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IS*CR2*, Another Vehicle for bla_{VEB} Gene Acquisition▽

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The expanded-spectrum β-lactamase (ESBL) gene bla_{VEB-1}, identified worldwide in *Enterobacteriaceae* and *Pseudomonas aeruginosa*, is associated with either class 1 integrons or repeated elements. We report here the first association of bla_{VEB-1a} with the insertion sequence IS*CR2* in six *Acinetobacter* species isolates recovered from Argentina. That genetic structure was likely at the origin of the mobilization of this ESBL gene.

Among the different Ambler class A expanded-spectrum β-lactamase (ESBL) genes, the bla_{VEB-1} gene is considered to be an emerging one since its presence has been detected in many gram-negative organisms from different parts of the world during the last decade (14, 25). It has been identified in *Enterobacteriaceae* and *Pseudomonas aeruginosa* in many countries, such as France, Spain, Algeria, Turkey, Canada, Korea, and Thailand (1, 2, 8, 23, 29). In addition, *P. aeruginosa* isolates producing the VEB-1a variant (differing from VEB-1 by just a single amino acid located in the leader peptide of the premature protein) have been found in Kuwait and India (3, 24). VEB-1-producing *Acinetobacter baumannii* strains have been identified in France, having disseminated on a nationwide scale (13, 20).

The bla_{VEB-1} gene is often part of a gene cassette located in class 1 integrons. In *A. baumannii* strain AYE from France, the corresponding class 1 integron was itself part of an 86-kb resistance island, the largest identified so far (7). However, in some cases, the bla_{VEB-1} and bla_{VEB-1a} genes have not been identified in the form of gene cassettes, being associated with peculiar genetic structures called repeated elements (Re) in *P. aeruginosa* and *Enterobacteriaceae* (3, 12, 22).

From 2002 to 2004, several *Acinetobacter* isolates producing the ESBL VEB-1a were recovered from different cities in Argentina (16). Six VEB-1a-positive isolates were retained: four *A. baumannii* isolates, one *Acinetobacter johnsonii* isolate, and one *Acinetobacter* genomospecies 10 isolate (17). Five of them were shown previously to produce the narrow-spectrum β-lactamase SCO-1 (17). All six isolates are resistant to all β-lactams except carbapenems, with the exception of *Acinetobacter* genomospecies 10, which coproduces the carbapenem-hydrolyzing oxacillinase OXA-58 and is resistant to imipenem (17). Preliminary experiments performed by PCR mapping using corresponding primers (3) showed that the bla_{VEB-1a} gene is neither part of a class 1 integron nor associated with Re elements in those isolates. Accordingly, we have examined the genetic context of the bla_{VEB-1a} gene in those six isolates in order to predict its acquisition mechanism.

First, cloning experiments were performed using DNA from a whole-cell preparation of one of the isolates, *A. johnsonii* isolate 7037, and pBK-CMV as a cloning vector as described previously (17). BamHI-restricted DNA fragments of *A. johnsonii* 7037 were ligated into a BamHI-digested plasmid and transferred into *Escherichia coli* DH10B by electroporation as described previously (18). Selection of recombinant clones was performed using Trypticase soy agar plates containing 100 μg of amoxicillin (amoxicilline) and 30 μg of kanamycin per ml. Recombinant plasmid p7037B1, expressing an ESBL phenotype, was retained for further analysis. Sequencing of the 3,630-bp insert in p7037B1 identified the bla_{VEB-1a} gene (Fig. 1). Upstream of bla_{VEB-1a}, sequences corresponding to insertion sequence IS*CR2* (formerly designated the CR2 element) were identified. In fact, the IS*CR2* right-end extremity defined as *ori*IS was located 223 bp upstream of bla_{VEB-1a}, those 223 bp including the GTTAGCG sequence usually defining the core site of the bla_{VEB-1a} gene cassette when bla_{VEB-1a} is present in class 1 integrons. Downstream of bla_{VEB-1a}, a truncated copy of IS*CR2* (ΔIS*CR2*) was present, the corresponding *tnpA* transposase gene being truncated at its 5′ extremity, resulting in a protein lacking 119 of its 497 amino acids at the N-terminal extremity. IS*CR2* belongs to the IS*CR* family, which currently includes 20 members, all presenting some similarities to IS*91*-like insertion sequences (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html). The IS*CR2* transposase shares less than 57% amino acid identity with all other IS*CR* transposases. IS*CR*s are peculiar since they do not possess inverted repeats, do not generate target site duplications upon transposition, and transpose through a process called rolling-circle transposition (28, 29).

