ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2008, p. 2943–2946 Vol. 52, No. 8
0066-4804/08/$08.00+0 doi:10.1128/AAC.00679-07

Metallo-β-Lactamase Gene *bla*$_{IMP-15}$ in a Class 1 Integron, In*95*, from *Pseudomonas aeruginosa* Clinical Isolates from a Hospital in Mexico▽

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Received 23 May 2007/Returned for modification 22 July 2007/Accepted 11 May 2008

During 2003, 40 carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates collected in a Mexican tertiary-care hospital were screened for metallo-β-lactamase production. Thirteen isolates produced IMP-15, and 12 had a single pulsed-field gel electrophoresis pattern. The *bla*$_{IMP-15}$ gene cassette was inserted in a plasmid-borne integron with a unique array of gene cassettes and was named In*95*.

Metallo-β-lactamase (MβL) production is an emerging mechanism of carbapenem resistance among enteric and nonfermenting gram-negative bacilli (11, 25). Five acquired MβL classes (IMP, VIM, SPM, GIM, and SIM) have been identified in various host organisms, most commonly, *Pseudomonas aeruginosa*, *Acinetobacter* species, and species of the family *Enterobacteriaceae* (4, 15, 17, 22, 28). MβL genetic determinants are usually associated with class 1 integron structures that may reside on mobile genetic elements, such as plasmids and transposons (10, 29).

Previous reports from the SENTRY Antimicrobial Surveillance Program have identified the SPM-1, IMP-16, VIM-2, and IMP-1 MβLs among *P. aeruginosa*, *Acinetobacter* spp., and *Pseudomonas fluorescens* isolates collected in South America (24). The same group has identified *P. aeruginosa* strains producing IMP-18 in Mexico. The gene encoding this MβL was found to be carried in a class 1 integron named In*96* (7). Reports from North America are still rare; however, VIM-2, IMP-7, IMP-18, and VIM-7 (1, 14, 23) have been identified in isolates from the United States and Canada. Recently, IMP-15 was identified in Kentucky in a *P. aeruginosa* isolate obtained from a patient who had previously been hospitalized in Mexico (19).

In the present study, we report on the characterization of *P. aeruginosa* clinical isolates producing the IMP-15 MβL from a Mexican tertiary-care hospital.

(This work was presented in part at the 45th Annual Meeting of the Infectious Diseases Society of America, 2007, San Diego, CA.)

A total of 255 nonduplicate *P. aeruginosa* isolates recovered from clinical specimens at the Hospital Civil de Guadalajara Fray Antonio Alcalde, Jalisco, Mexico, from January to December 2003 were initially tested for their antimicrobial susceptibilities by the broth microdilution method (Dade MicroScan Inc., Sacramento, CA). Fifty-six (22%) of these isolates were resistant to carbapenems, and 40 of them were available for further characterization. These 40 isolates were tested for their antimicrobial susceptibilities by agar dilution, and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (5). Their resistance profiles were as follows: imipenem, 100%; meropenem, 87%; ceftazidime, 61%; aztreonam, 24%, piperacillin, 19%; piperacillin-tazobactam, 14%, amikacin, 51%; gentamicin, 54%; and ciprofloxacin, 56%.

Molecular typing of the 40 carbapenem-resistant isolates was performed by pulsed-field gel electrophoresis (PFGE) (12, 27). Analysis of the restriction patterns showed the presence of one clone (clone A) with two subtypes that included 12 isolates. The remaining 28 isolates had unique PFGE patterns (data not shown).

The 40 carbapenem-resistant isolates were tested for MβL production by the double-disk synergy test (16) and Etest MBL (AB Biodisk, Solna, Sweden). In addition, they were tested by PCR with *bla*$_{VIM}$- and *bla*$_{IMP}$-specific primers (Table 1). Only 13 isolates displayed an MβL phenotype and yielded positive amplicons with the *bla*$_{IMP}$-specific primers. Twelve of the 13 isolates belonged to clone A (Table 2). These 13 isolates were also screened for class 1 integrons by using primers targeting the 5′ and 3′ conserved sequences (CSs) (2, 18), yielding products of 5.4 and 1.4 kb. The amplification products were digested with the restriction endonucleases DraIII and HaeI and always showed identical restriction profiles (data not shown), suggesting that all isolates harbored two integrons of identical structure.

