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A simple, robust and rapid approach to detect carbapenemases in Gram-negative isolates by MALDI-TOF mass spectrometry: validation with triple quadripole tandem mass spectrometry, microarray and PCR

C. Vogne[1], G. Prod'hom[1], K. Jaton[1], L. A. Decosterd[2] and G. Greub[1]

1) *Institute of Microbiology, University of Lausanne and University Hospital Center, and* 2) *Laboratory of Clinical Pharmacology, Innovation and Development Unit, Service of Biomedicine, Lausanne, Switzerland*

Abstract

Carbapenemases should be accurately and rapidly detected, given their possible epidemiological spread and their impact on treatment options. Here, we developed a simple, easy and rapid matrix-assisted laser desorption ionization-time of flight (MALDI-TOF)-based assay to detect carbapenemases and compared this innovative test with four other diagnostic approaches on 47 clinical isolates. Tandem mass spectrometry (MS-MS) was also used to determine accurately the amount of antibiotic present in the supernatant after 1 h of incubation and both MALDI-TOF and MS-MS approaches exhibited a 100% sensitivity and a 100% specificity. By comparison, molecular genetic techniques (Check-MDR Carba PCR and Check-MDR CT103 microarray) showed a 90.5% sensitivity and a 100% specificity, as two strains of *Aeromonas* were not detected because their chromosomal carbapenemase is not targeted by probes used in both kits. Altogether, this innovative MALDI-TOF-based approach that uses a stable 10-μg disk of ertapenem was highly efficient in detecting carbapenemase, with a sensitivity higher than that of PCR and microarray.

Keywords: Carbapenem resistance, Gram-negative bacilli, MALDI-TOF MS, microarray, MS-MS spectrometry, PCR
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Corresponding author: G. Greub, Institute of Microbiology, University Hospital of Lausanne, Bugnon 48, 1011 Lausanne, Switzerland
E-mail: gilbert.greub@chuv.ch

Introduction

Antibiotic multiresistance is a major problem for treatment of Gram-negative bacterial infections, especially those due to Enterobacteriaceae and non-fermentative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. The use of carbapenems was a good alternative to cephalosporins, but worldwide spread of carbapenem resistance may impair this therapeutic alternative [1]. Carbapenem resistance may be caused by a loss of porin or overexpression of active efflux [2], but can also be due to the bacterial production of carbapenemases. Identification of carbapenemase-producing strains is difficult and time-consuming in the diagnostic laboratory, because the gene encoding this enzyme could be carried on a plasmid often associated with other resistance genes. Misidentification of carbapenemase resistance could lead to inappropriate antibiotic treatment and development of multiresistance [3,4].

To investigate the Gram-negative bacterial isolates regarding expression of carbapenemase, we used a panel of approaches including various antibiotic susceptibility methods such as E-test, disk diffusion assay and MIC in broth, as well as a modified Hodge test and combined disk assays [5]. The specificity and the sensitivity of these carbapenemase identification tests are not precisely defined regarding the different carbapenem enzymes and the modified Hodge test may be difficult to interpret [6,7]. New promising biochemical methods for detection of carbapenemase have been

described, and their place in the screening of carbapenemases has to be better defined [8–10].

Recently, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was successfully used to detect the activity of carbapenemase in an isolated bacterial strain (i.e. its ability to degrade the antibiotic during a 2.5-h incubation with a carbapenem). Burckhardt *et al.* [11] followed *in vitro* the degradation of carbapenems into hydrolyzed and decarboxylated products measured in incubation supernatants with MALDI-TOF MS. The authors described the complete disappearance within 2.5 h of signal peaks of ertapenem at an *m/z* value of 476, 498 and 521 Da. Similarly, Hrabak *et al.* [12] also used MALDI-TOF MS to directly detect meropenem and its degradation products in supernatants of carbapenemase-positive or negative isolates. The presence or the absence of specific meropenem peaks allowed the identification of the enzymatic carbapenem resistance phenotype. A shorter and simpler MALDI-TOF-based technique is warranted.

