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Genetic Structures at the Origin of Acquisition and Expression of the Carbapenem-Hydrolyzing Oxacillinase Gene bla_{OXA-58} in *Acinetobacter baumannii*

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Genetic structures surrounding the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-58} were characterized in a series of OXA-58-positive *Acinetobacter baumannii* strains isolated from different countries. We showed that in most of the cases, acquisitions of the bla_{OXA-58}-containing overall structure, including insertion sequence elements, may be likely the results of recombination events. In type strain *A. baumannii* MAD, the genetic structure surrounding the bla_{OXA-58} gene was bracketed by two 27-bp repeated sequences. The isolation of a clonally related OXA-58-negative *A. baumannii* isolate that possessed the same plasmid backbone as *A. baumannii* MAD but lacked this genetic structure indicated that the mechanism of acquisition could be reversible. Parts of the structure identified in *A. baumannii* MAD were conserved in other bla_{OXA-58}-positive isolates from various European countries. Primer extension experiments showed that bla_{OXA-58} expression was related to promoter sequences brought by different insertion sequence elements, such as an IS*Aba3*-like element, IS*Aba1*, IS*Aba2*, and IS*18*. This work identified novel structures at the origin of acquisition and expression of a carbapenem-hydrolyzing β-lactamase identified in non-clonally related *A. baumannii* isolates.

The increasing number of worldwide reports of β-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii* raises the question of the genetic structures that may be at the origin of their acquisition (18, 29). Those carbapenem-hydrolyzing β-lactamases are metallo-β-lactamases (Ambler class B β-lactamases) and oxacillinases (Ambler class D β-lactamases). Integrons are common vehicles for acquisition of metallo-β-lactamase genes. However, carbapenem-hydrolyzing oxacillinase (CHβL) genes have not been reported in class 1 integrons. The bla_{OXA-48} gene identified in a carbapenem-resistant *Klebsiella pneumoniae* isolate was associated with an IS*1999* insertion sequence element (19) and bla_{OXA-23} with an IS*Aba1* element (12, 13, 17). In addition, genetic structures for acquisition of the bla_{OXA-24}, bla_{OXA-25}, bla_{OXA-26}, bla_{OXA-27}, and bla_{OXA-40} genes of *A. baumannii* isolates are unknown (1, 4, 10). β-Lactamases OXA-51 and OXA-69, which are very similar and share less than 63% amino acid identity with the two latter groups and which may be considered, in view of recent findings (11), as naturally produced enzymes, would define a third group of those oxacillinases (5, 6). In addition, we have recently characterized β-lactamase OXA-58, which belongs to a fourth group of CHβLs (20). The bla_{OXA-58} gene was found to be plasmid located in most cases. This oxacillinase gene has been identified in a carbapenem-resistant *A. baumannii* isolate that was at the origin of a nosocomial outbreak in Toulouse, France (9), and further studies showed that carbapenemase OXA-58 was very widespread, since it has been detected in *A. baumannii* isolates recovered from different countries from southern Europe, the Balkans, and central Turkey (16) and also from Argentina, Kuwait, and the United Kingdom (J. Coelho, N. Woodford, M. Afzal-Shah, and D. Livermore, 6th Int. Symp. Biol. Acinetobacter, abstr. pC7, 2004). The aim of the present study was to investigate the genetic structures associated with the bla_{OXA-58} genes, since they have been reported worldwide and may be the main cause for carbapenem resistance in *A. baumannii*.

This work has been in part previously presented (L. Poirel, S. Marqué, C. Héritier, and P. Nordmann, 15th Eur. Cong. Clin. Microbiol. Infect. Dis, abstr. P425, 2005).

MATERIALS AND METHODS

Bacterial isolates. Fourteen bla_{OXA-58}-positive *A. baumannii* isolates were included in this study (Table 1). The bla_{OXA-58} gene was searched for by using a PCR technique with primers OXA-58A and OXA-58B as described previously (16). In addition, two bla_{OXA-58}-negative *A. baumannii* isolates not listed in Table 1 were included. They had been recovered in Toulouse, France, where an outbreak due to bla_{OXA-58}-positive *A. baumannii* had occurred (9) from a patient and from a hospital environment. They have been included here, since they were of the same pulsotype as the epidemic strain (pulsotype E) (Table 1). Isolates had been identified by using the API32GN systems (bioMérieux, Marcy-l'Etoile, France). In this study, we have selected a single representant of each pulsotype as previously determined (16). In addition, strains belonging to the identical pulsotype but possessing different plasmids were also studied (Table 1). Electrocompetent *Escherichia coli* DH10B (GIBCO BRL, Life Technologies, Cergy Pontoise, France) was used as the recipient strain in transformation experiments.

