Evaluation of five commercial MIC methods for colistin antimicrobial susceptibility testing for Gram-negative bacteria

P 161

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Introduction

An accurate method for antimicrobial susceptibility testing for colistin is crucial in an era of increasing numbers of multi-resistant bacteria and the simultaneous increasing colistin resistance. EUCAST and CLSI have agreed on how to perform broth microdilution (BMD) for colistin (www.eucast.org). EUCAST breakpoints for Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. is S≤2, R>2 mg/L.

Objective

The objective of this study was to evaluate three commercial BMD methods and two gradient tests for colistin MIC determination using frozen BMD panel MICs as reference.

Methods

Antimicrobial susceptibility testing was performed on an international collection of Gram-negative bacteria (n=75) with colistin MICs of 0.25-128 mg/L: *Escherichia coli* (n=14), *Klebsiella pneumoniae* (n=18), *Pseudomonas aeruginosa* (n=21) and *Acinetobacter* spp. (n=22). Colistin MIC determination was performed according to the manufacturers' instructions on frozen BMD panels (Thermo Scientific), three BMD methods with freeze-dried antibiotics: SEMPA1 (custom Sensititre plate, Thermo Scientific), MICRONAUT-S and MICRONAUT MIC-Strip (MERLIN Diagnostika) and two gradient tests: Etest (bioMérieux) and MIC Test Strip (MTS, Liofilchem). Etest and MTS were tested on Oxoid (Thermo Fisher Scientific) and BBL (BD) Mueller-Hinton agar in parallel, and Etest also on the bioMérieux' MHE medium (as recommended by the manufacturer). Isolates with skipped wells for BMD were retested. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *E. coli* NCTC 13846 (*mcr-1* positive) were used as quality control (QC). Essential and categorical agreements were calculated according to ISO 20776-2 vs. EUCAST Breakpoint Tables v. 7.1 using colistin MICs on frozen panels as reference.

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	Organism	*E. coli* and *K. pneumoniae* (n=32)	*P. aeruginosa* (n=21)	*Acinetobacter* spp. (n=22)	All isolates (n=75)
	Colistin MIC range (mg/L)	0.25-32	0.25-128	0.5-32	0.25-128
Essential agreement (EA)	Sensititre custom plate*	27	19	20	**66 (96%)**
	MICRONAUT-S	31	21	20	**72 (96%)**
	MICRONAUT MIC-Strip	31	21	22	**74 (99%)**
	Etest, Oxoid MH	27	13	13	**53 (71%)**
	Etest, BBL MH	20	11	1	**32 (43%)**
	Etest, MHE	24	9	2	**35 (47%)**
	MTS, Oxoid MH	19	12	9	**40 (53%)**
	MTS, BBL MH	24	12	13	**49 (65%)**
Major Errors (ME)	Sensititre custom plate	1	1	2	**4**
	MICRONAUT-S	2	1	3	**6**
	MICRONAUT MIC-Strip	2	0	3	**5**
	Etest, Oxoid MH	2	0	0	**2**
	Etest, BBL MH	1	0	0	**1**
	Etest, MHE	2	0	0	**2**
	MTS, Oxoid MH	0	0	0	**0**
	MTS, BBL MH	0	0	0	**0**
Very Major Errors (VME)	Sensititre custom plate	0	0	0	**0**
	MICRONAUT-S	0	2	0	**2**
	MICRONAUT MIC-Strip	0	2	0	**2**
	Etest, Oxoid MH	0	6	6	**12**
	Etest, BBL MH	1	7	7	**15**
	Etest, MHE	0	5	4	**9**
	MTS, Oxoid MH	6	6	4	**16**
	MTS, BBL MH	5	6	7	**18**

Table 1. Essential and categorical agreements for colistin MIC tests for 75 Gram-negative bacteria with MICs on frozen broth microdilution panels as reference.
Essential agreement (EA) = MICs being within ± 1 dilution of reference MIC

* The total number of tests for calculation of EA was 28 for *E. coli*/*K. pneumoniae* and 19 for *P. aeruginosa* due to truncation at ≤0.25 and >32 mg/L.

