Integron-Borne Transmission of VEB-1 Extended-Spectrum β -Lactamase in *Pseudomonas aeruginosa* in a Tertiary Care Hospital in India

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A total 14 clinical isolates of *Pseudomonas aeruginosa* that produced VEB-1 and were susceptible only to polymyxin B were recovered from hospitalized patients. VEB-1 was located within variable regions of the class 1 integron, flanked by resistant genes, and was horizontally transferable as well as carried within the IncP-type plasmid. We conclude that the IncP-type plasmid is responsible for the horizontal transmission of VEB-1-mediated expanded-spectrum cephalosporin resistance in this medical center.

VEB-type beta-lactamases are a rare type of enzymes responsible for conferring expanded-spectrum cephalosporin resistance, complicating therapeutic options (1). They are usually integron borne and horizontally transmitted at the intra- and interspecific levels (1). The present study investigates the genetic context of VEB-1 extended-spectrum beta-lactamases (ESBL) in nosocomial isolates of *Pseudomonas aeruginosa*.

A total of 136 isolates that were resistant to at least one of the expanded-spectrum cephalosporins (cefotaxime, ceftazidime, or ceftriaxone) by the agar dilution method and nonduplicates were selected for the study. The isolates were collected for a period of 1 year from January 2012 to December 2012. PCR assays were performed for detection of ESBL genes using *bla*_{GES}, *bla*_{OXA-10}, *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{VEB} primers (2). Class 1 and class 2 integrons were characterized by integrase gene PCRs (3). Gene cassettes of the *bla*_{VEB} gene were amplified using 5'-CS and 3'-CS primers (3). Products were cloned on the pGEM-T vector (Promega, Madison, WI, USA) and sequenced. Plasmids from the parent strains were extracted using a GeneJET plasmid miniprep kit (Thermo Scientific, Lithuania). The transformation experiments were performed using *Escherichia coli* strain JM107 as the recipi-

ent. Transformants were selected on Luria-Bertani agar (Hi-Media, Mumbai, India) plates containing 1 μ g/ml of cefotaxime, and all the transformants were subjected to PCR assays for the presence of *bla*_{VEB}. Plasmid incompatibility typing of all the transformants was done, targeting 18 different replicon types (4), and amplicons were sequenced. Restriction mapping of plasmids was performed using EcoRI, HindIII, and XbaI (Promega, Madison, WI, USA), and digested products were separated by pulse-field gel electrophoresis (CHEF DR III system, Bio-Rad, USA). Antimicrobial susceptibility (Kirby-Bauer disc diffusion) and MICs were determined, using the agar dilution method of the parent strains and transformants with various antibiotics including beta-lactams

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TABLE 1 Clinical and molecular history of *bla*_{VEB-1}

Serial no.	Sample identification	Age of patient (yr)	Sex of patient	Source (ward/clinic)	Type of clinical specimen	Integron arrangement/genetic context	Location (Inc type)	Resistance profile ^a
1	153	20	Female	Surgery	Pus	2	P	CPD, AMK, CIP, GEN, CRO, ATM, FOX, FEP
2	182	23	Female	Surgery	Pus	1	P	CPD, AMK, CIP, FEP, CRO, ATM, FOX
3	264	70	Female	Surgery	Pus	2	P	CPD, AMK, CIP, GEN, CRO, ATM, FOX, FEP
4	303	65	Female	Orthopedic	Drain tip	4	P	CPD, AMK, GEN, CRO, ATM, FOX
5	306	55	Male	Surgery	Pus	3	P	CPD, CIP, CRO, ATM, FOX, FEP
6	310	35	Female	Surgery	Pus	1	P	CPD, AMK, CIP, CRO, ATM, FOX, FEP
7	361	40	Male	Surgery	Urine	3	P	CPD, CIP, CRO, ATM, FOX, FEP
8	517	20	Female	Surgery	Pus	2	P	CPD, AMK, CIP, GEN, CRO, ATM, FOX, FEP
9	518	60	Male	Surgery	Pus	3	P	CPD, AMK, CIP, FEP, ATM, CRO, FEP
10	543	80	Male	Medicine	Pus	Not found	P	CPD, FEP, ATM, FOX
11	544	19	Female	Surgery	Pus	2	P	CPD, AMK, CIP, GEN, CRO, ATM, FOX, FEP
12	545	20	Female	Surgery	Pus	2	P	CPD, AMK, CIP, GEN, CRO, ATM, FOX, FEP
13	571	56	Female	Medicine	Urine	Not found	P	CPD, AMK, CPM, ATM, FOX
14	584	17	Female	Medicine	Wound swab	2	P	CPD, AMK, CIP, GEN, CRO, ATM, CEP, FOX, FEP

^a AMK, amikacin; CPD, cefpodoxime; CIP, ciprofloxacin; FEP, cefepime; CRO, ceftriaxone; ATM, aztreonam; GEN, gentamicin; FOX, ceftiofur.