Cloning of the bla_{OXA-58}-surrounding sequences and sequencing. Total DNAs of the *A. baumannii* solates were extracted as described previously (10). Those DNAs were used as templates in standard PCR conditions for detection of bla_{OXA-58}-surrounding sequences. Total DNA of *A. baumannii* MAD was digested partially by Sau3AI restriction enzyme, ligated into the BamHI site of plasmid pBK-CMV, and transformed in the *E. coli* DH10B reference strain, as described previously (22). Recombinant plasmids were selected on Trypticase soy (TS) agar plates containing amoxicillin (50 μg/ml) and kanamycin (30 μg/ml). The cloned DNA fragments of several recombinant plasmids were sequenced on both strands with an ABI 3100 sequencer (Applied Biosystems, Foster City,

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TABLE 1. Features of the OXA-58-positive *A. baumannii* isolates used in this study

Strain	Pulsotype[a]	Geographical origin	Plasmid size (kb) or location	Structure as referred to in Fig. 1	Additional IS	Source or reference
A. baumannii MAD	E	Toulouse, France	30	2	IS*Aba2*	20
A. baumannii 328	I	Lille, France	30	6	IS*Aba2*	16
A. baumannii 203	J	Mâcon, France	40	3	IS*Aba2*	16
A. baumannii 253	K	K.-Bicêtre, France	40	3	IS*Aba2*	16
A. baumannii CH22	E	Seville, Spain	27	5	IS*Aba2*	16
A. baumannii CH17	F	Seville, Spain	40	4	IS*Aba2*	16
A. baumannii CH28	G	Seville, Spain	25	7	IS*Aba2*	16
A. baumannii 18	B	Iasi, Romania	C[b]	8	None	16
A. baumannii 73	C	Iasi, Romania	15	8	None	16
A. baumannii 24	D	Iasi, Romania	20	10	None	16
A. baumannii CH29	H	Ankara, Turkey	21	9	IS*18*	16
A. baumannii 10	N	Ankara, Turkey	20	10	None	This study
A. baumannii 3601	L	Istanbul, Turkey	20	10	None	This study
A. baumannii 13015	M	Istanbul, Turkey	22	11	IS*Aba1*	This study

[a] Pulsotypes are referring to reference 16, except for the two additional lanes, L and M, obtained in this present study.
[b] C, chromosomal location.

Calif.). The entire sequence provided in this study was made of sequences of several plasmids that contained overlapping cloned fragments. The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available on the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

PCR mapping of the bla_{OXA-58}-surrounding sequences and identification of insertion sequence (IS) elements. Precise determination of the genetic structures surrounding the bla_{OXA-58} gene in *A. baumannii* MAD allowed us to design a series of primers for PCR amplifications from the other *A. baumannii* isolates tested. The primers used in this study are listed in Table 2, and their locations are indicated in Fig. 1. The primers specific for the different IS elements identified are also listed in Table 2. Identification of the IS elements located in the 5′ end of the bla_{OXA-58} gene was performed by sequencing PCR products generated with primer SM2 in combination with OXA-58B.

Genetic support. Plasmid DNA extraction was attempted by the Kieser method (15). Plasmid extracts were subsequently analyzed by electrophoresis on a 0.7% agarose gel.

PFGE. Pulsed-field gel electrophoresis (PFGE) analysis was done according to the manufacturer's instructions (Bio-Rad, Ivry-sur-Seine, France). ApaI restriction enzyme (Amersham Pharmacia Biotech, Orsay, France) was used for genotyping of the *A. baumannii* isolates, as described previously (21, 28).

