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# Detection of SPM-1-Producing *Pseudomonas aeruginosa* and Class D β-Lactamase-Producing *Acinetobacter baumannii* Isolates by Use of Liquid Chromatography-Mass Spectrometry and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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**This study evaluates the accuracy of liquid chromatography-mass spectrometry (LC-MS) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) for detecting carbapenem hydrolytic activity among SPM-1-, GIM-1-, and GES-5-producing *Pseudomonas aeruginosa* isolates and OXA-143-, IMP-10-, and OXA-58-producing *Acinetobacter baumannii* isolates. Class A and B carbapenemase activities were rapidly detected by MALDI-TOF in a 2-h assay. However, an extended incubation time was necessary for detection of carbapenem-hydrolyzing class D β-lactamase (CHDL) activity in *Acinetobacter* spp.**

Over the last two decades, the therapeutic options to treat infections caused by Gram-negative rods have been narrowed by bacterial acquisition of carbapenemase-encoding genes (1). The rapid detection of clinically significant carbapenemases is important for establishing adequate therapy and controlling their spread. Recently, the detection of class A or B carbapenemase activity has been accomplished by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (2, 3). Although MALDI-TOF MS seems to be a promising tool, there is a lack of evidence showing its usefulness in testing a broader range of carbapenemases. To date, the published studies have mainly focused on KPC-2- and NDM-1-producing *Enterobacteriaceae*, IMP-1-, VIM-1-, and VIM-2-producing *Pseudomonas aeruginosa*, and OXA-23- and OXA-24-producing *Acinetobacter baumannii* isolates (2–4). In the current study, we evaluated the performance of the MALDI-TOF MS assay in detecting carbapenemase activity of GES-5-, GIM-1-, or SPM-1-producing *P. aerugi-*

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**TABLE 1** Characterization of the 73 clinical isolates and performance of carbapenemase detection by different methodologies[a]

| Bacterial species | Enzyme(s) produced | No. of isolates | MIC range (μg/ml) | | | % of isolates detected as carbapenemase producers (no. of positive isolates/total no. of isolates) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | MALDI-TOF | | LC-MS | |
| | | | ETP | IMP | MEM | MHT | IMP hydrolysis | 2 h | 4 h | 2 h | 4 h |
| *Acinetobacter baumannii* | | 41 | | | | | | | | | |
| | Oxa-23 | 16 | 16–>256 | 4–64 | 0.5–128 | 56 (9/16) | 100 (16/16) | 19 (3/16) | 100 (16/16) | 19 (3/16) | 100 (16/16) |
| | Oxa-23, Oxa-143 | 4 | 256–>256 | 32–256 | 32–256 | 100 (4/4) | 100 (4/4) | 75 (3/4) | 100 (4/4) | 75 (3/4) | 100 (4/4) |
| | Oxa-24, Oxa-143 | 1 | >256 | 128 | 256 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | Oxa-25 | 1 | >256 | 128 | 256 | 100 (1/1) | 100 (1/1) | 0 (0/1) | 100 (1/1) | 0 (0/1) | 100 (1/1) |
| | Oxa-26 | 1 | >256 | 128 | 256 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | Oxa-58 | 3 | 64–128 | 16 | 8–32 | 0 (0/3) | 100 (3/3) | 0 (0/3) | 100 (3/3) | 0 (0/3) | 100 (3/3) |
| | Oxa-23, Oxa-143, IMP-1 | 1 | >256 | 128 | 256 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | IMP-10 | 3 | 8–128 | 0.5–32 | 4–64 | 67 (2/3) | 100 (3/3) | 100 (3/3) | 100 (3/3) | 100 (3/3) | 100 (3/3) |
| | Negative control | 11 | 2–8 | <0.06–0.25 | 0.25–1 | 0 (0/11) | 0 (0/11) | 0 (0/11) | 0 (0/11) | 0 (0/11) | 0 (0/11) |
| *Klebsiella pneumoniae* | | 6 | | | | | | | | | |
| | KPC-2 | 1 | 32 | 8 | 8 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | NDM-1 | 1 | 128 | 64 | 32 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | ESBL/ΔOmpK36 | 4[b] | 0.25 | 0.25–2 | <0.06–16 | 0 (0/4) | 0 (0/4) | 0 (0/4) | 0 (0/4) | 0 (0/4) | 0 (0/4) |
| *Pseudomonas aeruginosa* | | 26 | | | | | | | | | |
| | GES-5 | 1 | 256 | 16 | 256 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | GES-1 | 1 | 64 | 1 | 4 | 0 (0/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) | 0 (0/1) |
| | SPM-1 | 11 | >256 | 128–>256 | 64–>256 | 64 (7/11) | 100 (11/11) | 100 (11/11) | 100 (11/11) | 100 (11/11) | 100 (11/11) |
| | IMP-1 | 1 | >256 | 32 | >256 | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) | 100 (1/1) |
| | VIM-1 | 2 | 256->256 | 256 | 256 | 0 (0/2) | 100 (2/2) | 100 (2/2) | 100 (2/2) | 100 (2/2) | 100 (2/2) |
| | GIM-1 | 4 | >256 | 128–256 | 256–>256 | 100 (4/4) | 100 (4/4) | 100 (4/4) | 100 (4/4) | 100 (4/4) | 100 (4/4) |
| | Negative control | 6 | 4–>256 | 0.5–16 | <0.06–32 | 0 (0/6) | 0 (0/6) | 0 (0/6) | 0 (0/6) | 0 (0/6) | 0 (0/6) |

