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ORIGINAL ARTICLE

Characterization of bla_{OxA-23} gene regions in isolates of *Acinetobacter baumannii*

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Tn2006;
Tn2007;
Tn2008

Background/purpose: To investigate the characterization of bla_{OxA-23} gene regions in isolates of *Acinetobacter baumannii* from Taizhou Municipal Hospital.
Methods: Fifty-nine non-repetitive, multiresistant (including imipenem-resistant) isolates of *A. baumannii* were recovered from clinical infections in hospitalized patients from January 2010 to August 2011 in Taizhou Municipal Hospital (affiliated with Taizhou University) in China. These isolates were genotyped using pulsed-field gel electrophoresis (PFGE). bla_{OxA-23} β-lactamase and associated genetic structures were analyzed using polymerase chain reaction (PCR), and recombination plasmids were analyzed by *Bam*HI- or *Sac*I- restriction enzyme digestion; predicted promoter structures of bla_{OxA-23} genes were determined and compared using protein-protein BLAST analysis.
Results: Fifteen out of 59 isolates expressing imipenem-resistant *A. baumannii* clinical isolates acquired either a bla_{OxA-23} β-lactamase gene. A new gene cluster (ISA*ba1*-bla_{OxA-23}-AMP) with three previously identified transposons (Tn2006, Tn2007, and Tn2008) and one previously identified gene cluster (ISA*ba1*- bla_{OxA-23}) was found in the isolates. Recombination plasmids were

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analyzed by restriction enzyme digestion.
Conclusion: Our results indicate that pattern A was the most prevalent molecular type based on PFGE, and that different clones might be widespread with a majority of ISA*ba1*-bla_{OxA-23} clonal lineages in the 15 PCR positive isolates of *A. baumannii* in the hospital.

Introduction

Acinetobacter baumannii, a typical opportunistic pathogen, is often involved in nosocomial outbreaks, and increasingly reported to be resistant to carbapenems.[1] The strains may be linked not only to the production of Ambler class B metallo-β-lactamases,[2] but also to the production of carbapenem-hydrolyzing class D β-lactamases (CHDLs).[3,4]

Several studies have shown the geographically widespread occurrence of three identified multidrug-resistant *A. baumannii* strains (European clones I, II, and III), suggesting their clonal relatedness.[5,6] According to literature reports, *A. baumannii* strains with increasing resistance to carbapenems have been observed worldwide in the past decade; the main cause of carbapenem resistance in *A. baumannii* clinical isolates worldwide is the production of CHDLs, also designated OXA-type carbapenemases.[7] From the different groups of OXA-type enzymes defined on basis of sequence comparisons, OXA-23, OXA-24, and OXA-58 (which can be either plasmid- or chromosomally- encoded) have been most frequently associated to carbapenem-resistant clinical strains of *A. baumannii*,[1] but OXA-23 has the higher dissemination worldwide. The β-lactamase OXA-23 was identified first in *A. baumannii* from Scotland and was found to confer transferable imipenem resistance in *A. baumannii*.[8]

Since then, OXA-23 producers have been identified as the source of nosocomial outbreaks worldwide, including Brazil, Colombia, the United Kingdom, Korea, Tahiti (France), and China.[9–15] Recently, transposons Tn2006, Tn2007, and Tn2008 have been described in *A. baumannii* isolates from Europe,[5] and Tn2008 is a major vehicle carrying bla_{OxA-23} in *A. baumannii* from China.[16] We describe here the characterization of the genetic structures in which bla_{OxA-23} genes are embedded in *A. baumannii* clinical isolates obtained in Taizhou Municipal Hospital of China, between January 2010 and August 2011. These structures included three transposons (Tn2006, Tn2007, and Tn2008) and two gene clusters (ISA*ba1*-bla_{OxA-23}-AMP and ISA*ba1*-bla_{OxA-23}). This is the first report that identifies bla_{OxA-23} genes harbored by Tn2006, Tn2007, and Tn2008 transposons in our region.

