JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1198–1204 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1198–1204.2005

Molecular Characterization of an Epidemic Clone of Panantibiotic-Resistant *Pseudomonas aeruginosa*

A. Deplano,[1]* O. Denis,[1] L. Poirel,[2] D. Hocquet,[3] C. Nonhoff,[1] B. Byl,[4] P. Nordmann,[2] J. L. Vincent,[5] and M. J. Struelens[1]

Department of Microbiology,[1] Infection Control,[4] and Intensive Care Unit,[5] Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium, and Service de Bactériologie-Virologie, Hôpital de Bicêtre, Faculté de Médecine, Paris,[2] and Laboratoire de Bactériologie, Hôpital Jean Minjoz, Besançon,[3] France

Received 15 September 2004/Returned for modification 20 October 2004/Accepted 13 November 2004

We describe the molecular characterization of a multiresistant *Pseudomonas aeruginosa* clone causing an outbreak in the intensive care unit (ICU) of a tertiary-care university hospital. Analysis included antimicrobial susceptibility profile, O-serotyping, pulsed-field gel electrophoresis, and amplified fragment length polymorphism. Resistance mechanisms were characterized, including production of naturally occurring and acquired β-lactamases, porin expression, and efflux pump systems. Eighteen patients were colonized or infected with multiresistant *P. aeruginosa*. Multiresistant *P. aeruginosa* was panresistant to penicillins, cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones and remained susceptible only to colistin. Sixteen isolates (89%) belonged to serotype O:11, pulsed-field gel electrophoresis type A1, and amplified fragment length polymorphism type A. Resistance characterization of this epidemic clone showed an overexpression of the chromosomal cephalosporinase AmpC combined with decreased expression of porin OprD and the absence of metallo-β-lactamase or extended-spectrum beta-lactamase. An upregulation of the MexXY efflux system due to an *agrZ* mutation in the *mexZ* repressor was detected. This epidemic clone was restricted to the ICU and was not found elsewhere in hospital. Contamination of the ICU environment and the hands of an ICU nurse with this clone suggests possible hand-borne transmission. Implementation of contact precautions effectively controlled transmission of the epidemic clone. This study illustrates the ability of multiresistant *P. aeruginosa* to cause an outbreak with significant morbidity and mortality and underscores the need to identify clonal outbreaks, which require targeted infection control measures.

Pseudomonas aeruginosa is an opportunistic pathogen that is able to cause severe invasive diseases in critically ill and immunocompromised patients. Because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics, *P. aeruginosa* is a common pathogen in hospitals and particularly in intensive care units (ICUs). A European survey on the prevalence of nosocomial infection in ICU patients showed that *P. aeruginosa* was one of the most frequent pathogens, isolated from 29% of ICU-acquired infections (27, 28). A multicentric study conducted in Belgium placed *P. aeruginosa* as the first gram-negative pathogen recovered in the ICU (6). Furthermore, the high frequency of multiple resistance among *P. aeruginosa* strains makes its eradication difficult (6, 27, 28), and mortality associated with *P. aeruginosa* infection is high compared to other bacteria.

P. aeruginosa exhibits intrinsic resistance to several β-lactams and may acquire additional resistance mechanisms, including decreased outer membrane permeability, penicillin binding protein modifications, production of extended-spectrum β-lactamases, acquisition of metallo-β-lactamases or other enzymes, increased expression of efflux pump systems, and decreased porin expression (5, 8, 16–19). These multiple-antibiotic-resistant *P. aeruginosa* strains caused sporadic nosocomial outbreaks (1, 8, 24), but several drug-resistant *P. aeruginosa* clones are now spreading among vulnerable patients, such as those with cystic fibrosis, as recently described (20).

Several typing methods have been used effectively for epidemiological studies of *P. aeruginosa* infection, including macrorestriction analysis resolved by pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP). We describe here the study of the emergence and propagation in the ICU of our tertiary-care hospital of multiresistant *P. aeruginosa* strains. These strains showed resistance to multiple antibiotics, including penicillins, cephalosporins, carbapenems, and aminoglycosides. Molecular studies were performed to study the clonal transmission and determine the antibiotic resistance mechanisms that could explain this panresistance.

MATERIALS AND METHODS

Nosocomial multiresistant *P. aeruginosa* case definition. Cases of nosocomial colonization and infection with multiresistant *P. aeruginosa* were defined as any non-cystic fibrosis patients with an isolate of *P. aeruginosa* that was resistant or intermediately susceptible to meropenem, gentamicin, and ciprofloxacin acquired by a patient ≥48 h after his hospital admission.

Setting. Erasme Hospital is a 858-bed tertiary-care university hospital located in Brussels, Belgium. The ICUs include four separate units with a total of 31 beds and had 2,355 admissions in 2002 (range, 166 to 222 admissions/month). In the ICUs, all patients were hospitalized in separate rooms.