[a] ETP, ertapenem; IPM, imipenem; MEM, meropenem; MHT, modified Hodge test; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight mass spectrometry; LC-MS, liquid chromatography-mass spectrometry.
[b] Isolates previously characterized by Carvalhaes et al. (5).

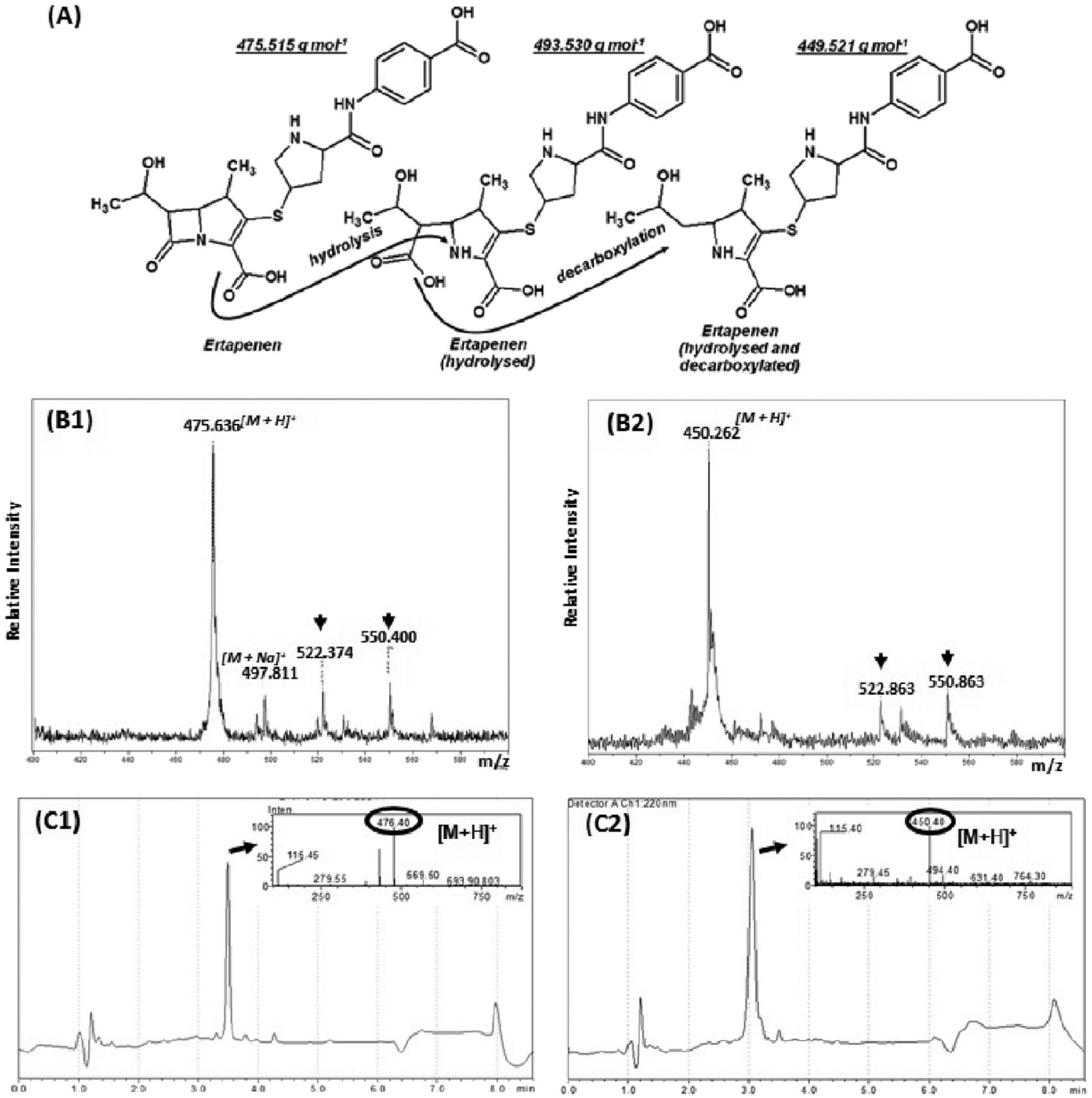


**FIG 1** (A) The hydrolysis of ETP mediated by β-lactamases occurs in two steps: in the first step, the ETP intact molecule (475 g $mol^{-1}$) suffers a rupture on its β-lactam ring, resulting in a hydrolyzed ETP molecule (493 g $mol^{-1}$). In the second step, the hydrolyzed ETP molecule loses a carboxyl group ($-CO_2$), resulting in a metabolite with a molecular mass of 450 g $mol^{-1}$ (hydrolyzed and decarboxylated ETP molecule). (B) Analysis of ETP degradation by MALDI-TOF MS. (B1) Results for a negative-control strain incubated with ETP solution which appears ionized with hydrogen ($[ETP + H]^+ = 475$ g $mol^{-1}$) and its sodium adduct ($[ETP + Na]^+ = 497$ g $mol^{-1}$). (B2) Results for a hydrolyzed and decarboxylated ETP after incubation with SPM-1-producing *P. aeruginosa*. Arrows represent HCCA matrix peaks. (C) Analysis of ETP degradation by LC-MS. (C1) Results for a negative-control strain incubated with ETP which appears ionized with hydrogen ($[EPT + H]^+ = 476$ g $mol^{-1}$). (C2) Hydrolyzed and decarboxylated ETP after incubation with SPM-1-producing *P. aeruginosa* ($[ETP + H]^+ = 450$ g $mol^{-1}$).

