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bla$_{VIM-2}$-Harboring Integrons Isolated in India, Russia, and the United States Arise from an Ancestral Class 1 Integron Predating the Formation of the 3′ Conserved Sequence[▽]

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The metallo-β-lactamase gene *bla*$_{VIM-2}$ was identified in a strain of *Pseudomonas aeruginosa* isolated in India. The integron encoding *bla*$_{VIM-2}$ was virtually identical to those recently found in the United States and Russia. These unusual structures are likely to have arisen from an ancestral integron predating the formation of the 3′ conserved sequence.

The increasing rates of antibiotic resistance among gram-negative bacteria, particularly *Pseudomonas aeruginosa* and *Acinetobacter* spp., is a serious cause for concern. The broad β-lactam resistance profile in these species may be mediated by metallo-β-lactamases (MBLs), which are capable of hydrolyzing most classes of β-lactams, and at present, there are currently no known effective clinical inhibitors. To date, there are five sub-major types of mobile MBL genes: *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SPM}$, *bla*$_{GIM}$, and *bla*$_{SIM}$ (2, 12, 14). However, it would appear that *bla*$_{VIM-2}$ has become the dominant genotype and has currently been reported from 23 counties, with the alleged "index" strain being a Portuguese *P. aeruginosa* isolate recovered in 1995 (1, 14). Most recently, *bla*$_{VIM-2}$ has been reported from a few isolates in China and an outbreak in the United States (6, 15). Herein, we report on the first characterization of an MBL (*bla*$_{VIM-2}$) from India that is carried on a unique integron but that shows genetic structures similar to those of integrons from the United States and Russia.

A clinical isolate (isolate 42) of *Pseudomonas aeruginosa* was collected from the Sri Ramachandra Medical College and Research Institute, Chennai, India, in 2003. The isolate displayed an MBL-like phenotype that was characterized by zone enhancement with EDTA-impregnated imipenem disks (750 μg) (16); and cell lysates also hydrolyzed imipenem and meropenem, as measured by spectrophotometry at 299 nm, as described previously (13). The resistance profile (MIC) of *P. aeruginosa* strain 42 from India was as follows: imipenem, >32 μg/ml; meropenem, >32 μg/ml; ceftazidime, 96 μg/ml; piperacillin, >256 μg/ml; piperacillin-tazobactam, >256 μg/ml; cefepime, >256 μg/ml; aztreonam, >256 μg/ml; colistin, 1 μg/ml; gentamicin, >1,024 μg/ml; amikacin, 8 μg/ml; and ciprofloxacin, >32 μg/ml. The isolate came from the bronchoalveolar lavage fluid of a 60-year-old man with ventilator-associated pneumonia. The patient was treated with meropenem in the intensive care unit of the hospital of the Sri Ramachandra Medical College and Research Institute but subsequently succumbed to the infection.

PCR with *bla*$_{VIM}$-specific primers was positive by using the Expand high-fidelity master mix containing a mixture of *Pfu* and nonproofreading *Taq* polymerases and deoxynucleoside triphosphates (ABgene; Epsom United Kingdom) and primers, as reported previously (13). The amplicon was sequenced to confirm the presence of the *bla*$_{VIM-2}$ gene cassette. Further PCR with class 1 integron conserved sequence (CS) primers 5′CS and 3′CS failed to amplify any class 1 integron genetic structures. However, subsequent PCRs with a combination of the 5′CS primer and a primer designed to detect the *tniC* gene of transposon Tn*5090* (primer *tni*CF) (Table 1) was successful and amplified an integron that harbored the *bla*$_{VIM-2}$ MBL gene but that lacked the normal 3′CS. This integron was sequenced in full by using a combination of primers 5′CS and *tni*CF and custom-made primers (Table 1). The integron had an unusual cassette structure consisting of a tandem array of *aacC7*, *bla*$_{VIM-2}$, *dhfrB5*, and *aacC6-II* gene cassettes (Fig. 1). The cassette array and integron structure were strikingly similar to those of two other *bla*$_{VIM-2}$-harboring integrons that have recently been sequenced from *P. aeruginosa* strains isolated in the United States and Russia (GenBank accession no. DQ522233) (6) (Fig. 1). In particular, all three integrons had the same three cassettes in positions 1 to 3 of their variable regions, i.e., *aacA7*, *bla*$_{VIM-2}$, and *dhfrB5* (previously called *dhfr*IIe), which confer resistance to aminoglycosides, β-lactams, and trimethoprim, respectively (4). Additionally, all three integrons lacked the 3′CS that is found in the vast majority of class 1 integrons in clinically relevant bacteria and that consists of fused *qacE* and *sul1* gene cassettes, termed *qacE*Δ*1*/*sul1*. Instead, the *tniC* gene encoding the resolvase of transposon Tn*5090* (also called *tniR* of Tn*402*) (7) was found 3′ adjacent to the variable region of each integron (Fig. 1). The Indian integron differs in only two respects from the integrons of the Russian and U.S. isolates. First, the fourth gene cassette is *aacC6-II*, an *N*-acetyltransferase gene that confers resistance to gentamicin, tobramycin, and netilmicin but not amikacin or isepamycin (9), rather than the *aacCA5* gene found in the integrons of the Russian and U.S. isolates, which confers resistance only to gentamicin (Fig. 1) (3). Second, the integron of

