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A Novel Integron Gene Cassette Harboring VIM-38 Metallo-β-lactamase in a Clinical *Pseudomonas aeruginosa* Isolate

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Dear Editor,

Multidrug resistance of *Pseudomonas aeruginosa* has been attributed to both intrinsic and acquired antibiotic-resistance mechanisms. Multidrug-resistant (MDR) *P. aeruginosa* isolates have become a serious healthcare problem worldwide because they are resistant to almost all β-lactams, aminoglycosides, and quinolones. Production of zinc-dependent metallo-β-lactamases (MBLs) has been identified as the most significant mechanism among carbapenem-resistant *P. aeruginosa* isolates [1]. MBLs are of particular clinical concern because of their broad-spectrum activities, and Imipenemase (IMP)-, Verona Integron-Encoded Metallo-β-lactamase (VIM)-, Sao Paulo metallo-β-lactamase (SPM)-, Germany imipenemase (GIM)-, and New Delhi Metallo-β-lactamase (NDM)-type MBLs have been identified in *P. aeruginosa* worldwide [2]. Forty-six variants of VIM enzymes have been identified to date (http://www.lahey.org/Studies/other.asp). VIM-38 was recently identified in *P. aeruginosa* isolates in Turkey and was shown to differ from VIM-5 by a single substitution (Ala-316Val) [3]. In *P. aeruginosa*, VIM-type MBLs have been reported within mobile genetic elements such as integrons, which contribute to the dissemination of antibiotic resistance [3].

We here report a new clinical *P. aeruginosa* strain isolated from a blood sample on January 2015 at Rize State Hospital in Turkey and identified by using the API 32GN system (bioMerieux, Marcy-l'Etoile, France). Minimal inhibitory concentrations were determined on a VITEK system for the following antibiotics: piperacillin/tazobactam, ceftazidime, cefepime, amikacin, netilmicin, ciprofloxacin, levofloxacin, imipenem, meropenem, cefoperazone-sulbactam, and inducible β-lactamase. 16S rDNA sequencing was used for molecular identification, performed according to Cicek *et al* [4].

The *P. aeruginosa* isolate was screened for β-lactamase-encoding genes and the class 1–class 2 integrases conserved region by PCR. The primers used for detection of β-lactamase-encoding genes and class 1 and class 2 integron gene cassettes are listed in Table 1 [4-8].

The positive PCR product of the class 1 integron was cloned into pGEM-T easy vector (Promega, Madison, WI, USA) and then sequenced by Macrogen (Amsterdam, The Netherlands). Sequencing results were analyzed by using the BLAST alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST) and the multiple sequence alignment program CLUSTALW2 (http://www.

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Table 1. Primers used in the amplification of selected genes

