Persistence of a Multidrug-Resistant *Pseudomonas aeruginosa* Clone in an Intensive Care Burn Unit

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Long-term colonization of various body sites with a multidrug-resistant *Pseudomonas aeruginosa* clone (resistant to piperacillin, cefoperazone, ceftazidime, aztreonam, imipenem, cefepime, cefpirome, ofloxacin, ciprofloxacin, minocycline, and aminoglycosides) with subsequent severe infections in burn patients has not been reported previously. Thirty-nine isolates of multidrug-resistant *P. aeruginosa* (resistant to ceftazidime and at least three of the agents listed above) recovered from various clinical samples from three patients in an intensive care burn unit from April 1997 to May 1997 and seven preserved isolates recovered from six patients in other medical wards at National Taiwan University Hospital from April 1996 to May 1997 were studied for their epidemiological relatedness. The epidemic could be attributed to a multidrug-resistant *P. aeruginosa* clone belonging to serogroup O:F (serogroup O:4) by means of antimicrobial susceptibility testing, O serogrouping, and analysis of the randomly amplified polymorphic DNA patterns generated by arbitrarily primed PCR of the isolates. The epidemic strain persisted in the three patients for weeks to months; in the meantime, these patients had received multiple antimicrobial agents for the management of intervening episodes of invasive infections (bacteremia, ventilator-associated pneumonia, and/or catheter-related sepsis) caused by this strain, as well as concomitant infections due to other organisms. The strain had been isolated only once previously, from a burn patient who was on the unit in December 1996. The present report, describing a small outbreak due to *P. aeruginosa*, documents the fact that a single clone of multidrug-resistant *P. aeruginosa* can cause long-term persistence in different body sites of burn patients and that the colonization can subsequently result in various severe infections.

Despite advances in surgical care and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life-threatening infections caused by *Pseudomonas aeruginosa* continue to be a common complication in burn patients and to contribute substantially to burn-related morbidity and mortality worldwide (6, 12, 13, 21, 22). Multidrug-resistant *P. aeruginosa* has frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of burn patients (13, 21, 22). Also, the long-term colonization of more than one *P. aeruginosa* clone in the respiratory tracts of patients with cystic fibrosis or bronchiectasis has been well demonstrated by various genotypic and phenotypic methods (4, 9, 11, 17, 18). However, the situation in which a multidrug-resistant *P. aeruginosa* clone colonizes various body sites of burn patients for weeks and months and causes intervening episodes of severe infection has not been described previously.

In our intensive care burn unit, an outbreak of infection due to a *P. aeruginosa* strain was initially suspected on the basis of the identification of an unusual antibiotype (resistance to all antimicrobial agents routinely tested for activity against *Pseudomonas* species) among three isolates from two burn patients. In the course of the investigation we used epidemiological surveillance methods coupled with appropriate microbiological studies to determine the relatedness of the isolates with regard to antimicrobial susceptibility, serogroup, and molecular type.

In the present report, we describe an outbreak of infections that was caused by one multidrug-resistant *P. aeruginosa* clone belonging to serogroup O:F as defined by The Serotyping Committee for the Japan *Pseudomonas aeruginosa* Society (Japanese Committee) (equivalent to U.S. serogroup O:4) and that was controlled by the maintenance of strict isolation of the patients.

MATERIALS AND METHODS

Background. The intensive care burn unit of National Taiwan University Hospital, a 2,000-bed tertiary-care teaching hospital, is a referral center for northern Taiwan. The unit has eight single-bed intensive care rooms. On average, 120 thermally injured adults and children are treated in the burn unit each year. From 4 April to 10 April 1997, three unusual isolates of *P. aeruginosa* recovered from various clinical specimens from two patients (patients 2 and 4) (Table 1) were found to be resistant to 12 antimicrobial agents (cefoperazone, ceftazidime, aztreonam, piperacillin, ticarcillin-clavulanic acid, imipenem, minocycline, gentamicin, tobramycin, amikacin, ofloxacin, and ciprofloxacin) by the routine disk diffusion method (16). It was noted upon reviewing the microbiological records that only one *P. aeruginosa* strain with the same antibiotype had previously been isolated, and that was from a patient who had been hospitalized in the intensive care burn unit in December 1996.

