneumoniae* isolates presented mutations in *pmrAB*, *phoPQ*,

Table 1. Features of the BMD panels

	Sensititre	UMIC	MicroScan
Manufacturer	ThermoFisher Diagnostics	Biocentric	Beckman Coulter
Reference of the panel	FRCOL (custom plate)	UM-COL-040	NM44
Range of colistin concentrations tested (mg/L)	0.12–128	0.06–64	2–4
Description of the panel	96-well microplate to test only colistin[a]	12-well panel to test only colistin	96-well microplate: 2 wells to test colistin, the others to test additional antibiotics[a]
Number of strains tested by panel	8	1	1
Medium	CAMHB with TES (ref. YT3462)	CAMHB	water with PLURONIC
Inoculation	manual or semi-automated with the AIM™ automated inoculation delivery system	manual	semi-automated with the RENOK inoculator system
Incubation time (h)	18–24	18–24	16–20
Reading	visual, semi-automated with Vizion® or automated with Optiread®	visual	visual, semi-automated with autoSCAN-4 or automated with the WalkAway *plus* system

[a] Other panels testing colistin are also available.

Table 2. MIC results obtained with the four BMD methods

		Colistin MIC (mg/L)			
Species (number of isolates)	Genotype	reference BMD	Sensititre	UMIC	MicroScan
Isolates naturally resistant to colistin					
Proteus mirabilis ($n = 1$), *Proteus vulgaris* ($n = 1$), *Providencia stuartii* ($n = 1$), *Morgenalla morganii* ($n = 1$), *Serratia marcescens* ($n = 1$)	NA	>128	>128	>64	>4
H. alvei ($n = 2$)	NA	8	4–8	**2**	4 to >4
H. alvei ($n = 1$)	NA	8	8	4–8	>4
H. alvei	NA	16	16	16	>4
Hafnia paralvei	NA	4	4	4	4
H. paralvei ($n = 9$)	NA	8	4–8	4–8	>4
Isolates with acquired colistin resistance					
K. pneumoniae	plasmid-mediated *mcr-1* gene	8	4	**2**	>4
K. pneumoniae	PmrA G53C	32	16	32	>4
K. pneumoniae	PmrA G53S	16	16	16	>4
K. pneumoniae	PmrA G53S	32	16	16	>4
K. pneumoniae	PmrB T157P	8	8	8	>4
K. pneumoniae	PmrB T157P	16	8	16	>4
K. pneumoniae	PhoP D191Y	64	32	64	>4
K. pneumoniae	PhoQ R16C	128	64	64	>4
K. pneumoniae	MgrB C28 (MgrB truncated)	128	64	64	>4
K. pneumoniae ($n = 2$)	MgrB Q30 (MgrB truncated)	64	32	64	>4
K. pneumoniae	MgrB W47 (MgrB truncated)	32	16	16	>4
K. pneumoniae	MgrB W20R	16	16	16	>4
K. pneumoniae	MgrB M27K	16	16	32	>4
K. pneumoniae	MgrB C39Y	4	4	8	>4
K. pneumoniae	MgrB N42Y and K43I	4	4	8	>4
K. pneumoniae	MgrB I45T	32	32	16	>4
K. pneumoniae	MgrB P46S	16	8	32	>4
K. pneumoniae	MgrB IS*Ecp1*/*bla*CTX-M-15	32	16	32	>4
K. pneumoniae	IS*10R* in *mgrB* promotor	64	64	64	>4
K. pneumoniae	IS*Kpn14* in *mgrB* promotor	32	32	16	>4
K. pneumoniae	IS*102*-like in *mgrB* gene	128	64	>64	>4
K. pneumoniae	IS*5*-like in *mgrB* gene	32	16	32	>4
K. pneumoniae	IS*5*-like in *mgrB* gene	32	16	64	>4
K. pneumoniae	IS*Kpn13* in *mgrB* gene	64	32	>64	>4
K. pneumoniae	IS*Kpn14* in *mgrB* gene	8	8	16	>4
K. pneumoniae	IS*903b*-like in *mgrB* gene	64	32	64	>4
K. pneumoniae	*mgrB* Δnt23 (frameshift)	8	8	8	>4
K. pneumoniae	*mgrB* Δnt74 (frameshift)	32	32	64	>4
K. pneumoniae	*mgrB* Δnt100 (frameshift)	32	16	32	>4
K. pneumoniae	*mgrB* Δnt23/33 (frameshift)	128	64	>64	>4
K. pneumoniae ($n = 2$)	Δ*mgrB*	32	16	32	>4
K. pneumoniae	CrrB N141Y	128	64	>64	>4
K. pneumoniae	CrrB P151L	>128	>128	>64	>4
K. pneumoniae	CrrB G183V	32	32	>64	>4
K. pneumoniae	—	4	4	8	>4
K. pneumoniae	—	8	4	4	>4
K. pneumoniae	—	16	16	16	>4
K. pneumoniae	—	64	32	32	>4
K. pneumoniae	—	128	64	>64	>4
Klebsiella oxytoca	IS*Kpn26*-like in *mgrB* promotor	32	16	32	>4

