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Letters to the Editor

First Isolation of bla_{VIM-2} in an Environmental Isolate of *Pseudomonas pseudoalcaligenes*

Since the first description of VIM-2, in France, in a *Pseudomonas aeruginosa* clinical isolate (7), this enzyme has been detected in clinical *Pseudomonas* isolates in several countries (6), thus highlighting the need for a detailed knowledge of its spreading dynamics. In Portugal, since the first clinical isolate of bla_{VIM-2}-producing *P. aeruginosa*, collected in 1995 (1), several carbapenemase producers have been recurrently observed in hospitals from the northern and central regions (8).

Concerns in the scientific community rapidly grew when, besides the rapid boost in the increase of this enzyme's geographical distribution area, VIM-2 started to be found in other species than *P. aeruginosa*, such as *Pseudomonas putida*, *Pseudomonas stutzeri* (11), and some *Enterobacteriaceae* (3, 4, 12). Nevertheless, all these isolates were found in the hospital setting, possibly suggesting a certain degree of confinement within this ecological niche. In this study we report the first environmentally isolated *Pseudomonas pseudoalcaligenes* carrying a metallo-β-lactamase (MBL).

P. pseudoalcaligenes E60 was recovered from a hospital wastewater-receiving urban sewage system, as a result of regular screening for MBL producers from several sources. Sampling procedure consisted of plating 100-μl aliquots of wastewater on MacConkey agar supplemented with imipenem (2 μg/ml). Samples were collected upstream and downstream from the point where hospital wastewaters were discharged in the sampled sewage. The identification of isolate E60 was based on the results of 16S rRNA sequencing. The MICs of β-lactams were determined by the Etest method (AB Biodisk, Solna, Sweden). The strain was resistant to carbapenems (MICs for imipenem and meropenem were >32 μg/ml) but was still susceptible to aztreonam (MIC, 2 μg/ml), ceftazidime (MIC, 8 μg/ml), cefepime (MIC, 4 μg/ml), piperacillin (MIC, 8 μg/ml), and piperacillin + tazobactam (MIC, 8 μg/ml). It was also susceptible to all four tested aminoglycosides (tobramycin, amikacin, gentamicin, and netilmicin) and to ciprofloxacin, tested by the disk diffusion method (5). The strain was positive by an MBL production screening test (1), and a multiplex PCR assay designed to amplify both the bla_{VIM} and bla_{IMP} genes (1, 10) was performed. Sequencing confirmed that this strain possessed bla_{VIM-2}.

The metallo-β-lactamase determinant was not transferred to rifampin-resistant *P. aeruginosa* strain PAO or rifampin-resistant *Escherichia coli* strain K802N in mating experiments in solid media (9). These results suggested the chromosomal location of bla_{VIM} which was later confirmed by total DNA digest with I-CeuI enzyme, its separation by pulsed-field gel electrophoresis, and hybridization with bla_{VIM-2} and rRNA probes, as described previously (9). Three chromosomal bands were visualized after I-CeuI digestion (ca. 430, 840, and 1,080 kb), and the bla_{VIM-2} probe hybridized with the 430- and 840-kb fragments of strain E60, indicating the chromosomal location of this gene (data not shown).

PCR amplification and sequencing with primers for the 5′ and 3′ conserved segments from class 1 integrons as previously described (9) revealed an integron almost identical to In56 previously reported in a *P. aeruginosa* clinical isolate (7). The only differences with In56 were those observed in the promoter region. The Pc promoter found in this *P. pseudoalcaligenes* E60 integron is a strong promoter (−35 [TTGACA]; −10 [TAAACT]) (2), a different configuration compared to In56 (with a weak-type promoter).

This is the first isolation of an environmental *P. pseudoalcaligenes* strain producing MBL. Like other species of the genus *Pseudomonas* (e.g., *P. putida* and *P. stutzeri*), *P. pseudoalcaligenes* is widely distributed in soil and aquatic habitats and rarely involved in clinical infections. It is important to note that no carbapenemase producers were detected upstream of the hospital wastewater discharge site and there were no previous recordings of *Pseudomonas* species, other than *P. aeruginosa*, infections in the hospital. Nevertheless, *P. aeruginosa* clinical isolates with VIM-2 were already detected in that same hospital (S. Quinteira, personal observation).

Our findings suggest that the ongoing spread of bla_{VIM-2} is currently occurring simultaneously in several dimensions, since it can now be found in distinct geographical locations, in several bacterial species, and in different environments. These enzymes seem not to be restricted to the hospital setting, a fact that emphasizes the importance of surveying environmental strains that might act as a source and/or reservoir of resistance genes with clinical relevance.

Nucleotide sequence accession number. The GenBank accession no. of the nucleotide sequence reported here is AY685199.

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