Determination of the transcription initiation sites by 5′-RACE. Total RNA was isolated from different *A. baumannii* isolates by using the RNeasy Midi kit (QIAGEN, Courtaboeuf, France). 5′-rapid amplification of cDNA ends (5′-RACE) reactions were performed using 5 μg of total RNA with the 5′-RACE system kit (version 2.0; Invitrogen, Life Technologies, Cergy Pontoise, France). The first-strand synthesis was primed with specific primer OXA-58-5′ext, and amplification of the target cDNA was performed with the dC-tailed cDNAs as templates by using the newly described primers AAP and AUAP (Table 2). The 5′-RACE PCR products were cloned into pCRBluntII-Topo (Invitrogen), and each clone with a distinct insert size was sequenced. Analysis of the cloned sequence allowed the determination of the transcription initiation site and subsequently the promoter sequences. For each transcription assay, at least 10 clones were analyzed, and the entire experiment was repeated twice for all strains studied.

Nucleotide sequence accession numbers. The nucleotide sequences of the insertion sequences reported in this paper have been submitted to the IS Finder website (http://www-is.biotoul.fr). The entire sequence identified for the *A. baumannii* MAD isolate and described in that study has been deposited in the EMBL/GenBank nucleotide sequence database and has been assigned accession no. AY665723.

TABLE 2. Oligonucleotide primers used in this study

Name	Nucleotide sequence (5′–3′)	Location/use	No. as referred to in Fig. 1[a]
OXA-58A	CGATCAGAATGTTCAAGCGC	bla_{OXA-58}	1
OXA-58B	ACGATTCTCCCCTCTGCGC	bla_{OXA-58}	2
ISAba1A	GTGCTTTGCGCTCATCATGC	*tnpA* of IS*Aba1*	16
ISAba1B	CATGTAAACCAATGCTCACC	*tnpA* of IS*Aba1*	17
ISAba2A	AATCCGAGATAGAGCGGTTC	*tnpA* of IS*Aba2*	12
ISAba2B	TGACACATAACCTAGTGCAC	*tnpA* of IS*Aba2*	13
ISAba3A	CAATCAAATGTCCAACCTGC	*tnpA* of IS*Aba3*	3
ISAba3B	CGTTTACCCCAAACATAAGC	*tnpA* of IS*Aba3* but not in IS*Aba3*-like	4
ISAba3C	AGCAATATCTCGTATACCGC	*tnpA of ISAba3*-like and IS*Aba3*	11
IS18A	CACCCAACTTTCTCAAGATG	*tnpA* of IS*18*	14
IS18B	ACCAGCCATAACTTCACTCG	*tnpA* of IS*18*	15
PreTh	ATCCAACCATTCATCAAACTCTGGC	Left-hand extremity of Re27-1	6
PreEt	CTATTTGGTTTTAAGGGGC	Right-hand extremity of Re27-2	5
Re27-1	TTCGTATAACCGCCATTATG	Inside the Re27-1 sequence	7
Re27-2	AACATAATGGCTGTTATACG	Inside the Re27-2 sequence	8
MAD-Th	AACAGCAATAGCCATCAAC	*Thr-Eff* gene	9
SM2	AAGTGTCTATATTCTCACC	Between Re27-1 and IS*Aba-3*-like	10
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	Used for 5′-RACE	–
AUAP	GGCCACGCGTCGACTAGTAC	Used for 5′-RACE	–
OXA-58-5′ext	CGACTCATACTATGCTCAGC	Used for 5′-RACE	–

[a] –, primer not indicated in Fig. 1.

RESULTS

Analysis of the plasmid-borne *bla*$_{OXA-58}$-surrounding sequences in reference OXA-58-positive *A. baumannii* MAD. Previous analysis of the *bla*$_{OXA-58}$ downstream-located sequences in *A. baumannii* MAD identified an IS*Aba3* element that was present 28 bp downstream of the stop codon of *bla*$_{OXA-58}$ (Fig. 1) (20). IS*Aba3* is a member of the IS*1* family and possesses a single open reading frame (ORF) corresponding to a 145-amino-acid transposase. We had showed that a 3-bp duplicated sequence bracketed this IS*Aba3* element, thus suggesting an independent insertion of that mobile structure.

Further sequencing of the right-hand extremity of IS*Aba3* by using several recombinant plasmids obtained in the present study revealed several genes. These genes were (i) *araC1*, encoding a putative 275-amino-acid-long protein sharing 42% amino acid identity with a transcription regulator of *Pseudomonas aeruginosa* belonging to the XylS family; (ii) *lysE*, encoding a putative 201-amino-acid-long protein exhibiting significant similarity with threonine efflux proteins of the LysE family (39% and 33% amino acid identity with those of *Ralstonia solanacearum* and *P. aeruginosa*, respectively); and (iii) *araC2*, encoding a 106-amino-acid-long putative transcription regulator exhibiting 61% amino acid identity with that of *Xylella fastidiosa* (Fig. 1).

