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IMP-27, a Unique Metallo-β-Lactamase Identified in Geographically Distinct Isolates of *Proteus mirabilis*

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A novel metallo-β-lactamase gene, bla_{IMP-27}, was identified in unrelated *Proteus mirabilis* isolates from two geographically distinct locations in the United States. Both isolates harbor bla_{IMP-27} as part of the first gene cassette in a class 2 integron. Antimicrobial susceptibility testing indicated susceptibility to aztreonam, piperacillin-tazobactam, and ceftazidime but resistance to ertapenem. However, hydrolysis assays indicated that ceftazidime was a substrate for IMP-27.

Metallo-β-lactamases (MBLs) are class B carbapenemases that hydrolyze most β-lactam antibiotics except aztreonam (1, 2, 3). Clinically relevant MBLs include IMP, VIM, GIM, SPM, SIM, KHM, AIM, and NDM family members (4, 5). The majority of IMP and VIM MBLs are found in isolates of *Pseudomonas aeruginosa* and, in some cases, *Klebsiella pneumoniae* and *Escherichia coli* (2, 5). The bla_{IMP} and bla_{VIM} genes have been discovered in isolates collected from Asia, Europe, Australia, and North and South America (2, 3, 5). One contribution to the spread of MBLs is the mobilization of the genes via integrons (5, 6). Most genes encoding IMP and VIM MBLs are found as gene cassettes in either class 1 or class 3 integrons (3, 4, 5). Here, we report a novel IMP MBL, IMP-27, that was found in a class 2 integron and isolated from two geographically distinct isolates of *Proteus mirabilis* from the United States. These isolates were collected in 2009 and 2015 from two different patients who were from two different states in the upper plains region of the United States. Pulsed-field gel electrophoresis (PFGE) and plasmid analysis showed that the two isolates were not highly related. NotI restriction analysis demonstrated a ≥8-band difference in the PFGE patterns between the isolates (7), as the isolate from 2009 contained no plasmids and the 2015 isolate contained 4 plasmids. Southern analysis using an 80-bp digoxigenin (DIG)-labeled probe specific for bla_{IMP-27} demonstrated that the 2009 isolate housed bla_{IMP-27} on the chromosome and the 2015 isolate housed the gene on both the chromosome and the high-molecular-weight plasmids (8). Antimicrobial susceptibilities were determined by a combination of Vitek 2 (bioMérieux, Hazelwood, MO), microbroth panel (Trek Diagnostic Systems, Cleveland, OH), and disk diffusion (9). Results were interpreted using Clinical and Laboratory Standards Institute guidelines (10). Both isolates were resistant to ceftriaxone (MIC, 64 μg/ml) and had MICs of >8 μg/ml to imipenem, meropenem, and doripenem. As expected, the isolates were susceptible to aztreonam by disk diffusion (≥21 mm) but atypically susceptible to ceftazidime (≤4 μg/ml) and piperacillin-tazobactam (≤4 μg/ml).

