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First Occurrence of an IMP Metallo-β-Lactamase in *Aeromonas caviae*: IMP-19 in an Isolate from France▿

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We describe the first IMP metallo-β-lactamase in *Aeromonas caviae*: IMP-19, which differed from IMP-2 by a single amino acid change (Arg to Ala at position 38). *bla*$_{IMP-19}$ was found within a class 1 integron located on a 35-kb plasmid. This is also the first description of an IMP producer in France.

In the last few years, many acquired metallo-β-lactamases (MBLs) have been detected worldwide; these IMP, VIM, SPM, and GIM types have very broad substrate profiles, including carbapenems (20). The first IMP-type MBL was described in Japan in 1988 (21). Since then, 23 IMP variant enzymes have been reported (http://www.lahey.org/studies/). IMP producers have now been detected worldwide: in Europe (3, 4, 14, 17, 19), South America (5), Australia (13), and Canada (7) and also, more recently, in the United States (8). So far, in France, IMP producers have not yet been detected. Nevertheless, the frequency of such isolates may be underestimated: several clinical isolates carrying a cryptic *bla*$_{IMP}$ gene demonstrated low-level carbapenem resistance (MIC ≤ 4 μg/ml) (16, 24). In this study, we describe the first isolate from France harboring an acquired *bla*$_{IMP}$ gene.

The *Aeromonas caviae* isolate (A324R) was recovered from a stool sample from an 8-year-old boy hospitalized for acute diarrhea, the final diagnosis being a celiac disease. The child had never been hospitalized before and had not received any antibiotic therapy for at least 6 months. The strain A324R was identified by using the API 20NE system (BioMérieux, Marne-la-Coquette, France) and by 16S rRNA and *rpoB* gene sequencing. Susceptibility testing by the disk diffusion method and the determination of the MICs by the standard broth dilution method were based on CLSI criteria (2). On the antibiogram from the disk diffusion method, A324R was characterized as being resistant to most β-lactams, except aztreonam and imipenem (inhibition zones of 24 and 27 mm in diameter, respectively). The MBL production was assessed by a positive double-disk test of synergy between a disk containing ceftazidime and a disk containing EDTA (10 μl; 500 mM) either alone or in combination with β-mercaptoethanol (2 μl) (Fig. 1) (1). The isoelectric point (pI) of the MBL was determined by analytical isoelectric focusing (12). The detection of β-lactamase activity was performed by a substrate-overlaying procedure (10). In all steps (from the bacterial growth to the gel preparation), 0.1 mM $ZnCl_2$ was added. A324R produced a β-lactamase with a pI of 8.2. A plasmid of 35 kb (pJDB2) was extracted by an alkaline lysis method (15), but all attempts at conjugation failed. *Escherichia coli* DH5α transformed with pJDB2 also produced a β-lactamase of pI 8.2. Acquired MBL genes are inserted mostly in integrons, especially class 1 integrons (20). To search for the presence of such a class 1 integron, we performed PCR analysis of the total DNA from A324R and *E. coli* DH5α(pJDB2) with primers L1 and R1 (11). A fragment of 2.8 kb was obtained, and both strands were sequenced with an Applied Biosystems 373A sequencer according to the manufacturer's instructions. By using a set of primers (Table 1), the structure of this class 1 integron was deduced. There was an insertion sequence (IS*Aeca1*) belonging to the IS*30* family located immediately downstream of the cassette integration site *attI1*. IS*Aeca1* was followed by a first cassette that carried an *aacA4* determinant identical to the cassette found in the integron In42 and in many integrons harboring *bla*$_{IMP}$ genes (14, 18). The *aacA4* determinant was located upstream of the *bla*$_{IMP-19}$ cassette. The 72-bp *attC* recombination site of the *bla*$_{IMP-19}$-containing cassette was identical to those of the cassettes carrying *bla*$_{IMP-2}$ and *bla*$_{IMP-8}$ (14, 22). The amino acid sequence deduced according to the numbering scheme of Galleni et al. (6) revealed that IMP-19 was similar to IMP-2 (Arg for IMP-2 and Ala for IMP-19 at position 38) and IMP-8 (Gly for IMP-8 and Val for IMP-19 at position 254).

