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Characterization of a Transmissible Plasmid Encoding VEB-1 and VIM-1 in *Proteus mirabilis*

VEB-1-producing *Klebsiella pneumoniae* strains have recently been identified in Greece (5). We report here on a VEB-1-positive and multidrug-resistant *Proteus mirabilis* isolate (PM91) recovered from a patient treated in a Greek hospital in 2010. PM91 was resistant to penicillins, penicillin-clavulanate combinations, ceftazidime, cefotaxime, cefoxitin, and imipenem but susceptible to piperacillin-tazobactam, cefepime, meropenem, and aztreonam. The isolate was also resistant to various non-β-lactam antibiotics (Table 1). PCR and sequencing showed that PM91 contained bla_{VEB-1} along with bla_{VIM-1}, bla_{OXA-10}, and bla_{TEM-1}. Production of the respective enzymes was confirmed by isoelectric focusing (IEF).

Most of the resistance traits of PM91 were readily transferred to a rifampin-resistant *Escherichia coli* strain by conjugation in broth cultures containing rifampin (400 μg/ml) and ampicillin (50 μg/ml) (Table 1). Resistance phenotypes, PCR, IEF, and plasmid content analysis showed a single transconjugant species (Trc-PM91) with a 120-kb plasmid (pPM91) encoding all four β-lactamases of the donor strain. PCR-based replicon typing and sequencing classified pPM91 as an A/C_2 plasmid (1). Notably, in the previously described *K. pneumoniae* 2741 (5), bla_{VEB-1}, as determined here, was also carried by a self-transmissible A/C_2 plasmid (pKP2741) conferring to *E. coli* recipients a multiresistant phenotype (Table 1). We therefore studied the genetic environment of the bla_{VEB-1} genes in both pPM91 and pKP2741 plasmids to gain information on their evolution, presuming that they were probably related. Additionally, the bla_{VIM-1}-carrying segment in pPM91 was determined. Characterization of the regions flanking *bla* genes was carried out by PCR mapping and sequencing.

bla_{VEB-1} in pKP2741 occurred as the first cassette of an integron that also included an array of *aadB*, *arr2*, *cmlA5*, bla_{OXA-10}, and *aadA1* gene cassettes. As is common in VEB-1-encoding integrons, an IS*1999* was located upstream of bla_{VEB-1} (2, 3, 6). The 3′ conserved segment (3′CS) of the integron was truncated 528 bp after the start codon of *sulI*. Moreover, downstream from Δ*sulI*, there was a *cmlA9*-*tetR*(G)-*tetA*(G) sequence associated with IS*CR6*. This structure appeared as a part of the bla_{VEB-1}-containing Tn*6061* from *Pseudomonas aeruginosa* (2) (Fig. 1). However, downstream from IS*1999*, instead of *intI1*, a ΔIS*26* was identified. Upstream of the latter element, there was a region comprising a restriction endonuclease fragment, an intact IS*26*, and a Δorf6-IS*6100* sequence (Fig. 1). Notably, the latter segment has been identified in several IncN plasmids, including the VIM-1-encoding pNL194 (4).

In pPM91, bla_{VIM-1}, along with the *aacA7*, *dfrA1*, and *aadA1* cassettes, comprised the variable region of an integron similar to In-e541 from pNL194 harbored by a *K. pneumoniae* clinical strain isolated in Greece (4). The 5′CS was disrupted by an IS*26*-Δorf6-IS*6100* sequence as in pNL194. Unlike In-e541, however, the integron did not include a 3′CS due to insertion at the *attC* site of *aadA1* of an IS*1* and a ΔIS*26* lacking 57 bp of the left inverted repeat (IRL) (Fig. 1). The bla_{VEB-1} gene in pPM91 was carried by an integron similar to that found in pKP2741. However, the sequences downstream from IS*1999* and *aadA1* in pPM91 resembled those found in the VIM-1-encoding region described above. The sole difference was the presence of a truncated IS*26* downstream from IS*1999* in the VEB-1-encoding region instead of an intact IS*26* found in the respective bla_{VIM-1}-containing structure. The fact that both IS*26* had the same boundary with the 3′CS (Δorf6-IS*6100*) may indicate that this difference was due to recombination between the two regions (Fig. 1).

The similarities of pPM91 and pKP2741 indicated a common ancestry. The presence of a ΔIS*26*-IS*1999* sequence in both plasmids further supports this notion. The differences in the left-hand extremities in the bla_{VEB-1}-containing regions could be explained by recombination events between IS*26* elements that may lead to deletion of the intervening sequence in pPM91. The carriage of at least three pNL194-derived parts (including a VIM-1-encoding region) by pPM91 suggests that the latter plasmid might have evolved through cointegration and resolution event(s) between a VEB A/C_2 replicon and the most common VIM IncN plasmid variant in this setting (1, 4). During this process, deletions and recombinations may have resulted in the observed structures.