Partial analysis revealed that at least part of IS*Aba3* was present 20 bp upstream of the start codon of *bla*$_{OXA-58}$. Further sequencing of recombinant plasmids revealed that, in *A. baumannii* MAD, this IS*Aba3* element was truncated by the insertion of IS*Aba2*, a novel insertion sequence belonging to the IS*3* family (IS*51* group). IS*Aba2* is 1,306 bp long and possesses two ORFs 74% and 66% identical to those encoding the transposase of IS*51*. The perfect inverted repeats of IS*Aba2* are 25 bp in length, and its insertion had generated a 5-bp duplication inside the transposase gene of IS*Aba3* (Fig. 1). At the left-hand extremity of IS*Aba2*, the C-terminal extremity of the TnpA transposase of IS*Aba3* element was identified (Fig. 1). However, this transposase sequence was interrupted, since the corresponding 20 last C-terminal amino acids were absent, as well as the right inverted repeat of IS*Aba3*. Thus, this incomplete insertion sequence will be named IS*Aba3*-like element in this work. Precise analysis of the site where the IS*Aba3*-like sequence differed from IS*Aba3* did not give a clue for an insertion mechanism of a foreign DNA. Consequently, the *bla*$_{OXA-58}$ gene was not part of a composite transposon made of two IS*Aba3* elements in *A. baumannii* MAD.

Interestingly, 89 bp downstream of the interrupted *tnpA* gene of the IS*Aba3*-like element, a 27-bp sequence termed Re27-1 (5′-TTTCGTATAACCGCCATTATGTTAAAT-3′) was identified that was identical to (except a single C→A mismatch at bp position 12) and in the same orientation as another 27-bp sequence (named Re27-2) identified 2,479 bp downstream of *bla*$_{OXA-58}$, located between the *lysE* and *araC2* genes (Fig. 1). This observation strongly suggested that a recombination process was at the origin of the acquisition of the *bla*$_{OXA-58}$ locus. This integration of foreign DNA could be the result of a recombination between a Re27 element initially present in the target sequence and another Re27 element carried by the donor molecule. On the left-hand extremity of Re27-1, a gene (*Est*) (Fig. 1) that encodes a putative 277-amino-acid esterase sharing 61% and 66% amino acid identities with esterases of *E. coli* and *Xanthomonas campestris*, respectively, was identified.

The *bla*$_{OXA-58}$ gene was embedded in various genetic structures. Analysis of the sequences bracketing the *bla*$_{OXA-58}$ gene was evaluated by PCR mapping in different *A. baumannii* isolates. Results indicated that some variability might be observed as indicated in Table 1 and drawn in Fig. 1. A constant feature was the plasmid location of the *bla*$_{OXA-58}$ gene (except for *A. baumannii* 18) even though a large variety of different plasmid sizes was noticed (Table 1). Another identified feature was the presence of IS*Aba3* downstream of *bla*$_{OXA-58}$ (Fig. 1). However, for several isolates, sequences located on both external extremities of the Re27 repeats lacked, but the overall *bla*$_{OXA-58}$ locus including only one Re27 (either Re27-1 or Re27-2) or the two Re27s were identified. This suggested that a recombination process had likely occurred in another target site location (Fig. 1). For some other isolates, both Re27 repeats lacked and did not give any PCR products using all the primers combinations.

Interestingly, for a single isolate (strain CH29) (Fig. 1, lane 9), PCR results indicated that an intact IS*Aba3* element was present upstream of the *bla*$_{OXA-58}$ gene. It was truncated by IS*18* (see below), but its right-end extremity was present by contrast to what is observed in other strains possessing the IS*Aba3*-like element. Since DNA sequences located downstream of the *bla*$_{OXA-58}$ gene were different just after the second copy of the IS*Aba3* element (Fig. 1), it is likely that this structure may correspond in that isolate to a composite transposon made of two IS*Aba3* elements flanking the *bla*$_{OXA-58}$ gene.

