

**TITLE:** EVALUATION OF THE MBT-ASTRA FOR THE DETERMINATION OF MEROPENEM SUSCEPTIBILITY IN ENTEROBACTERIACEAE BY RAPID MATRIX-ASSISTED LASER DESORPTION IONIZATION–TIME OF FLIGHT (MALDI-TOF MS)

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**ABSTRACT:**

Carbapenem-resistant Enterobacteriaceae (CRE) poses as a serious problem for public health. Rapid detection of resistance is crucial to initiate proper therapy. Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) is a revolutionary tool in the clinical microbiology laboratory as it allow to identify microorganisms very quickly. Furthermore, a semi-quantitative MALDI-TOF MS, which is called the MALDI Biotyper Antibiotic Susceptibility Test Rapid Assay (MBT-ASTRA), has been published as a new method for rapid susceptibility testing of different antibiotics. It has been indicated that the total time (including pre analysis setup, incubation, post incubation processing/extraction and data analysis) required to perform the assay is about 2 to 3 h. We have used this methodology to evaluate the meropenem susceptibility in Enterobacteriaceae. Five isolates resistant to meropenem (16-32 µg/mL); and four isolates susceptible to meropenem (<0.5µg/mL) were evaluated. These isolates were incubated in BHI for 2 h at a meropenem concentration of 1 µg/mL (for susceptible isolates) and 4 µg/mL (resistance isolates). After 2 h of incubation the cells were washed, lysed and supplemented with RNase B (internal standard necessary to semi quantify small proteins within a spectrum). The lysate was centrifuged and 1 µl of the supernatant was transferred to a steel target in quadruplicate. After air-drying, spots were overlaid with 1 µl of portioned matrix. Spectra were collected in a Microflex LT (Bruker Daltonics) using the same

configuration setup for microorganism identification. For data interpretation, spectra were baseline subtracted and peak picking was performed and normalized to the maximum peak resulting in relative intensity range between 0 and 100%. Subsequently, the relative growth was calculated as the ratio of the sum of the three most intense peaks from the microorganism profile (mass range from 4-5kDa, 6-7.5kDa and 11-15Da) versus the sum of the relative intensities of the peaks of RNase B. The MBT-ASTRA was able to identify all five isolates with high MICs as resistant, and the four isolates with low MICs as susceptible to meropenem. The semi quantitative MALDI TOF MS analysis was able to determine susceptibility of Enterobacteriaceae isolates, providing results after a short incubation time which reduced the time gap between the results of species identification and the standard antibiotic susceptibility.

**Keywords:** MALDI-TOF/MS, susceptibility profile, relative growth