TITLE: POLYMYXIN DROP TEST AND VITEK-2™: MODIFIED PROTOCOL AND AUTOMATION TO ENABLE DETECTION OF POLYMYXINS RESISTANCE IN GRAM-NEGATIVE BACILLI IN ROUTINE MICROBIOLOGY LAB

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ABSTRACT:
The CLSI and EUCAST only recommend broth microdilution (BMD) for polymyxins susceptibility testing, however this method is laborious, and supplies required are costly for many routine laboratories. Here we evaluated modifications of drop test developed by Jouy E. (2017) and Pasteran F. (2018), and the performance of Vitek-2 in comparison with gold standard BMD method for determining polymyxins susceptibility among Gram-negative bacilli (GNB). Polymyxin BMD was performed against carbapenem resistant GNB isolated between January and May 2018 at Complexo Hospital de Clínicas of the Universidade Federal do Paraná. BMD plates were prepared by investigators according to CLSI standards, and AST-N239 cards were tested as recommended by manufacturer using a Vitek-2 Compact instrument (bioMérieux, Marcy-L'Etoile, France). The drop test was performed as follows: 10 µL of 16 µg/mL polymyxin B solutions (POLY-SOL) dripped on the surface of an inoculated Mueller-Hinton agar (MHA) plate with an isolate on 0.5 McFarland. POLY-SOL were prepared by dissolving polymyxin B sulfate powder, and by elution of two 300 UI (30 µg) disks in 3,75 mL cations adjusted MH broth (MHB). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control. Isolates identification was also performed using Vitek-2. CLSI/EUCAST breakpoints (susceptible ≤ 2 µg/mL; resistant > 2 µg/mL) were used. The essential agreement was considered when Vitek-2 MIC was $\pm 1 \log_2$ in comparison with BMD MIC. Zone of total inhibition and no zone or zone with colonies inside were categorized as susceptible and resistant, respectively, for drop test. Seventy-one GNB clinical isolates were evaluated, including 43 *K. pneumoniae*, three *E. cloacae* complex, one *E. coli*, 20 *A. baumannii* complex, and four *P. aeruginosa*. Only 2.8% of isolates were resistant by reference method. The drop test showed 88.7% of categorical agreement (CA) and 11.2% of major error (ME). No very major error (VME) was observed. The Vitek-2 showed 87.3% of EA and 97.2% of CA. In addition, 1.4% of ME and VME were observed. Despite low resistance rate, most discrepancies occurred in K. pneumoniae isolates, especially in borderline 2 µg/mL. Drop test showed great agreement to *A. baumannii* isolates. Vitek-2 system and polymyxin drop test showed a suited performance among GNB to determine polymyxins susceptibility. However, Vitek-2 MICs 2 and 4 µg/mL and presence of pinpoint colonies within the zone must be confirmed by BMD for any GNB specie.

Keywords: Polymyxin B, Colistin, Drop test, Vitek-2, Broth microdilution