Occurrence of IMP-1 in non-baumannii Acinetobacter clinical isolates from Brazil

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Abstract
The aim of this study was to characterize the presence of carbapenemase-encoding genes in distinct species of Acinetobacter spp. isolated from Brazilian hospitals. Five carbapenem-resistant Acinetobacter spp. isolates (two Acinetobacter pittii, two Acinetobacter bereziniae and one Acinetobacter junii) recovered from two distinct hospitals between 2000 and 2016 were included in this study. All of the isolates harboured blaIMP-1, which was inserted into In86, a class 1 integron. Pulsed field gel electrophoresis analysis showed that both A. pittii were identical, while the two A. bereziniae isolates were considered to be clonally related. In this study, we demonstrated that mobile elements carrying carbapenemase-encoding genes such as In86 may persist for a long period, allowing their mobilization from A. baumannii to other Acinetobacter spp. that are usually susceptible to multiple antimicrobials.

In recent years, carbapenem resistance in Acinetobacter species has mainly been reported in Acinetobacter baumannii. However, other Acinetobacter species have emerged as important nosocomial pathogens and, in some cases, they present high levels of antimicrobial resistance [1]. The real prevalence of carbapenem resistance in non-baumannii species might be underestimated because of the inability of phenotypic tests to accurately identify Acinetobacter at the species level [2]. rpoB gene sequence analysis has been considered to be the gold standard methodology for species identification [2]. Carbapenem resistance in Acinetobacter spp. has mainly been attributed to the production of carbapenem-hydrolyzing class D β-lactamases [1, 3]. Here we reported the occurrence of three non-baumannii species carrying blaIMP-1, a metallo-β-lactamase-encoding gene, isolated from two Brazilian hospitals.

As part of a routine surveillance, only six non-baumannii Acinetobacter spp. were isolated that were resistant to carbapenem. Five of these isolates remained viable and were further characterized. These isolates were collected from the blood (n=3) and tracheal aspirate (n=2) of five distinct patients hospitalized in two tertiary hospitals located in the city of São Paulo, Brazil between 2000 and 2016 (Table 1). These isolates had originally identified as Acinetobacter spp. (A3146 and A4258) and Acinetobacter lwoffii (A64232, A67461 and A69575) by automated systems. rpoB gene sequencing [2] later identified the isolates as Acinetobacter pittii (n=2), Acinetobacter bereziniae (n=2) and Acinetobacter junii (n=1). Minimal inhibitory concentrations (MICs) were determined and interpreted by broth microdilution according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (http://www.eucast.org/clinical_breakpoints/), as shown in Table 1. All of the isolates showed high MICs for third- and fourth-generation cephalosporins (MICs >64 mg l⁻¹), imipenem (MICs 32 to >32 mg l⁻¹), meropenem (MICs >32 mg l⁻¹) and amikacin (MICs 32 to 128 mg l⁻¹). The A. bereziniae isolates were also resistant to gentamicin (MICs 8 to 16 mg l⁻¹), ciprofloxacin (MICs 8 mg l⁻¹) and levofloxacin (MICs 4 to 8 mg l⁻¹), while the A. pittii isolates remained susceptible to these drugs (MICs of 1, 0.5 and 0.25 mg l⁻¹, respectively). On the other hand, the A. junii was resistant to gentamicin (MIC 16 mg l⁻¹), but remained susceptible to both fluoroquinolones (MICs of 0.125 and 0.5 mg l⁻¹, respectively).
**Table 1.** Microbiological characterization of IMP-1-producing non-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Species</th>
<th>Date of isolation</th>
<th>Hospital</th>
<th>Source</th>
<th>Date of isolation</th>
<th>PFGE *</th>
<th>MICs (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3146</td>
<td>A. pittii</td>
<td>31 August 2000</td>
<td>A</td>
<td>Traqueal aspirate</td>
<td>A</td>
<td>8/4</td>
<td>&gt;64 &gt;128 &gt;64 32 &gt;32 64 1 0.5 0.25 0.125 1 0.25</td>
</tr>
<tr>
<td>A6243</td>
<td>A. pittii</td>
<td>31 January 2001</td>
<td>A</td>
<td>Blood</td>
<td>A</td>
<td>8/4</td>
<td>&gt;64 &gt;128 &gt;64 &gt;32 64 1 0.5 0.25 0.125 1 0.25</td>
</tr>
<tr>
<td>A6425</td>
<td>A. bereziniae</td>
<td>30 October 2014</td>
<td>A</td>
<td>Blood</td>
<td>A</td>
<td>8/4</td>
<td>&gt;64 &gt;128 &gt;64 &gt;32 &gt;32 64 8 8 8 8</td>
</tr>
<tr>
<td>A67461</td>
<td>A. bereziniae</td>
<td>27 May 2015</td>
<td>B</td>
<td>Blood</td>
<td>B</td>
<td>32/4</td>
<td>&gt;64 &gt;128 &gt;64 &gt;32 &gt;32 128 16 8 4 0.125 1 0.5</td>
</tr>
<tr>
<td>A69575</td>
<td>A. junii</td>
<td>10 February 2016</td>
<td>A</td>
<td>Blood</td>
<td>A</td>
<td>16/4</td>
<td>&gt;64 &gt;128 &gt;64 &gt;32 &gt;32 128 16 8 4 0.125 1 0.5</td>
</tr>
</tbody>
</table>