Primers	5′→3′	Amplicon size	T_m (°C)
bla_{TEM}	F: AGTATTCAACATTTYCGTGT	847	56
	R: TAATCAGTGAGGCACCTATCTC		
bla_{SHV}	F: ATGCGTTATATTCGCCTGTG	843	55
	R: TTAGCGTTGCCAGTGCTC		
bla_{CTX-M1}	F: GCGTGATACCACTTCACCTC	260	
	R: TGAAGTAAGTGACCAGAATC		
bla_{CTX-M2}	F: TGATACCACCACGCCGCTC	341	
	R: TATTGCATCAGAAACCGTGGG		
bla_{GES}	F: ATGCGCTTCATTCACGCAC	863	56
	R: CTATTTGTCCGTGCTCAGGA		
bla_{VEB}	F: ATTTCCCGATGCAAAGCGT	542	55
	R: TTATTCCGGAAGTCCCTGT		
bla_{PER-2}	F: ATGAATGTCATCACAAAATG	860	45
	R: ATAATAGCTTCATTGGTTC		
bla_{KPC}	F: ATGTCACTGTATCGCCGTCT	893	55
	R: TTTTCAGAGCCTTACTGCCC		
bla_{IMP}	F: CATGGTTTGGTGGTTCTTGT	488	56
	R: ATAATTTGGCGGACTTTGGC		
bla_{VIM}	F: ATTGGTCTATTTGACCGCGTC	780	58
	R: TGCTACTCAACGACTGAGCG		
bla_{NDM}	F: GAGATTGCCGAGCGACTTG	497	57
	R: CGAATGTCTGGCAGCACACTT		
bla_{OXA-51}	F: TAATGCTTTGATCGGCCTTG	353	52
	R: TGGATTGCACTTCATCTTGG		
bla_{OXA-23}	F: GATCGGATTGGAGAACCAGA	501	
	R: ATTTCTGACCGCATTTCCAT		
bla_{OXA-40}	F: GGTTAGTTGGCCCCCTTAAA	246	
	R: AGTTGAGCGAAAAGGGGATT		
bla_{OXA-58}	F: AAGTAT TGGGGCTTGTGCTG	599	
	R: CCCCTCTGCGCTCTACATAC		
bla_{GIM-1}	F: TCGACACACCTTGGTCTG AA	477	
	R: AACTTCCAACTTTGCCATGC		
bla_{SPM-1}	F: AAAATCTGGGTACGCAAACG	271	
	R: ACATTATCCGCTGGAACAGG		
bla_{SIM-1}	F: TACAAGGGATTCGGCATC G	570	
	R: TAATGGCCT GTTCCCATGTG		
bla_{CMY}	F: GACAGCCTCTTTCTCCACA	1,000	50
	R: TGGAACGAAGGCTA CGTA		
5′-CS	F: GGCATCCAAGCAGCAAG		56
3′-CS	R: AAGCAGACTTGACCTGA		
hep51	F: GATGCCATCGCAAGTACGAG		55
hep74	R: CGGGATCCCGGACGGATGCACGATTTGTA		

Abbreviations: Tm, melting temperature; CS, conserved segment of class-I integron; hep51, forward primer of of class-II integron; hep75, reverse primer of of class-II integron; CMY cephalomycinase coding gene.

ebi.ac.uk/Tools/msa/clustalw2/).

Transferability of antibiotic resistance was tested according to the previously defined protocol [9], by using the rifampin-resistant *Escherichia coli* K-12 strain J53-2 as a recipient [4]. Susceptibility testing of the MBL-producing integron-positive *P. aeruginosa* isolate showed that it was resistant to imipenem, meropenem, piperacillin/tazobactam, ceftazidime, cefepime, and cefoperazone-sulbactam. PCR analysis showed that the isolate did not harbor any of the antibiotic resistance genes listed in Table 1, except for bla_{VIM}-type MBL. Sequence analysis of the bla_{VIM}-variant identified it as bla_{VIM-38}. The *P. aeruginosa* isolate contained a class 1 integron gene cassette, but not a class 2 integron gene cassette. The bla_{VIM-38}-harboring class 1 integron gene cassette was sequenced and was found to be 3,239 bp long. DNA sequence analysis revealed that bla_{VIM-38} MBL was located on the class 1 integron gene cassette together with *AAC(6′)-Ib/EmrE/aadA1* (Fig. 1). The conjugation assay revealed that the class 1 integron cassette is not transferable.

The bla_{VIM-38} gene was identified in *P. aeruginosa* isolates in Turkey in 2014, and found to be located in a class 1 integron containing only two gene cassettes (bla_{VIM-38}/orfD) [3]. This genetic structure has also been associated with the bla_{VIM-5} gene in a clinical isolate of *Enterobacter cloaceae* from Turkey [9]. Moreover, steady-state kinetic analyses in a study on the enzymatic properties of VIM-38 showed that VIM-38 hydrolyzed all of the tested penicillins, cephalosporins, and carbapenems [10].