* Corresponding author. Mailing address: Department of Microbiology, Hôpital Erasme, 808, route de Lennik. 1070 Bruxelles. Belgium. Phone: 00 32 2 555 69 71. Fax: 00 32 2 555 31 10. E-mail: Ariane.deplano@ulb.ac.be.

Culture surveys. Multidrug-resistant bacteria screening by rectal swab was routinely performed on each patient admitted to the ICU. Rectal swabs were cultured on selective MacConkey agar medium (BBL, Becton Dickinson, Le Pont de Claire, France) containing 5 μg of ceftazidime per ml for 48 h. All gram-negative bacilli were identified, and antibiotic susceptibility testing was performed. From July to December 2002, all multidrug-resistant *P. aeruginosa* isolates collected from patients hospitalized at the ICU ($n = 18$) and clinical *P. aeruginosa* isolates from patients admitted to other wards during the same period ($n = 15$) were analyzed. Environmental specimens ($n = 102$) collected from surfaces, water, sink, tap, antiseptic solutions, soap, "sterile" solutions, and swabbing contact plates of medical instruments were sampled on five occasions from August to September 2002. These specimens were incubated in Letheen broth (Difco, Becton Dickinson) and blood agar before subculture on cetrimide agar selective medium (Bio-Rad, Marnes-La-Coquette, France). Health care worker hand specimens ($n = 10$) were also cultured in September 2002 with the broth-bag sampling technique (3).

Identification and antimicrobial susceptibility testing. Identification was based on Gram staining, oxidase test, arginine dihydrolase test, and growth on Kliegler medium (Merck, Darnstadt, Germany) and in brain heart infusion broth incubated at 42°C. Antibiotic susceptibility was determined for the following antimicrobial agents: ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, aztreonam, ceftazidime, cefepime, meropenem, imipenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and colistin with the disk diffusion method (Rosco, Taastrup, Denmark). Isolates were classified as susceptible, intermediate, or resistant according to the standard criteria (14). MICs of cefepime, imipenem, meropenem, amikacin, gentamicin, tobramycin, isepamicin, and ciprofloxacin were determined by the E-test method (AB Biodisk, Solna, Sweden).

Typing. O-serotyping was determined by slide agglutination test with polyvalent antisera and 16 monovalent antisera numbered O1 to O16 (Bio-Rad). Macrorestriction analysis resolved by PFGE was performed as previously described except that DNA was digested with SpeI and PFGE separation conditions were 2 to 40 s for 24 h (22). AFLP analysis was done on the ALF-Express sequencer (Amersham Biosciences, Roosendael, The Netherlands) as previously described (21). Computer analysis was achieved with the BioNumerics software (Applied Maths, Kortrijk, Belgium). For PFGE, DNA fragments ranging from 36 to 674 kb were considered, and analysis was performed with the Dice coefficient and the unweighted pair group method with average linkages clustering method. For AFLP, DNA fragments from 50 to 500 bp were analyzed with the Pearson correlation coefficient and unweighted pair group method with average linkages. The classification criteria used for PFGE analysis were described previously (22) and include a type, designated by a capital letter (e.g., A) and patterns showing zero to six DNA fragments. Any variant was indicated by a numerical suffix (e.g., A1). For AFLP, we classified a type designated by a capital letter and patterns showing ≥90% Pearson similarity as previously proposed (1).

Characterization of β-lactam resistance mechanisms. To detect the presence of metallo-β-lactamases, the MICs of imipenem in the presence or not of EDTA were determined by E-test (AB Biodisk) as previously described (30). Carbapenemase detection was also performed by measuring hydrolysis of imipenem with UV spectrophotometry and PCR for detection of the *imp* and *vim* genes as described by Poirel et al. (17, 18). Detection of extended-spectrum beta-lactamases was performed by the double-disk synergy test and isoelectric focusing analysis as previously described (16). To counteract the effect of high-level expression of the naturally produced AmpC β-lactamase, the double-disk synergy test was also analyzed on cloxacillin–containing plates (16).

PCR for detection of the TEM and SHV penicillinases as well as OXA-1, OXA-2, OXA-9, and OXA-10 oxacillinases was performed as previously described (5). Isolates P8 and P17, belonging to PFGE types A1 and K1, respectively, were randomly selected for further analyses. Production of the chromosomally encoded *ampC* cephalosporinase and *oprD* porin was quantified as previously described (8). The expression of the MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY efflux pump systems was determined by quantitative real-time reverse transcription-PCR as previously described (8, 10). DNA sequencing of the putative repressor gene *mexZ* adjacent to the *mexXY* operon was done as described by Hocquet et al. (8).