Continued

Table 2. *Continued*

		Colistin MIC (mg/L)			
Species (number of isolates)	Genotype	reference BMD	Sensititre	UMIC	MicroScan
E. coli	plasmid-mediated *mcr-1* gene	4	4	**2**	>4
E. coli	plasmid-mediated *mcr-1* gene	8	4	**2**	>4
E. coli (*n* = 13)	plasmid-mediated *mcr-1* gene	8	4–8	4–8	>4
E. coli (*n* = 2)	plasmid-mediated *mcr-1* gene	16	8–16	8	>4
E. coli	plasmid-mediated *mcr-1* gene	64	64	32	>4
E. coli	plasmid-mediated *mcr-2* gene	4	4	**2**	>4
E. coli	PmrA G15R	8	8	8	>4
E. coli	PmrB R11L	4	4	**2**	>4
E. coli	PmrB L13P	16	8	8	>4
E. coli	PmrB G19E	4	4	4	4
E. coli	PmrB G22E	8	8	4	>4
E. coli	PmrB T114P	4	4	4	>4
E. coli	PmrB R138H	8	4	8	>4
E. coli	PmrB R138H, G305R	8	4	8	>4
E. coli	PmrB D152V	8	8	4	>4
E. coli	PmrB D315N	16	16	8	>4
E. coli	PmrB T114P+plasmid-mediated *mcr-1* gene	16	16	16	>4
E. coli (*n* = 4)	—	8	4	4	>4
E. coli	—	16	16	8	>4
E. cloacae	—	4	**2**	4	4
E. cloacae	—	8	8	16	>4
E. cloacae (*n* = 2)	—	16	8	16	>4
E. cloacae	—	16	16	32	>4
E. cloacae	—	64	32	>64	4
E. cloacae (*n* = 2)	—	>128	>128	>64	>4
E. cloacae	—	>128	>128	>64	>4
E. aerogenes	—	4	**2**	4	>4
E. aerogenes	—	4	4	4	4
S. enterica[a]	ArcA T111P, EnvZ Del13nt	4	**2**	**2**	4
S. enterica[a]	*pmrD* gene amplification, ArcA V9L, MurD S161T	4	**2**	**2**	>4
S. enterica	—	4	4	**1**	>4
S. enterica	plasmid-mediated *mcr-4-like* gene	4	4	**2**	>4
S. enterica	—	4	4	4	>4
S. enterica	plasmid-mediated *mcr-1* gene	16	8	8	>4
S. enterica[a]	*pmrD* gene amplification, ArcA L50Q, MurD D418Y, FliM L54Q	64	32	32	>4
P. aeruginosa	—	16	8	16	>4
P. aeruginosa	—	64	16	>64	>4
P. aeruginosa	—	128	64	>64	>4
S. maltophilia	—	4	4	8	>4
S. maltophilia	—	8	4	**1**	>4
S. maltophilia	—	8	16	**2**	>4
S. maltophilia	—	16	16	**0.12**	>4
S. maltophilia	—	32	16	**0.5**	>4
S. maltophilia	—	32	16	64	>4
S. maltophilia	—	64	32	4	>4
S. maltophilia	—	128	64	>64	>4
A. baumannii	PmrA E8D, PmrB R263P	128	64	64	**≤2**
A. baumannii	PmrA E54G, PmrB E140V	>128	>128	>64	>4

