Hospital Outbreak of Carbapenem-Resistant *Pseudomonas aeruginosa*
Producing VIM-1, a Novel Transferable Metallo-β-Lactamase

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A total of 8 *Pseudomonas aeruginosa* isolates was collected from 7 different patients in different wards of the University Hospital of Verona, Italy, from February 1997 to February 1998. The high level of resistance to carbapenems (imipenem minimum inhibitory concentration was always >128 μg/mL) and other broad-spectrum β-lactams and the rate of imipenem hydrolysis and its inhibition by ethylenediamine–teta-acetic acid were all suggestive of production of a carbapenem-hydrolyzing metallo-β-lactamase. A specific DNA probe derived from the recently cloned *bla* _VIM_ gene hybridized to all the isolates. A genomic DNA fingerprinting profile revealed clonal relatedness for 7 of 8 isolates. A description of this hospital outbreak is reported, the occurrence of which confirms that proliferation of metallo-β-lactamase–producing strains multiply resistant to β-lactams is already a reality outside Japan. These findings emphasize the need for early recognition of similar isolates.

*Pseudomonas aeruginosa* are among the bacteria most commonly isolated from nosocomial infections. They exhibit intrinsic resistance to several antimicrobial agents, including most β-lactams, and they often carry acquired resistance determinants that further reduce their spectrum of susceptibility [1–5]. Carbapenems have the broadest antipseudomonal spectra of all β-lactams and play a fundamental role in the treatment of *P. aeruginosa* infections. Resistance to carbapenems is often associated with the loss of the 46–48-kDa outer-membrane protein D2 [6], which has been renamed OprD. A decrease in OprD expression regularly accompanies overexpression of the multidrug efflux system MexE-MexF-OprN, thus accounting for the carbapenem resistance observed in those strains [7]. In OprD-deficient strains, derepressed production of chromosomal β-lactamases significantly contributes to imipenem resistance [2], but of greater concern are the increasingly frequent reports of “carbapenemases,” that is, enzymes that are able to hydrolyze carbapenems with fast kinetics [8].

Until recently, the occurrence of *P. aeruginosa* isolates producing acquired carbapenemases had been reported only in the Far East, where the mobile *bla*_ _IMP_ determinant encoding the broad-spectrum metallo-β-lactamase IMP-1 has been spreading among *Enterobacteriaceae*, *P. aeruginosa*, and a number of other nonfastidious gram-negative nonfermenters since the late 1980s [9–10]. Recently, however, carbapenemase-producing *P. aeruginosa* isolates have also been detected in the United Kingdom, Italy, and Portugal [11–13], suggesting that this fearsome mechanism of resistance could soon become a global problem.

The carbapenemase-producing *P. aeruginosa* isolate from Italy (VR-143/97) was obtained from the Verona University Hospital (northern Italy) and was found to produce a novel metallo-β-lactamase, which was named VIM-1. Like IMP-1, it exhibits a very broad substrate specificity and is encoded by a determinant (*bla*_ _VIM_ ) carried on a mobile gene cassette inserted in an integron [11]. Following isolation of VR-143/97, systematic analysis of *P. aeruginosa* isolates was carried out at the University Hospital of Verona over a 1-year period in order to investigate the overall prevalence of carbapenem-resistant isolates from nonurinary clinical specimens and the presence of *bla*_ _IMP_, *bla*_ _VIM_, or other acquired carbapenemase determinants in the resistant strains. That survey detected additional *P. aeruginosa* isolates producing VIM-1 or closely related variants from the same hospital [14]. We carried out a retrospective analysis of the cases from which the metallo-β-lactamase–producing *P. aeruginosa* were isolated, including evaluation of various clinical and epidemiological parameters and of some relevant features of the microbial isolates.
Materials and Methods

Bacterial strains and screening procedures. A total of 541 clinical isolates of *P. aeruginosa* were collected from nonurinary specimens at the University Hospital of Verona, Italy, from February 1997 to February 1998. A number of these isolates were selected for further experiments on the basis of the following criteria, the simultaneous presence of which could be suggestive of production of a carbapenem-hydrolyzing metalloenzyme: resistance to imipenem; resistance to other broad-spectrum *β*-lactams, such as ceftazidime; and susceptibility to or an intermediate phenotype to aztreonam.

