First report of IS*Aba825* upstream of $bla_{OXA-143}$ gene in carbapenem-resistant *Acinetobacter baumannii* (Acb) clinical isolates

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Abstract

OBJECTIVES: The aim of this study was to describe a carbapenem-resistant Acb isolates producers of OXA-143, a carbapenem-hydrolyzing class D β-lactamase (CHDL), associated with the insertion sequence (IS) IS*Aba825* in Brazil. **METHODS**: Three carbapenem-resistant *Acinetobacter* spp. isolates were recovered from distinct patients hospitalized at a tertiary teaching hospital located at São Paulo city, between the years 1997 and 1998. The identification of Acb isolates was confirmed by *rpoB* sequence. Susceptibility testing was performed by broth microdilution according to CLSI guidelines. The genetic relatedness was evaluated by Rep-PCR and automated ribotyping. The production of CHDLs and its association with IS*Aba1*, as well as other β-lactamases encoding genes was confirmed by PCR followed by DNA sequencing. Outer membrane proteins encoding genes *carO*, *ompW*, *33-36kDa* and *oprD* were also evaluated by PCR and sequencing. **RESULTS:** All isolates were identified as Acb and showed the same Rep-PCR pattern and ribogroup (52-1). As expected, these isolates possessed the intrinsic bla_{OXA-51} gene and bla_{TEM-1}. The IS*Aba1* was only observed upstream to the bla_{ADC} gene. A PCR amplification analysis of $bla_{OXA-143-like}$ gene showed a single amplicon of nearly 1,822 bp instead of the expected fragment size of 889 bp. Sequencing analysis indicated the presence of an IS (876 bp), the IS*Aba825* (IS982 family), upstream of $bla_{OXA-143}$ gene. All Acb isolates showing IS*Aba825*-$bla_{OXA-143}$ were resistant to meropenem (MER; MICs, >8 mg/L), imipenem (IPM; MICs, 16 mg/L), levofloxacin (MICs, 16 mg/L), gentamicin (MICs,16 mg/L), amikacin (MICs, 64 mg/L), ciprofloxacin (MICs, 128 mg/L), cefepime (MICs, 128 mg/L), ampicillin/sulbactam (MICs, 256 mg/L), cefotaxime (MICs, 256 mg/L), ceftriaxone (MICs, 256 mg/L) and ceftazidime (MICs, 256 mg/L). In opposite, these isolates were susceptible only to tigecycline (MICs, 0.125 mg/L) and polymyxin B (MICs, 0.25 mg/L). PCR analysis showed that the *oprD* gene was disrupted by an IS in all Acb isolates. **CONCLUSION:** To date, similarly what occurs with the OXA-24/40 group, the expression of OXA-143 has not been associated with the presence of IS. In fact, the role of IS for OXA-143 expression has been poorly studied. To our knowledge this is the first report of the presence of IS*Aba825* upstream of $bla_{OXA-143}$ in a multidrug-resistant Acb isolates also showing the disruption of *oprD* gene. In the same way, the overexpression of ADC caused by IS*Aba1* contributed to the high MICs observed for the extended-spectrum cephalosporins among Brazilian OXA-143-producing Acb isolates. Although a clonal dissemination had been observed in our hospital between the years 1997 and 1998, none OXA-143-producing Acb isolate associated with IS*Aba825* had been observed subsequently, showing that this phenomenon rarely occur in this CHDL group.

Introduction

Nosocomial infections caused by carbapenem-resistant *Acinetobacter baumannii* isolates have reached epidemic levels in past decades **(1)**. Currently this microorganism is responsible for outbreaks of difficult eradication with high mortality rates worldwide **(2)**. The production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) is by far the most frequent mechanism of resistance to carbapenems in *A. baumannii* **(3)**. To date, five main groups of chromosomally encoded (OXA-51 group) and plasmid-encoded CHDLs (OXA-23-like, OXA-24/40-like, OXA-58-like and OXA-143-like groups) have been described, almost exclusively, in *A. baumannii* isolates. Firstly described in an *A. baumannii* strain isolated in Brazil in 2004 **(5)**, the OXA-143 is the second most frequently carbapenemase found among Brazilian *A. baumannii* isolates **(6)**. At this moment, four OXA-143 variants have been described worldwide: OXA-182, OXA-231, OXA-253 and 255 **(7)**, as showed on Figure 1.

