Dispersal of Carbapenemase *bla*_{VIM-1} Gene Associated with Different Tn402 Variants, Mercury Transposons, and Conjugative Plasmids in *Enterobacteriaceae* and *Pseudomonas aeruginosa*[▽]

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The emergence of *bla*_{VIM-1} within four different genetic platforms from distinct *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates in an area with a low prevalence of metallo- β -lactamase producers is reported. Forty-three VIM-1-producing isolates (including 19 *Enterobacter cloacae*, 2 *Escherichia coli*, and 2 *P. aeruginosa* isolates, 18 *Klebsiella pneumoniae* isolate, and 2 *Klebsiella oxytoca* isolate) recovered from 2005 to 2007 and corresponding to 15 pulsed-field gel electrophoresis types were studied. The *Enterobacteriaceae* isolates corresponded to a hospital outbreak, and the *P. aeruginosa* isolates were sporadically recovered. The genetic context of the integrons carrying *bla*_{VIM-1} (arbitrarily designated types A, B, C, and D) was characterized by PCR mapping based on known Tn402 and mercury transposons and further sequencing. Among *Enterobacteriaceae* isolates, *bla*_{VIM-1} was part of integrons located either in an In2-Tn402 element linked to Tn21 (type A; In110-*bla*_{VIM-1}-*aacA4-aadA1*) or in a Tn402 transposon lacking the whole *tni* module [type B; In113-*bla*_{VIM-1}-*aacA4-dhfrII* (also called *dfrB1*)-*aadA1-catB2*] and the transposon was associated with an IncHI2 or IncI1 plasmid, respectively. Among *P. aeruginosa* isolates, *bla*_{VIM-1} was part of a new gene cassette array located in a defective Tn402 transposon carrying either *tniB* Δ 3 and *tniA* (type C; *bla*_{VIM-1}-*aadA1*) or *tniC* and Δ *tniQ* (type D; *bla*_{VIM-1}-*aadB*), and both Tn402 variants were associated with conjugative plasmids of 30 kb. The dissemination of *bla*_{VIM-1} was associated with different genetic structures and bacterial hosts, depicting a complex emergence and evolutionary network scenario in our facility, Ramón y Cajal University Hospital, Madrid, Spain. Knowledge of the complex epidemiology of *bla*_{VIM-1} is necessary to control this emerging threat.

The emergence of acquired metallo- β -lactamases (MBLs) is of clinical concern since they confer resistance to all available β -lactams except aztreonam and they are not inhibited by class A β -lactamase inhibitors (42). MBLs have been categorized into different groups; IMP and VIM are the most commonly identified types, and they are predominant in Asia and Europe, respectively. VIM enzymes have been grouped into three main clusters designated VIM-1, VIM-2, and VIM-7 (42). Although they are more prevalent among nonfermenting gram-negative bacteria, they are increasingly recognized among members of the family *Enterobacteriaceae* (42). To date, VIM-2 is more widely spread among *Pseudomonas aeruginosa* isolates, whereas VIM-1 is normally confined to *Enterobacteriaceae* isolates (41, 42). MBL-producing isolates have been identified rarely in Spain, in contrast to other European countries such as Greece and Italy (28, 34, 41). Nevertheless, the prevalence of MBL producers in Spain may have been underestimated since *Enterobacteriaceae* and *P. aeruginosa* isolates with VIM-1 and VIM-2 enzymes have been identified in different Spanish cities (11, 25, 26, 32, 37).

The *bla*_{VIM} allelic variants are part of class 1 integrons which

are derivatives of Tn402 (also called Tn5090), a transposon characterized by the presence of a transposition module comprising a suite of four genes (*tniR/tniC*, *tniQ*, *tniB*, and *tniA*) (12, 21). Although diverse gene cassette arrays have been identified among class 1 integrons containing *bla*_{VIM} alleles, a detailed characterization of the whole Tn402-harboring structure has hardly been achieved. Recently, MBL genes have been identified as part of *sulI* integrons linked to a Tn402 element lacking *tniR/tniC* and *tniQ*, as reported for VIM-1- and VIM-2-producing *P. aeruginosa* isolates from Italy, Poland, and Australia (5, 29, 34, 35). Integrons lacking the 3' conserved sequence (3'-CS) and associated with either a complete Tn402 transposon, as described previously for an IMP-4-producing *Citrobacter youngae* isolate (GenBank accession no. AF288045), or a Tn402 element containing *tniC*, like the integrons in *P. aeruginosa* VIM-2 producers from Italy, Norway, Russia, Ghana, India, Taiwan, and the United States, have also been reported previously (13, 16, 31, 36, 44). Some of these Tn402 elements were identified within different Tn3-like transposons (5, 35, 40).

