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FIRST OCCURRENCE OF *BLA*$_{OXA-58}$ IN *ACINETOBACTER BAUMANNII* ISOLATED FROM A CLINICAL SAMPLE IN SOUTHERN BRAZIL

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ABSTRACT

This is the first report of an *Acinetobacter baumannii* from clinical origin carrying the *bla*$_{OXA-58}$ gene in Brazil. The isolate included in this study was from a patient during an outbreak in Porto Alegre, RS, Southern Brazil, in 2007. It was resistant to most of the beta-lactams tested, it has also the *bla*$_{OXA-65}$ gene and the IS*Aba1* sequence located upstream to both *bla*$_{OXA}$ genes detected and it has a MIC of imipenem of 64 μg/mL.

Key words: *bla*$_{OXA-58}$, IS*Aba1*, *bla*$_{OXA-65}$, carbapenemases, *Acinetobacter baumannii*.

The nosocomial infections caused by *A. baumannii* have been the target of intense research in recent years due to their great ability to rapidly acquire resistance to the drugs of choice used in antimicrobial therapy. The most common mechanism of drug resistance in these bacteria is the expression of OXA-carbapenemases, a class D carbapenem-hidrolysing-β-lactamase (1, 5, 12). Currently, there are four families of OXA carbapenemases in *Acinetobacter* sp.: OXA-23-like, OXA-24-like, OXA-58-like and OXA-51-like enzymes, the last being intrinsic to *A. baumannii* (9, 17). In addition, the presence of the insertion sequence IS*Aba1* immediately upstream of the *bla*$_{OXA-51}$ gene contributes to increased expression of resistance to carbapenems, since the presence of this gene is not necessarily related to resistance (20, 21).

Only carbapenemases OXA-23-like and OXA-51-like have been found in Brazil, so far (3, 6, 13). Carbapenemases OXA-58 were first described in Europe, where they are widely disseminated (15, 18). In South America, the presence of the gene was described in Argentina, commonly associated with resistance to carbapenems in *Acinetobacter* sp. outbreaks (5, 17). The occurrence of this carbapenemase has also been observed in Asia, showing that it is widely distributed throughout the world (5, 9, 17). Unlike *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ genes, broadly found across the country in different strains (13,19), the *bla*$_{OXA-58}$ gene has not been described in Brazil.

In a previous study (8), 74 clinical isolates were tested for their antimicrobial susceptibility and the presence of *bla*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$, *bla*$_{OXA-51-like}$ and *bla*$_{OXA-58-like}$ genes. The

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confirmation of *Acinetobacter* species was performed by PCR assay and sequencing of the 16S rRNA gene (7). The Minimum Inhibitory Concentration (MIC) of imipenem was determined by broth microdilution according to the guidelines of the Clinical and Laboratory Standards Institute (4). The detection of the OXA-carbapenemase genes ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$) was based on the MIC results. The PCR was carried out using a multiplex assay (22) for the isolates with MIC ≥ 8 µg/mL. The complete ORF of the gene bla_{OXA-51} and the insertion sequence IS*Aba*1 were amplified as previously described (20). The complete $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$ PCR products were sequenced by ABI-PRISM 3100 Genetic Analyzer and evaluated using BioEdit Version 7.0.5. All the partial sequences were deposited in GenBank under accession numbers (HM 626370, HM 626369 and HM 626368). In order to verify the position of the IS*Aba1* sequence, PCR mapping experiments using combinations of the IS*Aba1* forward, OXA-51-like and OXA-58-like reverse primers were performed (Figure 1a and 1b).

