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Letters to the Editor

First Isolation of a Carbapenem-Hydrolyzing β-Lactamase in *Pseudomonas aeruginosa* in Spain

Extended-spectrum β-lactamases capable of hydrolyzing carbapenems are increasingly being reported for *Pseudomonas aeruginosa* (3). These enzymes have the broadest spectrum, hydrolyzing substrates including all β-lactam antibiotics except the monobactam aztreonam (3). These metallo-β-lactamases are of the IMP or VIM series and share less than 30% amino acid identity (2, 8). The IMP enzymes have been reported mostly for Southeast Asia isolates (3, 9, 11). The VIM enzymes have been more recently reported: β-lactamase VIM-1 from *P. aeruginosa* isolates in Italy (1, 2); VIM-2 in Marseilles and Paris in France (7, 8), in Greece (5) in Italy (6), and in Korea (K. Lee, J. B. Lim, J. Yum, D. Yong, J. R. Choi, Y. Chong, and J. M. Kim, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2003, p. 123). The IMP and VIM genes are chromosome or plasmid located and are part of gene cassettes in class 1 integrons (2, 7–11).

A retrospective analysis of susceptibility to β-lactams was conducted with 52 imipenem- and ceftazidime-resistant *P. aeruginosa* strains that had been isolated at Sant Pau Hospital in Barcelona from January 1996 to June 2001. Preliminary antibiotic susceptibility testing by disk diffusion showed that one isolate (strain Ka. 209) was resistant to ceftazidime and imipenem and susceptible to aztreonam.

This strain was isolated from bronchoalveolar lavage of a 6-year-old boy with fever and respiratory distress syndrome who was diagnosed with acute lymphoblastic leukemia. He was admitted for allogeneic bone marrow transplantation. He had never travelled abroad. Despite first-line therapy with ceftazidime, amikacin, and amphotericin B, the patient died after 1 week.

The MICs of β-lactams for strain Ka. 209 were determined by agar dilution according to NCCLS guidelines (4). Strain Ka. 209 was resistant to all tested β-lactams but remained fully susceptible to aztreonam and of intermediate susceptibility to meropenem (Table 1).

TABLE 1. MICs of β-lactams for *P. aeruginosa* strain Ka. 209, *E. coli* DH10B harboring recombinant plasmid pPOI-1, and reference strain *E. coli* DH10B

β-Lactam(s)[a]	MIC (μg/ml)		
	P. aeruginosa Ka. 209	*E. coli* DH10B(pPOI-1)[b]	*E. coli* DH10B
Amoxicillin	>512	<512	4
Ticarcillin	>512	>512	4
Ticarcillin + CLA	>512	512	4
Piperacillin	>512	8	1
Piperacillin + TZB	>512	8	1
Cephalothin	>512	256	2
Cefoxitin	>512	128	1
Ceftazidime	256	32	0.5
Cefepime	64	0.12	0.03
Aztreonam	0.5	0.12	0.12
Meropenem	4	0.25	0.06
Imipenem	256	1	0.12

[a] CLA and TZB, clavulanic acid and tazobactam, at fixed concentrations of 2 and 4 μg/ml, respectively

[a] *E. coli* DH10B(pPOI-1), like *P. aeruginosa* strain Ka. 209, expressed the carbapenem-hydrolyzing β-lactamase VIM-2.

A β-lactamase extract from culture of strain Ka. 209 was subjected to analytical isoeletric focusing as described elsewhere (8), showing a β-lactamase with a pI of 5.6, revealed after imipenem hydrolysis detection (8).

PCR experiments were performed with primers specific for detecting IMP and VIM genes (8) with whole-cell and plasmid DNA extractions (8) of strain Ka. 209 as templates. Positive results were obtained with VIM-2 primers. A PCR product cloned in pPCRScript Cam (SK+) (8) was then sequenced on both strands from a recombinant plasmid (pPOI-1). The deduced amino acid sequence was 100% identical to that of VIM-2.

Whole-cell DNA and plasmid DNA analyses showed that strain Ka. 209 contained a single plasmid of ca. 50 kb (data not shown). Conjugation by mating strain Ka. 209 with ciprofloxacin-resistant *P. aeruginosa* PU21 and electrotransformation of its plasmid DNA into *E. coli* DH10B and *P. aeruginosa* PU21 failed (7, 8). Hybridizations with an 801-bp internal probe for bla_{VIM-2} (8) showed that bla_{VIM-2} was plasmid (not chromosome) encoded (data not shown).

This report constitutes a forewarning of the probable dissemination of plasmid-encoded carbapenem-hydrolyzing β-lactamases in *P. aeruginosa* at least in southern Europe.

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