Continued

Table 2. *Continued*

		Colistin MIC (mg/L)			
Species (number of isolates)	Genotype	reference BMD	Sensititre	UMIC	MicroScan
A. baumannii	PmrB I163S	32	32	32	>4
A. baumannii	PmrB P170L	16	16	32	>4
A. baumannii	PmrB G260D	>128	>128	>64	>4
A. baumannii	PmrB Q265P	>128	>128	>64	>4
A. baumannii	—	16	16	16	>4
A. baumannii	—	>128	>128	>64	>4
Colistin-susceptible isolates					
K. pneumoniae ($n = 5$)	NA	≤0.12–0.25	0.5	0.25–0.5	≤2
K. pneumoniae	NA	1	1	0.5	≤2
K. oxytoca	NA	≤0.12	0.25–0.5	0.12–0.25	≤2
E. coli ($n = 5$)	NA	≤0.12	0.25–0.5	0.12–0.5	≤2
E. coli ($n = 6$)	NA	0.25	0.25–0.5	0.25–0.5	≤2
E. coli ($n = 2$)	NA	0.5	0.5	0.25–0.5	≤2
E. cloacae ($n = 4$)	NA	≤0.12	0.25–0.5	0.12–0.5	≤2
E. cloacae	NA	0.25	0.5	0.25	**4**
E. cloacae	NA	0.25	0.5	0.5	≤2
E. cloacae	NA	0.5	0.5	0.5	≤2
E. aerogenes ($n = 2$)	NA	≤0.12	0.5	0.12–0.25	≤2
S. enterica	NA	0.5	1	0.5	≤2
S. enterica	NA	2	2	2	≤2
Citrobacter koseri	NA	0.25	0.5	0.5	≤2
P. aeruginosa ($n = 2$)	NA	0.25	0.5	0.12–0.25	≤2
P. aeruginosa ($n = 8$)	NA	1	1–2	1	**4**
P. aeruginosa ($n = 2$)	NA	1	2	1	≤2
P. aeruginosa	NA	2	2	1	≤2
S. maltophilia	NA	0.5	0.5	0.5	≤2
S. maltophilia	NA	1	1	1	>**4**
S. maltophilia	NA	1	2	0.12	**4**
A. baumannii	NA	0.5	1	2	**4**
A. baumannii ($n = 2$)	NA	1	1–2	0.5	**4**
A. baumannii	NA	1	1	0.5	≤2

NA, not applicable.

The discordant results compared with the reference method are in bold.

[a]These *S. enterica* strains were genotyped by Hjort *et al.*[22]

mgrB or *crrB* genes, and 11 *E. coli* and 6 *A. baumannii* isolates exhibited mutations in *pmrAB* genes. Twenty-three enterobacterial isolates recovered worldwide carried plasmid-mediated colistin resistance *mcr-1*, *mcr-2* or *mcr-4-like* genes.

Comparison of the UMIC system with the BMD reference method

The UMIC system did not detect two colistin-resistant *Hafnia alvei* isolates that presented low MIC values of colistin (8 mg/L). It failed also to detect five isolates (three *E. coli*, a single *K. pneumoniae* and a single *Salmonella enterica*) possessing plasmid-mediated colistin resistance genes (*mcr-1*, *mcr-2* or *mcr-4-like*) and exhibiting a low level of resistance (MICs from 4 to 8 mg/L). Several enterobacterial isolates (a single *E. coli* and three *S. enterica* isolates) also exhibiting low MICs of colistin (MIC of 4 mg/L), but lacking the plasmid-mediated *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* genes, were also falsely detected as susceptible by the UMIC system. For all these isolates, the UMIC system gave MICs of 1 or 2 mg/L. Finally, four *S. maltophilia* isolates with higher colistin MICs (ranging from 8 to 32 mg/L) were not detected as resistant by the UMIC system, which gave lower MICs ranging from 0.12 to 2 mg/L. For one *S. maltophilia* isolate, the CA was correct, but the colistin MIC found with the UMIC system was 4 mg/L, while the MIC was actually 64 mg/L by manual BMD.