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TABLE 1. Primer sequences used in this study

Primer name	Primer sequence (5′–3′)
*tni*CF	CGATCTCTGCGAAGAACTCG
5′CS	GCCTGTTCGGTTCGTAAGCT
3′CS	CGGATGTTGCGATTACTTCG
INDAM2638F	CGGTCTAGACTTGCTCAAGC
INDAM3059F	TTATCCTGTTGCGGCACTGG
INDAM3497F	CGCAGAGAAGGCATAGCTAC
INDAM4027F	TGGCGGCGGTCATCTTGAAG
INDAM3122R	GCCGTTGAGTGGAGCACTTC
INDAM3609R	GCTGGTTGGTCTTCTACGCC
ISPa21404F	GATATGTACAGGAGCAGCCG
ISPa21409R	CATATCGACCGAATGCCTCC

the Indian isolate contained an IS*Pa21*-like insertion sequence that has inserted within the 59-base element of the *aacC6-II* gene, an event that would "fix" this gene in the integron, making it refractory to integrase-mediated excision events (Fig. 1).

The lack of a 3′CS is characteristic of the class 1 integron harbored by transposon Tn*5090* (also called Tn*402*), the progenitor of the common type of class 1 integron structure that contains the 3′CS, as seen, for example, in transposon Tn*21* (5) (Fig. 1). The addition of the *sul1* gene cassette and its subsequent fusion to the Tn*5090*/Tn*402 qacE* gene cassette by integration and deletion events, respectively, gave rise to the common form of the class 1 integron (Fig. 1).

These three bla_{VIM-2}-harboring integrons found in *P. aeruginosa* strains isolated from widely separated geographical locations probably originated from a widely dispersed Tn*5090* transposon. This transposon has evolved by normal integrase-mediated acquisition and loss of gene cassettes to include the bla_{VIM-2} gene. The wide dispersal of this genetic structure with this particular gene array may be the reason that the bla_{VIM-2} MBL is reported more often than any other MBL gene (14). A hypothetical model of Tn*5090*/Tn*402* evolution that gives rise to the bla_{VIM-2}-harboring integrons described in this study as well as the more common form of class 1 integron found in Tn*21* is depicted in Fig. 1. Notably, the majority of integrons with a 3′CS are contained within Tn*5090*/Tn*402* transposons defective in transposition functions, often with the loss of *tniC* and a section of *tniB* (Fig. 1) (5). The Tn*5090*/Tn*402* transposon is fully functional (8), and therefore, it may be expected that these three class 1 integron structures harboring bla_{VIM-2} are also present on a functional transposon, enhancing mobility. Experiments are under way to determine if this is indeed the case. Tn*5090* was initially sequenced from the IncP plasmid R751, isolated from *Enterobacter aerogenes* (11). Plasmids were not detected in Indian *P. aeruginosa* strain 42 by the alkaline lysis procedure with a QIAGEN mini-prep kit (13) and could not be conjugated to *P. aeruginosa* PAO1 or *Escherichia coli* DH5α by standard methods (13).