Therefore, we developed a simplified technique compared with that of Burckhardt *et al.*, with a shorter incubation of the strains investigated and with the use of a 10-μg commercialized ertapenem impregnated disk, routinely used in clinical laboratories.

The ability of this new MALDI-TOF approach to detect carbapenem resistance was assessed by investigating strains (i) using phenotypic methods such as Vitek, e-tests and Hodge test, (ii) using a modified liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) assay (L.A. Decosterd, B. Ternon, S. Cruchon, N. Guignard, S. Lahrichi, B. Pesse, B. Rochat, T. Calandra, C. Csajka, T. Buclin, A. Mello, N. Widmer and O. Marchetti, unpublished data), [13] that allows the precise and accurate quantification of ertapenem concentrations in the incubation supernatant and (iii) using two rapid molecular genetics techniques, which both detect the presence of clinically relevant carbapenemase-encoding genes within a single reaction: (a) the Check-MDR Carba detects the plasmidic carbapenemases (i.e. KPC, OXA-48 and other metallo-beta-lactamases (MBL) such as VIM and IMP, as well as the rapidly emerging NDM-1); and (b) the Check-MDR CT103 microarray, which identifies KPC, NDM-1, OXA-48, VIM and IMP carbapenemase genes as well as TEM-, SHV- and CTX-M-type ESBLs.

Materials and Methods

Antimicrobial susceptibility

Carbapenem MICs (ERT, IMP and MEM) were determined with Etest (BioMérieux, Lyon, France) and interpreted according to EUCAST guidelines. (European Committee on Antimicrobial Susceptibility Testing. 2012. Breakpoint tables for interpretation of MICs and zone diameters, version 2.0. EUCAST, Växjö, Sweden. http://www.eucast.org/antimicrobial_susceptibility_testing). The two isolates used as references were the carbapenemase-positive *Klebsiella pneumoniae* (ATCC BAA-1705, *bla* $^{KPC+}$) and the negative carbapenemase strain *Klebsiella pneumoniae* (ATCC BAA-1706, (*bla* $^{KPC-}$).

Clinical isolates

We studied 47 non-repetitive clinical isolates presenting resistance to cephalosporins and/or carbapenems, isolated from January 2011 to December 2012 at our university hospital centre. The following 37 Enterobacteriaceae isolates were tested: *Klebsiella pneumoniae* (n = 10), *Escherichia coli* (n = 9), *Enterobacter cloacae* (n = 7), *Providencia stuartii* (n = 2), *Serratia marcescens* (n = 2), *Enterobacter aerogenes* (n = 3), *Hafnia alvei* (n = 1), *Klebsiella oxytoca* (n = 1), *Proteus vulgaris* (n = 1) and *Morganella morganii* (n = 1). We also tested 10 non-fermentative bacteria: *Pseudomonas aeruginosa* (n = 4), *Acinetobacter baumanii* (n = 4) and *Aeromonas* (n = 2).

Carbapenemase assays

Phenotypic detection of carbapenemase production was performed using the modified Hodge test recommended by the Clinical and Laboratory Standards Institute (CLSI) and Center for Disease Control (CDC) (CLSI. 19th Informational Supplement. CLSI document M100-S19, 2009 and CDC: http://www.cdc.gov/hai/). A suspension of *Escherichia coli* ATCC 25922, adjusted to 0.5 McFarland was inoculated on a Mueller-Hinton agar plate. Then, an ertapenem disk (10 μg, BBL) was placed at the centre of the agar plate. Control and test isolates were streaked from the edge of the disk to the periphery of the plate. The presence of a distorted inhibition zone after 16–18 h of incubation at 37°C was interpreted as a positive modified Hodge test. The imipenem/imipenem-inhibitor (IP/IPI) E-test was used to screen for the presence of metallo-beta-lactamase production. Interpretation of the results was carried out according to the manufacturer's instructions. After 16–18 h of incubation at 35°C, a reduction in MIC in the presence of EDTA of greater than or equal to eight-fold was interpreted as positive MBL activity.

