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Letter to the Editor

First Detection of a Carbapenem-Hydrolyzing Metalloenzyme in an *Enterobacteriaceae* Isolate in France

Although many β-lactamases with expanded spectra have been described for the *Enterobacteriaceae*, carbapenemases remain infrequent. Acquired carbapenemases of the metalloenzyme group (Ambler class B) are mostly either of the IMP or the VIM type. The VIM enzymes were reported first from Verona in Italy (VIM-1) and are scattered now in southern Europe, South America, and Southeast Asia (6). Our group had identified the first VIM enzyme (VIM-2) in France from *Pseudomonas aeruginosa* isolates (8), and we report now the first Ambler class B carbapenemase in a *Klebsiella pneumoniae* isolate from this same country.

K. pneumoniae MF was recovered from a 62-year-old man who had been hospitalized for liver transplantation. In the follow-up to the surgery, he developed liver abscesses and peritonitis. Two identical isolates were recovered from liver abscesses and peritoneal fluid, respectively. They were resistant to all available antibiotics except colimycin. After surgical peritoneal lavage and treatment with colimycin, gentamicin, and metronidazole, the patient recovered.

The MICs of β-lactams for *K. pneumoniae* MF were determined by agar dilution and interpreted as recommended elsewhere (5). Strain MF was resistant to all β-lactams (Table 1). A synergy was found between aztreonam and clavulanic acid on a double-disk synergy test (data not shown). A β-lactamase extract from a culture of strain MF subjected to analytical isoelectric focusing (8) showed three β-lactamases with pI values of 5.1, 7.6, and 8.2. This culture extract had significant imipenem hydrolysis activity (1.8 U/mg) (8).

Plasmid analysis detected four plasmids (160, 140, 130, and 3 kb) in strain MF. With the use of ampicillin (100 μg/ml) and sodium azide (100 μg/ml; Sigma-Aldrich, Saint-Quentin-Fallavier, France) as selective agents and *Escherichia coli* J53Az^r as the recipient strain (3), the 160-kb plasmid was only self-conjugative whereas the 140-kb plasmid carried also an ampicillin resistance determinant that was transferred only after electroporation. With the use of a series of primers for detection of class A and class B β-lactamase genes (7, 8), PCR experiments followed by sequencing identified a β-lactamase gene coding for the carbapenemase VIM-1 (pI 5.1) on the 160-kb plasmid whereas the gene coding for the class A expanded-spectrum β-lactamase SHV-5 (pI 8.2) was identified on the 140-kb plasmid. Both genes have been identified repeatedly in Greece (2, 4, 7, 9).

Then, identification of the sequences surrounding the bla_{VIM-1} gene found a class 1 integron that was similar to those reported for VIM-1-producing *E. coli* and *K. pneumoniae* isolates in Greece and different from those of other parts of the world (2, 4). In addition in the present case, an IS*26* element was identified just downstream of the integrase gene of the integron. IS*26* has been involved in expression and possible mobilization of a series of different β-lactamase genes (1).

Finally, this report underlines the requirement to analyze in detail the molecular mechanisms underlying a multidrug resistance phenotype. This is the first case of a simultaneous occurrence of β-lactamases VIM-1 and SHV-5, which may indicate the ongoing evolution of the spread of metallo-β-lactamase genes not only in the *Pseudomonaceae* but also in the *Enterobacteriaceae*.

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TABLE 1. MICs of β-lactams for *K. pneumoniae* MF and *E. coli* transformants

β-Lactam(s)[a]	MIC (μg/ml)			
	K. pneumoniae MF	*E. coli* DH10B (VIM-1)	*E. coli* DH10B (SHV-5)	*E. coli* DH10B
Amoxicillin	>256	>256	>256	4
Amoxicillin + CLA	>256	64	4	4
Ticarcillin	>256	>256	>256	4
Ticarcillin + CLA	>256	>256	16	4
Piperacillin	>256	64	256	2
Piperacillin + TZB	>256	64	4	2
Cephalothin	>256	>256	>256	4
Cefotaxime	>256	16	8	0.06
Ceftazidime	>256	128	>256	0.06
Cefepime	>256	0.5	0.12	0.06
Cefoxitin	>256	32	4	4
Moxalactam	>256	128	0.06	0.06
Aztreonam	>256	0.06	>256	0.06
Imipenem	64	4	0.06	0.06
Imipenem + EDTA	1	<1	0.06	0.06
Meropenem	>32	0.25	<0.06	0.06
Ertapenem	64	1	<0.06	0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml. EDTA was used at a fixed concentration of 320 μg/ml (2).

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