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IMP-33, a New IMP Variant Detected in *Pseudomonas aeruginosa* from Sicily

Lalitagauri M. Deshpande,[a] Todd A. Davies,[b] Giovanna Blandino,[c] Giuseppe Nicoletti,[c] Ronald N. Jones,[a] Mariana Castanheira[a]

JMI Laboratories, North Liberty, Iowa, USA[a]; Janssen Research & Development, Infectious Diseases & Vaccines, Raritan, New Jersey, USA[b]; Central Laboratory of Analysis of the Department of Bio-Medical Sciences, Section of Microbiology, University of Catania, Catania, Italy[c]

A variety of metallo-β-lactamase (MβL) enzymes have been reported from Gram-negative organisms collected in Italy, including VIM-1, the second acquired MβL to be reported; IMP-13; VIM-2; IMP-19; and FIM-1 (1–6). A survey conducted from September to December of 2004 in 12 Italian cities demonstrated a low overall prevalence of carbapenemase-producing organisms, but these strains were widespread across Italy, with a great genetic diversity of MβL genes and genetic elements (1). More recently, an outbreak of NDM-1 has been reported in Northern Italy, increasing the concerns of local health care authorities (3). In this study, we investigated the presence of MβL-encoding genes among *Pseudomonas aeruginosa* isolates from three Italian hospitals and describe a new IMP variant, named IMP-33. In addition, we performed a genetic characterization of the isolate carrying this enzyme.

During 2009 and 2010, 200 *P. aeruginosa* clinical isolates were recovered from three Italian hospitals located in Catania, Genoa, and Rome and submitted to the SENTRY Antimicrobial Surveillance Program. Isolates were susceptibility tested by reference broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (7). Carbapenem-nonsusceptible isolates (doripenem MIC, ≥1 μg/ml) were screened for the presence of the carbapenemase-encoding genes bla_{KPC}, bla_{SME}, bla_{GES}, bla_{NMC-A}, bla_{IMI}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{AIM-1}, bla_{KHM-1}, bla_{NDM}, bla_{DIM-1}, and bla_{BIC-1} by PCR (8, 9), and amplicons were subjected to sequencing. Nucleotides and deduced amino acid sequences were analyzed by using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available at the NCBI by BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Among 45 (22.4%) carbapenem-nonsusceptible *P. aeruginosa* isolates noted among the three hospitals surveyed, 5 (2.5% overall) carried MβL-encoding genes. Two VIM-1-producing strains were detected in Genoa and Catania, VIM-2 was noted in two strains from Rome, and an isolate displaying bla_{IMP}-positive amplification was observed from Catania. Sequencing revealed a new IMP variant, named IMP-33, that was most similar to IMP-13 (98.0% similarity). This new variant displayed five amino acid substitutions compared to IMP-13: A38S, A39S, E109K, I223V, and M302L (nomenclature according to Garau et al. [10]). The IMP-33-producing *P. aeruginosa* isolate was collected in April 2009 from a blood culture of a 79-year-old female patient who underwent surgery for hepatic carcinoma. After surgery, the patient presented with fever from an unidentified source and had several risk factors for infection, including a urinary catheter and prior use of antimicrobials and ventilation. This isolate was resistant to all β-lactam agents, including aztreonam and tobramycin, but was susceptible to amikacin, ciprofloxacin, and polymyxin B (using CLSI and/or EUCAST breakpoints) (11), with MICs of 4, ≤0.5, and 1 μg/ml, respectively.

All 45 carbapenem-nonsusceptible *P. aeruginosa* strains were typed by pulsed-field gel electrophoresis (PFGE) (12) as described elsewhere. Thirty-four PFGE profiles were observed among the isolates, and clusters of six (one cluster), three (one cluster), and two (four clusters) strains were detected within the hospitals. The IMP-33-producing *P. aeruginosa* strains displayed a unique profile compared to the other strains evaluated. Multilocus sequence typing (MLST) of the IMP-33-producing strain was performed according to the instructions at the website http://pubmlst.org/paeruginosa/, and this strain was found to belong to ST466, which, according to the MLST database, has been previously observed in Australia and Spain.

Evaluation of the bla_{IMP-33} genetic environment by primer walking sequence analysis showed that this gene was carried in the first position of a class 1 integron structure displaying standard 5′ and 3′ conserved regions. This MβL gene was followed by copies of *aac(6′)-1b*, bla_{OXA-2}, and *aadA1*. DNA digestion with S1 nuclease (13) and I-CeuI (14) was resolved by electrophoresis followed by Southern blotting, and hybridization with a bla_{IMP}-specific probe was performed to determine the genetic location of bla_{IMP-33}. A single hybridization signal was obtained from the I-CeuI preparation, demonstrating that this gene was chromosomally located. This result was confirmed by the absence of hybridization in the S1 nuclease preparations.

