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Arbitrarily Primed Polymerase Chain Reaction as a Rapid Method to Differentiate Crossed from Independent *Pseudomonas cepacia* Infections in Cystic Fibrosis Patients

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We used DNA fingerprinting by the arbitrarily primed polymerase chain reaction (AP-PCR) technique for an epidemiological investigation of 23 *Pseudomonas cepacia* isolates obtained from 11 cystic fibrosis (CF) patients attending our CF center. This approach was compared with ribotyping, pulsed-field gel electrophoresis (PFGE), and conventional phenotypic typing. AP-PCR and ribotyping were identical in resolving power, since the two methods generated four different profiles and identified the same group of strains. Six patients on the one hand and four on the other harbored strains of the same genotype, thus raising the possibility of either patient-to-patient transmission or acquisition from a common hospital environmental source. PFGE results were in good agreement with those of the other two methods, but PFGE seems more discriminative since it generated a fifth profile for a single strain in a group of four. Our results show in vivo stability for the three methods during a period extending from 3 to 41 months. These genotypic techniques are particularly promising for clinical laboratories to help to clarify the epidemiology of *P. cepacia* in CF patients. The AP-PCR method constitutes an easier alternative to the well-established ribotyping method. AP-PCR provides the quickest results with minimal technical complexity. However, our results suggest that it is less discriminative than the labor-intensive PFGE method.

Bacterial pulmonary infections are a major cause of morbidity and mortality in patients with cystic fibrosis (CF). Recently, workers at several CF centers have reported on the increasing frequency of *Pseudomonas cepacia* recovery from the respiratory tracts of patients with CF (30). Although many patients are only colonized with *P. cepacia* and show no noticeable ill effects, others experience severe, life-threatening infections. The clustering of colonization at some CF centers and the increased colonization rate among siblings with CF suggest a common environmental source or person-to-person transmission (30). However, the epidemiology of infection is unclear and the issue of cross-infection versus independent acquisition requires clarification in centers where *P. cepacia* is frequently encountered.

Efficient measures for the prevention of *P. cepacia* colonization depend on the ability to identify the source of contamination and to differentiate individual strains of *P. cepacia* accurately. Conventional methods for strain identification have relied on the analyses of phenotypic characteristics which may not be stably expressed (12, 13, 16). Genotypic characterization of strains may circumvent these difficulties. Indeed, analysis of the restriction fragment length polymorphism of total DNA by pulsed-field gel electrophoresis (PFGE) and analysis of the ribosomal DNA regions (ribotyping) have both been shown to be effective in distinguishing among *P. cepacia* strains (1, 20).

We report here on the use of DNA fingerprinting by the recently introduced arbitrarily primed (AP) polymerase chain reaction (PCR) technique (33, 35) for an epidemiological investigation of 23 *P. cepacia* isolates obtained from 11 CF patients attending our CF center. This approach was compared with ribotyping and PFGE and with conventional phenotypic systems, such as biochemical and antimicrobial susceptibility profiles.

MATERIALS AND METHODS

Bacterial strains. Thirty clinical *P. cepacia* strains and the type strain of the species, ATCC 25416, were studied. Twenty-three clinical *P. cepacia* strains were recovered between 1988 and 1992 from primary selective cultures from respiratory tract secretions of 11 CF patients with a mean age of 13 (range, 3 to 19) years. In addition, four epidemiologically unrelated non-CF isolates from the same hospital and three CF isolates from other hospital were analyzed. Isolates were identified as *P. cepacia* by the analytical profile index procedure (API 20NE System; Analytab Products, La Balme les Grottes, France). Disk agar tests for susceptibility to ceftazidime, imipenem, and ciprofloxacin were performed with Mueller-Hinton agar plates (Diagnostic Pasteur, Marnes-la-Coquette, France) (24).

