TITLE: Evaluation of the accuracy of the Policimbac® broth microdilution to polymyxin B MIC determination for Klebsiella pneumoniae

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ABSTRACT:

The susceptibility test to polymyxins is a challenge for clinical laboratories, due to the troubles in execution, reproducibility, and accuracy. The regulatory committees recommend only the broth microdilution method (BMD) for polymyxins. Policimbac® (Probac, Brazil) is a lyophilized microdilution plate, commercially available in Brazil. The aim of this study was to evaluate the accuracy of commercial Policimbac® plate and propose modifications to improve their results. The results were compared with gold-standard BMD, prepared according to ISO16782 standards, and the Policimbac® plate was performed according to the manufacturer's recommendations. The modified Policimbac® was performed with addition of sterile gauze moistened with sterile water. For the protocol as per the manufacturer's instructions, 56 *K. pneumoniae* clinical isolates were tested. Of these, 6 samples had to be excluded from the analysis due to the drying of the Policimbac® plate and 1 due to the "Skipped Well" phenomenon. Policimbac® had an excellent categorical agreement (CA) of 97.96%, but an unacceptable low essential agreement (EA) of 26.53%. The low MIC agreement was observed due to the most samples showed MICs around 2 to 3 logs higher than BMD. Due to the higher MICs, 2.04% of very major error (VME) was observed, which may be more expressive if strains with borderline MICs are tested. For the modified protocol, 48 samples were tested. Of these, 3 samples were lost due to the "Skipped well" phenomenon and were disregarded from the analysis. The addition of wet gauze improved the test performance, since there was no missing of samples due to drying. Compared with the standard protocol, we observed an average 1 log reduction in MICs with the addition of moistened gauze, mainly observed in sensitive strains, probably resulting from decreased evaporation of plaque content. In addition, the test showed 100% of CA, however, maintained an unacceptable low 24.4% EA. No ME or VME was observed with the modified protocol. In addition, the Policimbac® board is no easy execution, needing inoculum dilutions in saline solution, not provided in the kit. In conclusion, despite good CA, the Policimbac® present unacceptable MICs agreement for *K. pneumoniae*, in this way, their use is not justified, already the MICs determination is essential to correct polymyxin B therapy.

KEYWORDS: Polymyxins Susceptibility Test, Policimbac, broth microdiluition.