TITLE: EVALUATION OF CARBAPENEM HYDROLYSIS BY *ENTEROBACTERALES* DIRECTLY FROM POSITIVE BLOOD CULTURES USING MALDI-TOF/MS

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ABSTRACT:

Carbapenem-resistant *Enterobacterales* (CRE) are a major threat to human health and rapid and reliable detection of carbapenemase is an important matter. MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-of-Flight) has proven to be an accurate tool for rapid identification of bacteria and, recently, has been evaluated for resistance detection. The aim of this study was to detect meropenem hydrolysis directly from positive blood cultures. A total of 45 clinical isolates of *Enterobacterales* were selected (13 susceptible and 32 resistant to meropenem). A volume of 1 mL of bacterial suspension (10^4 CFU/mL) from fresh culture plus 4 mL of sterile blood were inoculated into a blood culture bottle which was incubated in the automated system BacT/ALERT®. After growth, a bacterial pellet was prepared according to MALDI Septityper Kit (Bruker Daltonics). The pellet was resuspended in 10 µL of antimicrobial solution: 1 mg/mL meropenem in water with 0.01% SDS. This suspension was incubated at 35-37ºC for 2-4 hours and centrifuged for 2 min at 14,000 rpm; the supernatant (1µL) was transferred to the MALDI-TOF plates. After drying, 1 µL of the matrix solution was added and allowed to dry. Each isolate was tested in duplicate. Intensity of peaks corresponding to non-hydrolyzed and hydrolyzed meropenem were used to calculate the logRQ value, which is the logarithm of the ratio of the sum of the intensity of the decarboxylated and hydrolyzed forms (358 m/z and 402 m/z) and the summed intensity of the non-hydrolyzed forms (384 m/z and 406 m/z). LogRQ values > 0.4 or < 0.2 indicate positive and negative results, respectively, for the hydrolysis of beta-lactams. Values between 0.2 and 0.4 are considered indeterminate. The 13 meropenem-susceptible isolates were negative for meropenem hydrolysis. Of the 32 meropenem-resistant isolates, only 14 presented hydrolysis in 2h; 11 isolates presented indeterminate or negative result in 2h but positive result in 4h; 7 isolates did not present hydrolysis, of these, 2 isolates were negative for carbapenemase, 3 were NDM (2 *Enterobacter* sp., MIC= 64 µg/mL; 1 *K. pneumoniae*, MIC= 16 µg/mL), 1 OXA-48-like (*Enterobacter* sp., MIC= 16 µg/mL) and 1 KPC (*Enterobacter* sp., MIC= 16 µg/mL). These results indicate an excellent specificity, however, a variable sensitivity according to the characteristics of each isolate (such as the bacterial genera and the enzyme type).

Keywords: Carbapenemase, *Enterobacterales*, MALDI-TOF

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