Journal of Microbiological Methods 137 (2017) 30–33

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Comparison of imipenem and meropenem antibiotics for the MALDI-TOF MS detection of carbapenemase activity

Veronika Rotova, Costas C. Papagiannitsis*, Anna Skalova, Katerina Chudejova, Jaroslav Hrabak

Department of Microbiology, Faculty of Medicine and University Hospital in Plzen,Charles University, Plzen, Czech Republic
Biomedical Center, Faculty of Medicine, Charles University, Plzen, Czech Republic

ARTICLE INFO

Keywords:
IMP
VIM
GES
Carbapenems
Pseudomonas aeruginosa
Enterobacteriaceae

ABSTRACT

A comparison of carbapenem molecules for the detection of carbapenemase-producing bacteria by MALDI-TOF MS showed that imipenem exhibited higher sensitivity (97%) and specificity (100%) scores for *Pseudomonas aeruginosa* than meropenem. However, meropenem was more efficient (98% sensitivity and 100% specificity) against *Enterobacteriaceae*.

Infections caused by carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas* spp. are increasing worldwide, and are associated with high rates of mortality. Therefore, methods like Carba NP test and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) carbapenem hydrolysis assay should be performed for the direct detection of carbapenemase-producing bacteria (Hrabak et al., 2014). Nowadays, several modifications of MALDI-TOF MS carbapenem hydrolysis assay have been described and validated (Hrabak et al., 2011; Burckhardt and Zimmermann, 2011; Kempf et al., 2012). However, most of these modifications have been validated only on *Enterobacteriaceae*, or they exhibit lower efficiency for the detection of carbapenemase-producing *Pseudomonas aeruginosa* mainly due to lower quality of their spectra (Hrabak et al., 2011).

In this study, we aimed to validate the efficiency of imipenem and meropenem, for the MALDI-TOF MS detection of carbapenemase-producing *Enterobacteriaeceae* and *P. aeruginosa*.

The methods were tested against a group of 250 *P. aeruginosa* isolates from the collection of the Czech National Reference Laboratory for Antibiotics. The isolates were previously characterized, as described below. For all isolates, susceptibility to carbapenems was determined by using imipenem and meropenem disks and interpreted according to the EUCAST criteria (http://www.eucast.org/). All isolates that were non-susceptible to at least one carbapenem were screened by PCR for the presence of the clinically important carbapenemase-encoding genes, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC}, bla_{GES} and bla_{OXA-48}, as reported previously (Papagiannitsis et al., 2015a). PCR products were sequenced on both strands using an ABI 3500 sequencer (Applied Biosystems, Foster City, CA). All carbapenem non-susceptible isolates that tested negative with molecular assays for the detection of carbapenemase genes were further investigated by a spectrophotometric assay with crude extracts, using imipenem as a substrate, as previously described (Lauretti et al., 1999), in order to exclude the presence of carbapenemase types not included in the molecular assay. The group included 142 isolates producing IMP- (n = 97), VIM- (n = 41), and GES-type (n = 4) carbapenemases (Table 1). The remaining 108 isolates were non-carbapenemase producers. Furthermore, a group of 124 *Enterobacteriaceae* isolates from collections of the Faculty of Medicine and University Hospital in Plzen (Czech Republic), the National Medicines Institute in Warsaw (Poland) and the Robert Koch Institute in Wernigerode (Germany) were also examined for the imipenem assay. These isolates were tested previously using meropenem assay (Papagiannitsis et al., 2015b).

The MALDI-TOF MS assays were performed essentially as described previously (Knox et al., 2014; Papagiannitsis et al., 2015b). Isolates were inoculated on Mueller-Hinton agar plates (Bio-Rad Laboratories, Prague, Czech Republic) and incubated overnight at 35 °C. A bacterial inoculum, equivalent to 3 of the McFarland scale, was prepared in a suspension buffer (20 mM Tris-HCl, 20 mM NaCl, and pH 7.0). Then, 1.0 ml of the bacterial inoculum was centrifuged. The pellet was resuspended in 50 µl of a reaction buffer (0.4 mM imipenem in 0.45% NaCl, 0.1 mM $ZnSO_4$ or 0.1 mM meropenem in 20 mM Tris-HCl [pH 7.0], 0.01% sodium dodecyl sulfate, 50 mM NH_4HCO_3). The reaction mixture was incubated at 35 °C for 2 h. Then, the reaction mixture was centrifuged, 1 µl of the supernatant was applied on a

* Corresponding author at: Department of Microbiology, Faculty of Medicine and University Hospital in Plzen, Alej Svobody 80, 304 60 Plzen, Czech Republic.
E-mail address: c.papagiannitsis@gmail.com (C.C. Papagiannitsis).

http://dx.doi.org/10.1016/j.mimet.2017.04.003
Received 1 March 2017; Received in revised form 4 April 2017; Accepted 4 April 2017
Available online 05 April 2017

Table 1
Results of the MALDI-TOF MS meropenem and imipenem hydrolysis assays.[a]