The downstream 1,170-bp fragment separating the bla_{VEB-1a} gene from the ΔIS*CR2* element contained two open reading

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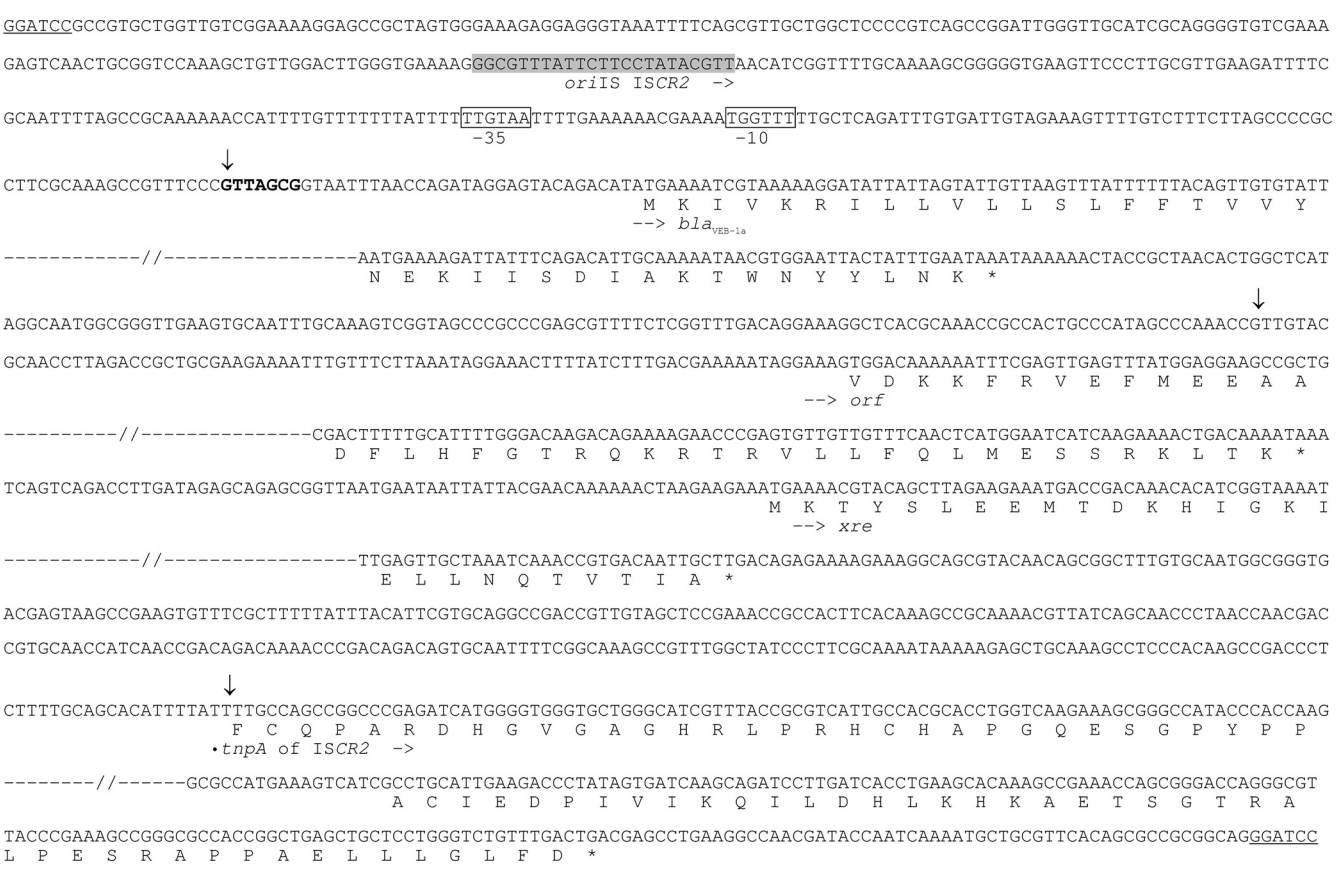

