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O0954 Can agar dilution be used for colistin MIC determination?

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Background: Colistin is a last resort agent for treatment of infections caused by multi-resistant Gram-negative bacteria, which makes it crucial that antimicrobial susceptibility testing (AST) results are correct. We have previously shown that colistin broth microdilution (BMD) is reliable, including several commercial products, whereas gradient tests perform poorly (ECCMID 2017, poster 161). The objective of this study was to evaluate agar dilution (AD) for colistin MIC determination of Gram-negative bacteria.

Materials/methods: Colistin AST was performed on the previously tested 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 reference MICs were determined using BMD according to ISO standard 20776-1. Agar dilution was performed on Mueller-Hinton agar (BBL/BD) according to EUCAST recommendations (E.Def 3.1) with two different inocula: 10^4 (standard) and 10^5 CFU/mL. Quality control (QC) was performed with E. coli ATCC 25922, P. aeruginosa ATCC 27853 and the colistin-resistant E. coli NCTC 13846 (mcr-1 positive). Essential (EA) and categorical (CA) agreements were calculated according to ISO 20776-2 vs. EUCAST Breakpoint Tables v. 7.1, 2017.

Results: The correlation between colistin agar dilution and reference BMD was better with the higher inoculum (10^5 CFU/mL) than with the standard inoculum (10^4 CFU/mL), Figure 1. EA was 73 and 80% for 10^4 and 10^5 CFU/mL, respectively, and the corresponding figures for CA was 85 and 88%. MICs tended to be lower with AD, with MICs being >1 dilution lower than BMD for 18/75 (24%) and 8/75 isolates (11%) with 10^4 and 10^5 CFU/mL, respectively. The number of false susceptible results (very major errors) were 9/75 with 10^4 and 6/75 with 10^5 CFU/mL. QC results were within ranges except for E. coli ATCC 25922 with the 10^4 inoculum.

Conclusions: Colistin agar dilution, using two different inocula, tended to underestimate colistin MICs, although the higher inoculum performed slightly better than standard inoculum. Unless further studies show improved results, EUCAST will not recommend agar dilution for colistin MIC determination. Our results also indicate that there may be problems developing screening agar plates for detection of colistin resistant isolates in patient samples.

Figure 1. Correlation between agar dilution and reference broth microdilution for a) 10^4 CFU/mL inoculum (standard) and b) 10^5 CFU/mL inoculum for 75 Gram-negative bacteria.

MICs identical with reference MICs are highlighted in green and MICs within essential agreement (EA, within ± 1 dilution of reference MICs) are highlighted in green and yellow. EUCAST breakpoints (susceptible ≤2, resistant >2 mg/L) are shown as lines.

a)

Colistin reference MIC (mg/L)

	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128
0.06			1									
0.125				3	1							
0.25			1	7								
0.5				4	5	1			2			
1					9	3	2		1			
2					1	1		1	2			1
4						1	3	2	1	1		
8						1	5	2	2	1		
16								1	5			
32								1		2		
64										1		
128												

b)

Colistin reference MIC (mg/L)

	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128
0.06												
0.125												
0.25			2	3								
0.5				8	2							
1				3	8	3	1		2			
2					6	1	1	1	1			
4						1			2			
8						1	8	2	1			1
16							2		2			
32						1		2	5			
64									2	2		
128										1		