ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1693–1697 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.01313-08

A bla_{VEB-1} Variant, bla_{VEB-6}, Associated with Repeated Elements in a Complex Genetic Structure▽

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Received 1 October 2008/Returned for modification 23 November 2008/Accepted 3 January 2009

bla_{VEB-6} was found on the *Proteus mirabilis* chromosome in a context similar to those of bla_{VEB-1a} and bla_{VEB-1b}, in a truncated gene cassette flanked by 135-bp elements and duplications of the 3′-conserved segment of class 1 integrons. A linked *aacA4-aadB-dfrA1-orfC* cassette array includes components of Tn*1331*, illustrating the complex mosaicism of multiresistance regions.

Resistance to extended-spectrum β-lactams is an important clinical problem. In addition to the large TEM, SHV, and CTX families, several minor extended-spectrum β-lactamases have been identified (22), including VEB. All VEB enzymes identified to date (Table 1) are minor variants of VEB-1, which confers a high level of resistance to ceftazidime, cefotaxime, and aztreonam (26). bla_{VEB} genes have been identified in a variety of species of *Enterobacteriaceae* and in nonfermenting bacilli from Asia, Europe, the Middle East, Africa, and North and South America (22) (Table 2) on both plasmids and the chromosome.

bla_{VEB-1} was first described in a gene cassette in a class 1 integron (26), and most other examples of bla_{VEB} genes where enough sequence data are available are also found in cassette arrays in class 1 integrons. These arrays are mostly related (Table 2), containing different combinations from a limited set of cassettes in different configurations, suggesting rearrangements mediated by both homologous and IntI-catalyzed recombination. The bla_{VEB} cassette is followed by the *aadB* cassette in almost all of these arrays, and the 5′-conserved segment (5′-CS) is interrupted in several cases by IS*1999*, with or without IS*2000* (21).

bla_{VEB-1a} and bla_{VEB-1b} cassettes missing the first 7 bp have been found outside arrays in regions containing one or more 135-bp repeat elements (Re1, Re2, Re3) (Fig. 1A). In both *Pseudomonas aeruginosa* 10.2 (GenBank accession no. AY444815) (2) and *Providencia stuartii* B1 (3), Re1 and Re2 (71% identical) flank a region containing a truncated bla_{VEB} cassette, and Re2 and Re3 (98% identical to Re1) flank a region apparently largely derived from Tn*1721* (GenBank accession no. X61367) that includes the *tetA*(A) gene truncated at both ends. This whole structure is flanked by duplications of the 3′-CS of class 1 integrons (3′-CS-a and 3′-CS-b) (Fig. 1A), and strain 10.2 has an extra copy of Re1. In *P. aeruginosa* TL-1 (GenBank accession no. DQ315788) (17), only Re1 is present, and bla_{VEB-1a} is linked to IS*CR1*, the *aphA6* gene, and the *aadA6-orfD* cassette array. A strong promoter in Re1 was found to drive expression of bla_{VEB-1a} (2), and the Re and/or the 3′-CS duplications in the first two structures may provide an alternative means for the movement of bla_{VEB} genes by homologous recombination.

We have previously reported a novel bla_{VEB} variant, designated bla_{VEB-6}, in a *Proteus mirabilis* clinical isolate (JIE273) that is resistant to cefotaxime and ceftazidime (37). VEB-6 is essentially identical to VEB-4 (1), the A52G variation, predicting only a conservative amino acid substitution (Ile18Val) in the leader peptide.

Repeated attempts to transfer bla_{VEB-6} from JIE273 to DH5αRf (a rifampin-resistant variant of *Escherichia coli* DH5α) by conjugation (38) and by electroporation with alkaline lysis preparations were unsuccessful. Whole-cell DNA from JIE273 was digested with I-CeuI (New England Biolabs, Ipswich, MA) (15), electrophoresed (for 36 h at a switch time of 5 to 60 s) (CHEF-DR II; Bio-Rad, Hercules, CA), and transferred to a Hybond-N+ membrane (GE Healthcare, Piscataway, NJ). Digoxigenin-labeled (DIG DNA labeling and detection kit; Roche, Penzberg, Germany) bla_{VEB} and 16S rRNA PCR amplicons (see Table 3) hybridized to the same (ca. 291-kb) I-CeuI fragment (data not shown), suggesting that bla_{VEB-6} is located on the chromosome of JIE273.