Materials and methods

Bacterial isolates

Fifty-nine non-repetitive, multiresistant (including imipenem-resistant) isolates of *A. baumannii* were recovered from clinical infections in hospitalized patients from January 2010 to August 2011 in Taizhou Municipal Hospital (affiliated with Taizhou University) in China. The patients were distributed among seven clinical units: intensive care unit (ICU) (31/59), neurosurgery (12/59), respiratory unit (6/59), urology surgery (4/59), neurology unit (3/59), infection unit (2/59), and chest surgery (1/59). The different bacterial isolates were obtained from sputum (44/59), blood (11/59), and venous cannula (4/59), and assigned to the *A. calcoaceticus-A. baumannii* complex using a Vitek GNI$^+$ card (bioMerieux, Lyon, France). Species identification was confirmed by sequence analysis of the 16S-23S rRNA gene intergenic spacer region.[17] The different *A. baumannii* isolates were designated from Ab1 to Ab59.

Susceptibility testing

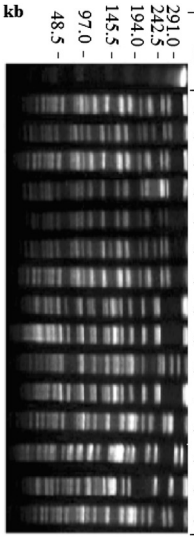
The minimum inhibitory concentration (MICs) of seven representative antimicrobial agents (including imipenem) for the different isolates, were determined by the MicroScan microdilution panel (Scott, Arizona, USA) broth dilution method. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Results were interpreted according to CLSI 2011 guidelines.[18]

Pulsed-field gel electrophoresis

DNA was prepared from 15 polymerase chain reaction (PCR)-positive isolates and cleaved with 40 U ApaI as previously described.[19] Electrophoresis was performed on a 1% agarose gel in 0.5 M Tris/borate/EDTA buffer on a CHEF-Mapper XA pulsed-field gel electrophoresis (PFGE) system (Bio-Rad, Hercules, California, USA) for 22 hours at 14°C, with run conditions of 6 V/cm, a pulse angle of 120 U and pulse times from 5 to 20 seconds. A λDNA ladder (Amersham Biosciences, Piscataway, New Jersey, USA) was used as molecular mass marker and bands were stained with ethidium bromide (0.5 mg/L) and photographed under UV light. Band profiles were interpreted using previous criteria.[20]

Identification of genetic structures bearing bla_{OxA-23} gene in different *A. baumannii* isolates

One of the aims of this study was to elucidate the mechanism(s) of acquisition and expression of the bla_{OxA-23} gene in the *A. baumannii* isolates recovered from different hospital areas (Fig. 1). For this purpose, we conducted PCR amplification following described procedures[11,21,22] using the primers listed in Table 1 in order to identify presumptive pre-bla_{OxA-23}, bla_{OxA-23}, $bla_{OxA-23-like}$, ATPase, ISA*ba1*, and ISA*ba4* genes. Total DNA was purified from isolates generating amplification products and digested with *Bam*HI



Strain	Date of isolation (mo/y)	Unit	MIC (μg/mL)							Transposon or genetic structure	PFGE
			IMI	AM	CFX	CAZ	LEV	AN	CFS		
M											
Ab53	05/2011	ICU	32	64	32	64	8	8	16	ISA*ba1*-bla_{OXA-23}-AMP	A
Ab31	02/2010	Neurosurgery	32	64	16	32	64	64	32	ISA*ba1*-bla_{OXA-23}	A
Ab22	03/2010	Urology surgery	16	32	64	16	32	32	16	ISA*ba1*-bla_{OXA-23}	A
Ab40	07/2010	Respiration unit	32	128	64	32	16	32	16	ISA*ba1*-bla_{OXA-23}	A
Ab9	05/2010	ICU	16	32	32	32	16	64	32	ISA*ba1*-bla_{OXA-23}	A
Ab15	08/2011	Neurosurgery	64	64	16	64	32	32	64	ISA*ba1*-bla_{OXA-23}	A
Ab24	04/2011	ICU	64	64	128	64	32	32	8	ISA*ba1*-bla_{OXA-23}	A
Ab34	10/2010	ICU	32	32	64	128	16	16	16	Tn2006	B
Ab41	07/2011	ICU	64	64	64	32	32	128	32	ISA*ba1*-bla_{OXA-23}	B
Ab12	07/2011	Respiration unit	32	64	32	64	16	16	16	Tn2008	C
Ab5	11/2010	ICU	32	128	64	32	32	16	32	ISA*ba1*-bla_{OXA-23}	B
Ab28	12/2010	Neurosurgery	64	32	32	32	16	32	8	Tn2008	A
Ab17	06/2010	ICU	32	64	64	32	16	32	16	ISA*ba1*-bla_{OXA-23}	D
Ab37	12/2010	ICU	64	64	64	64	16	64	32	Tn2007	E
Ab19	01/2011	Neurosurgery	64	64	32	128	64	32	16	ISA*ba1*-bla_{OXA-23}	A