A representative strain (strain 4677) from clone A was selected for further characterization of the MβL gene and the class 1 integrons. The 5.4- and 1.4-kb amplicons were separated by agarose gel electrophoresis, purified, and used for reamplification by PCR. Shotgun cloning of the 5.4-kb fragment was performed with the Zero Background cloning system (Invitrogen, Carlsbad, CA), according to the manufacturer's guidelines. The genetic library was sequenced by the chain termination method with a BigDye Terminator kit (Applied

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▽ Published ahead of print on 19 May 2008.

TABLE 1. Sequences of primers used in this study

Target	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Annealing temp (°C)	Reference or source
bla_{IMP}	IMP-F	GGAATAGAGTGGCTTAATTC	275	58	This study
	IMP-R	GCCAAGCTTCTATATTTGCG			
bla_{VIM}	VIM-F	GTGTTTGGTCGCATATCGC	380	58	This study
	VIM-R	CGCAGCACCAGGATAGAAG			
intI1	intl1[a]	CGTTCCATACAGAAGCTGG			1
	5′ CS[b]	GGCATCCAAGCAGCAAG	60		17
	3′ CS[b]	AAGCAGACTTGACCTGA			17
aadA1	aadA1-F	ATGAGGGAAGCGGTGATCG	792	60	This study
	aadA1-R	TTATTTGCGGACTACCTTG			
aacA4	aacA4-F	ATGACTGAGCATGACCTTG	508	56	This study
	aacA4-R	TGCGTGTTCGCTCGAATGCC			
aadA6	aadA6-F[b]	ATGAGTAACGCAGTACCCGC	634	54	This study
	aadA6-R[b]	CCCCAGTGGCAACGATATCC			
aacA7	aacA7-F	ATGGATAGTTCGCCGCTCGT	362	58	This study
	aacA7-R	GAGGCGAATTCGGTGCATCC			
qacH	qacH-F	CTGGCTCTTTCTGGCTATTG	325	60	This study
	qacH-R	TCAATGTGCGCTGACCTTGG			
oxa2	oxa2-F	ATGGCAATCCGAATCTTCGC	828	60	This study
	oxa2-R	TTATCGCGCAGCGTCCGAGT			
orfD	orfD-F[b]	CAGTATCTCAAACGCTGTG	223	56	This study
	orfD-R[b]	AATGTTAGAGCCAGAAGCC			
qacEΔ1	L1[a]	GCCCTACACAAATTGGGAGA	381	64	12
	R1[a]	AACACCGTCACCATGGCGCCG			12

[a] Primers used for PCR amplification and sequencing of *intI1* (partial sequence) and *qacEΔ1* genes of 5.4- and 1.4-kb class 1 integrons.
[b] Primers used for PCR amplification and sequencing of the variable region of 1.4-kb class 1 integron.

Biosystems, Foster City, CA), and analyses were carried out on an ABI Prism 3100 analyzer (Applied Biosystems). A total of 96 quality DNA sequences were obtained and assembled by using Phred-Phrap-Consed software (8). Sequence analysis revealed an integron containing seven gene cassettes (Fig. 1) that carried an aminoglycoside acetyltransferase-encoding gene, *aacA7* (3), in the first position. This cassette was followed by a bla_{IMP-15} cassette identical to that deposited in GenBank (GenBank accession number AY553333). The MβL gene cassette was located upstream of an array of gene cassettes con-

TABLE 2. Features of the bla_{IMP-15}-producing *Pseudomonas aeruginosa* clinical isolates

