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IMP-29, a Novel IMP-Type Metallo-β-Lactamase in *Pseudomonas aeruginosa*

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Analysis of two clonally related multiresistant *Pseudomonas aeruginosa* isolates led to the identification of a novel IMP-type metallo-β-lactamase. IMP-29 was significantly different from the other IMP variants (the closest variant being IMP-5 with 93% amino acid identity). The bla_{IMP-29} gene cassette was carried by a class 1 integron in strain 10.298, while in strain 10.266 it was located in a rearranged DNA region on a 30-kb conjugative plasmid. Biochemical analysis confirmed that IMP-29 efficiently hydrolyzed carbapenems.

Pseudomonas aeruginosa is a major opportunistic pathogen responsible for mild to severe nosocomial infections. Over the past decade, the emergence and spread of strains resistant to multiple antibiotics, including carbapenems, have become a serious clinical concern in many countries (12, 28). Resistance to carbapenems is usually due to mutational alteration of the imipenem-specific porin OprD but may also result from production of horizontally acquired β-lactamase genes (22). Most of the carbapenem-hydrolyzing enzymes (i.e., carbapenemases) found in *P. aeruginosa* belong to the Ambler class B of β-lactamases (27). These enzymes have been grouped into different types such as IMP, VIM, SPM, GIM, AIM, DIM, and NDM according to their amino acid sequences (4, 10). Of particular clinical importance are the IMP- and VIM-type metallo-β-lactamases (MBLs), which efficiently inactivate most of the antipseudomonal β-lactams except monobactams (7, 27). So far, 28 IMP-type β-lactamases have been identified in various Gram-negative species (K. Bush and G. Jacoby, http://www.lahey.org/Studies/).

Our study was initiated by the isolation of *P. aeruginosa* 10.266 (serotype O:12) from the blood culture of a 74-year-old male patient admitted in March 2010 to the medical intensive care unit of the teaching hospital of Besançon, France, for unstable diabetes associated with signs of pulmonary infection. The patient initially received a course of piperacillin-tazobactam, ciprofloxacin, rovamycin, and metronidazole for 16 days. Despite this aggressive therapy, he developed an acute respiratory distress syndrome, which prompted physicians to switch to imipenem and linezolid. This treatment failed, and the patient died from a septic shock 10 days later. A second serotype O:12 isolate, named 10.298, was recovered in June 2010 from a rectal swab of a 48-year-old male patient hospitalized for an acute myeloid leukemia in the hematology unit of the same hospital. The patient, who had no signs of infection, was not receiving antibiotics. Both isolates were highly resistant to all of the antipseudomonal β-lactams tested (ticarcillin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, and meropenem) except aztreonam (Table 1). They were also resistant to aminoglycosides (amikacin, tobramycin, and gentamicin) and ciprofloxacin but were susceptible to colistin (MIC of 0.5 μg/ml). Pulsed-field gel electrophoresis (PFGE) experiments using total DNA digested with endonuclease DraI (11) revealed that 10.266 and 10.298 were closely related (differing by only one band) and belonged to the same clone endemic in the hospital (23). The epidemiological link between the two patients carrying these bacteria could not be established (data not shown). The production of an MBL in both isolates was suggested by positive double-disk synergy tests between imipenem and EDTA (10 μl, 100 mM). Then, PCR experiments were carried out on purified DNA with primers specific for known MBL genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, and bla_{GIM}) (15, 18, 21, 26). A bla_{IMP} gene was amplified in both isolates, and sequencing identified a novel IMP-type MBL that was termed IMP-29 accordingly (http://www.lahey.org/Studies/).