Nucleotide sequence accession numbers. Sequences of bla_{VEB-1}-containing regions from *P. mirabilis* PM91 and *K. pneu-*

Published ahead of print 30 April 2012
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doi:10.1128/AAC.00470-12

TABLE 1 Antibiotic susceptibilities of strains harboring VEB-1-encoding plasmids

Strain	Etest MIC (μg/ml) for[a]:											Other resistance markers[b]
	Amp	Amc	Pip	Ptz	Fox	Ctx	Caz	Fep	Atm	Ipm	Mem	
P. mirabilis PM91	>256	32	64	4	16	8	32	2	2	16	1	Gen, Amk, Tob, Net, Sxt, Tet, Cip
E. coli Trc-PM91	>256	64	256	16	64	32	256	2	4	8	0.5	Gen, Amk, Tob, Net, Sxt, Tet
K. pneumoniae KP2741	NT[c]	24	>256	4	4	1.5	256	1	24	0.25	<0.12	Gen, Amk, Tob, Net, Sxt, Tet
E. coli Trc-KP2741	>256	24	>256	3	3	3	256	1.5	24	0.25	<0.12	Gen, Amk, Tob, Net, Tet
E. coli (recipient)	4	2	2	0.25	2	<0.12	0.25	<0.12	<0.12	<0.12	<0.12	

[a] Amp, ampicillin; Amc, amoxicillin-clavulanate (2:1); Pip, piperacillin; Ptz, piperacillin-tazobactam (inhibitor fixed at 4 mg/ml); Fox, cefoxitin; Ctx, cefotaxime; Caz, ceftazidime; Fep, cefepime; Atm, aztreonam; Ipm, Imipenem; Mem, meropenem.
[b] Gen, gentamicin; Amk, amikacin; Tob, tobramycin; Net, netlimicin; Sxt, trimethoprim-sulfamethoxazole; Tet, tetracycline; Cip; ciprofloxacin.
[c] NT, not tested.

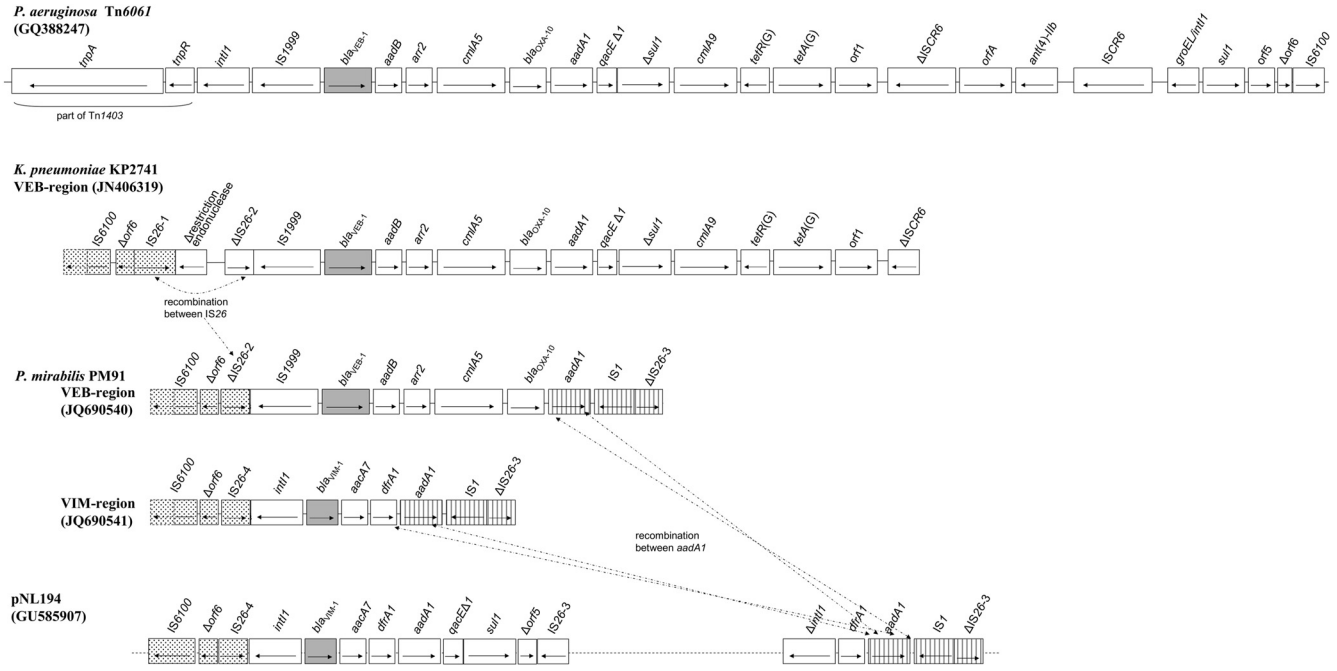

FIG 1 Schematic representation of the VEB-1- and VIM-1-encoding regions from *K. pneumoniae* KP2741 and *P. mirabilis* PM91. The structure of the Tn*6061* from *P. aeruginosa*, as well as parts of pNL194, has been included for comparison. Potential recombination sites are indicated by dashed lines.

moniae KP2741 have been assigned GenBank accession numbers JQ690540 and JN406319, respectively. The bla_{VIM-1}-carrying sequence from *P. mirabilis* PM91 has been assigned GenBank accession number JQ690541.

ACKNOWLEDGMENTS

This study was not funded by external sources.

The authors report no transparency declarations.

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