Multiplex PCR using the ARM-D for β-lactamase ID kit (Streck, Inc.) identified a bla_{IMP-1}-like gene in both isolates. Flanking primers were used to amplify the entire gene from the two isolates, and sequence analyses of these amplicons identified bla_{IMP-27} (GenBank accession number JF894248). The translated product of bla_{IMP-27}, IMP-27, had 87.4% identity to IMP-8 and 79.7% identity to IMP-1 (11). The isoelectric point for IMP-27 was determined to be 6.4 (12). To determine the kinetic parameters of IMP-27, bla_{IMP-27} was PCR amplified from total DNA extracted from the 2009 *P. mirabilis* isolate (13). The PCR product was subcloned into a pET-9a-positive (pET-9a$^+$) expression vector (Novagen, Darmstadt, Germany) and overexpressed in *E. coli* BL21(DE3)/pLysS (Promega, Madison, WI) using 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). IMP-27 was purified by anion-exchange chromatography, HiTrap Q FF column (GE Healthcare Life Sciences, Little Chalfont, United Kingdom), and hydrophobic interaction chromatography using the HiTrap Butyl HP (GE Healthcare Life Sciences). The purity of the final preparation was >98% as determined by SDS-PAGE. The kinetic properties of the purified IMP-27 (Table 1) were calculated for imipenem, meropenem, cefotaxime, ceftazidime, cefepime, aztreonam, piperacillin, and nitrocefin by measuring the initial hydrolysis rates using a UV-2550 spectrophotometer (Shimadzu Co., Kyoto, Japan). K_i values for EDTA and dipicolinic acid were determined using nitrocefin as the reporter substrate. The hydrolytic efficiencies of IMP-27 were experimentally compared to IMP-1. IMP-27 hydrolyzed all β-lactams tested with the exception of aztreonam and piperacillin. K_i values of IMP-27 for aztreonam and piperacillin were >23,000 μM and 3,000 μM, respectively, whereas IMP-1 showed a high affinity against piperacillin with a K_m value of 330 μM. The hydrolytic efficiency (k_{cat}/K_m) of imipenem for IMP-27 was 1.1×10^5 M^{-1} s^{-1} compared to 1.8×10^6

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TABLE 1 Kinetic parameters of purified IMP-27 and IMP-1

Antibiotic	IMP-27			IMP-1		
	K_m or K_i (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	K_m or K_i (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
Piperacillin	3,000 ± 390[a]	ND[b]	ND	330 ± 12[a]	40 ± 1.5[c]	1.2×10^5
Cefotaxime	24 ± 1.9	20 ± 0.66	8.6×10^5	9.8 ± 1.2	16 ± 0.34	1.6×10^6
Ceftazidime	54 ± 4.3	0.7 ± 0.03	1.3×10^4	46 ± 3.8	7.4 ± 0.10	1.6×10^5
Cefepime	53 ± 3.6	8.1 ± 0.37	1.5×10^5	42 ± 1.0	15 ± 0.36	3.6×10^5
Aztreonam	>23,000 NH[d]	NH	ND	>23,000 NH	NH	ND
Imipenem	310 ± 19[a]	34 ± 0.38	1.1×10^5	28 ± 2.0	52 ± 1.5	1.8×10^6
Meropenem	2.3 ± 0.8[a]	3.4 ± 0.53	1.5×10^6	4.3 ± 0.7	8.2 ± 0.71	2.0 ± 10^6
Nitrocefin	36 ± 3.1	370 ± 15	1.0×10^7	3.9 ± 0.37	270 ± 17	7.0×10^7

[a] The K_m values were measured as K_i with nitrocefin as the reporter substrate.
[b] ND, not determined.
[c] The k_{cat} values were derived from initial rate measurements at more than 5 times higher than the concentration of K_i.
[d] NH, no hydrolysis detected.

M^{-1} s^{-1} for IMP-1. IMP-27 and IMP-1 were efficiently inactivated by metal chelators showing K_i values for EDTA of 11 ± 0.9 mM and 1.5 ± 0.11 mM and dipicolinic acid K_i values of 4.6 ± 0.13 mM and 0.44 μM ± 0.017 mM, respectively. The combination of amino acid substitutions observed in IMP-27 resulted in a lower catalytic efficiency compared to IMP-1 for imipenem. In addition, the K_i values of EDTA and dipicolinic acid for IMP-27 were ~10 times higher than those observed for IMP-1.

To determine the genetic backbone of bla_{IMP-27} in these isolates, a series of PCR amplicons was generated using the GenomeWalker universal kit (Clontech, Mountain View, CA) with primers listed in Table 2. Sequence analysis of these PCR fragments identified the bla_{IMP-27} gene within a class 2 integron (Fig. 1A) (GenBank accession number KF501391). bla_{IMP-27} was identified within the first gene cassette instead of the dihydrofolate reductase (*dfrA1*) gene, which typically occupies this position (14, 15, 16). It has been shown experimentally that gene cassettes located immediately after the integron promoter have increased expression compared to subsequent gene cassettes (17, 18, 19). The putative class 2 integron −35 and −10 promoter sequences, the transcriptional start site (TSS), the core site, and the bla_{IMP-27} start codon, all of which are required for gene cassette transcription and site-specific recombination, are represented within the *attL2* recombination site (Fig. 1B) (20, 21, 22).

