In recent years, different resistance genes have been found in *Acinetobacter* spp., especially in the species *A. baumannii*. We describe two isolates of carbapenem-resistant *A. nosocomialis* harboring IS*Ab*a1-*bla*OXA-23* and *bla*OXA-51* found in patients from the city of Porto Alegre, southern Brazil. To the best of the authors’ knowledge, this is the first report of carbapenem-resistant *A. nosocomialis* in Latin America.

In recent years, *Acinetobacter* spp. have been described as important pathogens in outbreaks of nosocomial infection worldwide, especially in intensive care units (1). In particular, the species *A. baumannii* has presented an increased rate of antimicrobial resistance (2, 3). Carbapenems, once regarded as the treatment of choice for infections caused by *Acinetobacter* spp., are no longer effective in some cases (2). The main mechanism of carbapenem resistance among *Acinetobacter* spp. is the production of β-lactamases, in particular class D β-lactamases (oxacillinas), associated with promoter gene sequence IS*Ab*a1 (3). Among oxacillinas, the most prevalent one is *bla*OXA-23, identified in mobile genetic elements. Chromosomally located *bla*OXA-51 genes, in turn, do not always confer carbapenem resistance but are used to identify *A. baumannii*, as it is believed to be intrinsic to this species (4–6).

Traditionally, the *bla*OXA-23 and *bla*OXA-51 genes are associated with *A. baumannii* only, but recently some authors have described the presence of such genes in non-*A. baumannii* species. The *bla*OXA-23 gene was found in *A. pittii* (*Acinetobacter* genome species 3) in the Irish Republic in 2006 and in *A. nosocomialis* (*Acinetobacter* genome species 13TU) in South Korea and Thailand in 2012 (7, 8). Moreover, *bla*OXA-51 preceded by IS*Ab*a1 has been found in carbapenem-resistant *A. nosocomialis* in Taiwan (9).

In this study, we evaluated a set of non-*A. baumannii* species and found two isolates of carbapenem-resistant *A. nosocomialis* with the IS*Ab*a1-*bla*OXA-23 and *bla*OXA-51 genes, obtained from patients living in the city of Porto Alegre, southern Brazil.

A total of 118 isolates were evaluated, obtained over the year 2011 from clinical specimens of *Acinetobacter* spp. previously identified using conventional methods. Isolates were identified to the species level using *gyrB* multiplex PCR as described by Higgins et al., with few modifications (10). Briefly, we used seven primers at a total reaction volume of 25 μl, consisting of 0.2 μM each primer, 1.5 mM MgCl2, 1 X 0.2 mM each deoxynucleoside triphosphate (dNTP), and 1 U Taq DNA polymerase. The PCR program consisted of initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation (94°C for 1 min), annealing (56°C for 30 s), and extension (72°C for 1 min), with a final extension step at 72°C for 10 min. Species identification was also evaluated by PCR with primers targeting the 16S-23S rRNA intergenic transcribed spacer (ITS) region, followed by sequence analysis (11). Oxacillinas genes (*bla*OXA-23*, bla*OXA-24*, *bla*OXA-51*, *bla*OXA-58*, and *bla*OXA-143) were identified using multiplex PCR with specific primers. Isolates testing positive for oxacillinas genes were subjected to a PCR program for the promoter sequence IS*Ab*a1 (10, 12, 13).

Imipenem and meropenem MICs were determined in duplicate using the Clinical and Laboratory Standards Institute broth microdilution method (14). *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were used as controls.

