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IMP-51, a Novel IMP-Type Metallo-β-Lactamase with Increased Doripenem- and Meropenem-Hydrolyzing Activities, in a Carbapenem-Resistant *Pseudomonas aeruginosa* Clinical Isolate

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A meropenem-resistant *Pseudomonas aeruginosa* isolate was obtained from a patient in a medical setting in Hanoi, Vietnam. The isolate was found to have a novel IMP-type metallo-β-lactamase, IMP-51, which differed from IMP-7 by an amino acid substitution (Ser262Gly). *Escherichia coli* expressing bla_{IMP-51} showed greater resistance to cefoxitin, meropenem, and moxalactam than *E. coli* expressing bla_{IMP-7}. The amino acid residue at position 262 was located near the active site, proximal to the H263 Zn(II) ligand.

Metallo-β-lactamases (MBLs) confer resistance to all β-lactams, except for monobactams, and are characterized by their efficient hydrolysis of carbapenems (1). Acquired MBLs are produced by Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Acinetobacer* spp., and enterobacteria (1). The acquired MBLs are categorized by their amino acid sequences into various types (2–4), including AIM (5), DIM (6), FIM (7), GIM (8), IMPs (9), KHM (10), NDMs (11), SMB (12), SIM (13), SPM (14), TMBs (15) and VIMs (16). The most prevalent types of MBLs are the IMP-, VIM-, and NDM-type enzymes (1, 2, 17). We describe here a novel IMP-type MBL, IMP-51, produced by a clinical isolate of *P. aeruginosa* in a medical setting in Vietnam.

The *P. aeruginosa* clinical isolate NCGM3025 was obtained from a sputum sample of a patient in 2013 in an intensive care unit in a medical setting in Hanoi, Vietnam. MICs of various antibiotics were determined using the microdilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (18). IMP-type MBLs and an aminoglycoside modification enzyme, AAC(6′)-Ib, were detected using immunochromatographic assay kits (19, 20). DNA was extracted from the isolate using DNeasy blood and tissue kits (Qiagen, Tokyo, Japan), and

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TABLE 1 MICs of β-lactams for *P. aeruginosa* NCGM3025 and *E. coli* transformants containing bla_{IMP-7} and bla_{IMP-51}

Antibiotic(s)[a]	MIC (μg/ml) of antibiotic for:			
	P. aeruginosa NCGM3025	*E. coli* DH5α(pHSG398/IMP-7)	*E. coli* DH5α(pHSG398/IMP-51)	*E. coli* DH5α(pHSG398)
Ampicillin	>1,024	128	32	8
Ampicillin-sulbactam	512	64	8	4
Penicillin G	>1,024	128	32	32
Aztreonam	32	0.063	0.063	0.063
Cefepime	256	8	8	0.063
Cefotaxime	1,024	32	64	0.031
Cefoxitin	>1,024	512	>2,048	16
Cefozopran	256	16	8	0.125
Cefpirome	32	2	0.5	≤0.007
Ceftazidime	256	512	128	0.5
Ceftriaxone	>1,024	64	128	0.031
Cephradine	>1,024	512	64	16
Doripenem	256	2	4	0.031
Imipenem	16	0.25	0.25	0.031
Meropenem	512	1	4	0.015
Panipenem	16	0.25	0.25	0.063
Moxalactam	>1,024	256	1,024	0.125

[a] The ratio of ampicillin to sulbactam was 2:1.

the entire genome was sequenced by MiSeq (Illumina, San Diego, CA). Sequence data were analyzed using CLC Genomics Workbench version 8.0 (CLC bio, Tokyo, Japan). Multilocus sequence typing (MLST) was deduced as described by the protocols of the PubMLST databases (http://pubmlst.org/paeruginosa/). Sequences of drug resistance genes, including β-lactamase-encoding genes at the Lahey Clinic website (www.lahey.org/studies), aminoglycoside, chloramphenicol, and fosfomycin resistance genes registered in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/), and quinolone resistance genes (21), were determined using CLC Genomics Workbench version 8.0.