Genotyping techniques. Total *P. cepacia* DNA was prepared as previously described (5, 6). Bacterial DNA was studied by AP-PCR by using two PCR primers: 5′-TCAC GATGCA-3′ and 5′-GCCCCCAGGGGCACAGT-3′. The reactions took place in 50 μl of 100 mM Tris-HCl buffer (pH 8.3) containing 50 mM KCl, 4 mM $MgCl_2$, each deoxynucle-

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oside triphosphate at 0.4 mM, 3 μM primer, 50 ng of DNA, and 2.5 U of *Taq* DNA polymerase (Beckman, Fullerton, Calif.). The DNA thermal cycler (Perkin Elmer Cetus, Norwalk, Conn.) was programmed for 35 cycles as described previously (2, 3). Amplification products were resolved by electrophoresis in a 2% agarose gel and detected by staining with ethidium bromide.

The one-dimensional PFGE system used (Q-life System Inc., Kingston, Ontario, Canada) was developed on the basis of a theoretical model of the electrophoretic process (15, 25), with alternating fields applied by a computer-controlled power supply designed and built at the Xerox Research Center (Mississauga, Ontario, Canada). Genomic DNA from all *P. cepacia* isolates was prepared by a previously described method (29). Briefly, bacteria were embedded in agarose blocks and then incubated with proteinase K-EDTA. The DNA fragment size marker was yeast chromosomal DNA (Bio-Rad Laboratories, Richmond, Calif.). Total DNA was digested with *Spe*I. Voltage gradients, pulse intervals, and running times are built in parameters defined as those predicted by the model to give good resolution in the 8- to 500-kb size range. All gels were 0.8% agarose and were run in a conventional horizontal gel electrophoresis chamber (H1; Bethesda Research Laboratories, Gaithersburg, Md.) in either 1× TBE (89 mM Tris borate, 89 mM boric acid, 2 mM EDTA, pH 8.3) or 1.5× TBE.

For ribotyping, DNA (4 μg) was digested with restriction enzyme *Eco*RI (Boehringer, Mannheim, Germany) in accordance with the manufacturer's specifications and analyzed by electrophoresis on 0.8% ethidium bromide-containing submarine agarose gels in 40 mM Tris-acetate–1 mM EDTA buffer. The DNA fragment size marker Raoul I (Appligene, Strasbourg, France) was used. Size-separated restriction fragments were then transferred to a nylon membrane (GeneScreen Plus; New England Nuclear Products, Boston, Mass.) by the method of Southern. rRNA (16 and 23S) from *Escherichia coli* (Boehringer) was used as a probe. It was cold labelled by random priming with a mixture of hexanucleotides (Pharmacia, Uppsala, Sweden) and cloned mouse mammary leukemia virus reverse transcriptase (Bethesda Research Laboratories) in the presence of 0.35 mM digoxigenin-11-ddUTP (Boehringer). DNA hybridization temperature and chemiluminescence detection procedures were done as described previously (4).

RESULTS

Altogether, two biochemical patterns and four antibiotic susceptibility profiles were observed among the clinical isolates. Identical results were obtained with the two AP-PCR primers, and five profiles were identified among the 23 clinical isolates and the type strain of the species (Fig. 1A). But two predominant AP-PCR profiles accounted for more than half of the strains. Profiles I, II, III, and IV were identified in six, one, four, and one of the patients, respectively. Groups consisting of patients A, B, C, D, and E on the one hand and G, H, I, and J on the other were each respectively colonized with the same strain (Table 1). The remaining two patients (F and K) harbored two strains demonstrating two unique AP-PCR profiles. The profiles obtained with the seven epidemiologically unrelated strains were each different and different from the four profiles obtained from the 23 isolates from the 11 CF patients (Fig. 1B). In six patients (A, B, C, F, G, and J), the AP-PCR profiles of the *P. cepacia* strains remained unchanged throughout the study period. Intervals between recovery of

