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Two Novel Class I Integron Arrays Containing IMP-18 Metallo-β-Lactamase Gene in *Pseudomonas aeruginosa* Clinical Isolates from Puerto Rico

T. Martínez,[a] G. J. Vazquez,[a] E. E. Aquino,[a] R. V. Goering,[b] and I. E. Robledo[a]

Department of Microbiology and Medical Zoology, University of Puerto Rico, School of Medicine, San Juan, Puerto Rico,[a] and Department of Medical Microbiology, Creighton University, School of Medicine, Omaha, Nebraska, USA[b]

During a β-lactam resistance surveillance study, 12 IMP-18-positive *Pseudomonas aeruginosa* isolates belonging to 9 different pulsed-field gel electrophoresis groups were identified. In nine isolates, a class I integron with a novel gene array was identified that contained bla_{IMP-18} and $bla_{OXA-224}$, while in two isolates the class I integron contained bla_{IMP-18} and bla_{OXA-2} but in a new arrangement. Our findings show the dissemination of two novel class I integrons in *P. aeruginosa* from different regions of Puerto Rico.

Pseudomonas aeruginosa is an important nosocomial pathogen associated with high morbidity and mortality. Its treatment is, at times, very difficult because of the emergence of antibiotic-resistant isolates, for which the carbapenems are the treatment of choice. The metallo-β-lactamases (MBLs) are an important medical problem, because they can hydrolyze most β-lactam antibiotics, including the carbapenems (2, 13, 18). The MBLs include nine families with their respective variants: IMP, VIM, NDM, SPM-1, GIM-1, SIM-1, AIM-1, KHM-1 and DIM-1 (18, http://www.lahey.org). Although the IMP-type enzymes are more commonly identified in Asia, they have been reported sporadically worldwide (2, 18). The bla_{IMP} genes have been identified mainly in *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. and are frequently located on class I integrons, which can facilitate their horizontal spread (2, 18). In a recent review of IMP-type MBLs, 59 different class I integrons harboring various IMP gene cassettes were identified (18). The IMP-18 variant was first detected in the continental United States, followed by Mexico, Puerto Rico, and France (3, 6, 7, 14, 15). The objectives of this study were to characterize 12 IMP-18-positive *P. aeruginosa* isolates and to determine the genetic background of the bla_{IMP-18} isolates collected during a 6-month PCR-based surveillance study of β-lactam resistance in 17 hospitals from six different geographical regions of Puerto Rico (12).

Twelve of 272 multi-β-lactam-resistant *P. aeruginosa* isolates were identified as bla_{IMP} positive by PCR. The patients' basic epidemiological information and the susceptibility of the isolates to selected antibiotics, based on microdilution panels (TREK Diagnostic Systems), are shown in Table 1. Seven of the 12 isolates were obtained from a single hospital located in the Puerto Rico Medical Center. No bla_{IMP-18}-positive isolates were detected in the north and west regions. Only colistin demonstrated consistent antimicrobial activity (83%) against the isolates.

All organisms were screened by PCR using panels of family-specific β-lactamase primers for the detection of the following genes: KPC, IMP and VIM, TEM, SHV, OXA-1, -2, and -9, CTX-M extended-spectrum β-lactamases, and OXA carbapenemases. The bacterial DNA template, primers used, and the PCR conditions were as previously described (6, 8, 9, 15, 16, 17). Eleven isolates demonstrated additional β-lactamases: bla_{OXA-1} in 9, bla_{TEM} in 7, and bla_{OXA-2} in two isolates. After DNA sequencing of the PCR amplicon obtained with the primers for bla_{OXA-1}, the results obtained indicated that the amplicon differed from bla_{OXA-1} by 3 amino acid substitutions, making it a new variant, named $bla_{oxa-224}$. No additional β-lactamase genes were identified in one isolate (S1PA9-25).

The structure of the variable region of the class I integron containing the IMP-18 gene cassette was determined as previously described by Sánchez-Martínez et al. (14). All PCR amplicons generated were sequenced independently and bidirectionally at least twice. Sequence alignment and analysis were performed online using the BLAST program (www.ncbi.nlm.nih.gov). Two novel class I integrons with the following structures were identified: In706 (bla_{IMP-18}-*aadA43*Δ-bla_{OXA-2}-*gcuD*) in two isolates and In707 (bla_{IMP-18}-*aadA1*b-$bla_{OXA-224}$) in nine isolates (Fig. 1). The two new integron sequences were submitted to the INTEGRALL database (10; http://integrall.bio.ua.pt/) for integron (In) number assignment and to the GenBank database (see below).

