Rapid polymyxin B susceptibility test directly from positive blood cultures using the MALDI-TOF system

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Introduction: Sepsis is one of the leading causes of death worldwide, and most individuals with sepsis are patients admitted to Intensive Care Units. Currently, it is possible to perform rapid identification of the etiologic agent of sepsis directly from the blood culture flask using the MALDI-TOF system. To optimize the treatment of sepsis it is also important to provide rapid information regarding the susceptibility profile of the pathogen responsible for the sepsis. **Objective:** To evaluate a modified version of the 'direct on target microdroplet growth assay' from positive blood cultures by MALDI-TOF to assess the susceptibility of *Enterobacterales* to Polymyxin B. **Methodology:** 48 isolates of *Enterobacterales* (9 resistant and 39 susceptible to Polymyxin B) from patients attending at "Hospital de Clínicas de Porto Alegre" were tested. Positive blood cultures (3 mL) were centrifuged at 3000 rpm for 5 minutes, the supernatant was discarded and the pellet was added of 3 mL of saline. The same procedure was repeated and the bacterial pellet was adjusted to suspension of 10^7 UFC/mL in cation-adjusted Mueller Hinton broth. The inoculum was placed onto a MALDI-TOF target containing 2 ug/mL of Polymyxin B and incubated at 35 ± 1◦C for 4 hours. After incubation, formic acid 70% and HCCA matrix were added over the spots and, after drying, the plate was inserted into the MALDI-TOF equipment for reading. The tests were made in triplicate and a growth control without antibiotic was

also evaluated. The interpretation was made as follows: when the MALDI-TOF identified the bacteria at concentration of 2 ug/mL of Polymyxin B, the isolate was considered resistant to the antibiotic; absence of bacterial identification the isolate was considered susceptible. The results were compared to the standard broth microdilution (BMD). **Results:** The test of 5 isolates (4 *Enterobacter* sp. and 1 *Klebsiella pneumoniae*) was invalid as the positive control did not present growth. All other 43 isolates presented valid results and presented full concordance with the BMD. **Conclusion:** The method evaluated proved to be a rapid and easy alternative for the determination of the susceptibility to Polymyxin B directly from blood cultures, significantly reducing the time needed to report the result. This rapid MALDI-TOF method may play an important role to optimize the treatment of patients with sepsis by multidrug-resistant *Enterobacterales*.

Keywords: sepsis, maldi-tof, polymyxin, broth microdilution, enterobacterales

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