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Nosocomial Acquisition of *Pseudomonas aeruginosa* by Cystic Fibrosis Patients

BURKHARD TÜMMLER,[1,2]* UTA KOOPMANN,[1,2] DIETMAR GROTHUES,[1] HARTMUT WEISSBRODT,[3] GRATIANA STEINKAMP,[2] AND HORST VON DER HARDT[2]

Cystic Fibrosis Research Group, Abteilung Biophysikalische Chemie, OE 4350,[1] Abteilung Pädiatrische Pneumologie, OE 6710,[2] and Abteilung Medizinische Mikrobiologie, OE 5210,[3] Medizinische Hochschule Hannover, D-3000 Hannover 61, Germany

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During a 4-year period, at least 12 of 40 patients with cystic fibrosis (CF) who were newly colonized with *Pseudomonas aeruginosa* had acquired it at CF recreation camps, clinics, or rehabilitation centers. After introduction of hygienic precautions at the CF clinic, only a single episode of nosocomial transmission of *P. aeruginosa* was detected at the CF ward during the subsequent 2 years.

In patients with cystic fibrosis (CF), the chronic colonization of the airways with *Pseudomonas aeruginosa* is a major cause for morbidity and mortality (2, 7). Once this opportunistic pathogen has chronically colonized the patient's lungs, it is virtually impossible to eradicate the organism by antimicrobial chemotherapy (2, 7). So far, only a few groups have addressed the problem of nosocomial acquisition of *P. aeruginosa* in CF patients (4, 8–10, 12, 14, 17–19, 21, 24). In contrast to the Danish experience (9, 14, 24), most investigations of CF patients attending summer camps (10, 19, 21) and sharing hospital rooms (17) have failed to demonstrate any cross colonization. These data, however, may be biased by the short observation periods of less than a month, in which a slowly growing epidemic would have escaped notice. Hoiby and Pedersen calculated the risk of cross infection to increase with "contact density," i.e., the number of days a noninfected CF patient spent at a CF center (8). The Danish group observed that the yearly incidence of *P. aeruginosa* acquisition at their clinic had been increased during a period when there were more CF patients and their visits to the clinic were more frequent and that it had finally dropped from 17 to 3% after infected and noninfected CF patients were separated (8). Moreover, when the prevalence of chronic *P. aeruginosa* infection was compared for various CF centers, the percentage of patients colonized was found to be positively correlated with the population size (8). According to these epidemiological data, nosocomial transmission of *P. aeruginosa* seems to contribute significantly to the colonization of CF patients. This conclusion, however, needs to be substantiated by typing analysis of the respective isolates.

Using the novel technique of genome fingerprinting (5), we demonstrated the colonization of siblings with CF with either identical or closely related *P. aeruginosa* strains, indicating that cross infection is frequent when contact is intimate and prolonged (4). Thus we became suspicious whether nosocomial transmission has played a role at our CF clinic, where currently 60% of 250 patients being treated are colonized with *P. aeruginosa*. Here we report the results of the retrospective typing of our strain collection spanning the 6-year period from 1983 to 1988.

The *P. aeruginosa* strains were isolated from sputa or deep-throat swab specimens taken from CF patients who are treated at the CF clinic in Hannover, Germany. Second subcultures of the strains taken at 6-month intervals were stored in soy tryptone broth supplemented with 15% (vol/vol) glycerol at −70°C until used. Strains were serotyped by agglutination with commercial antisera (Pasteur Diagnostika, Munich, Germany). Pyocin typing was carried out by the spotting method (3). The phage typing pattern was assessed with the routine set of 20 bacteriophages described by Asheshov (1). Genome fingerprinting by pulsed-field gel electrophoresis was performed as described previously (4, 5). Briefly, the bacteria were embedded in agarose blocks and then incubated with proteinase K-EDTA. The unsheared genomic DNA was digested with the rarely cutting restriction endonuclease *Spe*I or *Dra*I and subsequently separated by field inversion or crossed-field gel electrophoresis.