Epidemiological surveillance. After the infections with the multidrug-resistant *P. aeruginosa* strains were discovered, bacterial cultures for multidrug-resistant *P. aeruginosa* isolates (resistance to ceftazidime and at least three of the other agents listed above) were performed with various samples from all patients in the burn unit. These samples included burn wound, nasal swab, sputum, urine, and stool samples. Environmental samples for surveillance cultures were obtained from 10 April to 16 April 1997 to detect the presence of multidrug-resistant *P. aeruginosa* from various sources; for this purpose, 100-ml samples of water from sink faucets and swabs of sink surfaces in each patient's room were obtained. Swab specimens from the hands and nares of the physicians and nurses in the unit were also collected for culture due to the concomitant occurrence of an outbreak due to oxacillin-resistant *Staphylococcus aureus*. All swab samples were inoculated directly onto blood agar plates (BBL Microbiology Systems, Cockeysville, Md.). Water specimens were processed as described previously (10).

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TABLE 1. Antibiotypes, O serogroups, and RAPD patterns of *P. aeruginosa* isolates from patients in a burn unit and other wards

Patient no.	Isolate	Location	Source of isolate	Date of isolation (day/mo/yr)	O serogroup[a]	Antibiotype	RAPD pattern (M13/H5)
1	A	Burn unit	Wound (chest wall)	27/12/1996	F	I	I/I
2	B1	Burn unit	Sputum	8/1/1997	B	II	II/II
	B2	Burn unit	Blood	4/4/1997	F	I	I/I
	B3	Burn unit	Sputum	21/4/1997	F	I	I/I
	B4	Burn unit	Sputum	28/4/1997	F	I	I/I
3	C1	Burn unit	Sputum	14/4/1997	F	I	I/I
	C2	Burn unit	Wound (right forearm)	23/4/1997	F	I	I/I
	C3	Burn unit	Sputum	28/4/1997	F	I	I/I
	C4	Burn unit	Sputum	5/5/1997	F	I	I/I
	C5	Burn unit	CVC[b] tip	5/5/1997	F	I	I/I
	C6	Burn unit	Blood	5/5/1997	F	I	I/I
	C7	Burn unit	Urine	12/5/1997	F	I	I/I
	C8	Burn unit	Sputum	12/5/1997	F	I	I/I
	C9	Burn unit	Wound (right arm)	12/5/1997	F	I	I/I
	C10	Burn unit	Wound (left arm)	12/5/1997	F	I	I/I
	C11	Burn unit	Wound (left arm)	15/5/1997	F	I	I/I
	C12	Burn unit	CVC tip	15/5/1997	F	I	I/I
	C13	Burn unit	Nasal swab	15/5/1997	F	I	I/I
	C14	Burn unit	Sputum	19/5/1997	F	I	I/I
	C15	Burn unit	Stool	23/5/1997	F	I	I/I
4	D1	Burn unit	Wound (right elbow)	10/4/1997	F	I	I/I
	D2	Burn unit	Wound (left forearm)	10/4/1997	E	III	III/III
	D3	Burn unit	Sputum	14/4/1997	F	I	I/I
	D4	Burn unit	Wound (right forearm)	14/4/1997	F	I	I/I
	D5	Burn unit	Wound (right arm)	14/4/1997	F	I	I/I
	D6	Burn unit	Wound (left arm)	14/4/1997	F	I	I/I
	D7	Burn unit	Wound (left arm)	14/4/1997	F	I	I/I
	D8	Burn unit	Sputum	21/4/1997	F	I	I/I
	D9	Burn unit	Wound (left leg)	23/4/1997	F	I	I/I
	D10	Burn unit	Stool	28/4/1997	F	I	I/I
	D11	Burn unit	Wound (left hand)	29/4/1997	F	I	I/I
	D12	Burn unit	Sputum	5/5/1997	F	I	I/I
	D13	Burn unit	Wound (neck)	5/5/1997	F	IV	IV/IV
	D14	Burn unit	CVC tip	6/5/1997	F	I	I/I
	D15	Burn unit	Sputum	12/5/1997	F	I	I/I
	D16	Burn unit	Wound (neck)	14/5/1997	E	V	V/V
	D17	Burn unit	Wound (right hand)	14/5/1997	F	I	I/I
	D18	Burn unit	Nasal swab	15/5/1997	F	I	I/I
	D19	Burn unit	Sputum	19/5/1997	N	VI	VI/VI
	D20	Burn unit	Stool	23/5/1997	F	I	I/I
5	E	Medical ward	Urine	20/4/1996	C	VII	VII/VII
6	F	Medical ward	Urine	10/8/1996	D	VIII	VIII/VIII
7	G1	Medical ICU[c]	Sputum	12/8/1996	A	IX	IX/IX
	G2	Medical ICU	Urine	12/8/1996	A	IX	IX/IX
8	H	Medical ward	Urine	3/9/1996	H	X	X/X
9	I	Medical ward	Drainage fluid	16/9/1996	I	XI	XI/XI
10	J	Medical ward	Sputum	5/2/1997	E	XII	XII/XII

