

**TITLE:** EXCELENT PERFORMANCE OF DROP TEST MODIFIED FOR DETERMINATION OF POLYMYXIN B SUSCEPTIBILITY

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**ABSTRACT:**

Polymyxins (polymyxin B-PMB and colistin) are often the last resort to treat infections due to multidrug-resistant Gram-negative bacteria. BrCAST (Brazilian version of EUCAST) and CLSI determine as reference method only broth microdilution (BMD) for polymyxins susceptibility testing, but this may not be practical in a routine laboratory. Although presently some rapid tests have been proposed, they are performed with the use of colistin and may not detect resistance in some bacterial groups. The present study proposes a method for determination of PMB susceptibility (drop test modified- DTM). A total of eighty one Gram-negative isolates, including 21 *Pseudomonas aeruginosa*, 16 *Acinetobacter baumannii*, 1 *Citrobacter freundii*, 1 *Klebsiella aerogenes*, 5 *Enterobacter cloacae*, 2 *Raoultella planticola*, 10 *Escherichia coli* and 25 *Klebsiella pneumoniae* with different resistance genes were included. These isolates were tested by BMD and the Minimum Inhibitory Concentration (MIC) values were interpreted according to BrCAST. The tests were performed with Cation-adjusted Mueller Hinton broth (Ca-MHB) in triplicate. For enterobacteria, the DTM was realized with PMB-concentration of the 16 µg/mL (one 300U PMB disk added in 1,875 mL of Ca-MHB) and for non-fermenters (NF) was conducted with 12 µg/mL of PMB (one 300U PMB disk added in 2,5 mL of Ca-MHB). After, 10 µL of these solutions were added on surface of a Mueller Hinton Agar plate inoculated (0.5 McFarland standard) of the each isolate and incubated at 35°C for 16–20h. The isolate was considered as PMB-susceptible when there was sharp zone edge with no colonies within halo and considered as PMB-resistant when no halo or colonies within the halo was observed. The results of DTM and the BMD were compared and the categorical agreement was evaluated. According to BMD, 21 isolates were PMB-resistant (MIC range 4-64 µg/mL), being 3 MCR-1-positive, and 60 were PMB-susceptible (MIC range 0.5-2 µg/mL). The use of 16 µg/mL of PMB (as proposed by the original drop test) showed 8% of very major error for NF, however the new proposal (16 µg/mL for enterobacteria and 12 µg/mL for NF) resulted in 100% categorical agreement between DMT and BMD. Considering the difficulty of performing BMD in routine laboratories, and the excellent performance of DTM, we believe that the proposed method may have great applicability, besides being a cheap, quick and easy method for determination of PMB susceptibility.

**Keywords:** microbial sensitivity tests, polymyxins, polymyxin B, Gram-negative bacteria

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