Isolate no.	Date of isolation (day/mo/yr)	Ward[a]	Origin	PFGE clone	Plasmid size(s) (kb)[b]	MIC (μg/ml)[c]								
						IMP	MER	CAZ	ATM	PIP	TZP	AMK	GEN	CIP
4667	13/02/2003	ICU	Blood	A	30	>128	128	>128	32	64	64	>128	128	32
4677	06/06/2003	CVS	Blood	A	30	>128	128	>128	16	64	64	>128	>128	64
4682-1	07/07/2003	S	Secretion	A	70, 30	128	128	>128	32	64	64	>128	>128	32
4696	29/09/2003	ICU	Catheter	A	30	128	128	>128	32	64	64	>128	>128	64
4698	11/10/2003	ICU	Urine	A	70, 30	>128	128	>128	16	64	64	>128	>128	64
4706	19/10/2003	ICU	Urine	A	30	32	128	>128	16	64	64	64	>128	64
4658	12/01/2003	IM	Secretion	A1	30	128	128	>128	16	64	64	>128	128	32
4679	26/06/2003	ICU	Pleural fluid	A1	30	128	128	>128	16	64	64	>128	128	64
4688	09/09/2003	PS	Secretion	A1	30	>128	128	>128	16	64	64	>128	>128	>128
4703	03/06/2003	IM	Secretion	A1	30	>128	128	>128	16	64	64	128	>128	64
4659	20/01/2003	ICU	Blood	A2	30	>128	128	>128	16	64	64	>128	>128	64
4680	04/07/2003	ICU	Catheter	A2	70, 30	128	128	>128	16	64	64	>128	128	32
4663	03/02/2003	IM	Urine	NR[d]	20	32	>128	>128	8	64	64	4	128	32

[a] CVS, cardiovascular surgery; ICU, intensive care unit; S, surgery; IM, internal medicine; PS, plastic surgery.
[b] Strains 4663 and 4703 each contain two additional plasmids of 1 and 3 kb.
[c] IMP, imipenem; MER, meropenem; CAZ, ceftazidime; ATM, aztreonam; PIP, piperacillin; TZP, piperacillin-tazobactam; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin.
[d] NR, nonrelated.

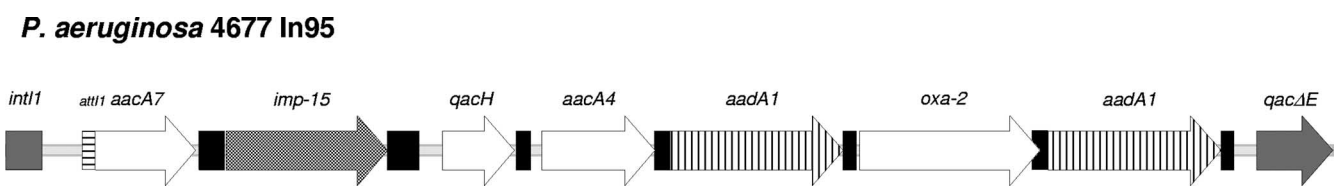

FIG. 1. Schematic representation of class 1 integron-containing clinical isolate *P. aeruginosa* 4677. In*95* carried bla_{IMP-15} and six additional gene cassettes. The open reading frames are indicated by arrows; the *attI* site is indicated, and the *attC* sites (59-base element) are indicated by filled rectangles.

taining *qacH*, *aacA4*, *aadA1*, bla_{OXA-2}, and another copy of *aadA1*, which was located upstream of *qacEΔ1*, which is usually found in the 3′ region of class 1 integrons (Fig. 1). The integron promoter region was sequenced and showed a single promoter (P_{ant}; −35 sequence TGGACA and −10 sequence TAAGCT) that was previously characterized as a weak promoter sequence (6). The structure of this unique integron, named In*95*, was confirmed by PCR with various combinations of primers (Table 1).

The structure of the 1.4-kb class 1 integron was revealed by overlapping PCR amplification and sequencing (Table 1). This additional conserved integron contained two gene cassettes, *aadA6* and *orfD*, inserted between *intI1* and *qacEΔ1* and was identical to In*51*, which has been reported in *P. aeruginosa* strains from China and India (9, 21).

The plasmid contents of the IMP-15-producing isolates were analyzed by the method of Kieser (13). All 12 isolates of clone A harbored a plasmid of 30 kb. Three of these isolates (isolates 4680, 4682-1, and 4698) also harbored a second plasmid of 70 kb. The IMP-15-producing strain (strain 4663) showing a PFGE pattern distinct from that of clone A and carried a 20-kb plasmid and smaller plasmids (Fig. 2A; Table 2). Southern blotting with a bla_{IMP-15}-specific DNA probe generated by PCR amplification with primers IMP-F and IMP-R (275 bp; Table 1) and labeled nonradioactively (ECL direct nucleic acid labeling and detection system; GE Healthcare, Piscataway, NJ) revealed that bla_{IMP-15} was carried on the 30-kb plasmid in all isolates of clone A and in the 20-kb plasmid in the genetically distinct isolate (Fig. 2B).