Escherichia coli transformants expressing bla_{IMP-7} and bla_{IMP-51} were produced, and the recombinant IMP-7 and IMP-51 were purified as previously described (22). During the purification process, β-lactamase activity was monitored using nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom). The initial rate of hydrolysis in 50 mM Tris-HCl (pH 7.4), 0.3 M NaCl, and 10 μM $Zn(NO_3)_2$ at 37°C was determined by UV-visible spectrophotometry (V-530; Jasco, Tokyo, Japan), with the reaction initiated by the addition of substrate into spectrophotometer cells, and UV absorption measured during the initial phase of the reaction. K_m, k_{cat}, and the k_{cat}/K_m ratio were determined using a Hanes-Woolf plot. Wavelengths and extinction coefficients were used for the analysis of β-lactam substrates (23–25). K_m and k_{cat} were determined using triplicate analyses.

A DNA plug of NCGM3025, digested with I-CeuI, was prepared, separated by pulsed-field gel electrophoresis, and subjected to Southern hybridization (26) using 16S rRNA and bla_{IMP-51} probes (12, 27).

P. aeruginosa NCGM3025 was resistant to all antibiotics tested, except for amikacin, colistin, and tigecycline. The isolate was susceptible to amikacin and intermediate to colistin and tigecycline. The MICs of β-lactams in NCGM3025 are shown in Table 1; the MICs of other antibiotics were 16 μg/ml for arbekacin, 16 μg/ml for amikacin, 1 μg/ml for colistin, 64 μg/ml for gentamicin, 16 μg/ml for ciprofloxacin, >1,024 μg/ml for fosfomycin, and 4 μg/ml for tigecycline. NCGM3025 was positive for IMP-type MBLs and AAC(6′)-Ib. Whole-genome sequencing revealed that the isolate had a novel bla_{IMP} variant, designated bla_{IMP-51}. Its predicted amino acid sequence revealed that IMP-51 differed from IMP-7 by an amino acid substitution (Ser262Gly) and from IMP-43 by two amino acid substitutions (Phe67Val and Ser262Gly). A phylogenetic tree showed that IMP-51 belonged to an IMP-7-like clade (Fig. 1). In addition to bla_{IMP-51}, NCGM3025 had several drug resistance genes, including *aac(6′)-Ib-cr*, *aac(6′)-Ib*, *aph(3′)-IIb*, bla_{PAO}, $bla_{OXA-246}$, bla_{OXA-50}, *cmlA1*, *catB7*, and *fosA*. The isolate had a point mutation in the quinolone-resistance-determining region of *gyrA* with an amino acid substitution of Ser83Ile in GyrA. The MLST of NCGM3025 was sequence type 235 (ST235).

E. coli DH5α, expressing bla_{IMP-7} or bla_{IMP-51}, showed a significant reduction in susceptibility to all tested β-lactams, except for aztreonam, compared with DH5α expressing a vector control (Table 1). *E. coli* DH5α expressing bla_{IMP-51} showed 4-fold higher MICs of cefoxitin, meropenem, and moxalactam, 4-fold lower MICs of ampicillin, ampicillin-sulbactam, penicillin G, cefpirome, and ceftazidime, and 8-fold lower MICs of cephradine than *E. coli* DH5α expressing bla_{IMP-7} (Table 1).

Recombinant IMP-7 and IMP-51 hydrolyzed all tested β-lactams, except for aztreonam (Table 2). IMP-51 showed markedly

