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Novel Genetic Structure Associated with an Extended-Spectrum β-Lactamase *bla*$_{VEB}$ Gene in a *Providencia stuartii* Clinical Isolate from Algeria

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A ceftazidime-resistant *Providencia stuartii* isolate from Algeria harbored a ca. 160-kb conjugative plasmid that contained a truncated *bla*$_{VEB-1b}$ gene flanked by three 135-bp repeated elements. This work gives further evidence of the worldwide spread of *bla*$_{VEB}$ genes that are associated with genetic structures other than class 1 integrons.

Providencia stuartii is frequently isolated from urinary tract infections of hospitalized patients (15). *P. stuartii* is naturally resistant to aminopenicillins and narrow-spectrum cephalosporins due to a chromosomally expressed Ambler class C cephalosporinase (AmpC) (1, 4). Resistance to expanded-spectrum cephalosporins in *P. stuartii* clinical isolates results mostly from overexpression of AmpC, but acquisition of extended spectrum β-lactamases (ESBL) such as TEM-, SHV-, or CTX-M-type enzymes have also been reported (3, 6, 19, 20).

The ESBL *bla*$_{VEB-1}$ gene has been reported as part of class 1 integrons in several gram-negative rods from France (16), the Near East (Kuwait [18]), and Far East (Asia [5, 7, 8, 10, 11, 13, 17]). However, a novel genetic environment of a *bla*$_{VEB-1a}$ gene has been characterized in a *Pseudomonas aeruginosa* clinical isolate from India (2). This *bla*$_{VEB-1a}$ gene was chromosomally located, and, instead of being surrounded by a class 1 integron structure, it was flanked by two 135-bp sequences, named repeated elements (Re), that were bracketed themselves by two truncated 3′-conserved sequences (3′-CS) (9) of class 1 integrons in direct-repeat orientation (2). These Re carried a strong promoter that drove the expression of the downstream *bla*$_{VEB-1a}$ gene (2).

In the present work, a peculiar genetic environment of the *bla*$_{VEB-1}$-like gene was characterized from a multidrug-resistant *P. stuartii* clinical isolate from a patient coming from Algeria.

Clinical *P. stuartii* isolate BI was recovered in 2004 from rectal and nasal swabs performed at admission at the Bicêtre Hospital of a 65-year-old patient that was directly transferred from a hospital located in Alger (Algeria). This patient suffered from septic shock with multiorgan failure. *P. stuartii* BI was identified by standard biochemical techniques (API-20E; bioMérieux, Marcy-l'Etoile, France).

A routine antibiogram, determined by disk diffusion method on Mueller-Hinton (MH) agar as previously described (17), revealed that *P. stuartii* BI was resistant to most β-lactams except imipenem and to chloramphenicol, fosfomycin, amikacin, gentamicin, kanamycin, netilmicin, tobramycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole and was susceptible to ciprofloxacin, nalidixic acid, and rifampin. A synergy image between cefepime- and clavulanate-containing disks on MH agar plates suggested the presence of an ESBL (Fig. 1A). In addition, a marked synergy image between cefepime-, aztreonam-, and cefoxitin- or imipenem-containing disks suggested the presence of a VEB-type β-lactamase, since VEB-type enzymes have an unusual synergy pattern due to very low K_m values for cefoxitin and imipenem (Fig. 1B) (13, 17).

Mating-out experiments, performed in liquid medium as previously described (17), between the *P. stuartii* BI isolate and a laboratory-obtained streptomycin- and rifampin-resistant *Escherichia coli* DH10B strain (Life Technologies, Eragny, France) yielded ticarcillin-resistant transconjugants at a frequency of ca. 2×10^{-4}. The transconjugants had identical resistance profiles, and *E. coli* DH10B(pNat-BI) was retained for further analysis. Plasmid DNA extraction as previously described (17) identified a plasmid in *P. stuartii* BI and in *E. coli* DH10B (pNat-BI) of ca. 160 kb (data not shown).

This plasmid conferred resistance to amino-, carboxy-, and ureidopenicillins; to narrow- and expanded-spectrum cephalosporins; to aztreonam; and to chloramphenicol, gentamicin, kanamycin, tetracycline, and trimethoprim-sulfamethoxazole. MICs of β-lactams for *P. stuartii* BI and *E. coli* DH10B(pNat-BI), determined and interpreted as described previously (14, 17), mirrored the results obtained with disk diffusion susceptibility testing (Table 1). The resistance to β-lactams was partially reduced by tazobactam and clavulanic acid addition.