*nosa* and IMP-10-, OXA-58-, or OXA-143-producing *A. baumannii* isolates which had not been tested previously. The detection of carbapenemase by MALDI-TOF MS was compared to that of the modified Hodge test (MHT) and hydrolysis assay. Additionally, liquid chromatography-mass spectrometry (LC-MS) was used to confirm the results of carbapenem hydrolysis obtained by MALDI-TOF MS.

A total of 73 carbapenemase-producing and non-carbapenemase-producing bacterial clinical isolates were studied and molecularly characterized as shown in Table 1. Generally, no carbapenemase-producing isolates were susceptible to imipenem (IPM) and meropenem (MEM), except for three *A. baumannii* isolates (one OXA-23 and two IMP-10 producers) that showed imipenem and/or meropenem MICs of 4 μg/ml by the agar dilution technique (5, 6). In our study, MHT using ertapenem (ETP) disks (10 μg), following CLSI guidelines, detected 55%, 100%, and 68% of carbapenem-hydrolyzing class D β-lactamase (CHDL) and class A and class B carbapenemases, respectively. No additional carbapenemase producers were identified by testing with meropenem disks (7). Recently, MHT was reported to have poor sensitivity

and specificity for detecting class B and D carbapenemase activity in carbapenem-resistant *A. baumannii* isolates (8). Indeterminate and false-positive results by MHT were observed in our study for non-carbapenemase-producing *P. aeruginosa* and *Klebsiella pneumoniae* ESBL/ΔOmpK36 isolates, respectively, as was observed previously (9, 10, 11). All carbapenemase isolates hydrolyzed imipenem according to a UV spectrometric assay using 100 mM phosphate buffer (pH 7.0) (9).