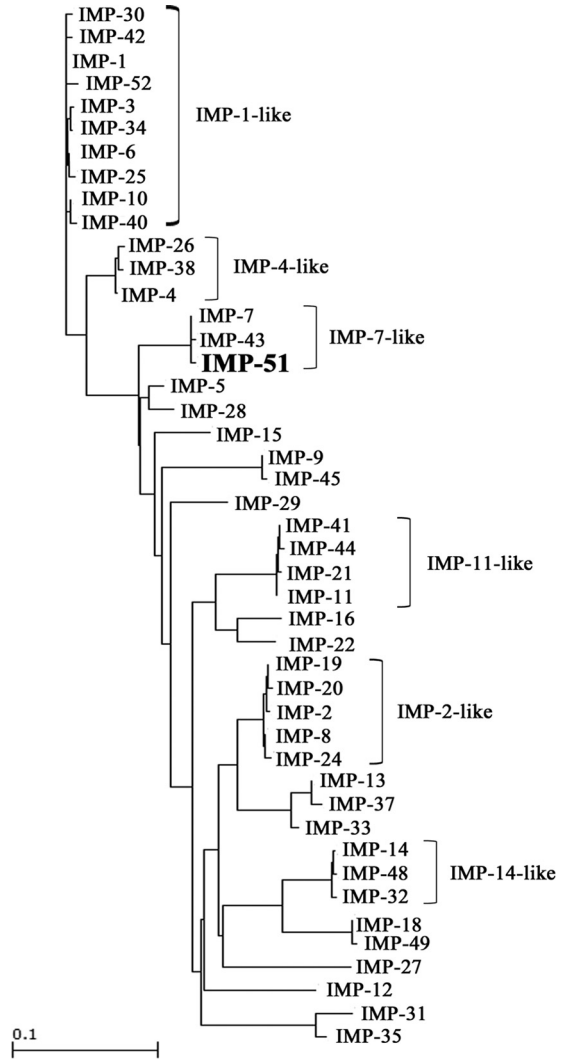

FIG 1 Dendrogram of 45 IMP-type MBLs for comparison with IMP-51. The dendrogram was calculated with the Clustal W2 program. Branch lengths correspond to the number of amino acid exchanges for IMP-type enzymes.

higher k_{cat}/K_m ratios for cefmetazole, cefotaxime, cefoxitin, doripenem, meropenem, and moxalactam and lower k_{cat}/K_m ratios for ampicillin, penicillin G, cefpirome, ceftazidime, cephradine, imipenem, and panipenem. In particular, the higher k_{cat} values of IMP-51 than those of IMP-7 for doripenem and meropenem resulted in the higher k_{cat}/K_m ratios for IMP-51 (Table 2). The k_{cat}/K_m values of IMP-51 against cefepime were similar to those of IMP-7 (Table 2).

The differences of the k_{cat}/K_m values between IMP-7 and IMP-51 were well-correlated to those of the MICs of antibiotics between *E. coli* expressing bla_{IMP-7} and *E. coli* expressing bla_{IMP-51}. Compared with IMP-7, IMP-51, which showed higher k_{cat}/K_m ratios for cefotaxime, cefoxitin, doripenem, meropenem, and moxalactam, conferred higher MICs for these antibiotics in *E. coli*, whereas IMP-51, which showed lower k_{cat}/K_m ratios for ampicillin, penicillin G, cefpirome, ceftazidime, and cephradine, conferred lower MICs for these antibiotics in *E. coli* (Table 1 and 2).

The sequence surrounding bla_{IMP-51} was determined to be *tnpA-tnpR-intI1-*bla_{IMP-51}*-aac(6′)-Ib-aac(6′)-cmlA1-*$bla_{OXA-246}$(9,797bp), which was obtained from a contig assembled by Genomic Work-

TABLE 2 Kinetic parameters of IMP-7 and IMP-51 enzymes[a]