PCR mapping (Table 3; Fig. 1A), including by long-range

TABLE 1. Amino acid differences among VEB variants

VEB variant	Amino acid position for indicated type of peptide[a]					GenBank accession no.[b]	Reference or source
	Leader		Mature				
	18	19	25	56	104		
1	Ile	Val	Thr	Leu	Thr	**AF010416**	26
1a	**Val**	Val	Thr	Leu	Thr	AF324833	28
1b	**Val**	**Glu**	Thr	Leu	Thr	AF324834	28
2	Ile	Val	**Ala**	Leu	Thr	**AY027870**	8
3	**Val**	Val	Thr	**Phe**	Thr	**AY536519**	11
4	Ile	Val	**Ala**	Leu	**Met**	**EF136375**	1
5	**Val**	**Glu**	Thr	Leu	**Met**	**EF420108**	
6	**Val**	Val	**Ala**	Leu	**Met**	**EU259884**	This work

[a] Positions were numbered consecutively from the start codon. Amino acids differing from those in VEB-1 are in boldface.
[b] Accession numbers in boldface type are listed at http://www.lahey.org/Studies.

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▽ Published ahead of print on 12 January 2009.

TABLE 2. Distribution and contexts of bla_{VEB} genes

bla_{VEB} variant	Species[a]	Country	GenBank accession no.	Genetic context[d]	Location[f]	Reference(s) or source[g]
1	*E. coli*	Vietnam	AF205943[b]	*qacI-aadB-aacA1/orfG-veb-aadB-arr2-cmlA-oxa10/aadA1*	P	20 and 26
	K. pneumoniae	Vietnam	Not available	Not available	P	26
	P. mirabilis	Vietnam	AF220758	*qacI-aadB-aacA1/orfG-veb-aadB-?-oxa10-?*	P	18
	A. xylosoxidans	France	DQ393569	*dfrA14-veb-aadB*	P	23
	A. baumannii	France	CT025832[c]	*veb-aadB-arr2-cmlA-oxa10/aadA1*	C	7
	A. baumannii	Belgium	Not available	*veb-aadB-arr2-cmlA-oxa10/aadA1*	C	19
	E. coli	Canada	Not available	*oxa10/aadA1-qacI-aadB-aacA1/orfG-veb-aadB-arr2-cmlA-oxa10/aadA1*	P	27
	E. coli	Canada	Not available	Not available	NA	25
	P. aeruginosa	China	AY536743	*veb-aadB-oxa10/aadA1*	NA	
	P. aeruginosa	China	DQ333895	Incomplete *veb* gene sequence	NA	
	P. aeruginosa	Thailand	AF133699	*veb-aadB*	C	21
	P. aeruginosa	Thailand	AF078527	*veb-aadB-arr2-cmlA-?*	NA	34
	P. aeruginosa	Thailand	Not available	*veb-aadB-arr2-cmlA-oxa10/aadA1*	C	8
	E. coli	Thailand	Not available	Various cassette arrays were partially characterized by PCR	P	9
	K. pneumoniae	Thailand				
	E. cloacae	Thailand				
	E. sakazakii	Thailand				
	P. aeruginosa	Bulgaria	Not available	Not available	NA	31
1a	*A. baumannii*	Argentina	Not available	PCR suggests that *veb* is not located in a cassette array or typical Re/3′-CS structure	C	24
	P. aeruginosa	Bangladesh	DQ315788	Re	C	17
	P. aeruginosa	India	AY444815	Re	C	2
	P. aeruginosa	Kuwait	AF324833	PCR suggests that *veb* is the last cassette in an array	P	28
	P. aeruginosa	United Kingdom	Not available	Not available	NA	36
1b	*P. aeruginosa*	Kuwait	AF324834	PCR suggests that *veb* is the last cassette in an array	C	28
	C. freundii	Kuwait	Not available	Not available	NA	4
	P. mirabilis	Korea	Not available	Cassette array; *aadB* also present	C	13
	P. stuartii	Algeria	Not available	Re	P	3
2	*P. aeruginosa*	Thailand	AY027870	*veb-aadB-arr2-cmlA-oxa10/aadA1*	C	8
	P. aeruginosa	Thailand	Not available	*?-veb-aadB-arr2-cmlA-oxa10/aadA1*	C	8
3	*E. cloacae*	China	AY536519	No array[e]	C	11
	P. aeruginosa	China	Not available	Not available	NA	12
	A. baumannii	Taiwan	Not available	Not in cassette array?		10
4	*P. mirabilis*	Spain	EF136375	*veb* gene sequence only	C	1
5	*E. coli*	United States	EF420108	Sequence includes start of *veb* cassette, suggesting a cassette array	NA	
6	*P. mirabilis*	Australia	EU259884	Re	C	This work

