TITLE: EVALUATION OF EDTA AND DIPICOLINIC ACID IN THE BROTH MICRODILUTION WITH POLYMYXIN B AS A PHENOTYPIC TEST TO DETECT *ENTEROBACTERALES* WITH *mcr*-1 GENE

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ABSTRACT:
Polymyxins (colistin and polymyxin B) have recently regained significant importance as last-line drugs to treat infectious diseases due to multidrug-resistant Gram-negative bacteria. However, resistance to polymyxins has increased and the recognition of the plasmid-mediated resistance (by the *mcr* gene) lead to an epidemiological concern. We aimed to evaluate the reduction of polymyxin B MIC in the presence of EDTA or dipicolinic acid (DPA) in the broth microdilution (BMD) method for the phenotypic screening of acquired polymyxin resistance mediated by the *mcr*-1 gene. The effect of 584.4 µg/mL (2 mM) EDTA and 225 µg/mL DPA was evaluated in the polymyxin B MIC compared to the absence of the chelators. Overall, 94 *Enterobacterales* (48 polymyxin-resistant and 46 polymyxin-susceptible) were evaluated: 47 *mcr*-1 positive (36 *Escherichia coli*, 2 *Klebsiella pneumoniae*, and 9 *Salmonella* spp.) and 47 *mcr*-1 negative (3*E. coli* and 44 *K. pneumoniae* - 27 isolates with MIC from ≤0.125 to 8 µg/mL and 20 isolates with MIC from 16 to 64 µg/mL) which included 30 isolates resistant to polymyxins by other mechanisms and 17 isolates polymyxin-susceptible. Results were categorized as positive whether the chelator decreased the original BMD MIC by ≥2 logs. The majority (95.7%) of the *mcr*-1 positive isolates displayed at least 3 log dilutions decrease of MIC values of polymyxin B with EDTA or DPA. The EDTA-based BMD assay detected 45 *mcr*-1 positive strains, with only one false-positive among the *mcr*-1 negative isolates (sensitivity [SN], 95.7%; specificity [SP], 97.9%), while the DPA-based BMD assay detected 44 *mcr*-1 positive isolates (SN, 93.6%; SP, 95.7%) with two false-positive results. The EDTA and DPA-based BMD assays presented accuracy equal to 97 and 95%, respectively. The EDTA and DPA-based assays demonstrated to be reliable methods to detect isolates *mcr*-1 positive, with excellent accuracy.

Keywords: Broth microdilution, DPA, EDTA, *mcr*-1, susceptibility testing

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