# Rapid protocol for routine detection of KPC-producing strains by using the Maldi-TOF Vitek-MS system

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## Background and Aim of the study

Several phenotypic methods have been proposed for carbapenemase routine detection. MALDI-TOF has recently been used to determine β-lactamase activity (1-2) but has not been used in a routine setting to detect carbapenemase activity in clinical isolates.

This study aims at quickly detect gram-negative bacteria producing KPC carbapenemase in the clinical lab routine by using the MALDI-TOF Vitek-MS system.

## Materials

10 *K. pneumoniae* resistant to carbapenems and KPC producers, and 10 strains of K. *pneumoniae* and *E. coli* ATCC 25922 (all susceptible to carbapenems) were included in the study.

## Methods

All strains were tested by hydrolysis assay by using the Maldi-ToF Vitek-MS system according to the following protocol: 0.5 McFarland suspension was made for each strain; 1ml suspension was centrifuged 1 min at 14000 rpm; the bacterial pellet was resuspended in 40 µl of imipenem solution 1 mg/ml and immediately divided in two aliquots. The first aliquot, worked as time 0, was immediately centrifuged 1 min for 14000 rpm, and 1 µl of clear supernatant was spotted onto a Vitek MS-DS slide and allowed to dry at room temperature. The second aliquot was incubated 20 min at 37°C, followed by 1 min centrifugation at 14000 rpm. 1 µl of clear supernatant was spotted onto a Vitek MS-DS slide and allowed to dry at room temperature. 1 µl of MH-CHCA routine matrix was added to both aliquots and allowed to dry before Maldi-TOF reading. Mass range 240,0 – 350,0; power laser 62.

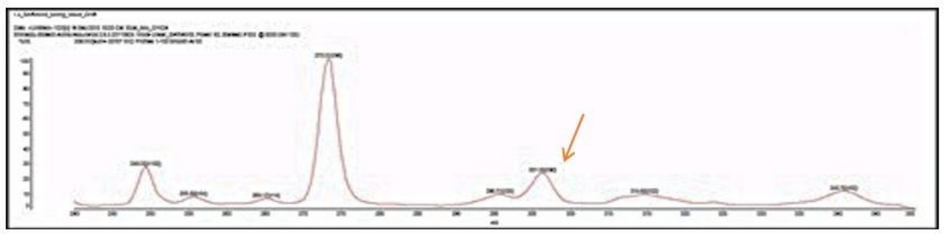
## Results

The mass range showed the presence or absence of the 300 Da peak corresponding to imipenem. When imipenem is hydrolised, the 300 Da peak disappeared. Since the MH-CHCA matrix presents a 254 Da peak, the occurrance of the peak correspondent to imipenem hydrolysis could not be seen. For this reason we included in our protocol a time 0, at which we could verify for each strain the actual imipenem presence.

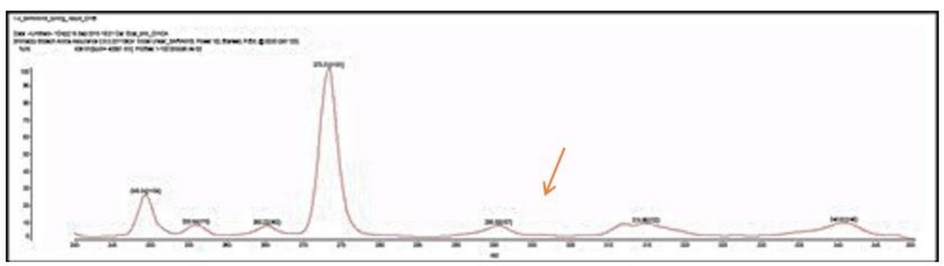
All the *K. pneumoniae* KPC-producer strains showed the 300 Da peak at the time 0 and this peak disappeared after 20 min of incubation at 37°C. (figure 1)

**Figure 1**

*K. pneumoniae* resistant to carbapenems and KPC producers, time 0:



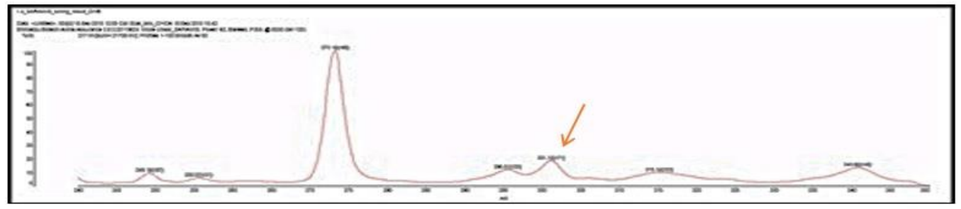
*K. pneumoniae* resistant to carbapenems and KPC producers, time 20:



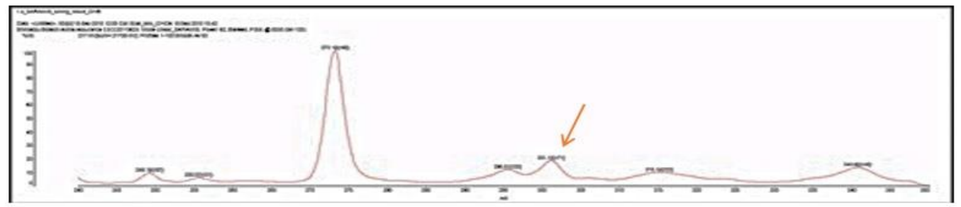
All the susceptible *K. pneumoniae* strains and E. coli ATCC 25922 presented the 300 Da peak both at time 0 and time 20, thus indicating that there was not imipenem hydrolysis (figure 2)

**Figure 2**

ECO ATCC 25922, time 0:



ECO ATCC 25922, time 20:



## Conclusions

This protocol allows to reveal the presence of KPC carbapenemase in the normal clinical lab routine by using the Maldi-TOF Vitek MS system, without changing parameters or matrix as compared with the routine procedure; in an estimated time of 45 min.

It is easily and rapidly method performed also in a routine set up.

Its use with other carbapenemases type and other carbapenems antibiotics is still under investigation.

1.Hrabak et al. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. JCM 2012:50:2441

2.Lasserre et al. Efficient Detection of Carbapenemase Activity in Enterobacteriaceae by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in Less Than 30 Minutes.JCM 2015: 53:2163



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