The bla_{IMP-33} and bla_{IMP-13} genes were cloned into the pPCRScriptCam SK+ plasmid vector (Stratagene Cloning Systems, La Jolla, CA) and transformed in *E. coli* XL10 Blue, and transformants were selected in 30 μg/ml of chloramphenicol. Plasmid constructs were sequenced, and recombinant strains were susceptibility tested as described above. The susceptibility profile of the recombinant strain carrying bla_{IMP-33} was almost identical to that of the strain with bla_{IMP-13} cloned into the same background, both displaying high MICs of penicillins alone or combined with currently available serine-β-lactamase inhibitor combinations and cephalosporins. Carbapenem MICs were modestly elevated (range, 0.5 to 2 μg/ml), and the aztreonam MIC was the same as that for the host strain carrying the cloning vector without insert (≤0.12 μg/ml; Table 1).

Quantitative reverse transcription-PCR was used to evaluate the

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Address correspondence to Mariana Castanheira, mariana-castanheira@jmilabs.com.

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TABLE 1 Antimicrobial susceptibility and gene expression of IMP-33-producing *P. aeruginosa* and susceptibility profiles of recombinant strains carrying the genes encoding IMP-13 and IMP-33 and the baseline strain carrying the vector with no insert

Antimicrobial or gene	MIC (μg/ml) or relative avg relative gene expression (SD)[a]			
	P. aeruginosa clinical isolate carrying bla_{IMP-33}	*E. coli* XL1-Blue PCRScript (bla_{IMP-33})	*E. coli* XL1-Blue PCRScript (bla_{IMP-13})	*E. coli* XL1-Blue PCRScript
Antimicrobial				
Doripenem	>32	1	2	≤0.06
Imipenem	>32	1	1	0.25
Meropenem	>32	0.5	1	≤0.12
Cefoxitin	NT[b]	>16	>16	4
Ceftriaxone	NT	>32	>8	≤0.25
Ceftazidime	>32	>32	>32	≤1
Cefepime	>16	4	2	≤0.12
Aztreonam	16	≤0.12	≤0.12	≤0.12
Ampicillin	NT	>8	>8	2
Ampicillin-sulbactam	NT	32	16	8
Amoxicillin-clavulanate	NT	32	16	8
Piperacillin-tazobactam	32	8	8	1
Amikacin	4	NT	NT	NT
Tobramycin	16	NT	NT	NT
Ciprofloxacin	≤0.5	NT	NT	NT
Polymyxin B	1	NT	NT	NT
Genes				
ampC	0.4 (0.2–0.6)	NT	NT	NT
mexA	1.0 (0.5–1.9)	NT	NT	NT
mexC	0.2 (0.1–0.3)	NT	NT	NT
mexE	0.5 (0.2–0.7)	NT	NT	NT
mexX	0.1 (0.0–0.3)	NT	NT	NT

[a] Results were normalized by using *rpsL* and compared to *P. aeruginosa* PAO1, except for *mexE*, which was normalized by using strain PAM1020 (kindly supplied by Olga Lomovskaya).
[b] NT, not tested.

expression of intrinsic carbapenem resistance mechanisms, including the efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM and chromosomal *ampC*. Assays were performed with high-quality, DNA-free RNA preparations as previously described (15), by using custom primers (sequences and conditions available upon request). Relative expression was determined as $2^{-\Delta\Delta T}$, where $\Delta\Delta T$ is the difference between the target gene and *rpsL* cycle threshold values, with custom-designed primers. Additionally, an OprD Western blot assay was performed as previously described (16). The expression of the efflux pump-encoding genes and *ampC* and phenotypic expression of OprD in IMP-33-producing *P. aeruginosa* were not significantly different from those of the *P. aeruginosa* PAO1 control (Table 1).

IMP-33 displayed high homology with IMP-13, which has been widely described in Italy during the last decade (1, 2, 4). Results from the SENTRY Program demonstrated that over the years, IMP-13 has been observed in hospitals in Rome and Genoa but not in Catania (2), and these results are similar to the literature reports that have not surveyed or detected elevated numbers of MβL-producing strains in Sicily. This suggests that IMP-33 is not derived from IMP-13 and both enzymes could have an ancestor in common but evolved separately.

The increase in carbapenemase-producing strains in Europe and the endemic spread of these organisms in the southern countries of Europe that include Italy (4) are matters of significant concern and should be the focus of a broader regional effort to prevent the further dissemination of these isolates within and between countries.

Nucleotide sequence accession number. The nucleotide sequence of bla_{IMP-33} and the integron carrying this gene has been submitted to GenBank and assigned accession number JN848782.

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