Resistance mechanism	No. of isolates	No. of carbapenemase producers detected using MALDI-TOF MS[b]:		Sensitivity		Specificity	
		Meropenem assay[c]	Imipenem assay	Meropenem assay[c]	Imipenem assay	Meropenem assay[c]	Imipenem assay
Pseudomonas aeruginosa				90%	97%	93%	100%
IMP	97	91	96				
VIM	41	33	38				
GES	4	4	4				
Total carbapenemase producers	**142**	**128**	**138**				
Non-carbapenemase producers	**108**	**8**	**0**				
Enterobacteriaceae				99%	96%	100%	100%
KPC	21	20	21				
VIM	18	18	16				
IMP	1	1	1				
NDM	24	24	24				
OXA-48-like	19	19	18				
Total carbapenemase producers	**83**	**82**	**80**				
Non-carbapenemase producers	**41**	**0**	**0**				

[a] Rows representing the total number of isolates per category are in bold.
[b] Numbers refer to the number of isolates that were interpreted as carbapenemase producers by each method.
[c] Data for the meropenem assay against *Enterobacteriaceae* isolates are from a previous study (MALDI-TOF BIC assay; Papagiannitsis et al., 2015b).

stainless steel MALDI target plate (MSP 96 target; Bruker Daltonics) and allowed to dry. Each sample was overlaid with 1 μl of matrix solution (3.3 mg/ml of a-cyano-4-hydroxycinnamic acid [HCCA] in 50% ethanol for imipenem assay; or 10 mg/ml of 2,5-dihydroxybenzoic acid [DHB] in 50% ethanol for meropenem assay). After air drying, spectra were measured within the *m/z* range 300–600 using a microflex LT mass spectrometer with the flexControl 3.4 software (Bruker Daltonics). The analysis of the spectra was performed using the flexAnalysis 3.4 software. For imipenem assay, a negative result was defined as the presence of imipenem (300-*m/z* peak), whereas a positive result was defined as the complete disappearance of imipenem (Knox et al., 2014). For meropenem assay, an isolate was interpreted as a non-carbapenemase producer if the absence of both decarboxylated products of meropenem (358.5-*m/z* and 380.5-*m/z* peaks) and the presence of meropenem and/or its sodium salt (384.5-*m/z* and 406.5-*m/z* peaks) were observed, while an isolate was interpreted as a carbapenemase producer if the presence of at least one of the decarboxylated products of meropenem was detected (Papagiannitsis et al., 2015b).

The results are summarized in Table 1. For *P. aeruginosa* isolates, the imipenem assay gave no false-positive results among non-carbapenemase producers (100% specificity), while it correctly detected 138 (97% sensitivity) carbapenemase-producing isolates. This assay missed one IMP- and three VIM-producing isolates. On the other hand, eight non-carbapenemase-producing isolates were falsely classified as positive by the meropenem assay (93% specificity). This assay correctly detected the production of a carbapenemase in 128 isolates, while it failed to detect five *P. aeruginosa* isolates expressing either IMP ($n = 3$) or VIM ($n = 2$). For nine carbapenemase-producing *P. aeruginosa*, meropenem hydrolysis spectra were of low quality precluding a definitive analysis (90% sensitivity). Imipenem hydrolysis spectra were generally better (Fig. 1), allowing an easier interpretation. However, in agreement with the findings of a previous study (Knox et al., 2014), no peaks corresponding to imipenem degradation products were identified.

For *Enterobacteriaceae* isolates, false-positive results were not observed with the imipenem assay (100% specificity) (Table 1). This assay correctly detected eighty carbapenemase-producing *Enterobacteriaceae*, including all KPC-, IMP- and NDM-producing isolates (96% sensitivity). The imipenem assay missed one OXA-48-producing *Klebsiella pneumoniae* and two *Enterobacter cloacae* expressing VIM metallo-β-lactamases.

To our knowledge, this is the first study validating the use of different carbapenem molecules for the MALDI-TOF MS detection of carbapenemase activity. Imipenem assay achieved the best sensitivity (97%) and specificity (100%) scores for *P. aeruginosa*. Whereas, based on the data previously published (Papagiannitsis et al., 2015b), the meropenem assay exhibiting 98% sensitivity and 100% specificity was more efficient against *Enterobacteriaceae*. For meropenem assay, addition of NH_4HCO_3 to the reaction buffer is crucial for the efficient detection of OXA-48-type producers (Papagiannitsis et al., 2015b). Imipenem assay didn't experience problems with the subset of the OXA-48-type producers. However, for this assay, addition of $ZnSO_4$ to the reaction buffer increased its sensitivity for the detection of VIM-producing bacteria (Papagiannitsis unpublished data). This is in accordance with the results of a recent study demonstrating that addition of zinc ions improved detection of metallo-β-lactamase producers by MALDI-TOF MS imipenem hydrolysis assay (Knox and Palombo, 2017). These findings may indicate that distinct strategies must be used for the detection of carbapenemase activity in different pathogen types.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We are thankful to Helena Zemlickova, Marek Gniadkowski and Yvonne Pfeifer for providing some of the isolates used in this study.

This work was supported by the National Sustainability Program I (NPU I; Nr. LO1503) provided by the Ministry of Education Youth and Sports of the Czech Republic.