Antibiotics. Working solutions were prepared on the day of use from laboratory standard powders of all compounds as specified by the manufacturers. Meropenem was provided by Zeneca (Milan, Italy). Cilastatin-free imipenem was provided by Merck Sharp & Dohme (Rome, Italy). All other antibiotics were from commercial sources.

Susceptibility tests. Disk susceptibility tests were performed and interpreted according to the latest National Committee for Clinical Laboratory Standards (NCCLS) documents [15–16]. Dilution antimicrobial susceptibility tests were performed by serial 2-fold dilution in Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD) and interpreted as described in the latest NCCLS documents [16–17].

*β*-lactamase assays. The hydrolysis rates of 100 μM antibiotic by sonic extracts of cells were obtained as previously described [18] with a Beckman DU-7 ultraviolet spectrophotometer (Palo Alto, CA), with readings recorded at 10-s intervals for 5 min, operating at the wavelength at which the difference between the extinction values of the hydrolyzed and nonhydrolyzed molecule was maximum. The exact wavelengths used were as follows: ceftazidime, 260 nm; and imipenem and meropenem, 299 nm. The millimolar differential absorbance value for all the *β*-lactams tested was 9.0 mM/cm for imipenem and ceftazidime and 6.5 mM/cm for meropenem, at the respective wavelengths. Inhibition of enzymatic activity by EDTA was determined by measuring the residual carbapenemase activity after incubation of the crude extract for 20 min at 25°C in the presence of 2 mM of EDTA. A control without EDTA was always run in parallel. Reactivation by Zn²⁺ was determined by measuring the carbapenemase activity after incubation of the EDTA-treated enzyme preparation for 20 min at 25°C in the presence of 2 mM Zn²⁺, as described previously [11]. Controls were performed for the effect of Zn²⁺ alone on enzyme activity and substrate stability were also included.

Isoelectric focusing analysis. Gel isoelectric focusing was performed in 5% polyacrylamide gels containing ampholytes (pH range, 5.5–9.5) with a Multiphor II apparatus (Pharmacia-LKB Biotechnology, Uppsala, Sweden). The gels were focused at 4°C and 25 W for 180 min. The isoelectric point (pI) value was determined by focusing 5 μg of each purified enzyme. *β*-Lactamases were detected by overlaying the gel with 0.5 μmol/mL nitrocefin in 0.1 mol/mL phosphate buffer, pH 7.0, applied as a nitrocefin-soaked filter paper. Carbapenem hydrolyzing activity was assayed by overlaying the gel with imipenem-containing nutrient agar and swabbing a susceptible indicator organism across the agar [19].

Characterization of outer-membrane proteins. Outer-membranes were prepared by a rapid procedure [6, 18] yielding appreciable degrees of purification. Cells were grown in Luria-Bertani broth up to the late exponential phase of growth, harvested by centrifugation, washed once with 20 mM HEPESS-NaOH buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, and resuspended in the same buffer. The suspension was sonicated with the above-mentioned ultrasonic disruptor by 4 30-s pulses of sonication with an intervening 30 s in ice. The unbroken cells were removed by centrifugation at 1500 g for 15 min, and membranes were pelleted by centrifugation of the supernatant at 19,000 g for 30 min. Finally, pellets were resuspended in 1 mL of HEPESS-NaOH buffer 20 mM, pH 7.4, and stored at −80°C. The protein content of the samples was determined with a protein assay kit (Bio-Rad Laboratories, Hercules, CA). Outer-membrane proteins were separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) essentially as described by Laemmli [20]. Proteins were detected by Coomassie blue staining.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared from each strain for analysis by PFGE by the method described by Smith and Cantor [21] as modified by Ichiyama et al. [22] and was digested with Xbal for 24 h at 37°C. DNA fragments were separated with a CHEF DR III apparatus (Bio-Rad). Half-strength Tris borate–EDTA running buffer was used, and the electrophoresis was run at 14°C for 23 h at 200 V. Pulse time was linearly ramped within an initial forward time of 5 s and a final forward time of 35 s. Interpretation of DNA restriction patterns generated by PFGE was according to the guidelines proposed by Tenover et al. [23].