[a] O serogroup as designated by Homma (7) (those designated by Lanyi and Bergan are given in parentheses): O:B (O:2), O:F (O:4), O:E (O:11), O:N (O:14), O:C (O:6), O:D (O:90), O:A (O:3), O:H (O:10), and O:I (O:1).
[b] CVC, central venous catheter.
[c] ICU, intensive care unit.

Samples from different body or environmental sites were collected once during the outbreak investigations. For a comparative study of the epidemiological relatedness of the isolates recovered in the burn unit, seven preserved multidrug-resistant isolates from six patients (patients 5 to 10) hospitalized on other medical wards geographically distant from the burn unit from April 1996 to May 1997 were also examined (Table 1).

Laboratory studies. The isolation and identification of *P. aeruginosa* from all specimens except stool specimens were performed by following conventional procedures (3). The stool samples, which were screened for the presence of ceftazidime-resistant *P. aeruginosa*, were inoculated and streaked onto Trypticase-based 5% sheep blood agar (BBL Microbiology Systems), and a 30-μg ceftazidime disk (BBL Microbiology Systems) was placed in the first quadrant.

TABLE 2. In vitro susceptibilities of 12 selected *P. aeruginosa* isolates to 12 antimicrobial agents

Isolate	MIC (μg/ml)[a]												Anti-biotype
	PP	CP	TZ	AT	IP	AK	NC	MC	OF	CI	PM	CR	
A	>256	>256	>256	48	>32	>256	>256	>256	>256	>256	>256	>256	I
B1	>256	>256	>256	64	6	8	24	>256	>256	>256	>256	>256	II
D2	24	64	6	4	4	24	32	>256	0.19	2	4	4	III
D13	>256	96	4	4	4	12	>256	>256	0.75	4	2	4	IV
D16	>256	128	3	4	3	12	>256	>256	4	16	2	4	V
D19	>256	>256	128	24	6	64	12	2	0.094	0.75	64	64	VI
E	>256	>256	>256	>256	>32	16	>256	24	>256	>256	>256	>256	VII
F	>256	>256	>256	48	>32	>256	>256	>256	>256	>256	>256	>256	VIII
G1	>256	>256	>256	96	3	16	16	>256	0.19	1	128	64	IX
H	>256	>256	24	24	>32	192	>256	>256	>256	>256	16	32	X
I	>256	>256	128	24	>32	8	16	>256	>256	>256	64	64	XI
J	48	96	48	12	2	24	96	>256	2	24	24	32	XII

[a] PP, piperacillin; CP, cefoperazone; TZ, ceftazidime; AT, aztreonam; IP, imipenem; AK, amikacin; NC, netilmicin; MC, minocycline; OF, ofloxacin; CI, ciprofloxacin; PM, cefepime; CR, cefpirome.

After 18 to 20 h of incubation at 35°C, any colonies within the inhibition zone of the ceftazidime disk were subcultured and identified to the species level by conventional methods, as indicated previously (3). The susceptibilities of the *Pseudomonas* species to 12 antimicrobial agents were determined by the disk diffusion method at the Microbiology Laboratory (National Taiwan University Hospital), and this test was used as a first-pass screening test for defining multidrug-resistant isolates.