The objective of this work was to characterize the genetic platforms linked to the *bla*_{VIM-1} gene from clinical isolates of different bacterial species recovered at Ramón y Cajal University Hospital over a period of 3 years (2005 to 2007). These isolates included the VIM-1-producing *Enterobacteriaceae* isolates involved in a recent outbreak (32) and two sporadic VIM-1-producing *P. aeruginosa* isolates contemporarily iden-

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TABLE 1. Epidemiological data for VIM-1-producing isolates at Ramón y Cajal University Hospital (2005 to 2007)

Species	PFGE type	No. of isolates	Ward(s) ^a and/or patient status (no. of isolates)	Date(s) (mo/yr) of isolation	Integron type and identity	Plasmid size(s) (kb) ^b	Inc group ^c	Conjugation frequency ^d
<i>E. cloacae</i>	ECMBL-1	2	Pediatric cardiology (1), pediatric cardiology ICU (1)	03/05–11/05	A, In110	300	ND	NT
	ECMBL-2	1	Outpatient (1)	03/06	A, In110	290, 300	HI2	10 ⁻⁷
		4	Internal medicine (1), neurology (3)	04/07–07/07	A, In110	40 , 290, 300	HI2	10 ⁻⁴
	ECMBL-3	1	Cardiovascular ICU (1)	05/05	A, In110	435	HI2	10 ⁻⁶
		1	Internal medicine (1)	05/05	A, In110	90, 300	HI2	10 ⁻⁷
	ECMBL-4	1	Gastroenterology (1)	08/05	A, In110	300	HI2	10 ⁻⁷
	ECMBL-5	1	Hematology (1)	01/06	A, In110	300	HI2	10 ⁻⁷
	ECMBL-6	5	Medical ICU (1), cardiovascular ICU (4)	02/06–12/06	A, In110	90, 300	ND	NT
	ECMBL-7	1	Pediatric cardiology ICU (1)	10/06	A, In110	190, 300	HI2	10 ⁻⁶
<i>K. oxytoca</i>		1	Cardiology (1)	11/06	B, In113	60 , 90, 200		10 ⁻³
		1	Surgical cardiology (1)	02/07	A, In110	90, 300	ND	NT
	KOMBL-1	2	Pneumology (1), medical ICU (1)	10/05–01/07	A, In110	300	ND	NT
<i>K. pneumoniae</i>	KPMBL-1	18	Cardiology (2), oncology (1), urology (1), surgical cardiology (2), cardiovascular ICU (5), surgical neurology ICU (2), general surgery ICU (1), general surgery (2), internal medicine (1), outpatient (1)	10/05–09/07	B, In113	40, 60 , 90, 200	I1	10 ⁻¹ –10 ⁻⁴
<i>E. coli</i>	ECOMBL-1	1	Urology (1)	02/06	B, In113	60	I1	10 ⁻⁴
	ECOMBL-2	1	General surgery (1)	12/06	B, In113	90 , 200		10 ⁻²
<i>P. aeruginosa</i>	PAMBL-1	1	Pneumology (1)	04/06	D	30	ND	10 ⁻⁵
	PAMBL-2	1	Urology (1)	01/07	C	30	ND	10 ⁻⁸

^a ICU, intensive care unit.^b Plasmid sizes corresponding to the indicated incompatibility groups for the transconjugants are in boldface. Replicon sequences corresponding to IncHI2 plasmids showed 100% identity with R478, the prototype of IncHI2 plasmids (GenBank accession no. BX664015).^c The plasmid incompatibility (Inc) group was studied only in the cases of transconjugants. ND, not determined.^d The conjugation frequency is calculated as the number of transconjugants per donor cell. NT, no transfer.

tified in the same hospital. In addition, these genetic platforms were compared with those already described.

