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# 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 <=2.0µg/ml; resistant >2.0µg/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 3.0µg/ml (final concentration) of colistin (Sigma) and swabbed within 1cm$^2$ spot of the tested strain (0.5 McFarland). 2-COLTEST, commercial SPOT (Britania). 3- PREDIF: 10µg colistin tablet (Rosco) following manufacture recommendations. 4-DROP: 10µl of a 16µg/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 10µg disks (BD) in 5ml CA-MHB (Difco). 5-DELU: 1, 2 or 4 10µg disks were added to respective glass tubes containing 10ml of CA-MHB (1, 2, 4µg/ml CSOL, respectively) and subsequently fractionated in 1ml glass tubes. 5µl 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 | validation (% resistance) | agreement (CA) | errors (VME) | Mayor errors (ME) | Minor errors | FDA criteria required[a] |
|---|---|---|---|---|---|---|
| SPOT 2.0µg/ml | 260 (53%) | 96.8 | 0 | 6.6[b] | NC | CA: >89.9 VME: <2.86 ME: <3.0 |
| SPOT 3.0µg/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.)