TITLE: ELUTION METHODS TO EVALUATE POLYMYXINS SUSCEPTIBILITY

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The polymyxins are antimicrobials considered the last resources against multidrug resistant Gram-negative but reports of polymyxin resistance have been increasing worldwide. The standard technique to determine the susceptibility profile of polymyxins is the broth microdilution (BMD), however it is difficult to perform due to high costs and time for execution. A new method (Colistin Broth Microelution) was developed which requires low cost materials easily obtained in routine microbiology laboratories. The aim of this study was to evaluate the Polymyxins Broth Microelution (PBM) in comparison to the BMD; we also developed a modified protocol using diminished volumes (Microelution-Plates Test - MPT) and a screening test (Colistin Suceptibility Test Tube- CSTT). A total of 53 clinical isolates of Gram negative rods were submitted to the BMD and the MIC results were interpreted according to BrCAST (resistance >2µg/ml). To perform the Microelution tests, 3 tubes with 10mL of Cation-adjusted Mueller– Hinton broth (CA-MHB) were prepared and colistin disks (10µg) were added as follow: one disk in tube 1 (1µg/mL), two in tube 2 (2µg/mL) and four in tube 3 (4µg/ml). One tube without antibiotic was used as the growth control. Colistin was allowed to elute from the disks for 60 minutes. The solutions were fractionated in tubes (1mL) for the PBM and in microtiter plates (200µL) for the MPT and a volume of 5µL and 3µL of the bacterium inoculum (10^8 UFC/mL), respectively, was added to each tube or well. For the CSTT one tube with 5mL of CAMHB with one colistin disks (10µg) was used (2µg/mL of colistin) which was inoculated with 25µL of the bacterium

inoculum (10^8 UFC/mL). CSTT was considered positive (colistin resistance) whether it presented bacterial growth (turbidity). Among the 53 isolates, 21 were susceptible and 32 were resistant to polymyxins according to BMD and all of them presented categorical agreement with the PBM and CSTT. Fifty isolates also presented categorical agreement with MPT but 3 isolates which were negative (susceptible) in the MPT assay were resistant by the BMD. PBM and CSTT exhibited excellent discrimination between polymyxin-resistance and polymyxin-susceptibility. The MPT presented categorical agreement for 94% of the isolates but 3 isolates presented very major errors in this assay. The tests proved to be easy to perform and to require materials commonly found in a routine microbiology laboratory.

Keywords: polymyxins, resistance, elution