TITLE: DEVELOPMENT OF AN ALGORITHM FOR REAL-TIME IDENTIFICATION OF KPC-PRODUCING K*lebsiella pneumoniae* BY MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

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ABSTRACT:

Klebsiella pneumoniae carbapenemase *(KPC)*-producing *Klebsiella pneumoniae (KP)* is an important public health problem as the KPC-KP are multiresistant to antibiotics. Recently, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was presented as capable of quick detection of KPC production which is mandatory to prevent the spread of this resistance mechanism. We aimed to develop an algorithm to detect KPC-KP using MALDI-TOF. A total of 85 isolates with reduced susceptibility to carbapenem, obtained from a molecular epidemiology study in Rio Grande do Sul state, were evaluated. Protein extraction for MALDI-TOF MS analysis was done using fresh bacterial isolates re-suspended in 1 mL of 70% ethanol, vortexed for 1 min and centrifuged at 13,000 rpm for 2 min. The supernatant was removed and the pellet was vortexed for 10s with 25 µl of 70% formic acid (FA) and 25 µl of acetonitrile (ACN). After 2 min of centrifugation at 13,000 rpm, 1 µl of supernatant was spotted onto the target plate and overlaid with 1 µl of alpha-cyano-4-hydroxycinnamic acid (α-CHCA). MALDI-TOF MS analysis was performed on a Bruker AutoFlex LT mass spectrometer (Bruker Daltonics, Billerica, MA). ClinProTools software (v3.0 Bruker Daltonics, Inc.) was used for peak analysis and development of the algorithm. In order to establish the best algorithm, we used 49 KPC-KP positive as group 1 and 36 KPC-KP negative as group 2. The Genetic Algorithm (GA) presented the best performance to classify the isolates as KPC-KP positive and negative. This algorithm was evaluated using 116 isolates, which were not used in the algorithm development, and presented a correct classification of 97.1% and 100% of KPC-positive KP (n = 94) and KPC-negative KP (n = 22) isolates, respectively. Single-peak analysis by the GA identified a total of 10 peaks (m/z of 3,153; 5,143; 5,725; 6,213; 7,161; 7,364; 7,707; 8,371; 8,995 and 11,434) that potentially differentiate between KPC-positive and KPC-negative KP isolates. However, visual analysis of these peaks indicated that most of them were present in both KPC-positive and KPC-negative isolates. The ClinProTools might be an important tool to identify KPC-KP using GA, however further evaluations are needed to identify possible single (or multiple) peaks as markers of KPC-KP in MALDI-TOF.

Keywords: *Klebsiella pneumoniae,* KPC, MALDI-TOF MS, plasmid

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