MALDI-TOF MASS SPECTROMETRY FOR THE DETECTION OF CARBAPENEMASE PRODUCTION IN GRAM-NEGATIVE BACTERIA

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Background. 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 (MEM) hydrolysis assay by MALDI-TOF mass spectrometry (MS) were applied in order to develop a valid tool for the phenotypic discrimination between carbapenemases-producing and -non-producing Gram-negative bacteria.

Materials and Methods. The 2 protocols (Table 1, workflow scheme) were initially evaluated on pure MEM and on 3 *Klebsiella pneumoniae* ATCC reference strains (strain BAA-1705 *bla*KPC+; strain BAA-2146 *bla*NDM+; strain BAA-1706 carbapenemase-non-producer) and on a well-characterised *bla*VIM carbapenemase-producing clinical isolate. After this preliminary evaluation, the most performing protocol was applied on 45 Gram-negative carbapenem non-susceptible clinical isolates, previously characterized by phaenotypical conventional assays for the carbapenemase production (modified Hodge and sinergy tests). The spectra obtained with Microflex LT mass spectrometer (Bruker Daltonics, Germany) were analysed by FlexAnalysis software.

Table 1. Comparison between the 2 protocols for the MEM hydrolysis evaluated in the study.

	PROTOCOL 1	PROTOCOL 2
Matrix dissolved in v/v acetonitrile 30:70 trifluoroacetic acid 0,1% in water	α-Cyano-4-hydroxycinnamic acid (HCCA) (10mg/ml)	2,5-Dihydroxybenzoic acid (2,5-DHB) (20mg/ml)
MEM diluent buffer/ bacterial suspension buffer	Ammonium citrate 100x pH 5-6	TRIS-HCl 20 mM pH 6,8
MEM concentration	23, 10, 5, 2.3, 1 mM	23, 10, 5, 2.3, 1 mM
Time of incubation	2h, 4h	30min, 1h, 2h, 3h, 4h
NaCl	No	Yes (0.45%)

WORKFLOW SCHEME

Results. On the preliminary evaluation, the spectra obtained with Protocol 1 applied to pure MEM showed only 2 peaks, one referring to HCCA matrix (only 1 Da of difference from one of the peaks of hydrolised MEM) and one non-specific peak (573 Da) with an intensity directly proportional to meropenem concentration. Only one peak referring to pure MEM was slightly observed at an antibiotic concentration of 23 mM. No other peaks were observed.
The spectra obtained with Protocol 2 showed the expected peaks. The optimal MEM concentration was assessed at 10 mM (Table 2, Figure 1A, B).
The evaluation of different incubation times showed a complete degradation of meropenem after 30 minutes for the KPC-producing reference strain and after 1h or 2h for the class B carbapenemase-producing strains, NDM and VIM, respectively (Figure 2).

Table 2. Comparison between the results obtained with the 2 protocols evaluated in the study.

	Pure MEM		BAA-1705 *bla*KPC +		BAA-2146 *bla*NDM +		BAA-1706 Carbapenemase non-producer		clinical isolate *bla*VIM+	
PROTOCOL	1	2	1	2	1	2	1	2	1	2
Peak of intact MEM (384, 406, 428 Da)	-	+	-	-	-	-	-	+	-	-
Peaks of hydrolised MEM (358, 380, 402, 424 Da)	-	-	-	+	-	+	-	-	-	+

Legend. +: presence; -: absence.

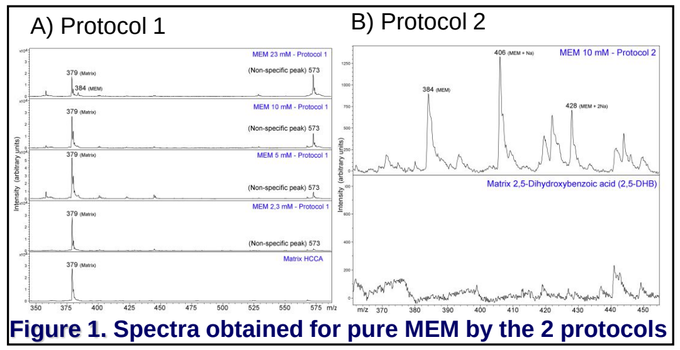

Figure 1. Spectra obtained for pure MEM by the 2 protocols

The Protocol 2 was applied on the 45 clinical isolates and the spectra obtained showed the peaks referred to intact meropenem in 9/45 cases and to that hydrolysed drug in 36/45 cases. Among the 12 *Acinetobacter baumannii strains* analysed, 10 were carbapemase-producers: 1/10 revealed after 2h and 9/10 after 4h incubation. The results obtained were in agreement with those of conventional assays.

