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

Aline Borges Teixeira,\textsuperscript{a} Andreza Francisco Martins,\textsuperscript{b} Juliana Barin,\textsuperscript{a} Djuli Milene Hermes,\textsuperscript{a} Caroline Pormann Pitt,\textsuperscript{c} Afonso Luis Barth\textsuperscript{a,c,d}

Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; Departamento de Vigilância em Saúde, Porto Alegre, Brazil; Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; Serviço de Patologia Clínica, Hospital de Clínicas de Porto Alegre, Brazil.

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\textsubscript{OXA-23} and bla\textsubscript{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 ISAba1 (3). Among oxacillinas, the most prevalent one is bla\textsubscript{OXA-23} identified in mobile genetic elements. Chromosomally located bla\textsubscript{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\textsubscript{OXA-23} and bla\textsubscript{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\textsubscript{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 (7, 8). Moreover, bla\textsubscript{OXA-51} preceded by ISAba1 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 ISAba1-bla\textsubscript{OXA-23} and bla\textsubscript{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 MgCl\textsubscript{2}, 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 (11). Oxacillinase genes (bla\textsubscript{OXA-23}, bla\textsubscript{OXA-24}, bla\textsubscript{OXA-58}, and bla\textsubscript{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 (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, with 100% concordance to species of the A. baumannii-A. calcoaceticus complex by two PCR methods tested. The bla\textsubscript{OXA-51} and bla\textsubscript{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\textsubscript{OXA-51}, four were A. nosocomialis and one was A. pittii. Among the four isolates positive for bla\textsubscript{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\textsubscript{OXA-51} and bla\textsubscript{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 (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\textsubscript{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.
to oxacillinase genes, as one Acinetobacter 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 bla\textsubscript{OXA-23}, bla\textsubscript{OXA-51} and bla\textsubscript{OXA-143}; bla\textsubscript{OXA-51} 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 ISA\textsubscript{Ab1} upstream of bla\textsubscript{OXA-23} and bla\textsubscript{OXA-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.

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