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, blaIMP-27, was identified in unrelated Proteus mirabilis isolates from two geographically distinct locations in the United States. Both isolates harbor blaIMP-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 determined 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 blaIMP and blaVIM 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 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 blaIMP-27 demonstrated that the 2009 isolate housed blaIMP-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 blaIMP-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 blaIMP-27 (GenBank accession number JF894248). The translated product of blaIMP-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, blaIMP-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<sub>i</sub> 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<sub>i</sub> values for 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<sub>i</sub> value of 330 μM. The hydrolytic efficiency (k<sub>catalytic</sub>/K<sub>i</sub>) of imipenem for IMP-27 was 1.1 × 10<sup>5</sup> M<sup>-1</sup> s<sup>-1</sup> compared to 1.8 × 10<sup>6</sup>
M\(^{-1}\) s\(^{-1}\) for IMP-1. IMP-27 and IMP-1 were efficiently inactivated by metal chelators showing \(K_v\) values for EDTA of 11 ± 0.9 mM and 1.5 ± 0.11 mM and dipicolinic acid \(K_v\) values of 4.6 ± 0.13 mM and 0.44 \(\mu\)M ± 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 \(K_v\) 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 \(att\)L2 recombination site (Fig. 1B) (20, 21, 22).

A cassette typically consists of a resistance gene linked to an \(att\)C site (23, 24, 25, 26). Each gene cassette’s \(att\)C 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 \(sat\)2 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 \(P\). \textit{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 \(P\). \textit{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 \(\beta\)-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 \(P\). \textit{mirabilis} isolate was identified in Ontario, Canada (30).

The identification of MBLs in \(P\). \textit{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 \(\beta\)-lactams.

### Table 1: Kinetic parameters of purified IMP-27 and IMP-1

| Antibiotic | \(K_m\) or \(K_v\) (\(\mu\)M) | \(k_{cat}\) (s\(^{-1}\)) | \(k_{cat}/K_m\) (M\(^{-1}\) s\(^{-1}\)) | \(K_m\) or \(K_v\) (\(\mu\)M) | \(k_{cat}\) (s\(^{-1}\)) | \(k_{cat}/K_m\) (M\(^{-1}\) s\(^{-1}\)) |
|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Piperacllin | 3,000 ± 390\(^a\) | ND\(^b\) | ND | 330 ± 12\(^a\) | 40 ± 1.5\(^c\) | 1.2 \(\times\) 10\(^5\) |
| Cefotaxime | 24 ± 1.9 | 20 ± 0.66 | 8.6 \(\times\) 10\(^5\) | 9.8 ± 1.2 | 16 ± 0.34 | 1.6 \(\times\) 10\(^6\) |
| Ceftazidime | 54 ± 4.3 | 0.7 ± 0.03 | 1.3 \(\times\) 10\(^5\) | 46 ± 3.8 | 7.4 ± 0.10 | 1.6 \(\times\) 10\(^5\) |
| Cefepime | 53 ± 3.6 | 8.1 ± 0.37 | 1.5 \(\times\) 10\(^5\) | 42 ± 1.0 | 15 ± 0.36 | 3.6 \(\times\) 10\(^8\) |
| Aztreonam | >23,000 NH\(^d\) | NH | ND | >23,000 NH | NH | ND |
| Imipenem | 310 ± 19\(^a\) | 34 ± 0.38 | 1.1 \(\times\) 10\(^5\) | 28 ± 2.0 | 52 ± 1.5 | 1.8 \(\times\) 10\(^4\) |
| Meropenem | 2.3 ± 0.8\(^a\) | 3.4 ± 0.53 | 1.5 \(\times\) 10\(^5\) | 4.3 ± 0.7 | 8.2 ± 0.71 | 2.0 ± 10\(^4\) |
| Nitrofisin | 36 ± 3.1 | 370 ± 15 | 1.0 \(\times\) 10\(^5\) | 3.9 ± 0.37 | 270 ± 17 | 7.0 \(\times\) 10\(^7\) |

\(\text{a}\) The \(K_m\) values were measured as \(K_v\) with nitrofisin as the reporter substrate.

\(\text{b}\) ND, not determined.

\(\text{c}\) The \(k_{cat}\) values were derived from initial rate measurements at more than 5 times higher than the concentration of \(K_v\).

\(\text{d}\) NH, no hydrolysis detected.