Figure 1. Characterization of *Acinetobacter baumannii* clinical isolates used in this work. The pulsed-field gel electrophoresis (PFGE) profiles of *Apa*I-digested genomic DNA of selected clinical *A. baumannii* isolates are shown on the left. The final positions and sizes (in kbp) of the molecular markers used are indicated at the top. The figure also includes the assigned PFGE clone (A to E, right column), and information regarding isolate denomination, date and unit of isolation, minimum inhibitory concentration (MIC) of imipenem, and the transposon or bla_{OxA-23} genetic structure identified in the particular isolate. AMP = AMP-acid ligase gene; M = molecular size markers; AM = ampicillin; AN = amikacin; CAZ = ceftazidime; CFS = cefoperazone/sulbactam; CFX = cefoxitin; ICU = intensive care unit; IMI = imipenem; LEV = levofloxacin.

or *Sac*I. The resulting fragments were ligated into either *Bam*HI- or *Sac*I-digested pBKCMV plasmid, and transformed into *E. coli* DH10B as previously described.[23]

Transformed bacteria were plated on trypticase soy agar plates containing amoxicillin (50 μg/mL) and kanamycin (30 μg/mL), and plasmids obtained from different colonies selected under these conditions were purified in order to sequence and analyze the cloned DNA fragments as described previously.[24] The deduced genetic structures associated with bla_{OxA-23} gene obtained from these studies are depicted in Fig. 2. Finally, the cloned DNA fragments were sequenced and analyzed as described previously.[24]

Predicted promoter structures of bla_{OxA-23} genes and comparison of partial amino acid sequences for OXA-23-like enzymes

The different promoter regions of bla_{OxA-23} or $bla_{OxA-23-like}$ genes identified in the *A. baumannii* isolates studied here were obtained by sequence analysis of PCR fragments obtained using primers described in Table 1, and the results are shown in Fig. 3. The deduced amino acid sequences of OXA-23 enzymes were subsequently compared using BLASTp. The sites of initiation of transcription of the bla_{OxA-23} gene were mapped also using real time PCR as the primer of pre-OXA-23 (Table 1).

Results

Bacteria source and antibiotic susceptibility

The majority of the 59 multiresistant (including imipenem-resistant) isolates of *A. baumannii* came from the ICU (52.2%), neurosurgery (22.0%), and respiratory unit (10.2%), with most samples isolated from sputum (74.6%). In addition, all of the imipenem-resistant isolates showed high resistance rates (> 99%) to ampicillin, cefoxitin, ceftazidime, levofloxacin, amikacin, and cefoperazone/sulbactam in 15 out of 59 isolates, respectively (Fig. 1).

Molecular typing

Fifteen out of 59 clinical isolates were grouped into five clonal patterns by PFGE. The two major patterns were pattern A represented by nine isolates and pattern B represented by three isolates (Fig. 1). Each of the patterns C, D, and E was represented by a single strain. Four molecular types, namely patterns A, B, D, and E, were from the ICU. Strains Ab34 and Ab37, both isolated from venous cannulas of patients, belonged to patterns B and E, respectively (Fig. 1). Meanwhile, strains Ab12 and Ab28, both from the blood of patients in neurosurgery, had patterns C and A, respectively (Fig. 1). Pattern A clonal lineage was shared by isolates Ab53, Ab22, Ab40, Ab9, Ab24, Ab31, Ab15, Ab28, and Ab19 based on PFGE analysis following Tenover et al[20] criteria (Fig. 1). According to the details, however, isolates Ab53, Ab22, Ab40, Ab9, and Ab24 were apparently identical on this basis, but isolates Ab31, Ab15, Ab28, and Ab19 were closely related to the above. Actually, isolates Ab31 and Ab15 were apparently identical among them, but differed in PFGE fragments of isolates Ab53, Ab22, Ab40, Ab9, and Ab24; simultaneously, isolates Ab28 and Ab19 were apparently identical among them but were different with PFGE fragments of isolates Ab31 or Ab15. Consequently, they were obviously of different subtypes of pattern A between isolate Ab28 or Ab19 and isolate Ab31 or Ab15, in the study.