PCR amplification experiments using internal primers specific for *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{PER-1}$, *bla*$_{VEB-1}$, and *bla*$_{GES-1}$ genes and whole-cell DNA of *P. stuartii* BI and *E. coli* DH10B(pNat-BI) as templates were as described previously (16). The *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{VEB-1}$ gene-specific primers yielded PCR products for *P. stuartii* BI and *E. coli* DH10B(pNat-BI). Sequencing of these PCR products revealed internal and partial sequences that were identical to those of the *bla*$_{TEM-2}$,

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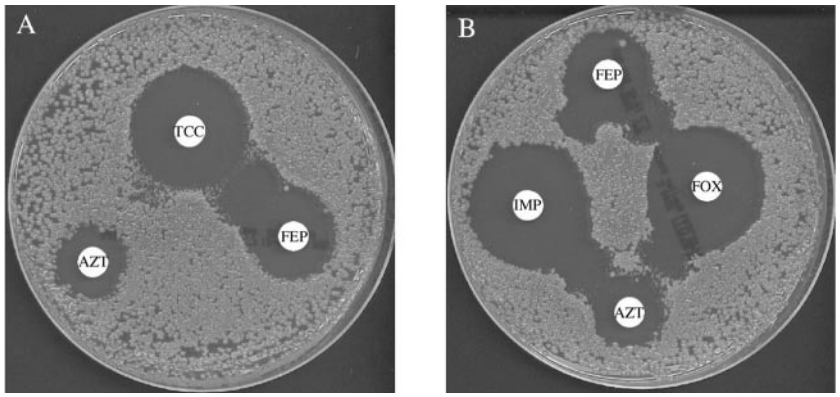

FIG. 1. Double-disk synergy test with bla_{VEB-1}-positive *P. stuartii* strain BI on MH agar plates with clavulanate (A) or imipenem and cefoxitin (B) as inhibitors. The disks tested contained ticarcillin plus clavulanate (TCC), imipenem (IMP), cefoxitin (FOX), cefepime (FEP), and aztreonam (AZT).

bla_{SHV-2}, and bla_{VEB-1} genes. This result indicated that these β-lactamase genes were located on the same conjugative plasmid, thus further supporting previous findings that bla_{VEB-1}-like genes are mostly plasmid located in *Enterobacteriaceae*, whereas they are chromosomally located in *P. aeruginosa* and *Acinetobacter baumannii* (7, 8, 16).

β-Lactamase extracts of cultures of *P. stuartii* BI and *E. coli* DH10B(pNat-BI) were prepared and subjected to analytical isoelectric focusing, as previously described (13). *P. stuartii* BI expressed four β-lactamases with pI values of 5.6, 7.4, 7.6, and 8.9, consistent with those of β-lactamases of TEM-2, VEB-1, SHV-2, and AmpC from *P. stuartii*, respectively (4, 13). The pI values of 5.6, 7.4, and 7.6 were also identified with culture extracts of *E. coli* DH10B(pNat-BI).

PCR amplification experiments failed using primers located in the bla_{VEB-1} gene and in the class 1 integron conserved sequences (5′-CS and 3′-CS [9]) or in genes known to be associated with the bla_{VEB-1} gene (e.g., *aadB*, *cmlA5*, and *arr-2*) (13), thus suggesting a different genetic environment.

TABLE 1. MICs of β-lactams for the *P. stuartii* BI clinical isolate, *E. coli* DH10B harboring natural plasmid pNat-BI and recombinant plasmid pRec-BI, and *E. coli* DH10B reference strain

β-Lactam[a]	MIC (μg/ml) for[b]:			
	P. stuartii BI	*E. coli* DH10B (pNat-BI)	*E. coli* DH10B (pRec-BI)	*E. coli* DH10B
Ticarcillin	>512	>512	>512	4
Ticarcillin-CLA	8	256	64	4
Piperacillin	512	>512	>512	2
Piperacillin-TZB	4	8	4	2
Cefoxitin	1	4	4	4
Cefotaxime	64	32	512	<0.06
Cefotaxime-CLA	0.5	<0.06	0.12	<0.06
Ceftazidime	>512	>512	>512	0.5
Ceftazidime-CLA	0.5	0.25	4	0.25
Cefuroxime	128	512	>512	4
Cefepime	32	16	>512	<0.06
Aztreonam	128	512	>512	0.12
Imipenem	1	0.12	0.12	0.12

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

[b] The natural plasmid pNat-BI and the recombinant plasmid pRec-BI contained bla_{VEB-1b}.

