

TITLE: A SIMPLE AND RAPID METHOD TO DETERMINE QUALITATIVE POLYMYXIN B SUSCEPTIBILITY BY MALDI-TOF MS

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ABSTRACT:

In recent years, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a mean to access antimicrobial susceptibility. The main methodologies that are able to detect polymyxins resistance by MALDI-TOF MS are related of modifications to lipid A, but the current lipid extraction methods require significant hands-on time and thus may not be suited to be performed in the routine of a clinical microbiology laboratory. This study aimed to evaluate the applicability of MALDI-TOF MS - MicroFlex LT mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) for assessing the in vitro qualitative polymyxin B susceptibility for *Enterobacteriales*. MALDI-TOF MS Polymyxin B Test (named MPT) procedure was performed as follows: (i) bacterial incubation in two liquid culture media, with and without polymyxin B; (ii) bacterial cells washing and extraction; (iii) qualitative MALDI-TOF MS data analysis. Broth microdilution (BMD) was used as the reference method and was interpreted according to the BrCAST guidelines. Results: A total of 95 isolates were evaluated and the MPT presented 95.8% of categorical agreement with BMD. Only three isolates (3.15%) presented Major Error and only one (1.05%) presented a Very Major Error. Conclusion: As the MPT presented a high agreement with BMD, it is an important alternative approach to detect early polymyxin B resistance. This study described a new polymyxin B susceptibility tests which presents several advantages as follows: (i) it is rapid (requires only 3 hours of incubation); (ii) it does not require special solvents; (iii) it is easy to perform; (iv) it is reliable and (v) does not depend of reading by human eye. Moreover, the MPT test proposed in this study was optimized for a conventional mass spectrometer of MALDI-TOF and uses the same standard organic matrix (HCCA) commonly used in clinical microbiology laboratories.

Keywords: Polymyxin B; MALDI-TOF MS; *Enterobacteriales*; Qualitative; Susceptibility

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