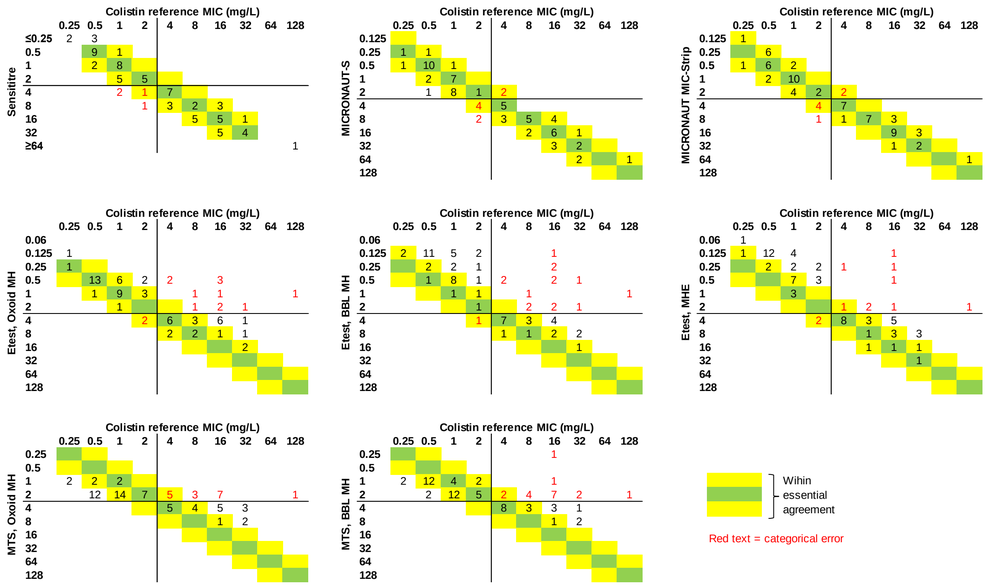

Figure 1. Correlation for colistin MIC values between test methods and reference broth microdilution for three BMD methods (Sensititre custom plate, MICRONAUT-S and MICRONAUT MIC-Strip) and gradient tests from two manufacturers (Etest and MTS). For gradient tests, results are shown per Mueller-Hinton (MH) agar.
MICs identical with reference MICs are highlighted in green. EUCAST breakpoints (S≤2, R>2 mg/L) are shown as lines.

Results

Essential and categorical agreements for the five methods are shown in **Table 1**. The correlation with reference MICs was good for all BMD methods but poor for gradient tests (**Figure 1**). Skipped wells occurred occasionally on all BMD panels and resulted in unreliable results unless retested. The BMD methods tended to overcall resistance to a small extent, resulting in a few major errors. Gradient tests generally underestimated MICs, resulting in a significant number of false susceptible results (very major errors). For Etest, very major errors were more abundant for *P. aeruginosa* and *Acinetobacter* spp. than for *E. coli* and *K. pneumoniae*.

For BMD methods, all QC results were within ranges, except for one reading below the range for MICRONAUT MIC-Strip with *E. coli* ATCC 25922 (**Table 2**). All MICs for MTS were within range for both QC strains. All Etest MICs were out of range for *E. coli* ATCC 25922 on BBL and MHE agar, and below range or in the lower part of the range for *P. aeruginosa* ATCC 27853. For *E. coli* NCTC 13846, all MICs were within ± 1 dilution of the expected 4 mg/L.

Table 2. Quality control results per MIC method and QC strain.
QC target values are highlighted in green and upper and lower limits in yellow.
Results out of range are shown in red text.

Colistin MIC method	Colistin MIC (mg/L)												
	Escherichia coli ATCC 25922					*Pseudomonas aeruginosa* ATCC 27853					*Escherichia coli* NCTC 13846**		
	0.125	0.25	0.5	1	2	0.25	0.5	1	2	4	2	4	8
Reference frozen panel			7	1				8			1	7	
Sensititre custom plate		4*	4				1	7				8	
MICRONAUT-S			5	3				4	4			7	1
MICRONAUT MIC-Strip	1		6	1				8			2	6	
Etest, Oxoid MH			2	5				7				8	
Etest, BBL MH	12						4	8				8	
Etest, MHE	7					3	4				5	3	
MTS, Oxoid MH				6	1			2	5			8	
MTS, BBL MH			1	6				4	3			8	

* ≤0.25 mg/L ** *mcr-1* positive

Conclusions

The commercial BMD methods reliably determined colistin MICs when no skipped wells were present. The correlation between gradient tests and reference MICs was poor, even when QC results were within range. This was probably related to the poor diffusion of colistin in agar. Based on the results of this study, EUCAST recommends laboratories to use BMD methods for colistin MIC determination and advice against the use of gradient tests at this point.

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