In 9 of 13 families with two or more children with CF, one to three closely related or identical *P. aeruginosa* strains were present in the CF siblings over the entire observation period from 3 to 6 years. Control throat swab specimens taken in 1987 from the other members of these nine families were negative for *P. aeruginosa*. In three families, resistance to β-lactam antibiotics or aminoglycosides or both emerged in one CF sibling during a 2-week course of intravenous antipseudomonal antibiotics. Within less than 6 months after discharge of this patient, closely related strains with the same patterns of susceptibility to antimicrobial agents were found in the other CF siblings who had not been hospitalized (Table 1). This finding demonstrates the spread of antibiotic-resistant *P. aeruginosa* strains among affected family members.

However, intimate contacts for long periods of time are not necessary for transmission. Looking at data from 1983, we detected a cluster of clonal variants in four unrelated CF patients (Fig. 1). At that time, only 33 of 89 patients treated for CF were colonized with *P. aeruginosa*. The four patients harboring these related strains lived in different areas of Lower Saxony, Germany. The families did not know each other, and the only common meeting place was our outpatient clinic. This example illustrates that CF centers are locations at risk for cross infection.

From 1984 to 1988, 10 noncolonized CF patients from our clinic received 14 courses of treatment at different CF rehabilitation centers. At each of these three institutions,

* Corresponding author.

TABLE 1. Transmission of antibiotic-resistant *P. aeruginosa* strains among siblings with CF: typing of strains isolated within 6 months after emergence of resistance in one sibling

Source of isolate (family and patient)	Drug resistance phenotype[a]	*Dra*I or *Spe*I fingerprint[b]	Serotype[c]	Pyocin type	Phage lysis pattern
A					
CF 37	Ami^r Gen^r Tob^r	A_1	PA	1l	PS44, 1214, Col21, PS31
	Ami^s Gen^s Tob^s	A_2	PA	1k	PS44, 1214, M4, Col21
CF 38	Ami^r Gen^r	A_1	PA	1l	PS44, 1214, Col21, PS31
	Ami^s Gen^s	A_1	PA	1l	PS44, 1214, M4, Col21, PS31
B					
CF 145	Azl^r Gen^r Tob^r	B_1	1	0	PS16, PS73, F7, F10, 119x, 1214, M6, Col11, Col21
	Azl^s Gen^s Tob^s	B_1	1	0	PS16, PS73, F10, 119x, 1214, M6, Col11, Col21
CF 146	Azl^r Gen^r Tob^r	B_2	1	0	PS16, PS73, F7, F8, 119x, 1214, Col21
	Azl^s Gen^s Tob^s	B_2	1	0	PS16, PS73, F7, F8, 119x, 1214, Col21
CF 147	Azl^s Gen^r Tob^r	B_2	1	0	PS16, PS73, 119x, 1214, Col21
	Gen^s Tob^s	B_2	1	0	PS16, PS73, 119x, 1214, Col21
C					
CF 260	Azl^s Pip^r	C_1	PA	1f	Negative
	Azl^s Pip^s	C_1	PA	1f	Negative
CF 261	Azl^r Pip^r	C_1	PA	1f	Negative
	Azl^s Pip^s	C_1	PA	1f	Negative

[a] Ami, amikacin; Azl, azlocillin; Gen, gentamicin; Pip, piperacillin; Tob, tobramycin.
[b] Related fingerprints are indicated by the same letter; fragment patterns are differentiated by number.
[c] PA, polyagglutinable.

techniques of chest physiotherapy were taught to CF patients from all over the country. Because patients shared accommodations and facilities, the stay at the rehabilitation center allowed a high degree of contact between noninfected and infected patients. After return from the 4- to 6-week stay, *P. aeruginosa* was detected for the first time in 8 of the 10 patients. As judged from genome fingerprinting and phage and pyocin typing of the isolates, each patient had acquired a different strain. Prior to the visit to the rehabilitation center, the patients had already been seen at our clinic for a period of 28 to 81 (median, 61) months, and 7 to 36 (median, 18) throat swabs or sputum specimens from each patient had been subjected to bacteriological examination. Before visiting the rehabilitation center, none of the patients had specimens positive for *P. aeruginosa*. Despite the fact that the patients were hospitalized for a 2-week course of intravenous antipseudomonal antibiotics, seven CF patients remained colonized with *P. aeruginosa*. This outcome of antimicrobial therapy is in contrast to our general experience that early treatment of the *P. aeruginosa* infection has a 60% chance for an at least temporary eradication of the bacterium from the patient's lungs (20). Three of the seven previously noncolonized patients were carrying mucoid lipopolysaccharide-deficient strains from the beginning, which are characteristic for the chronic stage of the *P. aeruginosa* lung infection in CF patients (6, 15). The evidence for nosocomial acquisition by patients at training centers for physiotherapy is supported by an independent study at one of these institutions. Wolz et al. monitored the epidemiology of the *P. aeruginosa* infection in 44 patients over a 6-week period (23). Of the 13 patients who were initially uninfected, 6 left the clinic harboring *P. aeruginosa* in their sputa. Cross colonization was proven in four patients.

