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LETTER TO THE EDITOR

VIM-2-Producing *Pseudomonas* spp. in Uruguay: Sequence Types, Pulsotypes, and Class 1 Integrons Including New Variable Regions Featuring bla_{VIM-2} and bla_{GES-7}

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Metallo-β-lactamase (MBL) production constitutes a global concern due to its dissemination, its wide spectrum of hydrolysis, and its association with multiple resistance determinants (1). The most frequently detected MBLs in *Pseudomonas* spp. correspond to VIM and IMP derivatives, usually associated with class 1 integrons (2).

We characterized the MBLs present in the *P. aeruginosa* and *P. putida* group, nonredundant clinical isolates obtained from three hospitals in Uruguay from 2011 to 2013. Two of the hospitals were located in our capital city, Montevideo, Uruguay (M1 and M2), and the remaining hospital was in Florida, Uruguay (F1), located 90 km north of the former.

Isolates were identified with the Vitek 2 system (bioMérieux), and their identities were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker). MIC determination was performed by agar dilution methods and interpreted according to CLSI guidelines (3). MBL detection was done by PCR and sequencing (4), whereas class 1 integrons and their variable regions (VR) were characterized by PCR mapping (5, 6).

Pulsed-field gel electrophoresis (PFGE) analysis was performed as previously described (7), albeit using SpeI and running at 14°C with pulse times of 4 to 40 s for 20 h. Multilocus sequence typing (MLST) was performed as previously described (8). Primers trpEnF (CTGCGCTGTTTCAATCCGAC) and trpEnR (TCACCGTTCTTGATCACCGC) were designed for those isolates belonging to pulsotype 1.

We studied 1,202 *P. aeruginosa* and 59 *P. putida* isolates; 289/1,202 (24%) and 32/59 (54.2%), respectively, were resistant to at least one carbapenem. Furthermore, 19/289 (6.6%) and 9/32 (28.1%), respectively, displayed positive synergy tests (antibiotic resistance profiles are depicted in Fig. 1). Three *P. aeruginosa* isolates and one *P. putida* isolate could not be recovered for further analysis.

We detected three different *P. aeruginosa* pulsotypes and seven different *P. putida* pulsotypes, six of which were represented by a single isolate (Fig. 1). The most frequent *P. aeruginosa* pulsotype belonged in F1 and was further typed as ST155; on the other hand, M1 and M2 yielded sequence types ST1565 (first described in this work) and ST1195 (Fig. 1). VIM-2-producing *P. aeruginosa* ST155 isolates have been detected in Spain (9), whereas non-MBL-producing ST155 isolates have been reported in Spain, Australia, Canada, Germany, France, and Brazil (10, 11). Our situation differs from that described in previous reports from Colombia, where Vim-2-producing *P. aeruginosa* strains belong mainly to ST111 (12).

Amplicons for *intI1* and VR were obtained from 22/24 isolates. We detected three different VR arrays spanning 1,000, 3,500, and 4,000 bp and harboring the following genes: 5′ conserved sequence (5′CS)-bla_{VIM-2}-3′CS, 5′CS-bla_{VIM-2}-*aacA4-cmlA6-catB11*-3′CS (INTEGRALL database name In1270), and 5′CS-bla_{VIM-2}-bla_{GES-7}-*aacA7-aacA4-aacA7*-3′CS (INTEGRALL database name In1271).

Although the 1,000-bp VR is identical to In899 (13), this is the first description of In1270 and In1271; both include amikacin and/or tobramycin resistance genes (*aacA4* and/or *aacA7*); furthermore, in In1271 (detected in *P. putida*) the occurrence of bla_{GES-7} also adds resistance to aztreonam.

The coexistence of bla_{VIM} and bla_{GES} derivatives in *P. aeruginosa* isolates has already been described (14); however, to the best of our knowledge, this is the first description of an integron featuring both genes. Additionally, bla_{GES-7} in *P. aeruginosa* ST111 was described previously in 2014 by Guzvinec et al. (15).

In899 was detected in *P. aeruginosa* and *P. putida* obtained from M1, M2 (*P. aeruginosa* isolates from both hospitals), and F1 (*P. putida* isolates). In contrast, In1270 was present in *P. aeruginosa* isolates obtained from F1 and in *P. putida* isolates from M1 and M2. This strongly suggests horizontal-transmission events of these genetic elements among different *Pseudomonas* species isolated in distinct Uruguayan hospitals, as previously indicated (9, 16).

The detection of new VRs and the occurrence of ST155, previously described as a successful clone (17), constitutes an alert for health care systems.

Accession number(s). The sequences of integrons In1270 and In1271 were deposited into the EMBL database under accession numbers LT222321 and LT222320, respectively.

