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Heterogeneity, Persistence, and Distribution of *Pseudomonas aeruginosa* Genotypes in Cystic Fibrosis Patients

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A collection of 222 isolates of *Pseudomonas aeruginosa* was obtained from the respiratory tract of 16 patients with cystic fibrosis over a 4- to 9-month period. Fourteen of these patients were unrelated, while the remaining two were siblings. Isolates were typed by conventional pyocin typing and also by the use of a DNA probe containing 741 bp immediately upstream of the exotoxin A structural gene and the initial 732 bp of the exotoxin A structural gene. By pyocin typing, 69% (11 of 16) of the patients were shown to harbor a single type that persisted in the lung throughout the study. By genotyping (DNA probe typing), all but three patients (13 of 16, 81%) harbored a single persistent genotype in their lungs. Six patients other than the sibling pair (6 of 14, 43%) shared a common genotype in their lungs as judged by DNA probing, and the pyocin type of these isolates was also identical. In four of these six patients, the shared genotype was also the persistent genotype. The sibling pair studied also carried a common genotype in their lungs as indicated by DNA probing, even though the pyocin type of these isolates varied. Results presented suggest that the majority of patients harbor a persistent strain in their lungs and that cross-colonization may occur.

Pseudomonas aeruginosa is the predominant bacterium associated with pulmonary infection in cystic fibrosis (CF) patients (6, 29). The organism persists in the airways of CF patients despite intensive antibiotic therapy and contributes to pulmonary failure, which is the major cause of morbidity and mortality in CF patients (9). Strains of *P. aeruginosa* colonizing the lung in CF are unique in their sensitivity to serum (6, 16, 24), presence of rough outer membrane lipopolysaccharide (5), ability to polyagglutinate in O antisera, and tendency to produce alginate exopolysaccharide.

Understanding the epidemiology of *P. aeruginosa* colonization of the CF respiratory tract has been hampered due to the predominance of polyagglutinable or nontypeable strains of *P. aeruginosa* from CF in conventional O-antigen serotyping reactions (13). Other conventional typing methods such as phage typing and pyocin typing have poor discriminative powers (18) or poor reproducibility (20), respectively. However, the DNA probe to the upstream region of the exotoxin A gene developed by Vasil et al. (27) has been shown to give good discrimination and reproducibility when used to type *P. aeruginosa* of CF origin (12).

A probe similar to that used by Ogle et al. (12) was used in combination with pyocin typing in the present study. Our results confirm earlier studies (8, 12, 14, 23, 28) that suggest carriage of a persistent genotype within the lungs of CF patients. This study also presents results that suggest that cross- or coinfection may occur in nonrelated patients.

MATERIALS AND METHODS

Patient group. Sixteen children attending the CF clinic, Royal Children's Hospital, Brisbane, Australia, and ranging in age from 6 to 19 years were studied. Sputum specimens were collected from the patients at home following physiotherapy administered by their parents. Specimens were collected one or two times every month over a 4- to 9-month period.

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Isolation and identification of *P. aeruginosa*. Each specimen was diluted 1:5 in a 1% pancreatin solution, vortexed for 1 min, and incubated at 37°C for 15 to 20 min to allow the pancreatin to act. Each specimen was again vortexed and diluted 1:2 to give an initial 1:10 dilution, and further 1:10 dilutions were performed to a final dilution of 10^{-6}. A 100-μl portion of each 10^{-6}, 10^{-5}, and 10^{-4} dilution was plated onto King A medium (10) supplemented with 200 mg of cetrimide and 15 mg of nalidixic acid per liter. One of each colony form present on the isolation plates was picked and purified on a King A plate. Gram-negative, rod-shaped, cytochrome *c* oxidase-positive organisms that utilized glucose oxidatively, gave a positive arginine dihydrolase reaction, and grew at 41°C were identified as *P. aeruginosa*. Colony form was assessed as by Philips (17).