A cassette typically consists of a resistance gene linked to an *attC* site (23, 24, 25, 26). Each gene cassette's *attC* site contains four core sites: 1L, 2L, 2R, and 1R. Core site 1L is typically the reverse complement of 1R and 2L is typically the reverse complement of 2R, resulting in the formation of a hairpin structure upon recombination. Within the 1R core site is a conserved GTT sequence, which is required for the binding of the IntI2 enzyme and thus recombination of a gene cassette between the G and T bases (22, 27, 28, 29). Within the bla_{IMP-27} *attC* site, this recombination site lies directly adjacent to the beginning of the *sat2* gene cassette (Fig. 1C).

To our knowledge, the data presented in this report describe several firsts. (i) This is the first publication identifying an IMP-type MBL in a *P. mirabilis* isolate. (ii) We report the genetic identification and kinetic analysis of a novel IMP MBL, IMP-27. The kinetic data support the susceptibility to both aztreonam and piperacillin. It is interesting that this enzyme did not confer resistance to ceftazidime for the *P. mirabilis* isolates even though the kinetic data indicated ceftazidime hydrolysis. (iii) bla_{IMP-27} was identified in a class 2 integron, which is very unusual, as class 2 integrons contain a premature stop codon in their integrase gene, leading to little genetic diversity among class 2 integrons (16, 19, 20, 21). (iv) This novel IMP MBL is distinct from most other IMP β-lactamases and has been identified in three geographically distinct locations within a 6-year period. Two of the unrelated isolates were described in this report, and the other *P. mirabilis* isolate was identified in Ontario, Canada (30).

The identification of MBLs in *P. mirabilis* is rare and may be due to the unique susceptibility profile of these isolates. Organisms expressing only a MBL are typically resistant to all β-lactams

TABLE 2 Primers utilized in combination with GenomeWalker to determine the genetic backbone of bla_{IMP-27}

Primer name	Primer type	Sequence (5′ to 3′)	Target	Source
IMP2813F	Primary	CGAGAAGCTTGAAGAAGGT	3′ bla_{IMP}	This work
AP1		GTAATACGACTCACTATAGGGC		GenomeWalker
IMP-PMF2	Nested	CAAGACAACGTAGTAGTTTGG	3′ bla_{IMP}	This work
AP2		ACTATAGGGCACGCGTGGT		GenomeWalker
IMP-PMR1	Primary	GTATCTTTAGCAGTAAATGG	5′ bla_{IMP}	This work
AP1		GTAATACGACTCACTATAGGGC		GenomeWalker
IMP-PMR5	Nested	CCACCAAACGTGTTTAGTAAC	5′ bla_{IMP}	This work
AP2		ACTATAGGGCACGCGTGGT		GenomeWalker
PmintI2F1	Primary	CCTGACCTCTTCACTGCCC	3′ *intI2*	This work
AP1		GTAATACGACTCACTATAGGGC		GenomeWalker
PmintI2F2	Nested	CAGCAGACATGTAGCCATAAACACGC	3′ *intI2*	This work
AP2		ACTATAGGGCACGCGTGGT		GenomeWalker

