

Occurrence of *bla*_{OXA-23} gene in imipenem-susceptible *Acinetobacter baumannii*

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The aim of the current study was to describe the occurrence of the bla_{OXA-23} gene and the ISAbal element in imipenem-susceptible Acinetobacter baumannii strains. By performing the polymerase chain reaction mapping using combinations of ISAbal forward primers and the bla_{OXA-23-like} gene reverse primers, we demonstrated that the ISAbal element did not occur upstream of the bla_{OXA-23} gene in five of 31 isolates, which explained the lack of resistance to imipenem despite the presence of the bla_{OXA-23} gene. All of the bla_{OXA-23}-positive isolates were susceptible to imipenem and meropenem with minimal inhibitory concentration ≤ 4 µg/mL. Pulsed-field gel electrophoresis analysis revealed four genotypes among the five bla_{OXA-23}-positive isolates. The current report of the bla_{OXA-23} gene in imipenem-susceptible isolates provided evidence that this gene may be silently spread in a hospital environment and highlighted the threat of undetected reservoirs of carbapenemase genes.

Key words: imipenem-susceptible - *Acinetobacter baumannii* - *bla*_{OXA-23}

Carbapenem resistance in *Acinetobacter baumannii* is predominantly caused by the carbapenem-hydrolysing class D β-lactamases (CHDLs). Sequence-based comparisons classify OXA-carbapenemases into eight subgroups as follows: four of the OXA-carbapenemases have been identified in *A. baumannii*, namely, OXA-23-like, OXA-24-like, OXA-51-like, OXA-58-like and OXA-143, which is a novel plasmid-mediated CHDL (Higgins et al. 2009). OXA-23 was identified as the first member of this enzyme group in 1985 in Scotland, which was before the wide availability of imipenem in clinical practice. Since 1985, a number of outbreaks of OXA-23-producing imipenem-resistant *Acinetobacter* spp have been reported worldwide (Mugnier et al. 2010). The OXA-23-encoding gene is mainly found on plasmids and has been associated with the *ISAbal* or *ISAbal4* elements (Corvec et al. 2007). The *ISAbal* or *ISAbal4* element that is upstream of *bla*_{OXA-23-like} genes provide promoter sequences that enhance their expression (Corvec et al. 2007). *ISAbal* regulates *bla*_{OXA-23} mobility and has two copies that surround the β-lactamase gene and form a composite transposon (defined as *Tn2006*) (Mugnier et al. 2010). *ISAbal4* belongs to the IS982 family, is 975 bp in length, possesses two 18-bp inverted repeats and encodes a 292-amino-acid putative transposase. These insertion elements may be involved in transposition processes at the origin of acquisition of the *bla*_{OXA-23} gene (Corvec et al. 2007).

The current study described the silent carriage of the *bla*_{OXA-23} gene in five imipenem-susceptible *A. baumannii* isolates.

From April 2005-September 2007, 31 clinical isolates of imipenem-susceptible *A. baumannii* were collected from non-related patients at five hospitals in Rio de Janeiro, Brazil. The isolates were identified using conventional techniques and confirmed using the amplified ribosomal DNA restriction analysis (Carvalho et al. 2009) and *bla*_{OXA-51} polymerase chain reaction (PCR) (Turton et al. 2006b).

Among the 31 imipenem-susceptible isolates, five were positive for *bla*_{OXA-23} by PCR and DNA sequencing using the following primers: OXA-23 F (5'-ACTTGC-TATGTGGTTGCTTCTC-3') and OXA-23-R (5'-TGT-CAAGCTCTTAAATAATATTCAGC-3'), which annealed at the region between 987 and 1,778-bp positions in GenBank isolate AJ132105 (Higgins et al. 2009). In addition, these isolates were positive for *ISAbal* (*ISAbal* F: 5'-CACGAATGCAGAAGTTG-3' and *ISAbal* R: 5'-CGACGAATACTATGACAC-3') (Turton et al. 2006a) but negative for *ISAbal4* (*ISAbal4* F 5'-ATTTGAAC-CCATCTATTGGC-3' and *ISAbal4* R 5'-ACTCTCAT-ATTTTTTCTTGG-3') (Corvec et al. 2007). The nucleotide sequences of *bla*_{OXA-23} and *ISAbal* have already been deposited in the GenBank under the accessions JF421124 and JF340121, respectively.