Determination of MICs. The MICs of 12 antimicrobial agents for all the isolates were determined by the Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's guidelines (see Table 2). A bacterial suspension of growth from a Trypticase soy agar plate (BBL Microbiology Systems) was prepared in 5 ml of Mueller-Hinton broth (BBL Microbiology Systems), and the turbidity was adjusted so that it was equivalent to that of a 0.5 McFarland standard. The bacterial suspension was streaked onto a 150-mm-diameter plate containing Mueller-Hinton agar (BBL Microbiology Systems); the plate was later incubated at 35°C in ambient air for 16 to 18 h. The MIC was read on the basis of the interception of the elliptical zone of growth inhibition with the graded Etest strip. For defining the categories of susceptibility and resistance of the isolates (17), any Etest MIC that fell between twofold dilutions was rounded up to the next twofold dilution. A pan-drug-resistant strain was defined as one that was resistant to all 12 antimicrobial agents tested. Antibiotypes were considered identical if the MICs of all agents tested were identical or within a twofold dilution discrepancy.

Determination of O serogroup. Group antisera against 14 O-serogroup antigens, designated A to N, were purchased from Denka Seiken Co., Ltd. (Tokyo, Japan). The O serogroup of each isolate was determined by using the slide agglutination method described previously (7). These 14 O serogroups alphabetically designated by the Japanese committee corresponded to those previously designated numerically by Lanyi and Bergan (7).

RAPD patterns. The randomly amplified polymorphic DNA (RAPD) patterns generated by arbitrarily primed PCR (AP-PCR) were determined essentially as described before (8). Amplification was performed in a PTC-100 thermocycler (MJ Research Inc., Watertown, Mass.) and consisted of the following steps: predenaturation at 94°C for 4 min and 40 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C, with a final extension for 5 min at 72°C. Two arbitrary oligonucleotide primers were used: M13 (5′-TTATGTAAAACGACGGCCAG T-3′; Gibco BRL Products, Gaithersburg, Md.) and H5 (5′-AGTCGTCCCC-3′; OPERON Technologies, Inc., Alameda, Calif.). The amplification products were separated by electrophoresis in 1.5% agarose gels. A 1-kb ladder (Gibco BRL Products) was used in each gel as a DNA fragment size marker. The AP-PCR analyses of these isolates were done in duplicate. Isolates with RAPD patterns that differed by one or more discrete bands were considered different; otherwise, the isolates were considered identical.

RESULTS

Characteristics of the patients. During the 2 months (April and May 1997) of investigation, 16 patients were treated in the intensive care burn unit. Table 1 presents the sources and dates of recovery of the 47 isolates of *P. aeruginosa* included in this study: 40 from various clinical samples from four patients in the burn unit and 7 from six patients in other medical wards. The four burn patients all had more than a 60% total-body-surface-area burn wound and had been exposed to various invasive procedures (central venous catheter insertion and ven-

tilator use) during their stays in the burn unit. None of these patients received hydrotherapy. All of these patients had been treated with various β-lactams, including those with antipseudomonal activity (piperacillin, ceftazidime, and imipenem), and aminoglycosides before the acquisition of *P. aeruginosa* infection or colonization. Other isolates (oxacillin-resistant *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, or yeast isolates) were recovered from burn wounds of all of these patients during their hospital stays. In addition to the colonization or infection of the burn wounds of the four patients by the epidemic multidrug-resistant *P. aeruginosa* strain, this organism caused other complicated infections: patient 2 had bacteremia and ventilator-associated pneumonia, and patients 3 and 4 both had central venous catheter-related sepsis and ventilator-associated pneumonia. After the notification of the presence of the multidrug-resistant *P. aeruginosa* strain, these four burn patients received a wide array of antimicrobial agents, including broad-spectrum cephalosporins, aminoglycosides, and ciprofloxacin, which were prescribed for the treatment of concomitant infections caused by pathogens other than *P. aeruginosa*. Patient 2 died of respiratory failure due to a fulminant pneumonia caused by oxacillin-resistant *S. aureus*. Patients 3 and 4 both survived, although strains of *P. aeruginosa* were repeatedly isolated from different clinical specimens during the course of care in the intensive care burn unit (Table 1).

Susceptibility testing. Table 2 presents the Etest MICs of 12 antimicrobial agents for 12 of the 47 *P. aeruginosa* isolates tested. For all isolates from patients 2 (all isolates except isolate B1), 3 (isolates C1 to C15), and 4 (all isolates except isolates D2, D13, D16, and D19), the MICs of all antimicrobial agents tested except aztreonam were identical to those for isolate A; for aztreonam we found no more than one twofold dilution discrepancy in the MICs (MICs, 32 to 64 μg/ml) for these isolates. A total of 12 antibiotypes were found (Table 2): six antibiotypes were found among the 40 isolates recovered in the burn unit and the other six were discovered among the seven isolates from patients on medical wards (Table 1).