A total of 106 (89.8%) isolates proved to be *A. baumannii*. Twelve non-*A. baumannii* isolates were identified, including 6 (5.1%) *A. nosocomialis* isolates, 5 (4.2%) *A. pittii* isolates, and 1 (0.8%) *Acinetobacter* genomic species 10 isolate, with 100% concordance to species of the *A. baumannii-A. calcoaceticus* complex by two PCR methods tested. The *bla*OXA-51 and *bla*OXA-23 genes were identified in 5 (4.3%) and 4 (3.4%) non-*A. baumannii* isolates, respectively. Of the five isolates that tested positive for *bla*OXA-51, four were *A. nosocomialis* and one was *A. pittii*. Among the four isolates positive for *bla*OXA-23, three were *A. nosocomialis* and one was *A. pittii*. No other oxacillinas were found. For the first time in Latin America, IS*Ab*a1 upstream of the *bla*OXA-51 and *bla*OXA-23 genes was identified in two isolates of carbapenem-resistant *A. nosocomialis* (Table 1). The presence of oxacillinas genes in non-*A. baumannii* isolates had already been described in studies from China, South Korea, and Singapore, which underscores the potential clinical significance of these species (7–9, 15).

It is worthy of note that two isolates of carbapenem-susceptible *A. nosocomialis* and one of *A. pittii* were found to harbor *bla*OXA-23*. Notwithstanding, these isolates did not present IS*Ab*a1 upstream of the oxacillinas genes. It is well established that the promoting sequence IS*Ab*a1 has to be present to ensure oxacillinas expression and, consequently, the development of resistance. We also found that resistance to carbapenems was not necessarily related.
to oxacillinase genes, as one A. nosocomialis isolate and one A. pittii isolate resistant to carbapenems did not present these genes. In fact, it has already been shown that carbapenem resistance may be mediated by other mechanisms, e.g., porin loss and hyperexpression of efflux pumps (2).

Several studies have identified a variety of oxacillinases in carbapenem-resistant A. baumannii isolates. The main oxacillinases described include blaOXA-51, blaOXA-23 and blaOXA-143, which is believed to be intrinsic to A. baumannii, whereas the two latter genes have been associated with carbapenem resistance (16–20).

In this study, we found two isolates of A. nosocomialis harboring the ISAba1 upstream of blaOXA-23, and blaOXA-51, which has proved to confer resistance to carbapenems. These findings reinforce the importance of species-level identification, as there may be horizontal transfer of oxacillinase genes among different species of the Acinetobacter genus, a phenomenon previously described by Poirel et al. (21). In fact, non-A. baumannii species cannot be considered homogeneously susceptible to carbapenems and may lead to an increased prevalence of nosocomial infections caused by carbapenem-resistant Acinetobacter spp.

To the best of our knowledge, this is the first study reporting the identification of oxacillinase genes in non-A. baumannii isolates in Latin America.

ACKNOWLEDGMENTS

This work was supported by Fundo de Incentivo à Pesquisa e Eventos—Hospital de Clínicas de Porto Alegre (FIPHE-HCPA), by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS), and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES


### TABLE 1 Characteristics of non-Acinetobacter baumannii isolates

<table>
<thead>
<tr>
<th>Acinetobacter species</th>
<th>PCR result&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ISAba1 upstream</th>
<th>ISAba1 upstream</th>
<th>MIC (µg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>A. pittii&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>≥0.5</td>
</tr>
<tr>
<td>A. nosocomialis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≥0.5</td>
</tr>
<tr>
<td>A. pittii</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≥0.5</td>
</tr>
<tr>
<td>A. pittii</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≥256</td>
</tr>
<tr>
<td>Acinetobacter genospecies 10</td>
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<td>–</td>
<td>–</td>
<td>≥0.5</td>
</tr>
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<td>+</td>
<td>≥0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> All isolates were identified using gyrB multiplex PCR and confirmed by 16S-23S intergenic transcribed spacer sequence analysis.

<sup>b</sup> MIC breakpoints for two carbapenems according to the Clinical and Laboratory Standards Institute broth microdilution method: resistant, ≥16 µg/ml; intermediate, 8 µg/ml; and susceptible, ≤4 µg/ml.

<sup>c</sup> Formerly Acinetobacter genomic species 3.

<sup>d</sup> Formerly Acinetobacter genomic species 13TU.