Table 1 The primers used in the study

Primer name	Nucleotide sequence (5′→3′)	Location	Reference
PreOXA-23 F	ACCTTTAGGGATTCTTTTA	bla_{OxA-23} gene, forward external primer	This study
PreOXA-23 R	GGCTTAGAGCATTACCATA	bla_{OxA-23} gene, reverse external primer	This study
OXA-23 F	GGAATTCCATGAATAAATATTTTACTTGC	bla_{OxA-23} gene, forward primer	31
OXA-23 R	CGGGATCCCGTTAAATAATATTCAGGTC	bla_{OxA-23} gene, reverse primer	31
OXA23-like F	GATGTGTCATAGTATTCGTCG	bla_{OxA-23}-like gene, forward primer	This study
OXA23-like R	TCACAACAACTAAAAGCACTG	bla_{OxA-23}-like gene, reverse primer	This study
ATPase R	GCTTCATCCAGAAGCGTCCGG	ATPase gene, reverse primer	34
IS*Aba1* F	ATGCAGCGCTTCTTTGCCAGG	*tnpA* gene of IS*Aba1*, forward primer	34
IS*Aba1* R	AATGATTGGTGACAATGAAG	*tnpA* gene of IS*Aba1*, reverse primer	34
IS*Aba1*-ext F	AAGCACTTGATGGGCAAGGC	*tnpA* of IS*Aba1*, forward external primer	30
IS*Aba4* F	ATTTGAACCCATCTATTGGC	*tnpA* gene of IS*Aba4*, forward primer	30
IS*Aba4* R	ACTCTCATATTTTTTCTTGG	*tnpA* gene of IS*Aba4*, reverse primer	30

Identification of genetic structures containing bla_{OxA-23} genes in the *A. baumannii* clinical isolates under study

Different genetic structures harboring bla_{OxA-23} genes were identified in the *A. baumannii* isolates under study by cloning *Bam*HI- or *Sac*I-digested DNA fragments in plasmid pBKCMV and selecting *E. coli* transformants bearing bla_{OxA-23} genes in amoxicillin-containing solid media. Further sequence analysis of different clones allowed the identification of five different genetic structures in which bla_{OxA-23} genes were present (Figs. 1 and 2). These were transposons Tn2006, Tn2008, Tn2007, IS*Aba1*-bla_{OxA-23}-AMP-acid ligase gene, and IS*Aba1*-bla_{OxA-23} (Fig. 2). The *A. baumannii* isolates in which these structures were identified are depicted in Fig. 1. As seen in this figure, the IS*Aba1*-bla_{OxA-23} arrangement was the most frequently found (10/15 isolates: Ab31, 22, 40, 9, 14, 24, 41, 5, 17, and 19). Tn2008 was found in two isolates (Ab12 and Ab18), Tn2006 in Ab34, Tn2007 in Ab37, and IS*Aba1*-bla_{OxA-23}-AMP-acid ligase gene in Ab53.

IS-derived hybrid promoters enhancing bla_{OxA-23} expression

Using BLASTp analysis, although the $bla_{OxA-23-like}$ amino acid sequence was approximately 99.9% identical to that of bla_{OxA-23} with a difference at site 104 (K104X), however, the later experiment proved that $bla_{OxA-23-like}$ was identified with bla_{OxA-23}. IS*Aba1* is an insertion sequence located upstream of *bla*ampC and bla_{OxA-51}-like genes in *A. baumannii* associated with overexpression of these genes.[25,26] As seen in Fig. 3, in isolates Ab31, 22, 40, 9, and 17 we found that an IS*Aba1* insertion upstream of the bla_{OxA-23} gene generated a hybrid promoter (-35 sequence, TTAGAA; -10 sequence, TTATTT) identical to that identified previously[26] that directs expression of this gene. Moreover, we also found in strain Ab37 an IS*Aba4* insertion upstream of the bla_{OxA-23} gene, also generating a hybrid promoter directing expression of this gene. In this case, the +1 transcription start was located 31 bp upstream of the bla_{OxA-23} start codon, and inside IS*Aba4* IRL. The identified -35 sequence (TAACTA) and -10 (TTTCTT) promoter sequences which were separated by 17 bp (Fig. 3) were identical to those described previously by other authors.[26]

