27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P007 MIC and disc diffusion methods - revisited

Category: 3c. Susceptibility testing methods

22 April 2017, 15:30 - 16:30
P0161

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

Erika Matuschek*[1], Jenny Ahman[1], Catherine Webster[1], Gunnar Kahlmeter[1]

[1]*Eucast Development Laboratory; Clinical Microbiology*

Background: 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. 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.

Material/methods: Antimicrobial susceptibility testing was performed on an international collection of Gram-negative bacteria (n=75) with colistin MICs 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 (TREK Diagnostics/Thermo Fisher Scientific), three BMD methods with freeze-dried antibiotics: SEMPA1 (custom Sensititre plate, TREK Diagnostics), Micronaut-S and Micronaut MIC-Strip (MERLIN Diagnostika) and two gradient tests: Etest (bioMerieux) 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 bioMerieux' MHE medium (as recommended by the manufacturer). Isolates with skipped wells for BMD were retested. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (≥7 tests per strain and method) were used as quality control (QC). Essential (EA) and categorical agreements were calculated according to ISO 20776-2 vs. EUCAST Breakpoint Tables v. 6.0, but with revised breakpoints for *Pseudomonas* spp. (S≤2, R>2 mg/L).

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 (EA 96-99%) but poor for gradient tests (43-71%). 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 (major errors). Gradient tests generally underestimated MICs, resulting in a significant number of false susceptible results (very major errors). 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. 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, whereas most MICs were within range for *P. aeruginosa* ATCC 27853.

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.

Table 1. Essential and categorical agreement for colistin MIC tests for 75 Gram-negative bacteria with MICs on frozen panels as reference.

Categorical agreement was calculated against EUCAST Breakpoint Tables v 6.0, but with the EUCAST revised breakpoint for *Pseudomonas* spp. (S≤2, R>2 mg/L).

	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
EA[1]	SEMPA1[4]	27	19	20	66 (96%)
	MICRONAUT-S	31	21	20	72 (96%)
	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	10	13	47 (63%)
ME[2]	SEMPA1	1	1	2	4
	MICRONAUT-S	2	1	3	6
	MIC-Strip	2	0	0	2
	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
VME[3]	SEMPA1	0	0	0	0
	MICRONAUT-S	0	2	0	2
	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

[1] Essential agreement (MICs being within ± 1 dilution of reference MICs)

[2] Major Errors (R with test method, S with reference method = false resistant)

[3] Very Major Errors (S with test method, R with reference method = false susceptible)

[4] 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.