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Letter to the Editor

VIM-4 in a Carbapenem-Resistant Strain of *Pseudomonas aeruginosa* Isolated in Sweden

The first VIM metallo-β-lactamase (MBL), VIM-1, was described in Verona in 1997 for a *Pseudomonas aeruginosa* isolate (2). Since then VIM-2 has been identified in Marseille in 2000 (1), VIM-3 has been identified in Taiwan (7) in 2001, and VIM-4 has been identified in Greece in 2001 (5). Worldwide spread of the VIM and IMP MBLs is now well documented (3), but MBLs have not been described in all parts of the world. In this letter we report a *P. aeruginosa* strain isolated in Sweden producing VIM-4. This is the first description of an MBL-producing *P. aeruginosa* isolate in Scandinavia.

Eighty imipenem-resistant clinical isolates of *P. aeruginosa* from the years 2000 to 2003 were collected from Karolinska Hospital to evaluate the presence of MBL production. Imipenem susceptibility was determined by the disk-diffusion method (4) (http://www.srga.org). All replicate isolates were excluded from the study. Seventy-three of eighty isolates (91.3%) were hospital derived, and out of these, 15 isolates (20.5%) came from intensive care units. Six of the isolates (7.5%) were derived from patients with cystic fibrosis. There were no reports of outbreaks of carbapenem-resistant *P. aeruginosa* at Karolinska Hospital during the three years when the strains were collected.

The strains were screened for MBL production, using the imipenem-EDTA E-test synergy test (6). E-tests were purchased from AB Biodisk (Solna, Sweden). Positive control strains were *P. aeruginosa* CP99-24 and *Serratia marcescens* CI 10-4-9, while *P. aeruginosa* ATCC 27983 was used as a negative control. One strain, *P. aeruginosa* PA 66, showed a reduction in the MIC for imipenem-MIC from >256 mg/liter to 6 mg/liter, indicating a strong inhibitory effect of EDTA.

The strain was further investigated with primers for IMP and VIM (Table 1). Primers were purchased from Cybergene (Huddinge, Sweden). One amplicon was generated with the VIM primers, and the amplicon was sequenced on the GeneAmp PCR System 9700 (AB Applied Biosystems, Foster City, Calif.), using the same VIM primers as for PCR. Sample electrophoresis was performed on an ABI PRISM 310 genetic analyzer (AB Applied Biosystems). Sequencing of both strands generated a 640-bp sequence, which was compared to sequence databases with BLAST (http://www.ncbi.nlm.nih.gov/blast), showing 100% identity to bla_{VIM-4}. Sequencing with a new set of primers based on published VIM-4 sequences (Table 1) generated a sequence of 874 bp, showing 100% identity to class 1 integron-borne bla_{VIM-4}.

TABLE 1. Primers for PCR detection of MBL genes and sequencing

Gene	Use	Primers (5′ to 3′)
bla_{IMP-1}	Amplification	CTACCGCAGCAGAGTCTTTG AACCAGTTTTGCCTTACCAT
bla_{VIM-2}	Amplification	ATTGGTCTATTTGACCGCGTC TGCTACTCAACGACTGAGCG
bla_{VIM-4}	Sequencing	CCCCTATGGAGTCTTGAGG TGAGTTGAGGGGCTTTTGC

TABLE 2. MICs for PA 66, as determined by NCCLS broth dilution

Antibiotic (concn)	MIC (μg/ml)
Imipenem	>256
Imipenem + EDTA	6
Meropenem	>256
Ceftazidime	64
Piperacillin-tazobactam (4 μg/ml)	64
Amikacin	>256
Ciprofloxacin	64

The VIM-4 strain found in our study, PA 66, was isolated in November 2001 in a urine sample from a Greek citizen, who had emigrated to Sweden in August 2001, the same year that bla_{VIM-4} was described for the first time in Greece. The isolate was resistant to imipenem, meropenem, piperacillin-tazobactam, ceftazidime, and ciprofloxacin (Table 2). In the same urine culture an *Escherichia coli* strain was also found that was susceptible to cephalosporins, carbapenems, and quinolones. The patient was treated successfully with a quinolone for the urinary tract infection, indicating that the *P. aeruginosa* isolate was probably a colonizing strain. Later urine cultures from the same patient have not resulted in isolation of *P. aeruginosa*. We have no data on previous carbapenem therapy or information of previous hospitalization in Greece.

The detection of MBLs in Scandinavia provides additional evidence for the assumption of worldwide spread of MBL enzymes. This emphasizes the need for awareness throughout the world with regard to emerging carbapenemases in carbapenem-resistant *P. aeruginosa*.

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