Pyocin typing. For pyocin typing, the method of Fyfe et al. (2) was used.

DNA extraction. For DNA extraction, the method of Samadpour et al. (19) was used with some modifications. *P. aeruginosa* isolates were grown in 1 ml of Luria broth (11) shaken in a 5-ml screw-capped tube at 37°C for 18 h. The bacteria were pelleted in a 1.5-ml microcentrifuge tube in a Microfuge for 5 to 10 min. The cells were washed in 1 ml of 50 mM Tris hydrochloride (pH 8.0), pelleted, resuspended in 0.7 ml of 50 mM Tris hydrochloride (pH 8.0)–50 mM EDTA containing 2 mg of lysozyme per ml, and incubated at room temperature for 10 min. Ten microliters of 20% sodium dodecyl sulfate and 50 μl of 1 mg of pronase E per ml in 10 mM Tris hydrochloride (pH 8.1)–0.2 mM EDTA were added to each tube, and the tubes were incubated at 37°C for 30 min. Phenol (0.7 ml) saturated with 50 mM Tris hydrochloride (pH 8.0) was added, and the tubes were shaken vigorously by hand and incubated at 37°C for 30 min with occasional mixing. The aqueous phase was separated by centrifuging for 5 min and transferred to a second tube; the tube was filled with phenol saturated with 50 mM Tris hydrochloride (pH 8.0) and shaken vigorously by hand. The aqueous phase was again separated and transferred to a third tube, chloroform was added to an approximately 1.2-ml total