The finding of a number of class 1 integron structures without a 3′CS draws attention to the fact that the frequency of class 1 integrons in clinically important bacterial pathogens is probably underestimated in the literature, since most studies use PCR analysis with primers designed to be specific for the 5′CSs and 3′CSs. Indeed, a recent study has also highlighted the fact that class 1 integrons are also commonly found in forest soil and lake sediments and that these integrons lack both antibiotic resistance gene cassettes and Tn*402* transposon genes (10).

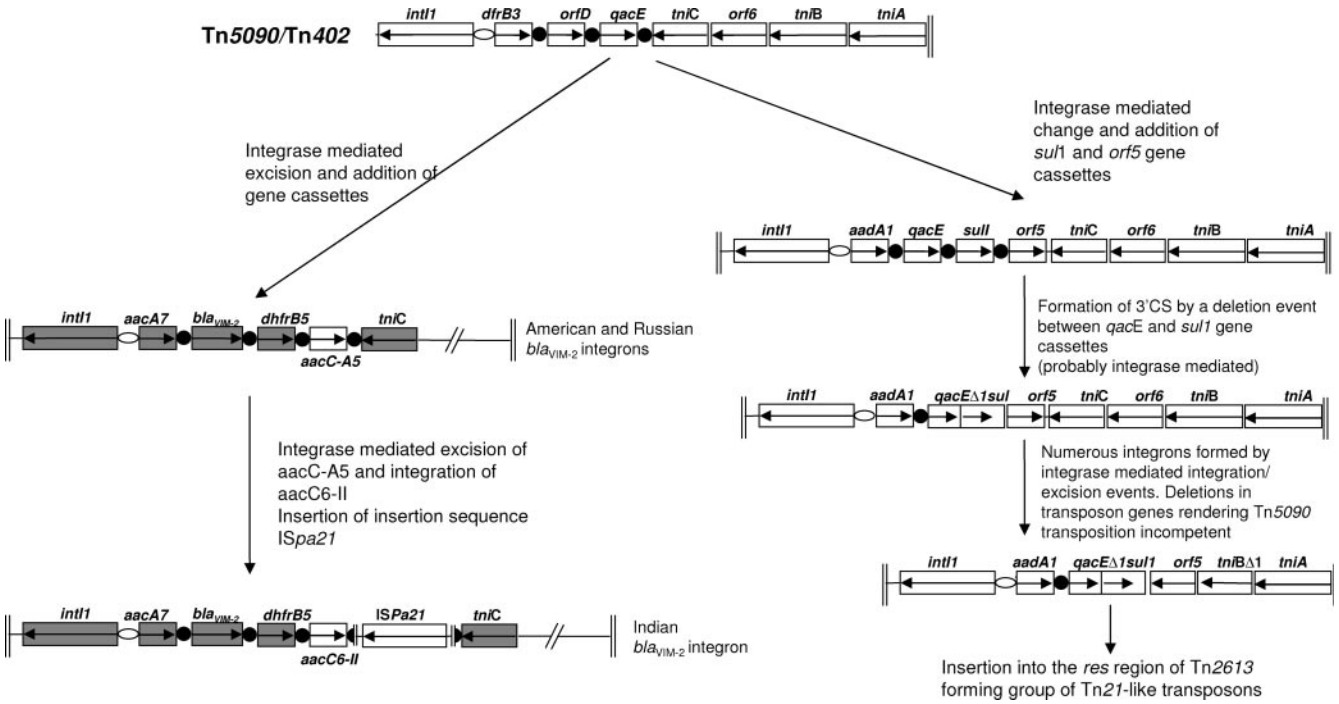

FIG. 1. Hypothetical model of class 1 integron evolution. Open reading frames are represented by open boxes, with the arrows indicating the direction of transcription. Solid black circles represent 59-base elements, and open ellipses represent the *att*I1 site of the integron. Inverted repeats are depicted as parallel vertical lines. Open reading frames that are identical in all three bla_{VIM-2}-containing integrons are shaded gray.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank under accession number AM296017.

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