TITLE: EVALUATION OF THE CEFTAZIDIME/AVIBACTAM SUSCEPTIBILITY BY MALDI-TOF MBT-ASTRA DIRECTLY FROM POSITIVE BLOOD CULTURES

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ABSTRACT:
Serine and metallo-beta-lactamase (MBL), as KPC and NDM, are the main resistance mechanisms in carbapenem-resistant *Enterobacterales* (CRE). Bacteremia caused by *Enterobacterales* producing NDM and/or KPC are associated to increased mortality rates. The antibiotic ceftazidime/avibactam (CZA) has activity against KPC, but not against MBL CRE producers. Therefore, the development of a rapid methodology, such as MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA), is of great interest for determining CZA susceptibility. The objective of this study was to evaluate the MBT-ASTRA in order to determine bacterial susceptibility to CZA directly from positive blood cultures (BC). A total of 35 clinical isolates of *Enterobacterales* were tested for CZA susceptibility by disk diffusion according to EUCAST. An adapted MBT-ASTRA from positive BC was performed as follows: 100 µL of the positive BC, adjusted to a 1.5 McFarland standard in brain heart infusion broth, was added to 100 µL of CZA solution (20/8 mg/L). The solutions were incubated for 1.5, 2, 2.5 and 3 h and a protein extraction was performed with the addition of the internal standard RNase B. The supernatant was deposited, in quadruplicates, into a steel plate and overlaid with HCCA matrix. The MALDI-TOF analysis were performed in a mass spectrometer Microflex LT. Spectra were acquired with flexControl and analyzed in flexAnalysis. Peaks were selected and normalized in relation to the internal standard. The relative growth (RG) was calculated for each isolate, considering peak intensities of bacteria with antibiotic (IntBac+ATB) and without antibiotic (IntBac), as follows: RG = Median (∑ IntBac+ATB) / Median (∑ IntBac). For incubation time of 1.5 and 2.5 h, respectively, isolates with RG ≤0.6 and ≤0.5 were considered susceptible and >0.6 and >0.5 were considered resistant to CZA. *Klebsiella* spp. (n=26) were correctly categorized as susceptible/resistant after 1.5 h incubation. *Citrobacter freundii* (n=1), *Enterobacter* spp. (n=5) and *Providencia stuartii* (n=1) needed 2.5 h of incubation to be categorized as susceptible/resistant. The two *Serratia marcescens* could not be correctly classified in any time of incubation. Except for *S. marcescens*, these results provide 100% total categorical agreement. The classification of isolates, after species identification, as resistant to CZA by MBT-ASTRA directly from positive BC indicates the possible presence of MBL and may optimize antibiotic therapy.

Keywords: antimicrobial susceptibility testing, blood culture, ceftazidime/avibactam, MBT-ASTRA

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