The LC-MS and MALDI-TOF MS assays were performed by incubating fresh bacterial inoculums from Mueller-Hinton agar plates (Oxoid, Basingstoke, United Kingdom) in a buffer-adjusted solution (20 mM Tris-HCl, pH 6.8) with or without serial dilutions of ertapenem (0.12 to 0.5 μg/ml; Merck Sharp & Dohme, NJ) for periods of 2 and 4 h. This buffer solution was selected since a NaCl solution could inhibit the CHDL activity. After centrifugation, 1-μl aliquots of each sample were overlaid with 1 μl of α-cyano-4-hydroxycinnamic acid matrix solution (HCCA; Bruker Daltonics, Bremen, Germany) and spotted onto a stainless steel MALDI target plate as recommended by the manufacturer. The mass spectrum was obtained by using a Bruker Daltonics Microflex LT instrument operating in linear, positive ion mode and using the Flex Control 3.3 software with the following instrument parameters: pulsed ion extraction delay, 30 ns; ion source voltage one, 20 kV; ion source voltage two, 18.65 kV; and ion source lens voltage, 7.00 kV. The mass spectrometer was calibrated using the HCCA matrix peaks ($[M+H]^+ = 189.17$; $[2M+H]^+ = 379.35$) and bradykinin fragment 1-7 ($[M+H]^+ = 757.40$). For each sample, mass spectra were acquired by accumulating 100 laser shots at 45% to 60% laser power in the *m/z* range of 200 to 600 Da. Carbapenem hydrolysis was considered positive if the ertapenem intact-molecule mass peak (475 *m/z*) and that of its monosodium salt (497 *m/z*) disappeared completely (Fig. 1A and B2) (2).

LC-MS was performed in an LCMS-2020 instrument equipped with an electrospray ionization probe (ESI-145; Shimadzu, Kyoto, Japan). Aliquots of 15 μl from each reaction tube were applied directly into the instrument. The analytical LC-MS conditions were as follows: an XR-ODS $C_{18}$ column (2 μm, 3.0 by 100 mm) was eluted with solvent system A (water/trifluoroacetic acid[TFA], 1:1,000) and B (acetonitrile/water/TFA 900:100:1) at a flow rate of 1 ml/min and a 0-to-80% gradient for 10 min, monitored by absorbance at 220 nm. The mass spectrum profile was considered positive using the same interpretative criteria established for the MALDI-TOF MS assay.

The performance of the MALDI-TOF MS, LC-MS and hydrolysis assays in detecting class A (KPC-2 and GES-5) and B (SPM-1, IMP-1, IMP-10, VIM-1, GIM-1, and NDM-1) carbapenemase activity was excellent for both 2- and 4-h incubation times (100% sensitivity and 100% specificity). A similar result was observed by Burckhardt and colleagues, who tested an ertapenem MALDI-TOF MS assay to detect carbapenemase activity of NDM-1, VIM-1, VIM-2, and KPC-2 enzymes in *P. aeruginosa* isolates and *Enterobacteriaceae* (2). In our study, an extended incubation period (4 h) was necessary to detect carbapenemase activity in 100% of the CHDL-producing *A. baumannii* isolates, mainly due to OXA-23-producing isolates (Table 1). Probably an extended incubation period was necessary for detection of the carbapenemase activity in CHDL-producing isolates because these enzymes usually show a low level of carbapenem hydrolysis due to poor turnover of these β-lactams, as previously noticed by Queenan and Bush (12). In addition, the expression of CHDL-encoding genes is driven by an upstream-inserted insertion sequence, IS*Aba1* (13). LC-MS confirmed all MALDI-TOF MS results. Recently, Kempf and colleagues observed a similar detection rate (95% sensitivity) in a collection of OXA-23- and/or OXA-24-producing *A. baumannii* isolates by MALDI-TOF MS using imipenem and HCCA as the substrate and matrix, respectively (4). In the present study, imipenem was not selected for the MALDI-TOF MS because the mass spectrum profile obtained did not show good resolution with overlap of mass peaks, even when different matrices were tested. It could be due to the MALDI-TOF MS system tested in our study, the Microflex LT instrument, since Kempf and colleagues tested the Ultraflex I mass spectrometer. However, the Microflex LT instrument has been the apparatus mostly available in the European clinical laboratories.