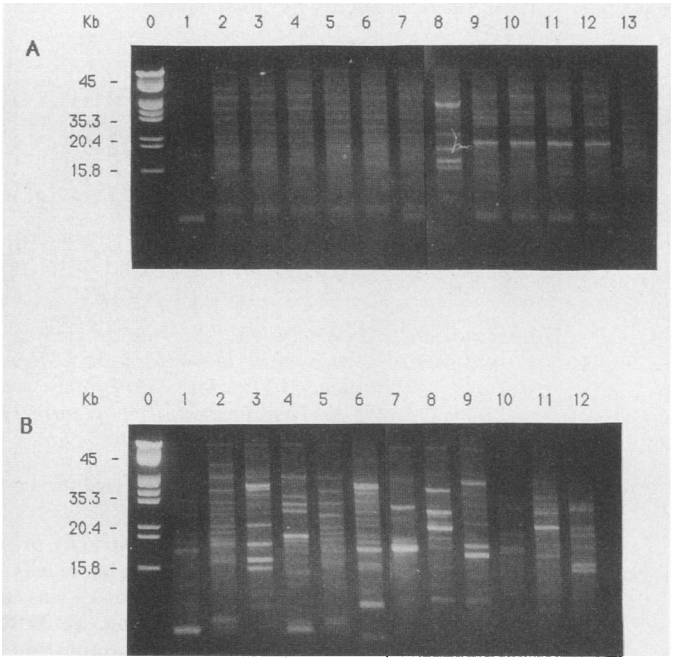

FIG. 1. *P. cepacia* DNA fingerprinting by AP-PCR. The primer was 5′-TCACGATGCA-3′. (A) Lanes: 0, size marker; 1, strain ATCC 25416; 2, strain A-1; 3, strain B-1; 4, strain C-1; 5, strain K-5; 6, strain D-1; 7, strain E-1; 8, strain F-1; 9, strain G-1; 10, strain H-1; 11, strain I-1; 12, strain J-1; 13, strain K-1. See Table 1 for data on the strains. (B) Lanes: 0, size marker; 1, strain ATCC 25416; 2 to 5, isolates representative of the four profiles observed (I, II, III, and IV); 6 to 12, profiles obtained with the seven epidemiologically nonrelated strains. All of the profiles were different.

the first strain and recovery of the subsequent strains from these six patients were 3, 5, 15, 16, 21, and 41 months, respectively. Patient K, however, who was the only patient colonized with a strain showing AP-PCR profile IV, which was isolated four times during a period of 36 months, showed a strain with AP-PCR profile I at 51 months; this was the most common profile among the strains from our patients.

Ribotyping generated four distinct patterns for the 23 clinical strains. Profiles a, b, c, and d were identified in six, one, four, and one of the patients, respectively (Table 1 and Fig. 2A). These results were in complete correlation with the AP-PCR typing results. The 12 different ribotypes obtained with the 23 clinical isolates, the seven epidemiologically unrelated strains, and the type strain of the species are shown in Fig. 2B. Indistinguishable patterns were observed for all of the strains obtained from patients A, B, C, F, G, and J, indicating chronic colonization (Table 1).

PFGE of *Spe*I digests yielded well-resolved patterns of 12 to 17 fragments in the 200- to 500-kb range (Fig. 3). The PFGE analysis results were also in good agreement with the other two genotyping methods. However, strains J-1 and J-2, which belonged to a group of seven strains isolated from three other patients and showed the same AP-PCR profile (III) (Fig. 1, lane 12) and the same ribotype (c) (Fig. 2, lane 12), exhibited a unique PFGE pattern (E) (Fig. 3, lane 12) (Table 1). Thus, PFGE provides the greatest resolving power since it was the only method capable of differentiating these strains. The successive isolates which were found to give stable AP-PCR profiles over time also gave stable PFGE (Fig. 3) and ribotype profiles.