RESULTS

Outbreak description. From July to August 2002, a cluster of multiresistant *P. aeruginosa* colonizations were detected in the ICU. The incidence of colonization was 16 of 381 (4.2%) compared to 3 of 1,175 (0.25%) in the previous 6-month period ($P < 0.001$). From 8 August to 30 September 2002, the infection control team asked ICU staff to implement precautions, including the wearing of gowns and gloves for the care of ICU patients colonized or infected with multiresistant *P. aeruginosa*. Standard procedures using alcoholic chlorhexidine handrub solution for hand disinfection were reinforced. During the outbreak, 18 non-cystic fibrosis ICU patients were colonized ($n = 9$) or infected ($n = 9$) with multiresistant *P. aeruginosa* (Table 1). Nine patients (50%) developed infections, including pneumonia ($n = 4$), urinary ($n = 1$) or wound infections ($n = 2$), bacteremia ($n = 1$), and peritonitis ($n = 1$). All patients received colistin intravenous treatment. Three of the nine infected patients died, and mortality was attributable to their *P. aeruginosa* infection.

Antimicrobial susceptibility. Most of the multiresistant *P. aeruginosa* isolates were panresistant to all antimicrobials tested except colistin (Table 1). The proportion of isolates showing resistance was as follows: cefepime, meropenem, imipenem, gentamicin, tobramycin, and ciprofloxacin, 100%; piperacillin, piperacillin/tazobactam, ceftazidime, and aztreonam, 94%; and ticarcillin/clavulanic acid, 83%. MIC determination confirmed the high-level resistance to cefepime (MIC = 16 to 32 μg/ml), imipenem (MIC > 32 μg/ml), meropenem (MIC = 8 to 32 μg/ml), amikacin (MIC = 32 to 64 μg/ml), gentamicin (MIC > 256 μg/ml), tobramycin (MIC = 128 to 256 μg/ml), isepamicin (MIC = 32 to 64 μg/ml), and ciprofloxacin (MIC > 256 μg/ml).

Typing. Multiresistant *P. aeruginosa* isolates clustered in the same epidemic profile: serotype O:11, PFGE type A1, and AFLP type A (Table 1 and Fig. 1). Two serotype O:11 isolates were classified in another PFGE type, K1, and two serotype O:1 isolates showed the epidemic PFGE type A1/AFLP type A, which is predominantly associated with serotype O:11 (Table 1). Typing of *P. aeruginosa* isolates recovered from cystic fibrosis patients ($n = 5$) and patients hospitalized in other units ($n = 10$) showed distinct PFGE (Fig. 1) and AFLP profiles (data not shown). The epidemic curve of ICU patients infected or colonized with multiresistant *P. aeruginosa* from July 2002 to December 2002 showed that a clonal outbreak had occurred due to PFGE type A1 which peaked with 14 cases from July to August 2002 (Fig. 2). After implementation of precautions in August, clonal transmission was partially controlled, with only two epidemic cases over the September to December period (incidence of 0.25%, $P < 0.001$) (Fig. 2). Surveillance of multiresistant *P. aeruginosa* acquired in the ICU in 2003 revealed five cases (incidence of 0.2%), and typing showed the disappearance of the epidemic clone A1 in 2003 (data not shown).

Environmental and health care worker culture surveys. *P. aeruginosa* was recovered from 11 (11%) environmental samples. Eight isolates from tap water ($n = 2$), taps ($n = 2$), sink trap ($n = 2$), and room surfaces ($n = 2$) were either fully susceptible or resistant to ≤2 antibiotics and showed PFGE types different from that of the epidemic clone (Fig. 1). Multiresistant *P. aeruginosa* was recovered from the sink trap ($n = 2$) and tap ($n = 1$). Of these, one sink trap isolate belonged to epidemic type O:11/A1/A (Table 1 and Fig. 1). This sink was located in the room of a patient colonized with multiresistant *P. aeruginosa*. Health care worker screening detected multiresistant *P. aeruginosa* epidemic type O:11/A1/A on the hands of a nurse who had taken care of infected patient P9 before

TABLE 1. Origin and characteristics of multiresistant *P. aeruginosa* strains isolated from patients admitted to Erasme Hospital ICU and the ICU environment, July to December 2002

Origin[a]	Isolation date (mmddyy)	Source	Resistant or intermediately susceptible[b] to:	Serotype	PFGE type	AFLP type[c]
P1	090702	Skin	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	A
P2	110702	Rectal swab	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	A
P4	180702	Sputum	PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	A
P5	220702	Rectal swab	TIM, PIP, TZP, ATM, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	A
P6	240702	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	A
P7	250702	Peritoneal fluid	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	A
P8	290702	Urine	PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	ND
P9	010802	Wound	PIP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	A
P10	050802	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND
P11	050802	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND
P12	120802	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	A
P13	130802	Sputum	TIM, PIP, TZP, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND
P14	180802	Rectal swab	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	01	A1	A
P15	190802	Rectal swab	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	01	A1	A
P16	210802	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	K1	A
P17	270802	Rectal swab	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	K1	A
P18	160902	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND
P19	101202	Sputum	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND
E1	070802	Sink drain ICU5	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	06	C1	C
E3	070802	Tap ICU5	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	06	C1	C
E4	070802	Sink drain ICU5	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, CIP	11	A1	A
H1	040902	Nurse's hands	TIM, PIP, TZP, ATM, CAZ, FEP, MEM, IPM, GEN, TOB, AMK, CIP	11	A1	ND