In the present study, the class 1 integron included four gene cassettes with bla_{VIM-38} followed by *AAC(6′)-Ib, EmrE* (multi-drug transporter), and *aadA1*. This is the first report in Turkey of the bla_{VIM-38}/*AAC(6′)-Ib/EmrE/aadA1* gene cassette array. Therefore, we report a novel gene cassette array with an MBL gene in a *P. aeruginosa* clinical isolate, and this is the second report for the detection of VIM-38 in a *P. aeruginosa* isolate in Turkey with different hospitalization and isolation times.

In conclusion, the presence of class 1 integrons in *P. aeruginosa* leads to increased resistance to antibiotics. The present study demonstrates the emergence of VIM-producing MDR *P. aeruginosa* strains harboring class 1 integrons and a gene cassette in Turkey. In particular, the bla_{VIM-38} MBL gene appears to be spreading among *P. aeruginosa* isolates in Turkey.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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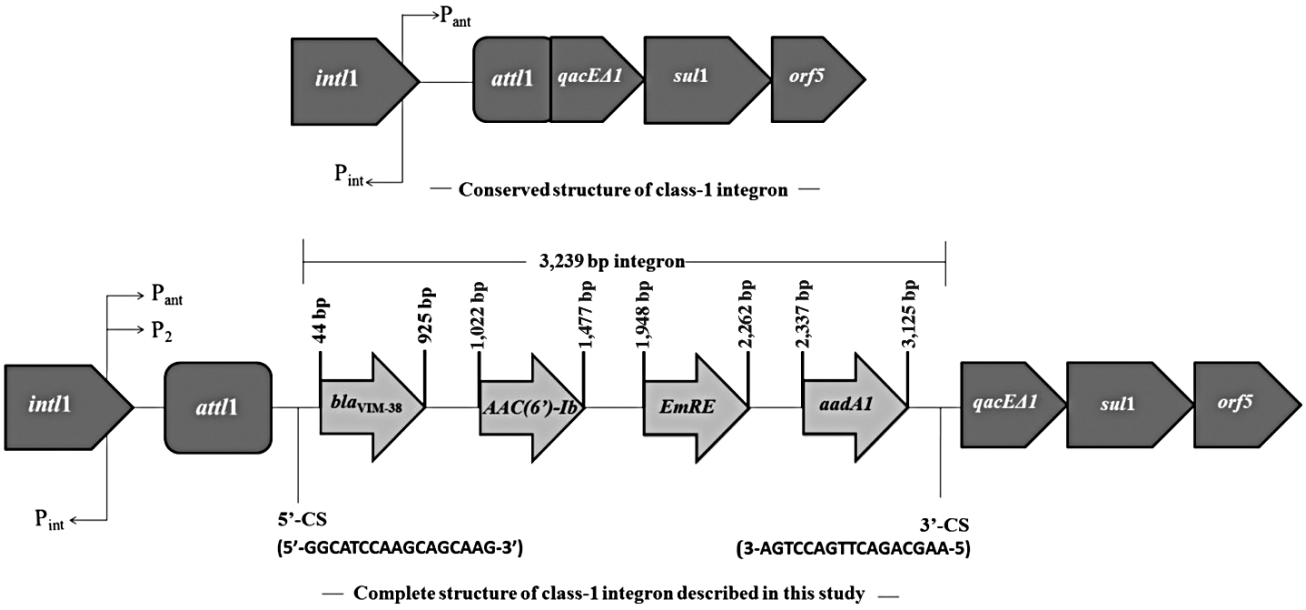

Fig. 1. Structure of the bla_{VIM-38}-carrying class 1 integron gene cassette in *Pseudomonas aeruginosa*.
Abbreviations: P_{int}, integrase promoter; P_{ant}, promoter of inserted gene(s); *intI*1, class 1 integrase; *attI*1, integron-associated recombination site; *qacEΔ1*, quaternary ammonium compound resistance gene cassette; *sul*1, sulfonamide resistance gene; bla_{VIM-38}, Verona Integron-Encoded Metallo-β-lactamase 38; CS, conserved segment.

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