The sizes of the class I integrons sequenced were 4,378 bp for In706 and 3,668 bp for In707, and their structures were compared to the three previously described *P. aeruginosa*-containing bla_{IMP-18} integrons isolated in Mexico (In96 and In169) and the United States (In133) (1, 3, 14). The location of bla_{IMP-18} in the first position in both integrons is similar to In96 and In169, but different from In133. The bla_{IMP-18} has been previously associated with bla_{OXA-2} in the In169 class I integron identified in Mexico, which harbors two copies of the *aadA1* gene cassette in the second and fourth positions with the bla_{OXA-2} gene between them (14). In In706, the bla_{OXA-2} gene was also identified in the third position as in In169, but with other gene cassettes. At the second position, a variant of the *aacA43* gene cassette (GenBank accession number HQ247816) was identified that differed by two amino acids (R60Q and R95K) and 6 nucleotide substitutions. Since it is not known if this variant is functional, the gene cassette was identified as *aacA43*Δ. A hypothetical *orfD* gene of unknown function, *gcuD*, located in the fourth position was previously found associated with a bla_{IMP-15} variant in a class I integron obtained from two *P.*

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Address correspondence to I. E. Robledo, iraida.robledo@upr.edu.

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TABLE 1 Baseline clinical information and antimicrobial susceptibilities to selected antibiotics of IMP-positive *P. aeruginosa* isolates

P. aeruginosa isolate	Geographical region[a]	Age (yrs)	Gender[b]	Anatomical site of collection[c]	Hospital unit[d]	Test agent[e] MIC (μg/ml)						
						FEP	ATM	TZP	IPM	MEM	DOR[f]	CST
M1PA9-12	San Juan metropolitan	73	M	RT	ICU	>16	>16	34/4	>8	>8	>2	2
M1PA9-15	San Juan metropolitan	66	M	UT	Gen Ward	>16	8	16/4	>8	>8	>2	2
S1PA9-25	South	0.08	M	RT	ICU	>16	<2	8/4	>8	<1	2	1
C1PA9-07	Central	56	M	UT	ICU	>16	>16	<8/4	>8	<1	2	4
E1PA9-8	East	92	M	RT	ICU	>16	>16	32/4	>8	>8	>2	1
MC1PA9-13	PRMC	27	F	RT	Gen Ward	>16	4	16/4	>8	>8	>2	4
MC1PA9-25	PRMC	63	M	UT	Gen Ward	>16	>16	>64/4	>8	>8	>2	2
MC1PA9-33	PRMC	58	M	Blood	Gen Ward	>16	16	>64/4	>8	>8	>2	2
MC1PA9-34	PRMC	60	F	Blood	ICU	>16	4	8/4	>8	>8	>2	2
MC1PA9-37	PRMC	65	M	UT	Gen Ward	>16	16	>64/4	>8	>8	>2	2
MC1PA9-38	PRMC	41	M	Blood	Gen Ward	>16	16	64/4	>8	>8	>2	2
MC7PA9-5	PRMC	89	M	UT	Gen Ward	>16	8	64/4	>8	>8	>2	2

[a] PRMC, Puerto Rico Medical Center.
[b] M, male; F, female.
[c] RT, respiratory tract; UT, urinary tract.
[d] ICU, intensive care unit; Gen Ward, general ward.
[e] Test agents and their Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints (in μg/ml): FEP, cefepime (≤8); ATM, aztreonam (≤8); TZP, piperacillin-tazobactam (≥64/4); IPM, imipenem (≤4); MEM, meropenem (≤4); DOR, doripenem (≤2); CST, colistin (≤2).
[f] The doripenem breakpoint value was obtained from the Doribax package insert.

aeruginosa isolates from Mexico (GenBank accession numbers GQ856540 and GQ856541) (11, 18). In all cases, *gcuD* was located near the 3′ conserved sequence (18). To our knowledge, this is the first time that the bla_{IMP-18} variant has been found associated with $bla_{OXA-224}$ in a class I integron (In707). $bla_{OXA-224}$ was recently identified in a class I integron (In662; GenBank accession number JN412067) but was not associated with an MBL gene (10; http://integrall.bio.ua.pt/). In isolate S1PA9-25, bla_{IMP-18} was located in the first position near the 5′ conserved region; however, the rest of the gene cassette(s), including the 3′ conserved sequence, was not detected.