The *bla*$_{IMP-19}$ gene was subcloned into vector pK18, and the recombinant strain *E. coli* DH5α(pIP19) was selected on kanamycin (30 μg/ml) and ceftazidime (4 μg/ml). The β-lactam MICs (determined by broth dilution) for A324R, *E. coli* DH5α(pIP19), *E. coli* DH5α(pJDB2), and *E.coli* DH5α are reported in Table 2. IMP-19 production conferred a high level of resistance to ceftazidime, cefoxitin, and cefazoline and only reduced susceptibility to carbapenems. A324R was much more resistant to ticarcillin than to piperacillin (MICs of 1,024 and 2 μg/ml, respectively). There was discordance between the results of susceptibility testing for imipenem: by the disk diffusion method, A324R was categorized as susceptible (27-mm-diameter zone of inhibition), whereas by the determination of the MIC by broth dilution, A324R was categorized as resistant (MIC, 16 μg/ml).

The difficulty of detecting IMP-2 variant producers has already been pointed out (23), and this characteristic is fully consistent with the findings of our study. The recombinant

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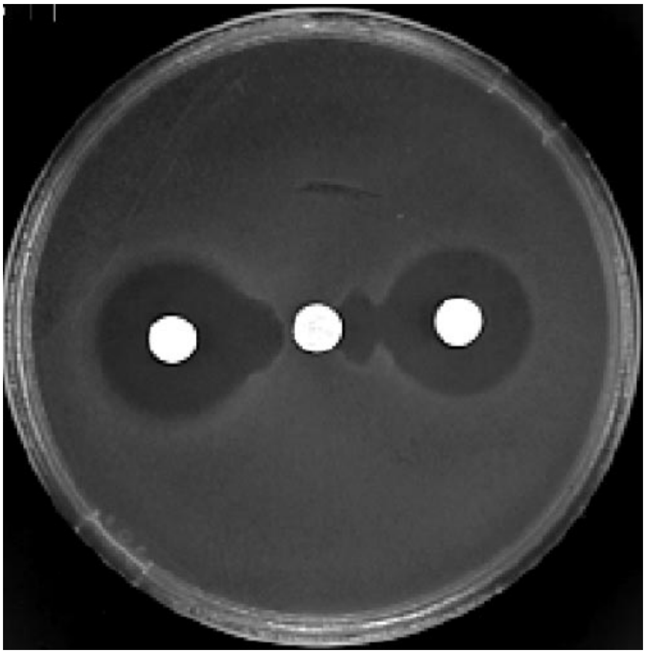

FIG. 1. A324R: synergy between disks containing ceftazidime (center) and EDTA (10 μl; 500 mM) alone (left) or in combination with β-mercaptoethanol (2 μl) (right).

TABLE 2. MICs of β-lactams for *A. caviae* A324R, the transformant *E. coli* DH5α(pJDB2), the recombinant *E. coli* DH5α(pIP19), and *E.coli* DH5α

β-lactam	MIC (μg/ml) for:			
	A. caviae A324R	*E. coli* DH5α(pJDB2)	*E. coli* DH5α(pIP19)	*E. coli* DH5α
Ticarcillin	2,048	512	1,024	4
Piperacillin	256	8	2	1
Cefazoline	512	256	512	2
Cefoxitin	1,024	128	512	4
Aztreonam	8	0.25	0.25	0.25
Ceftazidime	1,024	256	512	≤1
Clavulanate[a]	512	256	256	≤1
Tazobactam[a]	1,024	256	256	≤1
Imipenem	16	4	8	0.5
Meropenem	1	0.5	0.5	≤0.25

[a] Clavulanate and tazobactam were used at 2 and 4 μg/ml, respectively.