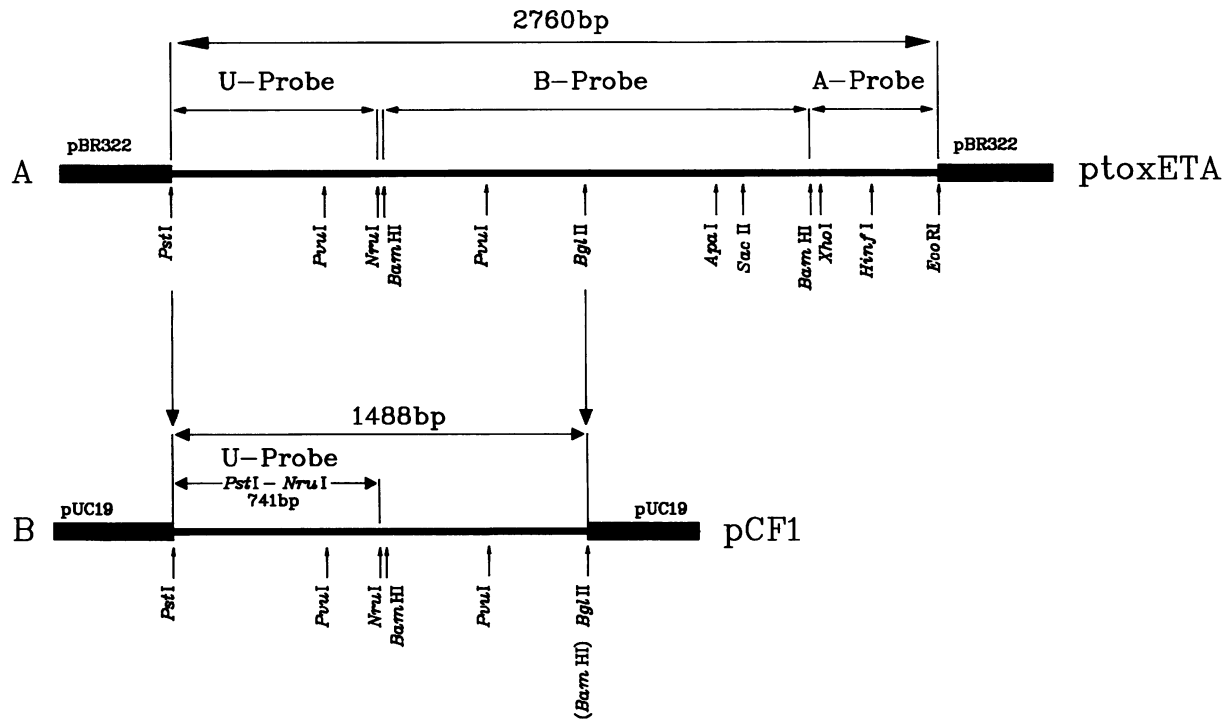

FIG. 1. Restriction maps of (A) the ptoxETA plasmid and (B) the pCF1 plasmid prepared by cloning the *Pst*I-*Bgl*II fragment of ptoxETA into the *Pst*I-*Bam*HI sites of pUC19.

volume, and the tubes were then vortexed for 1 min. The phases were separated by centrifugation for 5 min, the aqueous phase was transferred to a fourth tube, and 100 μl of 5 M ammonium acetate was added. The tubes were filled with absolute ethanol, and precipitated DNA was spooled on a Pasteur pipette, which was allowed to air dry and washed two to three times in 75% ethanol. Once dry, the DNA was resuspended in 200 to 400 μl of 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA depending on the amount of DNA present.

Probe preparation. The ptoxETA plasmid (3) was kindly supplied to us by M. L. Vasil, Department of Microbiology and Immunology, University of Colorado Medical School. A 1,488-bp *Bgl*II-*Pst*I fragment of the ptoxETA plasmid containing the 741 bp immediately upstream of the exotoxin A structural gene (which includes the promoter for the exotoxin A gene) and the initial 723 bp of the exotoxin A structural gene was subcloned into cloning vector pUC19, forming the new plasmid pCF1. This 1,488-bp fragment within pCF1 is a modification of the probe of Vasil et al. (27) in that it includes the U probe and part of the B probe as designated by those authors (27) (Fig. 1). Both U and B probes show restriction fragment length polymorphism when hybridized to restricted *P. aeruginosa* DNA (27). Plasmid pCF1 was labelled with biotin-7-dATP by nick translation and used directly for hybridization. Like the probe used by Ogle et al. (12), the probe used here revealed restriction fragment length polymorphism on Southern hybridization, but due to the longer sequence selected, a greater number of probe-reactive fragments per isolate were recognized, typically one or two (*Sal*I and *Bgl*II) or one to four (*Xho*I) unique fragments for each strain. Upon reading of the results, the molecular weight of the major probe-reactive fragment was measured with reference to biotinylated lambda *Hin*dIII molecular weight markers. The major probe-reactive fragment was taken to be the probe-reactive fragment present in the highest concentration, judged as the darkest fragment visualized on the blot. This was always equivalent to the smallest probe-reactive fragment. This is the fragment noted in Tables 1 and 2. In confirming the genotype, all probe-reactive fragments were used to deduce the genotype, and in all cases such an analysis confirmed the genotype as judged by the use of only the major probe-reactive fragment. Hence, only the major probe-reactive fragment is noted in Tables 1 and 2.

Southern blot analysis. Total chromosomal DNA was digested with the restriction enzymes *Xho*I, *Sal*I, and *Bgl*II, and each digest was electrophoresed on a 0.7% agarose gel for approximately 14 h at 30 V. Fragments from the three digests were transferred to Hybond-N (Amersham) nylon membrane by the method of Southern (21). The hybridization and visualization conditions used were those described by Samadpour et al. (19). Isolates were considered to represent the same genotype when the probe-reactive fragments from the three blots were identical in size. Nonidentical isolates were defined as those which exhibited different probe-reactive fragments or those with different total genomic digestion patterns, as determined on ethidium bromide-stained agarose gels, if the probe-reactive fragments were of identical size. However, in our work, if the probe-reactive fragments of isolates were identical, so were the total genomic digestion patterns.