Discussion

In this study, the 59 non-repetitive pan-resistant (including imipenem-resistant) isolates of *A. baumannii* were from the ICU unit (52.2%) and neurosurgery (22.0%). Fifteen out of 59 clinical isolates were grouped into five clonal patterns by PFGE: the two major patterns were pattern A (9 isolates) and pattern B (3 isolates); patterns A, B, D, and E were from the ICU unit (8 isolates) and pattern C was from the respiratory unit (1 isolate). It was suggested that pattern A might be prevalent in our hospital, and pattern B happened to appear in the ICU unit of our hospital. However, further investigation showed that pattern B also appeared in the neurosurgery and respiratory units in latter experiments.

Carbapenems have the most extended antimicrobial spectrum of antibacterial drug among all the β-lactams. However, reports of carbapenem-resistance have been increasing, especially in *P. aeruginosa* and *A. baumannii*.[15] A limited number of antimicrobial agents, including polymyxin, sulbactam, and minocycline, maintain a high activity against OXA-23-producing *A. baumannii*.[10] In particular, sulbactam has a direct antimicrobial and synergistic effect with ampicillin against *A. baumannii*.[27]

In silico analyses of GenBank databases and personal observations have indicated that an IS*Aba1* element, which belongs to the IS*4* family, is constantly identified upstream of bla_{OxA-23}, regardless of the geographical origin of the isolate. In addition, an IS*Aba4* element, which belongs to the IS*982* family, has been identified recently in *A. baumannii* clinical isolates from France and Algeria.[28] The presence of these IS elements upstream of β-lactamase genes suggests that they may play a role in bla_{OxA-23} expression by providing strong hybrid promoter sequences, and perhaps participate in the acquisition of carbapenem resistance.[1,28]

This study demonstrated that transposons Tn2006, Tn2007, and Tn2008, as well as genetic structures IS*Aba1*-bla_{OxA-23}-AMP and IS*Aba1*-bla_{OxA-23} were present in the clinical strains of *A. baumannii* studied here. There is an association between the presence of these β-lactamase-

