Metallo-β-lactamase-producing *Pseudomonas aeruginosa* in two hospitals from Southern Brazil

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This study determined the prevalence of metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* in two hospitals located in the Southern part of Brazil and compare the performance of two different phenotypic tests. Thirty-one non-repetitive *Pseudomonas aeruginosa* isolates from various clinical samples from patients admitted to two hospitals located in Rio Grande do Sul, Brazil (twenty-three from a hospital in Porto Alegre City and eight isolates from a hospital in Vale dos Sinos Region). All strains suggestive of possessing MBLs by phenotypic methods were included in this study. Phenotypic detection of MBLs was carried out simultaneously by using both the MBL Etest® and disk approximation test using 2-mercaptoethane sulfonic acid close to a ceftazidime disk. Strains positive were further confirmed using molecular techniques for *bla*<sub>VIM</sub>*<sup>+</sup>-like* and *bla*<sub>SPM-1</sub>*. The prevalence of MBLs from samples of inpatients from the hospital located in Porto Alegre was 30.4% and that of inpatients from Vale dos Sinos hospital was only 3.1%. Only MBL type SPM-1 was detected in these samples by molecular analysis and all were detected by the Etest® MBL strips. The prevalence of *P. aeruginosa* that produce MBLs can be markedly different in distinct geographical areas, even among different hospitals in the same area. In our study, the EDTA-based method was the only method able to detect all strains harboring the SPM-1 enzyme.

Key-Words: Metallo-beta-lactamase, *Pseudomonas aeruginosa*, multiresistance.

*Pseudomonas aeruginosa* is a Gram-negative rod unable to ferment glucose and is widely known as an opportunistic organism, frequently involved in infections of immunosuppressed patients, and also causes outbreaks of hospital-acquired infections [1]. *P. aeruginosa* is very commonly isolated from nosocomial infections and is the most prevalent bacterial agent isolated from cases of pneumonia in Brazilian hospitals. This organism is clinically important since it possesses several virulence factors and is intrinsically resistant to most antimicrobial and disinfectant agents, a feature that is also responsible for the difficulty in treating infected patients [2].

Carbapenems are generally used as an option to treat serious infections caused by *P. aeruginosa*, since these drugs present a good spectrum of activity and are stable to hydrolysis by most β-lactamases, including the extended spectrum β-lactamases (ESBL). However, the use of carbapenems has been hampered by the emergence of strains that produce metallo-β-lactamase (MBL), an enzyme that is able to hydrolyze and inactivate this class of antibiotics. Moreover, the spread of MBL-producing clones, even over distant regions, has been reported [2].

Among β-lactamases, MBLs are unique in requiring the presence of zinc ion in the active site of the enzyme, and are, thus, inhibited by chelating agents such as EDTA [3].

Since the early 90s, new genes coding for distinct MBLs have been described in clinical important pathogens like *Pseudomonas* spp., *Acinetobacter* spp. and even among members of the family *Enterobacteriaceae*. These genes are usually inserted in mobile elements facilitating the exchange of these resistance genes among several bacterial species [4]. Several MBLs were reported, including: *IMP, VIM, SPM, GIM* and, more recently, *SIM-1*, encoded by the *bla*<sub>SIM-1</sub> gene in isolates of *A. baumannii* from South Korea [5].

Prompt detection and recognition of the MBLs is important to implement adequate counter-measures to control the spread of the organisms bearing these enzymes, and proper treatment of infections caused by MBL-producing microorganisms. A few phenotypic methods have been published for MBL detection; however, the results have shown that no method alone is able to detect all these enzymes, probably due to the genetic variability of these enzymes [6]. Molecular methods are more sensitive and specific for the detection of MBLs, but they may still be technically demanding and non available in most clinical microbiology laboratories.

We sought to determine the prevalence of MBL in *P. aeruginosa* strains from two South Brazilian hospitals and compare the efficacy of two different phenotypic methods to detect MBLs in this organism.

Material and Methods

Thirty-one clinical isolates of carbapenem-resistant *P. aeruginosa* were collected from two hospitals in Southern Brazil. Identification of the isolates was confirmed using conventional techniques [7] and disk diffusion susceptibilities were determined according to the CLSI methodology [8]. Only one isolate per patient was included and those isolates from cystic fibrosis patients were excluded from this study.

The following antimicrobial agents were included: aztreonam (ATM), cefepime (CPM), ceftazidime (CAZ) and imipenem (IPM), all from Cecon, São Paulo, SP, Brazil. Strains classified as resistant or intermediate to these drugs were included in this study. The production of MBL was screened for using two different methods, simultaneously: (i) using the
were isolated from ICU patients (56.5%), and among these, 5 showed phenotypic evidences of MBL production and all were presented classical phenotypical features compatible with the tracheal aspirates (12.5%) and skin ulcers (12.5%). The MBL-diverse clinical samples, such as urine (50%), sputum (25%), aztreonam and only 1 (12.5%) showed resistance to imipenem. None of the isolates were from low (3.1%), and the single MBL-positive strain was detected by the Etest-MBL® alone. Among the 23 strains isolated in Porto Alegre, 7 (30.4%) were positive for MBL production; however, this finding was not considered statistically significant by the Qui-square test. The strains of P. aeruginosa isolated in Porto Alegre were from clinical samples such as tracheal aspirates (69.6%), sputum (17.4%), urine (8.7%) and one was from an unknown source (4.3%). Interestingly, none of the 7 MBL-producing strains could be detected by the CAZ-2-MPA disks, as proposed by Arakawa et al. (2000) [9]. However, all strains were considered positive for MBL production by the E-test strip.