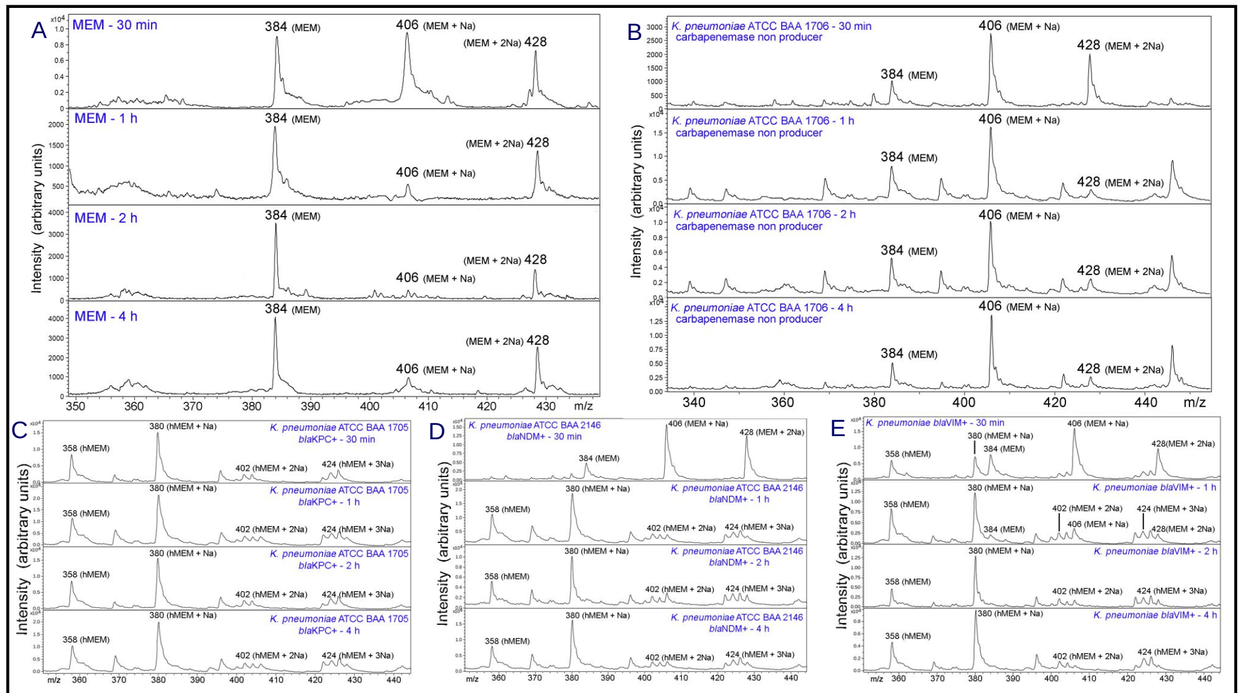

Figure 2. Degradation of MEM by different *K. pneumoniae* strains at different incubation time. MEM w/o bacteria (A); *K. pneumoniae* carbapenemase non-producing reference strain (B); *K. pneumoniae* reference strains *bla*KPC+ (C), *bla*NDM+ (D) and *K. pneumoniae* clinical isolate *bla*VIM+ (E).
Legend. hMEM: hydrolysed meropenem

Conclusion. Only the Protocol 2 for MEM hydrolysis assay by MALDI-TOF MS allowed to detect carbapenemases production in Gram-negative bacteria. The production of carbapenemases was rapidly (2 h for all species and 4 h for *A. baumannii*) detected. Moreover, the hydrolysis assay resulted easy to perform and to interpret with a low cost per reaction (less than 1.00 Euro).

References. Wang L, et al., 2013. MALDI-TOF MS applied to indirect carbapenemase detection: a validated procedure to clearly distinguish between carbapenemase-positive and carbapenemase-negative bacterial strains. *Anal Bioanal Chem*; 405:5259-66.

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