Since bla_{IMP} genes are usually associated with class 1 integron sequences, PCR experiments were performed with primers annealing to the 5′ conserved sequence (CS) and 3′ CS of class 1 integrons (3). This yielded a single amplicon in 10.266 (2.8 kb) and three amplicons in 10.298 (2.8, 2.1, and 1.2 kb, respectively). Sequencing of the 2.8-kb PCR products revealed the presence of the gene (bla_{PSE-1}) encoding narrow-spectrum penicillinase PSE-1 (also termed CARB-2), flanked by two gene cassettes, *aacA4* and *aadA2*, encoding the aminoglycoside-modifying enzymes AAC(6′)-Ib and ANT(3″), respectively (Fig. 1A). Production of the two latter enzymes is consistent with the high resistance of the isolates to aminoglycosides. The 1.2-kb fragment from 10.298 contained the bla_{OXA-2} gene encoding narrow-spectrum oxacillinase OXA-2 (Fig. 1B), while the 2-kb fragment appeared to carry the bla_{IMP-29} gene located downstream of the *aacA4* and *dfrII* gene cassettes encoding the AAC(6′)-Ib and dihydrofolate reductase enzymes, respectively (Fig. 1B).

Sequence alignments showed that IMP-29 was quite distant from other IMP-type MBLs (88.6% and 83.6% amino acid sequence identities with IMP-1 and IMP-8, respectively). Its closest

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TABLE 1 Susceptibilities to β-lactams of *P. aeruginosa* and *E. coli* strains harboring gene bla_{IMP-29}

	MIC (μg/ml) for strain						
	P. aeruginosa				*E. coli*		
β-Lactam	10.266	10.298	PU21(pIMP266)	PU21	DH5α(pK-IMP29)	DH5α(pK-IMP266)	DH5α(pK18)
Amoxicillin	>256	>256	>256	>256	64	128	0.5
Ticarcillin	>256	>256	256	8	256	>256	0.5
Piperacillin	256	256	8	2	1	2	≤0.06
Cefotaxime	>256	256	>256	8	4	16	≤0.06
Ceftazidime	>256	128	256	1	16	64	≤0.06
Cefepime	>256	64	256	1	0.5	4	≤0.06
Imipenem	128	64	8	1	0.5	1	0.12
Meropenem	>256	128	64	0.5	0.25	4	≤0.06
Doripenem	>256	64	64	0.25	0.25	4	≤0.06
Ertapenem	>256	256	256	8	0.12	4	≤0.06
Aztreonam	8	4	4	2	≤0.06	0.12	≤0.06

relatives were IMP-5, IMP-22, and IMP-16 with 93, 92, and 91% sequence identity, respectively (5, 16, 19).

Interestingly, PCR experiments failed to detect a class 1 integron structure associated with bla_{IMP-29} in strain 10.266. To get further insight into the genetic environment of that gene, the plasmid content of isolate 10.266 was analyzed (13). A plasmid of ca. 30 kb was detected, which was named pIMP266. The potential of pIMP266 for conjugative transfer was assessed between 10.266 and a rifampin-resistant *P. aeruginosa* strain, PU21 (9). Transconjugant PU21(pIMP266) was selected on Mueller-Hinton agar plates supplemented with 100 μg/ml ticarcillin and 200 μg/ml rifampin. All the clones tested exhibited a high resistance to ticarcillin, cefotaxime, ceftazidime, and cefepime, associated with a reduced susceptibility to piperacillin and almost unchanged susceptibility to aztreonam compared with PU21 (Table 1). Plasmid pIMP266 conferred a higher resistance to meropenem (64 μg/ml) and doripenem (64 μg/ml) than to imipenem (8 μg/ml). Similar results have been reported in *P. aeruginosa* PAO1, PAO4141 (an AmpC-deficient mutant of PAO1), and *Escherichia coli* transformed with plasmids carrying bla_{IMP-12}, bla_{IMP-10}, and bla_{IMP-6}, respectively (6, 8, 25). No coresistance marker was transferred by plasmid pIMP266.