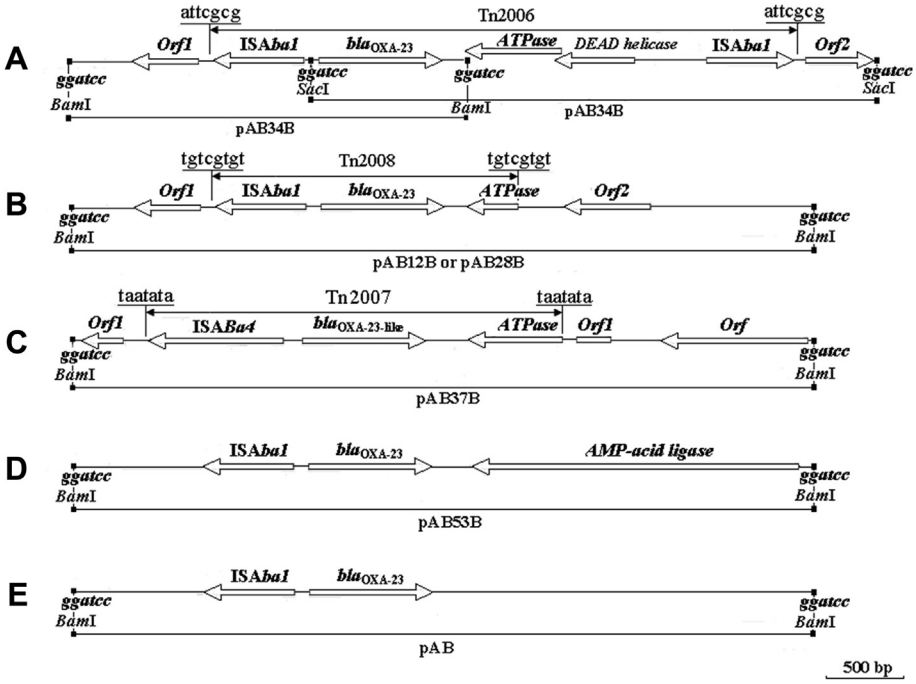


Figure 2. Schematic representation of the different genetic structures harboring bla_{OxA-23} genes found in the clinical *Acinetobacter baumannii* isolates studied: (A) transposon Tn2006, reconstructed from two overlapping DNA fragments of approximately 3.4 kbp and 5.4 kbp derived from *Bam*HI and *Sac*I digestion, respectively, of Ab34 DNA and cloned into recombinant plasmids pAB34B and pAB34S; (B) transposon Tn2008, present in an approximately 7.3 kbp fragment derived from *Bam*HI digestion of either Ab12 or Ab28 DNA cloned into plasmid pAB12B or pAB28B; (C) transpose Tn2007, present in an approximately 7.3 kbp fragment derived from *Bam*HI digestion of Ab37 DNA and cloned into plasmid pAB37B; (D) ISA*ba1*-bla_{OxA-23}-AMP acid ligase gene, present in an approximately 7.3 kbp fragment derived from *Bam*HI digestion of Ab53 DNA and cloned into plasmid pAB53B; (E) ISA*ba1*-bla_{OxA-23}, present in approximately 7.3 kbp fragments derived from *Bam*HI digestion of DNA obtained from isolates Ab31, Ab22, Ab40, Ab9, Ab14, Ab24, Ab41, Ab5, Ab17, or Ab19, and cloned into plasmids generally designated pAB. The *Bam*HI (*gagctc*) and *Sac*I (*ggatcc*) restriction sites are indicated. The boundaries of the different transposons are suggested by duplication of target sites likely generated by the transposition event: Tn2006 (*attcgcg*), Tn2007 (*taatata*), and Tn2008 (*tgtcgtgt*). Deduced open reading frames orf 1 and orf 2 of unknown function are indicated. ATPase = putative AAA *ATPase* gene; DEAD helicase = putative *DEAD* (Asp-Glu-Ala-Asp) *helicase* gene; AMP-acid ligase = *AMP-acid ligase* gene.

containing genetic elements and the increased MIC for imipenem, implying higher drug-resistance levels. Increased resistance to imipenem suggests that clinical isolates may contain one or more of the above transposons and structures. We hypothesize that carbapenem-hydrolyzing class D β-lactamase genes, belonging to the bla_{OxA-23}, serve as the actual resistance genes in the study.

An ISA*ba4* element was identified in the upstream region of a bla_{OxA-23} gene in *A. baumannii* isolate Ab37. This ISA*ba4* element belongs to the IS982 family, is 975 bp in length, possesses two 18 bp IRs, and encodes a 292 amino acid putative transposase. No target site duplication was observed on either end of ISA*ba4*. PCR mapping by different sets of primers did not detect any extra copy of ISA*ba4* downstream of the bla_{OxA-23} gene. Instead, a predicted AAA ATPase gene truncated at its 5′ terminus is located downstream of the bla_{OxA-23} gene. Detailed analysis of the truncation site identified a 7 bp sequence with an AT-rich content (TAATATA) at the extremity of the IRR of ISA*ba4* (Fig. 2), which most likely represented a transposition process mediated by ISA*ba4* that occurred at the origin of acquisition of the bla_{OxA-23} gene. This potential transposon, termed Tn2007, is 2471 bp long and included ISA*ba4* and bla_{OxA-23} genes. The ISA*ba4*-mediated mobilization process may be based on a one-ended transposition mechanism. This is unlike what has been observed for IS*Ecp1*, which uses a wide range of DNA sequences such as that of IRR, which are not absolutely random.[24,26,27] These data were consistent with previous findings.[26]

The Tn2006 insertion isolated from isolate Ab34 occurred inside a gene encoding a putative sulfonamide resistance protein sharing 89% amino acid identity to a protein identified in *A. baumannii* AYE (accession no.CAJ31116). Sequence analysis of strain Ab34 revealed a 2445 bp sequence containing the bla_{OxA-23} gene, as well as part of a putative AAA ATPase gene,[29] and part of a putative DEAD helicase gene.[26] These two latter genes were truncated at the exact same position, suggesting that a recombination event had occurred.[26] Simultaneously, ISA*ba1* has been shown to be very prevalent in *A. baumannii* and might be "customized" for that species.[30] It was described as an ISA*ba1*-based putative composite transposon in a previous reference,[26] and it was also proved to be identical in isolate Ab34 (Fig. 2A).