PCR mapping using the combination of the *ISAbal* forward primer and the *bla*_{OXA-23-like} reverse primer were negative. In five carbapenem-resistant control isolates, the *ISAbal* element was detected upstream of the *bla*_{OXA-23} gene (Carvalho et al. 2009).

According to antimicrobial susceptibility tests, such as the E-test (AB Biodisk, Solna, Sweden) and the agar dilution method, all *bla*_{OXA-23}-positive imipenem-susceptible isolates were susceptible to imipenem and meropenem [minimal inhibitory concentration (MIC) ≤ 4 µg/

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mL]. These results may be explained by the absence of the IS*Aba1* element upstream of the *bla*_{OXA-23-like} gene.

All 31 isolates were susceptible to polymyxin B (MIC ≤ 1 $\mu\text{g}/\text{mL}$). Using the disk diffusion method, four isolates were non-susceptible (i.e., intermediate or resistant) to aztreonam, ceftazidime, cefepime and ciprofloxacin, whereas three isolates were susceptible to ampicillin-sulbactam and amikacin. These findings suggest the presence of another resistance mechanism that has not been previously investigated, such as extended spectrum beta lactamases, the modification of penicillin-binding proteins and porins or the upregulation of the efflux system (Zarrilli et al. 2009).

Macrorestriction with *ApaI*-digestion following pulsed-field gel electrophoresis (PFGE) was used to determine the genetic relatedness of the *bla*_{OXA-23}-positive isolates. The *bla*_{OXA-23}-positive isolates were clustered into four different genotypes (B, C, G and I). The criteria for the classification of DNA band patterns included the following: undistinguishable (clonally related isolates), closely related (clonal variants, ≤ 3 different bands), possibly related (4-6 different bands) and unrelated (≥ 6 different bands) (Tenover et al. 1995). Two isolates proved to be genetically related (genotype C). Except for genotype I, all of the genotypes were detected among *bla*_{OXA-23}-negative isolates. Genotypes B and C were detected among imipenem-resistant *A. baumannii* isolates as described previously (Carvalho et al. 2009). Chromosomally encoded *bla*_{OXA-23-like} genes have been described in carbapenem-susceptible *Acinetobacter radioresistens* isolates, suggesting that this species is a source of these genes (Poirel et al. 2008, Boo & Crowley 2009, Mendes et al. 2009). Poirel et al. (2008) has identified a similar plasmid backbone in several *bla*_{OXA-23}-positive *A. baumannii* and *A. radioresistens* isolates. These results suggest that a plasmid-mediated IS*Aba1* element originating from *A. baumannii* may have entered *A. radioresistens* to be transposed and targeted to the regions that are upstream and downstream of the chromosomal *bla*_{OXA-23-like} genes. A transposon-like structure is then formed, which enhances the expression of the *bla*_{OXA-23-like} genes. This structure may transpose and target a plasmid inside the *A. radioresistens* genome and, finally, this plasmid conjugates into *A. baumannii* to spread the resistance determinant in the latter species (Poirel et al. 2008).

The current report of *bla*_{OXA-23} in imipenem-susceptible *A. baumannii* isolates provided evidence that the *bla*_{OXA-23} gene may be silently spread in a hospital environment and highlighted the threat of undetected reservoirs of carbapenemase genes. The undetected carbapenemase genes are challenging, because the laboratory detection of such genes and the subsequent measures

for infection control in hospital settings generally target only phenotypically multidrug-resistant organisms. The future control of multidrug resistance may require the identification of multidrug-resistant isolates and their reservoirs using molecular-based techniques.

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