No correlation between phenotypic characteristics, such

TABLE 1. Phenotypic and genotypic characterization of the *P. cepacia* clinical strains studied

Strain[a]	Period[b] (mo)	Biochemical pattern	Susceptibility[c] pattern	AP-PCR profile[d]	Ribotype[e]	PFGE profile[f]
A-1		I	a	I (2)[g]	a (2)	A (2)
A-2	5	I	b	I	a	A
B-1		I	b	I (3)	a (3)	A (3)
B-2	16	I	b	I	a	A
C-1		II	b	I (4)	a (4)	A (4)
C-2	41	II	b	I	a	A
D-1		II	b	I (6)	a (6)	A (6)
E-1		II	a	I (7)	a (7)	A (7)
F-1		II	b	II (8)	b (8)	B (8)
F-2	6	II	b	II	b	B (14)
F-3	21	I	a	II	b	B
G-1		I	b	III (9)	c (9)	C
G-2	14	I	b	III	c	C
G-3	15	II	c	III	c	C (9)
H-1		I	b	III (10)	c (10)	C
I-1		I	b	III (11)	c (11)	C (11)
J-1		II	d	III (12)	c (12)	E (12)
J-2	3	II	b	III	c	E
K-1		I	b	IV (13)	d (13)	D (13)
K-2	32	I	d	IV	d	D
K-3	33	II	a	IV	d	D
K-4	36	II	b	IV	d	D (13′)
K-5	51	II	a	I (5)	a (5)	A (5)
ATCC 25416				V (1)	e (1)	F (1)

[a] In the strain designations, identical first letters refer to the same patient and the numbers correspond to different isolates from the same individual.
[b] Time since the first isolation of *P. cepacia* from the same patient.
[c] Susceptibility patterns are defined as sensitivity (S) or resistance (R) to the antibiotics ceftazidime, imipenem, and ciprofloaxin, respectively, as follows: a, RRR; b, SRR; c, SRS; d, SSR.
[d] Based on the data in Fig. 1.
[e] Based on the data in Fig. 2.
[f] Based on the data in Fig. 3.
[g] The numbers in parentheses are lane numbers in the corresponding figures.

as biochemical profile or antibiotic susceptibility pattern, and the results of the genotypic studies was found.

DISCUSSION

The appearance of *P. cepacia* in the CF population has caused considerable alarm because some investigators have reported that colonization may be associated with accelerated clinical deterioration. Indeed, Thomassen et al. noted that a group of patients colonized with *P. cepacia* had more serious lung disease and poorer prognoses than patients colonized with *P. aeruginosa* alone (32). Fortunately, this is not always the case and many patients colonized with *P. cepacia* have no detectable change in their clinical course (32). However, it is not clear how patients with CF initially become colonized with *P. cepacia*, thus potentially acquiring a higher risk of deterioration. The high frequency of *P. cepacia* colonization in some CF centers contrasts with the low rate observed in others (30). This clustered distribution and the increased risk of colonization among siblings with CF suggest person-to-person transmission or a common source of infection (30, 32).

Until recently, epidemiological studies of *P. cepacia* have been based essentially upon the study of phenotypic traits such as biochemical profiles, bacteriocin, and serological typing. Although these techniques are often very useful, some problems are associated with them. Two biotyping systems have been proposed for *P. cepacia* (8, 28). The two systems separate *P. cepacia* into four (8) and eight biovars, respectively (28). The systems are appealing because each of them relies on a small number of biochemical reactions that can be performed easily in most laboratories. However, given the nutritional versatility of *P. cepacia*, commercial microidentification systems do not provide the degree of strain discrimination required to distinguish between outbreak and nonrelated strains (26). *P. cepacia* can also be characterized by bacteriocin production and sensitivity (11). Govan and Harris (12) reported that 95% of 400 strains were typeable, falling into 44 cepacian types. However, bacteriocin typing does not provide the reproducibility required for epidemiologic assessments, and its greater technical complexity also limits its usefulness for characterization of epidemic strains of *P. cepacia* (27). A number of serotyping systems using either heat-killed or heat-stable antigens of *P. cepacia* have been reported (10, 21, 26, 27). A French group (13) serotyped 285 isolates by using a combination of seven O (somatic) and five H (flagellar) antisera. Another system of 10 serogroups has been described by Nakamura et al. (23). With this system, all but 12% of 105 strains could be serotyped. However, these systems have not been standardized or made widely available. Multilocus enzyme electrophoresis has been used successfully to investigate *P. cepacia* strains involved in an outbreak (7). This technique, however, is labor intensive and technically too complex for most clinical laboratories.