Ertapenem degradation assay

The tested isolates were grown overnight on a blood agar plate at 37°C. An inoculum of 7 on the McFarland scale was resuspended in 1 mL of NaCl 0.45% in glass tubes. This bacterial suspension was then incubated for 60 min at 37°C with an ertapenem disk (10 μg) to reach a final concentration of 10 mg/L. After incubation, 500 μL were centrifuged at 12 000 $\times$ g for 2 min and 1 μL of the supernatant was used

for MALDI-TOF MS analysis. Then, we added 1 μL of MALDI matrix (a saturated solution of a-cyano-4-hydroxycinnamic acid in 5% acetonitrile – 2.5% trifluoroacetic acid).

MALDI-TOF and spectrum data analysis

The analysis was carried out with the Bruker Daltonics Microflex (Bremen, Germany). The detection parameter used for the analysis was the Antibiotics_500.par provided by Bruker, and the Mass Range was the 'low level (300–800 Da)' option. Flex analysis 3.0 software was used to analyse the mass spectrum, with windows ranging from 400 to 600 Da. Around 500 shots per spectrum were summed up, then all spectrums were smoothed and the baseline was subtracted. All spectra of the different incubation supernatants were analysed and compared with the ertapenem incubation control diluted at 10 mg/L and the supernatant of culture without ertapenem (blank).

MS-MS analysis

The ertapenem concentrations in enzymatic incubation medium have been measured with a specific and sensitive liquid chromatography-tandem mass spectrometry method (LC-MS/MS) using an adaptation of validated multiplex assays developed in our laboratory for the drug monitoring of antimicrobials (L.A. Decosterd, B. Ternon, S. Cruchon, N. Guignard, S. Lahrichi, B. Pesse, B. Rochat, T. Calandra, C. Csajka, T. Buclin, A. Mello, N. Widmer and O. Marchetti, unpublished data), [13]. Briefly, the enzymatic incubation aliquot (100 μL) was added to 50 μL of Internal Standard (I.S.) solution (cefazoline 18 mg/L) and was placed in ice at +4°C. The resulting sample was carefully vortex-mixed with methanol (300 μL). The mixture was then centrifuged at +4°C for 10 min at 20 000 *g* (14 000 rpm) and an 80-μL aliquot of the supernatant was diluted with 480 μL of ultrapure water prior to HPLC-MS/MS analysis. Processed samples were maintained at +4°C in the thermostat autosampler rack during the entire LC-MS/MS analysis.

Reverse-phase chromatographic separation of ertapenem (eluted at 2.6 min) was obtained using a gradient elution of the mobile phase of 10 mM ammonium formic acid solution with 4% formic acid (FA) (solution A) and acetonitrile (solution B) delivered onto the UPLC column Waters Acquity UPLC HSS T3 2.1 × 50 mm (Waters®, Milford, MA, USA), 1.8 μm, using the following stepwise gradient elution program: 4% of B at 0 min to 90% of B at 5.5 min, followed by a re-equilibration step to the initial solvent up to 9 min. Ertapenem quantification using matrix-matched calibration samples (NaCl 150 mM) was performed by electro-spray ionisation–triple quadrupole mass spectrometry on a Quantum Ultra mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), by selected reaction monitoring detection using the transitions for ertapenem at *m/z*: 476.2 → 432.7, 476.2 → 114.4 and 476.2 → 68.1, in the positive mode. The lower limit of quantification of ertapenem in incubation medium was 0.1 mg/L.

Molecular methods

Two molecular kits were used, which detect the presence of most clinically relevant carbapenemase-encoding genes, the Check-MDR Carba and the Check-MDR CT103 microarray. These kits, designed by Check-Points Health BV (Wageningen, the Netherlands), were used according to the manufacturer's instructions.