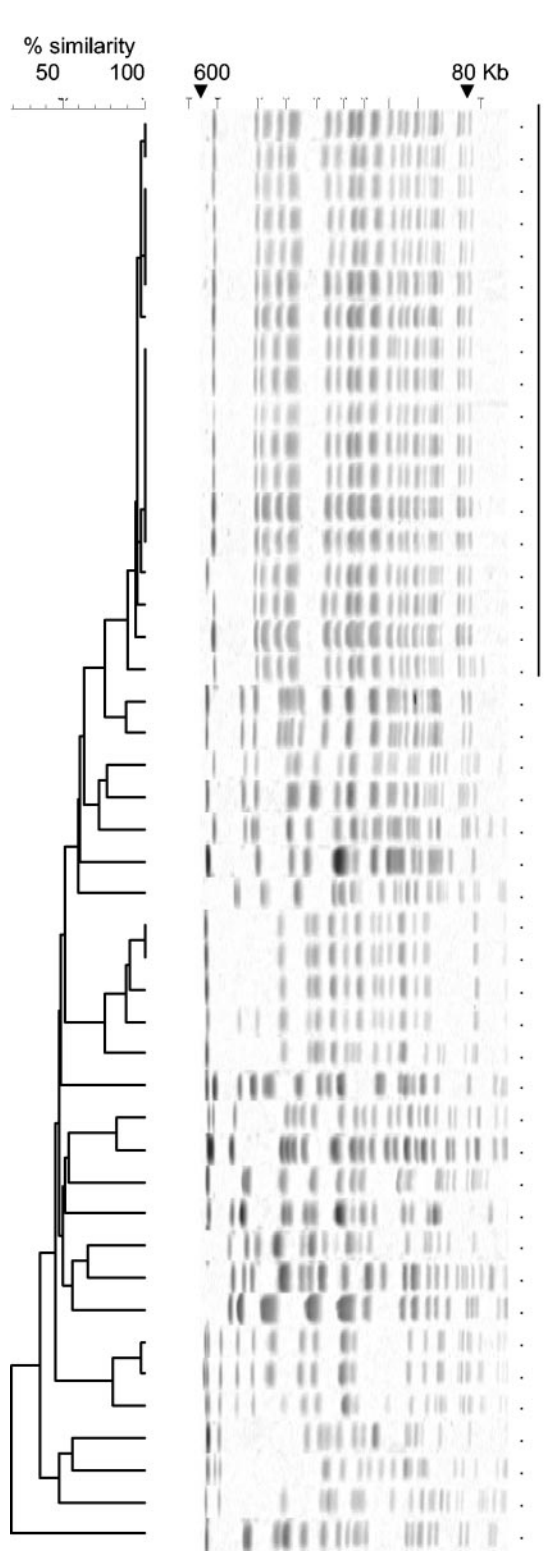
[a] P, patient; E, environment; H, health care worker.
[b] Antibiotic codes: TIM, ticarcillin/clavulanic acid; PIP, piperacillin; TZP, piperacillin/tazobactam; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; IPM, imipenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin; COL, colistin.
[c] ND, not done.

bacteriological screening (Table 1 and Fig. 1). This finding was used by the infection control team to emphasize the importance of careful hand protection and disinfection to the ICU staff.

β-Lactamase production. Analysis by MICs determined by E-test with and without EDTA, spectrophotometric analysis of imipenem hydrolysis, and PCR detection for the *vim* and *imp* genes showed the absence of metallo-β-lactamases in multiresistant *P. aeruginosa* isolates. No TEM or SHV extended-spectrum beta-lactamases or oxacillinases (OXA-1, OXA-2, OXA-9, and OXA-10) were detected by PCR. Nevertheless, phenotypic and quantitative reverse transcription-PCR analysis detected hyperproduction of AmpC cephalosporinase. The putative function of AmpC in the resistance was evaluated by analysis of PFGE types A1 and K1 that showed expression values >50-fold higher than that of the wild-type PAO1 strain (Table 2).

OprD porin. The transcription level of the OprD porin was analyzed by quantitative real-time RT-PCR and compared to that of the reference strain PAO1. OprD appeared to be expressed 5- to 50-fold less than the wild-type PAO1 OprD in multiresistant *P. aeruginosa* types A1 and K1 (Table 2).

