

TITLE: DETECTION OF RESISTANCE TO POLYMYXINS IN *ENTEROBACTERIACEAE* USING A SIMPLE SCREENING ELUTION TEST

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ABSTRACT:

Over recent years, the rate of polymyxin chromosomal resistance has more than duplicated, reaching 35% of carbapenemase-producing *Enterobacteriaceae*. Routine techniques such as disc diffusion, concentration gradient (E-test) and automation cannot be used to determine *in vitro* polymyxin's susceptibility properly. Only broth microdilution (BMD) is considered satisfactory in this scenario, as recommended by EUCAST. Nonetheless, BMD is a laborious and expensive method, and its application in many laboratories is not feasible. Given this context, establishing an alternative method to BMD becomes extremely important. Thereby, this project aims to evaluate, by a prospective study, polymyxin resistance in *Enterobacteriaceae*, employing elution tests (microelution and macroelution) and a colistin screening tube test (CSTT), and then comparing the results with BMD (as gold standard). Sixty-five strains were selected, including: *K. pneumoniae* (26), *E. coli* (11), *P. aeruginosa* (8), *A. baumannii* (7) and others (3). The methods compared here include BMD, macroelution and microelution (as preconized by Pasteran et al.). Moreover, based on the elution methods, our group has proposed a validation of the CSTT, which uses one colistin disk eluted in 5 ml of Cation-adjusted Mueller Hinton Broth (CAMHB) with a 25 μ L inoculum of 10^8 UFC/mL, resulting in a final concentration of 2 μ g/ml. If the bacteria develop in the broth (turbidity), the strain is considered resistant (MIC > 2 μ g/ml), and the absence of growth (limpid), sensitive (MIC \leq 2 μ g/ml). In the selected strains, 45 had complete accordance in all methods, whilst 20 presented some divergence when compared to the gold standard. The macroelution method demonstrated superiority when compared to the others, with only 6% of Very Major Errors (VME) and 1% of Major Errors (ME), according to BMD results. The CSTT, however, had similar results with 7% of VME and 6% of ME. Analyzing only *K. pneumoniae* isolates, all tests gave outstanding results: the microelution method, returned no VME nor ME; for both macroelution and CSTT, 3% VME and 0% ME. In conclusion, all methods tested have shown potential in determining polymyxin susceptibility in *Enterobacteriaceae*, but given its ease of preparation, the use of CSTT is strongly recommended. Nevertheless, all methods demonstrated relevant discrepancy when applied to nonfermentative bacteria, therefore not recommended for these strains.

Keywords: polymyxin, colistin, resistance, elution, Enterobacteriaceae.

Development Agency: none.