### 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 | CGAGAGCTTGAGAAGG | 3’ \(bla_{IMP}\) | This work |
| AP1 | | GTAATAGCTACATAGGCC | This work |
| IMP-PF2 | Nested | CAAGGACAGTGATTGTG | This work |
| AP2 | | ACTATAGGGCGACGGGT | This work |
| IMP-PMR1 | | GTATCGTTAGCAGTAAAGG | This work |
| AP1 | Primary | GTATAGAGCTCACTATAGGGC | This work |
| IMP-PMR5 | Nested | CCAACCAAGCTTATTAGAC | This work |
| AP2 | | ACTATAGGGCGACGGGT | This work |
| Pint12F1 | Primary | CCTAGCTCTTGACTGCC | 3’ intI2 | This work |
| AP1 | | GTATAGAGCTCACTATAGGGC | This work |
| Pint12F2 | Nested | CAGCAGACATGAGCCATATAAACGCG | 3’ intI2 | This work |
| AP2 | | ACTATAGGGCGACGGGT | This work |
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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**REFERENCES**

1. Bebrone C. 2007. Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol 74:1686–1701. [http://dx.doi.org/10.1016/j.bcp.2007.05.021](http://dx.doi.org/10.1016/j.bcp.2007.05.021).
2. Maltezou HC. 2009. Metallo-β-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? Int J Antimicrob Agents 33:405.e1–405.e7.
3. Walsh TR. 2005. The emergence and implications of metallo-β-lactamases in Gram-negative bacteria. Clin Microbiol Infect 11(Suppl):2–9. [http://dx.doi.org/10.1111/j.1469-0691.2005.01264.x](http://dx.doi.org/10.1111/j.1469-0691.2005.01264.x).
4. Khosravi Y, Tay ST, Vadivelu J. 2011. Analysis of integrons and associated gene cassettes of metallo-β-lactamase-positive *Pseudomonas aeruginosa* in Malaysia. J Med Microbiol 60:988–994. [http://dx.doi.org/10.1099/jmm.0.029868-0](http://dx.doi.org/10.1099/jmm.0.029868-0).
5. Walsh TR, Toleman MA, Poirel L, Nordmann P. 2005. Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev 18:306–325. [http://dx.doi.org/10.1128/CMR.18.2.306-325.2005](http://dx.doi.org/10.1128/CMR.18.2.306-325.2005).
6. Ramírez MS, Píneiro S, Argentinian Integron Study Group, Centron D. 2010. Novel insights about class 2 integrons from experimental and genomic epidemiology. Antimicrob Agents Chemother 54:699–706. [http://dx.doi.org/10.1128/AAC.01392-08](http://dx.doi.org/10.1128/AAC.01392-08).
7. Sabbuba NA, Mahenthiralingam E, Stickler DJ. 2003. Molecular epide-
miology of Proteus mirabilis infections of the catheterized urinary tract. J
Clin Microbiol 41:4961–4965.
8. Fowler RC, Hanson ND. 2014. Emergence of carbapenem resistance
due to the novel insertion sequence ISPa8 in Pseudomonas aeruginosa.
PLoS One 9(3):e91299. http://dx.doi.org/10.1371/journal.pone.0091299.
9. Moland ES, Kim S-Y, Hong SG, Thomson KS. 2008. Newer β-lactama-
es: clinical and laboratory implications, part II. Clin Microbiol NewsL
30:79–85. http://dx.doi.org/10.1111/j.1574-6976.2008.00001.
10. Clinical and Laboratory Standards Institute. 2012. Performance stan-
dards for antimicrobial susceptibility testing; 22nd informational supple-
ment. CLSI M100-S22. Clinical and Laboratory Standards Institute,
Wayne, PA.
11. Tada T, Huong PH, Miyoshi-Akiyama T, Shimada K, Phuong DM, Anh
NQ, Qhmagari N, Kirikae T. 2015. IMP-51, a novel IMP-type metallo-
β-lactamase with increased doripenem- and meropenem-hydrolyzing ac-
tivities, in a carbapenem-resistant Pseudomonas aeruginosa clinical isolate.
Antimicrob Agents Chemother 59:7090–7093. http://dx.doi.org/10.1128/
AAC.01611-15.
12. Sanders CC, Sanders WE, Jr, Moland ES. 1986. Characterization of
β-lactamas in situ on polyacrylamide gels. Antimicrob Agents Chemother
30:951–952. http://dx.doi.org/10.1128/AAC.30.6.951.