RESULTS

A Southern hybridization of DNA isolated from selected strains digested with a single restriction endonuclease, *Sal*I, is shown in Fig. 2. Sequential isolates from patients 5 (lanes

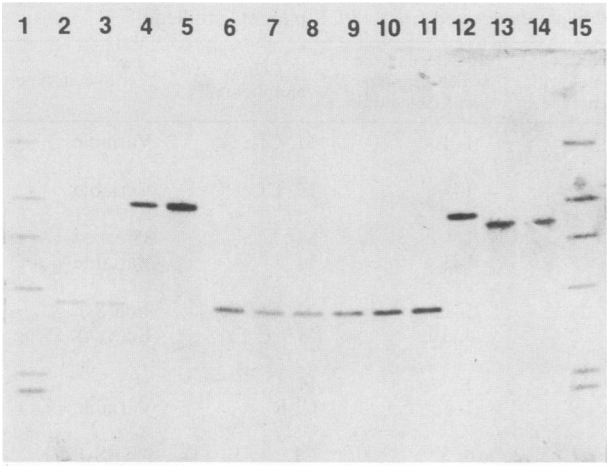

FIG. 2. Southern hybridization analysis of restriction digests of sequential *P. aeruginosa* isolates digested with *SalI* and probed with biotinylated pCF1. Lanes 1 and 15 are biotinylated *Hin*dIII-cut lambda DNA molecular size markers: 23, 9.4, 6.6, 4.4, 2.3, and 2.1 kb, from top to bottom. Lanes 2 and 3 are restricted DNA of sequential isolates from patient 5; lanes 4 and 5, patient 6; lanes 6 to 8, patient 7; lanes 9 to 12, patient 9; lanes 13 and 14, patient 13. All lanes show restricted DNA of persistent genotypes of the patient indicated except lane 12, which contains restricted DNA from a possible cocolonizing or reinfecting genotype.

2 and 3), 7 (lanes 6 to 8), and 9 (lanes 9 to 11) contained the same probe-reactive fragment. Patient 9 also harbored an isolate with a different probe-reactive fragment, as seen in lane 12. Sequential isolates from patient 6 (lanes 4 and 5) gave identical probe-reactive fragments, as did those from patient 13 (lanes 13 and 14). The genotype of these strains was derived from the size of the major probe-reactive fragments in Southern hybridization, using chromosomal DNA digests from three different restriction enzymes. Thus, the genotype of isolates from patient 3a is 11.3-9.1-7.9 (Table 1) as derived from the size, in kilobases, of the major probe-reactive fragments after digestion of chromosomal DNA with *Xho*I, *Sal*I, and *Bgl*II enzymes, respectively.

Tables 1 and 2 summarize the data obtained. When a pyocin type remained stable over the study period, that is, when serial isolates from a patient gave the same pyocin typing result, the genotype in most cases confirmed identity of the isolates (isolates from patients 5, 11, and 13; Table 1). For example, 11 isolates of pyocin type 22/e($S_{3,6,7}$) were isolated from patient 13, and all exhibited a genotype of 9.6-8.0-6.9 (Table 1). However, many isolates identified as identical by genotype displayed a variable pyocin type, for example, those from patients 3a and 3b (Tables 1 and 2). Genotypically identical isolates also exhibited a range of colony forms (isolates from patients 3a, 3b, 9, 12, 13, 15, 17, and 18; Tables 1 and 2). For example, isolates with the genotype 15.5-2.7-7.5 from patient 15 exhibited classic, mucoid, coliform, and rough colony forms.

Of the 16 patients studied over the 4- to 9-month period, all but 3 patients (patients 10, 11, and 16) exhibited a single persistent colonizing genotype (Table 1). A persistent colonizing genotype was defined as one isolated from all or all but one specimen collected over the study period and accounting for the majority of isolates from the patient.