Comparison of the Sensititre system with the BMD reference method

This comparison showed a high rate of agreement. The Sensititre system detected correctly the 19 isolates naturally resistant to colistin and all the 23 enterobacterial isolates harbouring plasmid-mediated colistin resistance. Except for a single *P. aeruginosa* isolate, the MICs determined by the Sensititre system for all the resistant isolates were equal or only differed by one dilution when compared with those determined by the BMD reference method. However, two *Enterobacter* spp. and two *S. enterica* isolates presenting low MICs (4 mg/L) were falsely detected as susceptible by the Sensititre system, which gave MICs of 2 mg/L.

Comparison of the MicroScan system with the BMD reference method

The MicroScan system detected all the colistin-resistant isolates, except a single *A. baumannii* isolate showing an MIC of colistin of 128 mg/L, whereas the MicroScan system gave an MIC of ≤2 mg/L. However, 13 out of the 20 colistin-susceptible non-fermenters (13 *P. aeruginosa*, 3 *S. maltophilia* and 4 *A. baumannii*) were found resistant to colistin with the MicroScan system. Moreover, a single *Enterobacter cloacae* isolate was also found falsely resistant with an MIC of 4 mg/L.

Discussion

In this study, we evaluated three marketed BMD panels (Sensititre, UMIC and MicroScan) to determine MICs of colistin for a collection of 185 isolates of Gram-negative bacilli.

The UMIC system is a novel BMD panel for colistin susceptibility testing. Its advantages are the absence of any need for specific equipment and the form of the panel allowing colistin susceptibility to be tested for a single strain. However, the UMIC system failed to detect 15 isolates among the 133 colistin-resistant isolates, giving a high rate of VMEs (11.3%) (Table 3). It failed to detect colistin resistance in two *H. alvei* isolates (Table 2). In fact, we recently showed that the *H. alvei* species exhibits intrinsic resistance to colistin, though the resistance was of low level (MICs ranging from 4 to 16 mg/L).[20] UMIC failed also to detect nine other enterobacterial isolates with low-level resistance to colistin (MICs ranging from 4 to 8 mg/L), whereas the MICs of colistin reported with this system were close to the breakpoints (MICs of 1 or 2 mg/L, respectively) (Table 2). Five of those isolates possessed a plasmid-mediated colistin resistance determinant (*mcr-1*, *mcr-2* or *mcr-4-like*). This misdetection could underestimate the carriage of plasmid-mediated colistin resistance isolates, and thus participate in the spread of this resistance trait by delaying the rapid implementation of adequate hygiene measures. For those enterobacterial isolates with MICs of 1 or 2 mg/L, a more sensitive method should be used for confirmation, such as the BMD reference method or the newly developed rapid test (Rapid Polymyxin NP test).[21] The UMIC system also widely underestimated the MICs for isolates belonging to the *S. maltophilia* species, and failed to detect high-level resistance (MICs from 8 to 32 mg/L).

The performance of the Sensititre system was much better, but this system failed to detect four colistin-resistant isolates (one *E. cloacae* isolate, one *Enterobacter aerogenes* isolate and two *S. enterica* isolates) (Table 2), giving an acceptable VME rate of 3% (Table 3). The two tested *Salmonella* isolates not detected as colistin resistant were characterized as heteroresistant by Hjort *et al.*[22] This lack of detection could therefore be due to the presence of colistin-resistant subpopulations. Guérin *et al.*[23] also showed that some subpopulations of *E. cloacae* isolates may exhibit heteroresistance to colistin, which could explain a misdetection of that resistance for one of the isolates in our study. Of the 133 colistin-resistant isolates, the Sensititre system detected 129, underlining the accuracy of this system for detecting colistin resistance with a high CA of 97.8%. However, the main disadvantage is that this system is not adapted to test only one strain (at least eight strains need to be tested per panel) (Table 1).

