P0951
Paper Poster Session
Recent studies of non-culture techniques for detection of resistance

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

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

Material/methods: 10 *Klebsiella pneumoniae* resistant to carbapenems and KPC producers, and 10 strains of K. *pneumoniae* and *Escherichia coli* ATCC 25922 (all susceptible to carbapenems) were included in the study.
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 worked as time 20 and 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 CHCA routine matrix was added to both aliquots and allowed to dry before Maldi-TOF reading.
Each slide spot was read with the manual system of Vitek MS: mass range 240.0-350.0, laser power 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 CHCA matrix presents a 254Da peak, the occurrance of the peak correspondent to imipenem hydrolysis (also at 254 Da) 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.
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.

Conclusions: This protocol allows one 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.