Recently, epidemiological studies have successfully used DNA analysis. Plasmid analysis has been used in outbreaks of *P. cepacia* infections that have involved plasmid-bearing strains (27). However, the frequency of plasmid carriage in the overall *P. cepacia* population has been estimated to be as low as 10%, which seriously limits the use of this technique as a general analytic tool (7). Ribotyping was found to be the

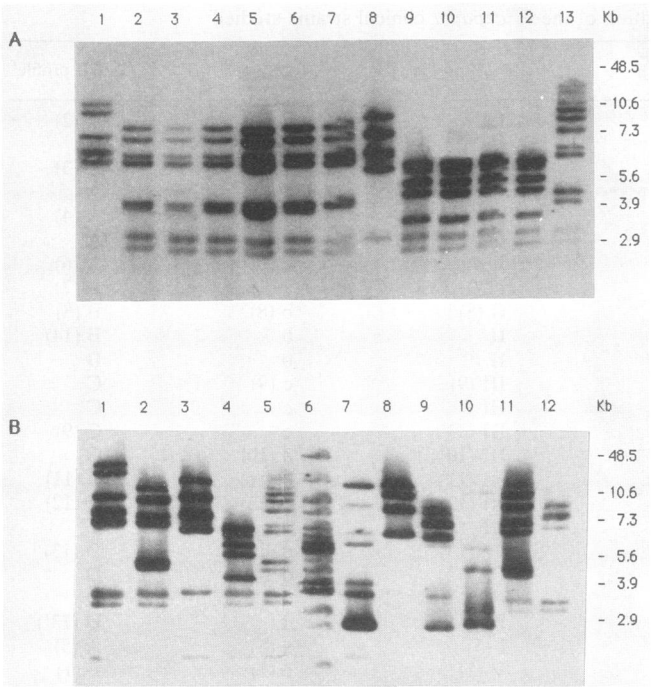

FIG. 2. *P. cepacia* ribosomal DNA restriction fragment length polymorphism ribotyping patterns obtained after *Eco*RI digestion. (A) Lanes: 1, strain ATCC 25416; 2, strain A-1; 3, strain B-1; 4, strain C-1; 5, strain K-5; 6, strain D-1; 7, strain E-1; 8, strain F-1; 9, strain G-1; 10, strain H-1; 11, strain I-1; 12, strain J-1; 13, strain K-1. (B) Lanes: 1, strain ATCC 25416; 2 to 5, isolates representative of the four profiles observed (a, b, c, and d); 6 to 12, profiles obtained with the seven epidemiologically nonrelated strains. All of the profiles were different.

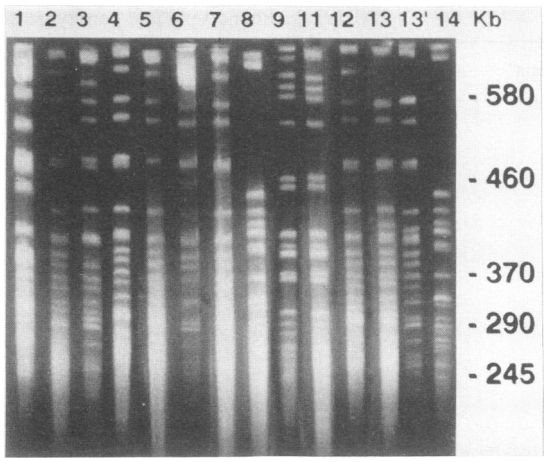

FIG. 3. *Spe*I-cleaved *P. cepacia* DNA after PFGE and ethidium bromide staining. Lanes: 1, strain ATCC 25416; 2, strain A-1; 3, strain B-1; 4, strain C-1; 5, strain K-5; 6, strain D-1; 7, strain E-1; 8, strain F-1; 9, strain G-3; 11, strain I-1; 12, strain J-1; 13, strain K-1; 13′, strain K-4; 14, strain F-2.