According to Marra et al. (2006) [12] and Magalhães et al. (2005) [13], the SPM-1 enzyme, which is highly prevalent in Brazil, could be equally detected by the 2-MPA method and the EDTA-based method. Zavascki et al. (2005) [14] reported that 77.1% of their P. aeruginosa isolates, resistant to IPM, could be detected by 2-MPA and 87.5% of the strains carried the blaSPM-1 gene. Similarly, another study by Zavascki et al. (2006) [15] reported the detection of 28.9% of MBL-positive P. aeruginosa, containing the blaSPM-1 gene, when the 2-MPA method was used to screen for the presence of this enzyme. In our study, 19.3% (6 strains) were positive for the presence of SPM-1, as confirmed by PCR analysis, but none could be detected using the phenotypic test proposed by Arakawa et al. (2000) [9]. Interestingly, in the remaining 2 strains, which were clearly MBL positive by phenotypic test (Etest), no other beta-lactamase gene could be found by PCR as specific primers (blaSPM-1, blaIMP or blaVIM), suggesting that maybe another kind of MBL gene(s) may be involved in these strains. It is known that EDTA may increase bacterial cell-wall permeability and that zinc (chelated by EDTA) accelerates imipenem decomposition and decreases OprD expression of P. aeruginosa [16]. Another alternative would be the presence of OXA-10 and/or OXA-14 stabilized in the dimeric form by metal ions (Zn²⁺). Dimeric forms are more active than the monomeric form, increasing its enzymatic activity turning the enzyme more efficient to inactivate carbapenems and other beta-lactam antibiotics. However, in the presence of a chelator agent, such as EDTA, the OXA class of enzymes is converted to their monovalent forms, which are less efficient in their activity against carbapenems. For these reasons, it is advisable to be cautious when interpreting the results of tests that use EDTA, as the E-Test MBL [17].

Detailed molecular knowledge of the MBLs involved may be necessary to better understand the reasons for success or failure of the phenotypic methods used for MBL screening.

Considering the susceptibility profile of our isolates, all MBL-positive samples were resistant to IPM, CAZ and CPM, including three of these samples also resistant to ATM. The possible explanation for this event is the concomitant presence of another extended-spectrum beta-lactamase (Such as the Ambler’s class A). Similar results were found in a work by Marra and coworkers (2006) [12], where, among four P. aeruginosa isolates positive for SPM-1, only two were resistant to ATM, while all strains were resistant to CPM and IPM. In this study, the authors also found isolates harboring the IMP-16, which were equally resistant to ATM.
EDTA-based tests, or a combination of tests, would be preferred detected by phenotypic tests based on the disk-approximation

**Conclusion**

In conclusion, we demonstrated here that some strains of *P. aeruginosa* producing the SPM-1 type of MBL are not detected by phenotypic tests based on the disk-approximation method (e.g. CAZ plus 2-MPA), suggesting that the use of EDTA-based tests, or a combination of tests, would be preferred to increase the sensitivity of these methods to detect the presence of MBL among clinical isolates of *P. aeruginosa*.

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**References**


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Table 1. Clinical and microbiological characteristics of imipenem-resistant clinical isolates of *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hospital</th>
<th>Sample</th>
<th>Diffusion diska</th>
<th>DDTb</th>
<th>E-Testc</th>
<th>PCR aBlagene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VS</td>
<td>skin ulcer</td>
<td>S/R/R/R</td>
<td>-</td>
<td>+</td>
<td>SPM</td>
</tr>
<tr>
<td>2</td>
<td>PA</td>
<td>tracheal aspirate</td>
<td>R/R/R/R</td>
<td>-</td>
<td>+</td>
<td>SPM</td>
</tr>
<tr>
<td>3</td>
<td>PA</td>
<td>tracheal aspirate</td>
<td>R/R/R/R</td>
<td>-</td>
<td>+</td>
<td>SPM</td>
</tr>
<tr>
<td>4</td>
<td>PA</td>
<td>sputum</td>
<td>S/R/R/R</td>
<td>-</td>
<td>“Ghost zone”</td>
<td>SPM</td>
</tr>
<tr>
<td>5</td>
<td>PA</td>
<td>tracheal aspirate</td>
<td>R/R/R/R</td>
<td>-</td>
<td>+</td>
<td>SPM</td>
</tr>
<tr>
<td>6</td>
<td>PA</td>
<td>tracheal aspirate</td>
<td>S/R/R/R</td>
<td>-</td>
<td>+</td>
<td>SPM</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>S/R/R/R</td>
<td>+</td>
<td>+</td>
<td>SPM</td>
</tr>
</tbody>
</table>

*aATM: aztreonam; CAZ: ceftazidime; CFP: cefepime; IMP: imipenem. S: susceptible, R: resistant. Double-disk test (DDT) was performed using disks containing the 2-mercaptoethanol acid and ceftazidime as inhibitor and substrate, respectively. (+) negative; (-) positive. The MBL presence is considered when MIC IP/IP+EDTA ≥ 8. Ghost zone: suggestive of MBL production.
VS: Vale dos Sinos; PA: Porto Alegre.

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