Dynamics of a Nosocomial Outbreak of Multidrug-Resistant *Pseudomonas aeruginosa* Producing the PER-1 Extended-Spectrum β -Lactamase

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From November 1998 to August 1999, a large outbreak occurred in the general intensive care unit of the Ospedale di Circolo in Varese (Italy), caused by *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β -lactamase. A total of 108 clinical isolates of *P. aeruginosa* resistant to broad-spectrum cephalosporins were recovered from 18 patients. Epidemic isolates were characterized by synergy between clavulanic acid and ceftazidime, cefepime, and aztreonam. Isoelectric focusing of crude bacterial extracts detected two nitrocefin-positive bands with pI values of 8.0 and 5.3. PCR amplification and characterization of the amplicons by restriction analysis and direct sequencing indicated that the epidemic isolates carried a *bla*_{PER-1} determinant. The outbreak was of clonal origin as shown by pulsed-field gel electrophoresis analysis. This technique also indicated that the epidemic strain was not related to three other PER-1-positive isolates obtained at the same hospital in 1997. Typing by enterobacterial repetitive intergenic consensus-PCR showed that minor genetic variations occurred during the outbreak. The epidemic strain was characterized by a multiple-drug-resistance phenotype that remained unchanged over the outbreak, including extended-spectrum cephalosporins, monobactams, aminoglycosides, and fluoroquinolones. Isolation of infected patients and appropriate carbapenem therapy were successful in ending the outbreak. Our report indicates that the *bla*_{PER-1} resistance determinant may become an emerging therapeutic problem in Europe.

Pseudomonas aeruginosa is an opportunistic pathogen that causes bacteremia in immunocompromised patients and burn victims, iatrogenic urinary tract infections, and hospital-acquired pneumonia, particularly in intensive-care settings (27). Infections are especially serious in intubated patients, with a reported mortality of up to 40 to 50% (28). A variety of mechanisms contribute to the virulence of this agent: expression of adhesins, production of biofilms, secretion of hydrolytic enzymes, and production of toxins that may be injected directly into host cells (30).

P. aeruginosa infections are often difficult or impossible to eradicate. In part, this is due to high-level resistance to many antibiotics and disinfectants as a result of both intrinsic and acquired mechanisms (11, 16). Acquired resistance to β -lactams, which are among the frontline drugs for *P. aeruginosa* infections, may result from the production of β -lactamases and/or from a reduced access to the target due to decreased outer membrane permeability (23). Concerning the former mechanism, mutations that cause constitutive overexpression of the resident class C β -lactamase are a common cause of acquired resistance to ceftazidime and other broad-spectrum cephalosporins (12). In addition, secondary β -lactamases of

classes A, B, and D can be responsible for the variable patterns of acquired β -lactam resistance (15, 20, 26).

The PER-1 enzyme is a secondary β -lactamase that was first detected in *P. aeruginosa* by Nordmann et al. in 1993 (18). It was reported as a chromosomally encoded class A enzyme produced by a urinary isolate of *P. aeruginosa* from a Turkish patient (18). PER-1 was later also detected in *Salmonella enterica* serovar Typhimurium (34). A multicenter study conducted in Turkey in 1996 revealed a high prevalence of this enzyme among nosocomial isolates of *Acinetobacter* spp. (46%) and *P. aeruginosa* (11%) (35).

In 1997, our group detected for the first time PER-1-positive *P. aeruginosa* isolates outside the geographic area of origin (F. Luzzaro, G. M. Rossolini, M. Perilli, L. Pagani, R. Belloni, L. Lauretti, G. Amicosante, and A. Toniolo, Abstr. 99th Gen. Meet. Am. Soc. Microbiol. 1999, abstr. A73, p. 15, 1999). The isolates were obtained from three different patients admitted to different wards of the Ospedale di Circolo in Varese, Italy. One year later, a large outbreak of PER-1-positive *P. aeruginosa* occurred in the general intensive-care unit (ICU) of the same hospital. Here we describe the dynamics of that nosocomial outbreak and the properties of the responsible multidrug-resistant strain.