From 1983 to 1986, the number of patients treated at the CF outpatient clinic in Hannover increased from 89 to 174. Within these 4 years, the prevalence of *P. aeruginosa* infection rose from 37% (33 patients) to 60% (104 patients). Forty patients became newly colonized with *P. aeruginosa*.

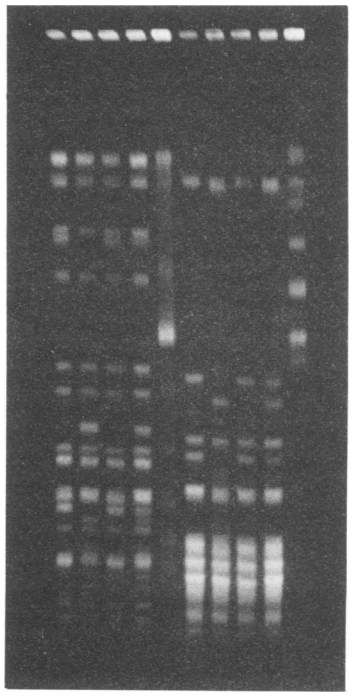

FIG. 1. Detection of a cluster of closely related *P. aeruginosa* strains in four unrelated CF patients. *Spe*I (lanes 1 through 4) and *Dra*I (lanes 6 through 9) digests of chromosomes of strains (from left to right) CF10839, CF89839, CF99839, and CF121838 were separated on 1% agarose gels by field inversion gel electrophoresis. λ oligomers as size markers (monomer, 48.5 kbp) were applied to lanes 5 and 10. Electrophoresis was run for 20 h at 14°C with a field strength of 5.6 V/cm. Pulse times were increased linearly from 1 to 30 s; the forward-to-reverse ratio was chosen to be 3:1. All strains were O serotype 4 and pyocin type 1h and had the phage lysis pattern PS31, M4, F8, PS24, PS2.

Nosocomial acquisition at CF recreation camps, rehabilitation centers, and clinics could be demonstrated for 12 cases. In order to limit the burden of nosocomial infection in the patient population, hygienic precautions were introduced at the CF clinic by May 1986. Infected and noninfected patients were hospitalized in separate wards. CF patients were not allowed to share rooms and were kept isolated if they were diagnosed with antibiotic-resistant *P. aeruginosa* strains. After discharge of the patients, the water taps and sinks in the patients' rooms were disinfected. During the next 2 years, only a single episode of nosocomial transmission was detected: two patients in the CF ward picked up an identical *P. aeruginosa* strain that neither of them had had previously. At the outpatient clinic, all patients continued to be seen in the same rooms, but infected and noninfected patients were separated for lung function testing and lessons in physiotherapy. The hands of medical personnel were examined for contamination with pseudomonads by the infection control nurse. By 1988, all unrelated CF patients were found to carry different *P. aeruginosa* strains, whereas mutual exchange of strains continued in families with two or more children with CF. Hence, the hygienic measures at the clinic seemed to be effective.

The data of this retrospective study confirm the conclusion of the Danish group (8) that nosocomial transmission contributes to a significant extent to the prevalence of *P. aeruginosa* in CF populations. During the last decade, another *Pseudomonas* species, *P. cepacia*, has become a serious problem in several CF centers in England (16) and North America (11, 22). Person-to-person transmission of this pathogen among CF patients was demonstrated by ribotyping of the isolates (13). By employing highly discriminatory DNA-based typing techniques such as Southern analysis of hypervariable sites (13, 23) and genome fingerprinting (reference 4 and this work), the more recent studies all demonstrate the communicability of *Pseudomonas* infections in patients with CF. Hence, avoidable social exposure of noninfected CF patients to *P. aeruginosa*- or *P. cepacia*-positive CF patients should be carefully evaluated on a case-to-case basis in order to minimize the risk of cross infection.

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