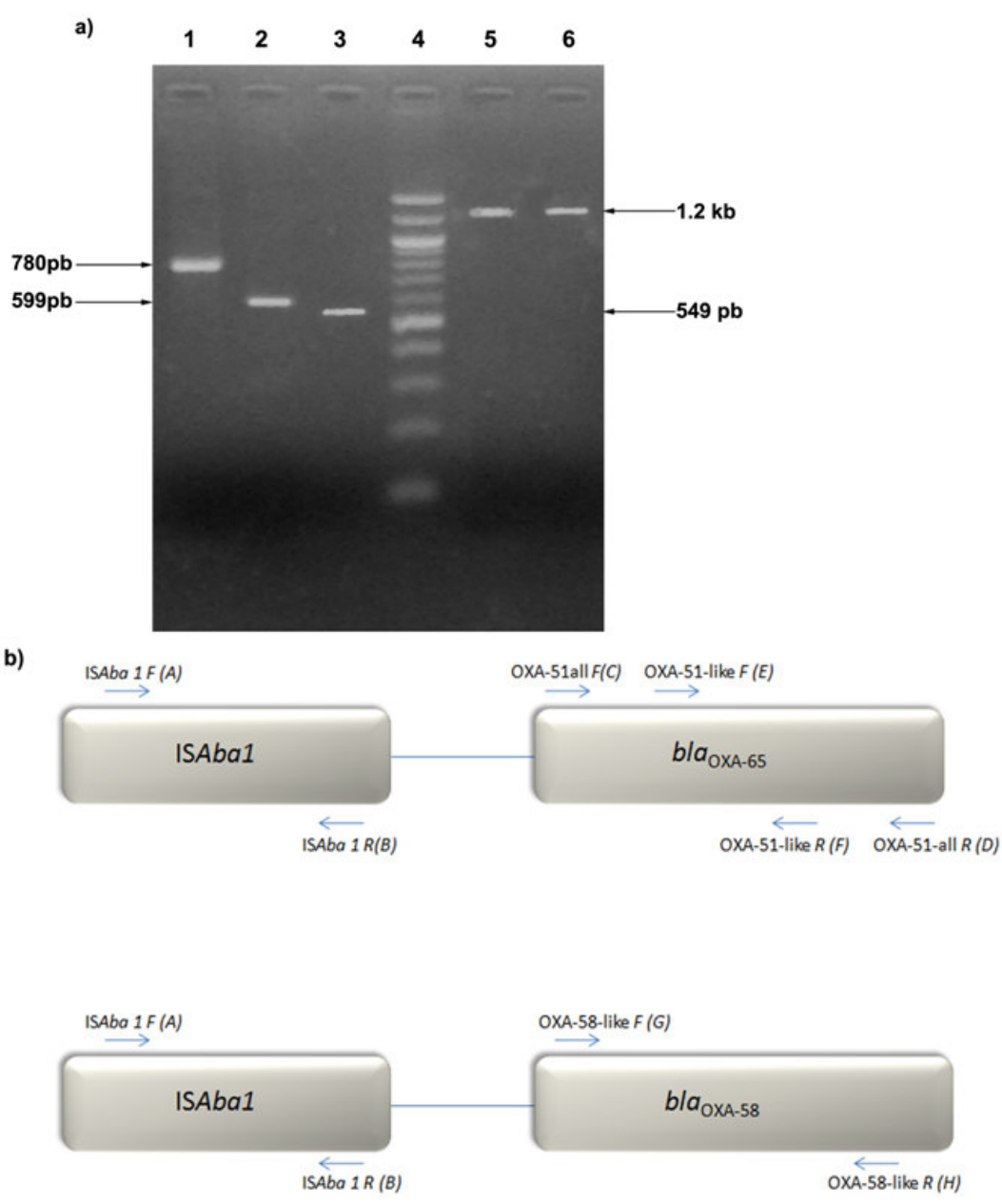

Figure 1. a) – Agarose gel with PCR products obtained using IS*Aba1*, OXA-51-like, OXA-51-likeALL and OXA-58-like primers. Lane 1 – PCR product of bla_{OXA-65} gene with OXA-51-likeALL F (C) and R (D)primers ; Lane 2 – PCR product of bla_{OXA-58} gene with OXA-58 F (G) and R (H) primers; Lane 3 – PCR product of IS*Aba1* sequence with IS*Aba1* F (A) and IS*Aba1* R (B) primers; Lane 4 – 100 bp molecular marker; Lane 5 – PCR mapping of IS*Aba1* sequence and bla_{OXA-65} gene with IS*Aba1*F (A) and OXA-51-like R (F) primers; Lane 6 – PCR mapping of IS*Aba*1 sequence and bla_{OXA-58} gene with IS*Aba*1F (A) and OXA-58-like R (H) primers.

b) – Schematic representation of PCR products obtained using IS*Aba1*,OXA-51-like, OXA-51-likeALL and OXA-58-like primers with the position of all the primers mentioned in figure 1a.

The IC-09 isolate included in this study showed resistance to imipenem, meropenem, amikacin, ciprofloxacin, gentamicin, cephalothin, ampicillin-sulbactam, trimetropim-sulfameto xazole and ticarcillin-clavulanate. The species of the isolate was confirmed as *A. baumannii* and the result of the MIC testing showed resistance to imipenem (64 µg/mL). The isolate was negative to the presence of $bla_{OXA-24-like}$ and $bla_{OXA-23-like}$ genes. The PCR reaction indicated the presence of OXA-58-like and OXA-51 like carbapenemases and PCR mapping indicated that the IS*Aba1* sequence is upstream of both genes but in separate positions of the genome (Figure 1a and 1b). Sequencing analysis of the $bla_{OXA-51-like-all}$ PCR product showed 99% homology with bla_{OXA-65} gene. The enzyme OXA-65 was first described in two isolates from Argentina and has 98% identity with OXA-51, thus belonging to the same subgroup primarily described in 2005 (2).

To our knowledge, for the first time in Brazil, a positive result was obtained for bla_{OXA-58} gene and the sequencing analysis showed 100% homology with the bla_{OXA-58} gene of *A. baumannii.* This enzyme was first described in France in an outbreak of hospital infection in 2003 and since then it has been found around the world. Corroborating with previous studies, this finding confirms the worldwide spread of the OXA-58 carbapenemase (15, 16). In South America this enzyme is prevalent and of public health concern in Argentina, a neighboring country to Rio Grande do Sul State, where the present study was conducted. In Argentina, in contrast to our findings, the MICs observed for imipenem were from 8 to 32µg/mL, indicating a low level of resistance to this antibiotic (5, 14). However, our results suggested that additional mechanisms of resistance may be present, since the MIC was higher. In the present study, the IS*Aba1* sequence is located upstream to both bla_{OXA-65} and bla_{OXA-58} genes (Figure 1a and 1b), therefore, its presence may be associated with the increased level of resistance observed (MIC = 64 µg/mL). As previously described, this IS, when located upstream to $bla_{OXA-51-like}$, may play a role of promoter and enhance resistance to carbapenems (10, 20, 21). Overexpression of the AdeABC efflux pump may be also associated with this resistance phenotype (11). In conclusion, our study reported the first occurrence of an *A. baumannii* from clinical origin, carrying the bla_{OXA-58} gene in Brazil and suggests that further studies are needed to identify if the expression levels of the bla_{OXA-65} and bla_{OXA-58} genes and/or efflux pumps may be responsible by the carbapenem resistance level observed.

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