To identify the genetic environment of the bla_{IMP-29} gene on plasmid pIMP266, a Sau3AI library of pIMP266 DNA was cloned into the BamHI-restricted plasmid pK18 (Km^r). Transformants of *E. coli* DH5α were selected on Mueller-Hinton agar plates containing 100 μg/ml ampicillin and 30 μg/ml kanamycin. In comparison to recipient strain DH5α, all the recombinant clones tested displayed a high level of resistance to β-lactams except aztreonam (Table 1). Sequence analysis of the 3-kb insert from one of these clones (plasmid pK-IMP266) revealed that the bla_{IMP-29} gene was flanked by several partial gene sequences belonging to loci distantly located on the *P. aeruginosa* genome such as *narK* (nitrite transport, PA3876) and *PA1212* (major facilitator super-

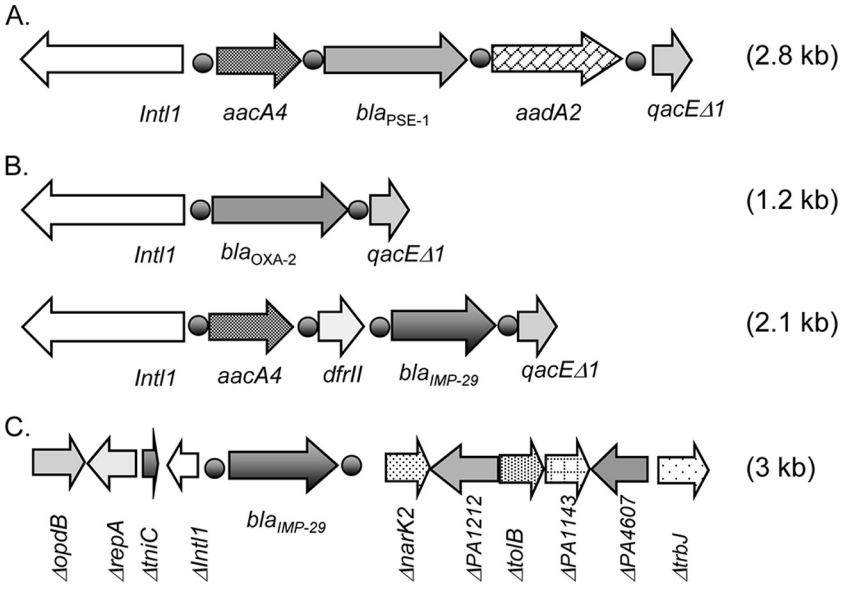

FIG 1 Schematic map of class 1 integrons identified in clinical strains 10.266 and 10.298. The genes and their transcriptional orientations are indicated by arrows. The 59-base elements are indicated by a solid circle. (A) Structure of class 1 integron identified in both 10.266 and 10.298. (B) Structures of the two integrons identified in *P. aeruginosa* 10.298. (C) Structure of the cloned region containing the bla_{IMP-29} gene from the plasmid pIMP266.

family [MFS] pump) (Fig. 1C). This complex genetic environment likely reflects multiple rearrangements of the region surrounding bla_{IMP-29}. Supporting this notion, the presence of a truncated *tniC/R* gene (encoding the resolvase of Tn*401*-like transposons), the integrase gene *intI1*, and a core site (59-base element) upstream of bla_{IMP-29} indicated that bla_{IMP-29} was initially associated with a mobile class 1 integron structure.

Since the MICs of carbapenems for isolate 10.266 were much higher than those for transconjugant PU21(pIMP266), the presence of an additional resistance mechanism was suspected (Table 1), such as the alteration of the OprD porin (17, 22, 24). Sequencing of the *oprD* gene revealed a premature stop codon in isolate 10.266 leading to a truncated peptide of 295 amino acid residues instead of 443 (wild-type OprD of *P. aeruginosa* PAO1). In 10.298, the *oprD* gene was also interrupted prematurely, resulting in a polypeptide lacking the last 28 C-terminal amino acids of OprD. However, the impact of the latter deletion on the porin function is likely to be modest since the MICs of carbapenems were relatively similar for 10.298 and PU21(pIMP266) (Table 1).