Results

Susceptibility to carbapenems

Thirteen of the 15 Enterobacteriaceae expressing carbapenemases were resistant to ertapenem with MIC >1 mg/L (Table 1). Two *Providencia stuartii* carbapenemase isolates exhibited no resistance to carbapenem (MIC ERT <0.064 mg/L). In contrast, among seven carbapenemases expressed by non-fermentative isolates, only three presented a high resistance to ertapenem. The two *Aeromonas* isolates presented a resistance to ertapenem even with concentrations lower than 1 mg/L.

Analysis of spectrum obtained with the two *Klebsiella pneumoniae* reference isolates in the presence of ertapenem

The first reference isolate was a carbapenemase producer (KPC strain) whereas the second one was resistant to carbapenem by another resistance mechanism (AmpC-type enzyme combined with porin loss). The analysis of the supernatant obtained with both strains using MALDI-TOF MS revealed two different molecular peaks (hereafter called peak A and peak B) that may be used to detect a carbapenemase producer strain (Fig. 1). The peak A appears at an *m/z* value of 476 Da in the absence of carbapenemase, corresponding to the non-hydrolyzed form (Ertapenem + H^+), whereas it is displaced to 478 Da when hydrolyzed. This 2 kDa increase probably reflects the presence of an intermediate compound, the precise molecular composition of which remains to be determined. Similarly, peak B is observed at an *m/z* value of 498 Da in the absence of a carbapenemase, corresponding to the monosodium ertapenem (Ertapenem + Na^+), whereas it is displaced to 496 Da in the presence of carbapenemase. However, for the KPC strain we observed in our conditions, there was no complete disappearance of 476, 498 and 521 Da peaks (corresponding to ertapenem degradation products), probably due to a relatively short incubation, which does not allow a complete ertapenem hydrolysis.

TABLE 1. Summary of MICs obtained for ertapenem, imipenem and meropenem, as well as results of the different tests used to define whether a carbapenemase is present in each isolate: (i) modified Hodge test and IP/IPI E-test, (ii) matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), (iii) Check-MDR CARBA PCR-based test, (iv) microarray and (v) MS/MS approach. In the last column, the presence (+) or absence (–) of a carbapenemase as derived from the results of these five different tests is provided (reference standard)

