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# Isolation of VIM-2-Producing *Pseudomonas monteilii* Clinical Strains Disseminated in a Tertiary Hospital in Northern Spain

**Alain A. Ocampo-Sosa,[a] Laura P. Guzmán-Gómez,[a] Marta Fernández-Martínez,[a] Elena Román,[a] Cristina Rodríguez,[a] Francesc Marco,[b] Jordi Vila,[b] Luis Martínez-Martínez[a,c]**

Service of Microbiology, University Hospital Marqués de Valdecilla-IDIVAL, Santander, Spain[a]; Department of Clinical Microbiology, Hospital Clinic, CRESIB, School of Medicine, University of Barcelona, Barcelona, Spain[b]; Department of Molecular Biology, University of Cantabria, Santander, Spain[c]

**We describe here the occurrence of *bla*$_{\text{VIM-2}}$ in 10 carbapenem-resistant *Pseudomonas monteilii* strains isolated from different clinical samples from patients at the University Hospital Marqués de Valdecilla in northern Spain. All the *bla*$_{\text{VIM-2}}$-harboring *P. monteilii* isolates possessed a class 1 integron, with the cassette array [*intI1_bla*$_{\text{VIM-2}}$*_aac(6′)-Ib_qacE*Δ*1_sul1*]. Our results show the emergence of VIM-2-producing multidrug-resistant species other than *Pseudomonas aeruginosa* or *Pseudomonas putida* in a Spanish hospital. *P. monteilii*, although sporadically isolated, should also be considered an important metallo-β-lactamase (MBL) reservoir.**

Metallo-β-lactamases (MBLs) have appeared worldwide as an important cause of acquired resistance to β-lactams in Gram-negative bacteria, especially in nonfermenting species such as *Pseudomonas aeruginosa*. The broad spectrum activities of these enzymes are of great concern for clinicians, as they confer resistance to the majority of available β-lactams (1). Twelve groups of MBLs (IMP, VIM, NDM, SPM-1, SIM-1, KHM-1, GIM-1, DIM-1, TMB-1, SMB-1, FIM-1, and AIM-1) have been identified in a number of nosocomial pathogens (2–6). IMP and VIM enzymes are among the β-lactamases that exhibit the widest range of substrate specificity (7).

IMP- and VIM-type MBLs usually form part of chromosomally or plasmid-encoded integrons. These integrons may be associated with mobile genetics elements such as insertion sequences or transposon-like structures, which contribute to their dispersion among different bacterial species (1). The presence of transferable MBLs is also important in pseudomonads other than *P. aeruginosa* since they can act as reservoirs and contribute to the spread of these resistance determinants (8).

*Pseudomonas monteilii* is closely related to *Pseudomonas putida*, but they can be differentiated by some biochemical tests, such as those for the assimilation of inositol and L- and D-tartrate, degradation of aromatic hydrocarbons, and arylamidase activity. *P. monteilii* differs from *P. aeruginosa* in pyocyanin production, lipase activity, denitrification, and some other physiological and biochemical features (9).

The occurrence of VIM and IMP has been documented in two *P. monteilii* strains of environmental and clinical origins, respectively (10, 11). In this study, we analyzed the presence of MBLs in 10 nonduplicated carbapenem-resistant strains initially identified as *P. putida* by the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). However, all the isolates were subsequently confirmed as

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Address correspondence to Alain A. Ocampo-Sosa, aocampo@humv.es.
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**TABLE 1** Characteristics of the *P. monteilii* strains isolated in this study