MATERIALS AND METHODS

Epidemiological and clinical data. From November 1998 to August 1999, 108 apparently identical isolates of *P. aeruginosa* were obtained from 18 patients admitted to the ICU of the Ospedale di Circolo in Varese, Italy. Isolates were characterized by multiple-drug resistance that included piperacillin, broad-spec-

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trum and "fourth-generation" cephalosporins, monobactams, aminoglycosides, and fluoroquinolones. Cultures were performed as requested by the clinical staff. Isolates were obtained most frequently from the respiratory tract (79 of 108) and urine (14 of 108) but also from blood (3 of 108). To better evaluate the dynamics of the outbreak, the following factors should be taken into consideration. Over the 1-year period from October 1998 to September 1999, 560 patients were admitted to the ICU. The mean hospitalization time was 7.6 days per patient. During the above period, 402 *P. aeruginosa* isolates were obtained from 87 (15.5%) of the 560 ICU patients. Subsequent studies revealed that 18 (3.2%) of the 560 patients were infected with epidemic multidrug-resistant PER-1-positive strains whereas 69 (12.3%) had infections caused by nonepidemic *bla*_{PER}-negative *P. aeruginosa* isolates.

Bacterial identification, antimicrobial susceptibility, and clavulanate synergy test. Bacteria were identified by the ATB System (ID32GN strips; Bio-Mérieux, Marcy L'Étoile, France). MIC of several antimicrobial agents (piperacillin, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, amikacin, and ciprofloxacin) were determined by the broth microdilution assay (Sceptor System custom MIC panels; Becton-Dickinson Microbiology Systems, Sparks, Md.). Data were interpreted according to the criteria of the National Committee for Clinical Laboratory Standards (17). Sensitivity to disinfectants (chlorhexidine, iodine povidone, toluen-*p*-sulfochloramide, mercurochrome, and silver nitrate) was tested by a broth dilution method in 96-well plates. The reference *P. aeruginosa* strain, ATCC 27853, was used as control. To detect strains producing extended-spectrum β -lactamases (ESBL), ceftazidime-resistant isolates were tested by a double-disk clavulanate synergy test on Mueller-Hinton (MH) agar. Disks containing 30 μ g of aztreonam, ceftazidime, or cefepime were placed 20 mm apart (center to center) around a disk containing amoxicillin plus clavulanic acid (20 and 10 μ g, respectively). Enhancement of the inhibition zone was taken as presumptive evidence of ESBL production.

β -Lactamase characterization by isoelectric focusing. As reported (24), supernatants (5 μ l) of crude bacterial lysates obtained by sonication were subjected to isoelectric focusing (IEF) on a polyacrylamide gel containing Pharmalyte ampholines (pH range, 3.5 to 10) (Pharmacia, Milan, Italy). The gel was run for 3 h at 10 W and 4°C using a Multiphor II system (Pharmacia); β -lactamase bands were visualized with the chromogenic substrate nitrocefin (Oxoid, Milan, Italy).

Molecular studies. Unless otherwise stated, molecular biology reagents and restriction enzymes were obtained from Sigma Chemical Co., St. Louis, Mo.; *Taq* polymerase and reagents for PCR were obtained from Perkin-Elmer, Monza, Italy; and PCR primers were obtained from Cruachem Inc., Dulles, Va. A Perkin-Elmer 2400 thermal cycler was used for gene amplification.

Colony blot hybridization was carried out as described previously (24) on bacteria grown directly on sterile nitrocellulose filters layered onto MH agar plates (Schleicher & Schuell, Dassel, Germany). *P. aeruginosa* VA-353/97 (one of the PER-1-positive strains isolated in our hospital in 1997) and *P. aeruginosa* ATCC 27853 were included as positive and negative hybridization controls, respectively. Southern blot hybridization was carried out using nitrocellulose membranes (Schleicher & Schuell). The probe used in filter hybridization experiments was a PCR-generated amplicon comprising the entire *bla*_{PER-1} open reading frame (19) labeled with ³²P by the random priming technique (24).

PCR amplification of *bla*_{PER} alleles was performed as described previously (24) on crude DNA extracts (prepared by boiling cells for 10 min in distilled water). The *bla*_{PER-1} and *bla*_{PER-2} genes were distinguished by digestion with *Pvu*II and *Stu*I. The amplicon sequence was determined on crude amplification products as described previously (24). The sequenced region included the coding sequence for the mature PER-1 enzyme and the last 10 amino acids of the signal peptide.

Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) was carried out as follows. Bacteria were cultured for 8 h in Trypticase soy broth. Genomic DNA was prepared from 10⁸ CFU suspended in 1 ml of PIV buffer (10 mM Tris-HCl [pH 7.6], 1 M NaCl) plus 1 ml of 1.3% PFGE-agarose in PIV buffer, essentially as described previously (25). After preincubation in the appropriate restriction buffer, DNA was digested overnight at 37°C with 10 U of *Spe*I or *Xba*I. DNA fragments were resolved in a 1.3% agarose gel with the Rotaphor system R23 apparatus (Biometra, Göttingen, Germany) using the following parameters: 12°C for 45 h; angle, 120° to 130°; voltage, 200 to 140 V; pulse time intervals, 10 to 40 s. Polymerized λ phage DNA was used as the size standard (48.5 to 1,018 kb). After staining with SYBR Gold (Molecular Probes, Eugene, Oreg.), gels were visualized with a Kodak CF440 camera (NEN Life Science Products, Boston, Mass.). PFGE patterns were interpreted according to the criteria of Tenover et al. (31).

Typing by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) was carried out as follows. Bacterial DNA was amplified with the ERIC2 primer (5'-AAG TAA GTG ACT GGG GTG AGC G-3') (37) using a standard reaction

mixture (1.5 mM MgCl₂, 2 μ M primer, 2.5 U of AmpliTaq, and 100 ng of template DNA) and the following parameters: 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 45 s, 58°C for 60 s, and 72°C for 45 s; and 72°C for 10 min. Amplicons were separated by electrophoresis on 1.4% agarose gels and stained with ethidium bromide.

Resistance transfer experiments. Conjugation experiments were performed on MH agar plates using *Escherichia coli* MKD-135 (*argH rpoB18 rpoB19 recA rpsL*) (kindly provided by G. Kholodii, Institute for Molecular Genetics, Russian Academy of Sciences, Moscow, Russia) and *P. aeruginosa* 10145/3 (an *rpoB his* derivative of reference strain ATCC 10145^T) as recipients. The initial donor/recipient ratio was 0.1. Mating plates were incubated at 30°C for 14 h. *E. coli* and *P. aeruginosa* transconjugants were selected on MH agar containing ceftazidime (100 mg/liter) plus rifampin (400 mg/liter). Under the above conditions, the detection sensitivity of the assay was $\geq 5 \times 10^{-8}$ transconjugant/recipient.

RESULTS

Description of the outbreak. In November 1998, a multidrug-resistant isolate of *P. aeruginosa* (VA-463/98) was obtained from respiratory secretions of a patient admitted to the ICU of the Ospedale di Circolo. The patient suffered severe injuries following a car accident, with closed abdominal trauma and spleen and anterior gastric wall rupture. After immediate surgical care and a 2-month hospitalization in two different hospitals, he developed a retropancreatic abscess, septic shock, and coma. He was then admitted to the ICU of the Ospedale di Circolo. At admission, cultures from the lower respiratory tract revealed high concentrations of multidrug-resistant *P. aeruginosa*. The resistance phenotype included piperacillin (MIC, ≥ 256 mg/liter), ceftazidime (MIC, ≥ 64 mg/liter), cefepime (MIC, 32 mg/liter), aztreonam (MIC, ≥ 64 mg/liter), amikacin (MIC, 64 mg/liter), gentamicin (MIC, ≥ 32 mg/liter), tobramycin (MIC, ≥ 32 mg/liter), and ciprofloxacin (MIC, ≥ 8 mg/liter). However, VA-463/98 was susceptible to imipenem (MIC, 1 mg/liter) and meropenem (MIC, 2 mg/liter). Treatment was then initiated with imipenem (1 g twice a day). Repeated cultures from different body sites over a 1-month period remained positive for *P. aeruginosa*. These subsequent isolates exhibited the same resistance profile as VA-463/98, except that the MIC of imipenem and meropenem were increased (4 and 8 mg/liter, respectively). After discharge from the ICU in mid-December, the patient was transferred to a fourth hospital, where he recovered uneventfully. Two days after discharge of the index patient, a second ICU patient developed a urinary tract infection due to a *P. aeruginosa* isolate showing the same multidrug resistance pattern of the isolates obtained from the index patient, as well as an increased carbapenem MIC.