Efflux pump systems. The contribution of the MexAB, MexCD, MexEF, and MexXY active efflux pump systems to the resistance of multiresistant *P. aeruginosa* was examined by comparison of PFGE types A1 and K1 with the reference strain PAO1. Transcription levels of *mexA*, *mexC*, and *mexE* were similar to those in wild-type PAO1 (data not shown). In



PFGE type	Origin	Unit	Resistance profile
	P1	ICU5	MR
	P2	ICU5	MR
	P4	ICU5	MR
	P5	ICU5	MR
	P6	ICU5	MR
	P7	ICU1	MR
	P8	ICU4	MR
	P9	ICU4	MR
	P10	ICU4	MR
A1	E4	ICU5	MR
	P11	ICU4	MR
	P12	ICU1	MR
	P13	ICU5	MR
	P14	ICU1	MR
	P15	ICU4	MR
	P18	ICU4	MR
	H1	ICU4	MR
	P19	ICU5	MR
K1	P17	ICU4	MR
K1	P16	ICU1	MR
I	P21	Other	MR
T	P30	Other	non MR
R	E2	ICU3	Multi S
U	CF P29	Other	non MR
J	E2	ICU5	Multi S
B2	E5	ICU4	Multi S
B2	E6	ICU4	Multi S
B2	E7	ICU4	Multi S
B2	E8	ICU5	Multi S
P	P22	Other	non MR
N	P23	Other	non MR
D1	CF P26	Other	MR
D2	CF P20	ICU1	MR
G	CF P28	Other	non MR
Q	P27	Other	non MR
M	P31	Other	non MR
O	E9	ICU3	Multi S
H	CF P28	Other	non MR
C1	E1	ICU5	MR
C1	E3	ICU5	MR
C2	P3	Other	MR
S	E10	ICU3	Multi S
E	P14	Other	non MR
F	P24	Other	MR
L	P15	Other	Multi S

FIG. 1. Dendrogram indicating the similarity of PFGE profiles of *P. aeruginosa* strains (n = 46) and correlation with origin and resistance profile. These strains were isolated from patients (P), cystic fibrosis patients (CF), the environment (E), a health care worker's hand (H), in different ICU units (ICU), and in other units (Other). Resistance profiles were classified as multiresistant (MR), multisusceptible (MS), or resistant to one to five antimicrobials (non-MR).

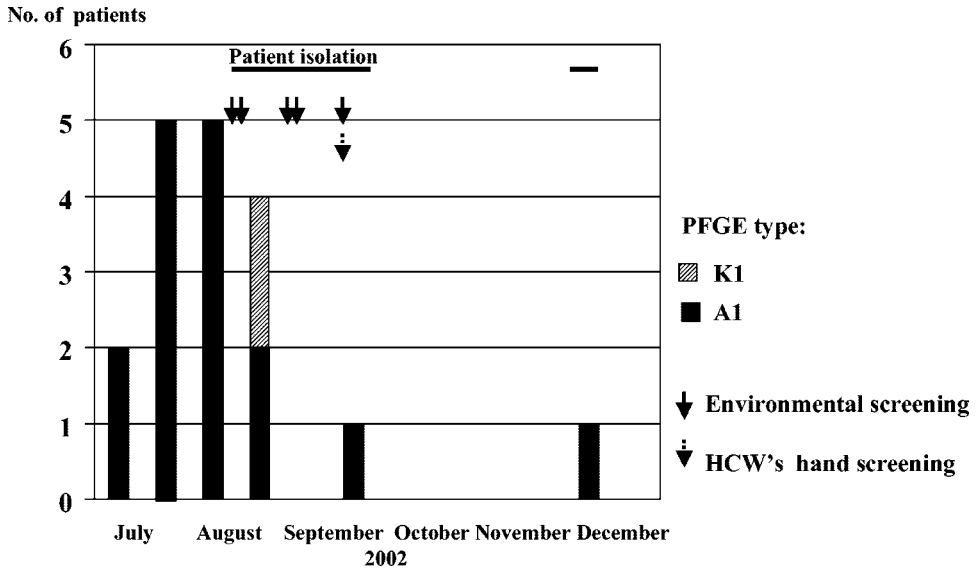


FIG. 2. Occurrence of new cases of nosocomially acquired multiresistant *P. aeruginosa* by PFGE type in the ICU, indicating the period when isolation precautions were implemented, July to December 2002. HCW, health care worker.

contrast, epidemic type A1 *P. aeruginosa* overproduced MexXY compared with the wild-type PAO1 (49-fold). This was not observed for the type K1 isolate (Table 2).

To identify the mutations responsible for this MexXY upregulation, sequencing of the *mexZ* regulator was performed, and the nucleotide sequence was compared to the PAO1 sequence (www.pseudomonas.com). Both type A1 and K1 multiresistant *P. aeruginosa* exhibited two silent mutations resulting from a single nucleotide substitution at the isoleucine at position 37 (ATC→ATT) and the glutamic acid at position 113 (GAA→ GAG) (data not shown). The epidemic type A1 multidrug-resistant *P. aeruginosa* had an additional base deletion at aspartic acid position 164 (GAC→ G-C), introducing a frameshift outside the DNA binding region of MexZ (Table 2).

DISCUSSION

This study describes a clonal outbreak of panresistant *P. aeruginosa* in an ICU during a 6-month period. Typing characterized this epidemic panresistant *P. aeruginosa* clone as O:11/A1/A. PFGE and AFLP analysis appeared to be discriminant typing methods as demonstrated by others (23, 32).