Colony blot hybridization. Colony blot hybridization was performed with cells grown directly on sterile nitrocellulose filters (Schleicher & Schuell, Keene, NH) layered on Mueller-Hinton agar. Cell lysis, prehybridization, and hybridization were carried out essentially as described elsewhere [24]. DNA probes were labeled with ³²P by the random priming technique with a commercial kit (Boehringer, Mannheim, Germany). The *bla*<sub>VIM</sub>-specific probe was a 0.5-kb HindIII fragment internal to the *bla*<sub>VIM</sub> gene [25]. The *bla*<sub>VIM</sub>-specific probe was a PCR-generated amplicon containing the entire *bla*<sub>VIM</sub> coding sequence from *P. aeruginosa* VR-143/97 [11].

Results

Origins and screening of the isolates. Of the 541 strains collected from nonurinary specimens over the period from February 1997 to February 1998, as many as 244 strains (45.1%) were isolated from lower respiratory tracts, whereas decidedly lower percentages of strains came from surgical wounds (11.6%), pharyngeal swabs of patients with hematological disorders (7.4%), and blood cultures (6.8%). Of the 541 isolates, 83 (15.3%) demonstrated imipenem resistance (MIC, ≥16 μg/mL). Of these, 62 (11.4% of all isolates) exhibited resistance also to ceftazidime (MIC, ≥32 μg/mL), and 35 of the latter group (6.5% of all isolates) also proved susceptible or intermediate to aztreonam (MIC, <32 μg/mL). These 35 isolates were selected for further experiments.

Analysis of carbapenemase production. In 7 of 35 strains selected in the previous step (the VIM-1–producing index strain VR-143/97 [11] was one of them), the crude cell-free extracts
showed fast hydrolysis of both imipenem and meropenem, confirming specific carbapenem-hydrolyzing activity, and also of the third-generation cephalosporin ceftazidime (data not shown). Carbapenemase activity was inhibited by treatment with EDTA and could be fully restored following addition of Zn$^{2+}$ to the EDTA-treated sample (see also Lauretti et al. [11]). On the basis of these data, all 7 isolates were judged to be eligible for further analysis. Because all these isolates lacked any inhibition zone in the Kirby-Bauer susceptibility test, a further (eighth) isolate sharing the same feature, namely VR-143/97, was also examined for metallo-$\beta$-lactamase production. The assay was also positive with this isolate, which was therefore considered for further analysis along with the other 7 strains.

After development with nitrocefin, analytic isoelectric focusing of the crude extracts of the 8 isolates revealed multiple bands of $\beta$-lactamase activity in all of them, including 1 large band plus minor ones at alkaline pH values ($\approx$7.5–8.8) and 1 band at acidic pH value ($\approx$5.3) (data not shown). The alkaline pI bands were probably contributed by the resident AmpC-like cephalosporinase of P. aeruginosa, whereas the acidic one (whose value was similar to that previously reported for VIM-1 [11]) was contributed by the carbapenemase, as demonstrated by its detection by means of the gel overlay procedure with imipenem.

A blavIM-specific probe derived from the blavIM gene cloned from VR-143/97 yielded a strong hybridization signal with all the carbapenemase-producing isolates in colony hybridization experiments, with the intensity of the signal similar to that obtained with VR-143/97. None of the above isolates was recognized by a blavIMP-specific probe in a similar hybridization assay.

Clinical associations of the selected isolates. The 8 isolates under investigation came from 7 different inpatients. Most of them had been isolated from patients given various combinations of antimicrobial agents, including broad-spectrum cepha-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Patient</th>
<th>Ward</th>
<th>Source</th>
<th>Collection date</th>
<th>Previous therapy with</th>
<th>Outcome of hospitalizationa</th>
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<tr>
<td>VR-143/97</td>
<td>NG</td>
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<td>Pus</td>
<td>1 February 1997</td>
<td>Imi</td>
<td>Death</td>
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<td>GB</td>
<td>ICU</td>
<td>Bronchial aspirate</td>
<td>25 March 1997</td>
<td>Imi</td>
<td>Discharge</td>
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<td>Death</td>
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<td>RS</td>
<td>ICU</td>
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<td>9 July 1997</td>
<td>Ctri</td>
<td>Death</td>
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<td>VR-170/97</td>
<td>RS</td>
<td>ICU</td>
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<td>CF</td>
<td>Hematology</td>
<td>Pharyngeal swab</td>
<td>20 February 1998</td>
<td>Czid</td>
<td>Discharge</td>
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NOTE. Amox/Clv, amoxicillin/clavulanate; Ctri, ceftriaxone; Czid, ceftazidime; ICU, intensive care unit; Imi, imipenem.

a Because of the retrospective nature of the analysis and because of the critical status of the patients, a definite correlation between Pseudomonas infection and death was in any case difficult to establish.