Overall, 108 apparently identical isolates were obtained from 18 patients admitted to the ICU from November 1998 to August 1999. Epidemic isolates were also resistant to high concentrations of several disinfectants, including chlorhexidine (MIC, 1.25 g/liter), iodine povidone (MIC, 19 g/liter), and toluen-*p*-sulfochloramide (MIC, 3.1 g/liter), while remaining susceptible to mercurochrome (MIC, 10 mg/liter) and silver nitrate (MIC, 6 mg/liter). Since epidemic isolates were characterized by the production of the PER-1 extended-spectrum β -lactamase, they were designed PER-1 positive.

To help evaluate the clinical impact of the resistance phenotype of epidemic isolates, Table 1 shows the drug susceptibility rates of nonepidemic *P. aeruginosa* isolates obtained from 69 of 560 patients hospitalized in the ICU over the time of the outbreak. These isolates were usually susceptible to extended-

TABLE 1. Drug susceptibility rates of PER-1-negative *P. aeruginosa* isolates from ICU^a

Antibiotic	% of isolates that were ^b :		
	Susceptible	Intermediate	Resistant
Piperacillin	83.3	0	16.7
Ceftazidime	87.1	6.1	6.8
Cefepime	89.4	5.4	5.2
Aztreonam	79.9	9.2	10.9
Imipenem	85.0	5.8	9.2
Meropenem	88.8	7.8	3.4
Gentamicin	76.1	2.8	21.1
Tobramycin	87.2	2.2	10.6
Amikacin	96.9	1.4	1.7
Ciprofloxacin	89.4	0.7	9.9

^a Nonepidemic PER-1-negative isolates obtained from 69 of 560 ICU patients (October 1998 to September 1999)

^b Susceptibility and resistance are based on National Committee for Clinical Laboratory Standards Criteria (17).

spectrum cephalosporins. The few ceftazidime-resistant strains gave no evidence of synergy between β -lactams and clavulanate and were shown not to carry *bla*_{PER}-related genes by colony blot hybridization. Thus, nonepidemic strains were designed PER-1 negative.

Figure 1 (upper panel) shows, month by month, the number of initial PER-1-positive *P. aeruginosa* isolates recovered from each of the 18 patients involved in the outbreak, together with the number of replicate isolates. Figure 1 (lower panel) shows the number of PER-1-negative *P. aeruginosa* isolates obtained per month (October 1998 to September 1999) from 69 patients hospitalized in the ICU.

Detection of PER-1 and sequencing of the *bla*_{PER-1} gene. The double-disk test showed that epidemic isolates were characterized by synergy between clavulanic acid and ceftazidime, aztreonam, and cefepime. The resistance phenotype and the results of the double-disk test suggested the presence of an ESBL. Analytical IEF revealed that double-disk-positive isolates had two nitrocefin-positive bands, at pIs of 5.3 and 8.0. The latter band is commonly found in *P. aeruginosa* as expression of chromosomal AmpC β -lactamase, whereas the band at pI 5.3 could be ascribed to a different enzyme, most probably representing the acquired ESBL (4; see also <http://www.lahey.org/studies/webt.htm>).

Based on IEF results and on the previous recovery of PER-1-producing *P. aeruginosa* isolates at our institution in 1997 (Luzzaro et al., Abstr. 99th Gen. Meet. Am. Soc. Microbiol. 1999), we suspected that the PER-1 enzyme could be involved in the outbreak. Thus, *bla*_{PER} alleles were searched for in all ceftazidime-resistant *P. aeruginosa* isolates by colony blot hybridization and PCR. A positive hybridization signal and an amplification product of the expected size (966 bp) were obtained with VA-463/98, its replicates, and subsequent epidemic isolates. Digestion of amplification products with *Pvu*II (which cuts into *bla*_{PER-1} but not into *bla*_{PER-2}) and with *Stu*I (which cuts into *bla*_{PER-2} but not into *bla*_{PER-1}) consistently revealed a restriction pattern typical of *bla*_{PER-1} (Fig. 2). Direct sequencing of the amplicon obtained from VA-463/98 revealed that the sequence of the resistance determinant was identical to that of *bla*_{PER-1} (reference 19 and data not shown).