FIG. 1. Nucleotide sequence of a 3,630-bp BamHI fragment of recombinant plasmid p7037B1 containing the bla_{VEB-1a} gene. The deduced amino acid sequence is designated in single-letter code below the nucleotide sequence. The transcription orientations of the ORFs are indicated by horizontal arrows (*orf* is the unknown gene, and *xre* is the gene encoding a putative transcriptional regulator). The right-end boundary of IS*CR2*, defined as *ori*IS, is shaded. The putative promoter sequences of the bla_{VEB-1a} gene, consisting of the −35 and −10 regions, are boxed. The Δ*tnpA* truncated transposase gene of IS*CR2* is indicated. The stars indicate stop codons. The vertical arrows indicate the boundaries of the bla_{VEB-1a} gene cassette as found in class 1 integrons (with the core site sequence in bold) and also the location where ΔIS*CR2* is identified. The BamHI sites of the cloned fragment are underlined.

frames (ORFs), in addition to the entire 59-bp sequence defining the bla_{VEB-1a} gene cassette when bla_{VEB-1a} is integron borne. One ORF corresponded to a 92-amino-acid putative protein of unknown function, and the second corresponded to a 103-amino-acid protein showing homology to helix-turn-helix XRE family transcriptional regulators (Fig. 1). The latter protein shared 84% amino acid identity with a protein identified by analysis of the genome of *Psychroflexus torquis* ATCC 700755 (GenBank accession number ZP_01253739).

We subsequently investigated the five other isolates of our collection for the IS*CR2*-bla_{VEB-1a} association. PCR assays performed using bla_{VEB-1}-specific primers (27) in combination with IS*CR2*-specific primer ISCR2A (5′-AAGAATTTCTCCA ATGCGGG-3′) or ISCR2B (5′-GCGGCTCCTTTTCCGACA AC-3′) showed that in all isolates the bla_{VEB-1a} gene was bracketed by the IS*CR2* elements, as found in isolate 7037. Attempts to identify plasmids by using the Kieser technique (10) permitted visualization of several plasmids in all the bla_{VEB-1a}-positive isolates. However, subsequent Southern hybridization performed with a probe specific for bla_{VEB-1a} indicated that this ESBL gene was very likely chromosomally located in all the isolates tested since a single hybridization signal corresponding only to the chromosomal band was obtained (data not shown). This result is in accordance with the results of electrotransformation assays, performed as described previously (17), that did not allow the transfer of any β-lactam resistance marker into an *A. baumannii* recipient strain.

The finding of two copies of the IS*CR2* element (including one truncated copy) at the extremities of bla_{VEB-1a} strongly suggests that IS*CR2* was at the origin of the gene's mobilization. According to the hypotheses raised by Toleman et al. (28), it is very likely that an intact IS*CR2* copy originally mobilized bla_{VEB-1a} by a rolling-circle transposition process and that a secondary process of homologous recombination between two IS*CR2* copies led to the observed structure. To confirm the hypothesis that such recombination events may occur, we performed a PCR assay using bla_{VEB-1a}-specific outward primers VEB-inv1 (5′-CAGTTTGAGCATTTGAATAC AC-3′) and VEB-inv2 (5′-AGCGTATTTGTTGCAGAGTC C-3′). Using DNA samples from all VEB-1a-positive isolates as templates, a ca. 2,900-bp amplicon from each isolate was obtained. Sequencing identified a structure encompassing the bla_{VEB-1a} gene and the corresponding downstream sequences, together with the downstream ΔIS*CR2* truncated element. However, the sequence of the upstream, intact IS*CR2* copy was not included in that amplicon. Therefore, homologous recom-