Table 1. Clinical associations of Pseudomonas aeruginosa strains carrying the metallo-$\beta$-lactamase gene.
During the following 2 months, several microorganisms underwent surgery in May 1997 for acute necrotic pancreatitis. After admission, death occurred on day 90 after admission. Isolates of Acinetobacter baumannii were isolated from bronchial aspirate on days 60 and 80, respectively, after admission to the ICU in late July 1997 because of severe shock. After frequent admissions to the ICU (day hospital) for placement of central venous catheters, the patient was finally transferred to the ICU in late January 1998 because of severe shock. On day 20 after the admission to the ICU, therapy with imipenem (0.5 g iv every 8 h) was initiated owing to onset of fever (38°C–39°C). Citrobacter freundii was isolated from bronchial aspirate on day 28 of ceftriaxone therapy (day 36 after admission). P. aeruginosa (isolates VR-158/97 and VR-170/97) were isolated from bronchial aspirate on days 60 and 80, respectively, after admission. Death occurred on day 90 after admission.

**Patient CE.** Patient CE was a 71-year-old woman who underwent surgery in May 1997 for acute necrotic pancreatitis. During the following 2 months, several microorganisms (namely Klebsiella pneumoniae, imipenem-susceptible *P. aeruginosa*, *Bacteroides fragilis*, Stenotrophomonas maltophilia, and Acinetobacter baumannii) were isolated from blood cultures and bronchial aspirates, in spite of consecutive therapies with imipenem (0.5 g iv every 8 h) and ceftazidine (2 g iv every 8 h). After frequent admissions to the ICU (day hospital) for placement of central venous catheters, the patient was finally transferred to the ICU in late July 1997 because of severe shock. On day 20 after the admission to the ICU, therapy with imipenem (0.5 g iv every 8 h) was initiated owing to onset of fever (38.8°C) and was continued for 6 days. *P. aeruginosa* (isolate VR-174/97) was isolated from blood culture on day 18 after discontinuation of the therapy (day 44 after admission). Death occurred on day 50 after admission to the ICU.

**Patient BC.** Patient BC was a 61-year-old man admitted to the ICU in late January 1998 because he suffered a cerebral vascular accident, which was followed by severe cerebral ischemia and coma. Therapy with amoxicillin plus clavulanic acid (1.2 g iv every 8 h) was started on the day of admission and was never discontinued. *P. aeruginosa* (isolate VR-155/97) was isolated from bronchial aspirate on day 6 after admission, and was never discontinued.

**Patient CF.** Patient CF was a 34-year-old man with acute leukemia admitted to the hematology department in February 1998 in order to receive bone marrow transplantation. On day 8 after transplantation, therapy was initiated with ceftazidime (2 g iv every 8 h) because of onset of fever (>38°C) and continued for 7 days. *P. aeruginosa* (isolate VR-193/98) was isolated from a pharyngeal swab on day 2 of ceftazidime therapy; all other specimens were negative. The transplantation proved successful, and the patient was discharged 30 days after admission.

**Susceptibility testing.** The results of susceptibility testing of 12 antimicrobial agents with the 8 *bla*<sub>IMP</sub>-positive isolates are shown in table 2. All isolates demonstrated high-level resistance to carbapenems, to all broad-spectrum cephalosporins including ceftazidime (MIC, ≥128 μg/mL), and to inhibitor-protected penicillins; MICs of aztreonam appeared to be lower and, apart from isolate VR-155/97, below the breakpoint for resistance (32 μg/mL). All isolates also demonstrated high-level resistance to non–β-lactam agents, including the quinolone ciprofloxacin and the aminoglycosides gentamicin and tobramycin; amikacin had similar MIC values that, however, would allow 4 strains to be classified as “intermediate” according to the NCCLS breakpoints (MIC, 32 μg/mL).