FIG 1 Structural variations of class 1 integron-carrying VEB-1.

and non-beta-lactams (Hi-Media), and results were interpreted according to the CLSI criteria (5). Isolates were typed by repetitive PCRs as described previously (6). All the VEB-1-positive isolates were further tested by the modified Hodge test and the imipenem-EDTA disk test (7) followed by PCR assays to detect production of class A (KPC), class B (NDM, VIM, and IMP), and class D (OXA-23, -24/40, and -58) carbapenemases (7–10).

A total of 14 isolates showed amplification with *bla*_{VEB} primers, which was further confirmed by sequencing as *bla*_{VEB-1}, and the rest of the isolates were harboring CTX-M-15 ($n = 5$), GES-5 ($n = 2$), OXA-10 ($n = 4$), SHV-148 ($n = 1$), and TEM ($n = 3$). Most of the VEB-bearing isolates were obtained from the surgery unit followed by the medicine and orthopedic wards and were recovered from pus, drain tip, urine, and wound swab samples collected from patients of different age groups (Table 1). We also confirmed that *bla*_{VEB-1} was located within the variable regions of class 1 integrons with four types of diverse arrangements (Fig. 1; Table 1). VEB-1 was flanked by *dfrA1* (arrangement 1), *dfrA12* (arrangement 2), and *dfrA17* (arrangement 4) in the upstream region, while in the downstream region *qacE* (arrangements 1, 2, 3, and 4), *aad2* (arrangement 2), and *aadA5* (arrangement 4) were found (Fig. 1). *bla*_{VEB-1} was successfully transferred on *E. coli* strain JM107 by transformation. PCR assays confirmed that *bla*_{VEB} was present in all the transformants and was found to be carried within the IncP-type plasmid according to the sequence results for all the transformants (Table 1). Restriction mapping of the plasmids showed similar banding patterns for all the transformants except two. The antimicrobial susceptibility results for the isolates showed that all were susceptible to polymyxin B. The MIC results showed that all the isolates and their transformants have high MIC values for oxymino cephalosporins and monobactam, for which the inhibitory concentrations ranged from 8 to 512 $\mu\text{g/ml}$. However, high MIC values for carbapenem drugs were also observed in all the isolates and their transformants (Table 2). DNA fingerprinting with repetitive PCRs showed that 11 different clones harboring *bla*_{VEB-1} were distributed within the hospital. Carbapenemase activity was observed only by the modified Hodge test. However, none of the clones could be characterized by our target primers.

Horizontally transferable *bla*_{VEB-1}-mediated expanded-spectrum cephalosporin resistance has been reported from different parts of the world (1, 11–13) and carriage within the integron gene cassette is well established (11–14). However, in India, there has as yet been no report regarding their genetic context and transferability. The presence of this rare type of ESBL with diverse gene

cassette arrangements within a single hospital setting signifies its epidemiological importance in terms of its origin, evolution, and source of acquisition and also its clinical implications for antimicrobial policy. In our study, the MIC data for carbapenem indicated that all the isolates and their transformants were not susceptible to carbapenems. Thus, a novel carbapenemase not identified by our primers may be coproduced and cotransferred with VEB-1 and could be contributing to both cephalosporin and carbapenem resistance in these strains.

Our study established transferability, within this hospital, of this resistant determinant through the IncP group plasmid. Thus, the present study is first report from this part of the world showing that the IncP-type plasmid acted as a genetic vehicle for horizontal transfer of VEB-1 cephalosporin resistance. We also established that the surgery ward was the origin for the dissemination of this rare type of enzyme in this hospital.