Variability of the 5′-end extremity of the *bla*$_{OXA-58}$ gene. Analysis of the region flanking the 5′ end of the *bla*$_{OXA-58}$ gene indicated that the IS*Aba2* element was not always present. IS*Aba2* was identified at the same position as in *A. baumannii* MAD in six other isolates belonging to different pulsotypes and from several cities in France and Spain (Table 1; Fig. 1). In the other isolates, IS*Aba2* was not identified at that location but was also absent of their whole genome (data not shown). In addition, an IS*18* isoform was identified upstream of the *bla*$_{OXA-58}$ gene in *A. baumannii* CH29 (Fig. 1, lane 9), and the IS*Aba1* element (13, 26) was identified upstream of the *bla*$_{OXA-58}$ gene in *A. baumannii* 13015 (Fig. 1, lane 11). Insertion sequence IS*18* belongs to the IS*30* family, is 1,074 bp long, possesses a 320-amino-acid transposase and inverted repeats of 20 bp, and generates a 3-bp duplication upon transposition (24). The IS*18* isoform we have identified possesses a transposase exhibiting five amino acid substitutions compared to that originally found in IS*18*. IS*Aba1* belongs to the IS*4* family, is 1,180 bp long, possesses two ORFs corresponding to 189 and 178 amino acids, respectively, and 16-bp inverted repeats, and generates a 9-bp duplication upon transposition (13).

Excision of the *bla*$_{OXA-58}$-borne locus. Shortly after the outbreak period that had occurred in Toulouse, France, in 2003–2004 (9), hygiene measures, including a systematic search for *A. baumannii* strains in the hospital environment and systematic rectal screening of patients, was implemented.