MATERIALS AND METHODS

Bacterial strains. Forty-three VIM-1-producing isolates (18 *Klebsiella pneumoniae*, 19 *Enterobacter cloacae*, 2 *Klebsiella oxytoca*, 2 *Escherichia coli*, and 2 *P. aeruginosa* isolates) recovered from 2005 to 2007 in our hospital were studied. Some of these isolates were detected at the time of the first report of VIM-1-producing *Enterobacteriaceae* in our hospital (32). Isolates were obtained from clinical and surveillance specimens from 38 patients (15 in intensive care units, 13 located in medical wards, 8 in surgical wards, and 2 outpatients) (Table 1).

Clonal relatedness. Clonal relatedness was established by comparison of XbaI and SpeI-digested genomic DNA patterns from *Enterobacteriaceae* and *P. aeruginosa* isolates, respectively, as described previously (32). The electrophoresis conditions used in the CHEF-DR III system (Bio-Rad, Hemel Hempstead, United Kingdom) were 6 V/cm² and 14°C for 24 h with initial and final pulse times of 10 and 40 s for XbaI and 20 h with initial and final pulse times of 1 and 36 s for SpeI.

Genetic environment of *bla*_{VIM-1}. The genetic environment of *bla*_{VIM-1} was evaluated by amplification of the integrase genes and the variable regions of class 1 integrons and sequences of the Tn402 backbone (20) (Fig. 1). The association with mercury transposons Tn21 and Tn5051 was preliminarily inferred by screening for the presence of *tnpA* from Tn21 (*tnpA*₂₁), *tnpR*₂₁, *IS1326*, *IS1353*, *merA*₂₁, *tnpA* from Tn5051 (*tnpA*₅₀₅₁), and *tnpR*₅₀₅₁ by PCR. The entire genetic environment of *bla*_{VIM-1} was fully identified by PCR mapping using primers based on the known Tn402 and Tn21 sequences (GenBank accession no. U67194 and AF071413.3) and further sequencing (20) (Fig. 1; Table 2).

Plasmid DNA analysis. Transfer of *bla*_{VIM-1} was screened for by filter mating at a 1:10 donor-recipient ratio using *E. coli* strain BM21 (a plasmid-free lactose fermenter resistant to rifampin [rifampicin] and fusidic acid) and *P. aeruginosa* strain PAO as recipients (32). Transconjugants were selected on Luria-Bertani agar plates containing ceftazidime (4 µg/ml) plus rifampin (300 µg/ml), the

plates were incubated at 37°C for 24 h, and the presence of *bla*_{VIM-1} in the transconjugants was confirmed by PCR. Plasmid sizes in *E. coli* transconjugants (or wild-type strains in the absence of transfer) were determined (20). Enterobacterial plasmids were classified according to their incompatibility group by using the PCR replicon-typing scheme described by Carattoli et al. (3). Confirmation of the incompatibility group was achieved by hybridizing the S1-digested plasmid DNA with probes specific for *bla*_{VIM-1} and the plasmid replicons amplified by PCR.

Nucleotide sequence accession numbers. The sequences corresponding to the genetic elements described in this work were assigned to the following NCBI accession numbers: GQ422826 (type A), GQ422827 (type B), GQ422828 (type C), and GQ422829 (type D).

RESULTS AND DISCUSSION

***bla*_{VIM-1} is spread by diverse *Enterobacteriaceae* and *P. aeruginosa* clones.** The increase in VIM-producing isolates in our institution was due to the spread of the *bla*_{VIM-1} cassette among multidrug-resistant strains of *Enterobacteriaceae*, mainly strains of *K. pneumoniae* and *P. aeruginosa* (Table 1). The 18 VIM-producing *K. pneumoniae* isolates showed indistinguishable pulsed-field gel electrophoresis (PFGE) patterns designated KPMBL-A, and both *K. oxytoca* strains showed the same KOMBL-1 type. Clonal diversity was detected among the 19 *E. cloacae* isolates (with nine PFGE types designated ECLMBL-1 to ECLMBL-9), the 2 *E. coli* isolates (with PFGE types ECOMBL-1 and ECOMBL-2), and the 2 *P. aeruginosa* isolates (with PFGE types PAMBL-1 and PAMBL-2). Clonal outbreaks caused by *K. pneumoniae* strains producing VIM-1