In order to better evaluate the impact of IMP-29 on the levels of resistance to β-lactams, the bla_{IMP-29} gene was expressed into *E. coli*. An 822-bp PCR fragment carrying bla_{IMP-29} was amplified by using specific primers cloIMP266F (5′-GTCGCCCGAAAACAAA GTTA-3′) and cloIMP266R (5′-AACTGTCCAAGGAGCGTAG C-3′). Once purified through a High Pure PCR product column (Roche Diagnostics, France), the amplicon was cloned into plasmid vector pCRScript CampR (Agilent Technologies) and subcloned into pK18 to yield recombinant plasmid pK-IMP29. As expected, transformant DH5α(pK-IMP29) exhibited an increased resistance to all the β-lactams tested except aztreonam (Table 1). However, the resistance levels conferred by pK-IMP29 were 2 to 32 times lower than those provided by pK-IMP266, suggesting a difference in bla_{IMP-29} expression between the two recombinant plasmids. *E. coli* DH5α(pK-IMP29) remained susceptible to imipenem, meropenem, doripenem, and ertapenem according to the current CLSI breakpoints (Table 1) (2).

To determine the catalytic properties of IMP-29, kinetic studies were carried out as previously described (20). Briefly, IMP-29 was purified from crude extracts of *E. coli* DH5α(pK-IMP29) by two-step anion-exchange chromatography in a Q-Sepharose column, first at pH 5.4 and then at pH 7.4. Since no enzymatic data have been reported for IMP-5, the closest relative to IMP-29, IMP-1 was used as the reference enzyme. Overall, the hydrolytic activities of IMP-29 were lower than those of IMP-1, in particular toward cefotaxime (35-fold lower), ceftazidime (72-fold lower), and cefepime (70-fold lower) (Table 2). IMP-29 hydrolyzed carbapenems at similar rates that were close to (meropenem) or lower than (imipenem, 20-fold; ertapenem, 17-fold) that of IMP-1. These assays confirmed that the enzyme has virtually no activity on aztreonam.

In conclusion, this work describes a novel transferable IMP variant acquired by an endemic O:12 clone. Both isolates were highly resistant to piperacillin because of production of penicillinase PSE-1 and to carbapenems as a result of alteration of porin OprD. While the characterization of IMP-29 was in progress, five other bla_{IMP-29}-positive, clonally related *P. aeruginosa* isolates were identified from January to July 2011 in patients hospitalized in the hematology unit of the hospital in Besançon. All of these isolates possessed the pIMP266 plasmid. This indicates an ongoing nosocomial dissemination of the multidrug-resistant *P. aeruginosa* strain 10.266 with a potential risk of transfer of pIMP266 to other Gram-negative bacteria.

TABLE 2 Kinetic parameters for β-lactamases IMP-29 and IMP-1[a]

Substrate	K_m (μM)		k_{cat} (s^{-1})		k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	
	IMP-29	IMP-1	IMP-29	IMP-1	IMP-29	IMP-1
Benzylpenicillin	130	520	140	320	1.1	0.62
Piperacillin	10	356	50		5	0.72
Cefotaxime	300	4	3	1.3	0.01	0.35
Ceftazidime	800	44	2	8	0.0025	0.18
Cefepime	110	11	1	7	0.01	0.63
Aztreonam	ND	>1,000	NH	<0.01	ND	ND
Imipenem	50	39	3	46	0.06	1.17
Meropenem	90	10	6	5	0.07	0.50
Ertapenem	150	16	7	21	0.045	0.76

[a] Data for IMP-1 are from references 14 and 1. Data are the means of three independent experiments. Standard deviations were within 10% of the means. NH, no detectable hydrolysis; ND, not determined.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this work have been deposited in the GenBank database under accession numbers JQ041634 and HQ438058.

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