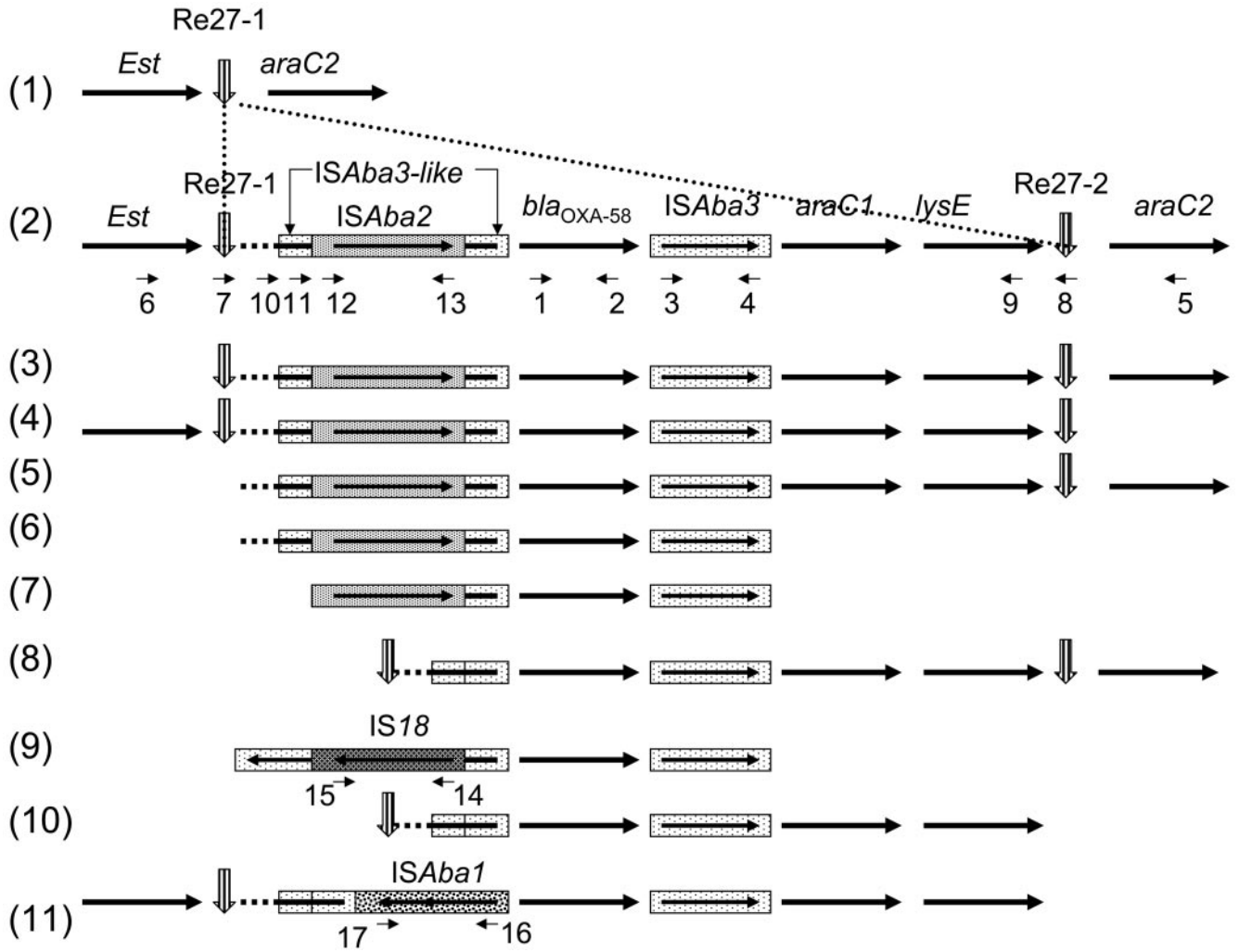

FIG. 1. Schematic map of bla_{OXA-58}-positive structures identified in *A. baumannii* isolates. (1), the structure identified in two bla_{OXA-58}-negative isolates; (2), the structure from *A. baumannii* MAD; (3) to (11), structures mapped in other isolates, as indicated in Fig. 1. Genes and their corresponding transcription orientations are indicated by horizontal arrows. The horizontal dotted line indicates the sequence separating the truncated IS*Aba3* element from the Re27-1 repeat sequence. The two different transcription regulator genes (*araC1* and *araC2*), the threonine efflux protein gene (*lysE*) and the esterase gene (*Est*) are indicated. Vertical arrows are for the two Re27 sequences. Positions of the primers are indicated as referred to Table 2. The whole genetic structure which has been acquired or lost by recombination is indicated with the dashed lines. The figure is not to scale.

As a result of this systematic screening, two carbapenem-susceptible *A. baumannii* isolates (MIC of imipenem, 0.5 μg/ml) were recovered from the urine of a patient who did not receive any antibiotic treatment (isolate 9) and from an environmental strain (alimentary peristaltic pump) (isolate 10). PFGE analysis showed that isolates 9 and 10 possessed the same genotype as opposed to the epidemic strain (data not shown). Plasmid analysis showed that they harbored the same ca. 25-kb plasmid, therefore suggesting that it could be related to the plasmid originally found in the epidemic strain (which was 30 kb [20]) without a bla_{OXA-58} locus. Thus, PCR experiments were performed in order to verify this hypothesis by using primers located on both ends of the repeated sequences (Table 2). Whereas a 5,547 bp-long PCR product was obtained with the bla_{OXA-58}-positive *A. baumannii* MAD, a 380-bp product was obtained by using DNAs of *A. baumannii* 9 and 10 as templates. Sequencing of the latter PCR product revealed that the plasmid had indeed lost the bla_{OXA-58} locus (Fig. 1, lane 1). Only the Re27-1 sequence was identified, indicating that this motif likely corresponded to the target sequence.

IS*Aba1*, *ISAba2*, and IS*Aba3*-like as enhancers for bla_{OXA-58} expression. Using the 5′-RACE PCR technique, the sites of initiation of transcription of the bla_{OXA-58} gene were mapped in four *A. baumannii* isolates, each having different sequences located upstream of bla_{OXA-58}, i.e., the presence of IS*Aba1*, of IS*Aba2*, of IS*18*, or of IS*Aba3*-like element only. In *A. baumannii* MAD that possessed an IS*Aba2* element, the +1 transcription start was located 120 bp upstream of the start codon of the bla_{OXA-58} gene, just inside the IS*Aba3*-like element (Fig. 2A). Interestingly, this promoter, named P_{Aba2}, was a hybrid, made of a −35 region located inside the IS*Aba2* element and a −10 region located inside the IS*Aba3* element (Fig. 2A). In