Although the hydrolysis assay yielded excellent results, this method is laborious to implement in routine clinical laboratories. To our knowledge, this is the first study to evaluate the performance of MALDI-TOF MS in detecting carbapenemase activity in a collection of SPM-1-, GES-5-, and GIM-1-producing *P. aeruginosa* isolates, as well as OXA-143-, IMP-10-, and OXA-58-producing *A. baumannii* isolates. In addition, the MALDI-TOF MS results were confirmed by using LC-MS. MALDI-TOF MS is a rapid tool to detect class A and B carbapenemase activity, including that of SPM-1-producing *P. aeruginosa*, in a 2-h assay. However, an extended incubation period of 4 h must be used for detection of CHDL activity. MALDI-TOF MS has been shown to be an easy, rapid, and cheap methodology for the detection of carbapenemase activity. It can yield results before those of antimicrobial susceptibility testing can be available and constitutes a very attractive methodology, especially for clinical laboratories that have already used this technique for bacterial identification.

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## REFERENCES

1. **Walsh TR.** 2008. Clinically significant carbapenemases: an update. Curr. Opin. Infect. Dis. **21**:367–371.
2. **Burckhardt I, Zimmermann S.** 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J. Clin. Microbiol. **49**:3321–3324.
3. **Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T.** 2011. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **49**:3222–3227.
4. **Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, Mesli E, Touati A, Rolain JM.** 2012. Rapid detection of carbapenem resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One **7**:e31676. doi:10.1371/journal.pone.0031676.
5. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. Approved standard M07-A8. CLSI, Wayne, PA.
6. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing, 22nd informational supplement M100-S22. CLSI, Wayne, PA.
7. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing, 19th informational supplement. CLSI document M100-S19. CLSI, Wayne, PA.

8. **Bonnin RA, Naas T, Poirel L, Nordmann P.** 2012. Phenotypic, biochemical, and molecular techniques for detection of metallo-β-lactamase NDM in *Acinetobacter baumannii.* J. Clin. Microbiol. **50**:1419–1421.
9. **Carvalhaes CG, Picão RC, Nicoletti AG, Xavier DE, Gales AC.** 2010. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results. J. Antimicrob. Chemother. **65**:249–251.
10. **Lee K, Kim MN, Choi TY, Cho SE, Lee S, Whang DH, Yong D, Chong Y, Woodford N, Livermore DM, KONSAR Group.** 2009. Wide dissemination of OXA-type carbapenemases in clinical *Acinetobacter* spp. isolates from South Korea. Int. J. Antimicrob. Agents. **33**: 520–524.
11. **Pasteran F, Veliz O, Faccone D, Guerriero L, Rapoport M, Mendez T, Corso A.** 2011. A simple test for the detection of KPC and metallo-β-lactamase carbapenemase-producing Pseudomonas aeruginosa isolates with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin. Microbiol. Infect. **17**: 1438–1441.
12. **Queenan AM, Bush K.** 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. **20**:440–458.
13. **Bogaerts P, Cuzon G, Naas T, Bauraing C, Deplano A, Lissoir B, Nordmann P, Glupczynski Y.** 2008. Carbapenem-resistant *Acinetobacter baumannii* isolates expressing the $bla_{\text{OXA-23}}$ gene associated with IS*Aba4* in Belgium. Antimicrob. Agents Chemother. **52**:4205–4206.