Tn2008, identified from isolate Ab12 or Ab28, was the major vehicle carrying bla_{OxA-23} and had been inserted into

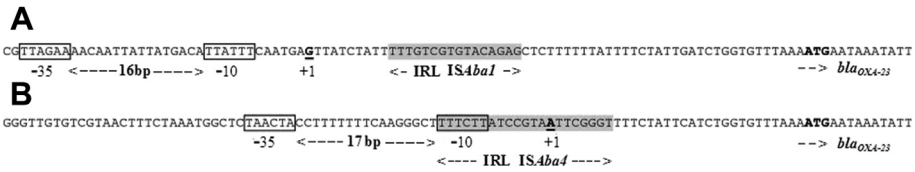


Figure 3. Promoter structures for expression of the bla_{OxA-23} in *Acinetobacter baumannii* strains: (A) the genetic structure of a plasmid in the representative strain Ab31, with insertion sequence ISA*ba1* and promoter elements located upstream of the bla_{OxA-23} gene, and it is of the same structures for strains Ab22, Ab40, Ab9, and Ab17; (B) the structure of a plasmid found in strain Ab37, with insertion sequence ISA*ba4* and promoter elements located upstream of the bla_{OxA-23} gene. The +1 initiation sites of transcription are shown in bold and underlined, while the promoter sequences are boxed. The ATG start codons of bla_{OxA-23} are shown in bold. The left IRL of ISA*ba1* and ISA*ba4* elements are shaded.

three different AT-rich locations (Fig. 2B). It is likely that this 16-bp sequence at the left end of Tn2008 served as IRL of ISA*ba1*. A single copy of ISA*ba1* could transpose bla_{OxA-23} and ATPase with itself by recognizing IRL as a new left boundary. The 16 bp sequence resembled the inverted repeat of ISA*ba1*, suggesting that ISA*ba1* might have utilized an alternative boundary to mobilize bla_{OxA-23}.[16] ISA*ba1* seems to be quite an important factor of genetic plasticity in *A. baumannii*,[30] and may facilitate the creation of composite transposons.[31] Together with our study that ISA*ba1* could be considered widespread in *A. baumannii* and a previous report,[32] this could suggest that bla_{OxA-23} originated from an *Acinetobacter* species *(A. radioresistens*). However, in recent studies[33–35] the genetic backgrounds of this enzyme were characterized, supporting its horizontal acquisition. It could also be hypothesized that the Tn2008 putative composite transposon might have been formed in *A. baumannii* after a previous acquisition process not related to ISA*ba1*.

To our knowledge, the genetic cluster ISA*ba1*-bla_{OxA-23}-AMP was the first to be identified in isolate Ab53 in the study. Its structure is similar to that of strain AcKOU1,[36] but with slight differences: the left side of structure ISA*ba1*-bla_{OxA-23} lacks a H^+ symporter gene, and the right side contains the full-length AMP-acid ligase gene. Meanwhile, our study also expresses that the ISA*ba1*-bla_{OxA-23} arrangement can be found in *A. baumannii clonal* lineages, namely patterns A, B, and D; Tn2008 can be found in clonal lineages, namely patterns A and C, and one group B or E isolate harbors Tn2006 or Tn2007. These findings probably suggest that different clones of *A. baumannii* might be widespread with the majority of ISA*ba1*-bla_{OxA-23} clonal lineages in our hospital.

The corresponding promoter structures for the bla_{OxA-23} gene in strains Ab31, 22, 40, 9, and 17, were made up of a -35 sequence (*TTAGAA*) and the -10 sequence (*TTATTT*), with a 16 bp spacer distance between them using bioinformatics procedures. These results agreed with previous work on ISA*ba1*,[26] but were not consistent with findings from other studies describing a 17 bp spacer region[37] and a -10 promoter sequence of *"TATTTC"*. Further studies are needed to investigate the nature of these inconsistencies.

Although all the strains were recovered from patients, drug-resistant bacteria are also found in hospital spots such as bed sheets, desks, floors, and cisterns. Numerous studies have shown that the hospital environment was a preferred habitat for *A. baumannii* isolates. *A. baumannii* appears to have unique characteristics among nosocomial Gram-negative bacteria with enhanced environmental persistence.[1] Our current findings warrant more extensive investigations into the occurrence of drug resistant bacteria.

Conflicts of interest

All authors declare no conflicts of interest.

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