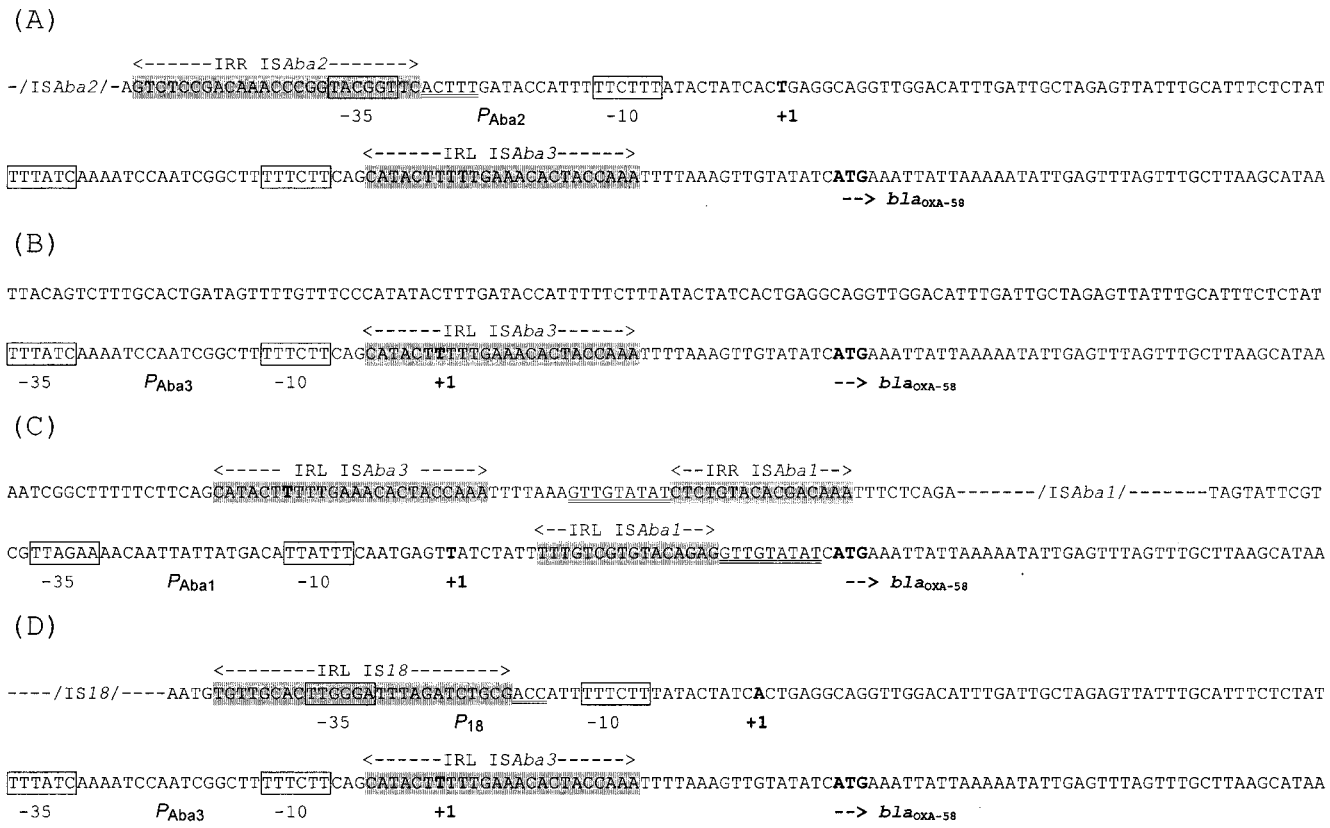

FIG. 2. Promoter structures for bla_{OXA-58} expression as determined by 5′-RACE experiment. The +1 initiation sites of transcription and the different start ATG codons are in bold. The inverted repeats of the IS elements are shaded in gray. The −35 and −10 motifs of the promoters are boxed. The target site duplications generated by IS transposition, if any, are double underlined. (A) Genetic structure identified from an IS*Aba2*-positive *A. baumannii* CLA isolate; (B) genetic structure identified from an IS*Aba1*-, IS*Aba2*-, and IS*18*-negative *A. baumannii* 10 isolate; (C) genetic structure identified from an IS*Aba1*-positive *A. baumannii* 13015 isolate; (D) genetic structure identified from an IS*18*-positive *A. baumannii* CH29 isolate.