[a] *K. pneumoniae*, *Klebsiella pneumoniae*; *A. baumannii*, *Acinetobacter baumannii*; *A. xylosoxidans*, *Achromobacter xylosoxidans*; *C. freundii*, *Citrobacter freundii*; *E. cloacae*, *Enterobacter cloacae; E. sakazakii*, *Enterobacter sakazakii.*

[b] GenBank accession no. AF205943 contains a longer sequence from the same strain as in AF010416 (Table 1).

[c] Partial genome sequence of *A. baumannii* AYE. The same sequence is also available from the whole-genome sequence of AYE under GenBank accession no. CU459141.

[d] Contexts available from the GenBank entry or a published paper are listed. ?, the remainder of the array was not determined; Re, a truncated *veb* cassette is found in a structure with repeat elements (Fig. 1A).

[e] The sequence includes the first 7 bp of the bla_{VEB-3} cassette, but the cassette is interrupted after 937 of 1,070 bp and the *attC* site is missing. A short region (34 bp) matching the right end of IS*CR1* immediately follows, and then the left end of IS*6100*, probably explaining why an amplicon carrying bla_{VEB-3} was not obtained using primers in the 5′-CS and 3′-CS (11).

[f] P, bla_{VEB} gene found on a plasmid obtained by conjugation or electroporation; C, bla_{VEB} gene in a whole-genome sequence or found on the chromosome by hybridization (in boldface type) or presumed to be on the chromosome from the absence of transconjugants/transformants and/or plasmid DNA; NA, information not available.

[g] Reference 8 includes several other cassette arrays, but the bla_{VEB} variant is not specified.

PCR (Expand Long Template PCR system; Roche, Mannheim, Germany), and sequencing revealed a genetic context most similar to bla_{VEB-1b} in *P. stuartii* B1. The *aacA4-aadB-dfrA1-orfC* array found adjacent to the 3′-CS-a was preceded by an unusual structure: the end of the 5′-CS is followed by the first 111 to 114 bp of the 3′-CS (Fig. 1) and then by 517 to 520 bp from the transposon Tn*3* (three A residues could be derived from either the 3′-CS or Tn*3*). The Tn*3* region includes the end