13. Roth AL, Hanson ND. 2013. Rapid detection and statistical differen-
tiation of KPC gene variants in Gram-negative pathogens by use of high-
resolution melting and ScreenClust analyses. J Clin Microbiol 51:61–65.
http://dx.doi.org/10.1128/JCM.02193-12.
14. Barlow RS, Gobius KS. 2006. Diverse class 2 integrons in bacteria from
beef cattle sources. J Antimicrob Chemother 58:1133–1138. http://dx.doi.
org/10.1093/jac/dkl423.
15. Hansson K, Sundstrom L, Pelletier A, Roy PH. 2002. IntI2 integron in-
grase in Tn7. J Bacteriol 184:1712–1721. http://dx.doi.org/10.1128/JB.
184.6.1712-1721.2002.
16. Wei Q, Hu Q, Li S, Lu H, Chen G, Shen B, Zhang P, Zhou Y. 2014.
A novel functional class 2 integron in clinical Proteus mirabilis isolates.
J Antimicrob Chemother 69:973–976. http://dx.doi.org/10.1093/jac/dkt456.
17. Partridge SR, Tsafnat G, Coiera E, Iredell JR. 2009. Gene cassettes and
cassette arrays in mobile resistance integrons. FEMS Microbiol Rev 33:
757–784. http://dx.doi.org/10.1111/j.1574-6976.2009.00175.s.
18. Collis CM, Hall RM. 1995. Expression of antibiotic resistance genes in the
integrated cassettes of integrons. Antimicrob Agents Chemother 39:155–
162. http://dx.doi.org/10.1128/AAC.39.1.155.
19. da Fonseca EL, dos Santos Freitas F, Vicente AC. 2011. Pce promoter
from class 2 integrons and the cassette transcription pattern it evokes.
J Antimicrob Chemother 66:797–801. http://dx.doi.org/10.1093/jac/
dk011.
20. Gillings MR. 2014. Integrons: past, present, and future. Microbiol Mol
Biol Rev 78:257–277. http://dx.doi.org/10.1128/MMBR.00056-13.
21. Cambray G, Guerout A, Mazel D. 2010. Integrons. Annu Rev Genet
44:141–166. http://dx.doi.org/10.1146/annurev-genet-102209-163504.
22. Stokes HW, O’Gorman DB, Recchia GD, Parsekhan M, Hall RM. 1997.
Structure and function of 59-base element recombination sites associated
with mobile gene cassettes. Mol Microbiol 26:731–745. http://dx.doi.org/
10.1046/j.1365-2958.1997.009180.x.
23. Hall RM. 2012. Integrons and gene cassettes: hotspots of diversity in
bacterial genomes. Ann N Y Acad Sci 1267:71–78. http://dx.doi.org/10.
1111/j.1749-6632.2012.06588.x.
24. Partridge SR. 2011. Analysis of antibiotic resistance regions in gram-
negative bacteria. FEMS Microbiol Rev 35:820–855. http://dx.doi.org/
10.1111/j.1574-6976.2011.00277.x.
25. Recchia G, Hall RM. 1995. Gene cassettes: a new class of mobile element.
Microbiology 141:3015–3027. http://dx.doi.org/10.1099/13500872-141
-12-3015.
26. Stokes HW, Hall RM. 1989. A novel family of potentially mobile DNA
elements encoding site-specific gene-integration function: integrons. Mol
Microbiol 3:1669–1683. http://dx.doi.org/10.1111/j.1365-2958.
1989.tb00153.x.
27. Hall RM, Brookes DE, Stokes HW. 1991. Site-specific insertion of genes
into integrons: role of the 59-base element and determination of the re-
combination cross-over point. Mol Microbiol 5:1941–1959. http://dx.doi.
org/10.1111/j.1365-2958.1991.tb00817.x.
28. Johansson C, Kamali-Moghaddam M, Sundstrom L. 2004. Integron
integrase binds to bulged hairpin DNA. Nucleic Acids Res 32:4033–4043.
http://dx.doi.org/10.1093/nar/gkh730.
29. Mazel D. 2006. Integrons: agents of bacterial evolution. Nat Rev Micro-
bio 4:608–620. http://dx.doi.org/10.1038/nrmicro1462.
30. Tijet N, Lo S, Siebert H, MacNeill M, Rawte P, Farrell DJ, Low DE,
Patel SN, Melano RG. 2012. Detection of IMP-27 metallo-β-lactamase in
Proteus mirabilis, ON, Canada, abstr C2-090. Abstr 52nd Intersci Conf
Antimicrob Agents Chemother.