Clinical isolate type	Isolate number	MIC (mg/L) for:			Hodge	IP/IPI	MALDI-TOF	Check-MDR CARBA	Results of microarray		MS/MS: ERT (mg/L)	Carbapenemase[a]
		ERT	IMP	MEM								
Klebsiella pneumoniae	Negative control				–	–	–	–	–	AmpC	9.8 ± 0.55	–
Klebsiella pneumoniae	Positive control				+	–	+	+	KPC	–	0	+
Klebsiella pneumoniae	7677	>32	0.75	0.5	+	–	+	+	VIM	ESBL	0	+
Klebsiella pneumoniae	7678	32	0.75	0.5	+	–	+	+	VIM	ESBL	0	+
Klebsiella pneumoniae	7708	0.047	0.38	0.125	–	–	–	–	–	ESBL	9.7 ± 1.22	–
Klebsiella pneumoniae	7732	>32	0.5	2	–	–	–	–	–	ESBL	6.5 ± 1.21	–
Klebsiella pneumoniae	7877	>32	>32	>32	+	+	+	+	NDM	ESBL	0	+
Klebsiella pneumoniae	7932	>32	>32	24	+	+	+	+	NDM	ESBL	0	+
Klebsiella pneumoniae	8052	6	4	2	+	–	+	+	KPC	ESBL	0	+
Klebsiella pneumoniae	8083	>32	>32	>32	+	–	+	+	KPC	ESBL	0	+
Klebsiella pneumoniae	8090	>32	2	8	–	–	–	–	–	ESBL	8.4 ± 0.35	–
Klebsiella pneumoniae	8161	>32	>32	>32	+	–	+	+	KPC	ESBL	0	+
Escherichia coli	7633	0.012	0.125	0.032	–	–	–	–	–	ESBL	9.5 ± 0.41	–
Escherichia coli	7645	0.064	0.19	0.047	–	–	–	–	–	ESBL	9.2 ± 0.25	–
Escherichia coli	7662	>32	0.38	0.19	–	–	–	–	–	AmpC	9.5 ± 1.35	–
Escherichia coli	7669	0.016	0.19	0.047	–	–	–	–	–	ESBL	9.3 ± 0.43	–
Escherichia coli	7680	>32	0.5	0.19	–	–	–	–	–	AmpC	8.7 ± 0.13	–
Escherichia coli	7715	0.023	0.19	0.032	–	–	–	–	–	ESBL	8.1 ± 0.55	–
Escherichia coli	7731	0.047	0.19	0.094	–	–	–	–	–	ESBL	8.9 ± 0.50	–
Escherichia coli	7469	4	2	2	+	–	+	+	OXA-48	–	2.4 ± 0.45	+
Escherichia coli	8160	1	1	0.25	–	–	–	–	–	–	8.2 ± 0.20	–
Klebsiella oxytoca	7064	0.064	0.25	0.094	–	–	–	–	–	–	9.8 ± 1.00	–
Enterobacter cloacae	6354	>32	8	8	–	–	–	–	–	AmpC	7.5 ± 0.64	–
Enterobacter cloacae	7456	>32	1	1	–	–	–	–	–	AmpC	9 ± 0.6	–
Enterobacter cloacae	7722	8	0.38	0.25	–	–	–	–	–	AmpC	9.40 ± 0.15	–
Enterobacter cloacae	7723	1.5	0.25	0.19	–	–	–	–	–	AmpC	9.6 ± 1.18	–
Enterobacter cloacae	7845	>32	0.75	1	+	–	–	–	–	AmpC	10.10 ± 0.35	–
Enterobacter cloacae	8088	3	0.25	0.12	+	–	+	+	OXA-48	AmpC	1.9 ± 1.68	+
Enterobacter cloacae	8121	12	4	2	+	–	+	+	VIM	–	0	+
Enterobacter aerogenes	6587	>32	32	3	–	–	–	–	–	–	8.8 ± 1.01	–
Enterobacter aerogenes	7548	32	1.5	0.75	+	–	–	–	–	–	9.5 ± 1.10	–
Enterobacter aerogenes	7801	3	0.5	0.19	+	–	–	–	–	–	9.2 ± 0.40	–
Serratia marcescens	5965	>32	24	>32	+	–	+	+	OXA-48	–	1.7 ± 0.15	+
Serratia marcescens	8057	>32	>32	32	+	–	+	+	KPC	–	0	+
Hafnia alvei	6884	0.19	0.25	0.094	–	–	–	–	–	AmpC	7.9 ± 1.65	–
Proteus vulgaris	8133	0.5	0.38	0.03	–	–	–	–	–	–	9 ± 0.78	–
Morganella morganii	7572	>32	>32	3	–	–	+	+	NDM	AmpC	0	+
Providencia stuartii	8117	0.045	0.5	0.06	+	+	+	+	VIM	–	0.10 ± 0.10	+
Providencia stuartii	8118	0.064	1	0.125	+	–	+	+	VIM	–	0 ± 0.03	+
Pseudomonas aeruginosa	7752	>32	>32	>32	–	+	–	–	–	–	10 ± 0.47	–
Pseudomonas aeruginosa	7622	>32	>32	>32	+	+	+	+	VIM	AmpC	0	+
Pseudomonas aeruginosa	6487	>32	>32	32	–	–	+	+	VIM	–	0	+
Pseudomonas aeruginosa	7072	>32	>32	>32	+	+	+	+	VIM	–	0	+
Aeromonas sp.	8053	1	>32	0.25	+	–	+	–	–	–	0	+
Aeromonas veronii	8113	0.75	0.5	0.25	+	–	+	–	–	–	0.04 ± 0.02	+
Acinetobacter baumanii	8119	>32	>32	>32	–	+	–	–	–	–	6.9 ± 0.45	–
Acinetobacter baumanii	8132	>32	>32	>32	–	+	–	–	–	–	8.3 ± 0.36	–
Acinetobacter baumanii	8061	>32	>32	>32	+	+	+	–	–	–	9 ± 0.55	–
Acinetobacter baumanii	7476	>32	0.38	0.5	–	–	–	–	–	–	10 ± 0.43	–