Molecular characterization of PER-1-positive *P. aeruginosa* isolates. *Spe*I macrorestriction analysis was conducted on rep-

FIG. 1. (Top) Number of PER-1-positive *P. aeruginosa* isolates obtained each month from the 18 ICU patients involved in the outbreak (November 1998 to August 1999). Columns show the number of initial isolates recovered from every patient (solid columns) and the number of replicate isolates (open columns). (Bottom) Number of PER-1-negative *P. aeruginosa* isolates obtained per month from 69 of 560 patients hospitalized in the ICU (October 1998 to September 1999). Columns show the number of initial isolates from every patient (solid columns) and the number of replicate isolates (open columns).

representative PER-1-positive epidemic isolates obtained from the 18 patients involved in the outbreak. For comparison, the three PER-1-producing *P. aeruginosa* isolates obtained in 1997 as well as selected PER-1-negative *P. aeruginosa* obtained from ICU patients during the outbreak were analyzed.

PFGE analysis showed that the epidemic PER-1-positive *P. aeruginosa* isolates recovered from November 1998 to August 1999 were of clonal origin. The macrorestriction pattern of the epidemic strain consisted of 28 discernible bands ranging from 10 to 710 kb (Fig. 3). Epidemic isolates showed closely related patterns, with the exception of isolate VA-139/99 (obtained at the end of the outbreak), which differed from the others in 4 of 28 bands. In contrast, the restriction pattern of epidemic strains differed substantially from those of the sporadic PER-1-positive strains isolated in 1997 (Fig. 3) and those of the PER-1-negative isolates obtained during the outbreak period (data not shown).

However, analysis by ERIC-PCR of the epidemic isolates showed two different banding patterns: the first consisted of 12 amplicons (size range, 250 to 2650 bp) and was characteristic of strains recovered from November 1998 to February 1999, whereas the second was characteristic of isolates obtained from April to August 1999. Figure 4 shows representative results for

FIG. 2. Amplification of the *bla*_{PER} gene from a representative epidemic *P. aeruginosa* isolate. Digestion of the amplicon (966 bp) (lane 1) with *Pvu*II produced the expected restriction pattern of the *bla*_{PER-1} gene (lane 2). Digestion with *Stu*I (which cuts into *bla*_{PER-2} but not into *bla*_{PER-1}) failed to digest the amplified product (lane 3). L, DNA size marker.

four strains belonging to the first period of the outbreak in comparison with the banding pattern of four strains obtained toward the end of the outbreak. Thus, ERIC-PCR revealed that minor genetic variations occurred during the outbreak.

Genetic location and transferability of the *bla*_{PER-1} determinant. Plasmid DNA was apparently not detectable either in plasmid or in whole genomic DNA preparations from VA-463/98 analyzed by agarose gel electrophoresis. In a Southern blot experiment carried out with genomic DNA of VA-463/98, the *bla*_{PER-1} probe hybridized to the band of chromosomal DNA, suggesting that the ESBL determinant was chromosomally located in this strain (data not shown).

Conjugation experiments failed to demonstrate the occurrence of transfer of the β -lactamase determinant from VA-463/98 (the initial isolate) and VA-139/99 (an isolate obtained

at the end of the outbreak) to either *E. coli* MKD-135 or *P. aeruginosa* 10145/3.

DISCUSSION

ICUs are high-risk areas for nosocomial infections because of the severity of underlying diseases, the duration of stay, and the use of invasive procedures. Surveillance studies have shown that 20 to 25% of nosocomial infections develop in intensive-care settings (38). Among gram-negative organisms, *P. aeruginosa* is an important pathogen, causing infections in 12 to 25% of ICU patients (10), and its prevalence is increasing (8). A limited number of antimicrobials maintain reliable activity against *P. aeruginosa*, including antipseudomonal penicillins and cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems (2, 3, 9). Thus, it is important to monitor and control the spread of genes conferring resistance to the above drugs.

The PER-1 ESBL is of particular interest in the management of severe infections for at least three reasons: (i) it confers resistance to most β -lactams, including aztreonam and newer antipseudomonal cephalosporins (i.e., ceftazidime and cefepime) (36); (ii) it may be carried on a plasmid that has been transferred in vitro from PER-1-positive *P. aeruginosa* to PER-1-negative strains of the same species (7); and (iii) unlike other class A β -lactamases of *P. aeruginosa*, PER-1 appeared to be transmissible among different species in Turkey (35).