However, eight patients (patients 4, 5, 6, 7, 10, 12, 13, and 14; Table 1) showed transient colonization with genotypes other than the persistent genotype. Transient colonizing genotypes were defined as those isolated only from one or two of the specimens collected during the study period. For example, patient 4 demonstrates a persistent genotype of 6.3-5.6-5.0 isolated from all five specimens obtained and a transient genotype of 15.5-2.7-7.5 isolated from only one of the specimens obtained from the patient. Patient 9 carried a persistent genotype (genotype 6.1-4.1-7.8) but also carried a recurring genotype (genotype 11.5-8.6-8.2) isolated from four specimens, suggesting reinfection or cocolonization (Table 1). Patients 10 and 16 each harbored three genotypes, none of which was seen to dominate, which may therefore indicate cocolonization (Table 1).

We studied one sibling pair (patients 3a and 3b; Table 2). The pyocin type of the 17 isolates of *P. aeruginosa* collected over a 7-month period from the sibling pair varied over time. However, the genotype of the isolates from the siblings (genotype 11.3-9.1-7.9) did not vary, indicating that the isolates from both siblings were identical (Table 2).

Of the 14 unrelated patients studied, patients 5, 7, 9, 11, 12, and 16 were found to share identical genotypes within their lungs (genotype 6.1-4.1-7.8; Table 1). Of these six patients, patients 5, 7, 9, and 12 harbored this genotype consistently within their lungs during repeated sampling over an 8- to 9-month period. Patient 11 was sampled only twice, and hence any conclusion about persistence cannot be drawn; patient 16 harbored three distinct genotypes, none of which was isolated from all specimens. Patient 16 also harbored a genotype common to patient 15 at times during the study period. This genotype was also isolated once from patient 4.

DISCUSSION

Respiratory tracts of CF patients are prone to colonization with *P. aeruginosa* (29). Due to the difficulties in typing isolates of *P. aeruginosa* from CF patients by conventional phenotypic typing techniques, the epidemiology of *P. aeruginosa* infection in CF patients is still not well-defined. We intensively studied isolates of *P. aeruginosa* from 16 patients over a 4- to 9-month period, using phenotypic and genotypic methods, to see whether the isolates of *P. aeruginosa* from individual patients changed over the study period.

Typing of *P. aeruginosa* isolates of CF origin by conventional phenotypic methods has been found difficult due to either the mucoid nature or the rough outer membrane of the isolates. Serotyping, the most commonly used typing method, gives many polyagglutinable, nonagglutinable, or autoagglutinable strains (5, 7, 22) and suffers from a lack of sensitivity (25). Phage typing is not based on stable characteristics, and pyocin type may change due to antibiotic therapy and changes in bacterial metabolism (25). It is unknown whether the phenotypic variation observed in *P. aeruginosa* isolates judged by such methods is related to any genotypic variation as determined by restriction fragment length polymorphism (23). More recently, genetic methods have been applied to study the epidemiology of *P. aeruginosa* in CF patients. A probe to the upstream region of the exotoxin A gene revealing restriction fragment length polymorphism in the region downstream of the exotoxin A gene was discovered by Vasil et al. (27) and has been applied (1, 12, 28) to epidemiological studies of *P. aeruginosa* isolates. A similar probe to the pilin genes has also been developed, but seems to have less discriminatory power when compared with probes derived from the exotoxin A gene region (23). Comparison of total genomic digests has also been used (4). These genotyping methods seem to be more reproducible

TABLE 1. Summary of typing results of *P. aeruginosa* isolates derived from all CF patients studied

