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Evaluation of EDTA addition to the broth microdilution using polymyxin B as a phenotypic test to detect the *mcr*-1 gene

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Background: Polymyxins are the last resort for the treatment of infections caused by multidrug-resistant Gram-negative bacteria. Nevertheless, polymyxin resistance has increased in the last few years and the development of specific screening methods to determine the susceptibility to polymyxins is a need in order to prevent dissemination of the *mcr*-1 gene. Since recent studies have demonstrated that the structure of the catalytic site of the phosphoethanolamine transferase MCR-1, the protein encoded by the gene *mcr*-1, is a zinc metalloprotein, we aimed to evaluate the reduction of polymyxin B minimum inhibitory concentration (MIC) based on the inhibition of the MCR-1 activity by the ethylenediaminetetraacetic acid (EDTA) in the broth microdilution (BMD). **Materials/methods:** We evaluated the effect of 2 mM EDTA in the polymyxin B MIC by the BMD. A total of 57 isolates were tested: 47 *mcr*-1 positive (36 *Escherichia coli*, 2 *Klebsiella pneumoniae*, 9 *Salmonella enterica*) and 10 *mcr*-1 negative polymyxin resistant (7 *K. pneumoniae*, 3 *E. coli*). The *E. coli* ATCC 25922 was used as quality control. Prior to perform the BMD with polymyxin B and EDTA, a BMD assay without antibiotic was carried out varying the EDTA concentration from 64 mM to 0.125 mM in order to establish the lowest sub inhibitory concentration of EDTA for the isolates included in the study. The BMD using polymyxin and polymyxin plus EDTA was based on the recommendations of the *European Committee on Antimicrobial Susceptibility Testing* (EUCAST). **Results:** All *mcr*-1 positive isolates displayed a decrease in the MIC value for polymyxin B with the addition of EDTA, where 96% (n=45) displayed a decrease of at least 2-fold dilutions. Two *mcr*-1 positive isolates presented a decrease of only 1-fold dilution with EDTA. None of the *mcr*-1 negative isolates presented difference in MIC values when the chelator was added to polymyxin B. **Conclusions:** Our results indicate that inhibition of MCR-1 by EDTA is a promising approach, which can be used for the presumptive detection of MCR-1-producing bacteria using the BMD.

Keywords: broth microdilution; EDTA; *mcr*-1; polymyxin resistance; susceptibility test.