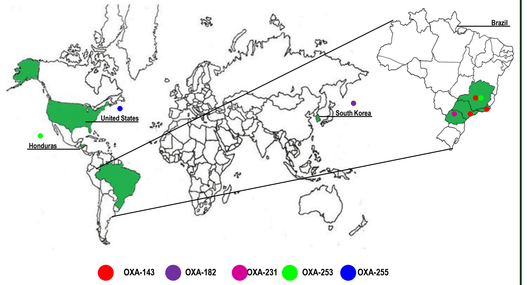

Figure 1. Worldwide distribution of OXA-143 variants among *Acinetobacter* spp. clinical isolates.

Until now, OXA-143 was only described among *A. baumannii* isolated in Brazil, especially from the Southeast **(6)**. In the same way, OXA-182 has been restricted to the South Korea, being described in *A. baumannii* as well as in *Acinetobacter nosocomialis* species **(7,8)**. Although rarely reported, other three variants have been described among *A. baumannii* isolates in Brazil (OXA-231 and OXA-253) **(9,10)** and Honduras (OXA-253) **(10)**, and in *Acinetobacter pittii* in USA (OXA-255) **(11)**.

The majority of CHDL groups (OXA-23-like, OXA-51-like and OXA-58-like) is associated with insertion sequences (IS), mainly the IS*Aba1*, that confer a strong promoter upstream of the oxacillinase encoding gene, increasing its expression **(3)**. However, it seems that the expression of other two related CHDLs groups, OXA-24/40 and OXA-143, is not mediated by these structures **(3,5)**, suggesting that these enzymes are by themselves capable of conferring high-level of carbapenem resistance.

The aim of this study was to describe for the first time the association of $bla_{OXA-143}$ gene with IS*Aba825* in carbapenem-resistant *A. baumannii* clinical isolates recovered from a tertiary teaching hospital located in the city of São Paulo, Brazil. The presence of IS*Aba125* and IS*Aba1* upstream of the *oprD* and $bla_{ADC-like}$ genes, respectively, were also observed in these strains showing that different IS concomitantly governed the expression of distinct β-lactam resistance genes.

Methods

❖ **Bacterial Isolates:** A total of three carbapenem-resistant *Acinetobacter* spp. isolates were recovered from distinct patients hospitalized at a tertiary teaching hospital located in the city of São Paulo, between the years 1997 and 1998. The amplification of the $bla_{OXA-143-like}$ gene by PCR showed an amplicon of unexpected size (nearly 1,822 bp instead of 889 b) for all isolates.

❖ **Species Identification.** The species identification of the *Acinetobacter* spp. isolates was confirmed by sequencing analysis of partial inner regions of RNA polymerase β subunit (rpoB) gene [zone 1 (350 bp, between positions 2,900 and 3,250) and zone 2 (450 bp, between positions 3,250 and 3,700)], as previously published **(12)**.

❖ **Molecular typing by Rep-PCR and automated ribotyping:** The clonal relationship of the *Acinetobacter* spp. isolates was initially performed by automated ribotyping. Ribotype patterns of all isolates were obtained by automated ribotyping using the RiboPrinter Microbial Characterization System (DuPont Qualicon) after digestion with the PvuII enzyme. Pattern analysis was carried out using the RiboPrinter software (version 2.2.012.0). Subsequently, the genetic similarity was also determined by Rep-PCR. The primers REP-1 (5´-IIIGCGCCGICATCAGGC-3´) and REP-2 (5´-ACGTCTTATCAGGCCTAC-3´) were used, as described previously **(13)**. The amplified fragments were separated on a 1.5% (w/v) agarose gels for 100 min.

❖ **Antimicrobial Susceptibility testing.** The minimum inhibitory concentrations (MIC) for many antimicrobials were determined by broth microdilution following the recommendations of the "Clinical and Laboratory Standards Institute - CLSI". The powder of antimicrobial agents were purchased from Sigma-Aldrich (Sigma, Steinheim, Germany). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested as quality control strains. The susceptibility results were interpreted according with the CLSI breakpoints (document MS-100-S24, 2014).

