TITLE: HAVE YOU EVER THOUGHT ABOUT SENDING BACTERIA BY CONVENTIONAL MAIL TO IDENTIFY THE KPC ENZYME USING MALDI-TOF MS?

AUTHORS: WILHELM, C.M.; MOREIRA, N.K.; CARNEIRO, M.S.; WINK, P.L.; CAIERÃO, J.; BARTH, A.L.

INSTITUTION: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL, PORTO ALEGRE, RS (R. RAMIRO BARCELOS, 2350, HCPA/CPE/LABRESIS, CEP 90035-903, PORTO ALEGRE – RS, BRAZIL)

ABSTRACT:
KPC is one of the most prevalent resistance mechanisms detected among carbapenem resistant *Enterobacterales*. In fact, apart from the epidemiological importance, the identification of the carbapenemase is now important to guide the antibiotic therapy. Therefore, it would be of interest to transport inactivated bacteria impregnated in filter paper for KPC identification using MALDI-TOF MS. Sixty-one *Enterobacterales* were evaluated: 20 isolates KPC positive and 41 isolates KPC negative as determined by High Resolution Melting or conventional PCR (gold standards). For KPC identification from filter paper, colonies were suspended in 300 µL distillated water and 900 µL absolute ethanol in propylene microtubes. They were vortexed and centrifuged for 2 minutes at 200 *g*. The supernatant was discarded and the remaining ethanol was left to air dry. The pellet was impregnated in filter paper, which remained 7 days in ambient temperature. Then, 200 µL solvent extraction (formic acid : isopropyl alcohol : water, 17:33:50) was added to microtubes containing the filter paper disks. The microtubes were vortexed and centrifuged for 2 minutes at 200 *g*. The double layer sinapinic acid (SA) technique was used: for the first layer a saturated solution of SA in ethanol was loaded onto a MALDI target plate; for the second layer, the supernatant was mixed with other saturated solution of SA (acetonitrile : 0.1% trifluoroacetic acid, 30:70); 1 µL of the mixture was deposited onto the first layer in triplicates. For MALDI-TOF MS analysis, spectra acquisition was performed in a Microflex LT mass spectrometer. Each triplicate was analyzed for the presence or absence of the KPC peak, which was approximately 28688 m/z, using flexAnalysis 3.4. From the 61 isolates, 16 were correctly identified for KPC and 41 confirmed the absence of KPC. Overall, our results demonstrated 80% sensitivity and 100% specificity. These preliminary results indicate that it is possible to transport bacteria in filter paper for KPC detection by MALDI-TOF MS. However, it is necessary to improve the technique in order to increase its sensitivity.

Keywords: KPC, carbapenemase, MALDI-TOF MS, identification, filter paper.

Development Agency: Fundo de Incentivo à Pesquisa e Eventos (FIPE/HCPA), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) Nº 001.