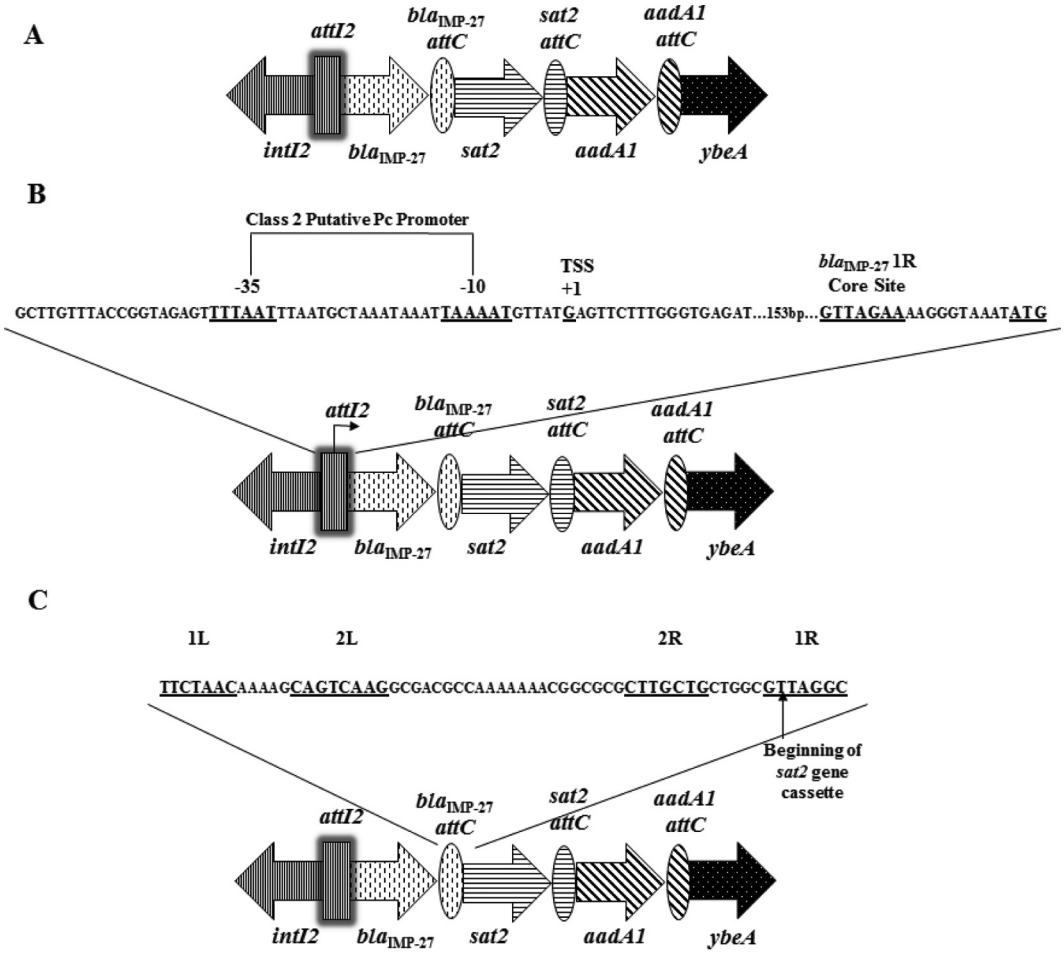

FIG 1 (A) Schematic representation of the class 2 integron harbored within the *P. mirabalis* isolates. The vertical rectangle represents the *attL2* site found in the *intL2* gene cassette. Each gene cassette consists of a gene, represented by an arrow, and its corresponding *attC* site, represented by ovals. (B) Schematic representation of the class 2 integron putative promoter sequence, transcriptional start site, core site, and bla_{IMP-27} start codon. (C) Schematic representation of the *P. mirabilis* integron 2 *dfrA1 attC* recombination site.

except aztreonam, but *P. mirabilis* isolates producing IMP-27 are also susceptible to ceftazidime and piperacillin-tazobactam; therefore, the identification of this novel MBL found in three geographically distinct *P. mirabilis* isolates is a concern. It is important for clinical microbiologists to be aware of this unique susceptibility profile when this MBL is harbored within *P. mirabilis*. Identification of this unique susceptibility profile by clinical microbiologists will aid in the surveillance and infection control measures needed to curb the spread of these types of resistance genes. It may also be prudent to closely monitor patients infected with an IMP-27-producing organism for the emergence of resistance if either ceftazidime or ceftazidime-avibactam are used to treat the infection.

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