Patient	Time (mo)[a]	Total no. of isolates[b]	No. of specimens	Genotype (kb)[c]			No. of isolates with genotype	Specimens from which genotype was isolated[d]	Colony morphology[e]	Pyocin type[f]
				*Xho*I	*Sal*I	*Bgl*II				
3a[g]	7	8	4	11.3	9.1	7.9*[h]	8	1–4	M, CL, R	Variable
3b[g]	7	9	5	11.3	9.1	7.9*	9	1–5	M, CL	Variable
4	9	6	5	15.5	2.7	7.5	1	1	M	35/c
				6.3	5.6	5.0*	5	1–5	M	Variable
5	8	26	12	6.1	4.1	7.8*	24	1–12	M, CL	9/ng(S_7)[i]
				8.6	8.6	4.6	2	4, 10	M	1/e($S_{4,7}$), 29/ng
6	6	12	3	5.3	3.9	7.4	3	1	M, C	3/c
				25.9	9.3	5.0*	9	1–3	C, R	Variable
7	8	30	11	8.6	8.8	7.3	1	6	CL	9/ng(S_7)
				14.2	2.8	6.9	1	6	M	9/ng(S_7)
				6.1	4.1	7.8*	28	1–11	CL, M	9/ng(S_7)[i]
9	9	32	11	6.1	4.1	7.8*	27	1–11	CL, M, R	9/ng(S_7)[i] 31/ng(S_7)
				11.5	8.6	8.2	5	4, 7, 10, 11	C, CL, M	3/k, 3/b
10	8	8	3	11.2	9.5	7.9	1	1	M	−/v
				15.0	8.8	9.1	2	2–3	M	1/a, 10/a
				12.7	9.9	8.8	5	2–3	CL, M	−/−, 5/ng
11	4	2	2	6.1	4.1	7.8	2	1–2	CL, R	9/ng(S_7)[i]
12	9	26	12	6.1	4.1	7.8*	24	1–12	CL, M, C, R	9/ng(S_7)[i]
				10.4	7.8	7.0	1	5	R	3/n
				6.3	5.4	5.3	1	11	CL	9/ng(S_7)
13	8	13	5	9.6	8.0	6.9*	11	1–5	CL, M, C	22/e($S_{3,6,7}$)
				14.1	9.4	5.2	2	1, 3	CL, C, R	3,e
14	8	4	4	6.2	4.0	8.2	1	1	M	10/a
				6.3	5.4	5.3*	3	2–4	M	6/c, 10/var
15	8	14	6	15.5	2.7	7.5*	14	1–6	CL, M, C, R	3/var, 1/var
16	8	10	7	15.5	2.7	7.5	4	1, 2, 3	M	Variable
				6.1	4.1	7.8	3	4, 6, 7	CL, C	9/ng(S_7)[i]
				8.6	9.4	8.2	4	1, 4, 5, 7	M	9/ng
17	8	10	4	11.5	9.5	8.5*	10	1–4	CL, M, C, R	NG/var
18	5	12	3	5.4	3.9	7.2*	12	1–3	CL, M, C, R	3/var

[a] Number of months over which specimens were collected.
[b] Total number of isolates derived from the patient indicated.
[c] Values indicate the size of the major probe-reactive fragment for each of the three blots from DNA digested with the restriction endonuclease indicated. The resulting pattern is the genotype.
[d] Each specimen was issued a number in chronological order of sampling; for example, 1–11 indicates that the genotype was isolated from all 11 specimens and 4, 6, 7 indicates that the genotype was isolated from specimens 4, 6, and 7.
[e] Colony morphology was assessed as by Philips (17): CL, classic; C, coliform; R, rough; M, mucoid.
[f] Pyocin type was assessed by the spot typing method of Fyfe et al. (2): Variable, total pyocin type exhibited variability in both typing sets; var, second pyocin indicator set exhibited variability; NG, does not conform to any published type using the first indicator set; ng, does not conform to any published type using the second indicator set; −, no reaction with the pyocin indicator set.
[g] Patients 3a and 3b are siblings.
[h] When a persistent genotype is evident, it is marked by *.
[i] The untypeable pattern found with the second pyocin typing indicator set with these patients was identical or varied by only a single indicator.

and give greater discrimination in comparison to phenotype based typing methods. Genotype is therefore a superior epidemiological marker because the genotype of a strain is a stable character in comparison to strain type as judged by nonstable phenotype.