| | | | | | | MIC (μg/ml) of[c]: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain[a] | Source[b] | PFGE | VIM-2 | AME | Integron | CAZ | FEP | PIP | TZP | ATM | IMP | MER | GEN | TOB | AMK | CIP | LVX | COL |
| 04/1103 | BAL | 1 | + | *aac(6′)-Ib* | Class 1 | 64 | 64 | 256 | 256 | 16 | >64 | >64 | >64 | 32 | 4 | 0.06 | 0.25 | 0.5 |
| 08/3472 | AD | 2A | + | *aac(6′)-Ib* | Class 1 | 128 | 32 | 256 | 256 | 64 | >64 | >64 | >64 | 64 | 4 | 16 | 16 | 0.25 |
| 09/1724 | BC | 3 | + | *aac(6′)-Ib* | Class 1 | 128 | 32 | 256 | 256 | 64 | >64 | >64 | >64 | 64 | 4 | 16 | 16 | 0.25 |
| 10/2871 | BC | 2A | + | *aac(6′)-Ib* | Class 1 | 128 | 128 | >256 | >256 | 128 | >64 | >64 | >64 | >64 | 8 | 16 | 32 | 0.25 |
| 11/3751 | UC | 4 | + | *aac(6′)-Ib* | Class 1 | >128 | 64 | 256 | 256 | 64 | >64 | >64 | >64 | 64 | 8 | 16 | 16 | 0.25 |
| 12/544 | UC | 2A | + | *aac(6′)-Ib* | Class 1 | 32 | 32 | 128 | 128 | 64 | >64 | >64 | >64 | 64 | 8 | 8 | 16 | 0.5 |
| 12/1411 | UC | 5 | + | *aac(6′)-Ib ant(2″)-Ia* | Class 1 | 64 | 32 | 128 | 128 | 64 | >64 | >64 | >64 | 64 | 8 | 8 | 16 | 0.5 |
| 12/2891 | BC | 2B | + | *aac(6′)-Ib* | Class 1 | 64 | 64 | 256 | 256 | 64 | >64 | >64 | >64 | >64 | 4 | 8 | 16 | 1 |
| 12/3475 | CV | 2A | + | *aac(6′)-Ib* | Class 1 | 32 | 32 | 128 | 128 | 64 | >64 | >64 | >64 | 64 | 8 | 8 | >32 | 0.5 |
| 12/3748 | BC | 2B | + | *aac(6′)-Ib* | Class 1 | 64 | 32 | 128 | 128 | 64 | >64 | >64 | >64 | 64 | 8 | 8 | 16 | 0.5 |

[a] Digits before the slash represent the year of isolation of the strain.
[b] BAL: bronchoalveolar lavage; AD: abdominal drainage; BC: blood culture; UC: urine culture; CV: cutaneous vesicle.
[c] CAZ: ceftazidime; FEP: cefepime; PIP: piperacillin; TZP: piperacillin-tazobactam; ATM: aztreonam; IMP: imipenem; MEM: meropenem; GEN: gentamicin; TOB: tobramycin; AMK: amikacin; CIP: ciprofloxacin; LVX: levofloxacin; COL: colistin.

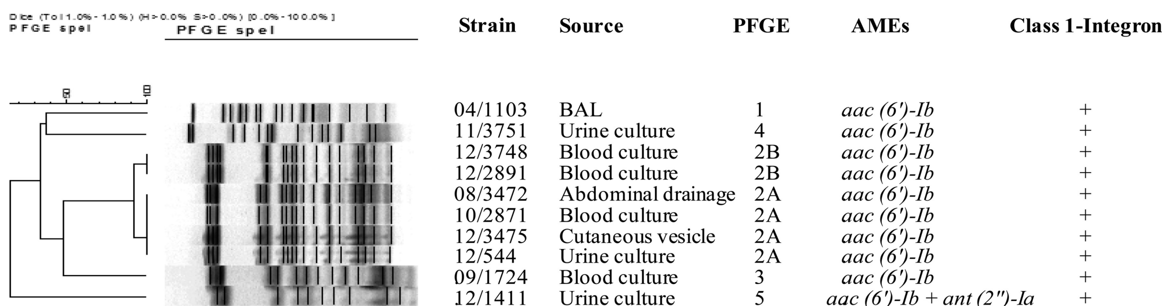


**FIG 1** PFGE profiles of the SpeI-digested genomic DNAs of the $bla_{\mathrm{VIM-2}}$-harboring *P. monteilii* isolates analyzed in this study.