Plasmid preparations of *P. aeruginosa* 4677 and 4663 were transformed by electroporation into *Escherichia coli* DH10B and *P. aeruginosa* PU21 as described by Smith and Iglewski (26), and the recipient strains were plated onto LB agar supplemented with ceftazidime (1 μg/ml) or imipenem (4 μg/ml). Conjugation experiments were performed in liquid medium, as described by Miller (20). The two clinical isolates used for transformations were mated with *E. coli* J53-2 and *P. aeruginosa* PAO1, and the conjugation mixture was plated on LB plates supplemented with rifampin (100 μg/ml) and ceftazidime or imipenem at the same concentration used in the transformation experiments. Neither transfer experiment yielded colonies, suggesting that these plasmids were nontransferable under these experimental conditions.

The gene encoding IMP-15 was previously described in Thailand in a class 1 integron with a different gene cassette array (GenBank accession no. AY553333); however, the characterization of the isolates carrying bla_{IMP-15} was not reported in the literature. Interestingly, an IMP-15-producing *P. aeruginosa* isolate obtained from a patient with wound drainage was recovered at University of Kentucky HealthCare in August 2005. This patient had previously been hospitalized in Mexico (in March 2005) (19). Molecular analysis of the class 1 integron encoding bla_{IMP-15} (In*95*) from that patient showed that it was identical to the one reported in this work (19). These results

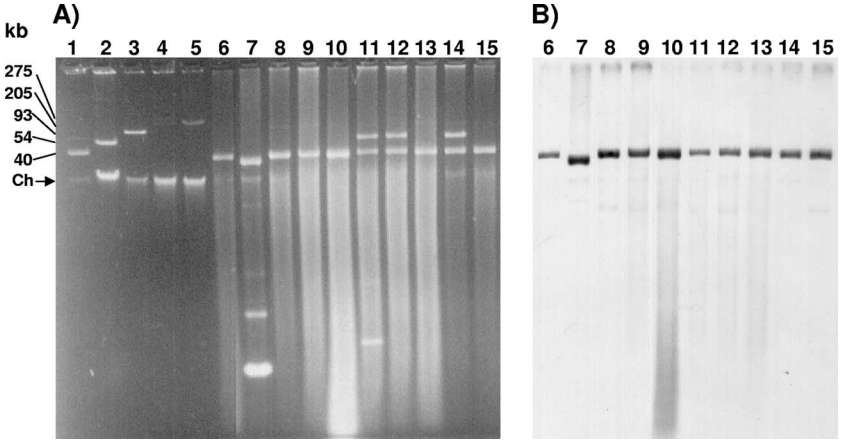

FIG. 2. Plasmid profile and Southern hybridization analysis of bla_{IMP-15}. (A) Plasmid profiles of the MβL-producing *P. aeruginosa* isolates. Plasmids were prepared from 10 isolates and were subjected to agarose gel electrophoresis. (B) Southern hybridization analysis. The 275-bp fragment amplified from bla_{IMP-15} by PCR was used as the DNA probe. Lanes: 1, plasmid R6K; 2, plasmid RP4; 3, plasmid R1; 4, pMG229; 5, pUD21; 6, strain 4658; 7, strain 4663; 8, 4667; 9, strain 4677; 10, strain 4679; 11, strain 4680; 12, strain 4682-1; 13, strain 4696; 14, strain 4698; 15, strain 4703. Ch, chromosomal DNA.

suggest that the In*95* class 1 integron could be broadly disseminated in Mexican hospitals.

Nucleotide sequence accession number. The sequence of integron In*95* carrying bla_{IMP-15} reported in this study has been deposited in the GenBank database and has been assigned accession number EF184216.

This work was supported by grant 37195-M from CONACYT and grant SALUD-2003-C01-009.

We thank T. Rojas and F. Reyna for excellent laboratory assistance. We thank Mariana Castanheira (JMI Laboratories) and Michael Dunn (Centro de Ciencias Genómicas, UNAM), Cuernavaca, Morelos, Mexico, for reviewing the manuscript.

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