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Screening culture method for reliable detection of colistin heteroresistance in clinical isolates of *Enterobacter cloacae* complex

poster 1768

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Background

Members of the *Enterobacter cloacae* complex (ECC) are major opportunistic pathogens, especially among ICU patients. Increasing antibiotic resistance in Gram-negative bacteria has renewed the clinical interest in colistin (CS).

It has been recently demonstrated that the CS heteroresistance (CS-HR) (Figure 1) in ECC could contribute to antibiotic treatment failure (1).

Detection of CS-HR is challenging with methods commonly used for in vitro susceptibility testing while the reference method (i.e. population analysis profiling [PAP]) is time-consuming and labor-intensive (2).

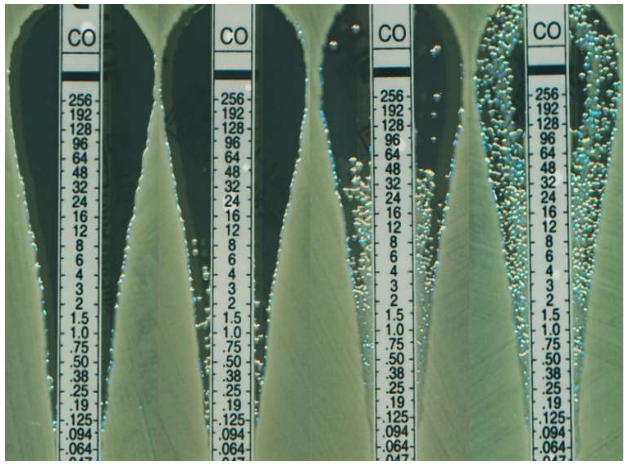


Figure 1 : Colistion E-Test of different isolates of ECC

The aim of this study was then to develop a screening assay for easy and reliable detection of CS-HR ECC isolates.

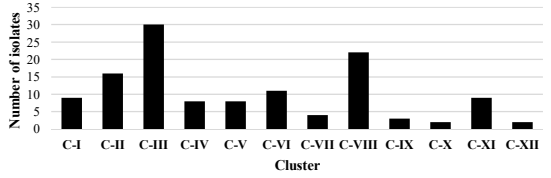
Material and methods

- ✓ A panel of 123 clonally-unrelated ECC clinical isolates was studied along with 2 reference strains used as negative and positive controls (CS-susceptible *Escherichia coli* ATCC 25922 and CS-HR *E. cloacae* subp. *cloacae* ATCC 13047).
- ✓ The strains were identified by MALDI-TOF mass spectrometry and ECC members were clustered by *hsp60* sequencing.
- ✓ Different methods to detect the CS-HR phenotype were compared to PAP: disc diffusion, E-test, broth microdilution method (BMD) and a novel screening culture method (SCM).
- ✓ For the latter, 0.1 ml of a 2-McFarland ($6 \pm 1 \times 10^8$ UFC/mL) bacterial suspension was spread over the surface of a Muller-Hinton agar supplemented with 4 mg/L (MH4) and 32 mg/L (MH32) of CS sulphate. Then, plates were incubated under ambient air at 37° C, and the number of viable colonies was counted after 24h.
- ✓ Isolates exhibited one or more colonies at 24h were considered as CS-HR after check of the presence of an image of subpopulation in the zone of inhibition.

Results

- ❖ The panel of 124 ECC strains was representative for the 12 different clusters (C-I to C-XII) (Table 1).

Table 1 : Distribution of number of isolates by cluster



- ❖ By comparison with PAP, the overall sensitivity, specificity, PPV and NPV values for SCM were for MH4 63.2%, 100%, 63.3% and 100%, and for MH32 100%, 94.1%, 100% and 96% respectively (Table 2).

Table 2 : Performance of the various techniques allowing the characterization of C-HR

	Sensitivity	Specificity	PPV	NPV
MH4	63,2	100,0	100,0	63,2
MH32	100,0	96,0	94,1	100,0
Disk	22,9	100,0	100,0	67,3
E-test	54,2	100,0	100,0	77,6
BMD	91,7	100,0	100,0	95,0

- ❖ Performances of other methods were significantly lower particularly in terms of sensitivity: disc diffusion (67.3%, 100%, 22.9% and 100%), E-test (77.6%, 100%, 54.2% and 100%), BMD (95%, 100%, 91.7%, 100%) (Table 2). It is worthy to note that the CS-HR phenotype was observed for all clusters except C-III and C-VI (that remained always susceptible).
- ❖ Concerning strains belonging to C-VIII and C-V clusters plated on MH4, we have obtained some mutants presenting MIC < 16 mg/L whereas they were miscategorised as CS-HR. On the other hand, we did not observe any mutant on MH32.

Conclusions

- ➢ **In this study, we propose a novel test MH32 for easy and reliable detection of CS-HR among ECC clinical isolates, which could be routinely used in order to prevent therapeutic failure.**

References:
1. Band VI *et al.* Antibiotic failure mediated by a resistant subpopulation in *Enterobacter cloacae*. Nat Microbiol. 2016 May9;1(6):16053.
2. Guérin F, *et al.* Cluster-dependent colistin hetero-resistance in *Enterobacter cloacae* complex. J Antimicrob Chemother. 2016 Nov;71(11):3058-3061.