Integron type	Species (n° isolates)	PFGE types	Tn402					Tn21		Tn5051		
			orf5	IS1326	IS1353	ΔtniB1-tniA	tniC	tnpA	tnpR	tnpA	tnpR	merA1
A	<i>E. cloacae</i> (19)	ECMBL-1 to 7, 9	+	+	+	+	-	+	+	-	-	+
	<i>K. oxytoca</i> (2)	KOMBL-1										
B	<i>K. pneumoniae</i> (18)	KPMBL-1										
	<i>E. cloacae</i> (1)	ECMBL-8	+	-	-	-	-	-	-	-	-	-
	<i>E. coli</i> (2)	ECOMBL-1,2										
C	<i>P. aeruginosa</i> (1)	PAMBL-2	+	-	-	+	-	-	-	-	-	+
D	<i>P. aeruginosa</i> (1)	PAMBL-1	-	-	-	-	+	-	-	-	-	-

TABLE 2. Oligonucleotides used in this study

Primer no.	Primer	Sequence (5'–3')	GenBank accession no.	Position (nt) ^a	Reference
1	VIM-F	ATGGTGTGTTGGTCGCATATC	AY987853	251–270	32
2	VIM-R	TGGGCCATTGAGCCAGATC	AY987853	760–742	32
3	5'CS	GGCATCCAAGCAGCAAG	AF17413	5298–5314	20
4	3'CS	AAGCAGACTTGACCTGAT	AF17413	6306–6289	20
5	orf5-F	CGATATCGACGAGGTTGTGC	AF17413	7712–7730	20
6	orf5-R	AGTTCTAGGCGTTCTGCG	AF17413	8157–8140	20
7	IS1326-F	TACCGGGTCTTATGACCGAGT	AF17413	10357–10337	20
8	IS1326-R	ACTGTCATAGCGGTTACGTT	AF17413	9141–9161	20
9	IS1353-F	ACACTACGGCAGCTGGGATA	AF17413	10830–10849	20
10	IS1353-R	TGCAGCATTGTCTTGCAGACA	AF17413	12113–12093	20
11	<i>tniA</i> -F	AGATGCGTGAGATTGTGACC	AF17413	13406–13387	This study
12	<i>tniBD1</i> -R	AGCATCATCGGCTCGAAGC	AF17413	12588–12606	This study
13	<i>tniC</i> -F	CGATCGGCTTGGCCGTGA	X72585	4421–4438	This study
14	<i>tniC</i> -R	CGCAGAGATCGCCACCT	X72585	4740–4723	This study
15	<i>tnpA</i> ₂₁ -F	CGGCAGTCGGTCATTCTGC	AF17413	2550–2532	This study
16	<i>tnpA</i> ₂₁ -R	CTTGGTGTGGAATGGCGCG	AF17413	835–853	This study
17	<i>tnpR</i> ₂₁ -F	CCGTGGTGGTGCATAGCAT	AF17413	3394–3376	20
18	<i>tnpR</i> ₂₁ -R	ATGCTATGCACCACCACGG	AF17413	3376–3394	20
19	<i>tnpA</i> ₅₀₅₁ -F	TGCCACGTCGTTCAATCCTG	Y17719	922–941	This study
20	<i>tnpA</i> ₅₀₅₁ -R	TCATGACCAGCTTCTGCCAG	Y17719	2282–2262	35
21	<i>tnpR</i> ₅₀₅₁ -F	ACGGTCAGCGCATCGGTTAC	Y17719	362–381	35
22	<i>tnpR</i> ₅₀₅₁ -R	GATACAGGGTTTCGCGACTG	Y17719	895–876	35
23	<i>merA1</i> -F	ACCATCGGCGGCACCTGCGT	AF17413	17597–17578	20
24	<i>merA1</i> -R	ACCATCGTCAGGTAGGGGAACAA	AF17413	16360–16382	20
25	IS5075-R	CGTAAGTTTGCTGTCGCGC	AF457211	286–304	This study
26	IS5075-R2	TCGCGATGGTTGTTGCCG	AF457211	1128–1146	This study
27	Tn21IR	GGGTCGTCTCAGAAAACGG	AF17413	2–20	20
28	<i>qacED1</i> -R	CAAGCTTTTGCCCATGAAGC	AF17413	6608–6588	20
29	<i>aadA1</i> -F	GCTGGCCGTACATTTGTACG	AY987853	2127–2146	32
30	IS1353-Rf	TGCTCGCCAGACAATGCTGCA	AF17413	12093–12113	20
31	TniA-F2	TCGTGCGGAGATCATCAGTCC	AF17413	14821–14801	20
32	Tn402IR	TTTTCAGAAGACGACTGCACCA	AF17413	15034–15013	This study
33	VIM-Rf	GATCTGGCTGAATGGCCCA	AY987853	742–760	32
34	<i>aacA4</i> -F	AGCGAACACGCAGTGATGC	AY987853	1541–1558	32
35	intI1-F	GGGTCAAGGATCTGGATTTTCG	AF17413	4774–4754	20
36	intI1-R	ACATGCGTGTAATCATCGTCC	AF17413	4312–4333	20
37	<i>qacED1</i> -F	ATCGCAATAGTTGGCGAAGT	AF17413	6383–6392	20
38	IS1326-R2	TGTTGTCGTAGATGCCGCG	AF17413	10035–10053	This study
39	<i>tniC</i> -F2	AGGTGGGCGATCTCTGCG	X72585	4723–4740	This study