Pulsed-field gel electrophoresis (PFGE) was performed as previously described (4, 5). A total of 9 distinct PFGE patterns, 2 related and 6 genetically unrelated groups, were identified in the 12 isolates (Fig. 2). For In707, the class I integron was found in 9 isolates (groups 1 to 6), and for In706 it was found in two (groups 8 and 9). Seven of the 12 isolates, harboring either In706 or In707, were obtained from a single Puerto Rico Medical Center hospital and belonged to one related and four unrelated PFGE groups. The presence of a bla_{IMP-18} class I integron in genetically related and unrelated isolates, either in a single institution or in several geographically unrelated hospitals, suggests that both clonal and horizontal transfer are contributing to its dissemination. However, Southern blot assays and PCR performed on genomic and plasmid

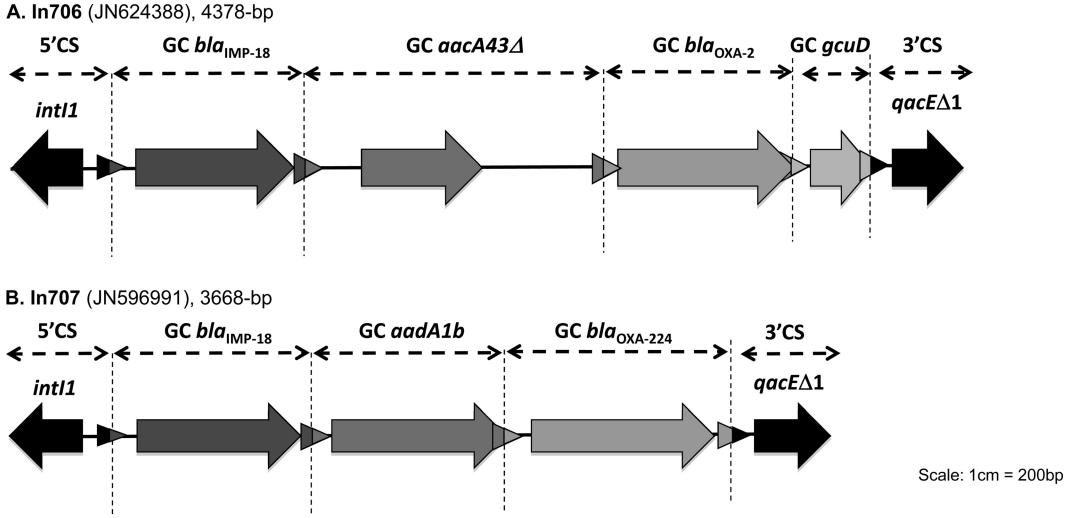

FIG 1 Schematic representation of two novel class I integrons containing bla_{IMP-18}. Two arrangements of the class 1 integron containing the bla_{IMP-18} gene were identified by a PCR mapping strategy described by Sánchez-Martínez et al. (14). In both class I integrons, the bla_{IMP-18} gene is located in the first position next to the 5′ conserved region. (A) In706, the disrupted *aacA43*Δ gene cassette (GC) was identified at the second position. bla_{OXA-2} and *gcuD* of a hypothetical *orfD* gene of unknown function were identified in the third and fourth positions, respectively. (B) The diagram of In707 shows the bla_{IMP-18} gene followed by *aadA1*b and $bla_{OXA-224}$. Open reading frames (ORFs) *attI1* and *attC* are indicated by arrows with different gray colors. Scale: 1 cm = 200 bp. Illustration courtesy of the INTEGRALL database (http://integrall.bio.ua.pt/).

FIG 2 Genetic relatedness determinations by pulsed-field gel electrophoresis. The In706 class I integron was detected in *P. aeruginosa* isolates with PFGE groups patterns 8 and 9 from a single hospital, while In707 was identified in groups 1 to 6 from either a single or several hospitals.

DNA with and without treatment with plasmid-safe DNase (Epicentre) to determine the integrons' locations gave inconclusive results (data not shown).

The finding of two new class I integrons containing bla_{IMP-18} are in agreement with previous reports that emphasized the plasticity and multiple arrangement of the class I metallo-β-lactamase integrons (1, 18).

Accession numbers. The PCR amplicon obtained with the primers for bla_{OXA-1} indicated that the amplicon differed from bla_{OXA-1} by 3 amino acid substitutions, making it a new variant, named $bla_{oxa-224}$. Its sequence was submitted to GenBank and assigned accession number JN596991. The two new integron sequences were submitted to the INTEGRALL database for integron number assignment and to the GenBank database, and the accession numbers JN624388 (In706) and JN596991 (In707) were assigned.

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