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# Letters to the Editor

## First Organisms with Acquired Metallo-β-Lactamases (IMP-13, IMP-22, and VIM-2) Reported in Austria▽

Metallo-β-lactamases (MBLs) hydrolyze penicillins, cephalosporins, and carbapenems. Organisms with acquired MBLs emerged in the late 1980s in Japan and since the mid-1990s have been identified worldwide. They include numerous species, with *Pseudomonas aeruginosa* playing the major role. Of several MBL families, VIM and IMP types are the most prevalent, being coded by gene cassettes inside class 1 integrons (4, 14, 17).

The first four MBL-producing *P. aeruginosa* isolates reported in Austria were identified in October to December 2007. Three isolates (22843, 26285, and 27135) were recovered in a hospital laboratory in Steyr which has tested all carbapenem-nonsusceptible *P. aeruginosa* isolates with Etest MBL (AB Biodisk, Solna, Sweden). The isolates gave the only positive results by the end of 2007 (774 *P. aeruginosa* isolates were recovered in 2007; 15% were carbapenem nonsusceptible). The last isolate (M136074) was identified in a laboratory in Salzburg collaborating with general practitioners and nine hospitals. All *P. aeruginosa* isolates with reduced susceptibilities to carbapenems, ticarcillin-clavulanate, and ceftazidime (MICs, ≥4, >16, and >8 μg/ml, respectively) have been analyzed with the MBL disk test (1), and M136074 has been the only positive isolate so far (361 *P. aeruginosa* isolates were recovered in 2007; 7% were carbapenem nonsusceptible). It was recovered from a wound of a nursing home resident with a hospitalization history in 2007. Susceptibility was determined by using Etest (Table 1). MBL detection was confirmed by the spectrophotometric assay (3) and the hyplex MBL ID system targeted at the $bla_{IMP}$ and $bla_{VIM}$ genes (BAG Health Care, Lich, Germany), which identified $bla_{IMP}$ genes in isolates 26285 and M136074 and $bla_{VIM}$ genes in isolates 22843 and 27135. Variable regions of class 1 integrons with MBL gene cassettes were sequenced as described previously (6). Isolate 26285 carried the $bla_{IMP-13}$ and *aacA4* cassettes, whereas isolate M136074 had a single cassette, $bla_{IMP-22}$. Both $bla_{IMP-13}$ and $bla_{IMP-22}$ were originally identified in Italy, which reported the highest rate of IMPs in Europe, especially IMP-13 (15). The $bla_{IMP-13}$-*aacA4* array was indistinguishable from those in *P. aeruginosa* from Rome (16) and San Giovanni Rotondo, Italy (11). The $bla_{IMP-22}$ cassette was identical to that in a *Pseudomonas fluorescens* strain from L'Aquila, Italy (EMBL/GenBank accession no. DQ361087) but was located in another integronic context. The isolates 22843 and 27135 contained the same array, *aacA29a*-$bla_{VIM-2}$-*aacA29b*, as in integron In59 in *P. aeruginosa* from France (13), later found in *P. aeruginosa* in Sweden (a Greek patient) (7). The isolates were subjected to multilocus sequence typing, using the procedure and the database available at http://pubmlst.org (5, 8). The isolates 26285 (with IMP-13) and M136074 (with IMP-22) represented new sequence types, ST621 and ST620, respectively. While ST621 is a singleton, ST620 has single-locus variants from other countries (ST320, ST338, and ST520). The isolates 22843 and 27135 (VIM-2) were classified into ST111, a founder of the clonal complex CC4, corresponding to the major European *P. aeruginosa* clone P12 (9). P12 members express various resistance mechanisms, including MBLs (2, 7, 9, 10, 12). Interestingly, the "Greek-Swedish" isolate with In59 represented ST229, which also belongs to CC4 (7). The conjugation and hybridization assays, performed as described previously (6), suggested the chromosomal location of the MBL genes.

This study revealed a remarkable diversity for the first MBL producers in Austria and a complex view of their genetic relationships with isolates from other European countries.

**Nucleotide sequence accession number.** The $bla_{IMP-22}$ gene cassette array sequence was assigned the EMBL database accession number FM876313.

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TABLE 1. Antimicrobial susceptibilities of MBL-producing *P. aeruginosa* isolates

| Isolate (MBL) | MIC (μg/ml) of drug[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TIC | TIM | PIP | TZP | CAZ | CTX | FEP | ATM | IPM | MEM | AMK | GEN | TOB | CIP | CST |
| M136074 (IMP-22) | >256 | >256 | 12 | 8 | >256 | >256 | 128 | 4 | 12 | 24 | 6 | 4 | 2 | 32 | 6 |
| 26285 (IMP-13) | >256 | >256 | 16 | 16 | >256 | >256 | 128 | 12 | >32 | >32 | 24 | >256 | 128 | >32 | 6 |
| 22843 (VIM-2) | >256 | >256 | 32 | 32 | 12 | >256 | 48 | 12 | >32 | >32 | >256 | 8 | >256 | >32 | 4 |
| 27135 (VIM-2) | >256 | >256 | 32 | 32 | 12 | >256 | 24 | 12 | >32 | >32 | >256 | 8 | >256 | >32 | 4 |

[a] Abbreviations: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; FEP, cefepime; GEN, gentamicin; IPM, imipenem; MEM, meropenem; PIP, piperacillin; TIC, ticarcillin; TIM, ticarcillin-clavulanate; TOB, tobramycin; TZP, piperacillin-tazobactam.

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