❖ **β-lactamases content analysis.** Multiplex-PCR experiments for the identification of CHDL encoding genes ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$), extended-spectrum (ESβLs) encoding genes ($bla_{CTX-M-like}$, $bla_{SHV-like}$, $bla_{TEM-like}$ and $bla_{GES-like}$) as well as metallo-β-lactamases (MβLs) encoding genes ($bla_{IMP-like}$, $bla_{VIM-like}$, bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1} and $bla_{NDM-like}$) were performed as described previously **(14,15,16)**. The sequencing reactions were carried out after purification of the amplicons using the "QIAquick Gel Extraction Kit" (Qiagen, Courtaboeuf, France) according to manufacturer instructions. Sequencing reactions were prepared using the "Kit BigDye Terminator Cycle Sequencing" (Applied Biosystems, Foster City, USA), and then performed on the machine "ABI 3500 Genetic Analyzer" (Applied Biosystems, Perkin Elmer, USA). The DNA sequences obtained and the derived protein sequences were analyzed using the "Lasergene Software Package" (DNASTAR, Madison, USA) and then compared with the sequences deposited in the "GenBank". The presence of IS*Aba1* was also evaluated upstream to the CHDLs and ADC encoding genes by PCR.

❖ **Sequencing of the outer membrane proteins encoding genes.** The outer membrane proteins encoding genes *carO*, *ompW*, *33-36kDa* and *oprD* were evaluated by PCR and sequencing using specific primers.

Conclusions

❖ We described for the first time the presence of IS (IS*Aba825)* upstream of the $bla_{OXA-143}$ gene in multidrug-resistant *A. baumannii* clinical isolates in Brazil. A putative promoter was found with -35 (TTGTCA) and -10 (TATGAA) located 17 bp apart from each other upstream of the $bla_{OXA-143}$ gene.

❖ Although this *A. baumannii* clone had been recovered during two consecutive years in our hospital, no other OXA-143-producing *A. baumannii* isolates associated with IS was subsequently observed, suggesting that this phenomenon rarely occur in OXA-143 group.

❖ The overexpression of ADC caused by IS*Aba1* contributed for the high MICs observed for the extended-spectrum cephalosporins among the three OXA-143-producing *A. baumannii* isolates evaluated in the study.

❖ The presence of the IS*Aba125* was also observed upstream of porin OprD-like encoding gene in all *A. baumannii* isolates. Additional studies are being carried out to understand the role of this IS in the expression of *oprD-like* gene.

Results

Table 1. Characterization of β-lactamase content, genetic similarity and antimicrobial susceptibility profile among carbapenem-resistant *A. baumannii* clinical isolates presenting the IS*Aba825* upstream to the $bla_{OXA-143}$ gene.

Strain	rpoB	Year	Clinical Specimen	Rep-PCR	Automated Ribotyping	MIC (mg/L)												β-lactamase content
						CAZ	CRO	FEP	IPM	MEM	AMK	GEN	CIP	LEV	TGC	MIN	PMB	
Acb58015	*A. baumannii*	1998	Catheter	A	52-1	>512	>512	>128	128	128	64	4	256	8	0.5	≤0.25	0.25	bla_{ADC}+IS*Aba1*, bla_{TEM-1}, $bla_{OXA-95-like}$, $bla_{OXA-143}$-IS*Aba825*
Acb58021	*A. baumannii*	1998	Sputum	A	52-1	>512	>512	>128	64	64	64	8	>256	16	0.5	≤0.25	0.25	bla_{ADC}+IS*Aba1*, bla_{TEM-1}, $bla_{OXA-95-like}$, $bla_{OXA-143}$-IS*Aba825*
Acb81062	*A. baumannii*	1997	Surgical Wound	A	52-1	>512	>512	>128	128	128	128	4	>256	16	0.5	≤0.25	0.25	bla_{ADC}+IS*Aba1*, bla_{TEM-1}, $bla_{OXA-95-like}$, $bla_{OXA-143}$-IS*Aba825*