A total of 222 isolates of *P. aeruginosa* were isolated from sputum specimens taken from the 16 patients. All isolates were typed by both a conventional phenotypic method of typing, pyocin typing, and genotyping, using the pCF1 plasmid probe based on the region of the exotoxin A gene of *P. aeruginosa*. Pyocin typing proved a valuable adjunct to the genotyping technique in this study. All isolates proved to be typeable, that is, reacted with the pyocin indicator strains, and isolates from patients with the genotype 6.1-4.1-

TABLE 2. Typing data on all isolates derived from the sibling pair studied

Patient	Isolate	Date of isolation	Genotype (kb)[a]			Colony type[b]	Pyocin type[c]
			*Xho*I	*Sal*I	*Bgl*II		
3a	61	25/8/87	11.3	9.1	7.9	M	NG/v
	62	25/8/87	11.3	9.1	7.9	M	26/v
	157	20/11/87	11.3	9.1	7.9	M	1/v
	158	20/11/87	11.3	9.1	7.9	M	NG/−
	164	20/11/87	11.3	9.1	7.9	C	NG/v
	208	5/2/88	11.3	9.1	7.9	M	31/−
	267	18/3/88	11.3	9.1	7.9	M	NG/v
	268	18/3/88	11.3	9.1	7.9	CL	NG/v
3b	47	25/8/87	11.3	9.1	7.9	M	NG/−
	48	25/8/87	11.3	9.1	7.9	M	55/ng
	107	8/10/87	11.3	9.1	7.9	M	5/ng
	161	20/11/87	11.3	9.1	7.9	M	−/−
	162	20/11/87	11.3	9.1	7.9	CL	−/−
	163	20/11/87	11.3	9.1	7.9	M	NG/v
	216	5/2/88	11.3	9.1	7.9	M	49/c
	251	18/3/88	11.3	9.1	7.9	M	NG/ng(S_8)
	252	18/3/88	11.3	9.1	7.9	M	NG/ng($S_{5,8}$)

[a] Values indicate size of the major probe-reactive fragment for each of the three blots from DNA digested using the restriction endonuclease indicated. The resulting pattern is the genotype.

[b] Colony morphology was assessed as by Philips (17): CL, classic; C, coliform; R, rough; M, mucoid.

[c] Pyocin type was assessed by the spot typing method of Fyfe et al. (2): NG, does not conform to any published pyocin type using the first indicator set; ng, does not conform to any published pyocin type using the second indicator set; −, no reaction with the pyocin indicator set.

7.8 proved to give an identical pyocin type [9/ng(S_7)]. However, with pyocin typing alone, the relationship of serial isolates from many patients became confused, with pyocin type indicating dissimilarity of isolates which were shown to be identical by genotyping. The intensive antibiotic therapy commonly used to treat pulmonary exacerbations may explain this variability in pyocin type (25).

Most recent research suggests that, once one strain colonizes the lung of the CF patient, this strain remains the dominant strain within the lung, and changes in the strain type are very rare (8, 12, 14, 23, 28). The results we obtained support this. As judged by the DNA probe, all but one patient studied exhibited a remarkably constant *P. aeruginosa* flora within their lungs, with one genotype predominating and only occasional transient genotypes being present; only two patients (patients 10 and 16) showed any marked variability in genotype composition of their sputum. However, both Speert et al. (23) and Wolz et al. (28) suggest that changes may occur in the persistent colonizing genotype. Speert and coworkers studied a group of 23 patients over a 7-year period, using a pilin gene probe, and discovered that in 43.5% (10 of 23) of patients a change in the persistent colonizing genotype occurred. Wolz and coworkers (28) found that, in 43% (12 of 32) of patients over a 6-month period, a change in the *P. aeruginosa* genotype or strain loss was found. The absence of any patient exhibiting a change in the persistent colonizing genotype in this study may be due to the limited time course or the limited patient group studied.

