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Outbreak of Infections Caused by *Pseudomonas aeruginosa* Producing VIM-1 Carbapenemase in Greece

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Resistance to imipenem and meropenem was observed in 211 (16.5%) isolates of *Pseudomonas aeruginosa* recovered in a Greek university hospital during 1996 to 1998. In six isolates selected from throughout this period, high-level resistance to both carbapenems (MICs ≥ 128 μg/ml) was associated with production of the class B β-lactamase VIM-1. *bla*$_{VIM}$-bearing isolates belonged to serotype O:12 and were indistinguishable by pulsed-field gel electrophoresis.

Pseudomonas aeruginosa is a common nosocomial pathogen, particularly among immunocompromised patients. Carbapenems, mainly imipenem and meropenem, are potent agents for the treatment of infections due to multiresistant pseudomonads. These drugs have considerable β-lactamase stability and overall have the broadest spectrum of activity compared with other β-lactams (9). Resistance to carbapenems in *P. aeruginosa* is mostly low level (MIC, 8 to 32 μg/ml): imipenem resistance typically reflects reduced uptake as a result of loss of the OprD porin, and resistance by this mechanism codepends on continued expression of the chromosomal AmpC β-lactamase; resistance to meropenem, but not to imipenem, can arise via overexpression of the MexA-MexB-OprM efflux system (9, 15). High-level resistance to carbapenems (MIC >32 μg/ml) is still uncommon in *P. aeruginosa* but can be caused by the presence of class B β-lactamases (2). IMP-1 was the first metallo-β-lactamase described in *P. aeruginosa* (20). Its gene, *bla*$_{IMP}$, has been dispersing among *P. aeruginosa* and other gram-negative rods in Japan and has also been reported from Singapore and South Korea (5, 16; K. Lee, Y. Chong, H. B. Shin, and D. Yong, Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-85, 1998). The association of *bla*$_{IMP}$ with integron elements and the intensive use of carbapenems in Japan apparently contributed to the dissemination of this resistance determinant (16). In Europe, IMP-1, or a close relative, has been recognized only in a highly imipenem-resistant strain of *Acinetobacter baumannii* isolated in Italy (3). A carbapenem-resistant *P. aeruginosa* clinical isolate from a patient at a second Italian hospital produced a novel class B β-lactamase, VIM-1, which conferred high-level resistance to imipenem and meropenem (6).

Between 1996 and 1998, 1,276 clinical isolates of *P. aeruginosa* were obtained from 973 inpatients at the AHEPA University Hospital, Thessaloniki, Greece. Identification was performed with the PASCO system (Difco, Detroit, Mich.), used according to the manufacturer's instructions. MICs of antipseudomonal drugs, except carbapenems, were determined with the PASCO microdilution system and applying the criteria prescribed by the National Committee for Clinical Laboratory Standards (NCCLS) (12). Susceptibility to imipenem and meropenem was determined by agar disk diffusion in accordance with NCCLS recommendations (13). For selected isolates, MICs of imipenem and meropenem and other antipseudomonal drugs were determined by an NCCLS-recommended agar dilution method using Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) containing serial twofold dilutions of antibiotic and a final inoculum of 10^4 CFU/spot. Most antibiotics were purchased from a commercial source (Sigma Chemical Co., St. Louis, Mo.), but imipenem was obtained from Merck Sharp & Dohme (West Point, Pa.) and meropenem was obtained from Zeneca (Wilmington, Del.). O serotypes were determined by the slide agglutination test of the International Antigenic Typing Scheme as previously described (7). Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA was performed with a CHEF-DRII system (Bio-Rad, Hemel Hempstead, United Kingdom) (11). Banding patterns were compared visually.