ERT, ertapenem; IMP, imipenem; MEM, meropenem.
[a]As defined using a reference standard based on five different approaches: true-positive carbapenemase producers were defined as 3/5 (n = 2), 4/5 (n = 2) or 5/5 (n = 17) positive tests and true-negatives were defined as 0/5 (n = 24) or 1/5 (n = 3) negative tests.

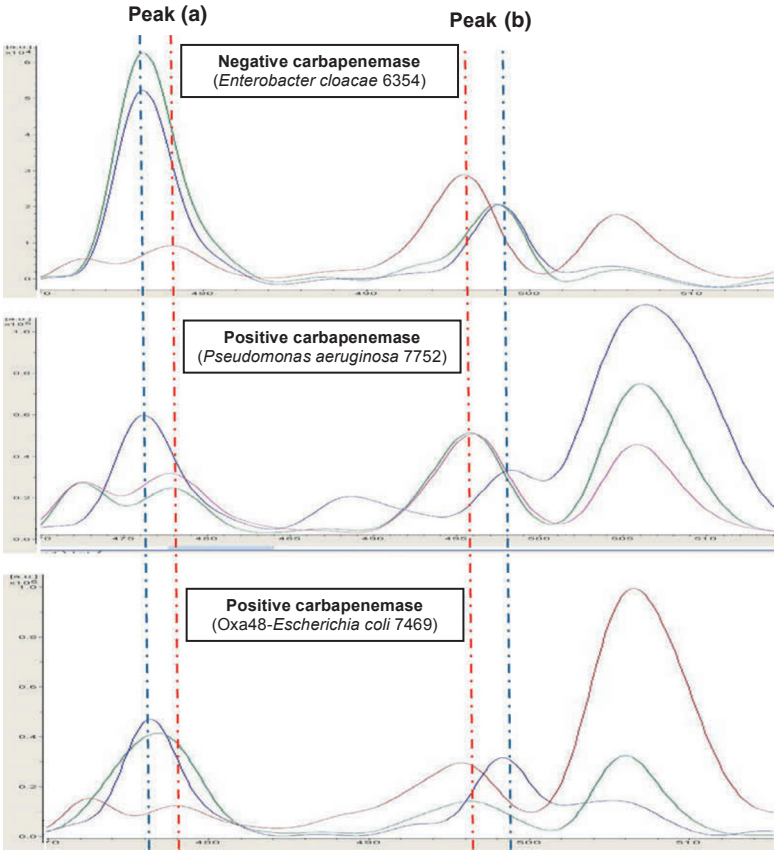

FIG. 1. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) spectra of supernatants after 1 h incubation of a KPC *Klebsiella pneumoniae* positive control (red line), a negative *K. pneumoniae* control (blue line) and the tested strain (green line), in the presence of ertapenem. Ertapenem degradation is shown when peak A (476 Da) and peak B (498 Da) are shifted. Dotted blue lines show the position of A and B peaks for negative strains whereas the dotted red line shows the position of A and B peaks for carbapenemase-producing strains. Please note that only the B peak obtained with the Oxa48 *Escherichia coli* 7469 strain exhibited a clear shift.