### Table 2. Susceptibility profiles of the *Pseudomonas aeruginosa* isolates carrying the *bla*<sub>IMP</sub> metallo-β-lactamase gene.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Imi</th>
<th>Mero</th>
<th>Ctri</th>
<th>Czid</th>
<th>CEP</th>
<th>Atm</th>
<th>Pip/Taz</th>
<th>Tic/Clv</th>
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**NOTE.** Amik, amikacin; Atm, aztreonam; CEP, cefepime; Cpx, ciprofloxacin; Ctri, ceftriaxone; Czid, ceftazidime; Gm, gentamicin; Imi, imipenem; Mero, meropenem; Pip/Taz, piperacillin/tazobactam; Tic/Clv, ticarcillin/clavulinate; Tm, tobramycin.

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**Discussion.**

The spread of metallo-β-lactamases capable of hydrolyzing carbapenems in *P. aeruginosa* and other major gram-negative...
the ICU before isolation of the bla\textsubscript{VIM} among most patients (6 of 7) consisted in outbreak and its subsequent spread, a common denominator considerations.

Analysis of the various parameters yielded the following overall clinical history, and antimicrobial chemotherapy), anal-

and epidemiological variables (ward, underlying diseases and of the survey permitted evaluation only of a number of clinical microbiological parameters. Although the retrospective nature of the outbreak by evaluating various clinical, epidemiological, and spread of the resistance gene.

The diffusion of metallo-\textbeta-lactamases is highly disturbing because, contrary to what occurred when permeability-related carbapenem resistance first emerged, the isolates bearing these enzymes usually do not remain susceptible to most broad-spectrum \textbeta-lactams [9–10, 13, 26]. The fact that the \textit{bla\textsubscript{VIM}} determinant was found to be carried on a mobile genetic element of the gene cassette type [11] could facilitate its rapid spread among major gram-negative pathogens. In the index strain (VR-143/97), the \textit{bla\textsubscript{VIM}} gene was apparently carried on the chromosome and was not transferable by conjugation to Es-
priate handwashing, which is crucial to avoid transferring multiresistant strains in ICUs, was usually assessed and probably contributed to limiting the diffusion of the \textit{bla\textsubscript{VIM}}-positive strain in such an epidemiological setting.

The clinical significance of the isolates was difficult to assess. One of them (VR-193/98, isolated from a pharyngeal swab) was clearly a colonizer. In the 6 remaining patients, a role in infection was reasonably clear for VR-143/97 (isolated from purulent material from a surgical wound), VR-155/97 (isolated from bile), and VR-174/97 (isolated from blood), whereas it remained more doubtful for VR-146/97, VR-158/97 + VR-170/97, and VR-186/98, because the source was bronchial and the isolates could have been mere colonizers. Although 5 patients died, it was difficult to establish the causes of death with certainty (owing to the critical conditions of the patients), and, at least in some cases, death occurred several days after isolation of multiresistant \textit{P. aeruginosa}. In any case, the presence of \textit{P. aeruginosa} carrying a similar metallo-\textbeta-lactase determinant in a nosocomial setting is a most worrying development from the epidemiological standpoint, because it could act as a reservoir for further spread of the resistance gene.

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Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of outer-membrane proteins from clinical isolates of \textit{Pseudomonas aeruginosa}. Lane 1, Molecular weight markers: \textbeta-galactosidase (116,000); phosphorylase B (97,400); bovine albumin (66,000), egg albumin (45,000), and carbonic anhydrase (29,000). Lane 2, Imipenem-resistant OprD-deficient strain 3B [27]. Lane 3, Imipenem-susceptible strain 3C [27]. Lane 4, Imipenem-susceptible strain VR-188/98. Lane 5, VR-143/97. Lanes 6 and 7, VR-146/97 (in duplicate). Lanes 8 and 9, VR-155/97 (in duplicate). Lane 10, VR-158/97. Lane 11, VR-170/97. Lanes 12 and 13, VR-174/97 (in duplicate). Lane 14, VR-186/98. Lane 15, VR-193/98.

