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MALDI-TOF: driving change in microbiology laboratories

MALDI-TOF mass spectrometry for the detection of carbapenemase production in Gram-negative bacteria

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Background: Carbapenem-hydrolysing β-lactamase can confer resistance to nearly all β-lactams. The rapid detection of carbapenemase-producing microorganisms is important to prevent dissemination of these strains. However, the detection methods for carbapenemase producers have not been standardized. In this study 2 different protocols of meropenem hydrolysis assay by MALDI-TOF MS were applied in order to develop a valid tool for the phenotypic discrimination between carbapenemases-non-producing and -producing Gram-negative bacteria.

Material/methods: Two different protocols of meropenem hydrolysis assay were applied on pure meropenem, on 3 *K. pneumoniae* reference strains (2 carbapenemase-producing strain BAA-1705, bla_{KPC} positive, and BAA-2146, bla_{NDM} positive and a carbapenemase-non-producing strain BAA-1706) and on 1 well-characterised carbapenemase-producing clinical isolate bla_{VIM} positive. In the first protocol, ammonium citrate and alpha-cyano-4-hydroxy-cinnamic acid (HCCA) were used as resuspension buffer and matrix, respectively; the second protocol required Tris-HCl, pH 6.8 as buffer and 2,5-dihydrossibenzoic acid as matrix. Different incubation times (from 30 min to 4 h) and different meropenem concentrations (from 2.3 to 23 mM) were also evaluated.
After this preliminary evaluation, 45 additional Gram-negative carbapenem nonsusceptible clinical isolates were submitted to the most performing protocol. The spectra obtained with Microflex LT mass spectrometer (Bruker Daltonics, Germany) were analysed by FlexAnalysis software.

Results: The spectra obtained by MALDI-TOF MS using the first protocol did not showed the peaks referring to both intact and/or hydrolysed meropenem at each concentrations tested. However, other 2 peaks were revealed: one referred to HCCA matrix with 1 Da of difference compared to pure meropenem and one non-specific peak with an intensity directly proportional to meropenem concentrations.
The spectra obtained by MALDI-TOF MS using the second protocol showed for pure meropenem and for the carbapenemase-non-producing reference strain the peaks of intact meropenem and for the carbapenemase-producing reference strains the peaks of hydrolysed drug. Using the second protocol the evaluation of different incubation times showed a complete degradation of meropenem after 30 min for the KPC-producing reference strain and after 1 h for the class B carbapenemase-producing strains (NDM and VIM).
The second protocol was applied on the 45 clinical isolates and the spectra obtained showed the peaks referred to intact meropenem in 19 cases and to that hydrolysed drug in 36 cases. Among the 12 *Acinetobacter baumannii* analysed, 10 were carbapemase-producers: 1 revealed after 2 h of incubation and 9 after 4 h.

Conclusions: Only one of the 2 different protocol for meropenem hydrolysis assay by MALDI-TOF MS evaluated in this study allowed to detect carbapenemases production in Gram-negative bacteria. The production of carbapenemases was rapidly (2 h for all species or 4 h for *A. baumannii*) detected. Moreover, the hydrolysis assay resulted easy to perform and to interpret with a low cost per determination (less than 1.00 Euro).