most accurate method of ascertaining strain identity among several systems based on phenotypic parameters (26). PFGE has been shown to provide data of equal discrimination and in good correlation with ribotyping (1). Kostman et al. have reported a new PCR-based ribotyping strategy which explores polymorphism of the 16S and 23S spacer regions of the rRNA genes (14). On *P. cepacia* strains, they found it to be as discriminative as conventional ribotyping but quicker. Along the same line, in the present study, we assessed the potential of the newly introduced AP-PCR technique for epidemiological evaluation of 23 *P. cepacia* strains isolated from 11 CF patients.

The AP-PCR method independently devised by Welsh and McClelland (33) and by Williams et al. (35) is based on amplification of random DNA segments with single primers of arbitrary nucleotide sequences. Like ribotyping, it is a method with general applicability and has already been used to type several different organisms (2, 3, 9, 17, 22, 34). It has the advantages of simplicity and rapidity conferred by the PCR procedure. Thus, it might very well constitute an easier alternative to the now well-established ribotyping and PFGE procedures. Typing of *P. cepacia* isolates by AP-PCR has not been reported before.

In this study, AP-PCR and ribotyping were identical in resolving power since the two methods generated the same number of different profiles and identified the same group of strains. PFGE results were in good agreement with those of the two other methods, but the method seems more discriminative since it generated a fifth profile for a single strain in a group of four. Our results show in vivo stability for the three methods during periods extending from 3 to 41 months.

On the basis of the results of the genotypic studies, four different groups were found for the clinical strains studied. Six patients on the one hand and four on the other harbored strains of the same genotype, thus raising the possibility of either patient-to-patient transmission or acquisition from a common hospital environmental source. Our attempts to identify a common environmental source of transmission of *P. cepacia* was unsuccessful. Microbiological and epidemiological studies did not support the hypothesis that respiratory therapy or pulmonary function testing equipment, water, or inhalant solutions were the vehicles of *P. cepacia* transmission. Our study, failing to identify an environmental reservoir for acquisition of *P. cepacia*, supports the possibility that nosocomial transmission occurs by direct contact between CF patients. All of the patients were in the same physical location and were treated by the same health care personnel. Patients A to D on the one hand and patients G to I on the other were hospitalized during overlapping periods. Our data support previous epidemiological findings which also suggest nosocomial acquisition of *P. cepacia* (19, 20, 31). Lipuma et al. reported that strains of different ribotypes predominate at different CF centers (20). Recently, the same investigators documented person-to-person transmission of *P. cepacia* between patients with CF (18). After acquiring *P. cepacia*, our seven patients remained colonized with a single strain for a prolonged period (as long as 3 years), indicating chronic colonization. Interestingly one patient acquired the most commonly encountered strain after having been colonized for 3 years with a strain that was unique in our series. Thus, further studies are needed to determine the precise mode of transmission between patients to set up prevention strategies. Development of rapid and discriminative typing systems is essential to this task.

In conclusion, we found ribotyping and AP-PCR to be very similar in effectiveness for distinguishing between *P. cepacia* strains. Because of their general applicability, these genotyping techniques are particularly promising for clinical laboratories, especially to help to clarify the epidemiology of *P. cepacia* among CF patients. Use of a nonradioactive probe in ribotyping has been a major step toward this aim,

but of the two methods, AP-PCR certainly provides results quicker and with minimal technical complexity. However, our results suggest that both ribotyping and AP-PCR are less discriminative than the labor-intensive PFGE method.

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