Analysis of the MALDI-TOF spectrum profiles of the clinical isolates

The 37 Enterobacteriaceae and the 10 non-fermentative bacteria presenting cephalosporins and/or carbapenems resistance were tested for their MALDI-TOF profiles spectrum in the presence of ertapenem. The analysis focusing on the two peaks A and B described above allowed the identification of 22 isolates exhibiting the typical shifts of peaks A-476 and B-498, compatible with carbapenemase degradation (Table 1). For the Oxa48-positive *Escherichia coli* strain 7469, we observed a clear shift of peak B, while peak A was only partially shifted to the right. This modification possibly reflects the lower activity of the Oxa-48-like enzyme (Fig. 1), a lower activity confirmed by MS-MS (see below).

MS-MS analysis

In order to confirm the positive carbapenemase producers, the degradation of ertapenem was assessed by LC-MS-MS in all supernatants and compared with the MALDI-TOF results. For all isolates identified as carbapenemase-positive by MALDI-TOF, ertapenem concentration was around 0 mg/L, except for three OXA-48-like producer isolates (*Enterobacter cloacae* 8088, *Serratia marcescens* 5965 and *Escherichia coli* 7469), in which 1.9, 1.7 and 2.4 mg/L of ertapenem, respectively, were detected in the culture media after 60 min of incubation with these isolates. Conversely, for all non-carbapenemase producers, the concentration of ertapenem after 60 min of culture in the presence of the antibiotic was always more than 6 mg/L and generally about 10 mg/L (Fig. 2, Table 1).

Rapid molecular detection of clinically important carbapenemase genes

The Check-MDR Carba was used to identify the most prevalent carbapenemase-encoding genes [14]. *Klebsiella pneumoniae* carbapenemase KPC, and metallo-beta-lactamase including IMP, VIM and NDM 1, class D Oxa-48-like genes, could be detected in a simple real-time PCR reaction. Of 47 isolates tested, a total of 19 isolates presented a carbapenemase gene, all belonging to the 22 strains identified to be carbapenemase producers by using the MALDI-TOF and the MS-MS approach (see below). Results were confirmed by the Check-MDR CT103 microarray. Eleven of the 47 isolates were AmpC producers (MIR-ACT group, CMY II, DHA and ACC), with three associated with a carbapenemase gene (Oxa-48, VIM and NDM). Among 16 extended-spectrum beta-lactamase (ESBL) isolates

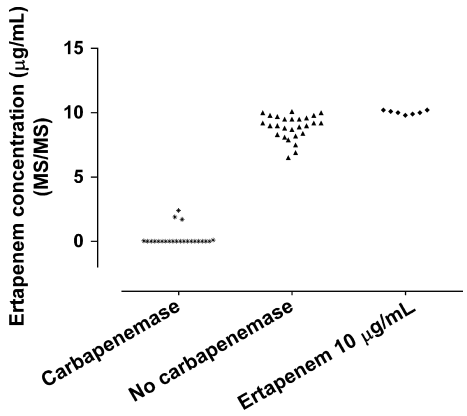

FIG. 2. Ertapenem concentration as measured by MS/MS according to the presence of carbapenemase as determined by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Please note that the positive control obtained with the 10-mg disk in the absence of any bacterial strain is shown in the right column.

(CTX and SHV), eight co-expressed carbapenemase genes (KPC, VIM and NDM). Finally, a total of five KPC, 12 MBL producers (nine VIM and three NDM) and three Oxa-48-like encoding genes were detected. For 10 beta-lactamine-resistant isolates, no expression of beta-lactamase was observed with molecular approaches (Check MDR 103 and microarray (Table 1)). Moreover, no carbapenemase genes were detected by PCR and microarray in four isolates presenting a modified Hodge test and/or an IP/IPI positive test. In addition, two *Aeromonas* strains with a natural resistance to carbapenems encoded on the bacterial chromosome were not detected using the Check-MDR Carba, as expected [15].