O serogrouping. Nine O serogroups among the 40 isolates recovered from patients in the burn unit and six O serogroups among the seven isolates from patients on medical wards were defined. The epidemic multidrug-resistant strains all belonged to serogroup O:F (serogroup O:4). Isolates belonging to the same antibiotype belonged to identical serogroups. However, isolates D2, D16, and J, which belonged to the same serogroup (serogroup O:E), had different antibiotypes. Isolates G1 and
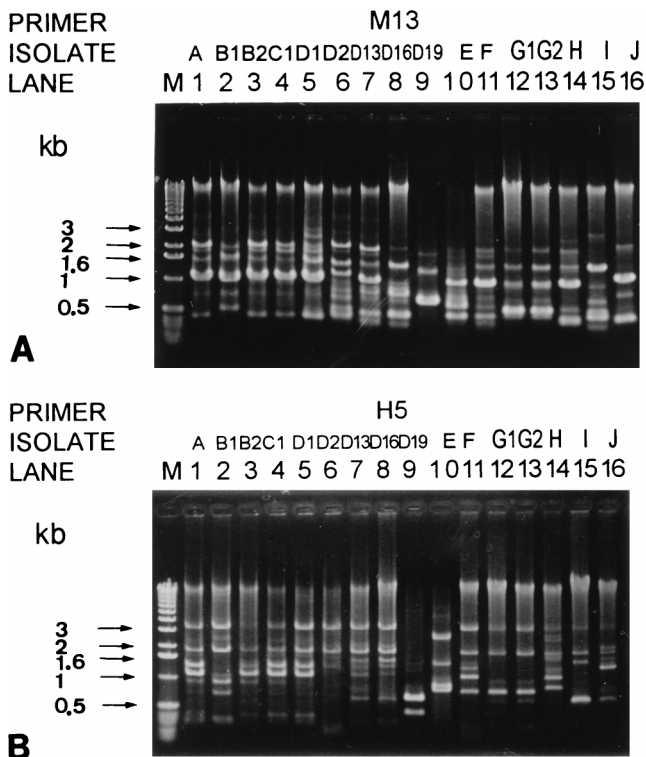
FIG. 1. RAPD patterns of 16 isolates of *P. aeruginosa* generated by AP-PCR with two primers, M13 (A) and H5 (B). Lanes: M, molecular size marker; 1 to 16, the isolates designated in Table 1.

G2 from patient 7 had the same antibiotype and both belonged to O serogroup A.

RAPD patterns. AP-PCR with two primers (primers M13 and H5) indicated that the 47 isolates recovered from the 10 patients had 12 RAPD patterns (Fig. 1). All of the epidemic multidrug-resistant serogroup O:F *P. aeruginosa* isolates possessed identical RAPD patterns. Isolates with the same antibiotype and belonging to the same O serogroup also had the same RAPD patterns.

Infection control measures. All staff members in the intensive care burn unit were notified of the need for strict compliance with the recommended isolation procedures during the outbreak investigation period (12). Samples from health care workers (three physicians and two nurses) were culture positive for oxacillin-resistant *S. aureus*, but all of the samples were negative for *P. aeruginosa*. None of the samples from environmental sites were positive for *P. aeruginosa*. Except for one patient (patient 3) who was found to be harboring the epidemic multidrug-resistant strain shortly after (2 days) the unit became aware of the outbreak, no additional cases of infection with the clone occurred. Environmental decontamination measures were not undertaken. The physicians in the unit were not told to restrict their use of broad-spectrum cephalosporins, aztreonam, or imipenem or to change the dosages of antimicrobial agents administered. The follow-up cultures of samples from various body sites (wound, sputum, and stool samples) from the two surviving patients 2 months after the completion of the investigation did not reveal the presence of the epidemic strain.

DISCUSSION

The present study, in which 40 isolates of multidrug-resistant *P. aeruginosa* recovered from four patients in an intensive care

burn unit were characterized phenotypically and genotypically, disclosed two important points. First, more than one clone of multidrug-resistant *P. aeruginosa* can colonize not only burn wounds but also other sites of the bodies of burn patients. Second, a single clone of multidrug-resistant *P. aeruginosa* can persist in different body sites of burn patients for weeks and months and can subsequently cause various severe infections.