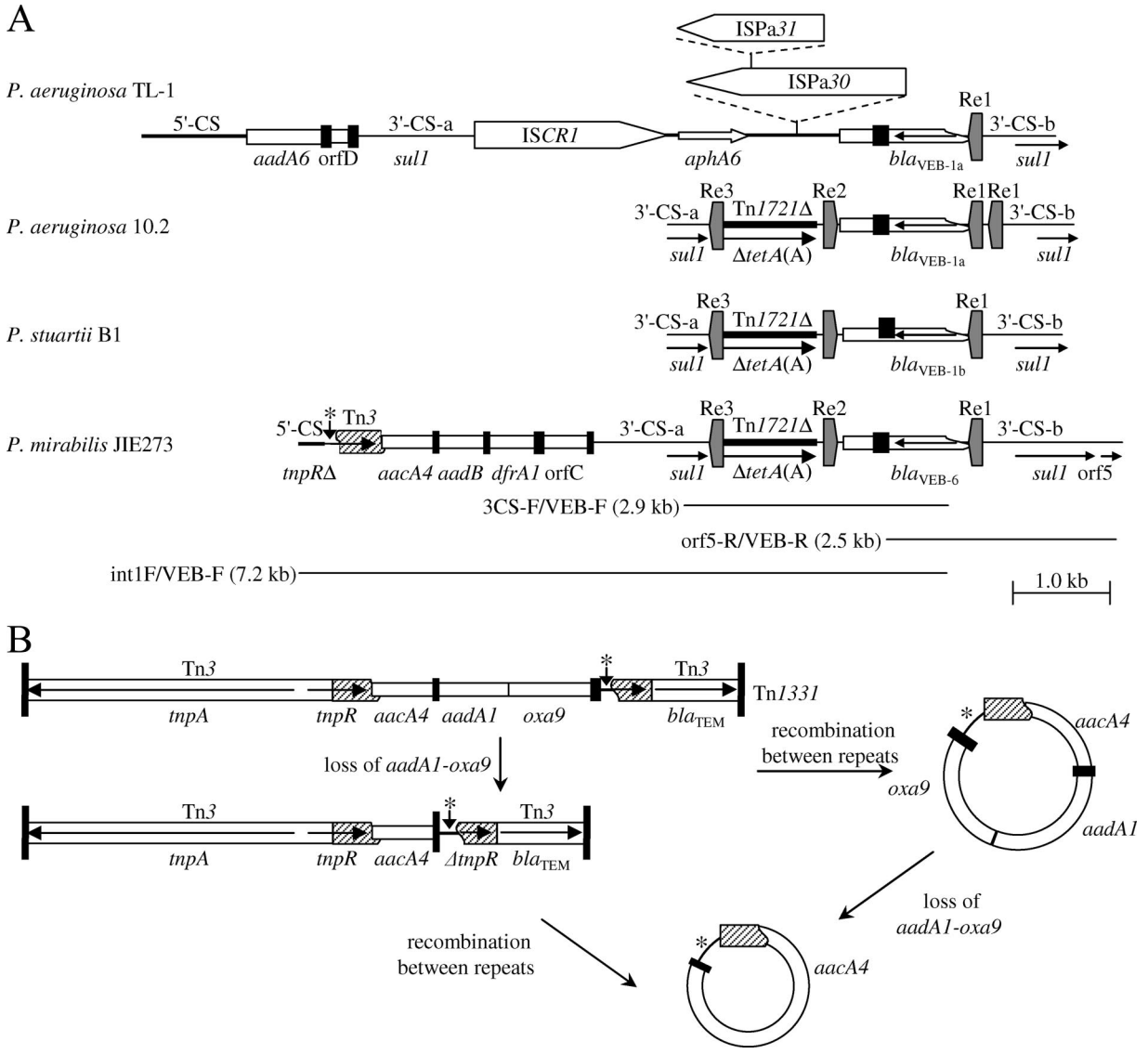

FIG. 1. (A) Genetic structures containing bla_{VEB} genes and repeat elements. The species and isolate names are shown on the left. Insertion sequences are shown as pointed boxes labeled with the insertion sequence number, and Re1, Re2, and Re3 and their orientations are indicated. Gene cassettes are shown as open boxes, with small, filled boxes representing their *attC* sites. The extents of other resistance genes are shown, and the 5′-CS and 3′-CS of class 1 integrons and transposon fragments are labeled. Selected PCR products used for mapping are indicated by lines labeled with the primers used to amplify them. The region marked with an asterisk represents the first 1 to 111/114 bp of the 3′-CS. The structures for *P. aeruginosa* strains TL-1 and 10. 2 were drawn from sequences available under GenBank accession no. DQ315788 and AY444815, respectively, and *P. stuartii* B1 was drawn from the work of Aubert et al. (3). No additional information about flanking regions is available for 10.2 or B1. (B) Structure of Tn*1331* and model for creation of a circular cassette containing *aacA4* fused to the start of the bla_{TEM-1a} gene. Circular molecules are not shown to scale. Tn*1331* was drawn from the sequence available under GenBank accession no. AF479774.

of *tnpR* and the start of bla_{TEM-1a} and is followed by a short sequence (AAACAAAG) derived from the *attI1* sites of class 1 integrons (Fig. 1A).

These components are all found in a different configuration in Tn*1331* (32) and related transposons. Tn*1331* (Fig. 1B) is a derivative of Tn*3*, with a 517- to 520-bp duplication at the end of the Tn*3* *tnpR* gene and at the start of the bla_{TEM-1a} gene flanking the *aacA4–aadA1–*bla_{OXA-9} cassette array and the first 111 to 114 bp of the 3′-CS (Fig. 1B). The sequence AAAC AAAG derived from the *attI1* site links the first 19 bp of bla_{TEM} to the start of the *aacA4* cassette, providing both a promoter and a ribosome binding site for *aacA4* expression and giving an AacA4 protein with a 17-amino-acid N-terminal extension (33). The *aadA1* and bla_{OXA-9} cassettes are separated by part of the *attI1* site rather than a complete *aadA1* *attC* site.