Test performance

Using a reference standard based on the results from five different approaches, true-positive carbapenemase producers were defined as 3/5 ($n = 2$), 4/5 ($n = 2$) or 5/5 ($n = 17$) positive tests and true-negatives were defined as 0/5 ($n = 24$) or 1/5 ($n = 3$) negative tests. Thus, in this work, a total of 21 positive and 28 negative isolates were included (Table 1). The sensitivities of the different diagnostic approaches ranged from 90% to 100%, whereas the specificities ranged from 86% to 100% (Table 2). The two spectrometry approaches (MALDI-TOF and MS-MS) exhibited the best sensitivities and specificities (100%). Although a specificity of 100% was obtained with both molecular approaches (MDR Carba and Check MDR 103), the specificities of these techniques exhibited only 90% (19/21). The modified Hodge test using ertapenem successfully identified 19/21 (90%) of the carbapenemase producers. False-negative modified Hodge test results were obtained for two isolates producing metallo-beta-lactamases (i.e. NDM and VIM, respectively).

TABLE 2. Sensitivity, specificity of different diagnostic approaches, phenotypic, PCR, microarray, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) and MS/MS

Diagnostic approach	Sensitivity	Specificity
Phenotypic detection (modified Hodge test)	90.5% (19/21)	86% (24/28)
Phenotypic detection (IP/IPI)[a]	54.5% (6/11)	92.1% (35/38)
PCR-Check MDR Carba	90.5% (19/21)	100% (28/28)
Microarray	90.5% (19/21)	100% (28/28)
MALDI-TOF	100% (21/21)	100% (28/28)
MS/MS	100% (21/21)	100% (28/28)

[a]Phenotypic detection of metallo-beta-lactamases (VIM, IMP and NDM).

False-positive results were obtained for three *Enterobacter* spp., including one AmpC hyperproducer.

Discussion

It is now well established that the MALDI-TOF can be used not only for bacterial identification [16,17] but also can be applied in other important areas of diagnostic bacteriology, such as the detection of antibiotic modifications mediated by enzymatic expression of different enzymes (beta-lactamase and carbapenemase) [11,18].

In this study, we developed an easy, robust and rapid technique for identification of carbapenemase in Gram-negative isolates by MALDI-TOF mass spectroscopy in 1 h. The degradation of the ertapenem antibiotic was analysed by MALDI-TOF mass spectrometry by comparing the pattern of peak profiles obtained between 300 and 800 Da *(m/z)*.

Ertapenem was used in our experiment because it appears to be the most promising Carbapenemase for detection of KPC and carbapenem producers. Our new approach is highly efficient for detecting carbapenemase activity, exhibiting a 100% congruency with quadrupole mass spectrometry and overall exhibiting 100% sensitivity and 100% specificity on the 47 strains investigated here. Check-point PCR and check-point microarray have been shown to be sensitive and specific techniques. However, the longer time to results and higher cost make them relatively useless. In our MALDI-TOF assay, the use of disk diffusion has the advantage of having a stable and calibrated carbapenem concentration to ease testing in the setting of a routine diagnostic laboratory.

In conclusion, we modified a previously described MALDI-TOF technique to detect the presence of carbapenemases, using a simple 1-h MALDI-TOF-based test. Our innovative approach, which uses a 10-μg disk of ertapenem (available in any laboratory using this disk diffusion test) was highly efficient in detecting carbapenemases, with a sensitivity higher than that of

PCR and microarray. MS-MS measurement of ertapenem after incubation of the 10-μg disk with the investigated strain is an alternative that showed 100% congruency with MALDI-TOF. Despite allowing more precise quantification, it has the limitation, unlike MALDI-TOF, of not always being available in clinical bacteriology laboratories, and necessitates a costly MS-MS infrastructure and the specific know-how of expert chemistry technicians.

Conversely, the MALDI-TOF-based approach that we report here has the advantage of being cheap, highly accurate and rapid (overall TAT about 1 h 15 min) and can be performed in any laboratory equipped with a MALDI-TOF, which is now part of the routine equipment of any diagnostic laboratory. However, the spectra should be manually interpreted by a specifically trained technician, at least until an automated interpretation algorithm is developed and implemented in the Bruker software.

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Transparency Declaration

The authors have no conflicts of interest.

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