TABLE 2. Variations of resistance mechanisms in multiresistant *P. aeruginosa* isolates

Strain	β-Lactamase activity (nmol/min/mg of protein)	Expression[a]		Sequence of *mexZ*[b]
		oprD	*mexX*	
PAO1	72	1.00	1.00	
Positive control			>22[c]	
PFGE type A1	5,100	0.02	49.4	GAC→G_C (164)
PFGE type K1	3,500	0.2	3.6	

[a] mRNA quantification by RT-PCR. Numerical value are expressed relative to PAO1, which is set at 1.00.

[b] Nucleotide sequence compared with the PAO1 genome (www.pseudomonas.com).

[c] Mut-Gr1 strain overproducing MexXY was used as a positive control (29).

Transmission of this epidemic clone in the ICU was followed by its complete disappearance in 2003 following implementation of contact isolation measures. During the outbreak, this epidemic clone was recovered from ICU patients, from the sink of a colonized patient who was probably the source of this environmental contamination, and from the hands of a nurse, suggesting that transmission between patients could have occurred via health care worker hands.

P. aeruginosa is known to colonize the hospital environment, particularly moist sites (2). Sources such as tap water, sink, antiseptic solutions, respiratory equipment, and bronchoscopes are the most commonly incriminated nosocomial reservoirs of *P. aeruginosa* (7, 22, 25). Recent studies also reported health care workers as a transient reservoir and possible vehicle of nosocomial outbreaks (4, 12, 13, 31, 32). *P. aeruginosa* is not typically considered a skin-colonizing organism. The mechanism or source of hand colonization is presumably through contact with colonized patients or water. This mechanism has been linked to skin lesions and use of artificial nails (4, 13). Compliance with hand protection by gloves, washing, and hand disinfection after glove removal was emphasized as part of the effort to control the epidemic.

This particular multiresistant *P. aeruginosa* clone appeared to be very stable, with 90% of isolates showing identical PFGE/AFLP/serotype/antibiotic susceptibility profiles. It was recovered only in the ICU department. In contrast, an outbreak reported in Greece as being caused by multiresistant *P. aeruginosa* serotype O:11 showed distinct PFGE subtypes, which were also recovered from different units of the hospital (24). A recent study from France described the wide dissemination of an epidemic clone of multiresistant *P. aeruginosa* serotype O:6 inside and outside the hospital (8). Nosocomial *P. aeruginosa* strains are often multidrug resistant and acquire resistance determinants during the course of antimicrobial therapy in heavily colonized patients. This property probably contributes to their maintenance in the clinical environment under high

antibiotic pressure, as was the case in the present study of a nosocomial outbreak limited to ICUs.

In the present study, multiresistant *P. aeruginosa* strains showed coresistance to 11 of the 12 antimicrobials tested and remained susceptible only to colistin. This broad-spectrum coresistance drastically restricted the choice of antimicrobial therapy, leaving colistin as the drug of last resort. Panresistance can be explained by accumulation of multiple resistance mechanisms, including gene mutation, overexpression of efflux pumps, loss or modification of porins, and acquired extended spectrum β-lactamases. In this study, overexpression of the AmpC chromosomal cephalosporinase was documented in the epidemic multiresistant *P. aeruginosa*. This mechanism contributed to resistance to β-lactams, as no acquired extended-spectrum beta-lactamase or oxacillinase was detected in these multiresistant *P. aeruginosa* isolates. However, differences in resistance to β-lactams between isolates were observed (for example, strain P4 was susceptible to ticarcillin/clavulanic acid and strain P5 was susceptible to ceftazidime) and these were probably due to various levels of AmpC cephalosporinase expression (8).

The carbapenem resistance of this epidemic strain could be explained by its decreased OprD porin expression rather than by AmpC overexpression (8). In contrast with European studies that showed the spread of the VIM metallo β-lactamase as the determinant of carbapenem resistance in *P. aeruginosa* isolates (9, 11, 15, 17), no VIM or IMP carbapenemase was detected in the strains in this investigation. A low prevalence of metallo-β-lactamase production was also observed among carbapenem-resistant *P. aeruginosa* in a Greek hospital (26).

We demonstrated by analysis of efflux pump systems that stable overexpression of the inducible *mexXY* system was present in the epidemic type A1. This altered phenotype could explain resistance to β-lactams and account for low-level coresistance to cefepime, aminoglycosides, and fluoroquinolones but also contribute to high-level resistance to these antibiotics in combination with other mechanisms (D. Hocquet, P. Plésiat, and the GESPA Group, Abstr. Int, Meet. Pseudomonas, abstr. 107, 2003). Sequencing analysis suggested that this overexpression was due to mutations in the *mexZ* repressor, recently described as *agrZ* (for aminoglycoside resistance dependent on *mexZ*) mutants by Llanes et al. (10). Double overexpression of the AmpC cephalosporinase and the inducible MexXY system combined with decreased expression of OprD could explain the panresistance displayed by our epidemic *P. aeruginosa*. Other mechanisms such as the presence of aminoglycoside-modifying enzymes or mutations in the quinolone-determining resistance region of topoisomerases can also contribute to resistance to aminoglycosides and fluoroquinolones, respectively, but were not explored in this study.