As mentioned above, in 1997 we detected three different isolates of PER-1-positive *P. aeruginosa* from Italian patients who had never traveled to Turkey (Luzzaro et al., Abstr. 99th Gen. Meet. Am. Soc. Microbiol. 1999). Macrorestriction analysis of the chromosomal DNA showed that those sporadic strains were different from each other and from that responsible for the outbreak described in this paper. It is noteworthy that the index patient in the outbreak was already infected with the PER-1-positive VA-463/98 strain at the time he was ad-

FIG. 3. PFGE banding patterns after *Spe*I digestion of representative PER-1-positive *P. aeruginosa* isolates. Epidemic isolates (lanes 1 to 8) show closely related patterns, with the exception of isolate VA-139/99 (lane 8), which differs from the other epidemic strains in 4 of 28 discernible bands. Lanes 9 to 11 show the different restriction patterns of nonepidemic PER-1-positive isolates obtained in 1997 before the beginning of the outbreak. L, λ -phage DNA pulse marker.

FIG. 4. ERIC-PCR analysis of representative epidemic PER-1-positive *P. aeruginosa* isolates obtained from November 1998 to February 1999 (lanes 1 to 4) and from April to August 1999 (lanes 5 to 8). Two clearly different banding patterns were seen, indicating that minor genetic rearrangements took place during the outbreak. L, DNA size marker.

mitted to our institution. Thus, the multidrug-resistant epidemic strain had presumably been acquired during a previous hospitalization(s). Interestingly, a clinical isolate of *Alcaligenes faecalis* producing the PER-1 enzyme was detected in an ICU patient over the course of the outbreak (24). Taken together, our epidemiological observations confirm the circulation of the *bla*_{PER-1} gene between different hospitals and bacterial species in our area. The mechanism of interspecies transmission is, however, unclear. The apparent lack of transferability of the *bla*_{PER-1} determinant from the epidemic strain is consistent with its location on a nonconjugative element.

The strain responsible for the outbreak was highly resistant to piperacillin, aztreonam, ceftazidime, and cefepime. Piperacillin resistance was presumably mediated by overexpression of chromosomal AmpC (detected by IEF as a nitrocefin-positive band with pI 8.0), since PER-1 shows a poor activity on this substrate (18). In addition, the epidemic strain was highly resistant to aminoglycosides and fluoroquinolones, and, with the exception of the first isolate, the MIC of imipenem and meropenem were increased. The latter property probably reflected an early selection of an OprD mutant of VA-463/98 following carbapenem treatment. Therapeutic management of infections due to multidrug-resistant PER-1-positive *P. aeruginosa* isolates was overall more problematic than was management of infections due to PER-1-negative *P. aeruginosa* isolates.

The adoption of strict hygienic measures, carbapenem therapy, and disinfection of decubitus ulcers and surgical wounds with mercurochrome or silver nitrate avoided disseminated infections and resulted in control the outbreak. However, the widespread use of carbapenems was associated with the subsequent emergence of *Stenotrophomonas maltophilia* (a species naturally resistant to these drugs) and with a few cases of *Pseudomonas putida* infections producing VIM-1 (a hydrolytic enzyme active on carbapenems [unpublished data]). Consistent with the latter observation, circulation of *P. aeruginosa* isolates carrying the *bla*_{VIM} metallo- β -lactamase determinant has been recently reported in Italy (29) and an outbreak has been described in Greece (33).

The outbreak reported in our study was apparently of clonal origin, as shown by PFGE analysis. This was reinforced by the observation that both drug resistance phenotype and MIC remained unchanged over the outbreak. PFGE also indicated that the epidemic strain was not related to the PER-1-positive isolates of 1997 or to the PER-1-negative isolates obtained during the outbreak period from ICU patients. Further investigation of epidemic isolates by ERIC-PCR, which is known to have a good correlation with restriction analysis (21), showed that two different banding patterns could be distinguished within the outbreak. The appearance of the second ERIC-PCR type could be due to the occurrence of minor genetic variations within the epidemic strain during the outbreak (13).

The clinical relevance of multidrug-resistant *P. aeruginosa* has recently been stressed by reports of nosocomial infections and outbreaks in several countries (1, 22, 32). Our report, together with more limited observations from Belgium (6), indicates that the *bla*_{PER-1} gene and associated resistance determinants may become an emerging therapeutic problem in Europe. New drugs and β -lactam/ β -lactamase inhibitor combinations will be needed to counteract *P. aeruginosa* resistance (5). In addition, new immunological tools will probably play an important role in future combination therapy (14).

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