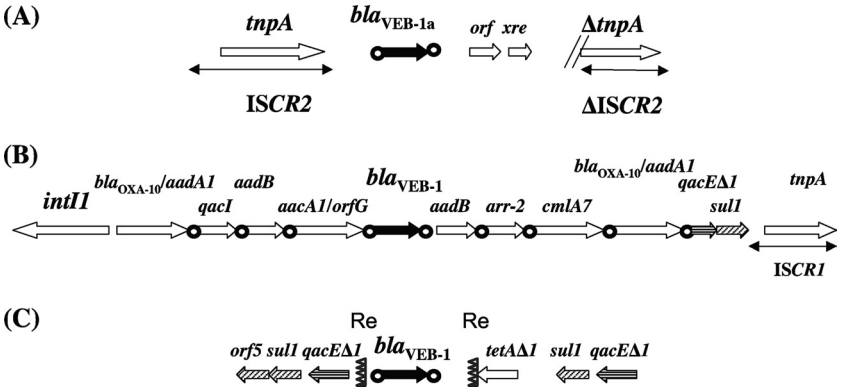

FIG. 2. Comparison of the different genetic structures in which the bla_{VEB-1}-like genes have been identified. The diagram in panel A corresponds to the present description of an IS*CR2*-associated bla_{VEB-1a} gene, that in panel B shows the bla_{VEB-1a} gene in the form of a gene cassette inside a class 1 integron as reported previously (21), and the diagram in panel C depicts the bla_{VEB-1a} gene bracketed by the Re1 and Re2 elements as described elsewhere (3, 12). The core and inverse core sites bracketing the bla_{VEB-1a} gene and defining its corresponding cassette are indicated by black circles.

bination may have been at the source of the mobilization of the bla_{VEB-1a} gene in its present genetic context. Further studies will be conducted to explore the possible role of the IS*CR2* transposase in that recombination process, with regard to the fact that the IS*CR* transposases were initially thought to be recombinases (15).

The genetic structures involved in the mobilization process for resistance genes often play an additional role in the genes' expression by providing promoter sequences, as demonstrated previously for many IS elements and class 1 integrons (11, 21) and in particular for IS*CR1* and IS*CR4* (19, 27). In the IS*CR2*-bla_{VEB-1a} configuration in evidence here, no obvious feature indicates a possible role of IS*CR2* in bla_{VEB-1a} expression. Indeed, the distance separating the *ori*IS extremity of IS*CR2* from the bla_{VEB-1a} start codon is quite long and no putative promoter is found in silico inside the IS*CR2* sequence. In contrast, a precise analysis of the sequences separating IS*CR2* from the bla_{VEB-1a} gene revealed putative promoter sequences made of a −35 motif (TTGTAA) and a −10 motif (TGGTTT) separated by the optimal 17-bp distance (Fig. 1), thus suggesting that bla_{VEB-1a} gene expression is driven by its original promoter in those *Acinetobacter* isolates.

This study demonstrates further that bla_{VEB}-like gene acquisition may be linked to a variety of genetic elements (Fig. 2). This is an uncommon observation since the widespread ESBL genes of the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} types are usually associated with very conserved genetic structures (28). In addition, this finding is the first evidence of IS*CR2*-mediated acquisition of a β-lactamase gene, while IS*CR2* has been identified previously in association with the *sul2* sulfonamide resistance gene (26), the *dfr18* trimethoprim resistance gene (4, 9), and the *floR* florfenicol resistance gene (5, 6). Interestingly, in the isolates described herein, a fragment larger than the known bla_{VEB-1} gene cassette had been mobilized by IS*CR2*, likely directly from the bacterial progenitor.

Finally, the origin of this IS*CR2* element remains be determined to better understand where and how the capture of bla_{VEB-1a} from its natural reservoir has occurred. Although the bla_{VEB-1a} gene has a GC content of 31.3%, that of IS*CR2* is 59.5%, clearly not consistent with the elements' having the same origin. IS*CR2* has been so far identified in diverse bacterial species, including *A. baumannii* (1).

Nucleotide sequence accession number. The sequences determined in this study have been assigned GenBank accession number FJ808975.

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