In conclusion, we demonstrated the epidemic spread of a panresistant *P. aeruginosa* clone involving 16 patients over a 6-month period in the ICUs of a university hospital. Increased expression of chromosomal AmpC cephalosporinase and the MexXY efflux pump system associated with a deficiency of OprD porin was implicated in this panresistance phenotype. Hand carriage of epidemic multidrug-resistant *P. aeruginosa* by a health care worker during the outbreak underlines the importance of careful hand hygiene in this high-risk ICU setting. This study illustrates the ability of multigene antibiotic-resistant mutants of *P. aeruginosa* to cause extensive outbreaks associated with significant morbidity and mortality. Our findings underscore the need to differentiate polyclonal emergence of multiple-antibiotic-resistant *P. aeruginosa* from clonal outbreaks, which require targeted infection control measures.

ACKNOWLEDGMENTS

We thank R. De Ryck and S. Rottiers for their technical contribution and F. Rost for help in collection of demographic data.

REFERENCES

1. **Bukholm, G., T. Tannaes, A. B. Kjelsberg, and N. Smith-Erichsen.** 2002. An outbreak of multidrug-resistant *Pseudomonas aeruginosa* associated with increased risk of patient death in an intensive care unit. Infect. Control Hosp. Epidemiol. **23:**441–446.
2. **Cortes, P., D. Mariscal, J. Valles, J. Rello, and P. Coll.** 2001. Presence of polyclonal *Pseudomonas aeruginosa* in an intensive care unit: a 27-month prospective study on molecular epidemiology. Infect. Control Hosp. Epidemiol. **22:**720–723.
3. **Doebbeling, B. N., M. A. Pfaller, A. K. Houston, and R. P. Wenzel.** 1988. Removal of nosocomial pathogens from the contaminated glove. Implications for glove reuse and handwashing. Ann. Intern. Med. **109:**394–398.
4. **Foca, M., K. Jakob, S. Whittier, L. P. Della, S. Factor, D. Rubenstein, and L. Saiman.** 2000. Endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit. N. Engl. J. Med. **343:**695–700.
5. **Girlich, D., L. Poirel, A. Leelaporn, A. Karim, C. Tribuddharat, M. Fennewald, and P. Nordmann.** 2001. Molecular epidemiology of the integron-located VEB-1 extended-spectrum beta-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. J. Clin. Microbiol. **39:**175–182.
6. **Glupczynski, Y., M. Delmee, H. Goossens, and M. J. Struelens.** 2001. Distribution and prevalence of antimicrobial resistance among gram-negative isolates in intensive care units (ICU) in Belgian hospitals between 1996 and 1999. Acta Clin. Belg. **56:**297–306.
7. **Grundmann, H., A. Kropec, D. Hartung, R. Berner, and F. Daschner.** 1993. *Pseudomonas aeruginosa* in a neonatal intensive care unit: reservoirs and ecology of the nosocomial pathogen. J. Infect. Dis. **168:**943–947.
8. **Hocquet, D., X. Bertrand, T. Kohler, D. Talon, and P. Plesiat.** 2003. Genetic and phenotypic variations of a resistant *Pseudomonas aeruginosa* epidemic clone. Antimicrob. Agents Chemother. **47:**1887–1894.
9. **Lagatolla, C., E. A. Tonin, C. Monti-Bragadin, L. Dolzani, F. Gombac, C. Bearzi, E. Edalucci, F. Gionechetti, and G. M. Rossolini.** 2004. Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired metallo-beta-lactamase determinants in European hospital. Emerg. Infect. Dis. **10:**535–538.
10. **Llanes, C., D. Hocquet, C. Vogne, D. Benali-Baitich, C. Neuwirth, and P. Plesiat.** 2004. Clinical strains of *Pseudomonas aeruginosa* overproducing MexAB-OprM and MexXY efflux pumps simultaneously. Antimicrob. Agents Chemother. **48:**1797–1802.
11. **Mavroidi, A., A. Tsakris, E. Tzelepi, S. Pournaras, V. Loukova, and L. S. Tzouvelekis.** 2000. Carbapenem-hydrolysing VIM-2 metallo- beta-lactamase in *Pseudomonas aeruginosa* from Greece. J. Antimicrob. Chemother. **46:**1041–1042.
12. **McNeil, S. A., L. Nordstrom-Lerner, P. N. Malani, M. Zervos, and C. A. Kauffman.** 2001. Outbreak of sternal surgical site infections due to *Pseudomonas aeruginosa* traced to a scrub nurse with onychomycosis. Clin. Infect. Dis. **33:**317–323.
13. **Moolenaar, R. L., J. M. Crutcher, V. H. San Joaquin, L. V. Sewell, L. C. Hutwagner, L. A. Carson, D. A. Robison, L. M. Smithee, and W. R. Jarvis.** 2000. A prolonged outbreak of *Pseudomonas aeruginosa* in a neonatal intensive care unit: did staff fingernails play a role in disease transmission? Infect. Control Hosp. Epidemiol. **21:**80–85.
14. **National Committee for Clinical Laboratory Standards.** 2002. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 5th ed. Approved standard M7–A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
15. **Poirel, L., L. Collet, and P. Nordmann.** 2000. Carbapenem-hydrolyzing metallo-beta-lactamase from a nosocomial isolate of *Pseudomonas aeruginosa* in France. Emerg. Infect. Dis. **6:**84–85.
16. **Poirel, L., O. Menuteau, N. Agoli, C. Cattoen, and P. Nordmann.** 2003. Outbreak of extended-spectrum beta-lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. J. Clin. Microbiol. **41:**3542–3547.
17. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J. D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
18. **Poirel, L., G. F. Weldhagen, T. Naas, C. De Champs, M. G. Dove, and P.**

