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ISCR2, Another Vehicle for blaVEB Gene Acquisition

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The expanded-spectrum β-lactamase (ESBL) gene blaVEB-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 blaVEB-1a, with the insertion sequence ISCR2 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 blaVEB-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-1 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).

The blaVEB-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 blaVEB-1 and blaVEB-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 A. johnsonii isolate, and one A. johnsonii genospecies 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-lactam antibiotics except carbapenems, with the exception of Acinetobacter genospecies 10, which coproduce 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 blaVEB-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 blaVEB-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 blaVEB-1a gene (Fig. 1). Upstream of blaVEB-1a, sequences corresponding to insertion sequence ISCR2 (formerly designated the CR2 element) were identified. In fact, the ISCR2 right-end extremity defined as oriIS was located 223 bp upstream of blaVEB-1a, those 223 bp including the GTTAGCG sequence usually defining the core site of the blaVEB-1a gene cassette when blaVEB-1a is present in class 1 integrons. Downstream of blaVEB-1a, a truncated copy of ISCR2 (ΔISCR2) was present, the corresponding intA transposase gene being truncated at its 5′ extremity, resulting in a protein lacking 119 of its 497 amino acids at the N-terminal extremity. ISCR2 belongs to the ISCR family, which currently includes 20 members, all presenting some similarities to IS91-like insertion sequences (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html). The ΔISCR2 transposase shares less than 57% amino acid identity with all other ISCR transposases. ISCRs 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 blaVEB-1a gene from the ΔISCR2 element contained two open reading
frames (ORFs), in addition to the entire 59-bp sequence defining the bla\textsubscript{VEB-1a} gene cassette when bla\textsubscript{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 \textit{Psychroflexus torquis} ATCC 700755 (GenBank accession number ZP_01253739).

We subsequently investigated the five other isolates of our collection for the IS\textsubscript{CR2}-bla\textsubscript{VEB-1a} association. PCR assays performed using bla\textsubscript{VEB-1a}-specific primers (27) in combination with IS\textsubscript{CR2}-specific primer IS\textsubscript{CR2A} (5'-H11032-AAGAATTTCTCCAATGCGGG-3'//H11032-GCGGCTCCTTTTCCGACAACCGGAC-3') or IS\textsubscript{CR2B} (5'-H11032-GCGGCTCCTTTTCCGACAACCGGAC-3'//H11032-CGCGGCTCCTTTTCCGACAACCGGAC-3') showed that in all isolates the bla\textsubscript{VEB-1a} gene was bracketed by the IS\textsubscript{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\textsubscript{VEB-1a}-positive isolates. However, subsequent Southern hybridization performed with a probe specific for bla\textsubscript{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 electrottransformation assays, performed as described previously (17), that did not allow the transfer of any \beta-lactam resistance marker into an \textit{A. baumannii} recipient strain.

The finding of two copies of the IS\textsubscript{CR2} element (including one truncated copy) at the extremities of bla\textsubscript{VEB-1a} strongly suggests that IS\textsubscript{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\textsubscript{CR2} copy originally mobilized bla\textsubscript{VEB-1a} by a rolling-circle transposition process and that a secondary process of homologous recombination between two IS\textsubscript{CR2} copies led to the observed structure. To confirm the hypothesis that such recombination events may occur, we performed a PCR assay using bla\textsubscript{VEB-1a}-specific outward primers VEB-inv1 (5'-H11032-CAGTTTGAGCATTTGAATACAC-3'//H11032-AGCGTATTTGTTGCAGAGTCAC-3') and VEB-inv2 (5'-H11032-AGCGTATTTGTTGCAGAGTCAC-3'//H11032-CGCGGCTCCTTTTCCGACAACCGGAC-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\textsubscript{VEB-1a} gene and the corresponding downstream sequences, together with the downstream \textit{IS\textsubscript{CR2}} truncated element. However, the sequence of the upstream, intact IS\textsubscript{CR2} copy was not included in that amplicon. Therefore, homologous recom
bination may have been at the source of the mobilization of the \textit{bla}_{\text{VEB-1a}} gene in its present genetic context. Further studies will be conducted to explore the possible role of the ISCR2 transposase in that recombination process, with regard to the fact that the ISCR 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 for resistance genes often play an additional role in the genes’ recombinases (15).

Contrast, a precise analysis of the sequences separating IS elements as described elsewhere (3, 12). The core and inverse core sites bracketing the core and inverse core sites bracketing the present description of an ISCR2-associated \textit{bla}_{\text{VEB-1a}} gene, that in panel B shows the \textit{bla}_{\text{VEB-1a}} gene in the form of a gene cassette inside a class I integron as reported previously (21), and the diagram in panel C depicts the \textit{bla}_{\text{VEB-1a}} gene bracketed by the Re1 and Re2 elements as described elsewhere (3, 12). The core and inverse core sites bracketing the \textit{bla}_{\text{VEB-1a}} gene defining its corresponding cassette are indicated by black circles.

**FIG. 2.** Comparison of the different genetic structures in which the \textit{bla}_{\text{VEB-1a}}-like genes have been identified. The diagram in panel A corresponds to the present description of an ISCR2-associated \textit{bla}_{\text{VEB-1a}} gene, that in panel B shows the \textit{bla}_{\text{VEB-1a}} gene in the form of a gene cassette inside a class I integron as reported previously (21), and the diagram in panel C depicts the \textit{bla}_{\text{VEB-1a}} gene bracketed by the Re1 and Re2 elements as described elsewhere (3, 12). The core and inverse core sites bracketing the \textit{bla}_{\text{VEB-1a}} gene and defining its corresponding cassette are indicated by black circles.

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