*P. monteilii* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Microflex LT; Bruker Daltonics, Leipzig, Germany) and sequencing of the gene encoding 16S rRNA using primers described before (12). The strains were collected during a period of 8 years (2004 to 2012) in different settings of our hospital in northern Spain. The isolates were recovered from a variety of clinical specimens, including urine culture ($n = 3$), bronchoalveolar lavage fluid ($n = 1$), blood culture ($n = 4$), abdominal drainage ($n = 1$), and a cutaneous vesicle ($n = 1$).

The MICs of 13 antipseudomonal agents were determined by broth microdilution according to the current Clinical and Laboratory Standards Institute (CLSI) guidelines (13). All the isolates were resistant to cephalosporins, aztreonam, carbapenems, gentamicin, and tobramycin, but they were susceptible to amikacin and colistin. In addition, all but one isolate (*P. monteilii* strain 04/1103) showed resistance to ciprofloxacin and levofloxacin (Table 1). Double-sided Etest (imipenem/imipenem-EDTA; AB Biodisk, Solna, Sweden) results suggested the presence of an MBL (data not shown). PCR amplification and DNA sequencing using the primers and conditions described before were performed to confirm the existence of $bla_{\mathrm{VIM-2}}$ (14).

The presence of aminoglycoside-modifying enzymes (AMEs) was also assessed by PCR according to previously described protocols (15–17). In all cases, the acetyltransferase gene *aac(6′)-Ib* was amplified. The nucleotidyltransferase gene *ant(2″)-Ia* was detected in one isolate (*P. monteilii* strain 12/1411) (Table 1). The $bla_{\mathrm{VIM-2}}$-containing integrons were detected by PCR and characterized by DNA sequencing using specific primers targeting the 5′ conserved sequences (CS) and 3′ CS of class 1 integrons (14, 18). All the isolates presented the same class 1 integron cassette arrangement [*intI1_*$bla_{\mathrm{VIM-2}}$*_aac(6′)-Ib_qacE*$\Delta$*1_sul1*], identical to that of the integron of *P. aeruginosa* strain 81-11963A (GenBank accession number AJ515707).

Several attempts to conjugate the putative $bla_{\mathrm{VIM-2}}$-carrying plasmids from *P. monteilii* strains to *Escherichia coli*, as described before (19), were unsuccessful. Therefore, we performed an alkaline lysis extraction, and plasmids were transformed by electroporation into *P. aeruginosa* strain PAO1. In each case, plasmid DNA (>50 kb) was observed. PCRs with VIM-2 and AME-specific primers (13–16) using the total DNA of PAO1 transformants as the template showed that the $bla_{\mathrm{VIM-2}}$ and *aac(6′)-Ib* genes were present. No amplification of *ant(2″)-Ia* was observed, suggesting that this gene might have a chromosomal location. According to PCR-based replicon typing, each *P. monteilii* strain contained an untypeable plasmid (20).

Finally, the clonal relatedness of the isolates was evaluated using pulsed-field gel electrophoresis (PFGE) analysis as previously described (21). Enzymatic restriction was carried out overnight at 37°C with 10 U of SpeI (TaKaRa). Five different clones were observed among the 10 $bla_{\mathrm{VIM-2}}$-carrying *P. monteilii* isolates. Six of the isolates were of clonal origin, as they differed from each other in about 3 bands. As shown in Table 1, pulsotype 2A intermittently appeared during the period of study. The rest of the strains were not clonally related (Table 1, Fig. 1).

*Pseudomonas monteilii* is a versatile bacterium that can be isolated from different natural environments (10, 22). Some strains have been isolated from clinical specimens (9, 11). The presence of VIM-2 was previously described in a *P. monteilii* isolate from an environmental origin (10). To our knowledge, this is the first study to report the occurrence of $bla_{\mathrm{VIM-2}}$ in strains of *P. monteilii* isolated from different clinical settings in a Spanish hospital. According to our results, the persistence and spread of $bla_{\mathrm{VIM-2}}$-mediated resistance in *P. monteilii* reflect clonal and horizontal dissemination. Further spread of these carbapenem resistance determinants from *P. monteilii* to other similar or unrelated species represents an important threat. Moreover, our results reinforce the idea of the relevant role of pseudomonads other than *P. aeruginosa* or *P. putida* as nosocomial reservoirs of MBLs as is the case of *P. monteilii*.

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