\*PFGE: pulsed-field gel electrophoresis. MICs, minimum inhibitory concentrations determined by E Test. SAM, ampicillin/sulbactam; IPM, imipenem; MEM, meropenem; FEP, cefepime; CAZ, ceftazidime; CRO, ceftriaxone; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; LEV, levofloxacin; MIN, minocycline; TGC, tigecycline; PMB, polymyxin B.

The ampicillin/sulbactam MICs ranged from 8/4 to 32/4 µg ml⁻¹. The most active antimicrobial agents tested against all isolates were minocycline (MICs ≤0.06 to 0.125 µg ml⁻¹), followed by tigecycline (MICs 0.125 to 1 µg ml⁻¹) and polymyxin B (MICs 0.25 to 2 µg ml⁻¹).

The presence of carbapenemase-encoding genes was confirmed by PCR followed by sequencing using specific primers [3, 4], and demonstrated that all strains carried the blaIMP-1 gene. To elucidate the genetic context of blaIMP-1, DNA amplification and sequencing were performed as previously described [3], and revealed that this gene was inserted into a class 1 integron, In86, that also carried two aminoglycoside-modifying enzymes encoding the genes aacA31 and adaA1a. The clonal relatedness of the two A. pittii isolates, as well as the two A. bereziniae isolates, was evaluated by pulsed field gel electrophoresis (PFGE) using the Apal restriction enzyme [3]. PFGE analysis showed that the A. pittii isolates were identical, while the two A. bereziniae isolates were clonally related.

Since its first description in *Serratia marcescens* isolated in Japan, blaIMP-1 has been reported in several Gram-negative rods [4]. Although IMP-1 has become endemic in eastern Asia, it has been sporadically reported in non-*baumannii* Acinetobacter species in that region [1], as well as in Europe and South America, where IMP variants are more commonly reported in *A. baumannii* [3, 5]. IMP-1-producing *A. baumannii* were initially detected in our institution in 1998, and since then the frequency of this microorganism has increased [5]. The class 1 integron In86 has been described in different Gram-negative bacilli among distinct hospitals located in the state of São Paulo [4]. The two IMP-1-producing *A. pittii* isolates were recovered in our institution in the early 2000s, and had probably acquired this resistant determinant from *A. baumannii*. Interestingly, we previously reported two *A. baumannii* isolates recovered in hospital A that harboured a new plasmid-mediated class 1 integron named In990, which was derived from In86 and carried blaIMP-10. This suggests that this genetic element has also undergone an evolution, probably due to selective pressure exerted by the local use of antimicrobials [3]. Park and colleagues also identified blaIMP-1 in *A. pittii* and *A. bereziniae* in South Korea, highlighting the spread of this resistance gene among non-*baumannii* species of *Acinetobacter* [6]. In addition, the presence of blaIMP-1 in two *A. bereziniae* isolates and an *A. junii*, more than 13 years apart and in a different hospital, emphasizes the persistence and spread of blaIMP-1-borne In86 over the years.

Although *A. non-baumannii* isolates are usually susceptible to many antimicrobial agents [1], additional caution should be taken due to the possibility of the acquisition of mobile genetic elements carrying resistance genes, as exemplified in this report. Our results emphasize the need for the proper implementation of infection control measures and accurate species identification in order to avoid patient-to-patient spread of this resistance determinant.
spread and determine the epidemiological role exerted by non-
baumannii species.

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Conflicts of interest
A. C. G. recently received research funding and/or consultation fees from BD, Bayer, Pfizer and MSD. The other authors have nothing to declare. This study has not been financially supported by any diagnostic/pharmaceutical company.

References


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