For β-lactamase studies, freeze-thawed cell extracts (10) were clarified by centrifugation and tested for their ability to hydrolyze imipenem by UV spectrophotometry at 297 nm and 37°C. Transfer of imipenem resistance was attempted by conjugation using recipient strains of *Escherichia coli*, 26R793 (Lac$^-$ Rif^r; kindly provided by E. J. Threlfall, Laboratory of Enteric Pathogens, Central Public Health Laboratory), and *P. aeruginosa*, PU21 (Rif^r) (4). *P. aeruginosa* clinical strains carrying transferable plasmids were used as controls (19). Transconjugants were selected on Mueller-Hinton agar containing imipenem, 4 μg/ml, and rifampin, 400 μg/ml. *bla*$_{IMP}$ and integron-associated gene cassettes were amplified with published primers and conditions (8, 16). In order to investigate the presence of *bla*$_{VIM}$, a 261-bp internal fragment of the gene was amplified with the primers 5′-AGT GGT GAG TAT CCG ACA G-3′ (forward) and 5′-ATG AAA GTG CGT GGA GAC-3′ (reverse), corresponding to nucleotides 1339 to 1599 of the published sequence (6). The conditions were an initial denaturation of 94°C for 5 min; 30 cycles of amplification at 94°C for 25 s, 52°C for 40 s, and 72°C for 50 s; and a final extension at 72°C for 6 min. The *bla*$_{VIM}$ product from one isolate was labeled with digoxigenin-11-dUTP (Roche, Lewes,

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TABLE 1. Resistance rates to carbapenems and other antipseudomonal drugs among *P. aeruginosa* isolates recovered in AHEPA University Hospital

Antibiotic(s)	No. (%) of resistant[a] isolates in:			
	1996	1997	1998	Total
Imipenem only	19 (4.2)	11 (2.4)	8 (2.2)	38 (3.0)
Meropenem only	5 (1.1)	3 (0.7)	3 (0.8)	11 (0.9)
Imipenem and meropenem	83 (18.5)	94 (20.5)	34 (9.2)	211 (16.5)
Ceftazidime	121 (27.0)	138 (30.1)	86 (23.3)	345 (27.0)
Aztreonam	97 (21.7)	136 (29.6)	78 (21.1)	311 (24.4)
Ciprofloxacin	182 (40.6)	163 (35.5)	101 (27.4)	446 (35.0)
Gentamicin	115 (25.7)	124 (27.0)	79 (21.4)	318 (24.9)
Amikacin	91 (20.3)	100 (21.8)	61 (16.5)	252 (19.7)
Piperacillin-tazobactam[b]	122 (27.2)	141 (30.7)	79 (21.4)	342 (26.8)
Total isolates	448	459	369	1,276

[a] Resistance was defined in accordance with NCCLS interpretive criteria.
[b] Tazobactam tested at a fixed concentration of 4 μg/ml.

United Kingdom) in a second-round PCR and used as a probe in hybridization experiments with hybrids detected colorimetrically on Southern blots of DNA, as recommended by the manufacturer.

The proportions of *P. aeruginosa* isolates from the AHEPA Hospital resistant to antipseudomonal compounds during each of the three study years are listed in Table 1. Most carbapenem-resistant isolates (81.2%) were resistant to imipenem and meropenem in the disk tests routinely used, but some were resistant only to imipenem (14.6%), and a few were resistant to meropenem alone (4.2%). Since up-regulation of *mexAB oprM* would compromise only meropenem and since loss of OprD generally gives resistance only to imipenem, it seemed possible that most carbapenem resistance at the hospital during this period was associated with production of a carbapenemase(s). Seven imipenem-resistant isolates were selected randomly for further study; three were isolated during 1996, three were isolated during 1997, and one was isolated during 1998. They were recovered from separate patients in five different hospital departments (Table 2). Six of these isolates exhibited high-level resistance to both carbapenems (MICs $\geq$ 128 μg/ml), whereas one was highly resistant to imipenem but susceptible to meropenem (MIC, 0.5 μg/ml). Crude cell extracts prepared from the six isolates highly resistant to both carbapenems hydrolyzed imipenem rapidly; those of the imipenem-resistant, meropenem-sensitive isolate did not hydrolyze imipenem. bla_{IMP} was not detected in any of the seven isolates, whereas bla_{VIM} was detected in the six isolates resistant to both carbapenems but not in the meropenem-susceptible isolate. Also, PCR testing of two more imipenem-resistant but meropenem-susceptible isolates, as well as of two imipenem-susceptible but meropenem-resistant isolates, failed to amplify bla_{IMP} and bla_{VIM} genes (data not shown). In the six bla_{VIM}-positive isolates, amplification of integron-associated gene cassettes using primers 5′-CS and 3′-CS (8), corresponding to conserved segments surrounding the variable cassette region, gave products of ca. 800 bp which, when Southern blotted, were found to hybridize with a bla_{VIM}-specific probe. All six isolates belonged to serotype O:12 and were indistinguishable by PFGE, indicating that they represented a single strain (not shown). The bla_{VIM}-negative isolate was nontypeable by serotyping and had a distinct PFGE pattern, indicating that it represented a distinct strain. Conjugation experiments failed to demonstrate transfer of carbapenem resistance from any of the isolates.

