Phenotypic detection of metallo-β-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from hospitalized patients in São Luis, State of Maranhão, Brazil

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**ABSTRACT**

**Introduction:** Acquired metallo-β-lactamases (MβL) are emerging determinants of resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The objectives of this study were to phenotypically detect MβL in imipenem-resistant *P. aeruginosa* and *A. baumannii*, to investigate the association between MβL-positive strains and hospitals, and to compare the resistance profiles of MβL-producing and non-MβL-producing strains. **Methods:** The approximation disk and combined disk assay methods were used in this study. **Results:** A total of 18 (38.3%) *P. aeruginosa* isolates and 1 (5.6%) *A. baumannii* isolate tested positive for the presence of MβL. **Conclusions:** These results demonstrate the need for strict surveillance and for the adoption of preventive measures to reduce the spread of infection and potential outbreaks of disease caused by MβL-producing microorganisms.

**Keywords:** Metallo-β-lactamases. *Pseudomonas aeruginosa*. *Acinetobacter baumannii*.

Metallo-β-lactamase (MβL) activity has emerged as one of the most feared resistance mechanisms because of the ability of MβLs to hydrolyze virtually all β-lactam agents, including carbapenems. However, MβLs are unable to hydrolyze monobactams because their genes are carried on highly mobile elements. The prevalence of metallo-β-lactamase-producing *Pseudomonas aeruginosa* (MPPa) causing nosocomial infections has been increasing worldwide.

Data from the SENTRY Antimicrobial Surveillance Program suggest 44.8% of *P. aeruginosa* isolates in Brazil are resistant to imipenem, and 43.9% of these isolates produce MβL.

The objectives of the present study were to phenotypically detect MβL in imipenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates, to investigate the association between MβL-positive strains and the hospitals studied, and to compare the resistance profiles of MβL-producing and non-MβL-producing strains.

A total of 129 consecutive *P. aeruginosa* and 71 *A. baumannii* isolates were recovered between June and November 2008. The strains were isolated from different clinical samples obtained from 2 private hospitals (Hospital 1: 100 beds and Hospital 3: 164 beds) and 1 public hospital (Hospital 2: 121 beds) in São Luis, State of Maranhão, northeastern Brazil. All isolates were identified both by conventional techniques and by the automated Vitek 2 system (BioMérieux®, Marcy l’Etoile, France). Among those isolates, 47 (36.4%) *P. aeruginosa* isolates and 18 (25.4%) *A. baumannii* isolates were resistant to imipenem as determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (inhibition zone of ≤13mm or MIC ≥16µg/mL); these isolates were characterized.

The disk diffusion method (Kirby-Bauer) was used for antimicrobial susceptibility testing according to the recommendations of the CLSI found in performance standard M100-S22 (2012). Quality control testing was performed using *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

The disk approximation test was performed according to the method of Arakawa et al.[1], with modifications to the inhibitor volumes and concentrations. For the detection of MβL production by *P. aeruginosa*, 2 cefazidime disks (30µg; Oxoid®, Basingstoke, England) were used as the substrates and were placed at center-to-center distances of 2.0 and 1.5cm from 2 un-impregnated disks. Next, 4µL of 2-mercaptopropionic acid (2-MPA, 1.4mM; Sigma-Aldrich®, Steinheim, Germany) was added to the first disk, and 4µL of ethylenediaminetetraacetic acid (EDTA, 400mM; Sigma-Aldrich®, Steinheim, Germany) was added to the second disk. This assay was repeated for *A. baumannii* using imipenem (10µg) as the substrate and 4µL of 2-MPA (1.4mM) and 8µL of EDTA (200mM) as inhibitors. The plates were incubated for 18-24h at 35°C.

For the combined disk assay, the inhibitor concentrations, pipetted volumes, and cut-off values for the differentiation of producers and non-producers of MβL were determined.
other biological samples. For isolates of samples, 7 (5.4%) samples from sores/wounds, and 17 (13.1%) urine samples, 10 (7.8%) catheter tip samples, 9 (7%) blood recovered from 68 (52.7%) tracheal secretion samples, 18 (14%) 12 (16.9%); blood, 5 (7%); urine, 5 (7%); and other biological was tracheal secretion, 40 (56.3%); followed by the catheter tip, resistant to imipenem, the most frequent site of strain isolation.

A level of significance of 5% (p<0.05) was adopted for all tests. (NPPa) strains with each hospital and with antibiotic resistance. The association of MPPa or non-MβL-producing chi-squared test for independent samples was applied to evaluate Social Sciences (SPSS) for Windows, version 20.0 (2012). The results were analyzed using the Statistical Package for the positive controls for MβL production. Hospital of Universidade Federal de Santa Catarina, respectively, were used as positive controls for MβL production.

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 20.0 (2012). The chi-squared test for independent samples was applied to evaluate the association of MPPa and non-MβL-producing P. aeruginosa (NPPa) strains with each hospital and with antibiotic resistance. A level of significance of 5% (p<0.05) was adopted for all tests.