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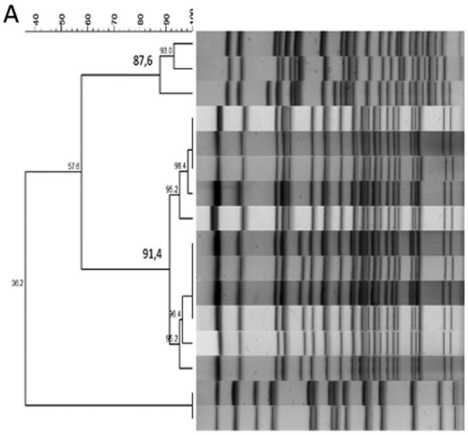
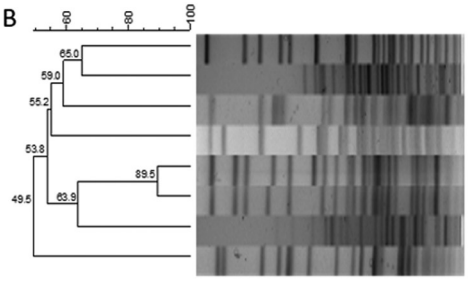


Strain	Source	MIC µ/ml							Integron	ST	PT	Date
		IPM	MEM	CAZ	GM	AK	CIP	CL				
M2-115	DFW	64	4 (I)	64	>64	32 (I)	128	4 (I)	In899	1195	1	05/2011
M2-213	FW	64	4 (I)	128	64	8 (S)	128	4 (I)	In899	1195	1	02/2013
M1-926	Urine	>64	16	16(I)	32	32 (I)	128	0.5	In899	1195	1	02/2013
F1-318	Resp. sec.	64	16	128	>64	8 (S)	0.125 (S)	2	In1270	155	2	07/2013
F1-663	Urine	64	16	64	2 (S)	2 (S)	0.125 (S)	2	In1270	155	2	09/2012
F1-782	Resp. sec.	64	16	32	1 (S)	1 (S)	0.125 (S)	2	In1270	155	2	07/2013
F1-523	Resp. sec.	64	16	32	4 (S)	8 (S)	0.125 (S)	2	In1270	155	2	08/2012
F1-089	Resp. sec.	64	16	32	32	8 (S)	0.125 (S)	2	In1270	155	2	07/2013
F1-149	Resp. sec.	>64	>64	128	2 (S)	2 (S)	0.125 (S)	2	In1270	155	2	03/2013
F1-356	Resp. sec.	64	16	32	32	4 (S)	0.125 (S)	2	In1270	155	2	11/2011
F1-517	Resp. sec.	64	16	64	32	8 (S)	0.5 (S)	2	In1270	155	2	04/2013
F1-931	Resp. sec.	>64	16	64	32	4 (S)	0.125 (S)	2	In1270	155	2	12/2013
F1-794	Resp. sec.	>64	16	32	64	8 (S)	0.125 (S)	2	In1270	155	2	11/2013
F1-559	Resp. sec.	>64	64	64	>64	8 (S)	0.5 (S)	4 (I)	In1270	155	2	05/2013
M2-602	Unknown	64	4 (I)	16 (I)	1 (S)	1 (S)	1 (S)	2	In899	1565	3	04/2011
M1-V2	PSW	64	4 (I)	16 (I)	1 (S)	1 (S)	1 (S)	2	In899	1565	3	03/2011



M1-144	Urine	>64	>64	64	32	8 (S)	0,125 (S)	4 (I)	In1270	nd	1	10/2012
M2-166	Unknown	>64	64	32	0,5 (S)	0,5(S)	256	4 (I)	In1270	nd	2	04/2012
F1-829	Pleural L.	>64	>64	32	32	0,5(S)	256	4 (I)	In899	nd	3	11/2011
F1-621	Resp. sec	64	16	32	64	8 (S)	0,125 (S)	4 (I)	In1270	nd	4	11/2012
M2-114	Urine	>64	>64	64	>64	0,5(S)	256	2 (S)	In1270	nd	5	02/2013
M1-189	Resp. sec.	>64	64	64	>64	0,5(S)	256	4 (I)	In1270	nd	5	12/2013
M2-717	Unknown	>64	64	>256	0,5 (S)	0,5(S)	0,125 (S)	2 (S)	In1271	nd	6	05/2012
F1-155	Urine	>64	>64	32	>64	8 (S)	0,125 (S)	4 (I)	In899	nd	7	07/2012

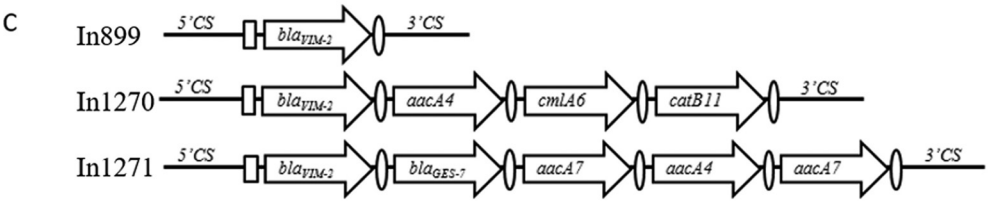


FIG 1 Dendrogram of PFGE results for *P. aeruginosa* (A) and *P. putida* (B). The name of each strain indicates the hospital of isolation. Abbreviations: DFW, diabetic foot wound; FW, foot wound; Resp. sec., respiratory secretions; PSW, plastic surgery wound; Pleural L, pleural liquid; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; GM, gentamicin; AK, amikacin; CIP, ciprofloxacin; CL, colistin; nd, not determined; ST, sequence type; PT, pulsotype; I, intermediate susceptibility; S, susceptible. MICs that do not have the letter "S" or "I" beside them are interpreted as resistant. (C) Genetic rearrangement of the three different variable regions found. Variable regions are not to scale. CS, conserved sequence. Each arrow represents a gene cassette, and ovals represent the 59-bp element.

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