β-Lactam	IMP-7			IMP-51		
	K_m (μM)[b]	k_{cat} (s^{-1})[b]	k_{cat}/K_m ($\mu M^{-1}\ s^{-1}$)	K_m (μM)[b]	k_{cat} (s^{-1})[b]	k_{cat}/K_m ($\mu M^{-1}\ s^{-1}$)
Ampicillin	116 ± 18	8.5 ± 1.3	0.02	872 ± 153	3.5 ± 0.6	0.004
Penicillin G	212 ± 18	17.5 ± 1.3	0.081	976 ± 188	4.6 ± 0.7	0.0048
Aztreonam	NH[c]	NH	NH	NH	NH	NH
Cefepime	58 ± 3	1.2 ± 0.1	0.020	56 ± 4	1.4 ± 0.1	0.025
Cefmetazole	47 ± 5	3.7 ± 0.1	0.078	1.8 ± 0.4	2.78 ± 0.01	1.5
Cefotaxime	12 ± 2	1.7 ± 0.1	0.15	5.7 ± 1.8	4.4 ± 0.2	0.93
Cefoxitin	120 ± 13	4.9 ± 0.2	0.041	2.2 ± 0.6	1.91 ± 0.02	0.88
Cefpirome	57 ± 5	2.0 ± 0.1	0.035	182 ± 25	3.4 ± 0.4	0.019
Ceftazidime	19 ± 3	0.34 ± 0.01	0.018	35 ± 4	0.03 ± 0.01	0.0085
Cephradine	55 ± 8	12 ± 1	0.22	75 ± 21	0.80 ± 0.08	0.011
Doripenem	46 ± 7	2.7 ± 0.2	0.059	61 ± 7	10.7 ± 0.4	0.18
Imipenem	104 ± 13	5.0 ± 0.2	0.048	312 ± 29	5.5 ± 0.3	0.018
Meropenem	59 ± 8	0.99 ± 0.07	0.017	51 ± 8	2.7 ± 0.1	0.053
Panipenem	40 ± 5	4.0 ± 0.2	0.099	230 ± 7	10.6 ± 0.2	0.046
Moxalactam	57 ± 6	4.6 ± 0.2	0.081	24 ± 3	5.0 ± 0.1	0.21

[a] The proteins were initially modified by a His tag, which was removed after purification.
[b] K_m and k_{cat} values represent the means ± standard deviations from three independent experiments.
[c] NH, no hydrolysis was detected at substrate concentrations up to 1 mM and enzyme concentration up to 700 nM.

bench. The bla_{IMP-51} gene was located within a class I integron, of which the downstream region was not determined because it was not contained in the sequence of the contig. The genetic structure that included bla_{IMP-51} had a unique gene cassette array and was located on the chromosome by Southern hybridization (data not shown). In the structure, *tnpA-tnpR* (nucleotide 1 [nt 1] to nt 5,059) was identical to the sequence of the Tn*1403*-like transposon in a plasmid pOZ176 from *P. aeruginosa* PA96 isolated in China (28). The *cmlA1-*$bla_{OXA-246}$ (nt 7,321 to nt 9,786) was similar to a part of the DK45-2 class 1 integron (nt 669 to nt 3,134) in *P. aeruginosa* DK45 isolated in South Korea (GenBank accession number GQ853420). The $bla_{OXA-246}$ was first identified in a plasmid from *P. aeruginosa* pae943 isolated in China (GenBank accession number EU886980).

The Ser262Gly substitution in IMP-51 markedly affected the catalytic activities of the enzyme against β-lactams, especially against carbapenems. IMP-51 had higher k_{cat}/K_m ratios against doripenem and meropenem but lower k_{cat}/K_m ratios against imipenem and panipenem than those of IMP-7. These differences in catalytic activities may explain the high resistance of NCGM3025 against doripenem and meropenem (Table 1). Similarly, IMP-6 with a Ser262Gly substitution had higher activity against meropenem and panipenem than that of IMP-1 (29). Residue 262 is located near the Zn(II) binding site, which plays an important role in β-lactam turnover catalyzed by IMP-type MBLs (30). The Ser262Gly substitution in IMP-6 compared with that in IMP-1 (29) was found to stabilize the anionic intermediate of certain β-lactam substrates bound to IMP-6, enhancing catalysis (31).

In conclusion, a doripenem- and meropenem-resistant *P. aeruginosa* isolate producing IMP-51 has emerged in Vietnam. The Ser262Gly amino acid substitution in IMP-51 appeared to significantly increase its hydrolytic activity for doripenem and meropenem. This substitution may have arisen due to the selective pressure caused by the use of doripenem and meropenem.

Nucleotide sequence accession number. The genomic environment surrounding bla_{IMP-51} was identified and deposited in GenBank under the accession number LC031883.

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