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TABLE 2 MICs of the parent strain versus those of transformants

Sample identification ^a	MIC of indicated antibiotic for the donor and transformants harboring VEB-1 ^b :										Sample identification (<i>bla</i> gene)	MIC of indicated antibiotic for the strain and transformants with other known β-lactamases:									
	CTX	CAZ	CRO	FEP	IPM	MEM	ETP	ATM	PMB	CTX		CAZ	CRO	FEP	IPM	MEM	ETP	ATM	PMB		
153 P	128	128	256	512	16	16	8	512	0.125	154 (OXA-10)	128	64	128	32	2	2	2	64	<0.125		
153 TF	128	128	256	512	8	2	2	256	0.125	TF	128	64	128	32	2	2	2	64	<0.125		
182 P	256	512	512	256	256	256	256	512	0.25	176 (OXA-10)	128	128	128	128	2	2	<2	256	0.125		
182 TF	128	256	128	128	64	32	8	256	0.25	TF	128	128	128	128	2	<2	<2	256	<0.125		
264 P	256	512	512	64	4	4	4	32	<0.125	188 (OXA-10)	256	128	256	128	4	2	2	512	0.125		
264 TF	128	128	64	64	2	4	2	8	<0.125	TF	256	128	256	128	4	2	<2	512	0.125		
303 P	256	256	512	256	256	32	4	512	0.125	283 (OXA-10)	256	256	256	256	4	2	<2	512	0.125		
303 TF	128	256	512	256	256	8	2	512	0.125	TF	256	256	256	256	2	2	<2	512	0.125		
306 P	16	32	256	256	8	64	8	256	0.125	270 (CTX-M-15)	64	128	128	256	<2	<2	<2	128	<0.125		
306 TF	16	16	128	256	4	16	2	128	0.125	TF	64	128	128	256	<2	<2	<2	128	<0.125		
310 P	512	512	512	512	512	512	512	512	0.25	430 (CTX-M-15)	64	64	64	128	2	2	<2	128	<0.125		
310 TF	512	256	256	128	32	16	4	256	0.25	TF	64	64	64	128	2	2	<2	128	<0.125		
361 P	128	16	32	256	64	8	8	64	<0.125	486 (CTX-M-15)	64	64	64	64	<2	<2	<2	128	0.125		
361 TF	16	8	32	128	4	16	2	16	0.125	TF	64	64	64	64	<2	<2	<2	128	0.125		
517 P	256	256	512	64	512	256	128	32	0.25	551 (CTX-M-15)	64	64	64	64	<2	<2	<2	32	<0.125		
517 TF	128	128	256	32	8	16	4	32	0.25	TF	64	64	64	64	<2	<2	<2	32	<0.125		
518 P	256	512	512	64	64	8	64	256	<0.125	562 (CTX-M-15)	32	64	256	256	2	<2	<2	128	<0.125		
518 TF	64	128	256	16	8	4	2	32	<0.125	TF	32	64	256	256	<2	<2	<2	128	<0.125		
543 P	128	16	128	16	128	8	16	32	<0.125	527 (SHV-148)	32	32	256	128	2	<2	<2	256	<0.125		
543 TF	128	16	128	8	32	4	4	32	<0.125	TF	32	32	256	128	<2	<2	<2	256	<0.125		
544 P	256	16	16	16	512	512	256	16	0.25	8 (TEM)	32	32	32	32	<2	<2	<2	64	<0.125		
544 TF	128	16	16	16	16	8	4	16	0.25	TF	32	32	32	32	<2	<2	<2	64	<0.125		
545 P	512	128	512	128	64	16	8	256	0.125	87 (TEM)	32	32	32	32	<2	<2	<2	64	<0.125		
545 TF	128	64	128	64	8	4	2	16	0.125	TF	32	32	32	32	<2	<2	<2	64	<0.125		
571 P	256	256	256	64	8	8	8	512	<0.125	95 (TEM)	32	32	32	128	<2	<2	<2	64	<0.125		
571 TF	128	256	64	64	2	2	2	256	<0.125	TF	32	32	32	128	<2	<2	<2	64	<0.125		
584 P	256	256	256	256	16	8	8	256	0.125	123 (GES-5)	256	256	256	256	2	2	<2	256	<0.125		
584 TF	257	256	128	256	8	8	2	128	0.125	TF	256	256	256	256	2	<2	<2	256	<0.125		
<i>E. coli</i> JM107 without plasmid	0.125	<0.125	0.125	<0.125	<0.125	<0.125	<0.125	0.125	<0.125	412 (GES-5)	256	256	256	512	4	2	<2	256	0.125		
										TF	256	256	256	512	4	2	<2	256	0.125		

^a P, parent strain; TF, transformant.

^b CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftioxaime; FEP, ceftioxaime; IPM, imipenem; MEM, meropenem; ETP, ertapenem; ATM, aztreonam; PMB, polymyxin B.

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