Figure 2. Pulsed-field gel electrophoresis of \textit{XbaI}-cleaved genomic DNA of \textit{Pseudomonas aeruginosa} clinical isolates. \lambda, \lambda Ladder DNA marker (DNA sizes indicated). Lane 1, VR-143/97. Lane 2, VR-146/97. Lane 3, VR-155/97. Lane 4, VR-158/97. Lane 5, VR-170/97. Lane 6, VR-174/97. Lane 7, VR-186/98. Lane 8, VR-193/98. Lane 9, VR-148/98 (control strain, not related to the outbreak).
It would be interesting to investigate whether a similar genetic background is also found in the other bla\text{VIM}\textsuperscript{-}positive isolates and to perform transfer experiments with them. In addition, because detection of the bla\text{VIM}\textsuperscript{-}determinant was carried out by means of filter hybridization, it would also be interesting to sequence the bla\text{VIM}\textsuperscript{-}determinants of the various isolates, and especially that of the genetically unrelated strain, to ascertain the existence of allelic variants of this resistance determinant.

Analysis of the antimicrobial chemotherapy received by the patients before isolation of the bla\text{VIM}\textsuperscript{-}positive \textit{P. aeruginosa} revealed that only 3 of the patients had received therapy with imipenem, whereas the others had been given noncarbapenem \(\beta\)-lactams, including expanded-spectrum cephalosporins or, in one case, amoxicillin/clavulanate. Similar findings were also reported in the Japanese multifocal epidemics of bla\text{IMI}\textsuperscript{-}positive gram-negative rods, where previous use of carbapenems could be confirmed for only 15% of the patients, whereas 38.8% of patients were administered cephalosporins before the isolation of bla\text{IMI}\textsuperscript{-}positive isolates and such strains were also isolated from patients who had not been administered antibiotics, suggesting that metallo-enzyme–bearing \textit{P. aeruginosa} can spread as hospital infections without the use of antibiotics [26]. It should be recalled that also on the occasion of the emergence of OprD-positive strains, 1 of 3 imipenem-resistant strains was not isolated during therapy with imipenem [27].

All the bla\text{VIM}\textsuperscript{-}positive \textit{P. aeruginosa} isolates in this outbreak exhibited high-level resistance not only to carbapenems and most other \(\beta\)-lactams but also to fluoroquinolones and several aminoglycosides. The cross-resistance to unrelated classes of antibiotics indicates the simultaneous presence of additional resistance determinants such as genes for aminoglycoside-modifying enzymes, mutations that upregulate efflux systems, or both, which are common among nosocomial isolates of \textit{P. aeruginosa}. The different gentamicin MICs for VR-158/19 (32 \(\mu\)g/mL) and VR-170/97 (>128 \(\mu\)g/mL), which were isolated from the same patient with a 20-day difference in isolation, were probably related to the acquisition of a novel aminoglycoside resistance determinant by the latter strain, in spite of the fact that no antimicrobial chemotherapy was administered between the 2 isolations.

It is worth noting that all the bla\text{VIM}\textsuperscript{-}positive \textit{P. aeruginosa} lacked the outer-membrane protein OprD and that the absence of this channel results in the acquisition of imipenem resistance [6]. Thus, the simultaneous presence of at least 2 resistance mechanisms in all isolates prevented us from assessing whether the acquisition of this novel metallo-\(\beta\)-lactamase gene alone is sufficient to confer high-level resistance on carbapenems or whether the simultaneous presence of an outer-membrane permeability modification is necessary for expression of high-level carbapenem resistance, as reported with IMP-1 [9]. The latter case would imply the potential existence, among metallo-\(\beta\)-lactamase producers, of low-level resistant strains or even of strains that, although exhibiting a reduced carbapenem susceptibility, are still classified as “susceptible” according to the interpretative breakpoints and would be hard to detect in routine susceptibility tests. Similar strains would undoubtedly have been missed because of the protocol of our study and its requirement of imipenem resistance as a preliminary screening criterion.

During the screening for metallo-\(\beta\)-lactamase producers, 28 isolates were found that were resistant to imipenem and ceftazidime, that were susceptible or intermediate to aztreonam, and that did not produce any detectable carbapenemase activity. A similar phenotype could reflect the contribution of different resistance mechanisms, such as a combination of permeability defects and active efflux. These isolates undoubtedly deserve further investigation to ascertain the molecular basis of their resistance phenotype.

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References