Nordmann. 2001. GES-2, a class A beta-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. **45:**2598–2603.
19. **Poole, K.** 2004. Efflux-mediated multiresistance in Gram-negative bacteria. Clin. Microbiol. Infect. **10:**12–26.
20. **Scott, F. W., and T. L. Pitt.** 2004. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. J. Med. Microbiol. **53:**609–615.
21. **Speijer, H., P. H. Savelkoul, M. J. Bonten, E. E. Stobberingh, and J. H. Tjhie.** 1999. Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit. J. Clin. Microbiol. **37:**3654–3661.
22. **Struelens, M. J., F. Rost, A. Deplano, A. Maas, V. Schwam, E. Serruys, and M. Cremer.** 1993. *Pseudomonas aeruginosa* and *Enterobacteriaceae* bacteremia after biliary endoscopy: an outbreak investigation using DNA macrorestriction analysis. Am. J. Med. **95:**489–498.
23. **Talon, D., V. Cailleaux, M. Thouverez, and Y. Michel-Briand.** 1996. Discriminatory power and usefulness of pulsed-field gel electrophoresis in epidemiological studies of *Pseudomonas aeruginosa.* J. Hosp. Infect. **32:**135–145.
24. **Tassios, P. T., V. Gennimata, A. N. Maniatis, C. Fock, and N. J. Legakis.** 1998. Emergence of multidrug resistance in ubiquitous and dominant *Pseudomonas aeruginosa* serogroup O:11. The Greek *Pseudomonas aeruginosa* Study Group. J. Clin. Microbiol. **36:**897–901.
25. **Trautmann, M., T. Michalsky, H. Wiedeck, V. Radosavljevic, and M. Ruhnke.** 2001. Tap water colonization with *Pseudomonas aeruginosa* in a surgical intensive care unit (ICU) and relation to Pseudomonas infections of ICU patients. Infect. Control Hosp. Epidemiol. **22:**49–52.
26. **Tsakris, A., P. T. Tassios, F. Polydorou, A. Papa, E. Malaka, A. Antoniadis, and N. J. Legakis.** 2003. Infrequent detection of acquired metallo-beta-lactamases among carbapenem-resistant *Pseudomonas* isolates in a Greek hospital. Clin. Microbiol. Infect. **9:**846–851.
27. **Vincent, J. L.** 2000. Microbial resistance: lessons from the EPIC study. European prevalence of infection. Intensive Care Med. **26**(Suppl. 1)**:**S3–S8.
28. **Vincent, J. L., D. J. Bihari, P. M. Suter, H. A. Bruining, J. White, M. H. Nicolas-Chanoine, M. Wolff, R. C. Spencer, and M. Hemmer.** 1995. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA **274:**639–644.
29. **Vogne, C., J. R. Aires, C. Bailly, D. Hocquet, and P. Plesiat.** 2004. Role of the multidrug efflux system MexXY in the emergence of moderate resistance to aminoglycosides among *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother. **48:**1676–1680.
30. **Walsh, T. R., A. Bolmstrom, A. Qwarnstrom, and A. Gales.** 2002. Evaluation of a new E-test for detecting metallo-beta-lactamases in routine clinical testing. J. Clin. Microbiol. **40:**2755–2759.
31. **Wenzel, R. P.** 1994. Healthcare workers and the incidence of nosocomial infection: can treatment of one influence the other? a brief review. J. Chemother. 6(Suppl. 4)**:**33–37.
32. **Widmer, A. F., R. P. Wenzel, A. Trilla, M. J. Bale, R. N. Jones, and B. N. Doebbeling.** 1993. Outbreak of *Pseudomonas aeruginosa* infections in a surgical intensive care unit: probable transmission via hands of a health care worker. Clin. Infect. Dis. **16:**372–376.