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Dissemination of a Class I Integron Carrying VIM-2 Carbapenemase in *Pseudomonas aeruginosa* Clinical Isolates from a Hospital Intensive Care Unit in Annaba, Algeria

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Over the last decade, the increase of carbapenem resistance in *Pseudomonas aeruginosa* has been due mostly to impermeability because of OprD loss and the production of metallo-β-lactamases (MBL), including those of the IMP, VIM, SPM, GIM, SIM, AIM-1, FIM-1, and NDM families (1–4); also, active efflux and serine carbapenemases may contribute to carbapenem resistance in *P. aeruginosa* in some cases. VIM-1 was reported in the Mediterranean area in 1997 in a clinical isolate of *P. aeruginosa* in Verona, Italy (5), and in a clinical isolate of *Pseudomonas mosselii* isolated in 1994 in Genoa, Italy (6). A VIM-2 variant appeared in Marseille, France, in 1996 (7). VIM-2 has now spread as the predominant MBL variant among *P. aeruginosa* in all European Mediterranean countries (1), but in North Africa, the presence of VIM-2 has been reported only recently in Tunisia (2, 8, 9). Here we report the first molecular characterization of VIM-2-producing *P. aeruginosa* clinical isolates from Algeria that harbored a novel class I integron that also contained two gene cassettes encoding resistance to aminoglycosides (*aadB* and *aacA4*).

A total of 17 nonreplicate imipenem-resistant *P. aeruginosa* clinical isolates (imipenem MIC, >8 μg/ml, as determined by the Etest) recovered from December 2010 to September 2011 in a surgical intensive care unit at the University Hospital of Annaba, Algeria, were screened by PCR for the presence of MBL-encoding genes using primers previously described (10–12). Antibiotic susceptibility testing was performed using disk diffusion with the breakpoints and according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfm-microbiologie.org), and results are summarized in Table 1. An imipenem-EDTA synergy test (13) was positive for 14 out of 17 strains that were PCR positive for VIM-2 carbapenemase using the universal primers VIM-all-F (5′-TGGTCTACATGACC GCGTCT-3′) and VIM-all-R (3′-CGACTGAGCGATTTGTGT G-5′), with an expected PCR size of 766 bp. The characterization of class I integrons reported previously (11) showed a novel class I

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TABLE 1 Phenotypic and genotypic features of the 17 imipenem-resistant *P. aeruginosa* clinical isolates[a]

Strain	Date of isolation (day/mo/yr)	Patient age	Sex	Source	IMP MIC (μg/ml)	Resistance phenotype								VIM-2 production	Sequence type
						TIC	TCC	TZP	CAZ	GEN	CIP	FOS	COL		
1	15/12/2010	42 yr	M	Bronchial aspirate	12	R	R	R	R	R	S	S	S	+	ST1420
2	15/12/2010	18 yr	F	Blood	32	R	R	R	R	R	S	S	S	+	ST1420
3	28/12/2010	18 mo	M	Bronchial aspirate	12	R	R	R	R	R	S	S	S	+	ST162
4	25/02/2011	2 mo	M	Bronchial aspirate	8	R	R	R	R	R	R	R	S	+	ST1420
5	27/02/2011	7 yr	M	Bronchial aspirate	16	R	R	R	R	R	R	R	S	+	ST1420
6	08/03/2011	7 days	M	Bronchial aspirate	32	R	R	R	R	S	S	R	S	+	ST1420
7	22/03/2011	57 yr	M	Urinary catheter	12	R	R	R	R	R	R	S	S	+	ST1420
8	23/03/2011	43 yr	M	Urinary catheter	32	R	R	R	R	S	S	R	S	+	ST1420
9	18/04/2011	35 yr	M	Urine	12	R	R	R	R	S	S	R	S	+	ST1420
10	25/05/2011	7 yr	M	Pus	16	R	R	R	R	R	S	R	S	+	ST1420
11	19/06/2011	46 yr	M	Urine	16	R	R	R	R	S	R	S	S	+	ST1420
12[b]	22/06/2011	58 yr	F	Bronchial aspirate	32	R	R	R	R	R	S	S	S	−	ST1175
13[c]	22/07/2011	40 yr	M	Bronchial aspirate	32	R	R	R	R	S	S	R	S	−	ST1420
14	18/08/2011	54 yr	M	Wound	16	R	R	R	R	R	S	S	S	+	ST1420
15	27/08/2011	5 yr	M	Wound	12	R	R	R	R	S	S	R	S	+	ST1420
16[d]	10/09/2011	10 yr	M	Urine	16	R	R	R	R	R	R	R	S	−	ST654
17	27/08/2011	45 yr	M	Wound	12	R	R	R	R	R	S	S	S	+	ST1420

[a] CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; TCC, ticarcillin-clavulanic acid; TIC, ticarcillin; TZP, piperacillin-tazobactam; M, male; F, female; R, resistant; S, susceptible. MICs were determined by the Etest.

[b] Contains a $_{529}$CGA-to-TGA mutation, leading to a stop codon in the *oprD* gene.

[c] Exhibits a $_{193}$TGGA-to-TG:A deletion, leading to a stop codon in the *oprD* gene.

[d] Contains a $_{757}$TCG-to-TAG mutation, leading to a stop codon in the *oprD* gene.

integron that harbored the VIM-2 gene associated with two gene cassettes encoding aminoglycosides resistance (*aadB* and *aacA4*). However, the molecular investigation of the remaining three strains using primers previously described (3) revealed chromosomic mutations that created premature stop codons in the *oprD* gene. Complete multilocus sequence typing (MLST) (14) revealed that the 17 strains belong to 4 different sequence types (ST), including ST162 (one isolate), ST654 (one isolate), ST1175 (one isolate), and a new ST, ST1420 (14 isolates), recently submitted to the PubMLST database (http://pubmlst.org/paeruginosa) from China (January, 2013).

To the best of our knowledge, in North Africa, VIM-2-MBL-producing *P. aeruginosa* isolates have been reported only in Tunisia (8, 9, 11). VIM-2-producing *P. aeruginosa* clinical isolates have been reported in other countries in Africa, including 1 from a Hungarian tourist who was hospitalized in Egypt (15), 1 from a single patient who was transferred to Norway after being hospitalized in Ghana (16), 57 recovered in 2006 and 2007 in Kenya (17), and 15 from South Africa (18).

Our results demonstrate that national surveillance should be urgently implemented in Algeria to monitor and control the emergence and spread of carbapenemase-encoding genes.

Nucleotide sequence accession numbers. The full sequence of the class 1 integron has been deposited in the GenBank database under accession number JX120362.

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