Strains of P. aeruginosa resistant to imipenem were recovered from 68 (52.7%) tracheal secretion samples, 18 (14%) urine samples, 10 (7.8%) catheter tip samples, 9 (7%) blood samples, 7 (5.4%) samples from sores/wounds, and 17 (13.1%) other biological samples. For isolates of A. baumannii that were resistant to imipenem, the most frequent site of strain isolation was tracheal secretion, 40 (56.3%); followed by the catheter tip, 12 (16.9%); blood, 5 (7%); urine, 5 (7%); and other biological samples, 9 (12.8%). The imipenem resistance profiles of P. aeruginosa and A. baumannii did not differ significantly among the hospitals studied (Table 1), and the phenotypic detection of MβL was positive in 18 (38.3%) P. aeruginosa isolates and 1 (5.6%) A. baumannii isolate. The results from the 2 methods used were in agreement.

A total of 12 (66.7%) MPPa isolates were collected from Hospital 1, and 6 (33.3%) were collected from Hospital 3. Furthermore, there was a significant difference in the proportion of MPPa and NPPa among the 3 hospitals (p=0.0016).

The highest percentage of MPPa isolation was derived from catheter tips, 6 (33.3%); followed by urine, 5 (27.8%); tracheal secretions, 3 (16.7%); bronchoalveolar lavage fluid, 1 (5.6%); peritoneal fluids, 1 (5.6%); blood, 1 (5.6%); and nose secretions, 1 (5.6%).

In total, 62.1%, 48.3 and 89.7% of the NPPa isolates were resistant to cefepime, ceftazidime, and meropenem, respectively; all MPPa isolates were resistant to these three compounds. The resistance rate was higher among MPPa isolates, with significant differences for amikacin (p=0.0264), cefepime (p=0.0085), ceftazidime (p=0.0007), and piperacillin/tazobactam (p=0.0119). Aztreonam resistance was higher in the NPPa group (p=0.0196). None of the P. aeruginosa isolates were resistant to polymyxin B (Table 2).

The only strain of MβL-producing A. baumannii (MPAb) was isolated from the blood sample of an Intensive Care Unit patient (ICU) from Hospital 1. This strain was resistant to amikacin, gentamicin, cefepime, ceftazidime, imipenem, meropenem, and ciprofloxacin and was susceptible to ampicillin/sublactam and piperacillin/tazobactam.

Multidrug-resistant P. aeruginosa and Acinetobacter baumannii strains are becoming a worldwide problem. SENTRY data have revealed that the prevalence of antimicrobial resistance among P. aeruginosa isolates has been increasing in Latin American medical centers. The arrival of MβL emphasizes the importance of phenotypic investigations for the presence of MβL in routine laboratory tests.

Monitoring programs in Brazil have indicated that the prevalence of P. aeruginosa isolates that are resistant to

### Table 1 - Imipenem resistance profiles of Pseudomonas aeruginosa and Acinetobacter baumannii strains isolated between June and November 2008, from 3 hospitals in São Luis, State of Maranhão, Brazil.

<table>
<thead>
<tr>
<th></th>
<th>Pseudomonas aeruginosa</th>
<th></th>
<th>Acinetobacter baumannii</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>resistant</td>
<td>intermediate</td>
<td>susceptible</td>
<td>total</td>
</tr>
<tr>
<td><strong>Hospital 1</strong></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Hospital 1</td>
<td>19</td>
<td>35.2</td>
<td>6</td>
<td>11.1</td>
</tr>
<tr>
<td>Hospital 2</td>
<td>19</td>
<td>38.8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hospital 3</td>
<td>9</td>
<td>34.6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>47</td>
<td>36.4</td>
<td>6</td>
<td>4.7</td>
</tr>
</tbody>
</table>

$\chi^2$ | 8.93 | 1.96 |

P | 0.0628 | 0.3748 |
resistance among non-MβL-producing strains that was observed may explain the high prevalence of agents to their intracellular targets. These resistance mechanisms play important roles by restricting the access of antimicrobial pump and the alteration of the outer membrane permeability may resistant to β-lactam antibiotics because of the hyperproduction reported by these authors. Normally, or southern regions of the country Brazil, but all such studies were restricted to the southeastern study. Regarding infections to polymyxins and ampicillin/sulbactam14. MPPa and MPAb are isolated with increasing frequency in Brazil and worldwide; therefore, understanding the prevalence and resistance of these strains has become critically important. These microorganisms are the etiological agents of most ICU-acquired infections, particularly infections of the respiratory tract; these infections are typically severe, and there are limited therapeutic options to treat them. These infections are directly responsible for the high rates of morbidity and mortality and the prolongation of hospital stays with concomitant high hospital costs. This scenario demonstrates the need for strict surveillance by infection control committees in the health care community and for the adoption of preventive measures to reduce the spread of infection and potential outbreaks caused by MβL-producing microorganisms.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

### REFERENCES


