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VIM-1-Producing Pseudomonas mosselii Isolates in Italy, Predating Known VIM-Producing Index Strains

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VIM-1-Producing *Pseudomonas mosselii* Isolates in Italy, Predating Known VIM-Producing Index Strains

Acquired metallo-β-lactamases (MBLs) are emerging resistance determinants of great clinical concern. They are found in *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and other Gram-negative nonfermenters, in which they can confer a very broad spectrum of β-lactam resistance, including the expanded-spectrum cephalosporins and carbapenems, that cannot be reverted by β-lactamase inhibitors currently available for clinical use (2).

The VIM-type enzymes were the second acquired MBLs to be detected, in the late 1990s in Europe (5, 10), and are currently the most widespread in terms of geographical distribution and range of host bacterial species (2). VIM-1 and VIM-2 were the first reported VIM-type allelic variants, in *P. aeruginosa* isolated in 1997 in Verona, Italy (index strain VR-143/97) (5) and in 1996 in Marseilles, France, respectively (10).

We report here on the characterization of a *Pseudomonas* clinical isolate producing the VIM-1 MBL, isolated in Genoa, Italy, in 1994, which to our best knowledge represents the earliest known VIM-producing strain.

Pseudomonas sp. AM/94 was isolated in November 1994 from the lower respiratory tract of an inpatient admitted in the general intensive care unit of Genoa University Hospital. The isolate was at the time identified as *Pseudomonas fluorescens* by the API 20E system (bioMérieux, Marcy l'Etoile, France), with a low grade of discrimination (62%). Reidentification of the isolate by Vitek-2 (bioMérieux) yielded a result of low discrimination between *P. aeruginosa* (34%), *P. fluorescens* (33%), and *Pseudomonas putida* (33%). Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) analysis, using a Microflex LT benchtop MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany), identified the isolate as *Pseudomonas monteilii* (score, 2.03). Molecular identification by amplification and sequencing of 16S rRNA, *rpoD*, *rpoB*, and *gyrB* genes unambiguously identified AM/94 as *Pseudomonas mosselii*, a recently recognized species of the *P. putida* group (3, 7).

Susceptibility testing, carried out with Etest (bioMérieux) or with broth microdilution (1) and interpreted according to the EUCAST breakpoints for *Pseudomonas* spp. (document version 1.3, 2011-01-05, http://www.eucast.org/clinical_breakpoints, accessed October 2011), revealed that AM/94 was resistant to all tested antibiotics except amikacin and colistin (Table 1).

MBL activity, assayed spectrophotometrically (5), was detected in a crude extract of AM/94 (specific imipenemase activity was 120 ± 1 nmol/min/mg protein, inhibited by >80% after exposure to 2 mM EDTA).

Analysis of acquired MBL genes, including bla_{VIM}, bla_{IMP}, and bla_{NDM}, using PCR and sequencing (4) revealed the presence of the bla_{VIM-1} allele. PCR mapping and sequencing of the genetic context of the bla_{VIM-1} gene (12) revealed that the gene was carried on a gene cassette inserted into a class 1 integron whose variable region contained four cassettes (bla_{VIM-1}, *aacA4*, *aphA15*, and *aadA1*) and was identical to that of In70-type integrons previously described in other VIM-1-producing strains from Italy (12). Mapping the region of the cognate Tn*402*-like transposon flanking the integrase gene, however, revealed that the structure was identical to that flanking In70 detected in plasmid pAX22 from *Achromobacter xylosoxidans* AX22 (11): it was lacking the IS*Pa7* insertion sequence present downstream of the *intI1* gene in In70.2, the bla_{VIM-1}-containing integron from the VR-143/97 *P. aeruginosa* index strain (12) (Fig. 1).

TABLE 1 Antimicrobial susceptibility profile of the *P. mosselii* strain AM/94

Antibiotic[a]	MIC (mg/liter)
Piperacillin-tazobactam*	>128
Trimethoprim-sulfamethoxazole	>128
Aztreonam	32
Ceftazidime	>128
Cefepime	>128
Imipenem	64
Meropenem	64
Amikacin*	2
Gentamicin*	16
Ciprofloxacin	2
Levofloxacin	4
Colistin	0.5

[a] MICs were determined by broth microdilution or by Etest (*).

All together, present results indicate that influx of the bla_{VIM-1} MBL gene in the clinical setting started at least since the early 1990s and that In70 might have been the original genetic element involved in this influx.

P. mosselii is an environmental species detected in rhizospheric soil (8, 9) and is an overall unusual human opportunistic pathogen. In literature, only one report describes *P. mosselii* as a cause of infection (a prosthetic valve endocarditis) in human (6). Similar environmental species occasionally acting as opportunistic pathogens most probably play a role as shuttles for acquired MBL genes from their as-yet-unknown natural reservoirs to the clinical setting.

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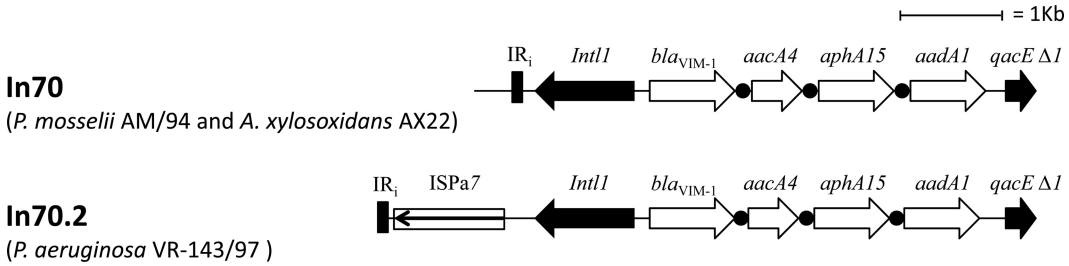

FIG 1 Map of the integron In70 of *P. mosselii* AM/94, identical to In70 of *A. xylosoxidans* AX22 (11), compared with the In70.2 variant carried by *P. aeruginosa* VR-143/97 (12). Open reading frames are indicated by arrows; the *attC* recombination sites of gene cassettes are indicated by circles.

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