The performance of the MicroScan system for detection of colistin resistance in Gram-negative bacilli was excellent regardless of the nature of the resistance mechanism. Only a single colistin-resistant *A. baumannii* isolate was not detected (Table 2), giving the lowest VME rate of 0.8% (Table 3). The main inconvenience of this panel was the narrow range of colistin concentrations tested, the absence of a panel to test colistin only, and the high rate of

Table 3. Performances of the BMD panels

						MicroScan[a]	
	Sensititre		UMIC			all the isolates	all isolates excluding *E. cloacae*, *Salmonella*, *Acinetobacter* and *Stenotrophomonas* isolates[a]
VME rate	Enterobacteriaceae	3.5% (4/114)	Enterobacteriaceae	9.6% (11/114)	Enterobacteriaceae	0% (0/114)	0% (0/105)
	NFGNB	0% (0/19)	NFGNB	21.1% (4/19)	NFGNB	5.3% (1/19)	0% (0/3)
	all species	3.0% (4/133)	all species	11.3% (15/133)	all species	0.8% (1/133)	0% (0/98)
ME rate	Enterobacteriaceae	0% (0/32)	Enterobacteriaceae	0% (0/32)	Enterobacteriaceae	3.1% (1/32)	0% (0/23)
	NFGNB	0% (0/20)	NFGNB	0% (0/20)	NFGNB	65.0% (13/20)	61.5% (8/13)
	all species	0% (0/52)	all species	0% (0/52)	all species	26.9% (14/52)	22.2% (8/36)
CA rate	Enterobacteriaceae	97.3% (142/146)	Enterobacteriaceae	92.5% (135/146)	Enterobacteriaceae	99.3% (145/146)	100% (128/128)
	NFGNB	100% (39/39)	NFGNB	89.7% (35/39)	NFGNB	64.1% (25/39)	50% (8/16)
	all species	97.8% (181/185)	all species	91.9% (170/185)	all species	91.9% (170/185)	94.4% (136/144)

NFGNB, non-fermenting Gram-negative bacteria.

[a] The procedural manual of the MicroScan system indicates that results for *E. cloacae*, *Salmonella* and non-Enterobacteriaceae (except *Pseudomonas*) should not be reported for colistin.

false-resistance results found for non-fermenters (65%) (Table 3). This finding supports a previous report showing a high rate of false-resistance results in *Acinetobacter* species.[4] The global rate of MEs for the MicroScan system was thus 26.9%, whereas Sensititre and UMIC systems did not give MEs (Table 3). However, the procedural manual of the MicroScan panel indicates that results for *E. cloacae*, *Salmonella* and non-fermenting Gram-negative bacilli except *Pseudomonas* spp. should not be reported, and hence the MICs and categorization results are not provided for those species. By excluding the results for those species, the ME rate was lower (22.2% instead of 26.9%) (Table 3), but still not acceptable (>3%) because of a high rate of false resistance found for *P. aeruginosa* isolates (8/13). Therefore, non-susceptibility results for non-fermenters including *P. aeruginosa* should be confirmed by the BMD reference method.

Conclusions

This study showed that variable results of colistin MICs can occur depending on the BMD panels used. It revealed that the UMIC system is not reliable for detection of colistin resistance, especially for isolates with a low level of colistin resistance and for *S. maltophilia* isolates. The performance of the MicroScan system was excellent, but this system is not suited for testing the colistin susceptibility of non-fermenters because of a high rate of false resistance. The Sensititre system showed excellent concordance with the BMD reference method and was reliable for testing colistin susceptibility for all the species of Gram-negative bacilli tested.

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Transparency declarations

None to declare.

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