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Simple screening culture method for reliable detection of colistin heteroresistance in clinical isolates of Enterobacter cloacae complex

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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) in ECC could contribute to antibiotic treatment failure. Detection of CS-HR is challenging using common methods of in vitro susceptibility testing while the reference method (i.e. population analysis profiling [PAP]) is time-consuming and labour-intensive. The aim of this study was then to develop a screening assay for easy and reliable detection of CS-HR ECC isolates.

Material/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 ($6 \pm 1 .10^8$ UFC/mL) bacterial suspension was spread over the surface of a Muller-Hinton agar supplemented with 4 mg/L (MH4) or 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. The phenotype of CS-HR was verified for all colonies.

Results: The panel of 124 ECC strains was representative with 12 different clusters (C-I to C-XII). By comparison with PAP, the overall sensitivity, specificity, PPV and NPV values for SCM were 63%, 100%, 100% and 63% on MH4 and 100%, 96%, 94% and 100% on MH32, respectively. Performances of other methods were as follows: disc diffusion (23%, 100%, 100% and 67%), E-test (54%, 100%, 100% and 78%), BMD (92%, 100%, 100%, 95%). 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-V and C-VIII clusters, some in vitro mutants (false-positive results) were obtained on MH4 whereas none were selected on MH32.

Conclusions: In this study, we propose a simple approach (SCM with MH32) for reliable detection of CS-HR among ECC clinical isolates, which could be routinely used in order to prevent therapeutic failure.