

Development and validation of simple tests (agar spot, colistin drop, 1ml-broth disk elution MIC and tablet pre-diffusion) as an alternative to improve accuracy in screening chromosomal and plasmid-mediated colistin resistance in gram-negative bacilli.

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Background

For routine colistin susceptibility testing, CLSI/EUCAST recommends that only broth microdilution (BMD) should be used, however it could be difficult to perform in resource-poor settings. Aims: to develop and validate tests for colistin resistance screening: an agar spot test (SPOT) (addition of a unique concentration of colistin solution-CSOL- on an agar plate), colistin drop (DROP) test (application of a single drop of CSOL on the agar surface), a 1ml volume-based broth disk elution (DELU) MIC and the tablet pre-diffusion (PREDIF) test.

Material/Methods

CLSI/EUCAST reference colistin BMD was performed against 260 clinical isolates (% resistance): 30 *Acinetobacter*-ACI- (43%), 30 *P. aeruginosa*-PAE- (37%), 200 Enterobacteriaceae (57.5%) (5 *Citrobacter*, 19 *Enterobacter*-ENT-, 106 *E. coli*-ECO-, 59 *K. pneumoniae*-KPN-, 5 *K. oxytoca*, 6 *Salmonella*-SAL-). EUCAST breakpoints (susceptible $\leq 2.0\mu\text{g/ml}$; resistant $> 2.0\mu\text{g/ml}$) were used. Isolates were screened for *mcr-1* by PCR (63 positive, 1/63 BMD susceptible). Screening tests: 1-SPOT: Mueller-Hinton agar (MHA) plate (Difco) supplemented with 2.0 or 3.0 $\mu\text{g/ml}$ (final concentration) of colistin (Sigma) and swabbed within 1 cm^2 spot of the tested strain (0.5 McFarland). 2-COLTEST, commercial SPOT (Britania). 3- PREDIF: 10 μg colistin tablet (Rosco) following manufacture recommendations. 4-DROP: 10 μl of a 16 $\mu\text{g/ml}$ CSOL dripped on the surface of a MHA plate, previously inoculated with a lawn of tested strain (0.5 McFarland). CSOL was made by dissolving powder or by elution of eight 10 μg disks (BD) in 5ml CA-MHB (Difco). 5-DELU: 1, 2 or 4 10 μg disks were added to respective glass tubes containing 10ml of CA-MHB (1, 2, 4 $\mu\text{g/ml}$ CSOL, respectively) and subsequently fractionated in 1ml glass tubes. 5 μl of 0.5 McFarland inoculum was added to each 1ml CSOL tubes and to one control (1ml CA-MHB). Performances against BMD were evaluated (FDA criteria).

Results. Fig:

Test	No. of strains included in the validation (% resistance)	Categorical agreement (CA)	Very mayor errors (VME)	Mayor errors (ME)	Minor errors	FDA criteria required ^a
SPOT 2.0 $\mu\text{g/ml}$	260 (53%)	96.8	0	6.6 ^b	NC	CA: >89.9 VME: <2.86 ME: <3.0
SPOT 3.0 $\mu\text{g/ml}$	260 (53%)	99.6	0.7 ^c	0	NC	
COLTEST	260 (53%)	100	0	0	NC	
PREDIF	260 (53%)	98.8	0	0	1,2 ^d	
DROP (powder)	225 (57%)	98.2	2.15 ^e	1 ^f	NC	CA: >89.9 VME: <2.31 ME: <3.0
DROP (disk elution)	225 (57%)	97.8	2.15 ^e	2 ^g	NC	
DELU	94 (50%)	98.9	0	2.12 ^h	NC	CA: >89.9 VME: 0 ME: <3.0

NC: not correspond. Test interpretation: SPOT/COLTEST: susceptible, no growth; resistant, >1 colony growth. PREDIF: manufacture breakpoints. DROP: susceptible, halo; resistant, no halo or halo with colonies within the inhibition zone. DELU: MIC as the lower colistin concentration that prevents visible growth.

a: number of VME as a function of the total number of resistant organisms tested. b: 2 ACI, 4 PAE, 2 KPN. c: 1 ECO (*mcr-1*, BMD susceptible). d: 1 KPN, 1 SAL, 1 PAE intermediate by pre-diffusion, resistant by BMD. e: 2 ENT (hetero-resistant), 1 PAE. f: 1 PAE. g: 2 PAE. h: 1 ECO (*mcr-1*, BMD susceptible).

Conclusions. Only SPOT-2.0µg/ml did not achieve adequate performance due to false-resistant non-fermenting bacilli, but could be used safely in Enterobacteriaceae. We have developed/validated five methods, SPOT-3.0µg/ml, COLTEST, DROP, DELU and PREDIF, suited to the systematic screening of resistance to colistin in gram negative bacilli with a performance similar to the reference BMD. These screening tests can be routinely performed in addition to the tests currently in use (Vitek, Phoenix, etc.)