^a nt, nucleotides.

have recently been reported in different southern European countries such as Greece, France, Italy, and Spain, although the lack of information about the relatedness among strains precludes conclusions about international clonal expansion (2, 13, 27, 30, 32, 41). Outbreaks caused by VIM producers of other enteric species such as *E. coli*, *Proteus mirabilis*, or *P. aeruginosa* have been documented less frequently (7, 13, 38, 39, 41). Horizontal transfer of particular genetic elements among

strains seems to have greatly contributed to the recent increase in the prevalence of VIM-1 producers among European hospitals, giving rise to multiclonal epidemics. However, the full characterization of the involved mobile elements is not addressed in most studies (14, 19, 26, 27, 32, 41).

The *bla*_{VIM-1} gene is linked to different Tn402 elements associated with different hosts. The *bla*_{VIM-1} gene was detected in four class 1 integrons arbitrarily designated types A, B, C,

FIG. 1. Schematic representation of *bla*_{VIM-1}-containing elements characterized by PCR mapping on the basis of Tn402 and Tn21 sequences. Findings from a preliminary screening of *bla*_{VIM-1} elements related to Tn402, Tn21, and Tn5051 are shown in the table at the bottom. The locations of the primers used (designated P followed by a number) for the PCR mapping assay are represented by black arrowheads. Black circles represent the *attI* sites. White circles represent *attC* sites or 59-base elements of the corresponding gene cassettes. *, PCR products had different molecular sizes for the different integron types.

FIG. 2. Tn402 derivatives associated with MBLs. ND, not determined (sequences downstream from *tniC* were not determined or were not provided in any of the studies corresponding to references 13, 16, 31, 36, and 44). NA, not applicable.

and D (Fig. 1) (32). Our analysis not only enlarges the list of MBL integrons (to include types C and D) but also confirms the wide distribution of particular variants such as In110 (type A), previously found among *Pseudomonas* isolates from Italy and Spain and now identified in *Enterobacteriaceae*, and In113 (type B), previously detected among *Enterobacteriaceae* isolates from another Spanish hospital (11, 17, 28, 37) (see Fig. 3). Detailed molecular characterization of the integron genetic environment revealed a level of heterogeneity not made obvious by the analysis of gene cassette arrays as highlighted previously (18).

Enterobacteriaceae. The *bla*_{VIM-1} gene was identified in integrons located either in an In2-Tn402 element within Tn21 (type A) or in a Tn402 transposon lacking the whole *tni* module (type B).