A. baumannii 24 that possessed the IS*Aba3*-like element upstream of bla_{OXA-58}, the +1 transcription start was located 35 bp upstream of the start codon of the bla_{OXA-58} gene, just inside the inverted repeat left (IRL) of the IS*Aba3*-like element (Fig. 2B). A −35 sequence and a −10 sequence separated by 16 bp defined a P_{Aba3} promoter (Fig. 2B). In *A. baumannii* 13015 that possessed an IS*Aba1* element, the +1 transcription start was located 34 bp upstream of the start codon of the bla_{OXA-58} gene, just inside the IRL of IS*Aba1* (Fig. 2C). This P_3 promoter was made of a −35 sequence separated by 16 bp from the −10 sequence (Fig. 2C). This promoter was identical to that brought by IS*Aba1* when located upstream of bla_{ampC} gene in *A. baumannii* isolates leading to overproduction of chromosome-encoded AmpC (7, 13). In *A. baumannii* CH29 that possessed an IS*18* element, two +1 transcription starts were identified. A +1 transcription start and, consequently, the −35 and −10 sequences were identical to those identified in *A. baumannii* 24, thus indicating that the expression of bla_{OXA-58} might be in part under the control of promoter P_{Aba3}, whereas the other +1 signal was located 122 bp upstream of bla_{OXA-58}, indicating that the −35 (TTG GGA) and −10 (TTTCTT) sequences separated by 17 bp were also acting as promoter sequences (Fig. 2D). Interestingly, as observed with IS*Aba2*, these promoter sequences were hybrids, since the −35 region is part of the IRL of IS*18* whereas the −10 region was part of the IS*Aba3*-like element (Fig. 2).

DISCUSSION

Upstream and downstream of the bla_{OXA-58} gene, a previous partial analysis had identified two IS*Aba3*-like elements that could have been related to a composite transposon. We identified here an unusual structure indicating that the acquisition of the bla_{OXA-58} gene was likely not the result of a transposition process. The mechanism at the origin of such acquisition should likely correspond to homologous recombination. However, it is unlikely that this mechanism could correspond to homology-facilitated illegitimate recombination known to be at the origin of integration of foreign DNA in *Acinetobacter* spp. (8), since no duplication of the target sequence results from this process. To the best of our knowledge, this is the first report of a β-lactamase acquisition process not related to integron, transposon, or insertion sequence features but based on a recombination event. Some interesting features designated as Re elements have been recently identified in association with the bla_{VEB-1a} gene. These 135-bp sequences were identified upstream and downstream of that β-lactamase gene

in a *Pseudomonas aeruginosa* isolate and in a *Providencia stuartii* isolate (2, 3), but it is not clear whether these unusual structures may result from recombination. Similarly, DR2 direct repeats of 145 bp were identified in the close vicinity of the bla_{TLA-2} expanded-spectrum β-lactamase gene identified on an antibiotic multiresistance plasmid from a wastewater treatment plant that may indicate an unusual integration process (27).

The lack of the bla_{OXA-58} gene and its close surrounding sequences in two *A. baumannii* isolates with the sequence defined as a target site being, however, still present suggested strongly a reversibility of that recombination process. Since these structures have been recovered from antibiotic-free samples, it is tempting to speculate that the absence of antibiotic selection pressure may enhance this spontaneous deletion. Despite several attempts, we did not reproduce in vitro the loss of the bla_{OXA-58}-containing genetic structure in *A. baumannii* MAD.

The variability of the promoter sequences identified upstream of the bla_{OXA-58} gene among the analyzed isolates was noteworthy. IS*Aba2*, a novel insertion sequence, and IS*18* provided hybrid promoters enhancing bla_{OXA-58} expression. Such hybrid promoters generated upon transposition have been already observed, for instance, with IS*1* and IS*2* (14, 23) and also with IS*18*. Indeed, similar to our observations, IS*18* identified previously also from an *A. baumannii* isolate activated a silent aminoglycoside resistance gene by providing a −35 promoter sequence of an hybrid promoter (24). In the case of IS*Aba1*, this element has provided the entire promoter sequences for bla_{OXA-58} expression, as demonstrated in different *A. baumannii* isolates in which IS*Aba1* enhanced expression of the bla_{AmpC} cephalosporinase gene or the *aac(3)-IIa* acetyltransferase gene (7, 13, 26). The exact same motifs belonging to IS*Aba1* were acting as promoter sequences in these latter cases, thus confirming that IS*Aba1* is a useful tool for gene expression in *A. baumannii* and might be customized for that species, as suggested by Segal et al. (25). The frequent association between bla_{OXA-58} gene and IS elements could be the consequence of low-level expression of this oxacillinase gene in a wild-type position.

Finally, analysis of several *Acinetobacter* sp. isolates from different geographical origins revealed variable genetic structures sustaining a bla_{OXA-58} acquisition which could likely contribute similarly to the worldwide spread of this CHβL gene in *A. baumannii*.

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