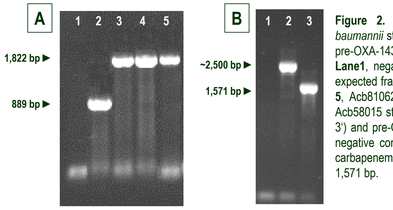

Figure 2. A - PCR amplification of the $bla_{OXA-143-like}$ gene of the studied *A. baumannii* strains, using pair primers pre-OXA-143A (5-AGTTAACTTTCAATAATTG-3) and pre-OXA-143B (5-TTGGAAAATTATATAATCCC-3) described by Higgins and colleagues **(5)**. **Lane1**, negative control; **Lane 2**, OXA-143-producing *A. baumannii* clinical isolate with expected fragment size of 889 bp; **Line 3**, Acb58015 strain; **Line 4**, Acb58021 strain; **Line 5**, Acb81062 strain. **B –** PCR amplification of the *oprD-like* gene in the *A. baumannii* Acb58015 strain using the pair primer pre-OprD.F (5'-CACATTAATCAAATCCTGCATAGC-3') and pre-OprD.R (5'-TAAGCCTAGAGYGTCGCAAT-3'), designed for this study. **Line 1**, negative control; **Line 2**, Acb58015 strain showing a fragment size >1,000 bp; **Line 3**, carbapenem-susceptible *A. baumannii* ATCC19606 strain with expected fragment size of 1,571 bp.

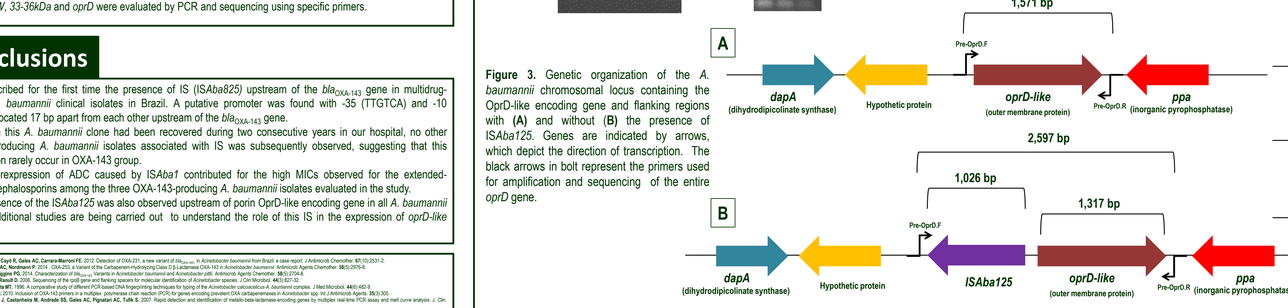

Figure 3. Genetic organization of the *A. baumannii* chromosomal locus containing the OprD-like encoding gene and flanking regions with **(A)** and without **(B)** the presence of IS*Aba125*. Genes are indicated by arrows, which depict the direction of transcription. The black arrows in bolt represent the primers used for amplification and sequencing of the entire *oprD* gene.

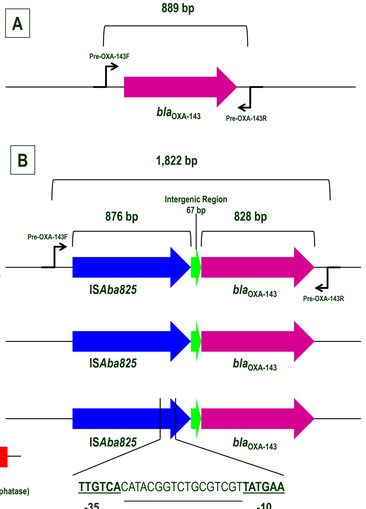

Figure 4. Schematic representation of the genetic arrangement of IS*Aba825* adjacent to the CHDL encoding $bla_{OXA-143}$ gene in three *A. baumannii* clinical isolates. **A -** The expected fragment of 889 bp in a OXA-143-producing *A. baumannii* isolate. **B** The presence of IS*Aba825* upstream of the $bla_{OXA-143}$ gene in all isolates showing a fragment of 1,822 bp. A putative promoter was found with -35 (TTGTCA) and -10 (TATGAA) located 17 bp apart from each other located 136 and 113 bp upstream of the $bla_{OXA-143}$ gene, respectively. Arrows designate transcription directions of genes. The black arrows in bolt represent the primers used for detection and sequencing the entire $bla_{OXA-143}$ gene. Figure is not to scale.

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