First Report of Carbapenem-Resistant Acinetobacter nosocomialis Isolates Harboring ISAba1-bla_{OXA-23} Genes in Latin America

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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 ISAba1-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. In particular, the species A. baumannii has presented an increased rate of antimicrobial resistance. Carbapenems, once regarded as the treatment of choice for infections caused by Acinetobacter spp., are no longer effective in some cases. The main mechanism of carbapenem resistance among Acinetobacter spp. is the production of β-lactamasises, in particular class D β-lactamasises (oxacillinas), associated with promoter gene sequence ISAba1. Among oxacillinases, 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.

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 genomic species 3) in the Irish Republic in 2006 and in A. nosocomialis (Acinetobacter genomic species 13TU) in South Korea and Thailand in 2012. Moreover, bla_{OXA-51} preceded by ISAba1 has been found in carbapenem-resistant A. nosocomialis in Taiwan.

In this study, we evaluated a set of non-A. baumannii species and found two isolates of carbapenem-resistant A. nosocomialis with the ISAba1-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. 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× 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.

Oxacillinase 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 oxacillinase genes were subjected to a PCR program for the promoter sequence ISAba1. Imipenem and meropenem MICs were determined in duplicate using the Clinical and Laboratory Standards Institute broth microdilution method. *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 oxacillinases were found. For the first time in Latin America, ISAba1 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 oxacillinase 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.

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 ISAba1 upstream of the oxacillinase genes. It is well established that the promoting sequence ISAba1 has to be present to ensure oxacillinase expression and, consequently, the development of resistance. We also found that resistance to carbapenems was not necessarily related...
The identification of oxacillinase genes in non-
A. baumannii
and may lead to an increased prevalence of nosocomial infections
cannot be considered homogeneously susceptible to carbapenems
described by Poirel et al. (21). In fact, non-A. baumannii
be horizontal transfer of oxacillinase genes among different spe-
proved to confer resistance to carbapenems. These findings rein-
ABa
believed to be intrinsic to
genes have been associated with carbapenem resistance (16–20).

REFERENCES

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REFERENCES


Aba
IS
1 upstream
harboring a
AbaR4-type resistance island including the
emase in clinical isolates of


8. Kim DH, Choi JY, Jung SI, Thamlikitkul V, Song JH, Ko KS. 2012. AbaR4-type resistance island including the
bla
OXA-23

9. Lee YT, Kuo SC, Chang MC, Yang SP, Chen CP, Chen TL, Fung CP. 2012. Emergence of carbapenem-resistant non-
baumannii
species of Acinetobacter harboring a
bla
OXA-23-like

Acinetobacter calcoaceticus
and

Acinetobacter calcoaceticus


Acinetobacter baumannii
carrying the ISAb1 upstream
bla
OXA-23


<table>
<thead>
<tr>
<th>Acinetobacter species (A. pittii, A. nosocomialis, A. pittii)</th>
<th>PCR result*</th>
<th>ISAba1 upstream</th>
<th>ISAba1 upstream</th>
<th>MIC (µg/ml)**</th>
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<tbody>
<tr>
<td>A. pittii d</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≤0.5</td>
</tr>
<tr>
<td>A. nosocomialis e</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≤0.5</td>
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<tr>
<td>A. pittii</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≤256</td>
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<tr>
<td>Acinetobacter genospecies 10</td>
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<td>–</td>
<td>–</td>
<td>≤0.5</td>
</tr>
<tr>
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<td>64</td>
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<tr>
<td>A. pittii</td>
<td>–</td>
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<td>≤0.5</td>
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</tbody>
</table>

*All isolates were identified using gyrB multiplex PCR and confirmed by 16S-23S intergenic transcribed spacer sequence analysis.

** 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.

† Formerly Acinetobacter genomic species 3.

‡ Formerly Acinetobacter genomic species 13TU.