The sibling pair studied harbored an identical, persistently colonizing genotype within their lungs, indicating a high probability of cross-colonization. Grothues et al. (4) used pulse-field gel electrophoresis of the whole *P. aeruginosa* chromosome in a genome fingerprinting technique to genotype isolates of *P. aeruginosa* from 22 siblings from eight families. They reported that colonization with identical or closely related genotypes is common. Wolz et al. (28), using a probe similar to that used in this study, reported that siblings predominantly harbor identical genotypes within their lungs. Ogle et al. (12a) reported that, over a 4- to 6-year period, two siblings remained nearly constantly colonized with the same persistent genotype. Cocolonizing genotypes were also found in the sibling pair studied by Ogle et al. (12a), similar to the possible cocolonizing genotype found in patient 9 in this study. From 4 of the 11 sampling occasions of patient 9, a genotype other than the persistent genotype was found. Frequency of reisolation of this genotype suggests reinfection or cocolonization with this genotype.

The question of cross-colonization in CF patients has not been conclusively resolved. Høiby and Rosendal in 1980 (7) described the presence of a dominant strain in Denmark, using serotyping and phage typing as the typing methods. These authors suggested a possible cross-infection with the endemic serotype in the CF treatment center. The same clinic was still experiencing colonization and possible cross-infection with this endemic serotype in 1983 (30). This serotype became resistant to the antibiotics commonly used to treat CF patients (15). Isolation of patients with this endemic serotype seemed to stop further spread. Speert and Campbell in 1987 (22), using serotyping to type *P. aeruginosa* isolates, concluded that transient cross-colonization occurred between pairs of patients, but the cross-colonizing serotype had usually disappeared from subsequent specimens. However, due to the inadequacies of serotyping and phage typing, interpretation of these results is difficult. Grothues et al. (4), using genome fingerprinting, found no cross-colonization between unrelated patients and concluded that cross-colonization is rare unless contact is prolonged or intimate. However, Wolz et al. (28), using a DNA probe technique, suggested that cross-colonization of patients occurred at home and in the clinic studied. Data presented here support possible cross-colonization of patients. Twenty-five percent (4 of 16, patients 5, 7, 9, and 12; Table 1) of patients harbored the same persistent genotype, which exhibited the same pyocin type, 9/ng(S_7). Also, one patient (patient 11) from whom only two isolates were typed

harbored this genotype, and another patient (patient 16) in whom the genotype composition of the sputum varied also harbored the same genotype. Neither of these patients, however, appeared to harbor this genotype as a persistent genotype. Therefore, it is possible that at some time in the past contact between these patients allowed cross-colonization.

A further explanation of the predilection of the 6.1-4.1-7.8 genotype for the CF lung may be that this genotype may outcompete other genotypes within the lung environment. It has been hypothesized that the high incidence of uncommon pyocin types may be related to an enhanced ability of these *P. aeruginosa* pyocin types to colonize the lung (26). One such uncommon pyocin type, 9ng(S_7), has been correlated with a persistent genotype isolated from four unrelated patients.

In conclusion, the phenotypically based pyocin typing method used in this study showed too great a variability in comparison to the genotypic typing method to be used as the only typing method in epidemiological investigations. However, when used in combination with genotypic methods, pyocin typing may provide valuable supplementary information to support genotypic epidemiological evidence.

Data presented here support previous investigations into the epidemiology of isolates of *P. aeruginosa* from the CF lung. Serial specimens from patients showed one persistent colonizing genotype of *P. aeruginosa*, and the sibling pair studied harbored an identical genotype of *P. aeruginosa* in their lungs. Results suggest that cross-colonization or cocolonization of some of the CF patients studied may have taken place, and possible environmental sources for such a strain are being investigated.

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