strain *E. coli* DH5α(pIP19) was used to determine the enzymatic parameters of IMP-19. The bacteria were disrupted by ultrasonic treatment. The supernatant was loaded onto an SP Sepharose column (Amersham Pharmacia Biotech) equilibrated with 50 mM MES (morpholineethanesulfonic acid)-NaOH (pH 6.0). The elution was performed with a linear NaCl gradient (0 to 500 mM). The β-lactamase-containing elution peak fraction was supplemented with 5 mM $ZnCl_2$, loaded onto a Superose 12 column (Amersham Pharmacia Biotech), and eluted with the buffer 20 mM MES-NaOH–100 mM NaCl (pH 6.0). The level of purity was estimated at >97% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The Michaelis constant (K_m) and catalytic activity (k_{cat}) were determined three times with purified extracts by using a computerized microacidimetric method (9). The variation coefficients showed a maximum variation of 10%. The enzymatic parameters of IMP-19 (Table 3) were overall very different from those of IMP-2. The hydrolytic activities (k_{cat}s) of IMP-19 were much higher for penicillins than those of IMP-2, especially for amoxicillin (k_{cat} of 456 versus 23 s^{-1}). The hydrolytic efficiency of IMP-19 for amoxicillin and ticarcillin was 10- to 15-fold higher than that of IMP-2. IMP-19 had greater affinity for ceftazidime than IMP-2 (K_m of 20 versus 111 μM) but

TABLE 1. Primers used for PCRs

Amplified DNA	Primer	Oligonucleotide sequence (5′ to 3′)	Accession no.
Variable region of class 1 integrons	L1	GGCATCCAAGCAGCAAGC	U49101
	R1	AAGCAGACTTGACCTGAT	
intI1	Int-IN	TGTCGTTTTCAGAAGACGG	U49101
	IntA-R	ATCATCGTCGTAGAGACG	
	IntB-F	GTCAAGGTTCTGGACCAG	
	Int-out-F	GTAGAACAAGCAGGCATC	
	Int-out-R	GAAACGGATGAAGGCACG	
aac(6′)-Ib	Aac6′Ib-F	ACTGAGCATGACCTTGCG	AY878717
	Aac6′Ib-R	TGTTCGCTCGAATGCCTG	
bla_{IMP}	Imp-F	GTTTTATGTGTATGCTTCC	AB184976
	Imp-R	AGCCTGTTCCCATGTAC	
	Imp-out-R	CCTTCTTCAAGCTTCTCG	
3′ conserved segment region	Qac-F	TCGCAATAGTTGGCGAAG	U49101
	Qac-R	AGCTTTTGCCCATGATGC	
	Sul-F	GACGGTGTTCGGCATTCT	
	Sul-R	TGAAGGTTCGACAGCACG	
	Orf5-F	GGTGATATCGACGAGGTT	
	Orf5-R	GATTTCGAGTTCTAGGCG	
bla_{IMP-19} cloning primers	Sub-IMP19-EcoRI-F	GGGGAATTCTTAGAAAAGGGCAAGTATG	
	Sub-IMP19-XbaI-R	GGGTCTAGATCACCGCCTTGTTAGAAAT	

TABLE 3. Kinetic parameters of purified IMP-19[a]

Substrate	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (μM^{-1} s^{-1})
Benzylpenicillin	1,011	206	4.91
Amoxicillin	456 (23)	207 (110)	2.20 (0.21)
Ticarcillin	683 (252)	140 (700)	4.88 (0.36)
Piperacillin	41.2	148	0.28
Cephalothin	11.0	76	0.14
Cefuroxime	16.4	95	0.17
Cefoxitin	9.7 (7)	33 (7)	0.29 (1.0)
Cefotaxime	20.1	61	0.33
Cefpirome	14.3	48	0.30
Ceftazidime	6.4 (21)	20 (111)	0.32 (0.19)
Imipenem	26.5 (22)	100 (24)	0.26 (0.92)
Meropenem	1.0 (1)	7 (0.3)	0.14 (3.3)
Aztreonam	<0.1	ND[b]	ND

[a] Values in parentheses are those for IMP-2 (14).
[b] ND, not determined.

lower hydrolytic activity, resulting in a twofold-higher k_{cat}/K_m ratio for IMP-19. Compared to that of IMP-2, the hydrolytic efficiency of IMP-19 was rather poor for carbapenems, despite an excellent affinity for meropenem (7 μM). Unfortunately, the IMP-8 enzymatic parameters are not available for comparison.

A324R had no clinical significance. Nevertheless, this is the first report of an IMP producer in France and the first report of IMP production by *Aeromonas*. The present findings confirm that the environmental reservoir of bla_{IMP} genes is widespread.

Nucleotide sequence accession number. The nucleotide sequence of the integron reported in this paper has been assigned the GenBank accession number EF118171.

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