Type A (*bla*_{VIM-1}-*aacA4*-*aadA1*), recovered from *K. oxytoca* isolates and 11 *E. cloacae* isolates, was identical to In110 integrons from *P. aeruginosa* and *Pseudomonas putida* isolates from Italy and Spain (11, 28) (GenBank accession no. AJ867812 and EF577408.1). The type A integron comprises a defective Tn402 *tni* (*tni*₄₀₂) module (*tniBΔ1 tniA*), truncated by the insertion of IS1326 and IS1353, which is usually associated with the In2 family of class 1 integrons (21). The sequences corresponding to this *tni*₄₀₂ module and the region beyond inverted repeat IRI of this In2-Tn402 element display 100% identity to the sequence of In2 found in Tn21 (GenBank accession no. AF071413). Moreover, the inverted repeat of *tnp*₂₁ was interrupted by a copy of IS5075 identical to that in Tn21 of pRMH760 from *K. pneumoniae* (GenBank accession

no. AY123253.3). Sequences beyond the *tni*₄₀₂ module were not characterized, although *merA*₂₁ was identified in isolates carrying type A integrons. This study constitutes the first complete characterization of a class 1 integron containing MBL genes in *Enterobacteriaceae*. Until now, the location of MBL integrons within Tn3/mercury transposons had been observed only in *P. aeruginosa*, as is the case for integrons containing *bla*_{VIM-1}, *bla*_{VIM-2}, *bla*_{VIM-3}, and *bla*_{IMP-13}, which are linked to Tn5051, a member of the Tn501 family, or Tn1403 (5, 28, 34, 35) (Fig. 2). Despite the host preferences (*Enterobacteriaceae* versus *Pseudomonadaceae*) exhibited by the distribution of Tn21 and Tn501 or Tn1403 subfamily elements, it is of interest to highlight that Tn21 variants with the inverted repeat of *tnp* interrupted by copies of IS5075/IS4321 may be located in broad-host-range conjugative plasmids able to spread among different genera or to be integrated into chromosomes (6, 9, 19a, 22, 23). In our case, plasmids of the incompatibility group HI2 with variable sizes (300 to 435 kb) and transfer properties were identified among isolates carrying a type A integron (Table 2).

Type B [*bla*_{VIM-1}-*aacA4*-*dhfrII* (also called *dhfrB1*)-*aadA1*-*catB2*], recovered from one *E. cloacae* clone, a *K. pneumoniae* clone, and both *E. coli* clones, showed 100% identity to In113 found in the first VIM-1-producing *K. pneumoniae* and *E. coli* isolates reported in Spain (37). An *intI1* gene with a hybrid P1 promoter (comprising the TGGACA -35 hexamer of the weak version of the P1/P_{ant} promoter and the TAAACT -10 hexamer of the strong version of the P1/P_{ant} promoter) similar to that of In1 was identified. The lack of association of type B

FIG. 3. Class 1 integrons containing *bla*_{VIM-1}. Black circles represent unusual *attC* sites of *aadA1* cassettes (see the text). The complete loss of the *attC* site of *aadA1* was detected in the type C integron, which is identical to In113. The integron listed herein as In105 is designated either In105 (10) or In115 (GenBank accession no. AJ786649). *A. xylosoxidans*, *Achromobacter xylosoxidans*; *C. freundii*, *Citrobacter freundii*; *M. morganii*, *Morganella morganii*; *A. baumannii*, *Acinetobacter baumannii*; *E. aerogenes*, *Enterobacter aerogenes*.

with *tni*₄₀₂ and Tn3-like elements was not surprising since MBL integrons in *Enterobacteriaceae* may be flanked by particular insertion sequences such as IS26 or IS1, creating a composite element able to recombine among plasmids of different incompatibility groups as demonstrated in Greece (19, 27). In our case, a common IncI1 plasmid of approximately 60 kb was detected in all transconjugants carrying the type B integron.

***P. aeruginosa*.** We detected two new integrons linked to defective Tn402 variants containing either *tmiB*Δ3 and *tmiA* (type C) or *tmiC* and Δ*tmiQ* (type D). Both variants had a typical 5'-CS containing an *intI1* gene with a weak P1 promoter followed by an inactive P2 promoter (lacking the GGG insertion prior to the -10 hexamer) and an *attI1* site. They were both located on 30-kb plasmids which were not amplified with any of the primers included in the scheme used.

Type C (*bla*_{VIM-1}-*aadA1*) was part of a Tn402 transposon with a deleted *tmi*₄₀₂ module (*tmiB*Δ3 *tmiA*) which is identical to the modules of In70 and In70.2 (5, 28, 29). We could not detect the presence of ISpA7 or Tn5051-like transposons, often associated with MBL integrons in *Pseudomonas* (28, 29, 35). However, the location of the type C integron in other elements such as Tn1403 transposons (GenBank accession no. AF313470.2)

currently spread in Italy, Tunisia (GenBank accession no. FM897214), Taiwan, and Australia cannot be discarded (5, 28, 40). Interestingly, most of these transposons are chromosomally located. Clonal expansion may explain chromosomal predominance in some instances (10, 11). Nevertheless, a plasmid origin for these Tn402 derivatives, the elements of which could have been transposed into the *res* sites of different transposon backbones in the *P. aeruginosa* chromosome, should be considered.