Previous studies suggest that the selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains (3, 15, 21). The subinhibitory antibiotic concentration in burn wounds, due to the administration of an inappropriate dosage of a β-lactam antibiotic or the regular administration of an aminoglycoside in combination with a β-lactam, provides optimal conditions for the selection and persistence of multidrug-resistant *P. aeruginosa* strains and their subsequent local invasion and hematogenous dissemination in burn patients (1, 21). Successful control of an outbreak caused by a multiresistant clone of *P. aeruginosa* by increasing the daily dosage of antimicrobial agents that are active in vitro was reported previously (21), although it has been recommended that this approach not be overused. The concomitant use of multiple antimicrobial agents, including those with good activity against *P. aeruginosa*, might have contributed to the emergence of the epidemic strain in patient 1. This also contributed to the long-term colonization and the subsequent spread of the epidemic multidrug-resistant strain in our burn patients.

In addition to the use of auxanograms and antibiograms, various conventional typing systems, including serotyping and pyocin and phage typing, have been extensively used to study the epidemiology of *P. aeruginosa* infections (20). However, the usefulness of these methods is limited by the high frequency of both nonserotypeable and polyagglutinable strains and the low reproducibility of pyocin typing, especially for strains from cystic fibrosis patients (20). Among the many DNA-based typing methods, restriction endonuclease analysis achieved by pulsed-field gel electrophoresis may be the most discriminatory (2, 5, 6, 9, 14, 19). However, fingerprinting by AP-PCR is documented as having discriminatory power comparable to that of pulsed-field gel electrophoresis for the epidemiological study of *P. aeruginosa* strains from cystic fibrosis patients (9). In the present study, all isolates with identical antibiotypes which all belonged to serogroup O:F (serogroup O:4) had identical RAPD patterns, suggesting the clonal origin of the epidemic strain. The good concordance of the discriminatory power of the antibiotyping and RAPD patterns for the outbreak-related and non-outbreak-related strains of multidrug-resistant *P. aeruginosa* might be due to the limited number of isolates studied.

Routine culture of samples from burn patients for surveillance and microbiological typing of *P. aeruginosa* is not recommended because of the high frequency of burn wound colonization and the organism's polyclonal origin (13). In addition, surveillance cultures of environmental samples are notorious for being nonproductive and for yielding results which usually are not clinically relevant. In the present study, the outbreak-related strain could be isolated from various body sites other than burn wounds and subsequently caused invasive infections. It is suggested that if a multidrug-resistant *P. aeruginosa* strain is isolated and an infection caused by this strain is documented, extensive cultures of samples from all body sites of all patients hospitalized in the same unit and an environmental survey for the strain, as well as the use of isolation precautions, may be crucial for the early control of an ongoing outbreak.

The route of transfer of the O:F strain was not identified. Previous studies suggested that surveys for the identification of

reservoirs of *P. aeruginosa* in burn units performed by obtaining samples for culture have yielded samples from sinks and hydrotherapy equipment that are positive by culture (21, 22). However, only hydrotherapy equipment is strongly linked to the epidemic of *P. aeruginosa* burn wound infections (22). Consequently, dispersal of *P. aeruginosa* from colonized or infected patients in the burn unit will result in further contamination of the environment of the unit as well as the hands of medical personnel. In the present investigation, all cultures of the environmental samples and hands and nasal swabs from medical personnel were negative for the epidemic strain. *P. aeruginosa* colonization of the rectums of burn patients is considered to be a potential source of subsequent burn wound infection. Only two of the cultures of stool samples obtained from the patients examined in the present study yielded the epidemic strain during surveillance of the outbreak. Whether the epidemic *P. aeruginosa* strains from the two patients were acquired from the alimentary tract or were merely contaminants in the stool specimens from nearby infected skin is unclear, because stool or rectal swab specimens were not obtained before the first positive epidemic strain was recovered from other body sites.

The present study, in which phenotypic and molecular biology-based studies were performed, highlights the fact that a single clone of multidrug-resistant *P. aeruginosa* can persist at different body sites of burn patients for weeks and months and can subsequently cause various severe infections. The eradication of the pan-drug-resistant strains from these patients is difficult. The extensive use of broad-spectrum cephalosporins in the unit was probably responsible for the emergence and selection of this multidrug-resistant strain.

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