P. aeruginosa isolates exhibiting high-level resistance to carbapenems as well as to other antipseudomonal drugs have been frequently isolated in our region (17). The association of the metallo-β-lactamase genes, bla_{IMP} and bla_{VIM}, with integrons has been previously recognized (1, 6), and our data have confirmed this association for bla_{VIM} in the Greek O:12 strain of *P. aeruginosa*. However, the dissemination of bla_{VIM} in the AHEPA Hospital might be due to spread of an O:12 strain rather than spread of the resistance gene. Its success may reflect heavy use of carbapenems in Greece and/or the inherent properties of this strain as a pathogen or colonizer. Isolates of serotype O:12 seem to dominate among multiresistant *P. aeruginosa* strains in Greece (18). A previous serotype O:12 strain has become widespread, if still infrequent, across much of Europe (14). This strain had the carbenicillin-hydrolyzing PSE-1 β-lactamase (14) and, apparently, not the VIM-1. The present bla_{VIM}-carrying Greek strain did not have bla_{PSE-1} by PCR and had a distinct DNA macrorestriction banding pattern from the European epidemic strain when investigated by PFGE (not shown).

bla_{VIM}-positive isolates were recovered in Greece during 1996, while VIM-1-producing pseudomonads were detected the next year in Italy (6; G. Corneglia, Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1482, 1999). The appearance of bla_{VIM} in *P. aeruginosa* isolated in nearby European countries may reflect strain or gene dissemination through people traveling between these countries. Further spread of carbapenemase-producing *P. aeruginosa* strains would represent a significant threat for the future of β-lactams.

TABLE 2. Characteristics of seven *P. aeruginosa* isolates tested for carbapenemase production

No of isolate	Date of isolation (mo/day/yr)	Department	Material	MIC (μg/ml) of[a]:									bla_{VIM} status
				IM	ME	CZ	CF	AT	CP	GM	AN	PI-TZ[b]	
4784	06/20/96	C-Surgical	Blood	>128	>128	>128	>128	32	64	>128	>128	>128	+
4824	08/18/96	A-ICU[c]	Wound	>128	>128	>128	>128	64	64	128	>128	>128	+
4880	10/11/96	C-ICU	Wound	128	0.5	>128	>128	32	16	128	128	>128	−
4956	01/25/97	A-ICU	Bronchial	>128	>128	>128	>128	64	128	>128	>128	>128	+
174	06/12/97	B-Surgical	Urine	>128	>128	>128	>128	32	64	128	128	>128	+
388	10/03/97	Dialysis Unit	Blood	>128	128	>128	>128	32	64	128	128	128	+
556	03/02/98	C-Surgical	Wound	>128	>128	>128	>128	32	128	128	128	>128	+

[a] IM, imipenem; ME, meropenem; CZ, ceftazidime; CF, cefepime; AT, aztreonam; CP, ciprofloxacin; GM, gentamicin; AN, amikacin; PI-TZ, piperacillin-tazobactam.
[b] Tazobactam tested at a fixed concentration of 4 μg/ml.
[c] ICU, intensive-care unit.

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