The configuration seen in pJIE273 may be derived from Tn*1331* or a related transposon, but in Tn*1331*, the 111- to 114-bp 3′-CS region follows the cassette array, while in JIE273, it precedes the cassette array. This region would be brought to the start of the *aacA4* cassette in a circular molecule created by homologous recombination between the duplicated regions of

TABLE 3. Primers used in this work

Primer	Sequence (5′–3′)	Target	Reference or source
VEB-F	CGACTTCCATTTCCCGATGC	bla_{VEB}	19
VEB-R	GGACTCTGCAACAAATACGC		
hep58	TCATGGCTTGTTATGACTGT	5′-CS	35
hep59	GTAGGGCTTATTATGCACGC	3′-CS	
Int1F	CAGTGGACATAAGCCTGTTC	*intI1*	14
3CS-F	CTATTGGTCTCGGTGTCG	3′-CS	6
3CS-R	ATCGTTCAGGTAGCCCAC	3′-CS	
orf5-R	ACGAAGGTCTCCGCGAA TGTC	3′-CS	This work
A	AGAGTTTGATCHTGGYT YAGA	16S rRNA	16
B	ACGGYTACCTTGTTACG ACTT		

Tn*1331* (Fig. 1B). Tn*1331.2* has a duplication of the *tnpR–aacA4–aadA1–*bla_{OXA-9} region, and experimentally observed conversion to Tn*1331* (30) could also occur by loss of this circular molecule. Loss of *aadA1–*bla_{OXA-9} from the circular molecule would effectively give an extended *aacA4* cassette and could occur either before or after circularization. It has recently been demonstrated that *aadA1–*bla_{OXA-9} can be readily excised from Tn*1331* (29), and circles containing more than one cassette were found to separate into individual cassettes before insertion into class 1 integrons (5). The extended *aacA4* cassette could then be inserted in front of an *aadB-dfrA1-orfC* array by IntI1-mediated recombination to give the configuration seen in pJIE273.

The only example of an *aadB-dfrA1-orfC* array currently in GenBank has three nucleotide differences from the corresponding part of the *aacA4-aadB-dfrA1-orfC* array in JIE273 and is found in *P. aeruginosa* TL-1 (GenBank accession no. DQ315789, where *orfC* is referred to as *orfX*), although it is not linked to bla_{VEB-1a} (17). It is also interesting that in *P. aeruginosa* TL-1, the *aadA6-orfD* array is linked to bla_{VEB-1a}, while a related array, *aacA8–*bla_{OXA-2a}*–aacA7–aadA6–orfD*, was found in *P. aeruginosa* 10.2 but could not be linked to bla_{VEB-1a} by PCR. These similarities suggest multiple recombination events. The association of bla_{VEB-6} with a class 1 integron and components of both Tn*1721* and Tn*1331* illustrates recombinations and rearrangements of a limited set of different components which, in addition to the actions of individual mobile elements, all contribute to the mosaicism characteristic of many complex multiresistance regions.

The patient carrying JIE273 was born in and had recently traveled to India, and bla_{VEB} was not detected in other enterobacterial isolates from Sydney (38). *P. aeruginosa* strains 10.2 and TL-1 were also isolated from patients in India/Bangladesh (2, 17), and the similarities between the genetic contexts of bla_{VEB-6} in JIE273 and bla_{VEB-1a} in these strains suggest that this structure, if not this bacterial strain, was acquired in that region.

Nucleotide sequence accession number. The nucleotide sequence surrounding bla_{VEB-6} has been added to GenBank accession no. EU259884.

We are grateful to Lee Thomas for collecting isolate JIE273. We also thank Laurent Poirel and Thierry Naas (Hôpital de Bicêtre, Paris, France) for helpful discussions.

Z.Z. is supported by an Endeavor International Postgraduate Student Scholarship from the Australian Government Department of Education, Science and Training. S.R.P. is supported by grants from the National Health and Medical Research Council of Australia.

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