The type D integron (*bla*_{VIM-1}-*aadB*) lacks the 3'-CS region (including *qacEΔI1*, *sulI*, and *orf5*), *tmiB*, and *tmiA*. The incomplete *tmi* module comprises a region displaying 100% identity to the *tmiR/tmiC* gene and a sequence of 161 bp showing 86% similarity to *tmiQ* of Tn402 from IncP-1β plasmid R751 (GenBank accession no. U67194). Although integrons lacking the 3'-CS and containing *tmiC* are spread among *P. aeruginosa* VIM-2 producers from the United States, Italy, Norway, Russia, India, Taiwan, and Ghana, this study constitutes the first documentation involving *bla*_{VIM-1} (13, 16, 31, 36, 44) (Fig. 2).

Widespread integrons carrying *bla*_{VIM-1} show unusual and common features. The *aadA1* gene cassettes present in type A, B, and C integrons showed differences from previously de-

scribed *aadA1* gene cassettes. The variant present in types A and C had a shorter *attC* site than previously described cassettes, containing the heptanucleotide boxes called R' and R'' but lacking most of the central region, including parts of the recombination core sites known as L' and L'' (24). This variant constitutes a new alternative form of the *aadA1* gene cassette which was previously observed in the widespread In70/In70.2 integrons (22, 29). The identity and synteny of the gene cassettes present in In70 and In110, as well as the unusual hybrid configuration of their *intI1* promoters (with the TTGACA –35 hexamer of the strong version of the P1/P_{ant} promoter and the TAAGCT –10 hexamer of the weak P1/P_{ant} promoter), suggest a common origin for gene cassette arrays of In70 and In110. However, differences in the Tn402 variants linked to In70 (*tniBΔ3 tniA*) and In110 (*tniBΔ1 tniA*) may reflect recombination of these integrons with different platforms (Fig. 2). Interestingly, the *attC* site of *bla*_{VIM-1} located in integron type C showed a deletion identical to that observed in the *attC* sites of the *aadA1* cassettes in types A and C and may represent a particular recombination crossover event in this area.

Concluding remarks. We describe the emergence and dispersal of *bla*_{VIM-1} in an allodemic scenario (1) in which both clonal expansion and the transfer of conjugative plasmids carrying different integrons/transposons among distinct clones of *Enterobacteriaceae* and *P. aeruginosa* have contributed to increasing the presence of this MBL gene. At first glance, the complex epidemiology of *bla*_{VIM-1} in our institution would suggest independent events of selection in the hospital environment. However, recombination between different *bla*_{VIM-1}-carrying platforms with unusual gene cassettes might also have occurred. The capture of *bla*_{VIM} or *bla*_{IMP} mostly by integrons containing *aacA4* and *aadA1* cassettes, which are overrepresented in environmental plasmids of *Enterobacteriaceae* or *Pseudomonas*, may have influenced the recent and fast dissemination of *bla*_{VIM-1} (6, 15, 19, 19a, 33, 43) (Fig. 3). Although *bla*_{VIM-2} and *bla*_{VIM-1} have been predominant among *P. aeruginosa* and *Enterobacteriaceae* strains, respectively, our results show that both genes have the potential to be located in different platforms suitable for successful dissemination and eventually favored by recombination events. Moreover, the diversity of platforms associated with *bla*_{VIM-1} may facilitate the rapid spread of this allele among *P. aeruginosa* isolates (7, 8, 10, 13, 28, 39).

To date, the epidemiological studies of MBL producers have been focused on the description of gene cassette arrays containing MBL genes. Further studies addressing the full characterization of MBL genetic elements are needed for the better understanding of their spread, as this and other studies reveal (5, 18). The roles of selective pressures for clonal expansion of certain strains, genetic platform rearrangement, and/or possible plasmid dissemination among different clones need to be clarified in order to establish infection control measures for this emerging threat.

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