To determine the surrounding sequences of the bla_{VEB-1}-like gene in *P. stuartii* BI, PstI-restricted fragments of whole-cell DNA of *P. stuartii* BI were ligated into PstI-restricted vector pBBR1MCS.3 (12), followed by electroporation into *E. coli* DH10B. Recombinant clones were selected on ceftazidime (4 μg/ml)- and tetracycline (15 μg/ml)-containing plates. Recombinant plasmid pRec-BI, expressing the bla_{VEB-1}-like gene, contained a 4.3-kb PstI insert. Sequencing of the entire bla_{VEB-1}-like gene revealed 100% nucleotide identity with the bla_{VEB-1b} gene reported previously in a *P. aeruginosa* isolate

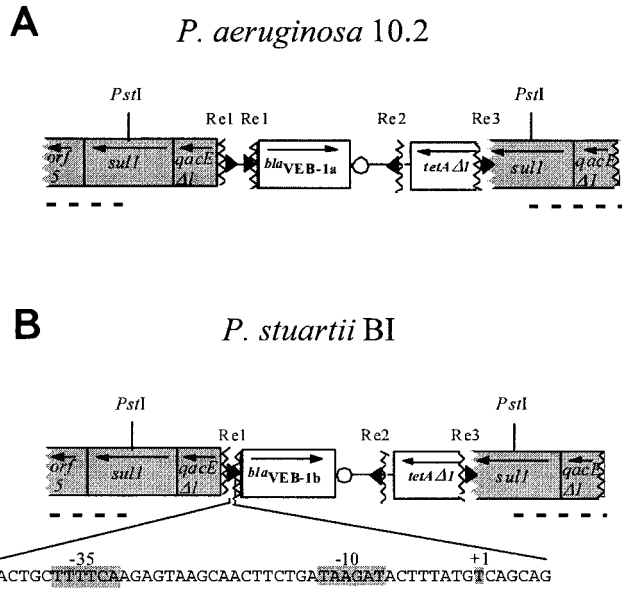

FIG. 2. Schematic representations of the genetic environment of the bla_{VEB-1a} gene in *P. aeruginosa* 10.2 (A) and of the bla_{VEB-1b} in *P. stuartii* BI (B) (2). The breakpoints and the Re in the bla_{VEB-1}-like gene environment are designated by broken lines and black triangles, respectively. The coding regions are shown as boxes, with an arrow indicating the orientation of transcription and white circles indicating the 59-bp element (59-be). Restriction sites that were used for cloning are indicated. Dashed lines in boldface indicate regions that were identified using PCR (the sequences of primers used are available upon request). The −10 and −35 promoter sequences of Re1 as well as the transcriptional initiation site (+1) are indicated by shaded boxes (2).

from Kuwait (18) that differed from the bla_{VEB-1} gene sequence (17) by two nucleotide substitutions that led to two amino acid changes (I18V and V19E) located in the leader peptide sequence.

Sequence analysis on both sides of the bla_{VEB-1b} gene revealed genetic structures identical to those found in *P. aeruginosa* 10.2 from India (2) (Fig. 2A). Indeed, the bla_{VEB-1b} gene cassette and *qacEΔ1* from the upstream 3′-CS region were truncated since they were not preceded by their typical recombination core site sequence (Fig. 2B). The breakpoints were at the same location compared to *P. aeruginosa* 10.2 (Fig. 2A). However, instead of a 336-bp DNA stretch that was flanked by two Re1 sequences in *P. aeruginosa* 10.2, a single 135-bp Re1 DNA sequence was found in *P. stuartii* BI (Fig. 2B). The region located downstream of the bla_{VEB-1b} gene in *P. stuartii* BI was identical to that of bla_{VEB-1a} in *P. aeruginosa* 10.2, including the truncated tetracycline resistance gene (*tetAΔ1*), the two 135-bp DNA stretches in opposite orientations (Re2 and Re3), and, at the outermost right hand, part of a second 3′-CS region containing a *sul1* gene (Fig. 2B). These Re (Re1, Re2, and Re3) were identical to those described in *P. aeruginosa* 10.2 (2).

This is the first report of a bla_{VEB}-like gene from Africa, further illustrating the worldwide spread of VEB-type β-lactamases. Moreover, it is the first report of that ESBL in *P. stuartii* and the second report of bla_{VEB}-like genes being flanked by Re. The function of these Re in gene mobilization will be further investigated. Finally, this report underlines that cassette-associated β-lactamase genes may be found on genetic structures that are different from typical class 1 integrons and that may contribute to their spread. The bla_{VEB-1} gene cassette is the first example of an ESBL gene associated with both integrons and Re sequences.

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