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DNA Fingerprinting by Pulsed Field Gel Electrophoresis and Ribotyping To Distinguish *Pseudomonas cepacia* Isolates from a Nosocomial Outbreak

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We typed 40 isolates of *Pseudomonas cepacia* obtained from patients involved in a single outbreak using pulsed field gel electrophoresis and ribotyping. All isolates from the majority of the patients, 16 of 18 (89%), were included in a single group. These typing methods should aid in the clarification of the epidemiology of infection with *P. cepacia*.

Pseudomonas cepacia is increasingly recognized as an important pathogen (1, 3, 6). The epidemiology of infection with *P. cepacia* is not well understood due, in part, to the lack of a standardized method of typing. We examined the utility of DNA fingerprinting by transverse alternating pulsed field gel electrophoresis and ribotyping in classifying the relatedness of *P. cepacia* isolates obtained from patients involved in an outbreak at the Minneapolis Veterans Affairs Medical Center (VAMC).

Routine cultures for management of febrile illnesses at the Minneapolis VAMC during 1987 identified isolates of *P. cepacia* from 38 patients. All isolates identified from July to December 1987, when the study was conducted, were saved. Isolates obtained prior to July 1987, before the study had begun, were not available for study.

Bacterial isolates were identified and determined to the species level by using MicroScan panels (American MicroScan Systems, American Hospital Supply Corp., West Sacramento, Calif.) according to the guidelines of the manufacturer. All isolates, which were regrown overnight at 32°C, were typed blindly.

DNA fingerprinting by transverse alternating pulsed field gel electrophoresis was carried out exactly by the method of Vasil et al. (8). Briefly, cells were spun down, resuspended in 75 mM NaCl–25 mM disodium EDTA (SE buffer), and mixed with 2% low-melting-point agarose (Sea Plaque Agarose; FMC Corp.), poured into a Beckman plug mold, and allowed to cool. Cells were lysed by incubating the agarose plug with sodium lauryl sarcosine-protease K, washed, and stored at 4°C. DNA was digested with *Spe*I, and the electrophoresis was performed with a Beckman Geneline transverse alternating field electrophoresis (TAFE) system. Electrophoresis runs were done at 240 V for 19 h in 1× TAFE running buffer (20× TAFE buffer is 200 mM Tris hydrochloride, 0.5 mM free acid EDTA, 87 mM acetic acid). Each run was begun with 5-s pulses for 1 h followed by three 30-s pulses for 18 h, depending on the size of the DNA fragment to be resolved. Gels were stained with ethidium bromide. DNA fingerprint patterns are designated by capital letters.

Ribotyping was done as described by the method of Stull et al. (7). *Escherichia coli* rRNA (Boehringer Mannheim) was dephosphorylated with bacterial alkaline phosphatase (Bethesda Research Laboratories, Gaithersburg, Md.), tested for purity by polyacrylamide gel electrophoresis of a representative sample, and labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. The specific activities of the probes were typically 10^7 to 10^8 dpm/μg of RNA. Approximately 3 μg of whole-cell DNA isolated as described above was digested with *Eco*RI restriction endonuclease (Boehringer Mannheim) according to the guidelines of the manufacturer. Restriction fragments were separated by electrophoresis overnight (0.5 V/cm) on a 0.7% agarose gel in TAE and transferred to a nitrocellulose membrane by the method of Southern (5). Prehybridization and hybridization buffers contained 50% formamide, 5× SSPE (0.9% NaCl, 0.05 M sodium phosphate [pH 7], 5 mM EDTA), 2× Denhardt solution (0.04% bovine serum albumin, 0.04% polyvinylpyrrolidone, 0.04% Ficoll), 100 μg of denatured salmon sperm DNA per ml, and 200 μg of yeast tRNA per ml. After prehybridization, membranes were hybridized with *E. coli* [^{32}P]rRNA (10^6 dpm/ml) under moderately stringent conditions at 42°C for 12 to 24 h. The blots were then washed in 0.1× SSPE–0.1% sodium dodecyl sulfate (preheated to 50°C) twice at 50°C for 15 min and once at 60°C for 15 min. Hybridized bands were visualized by autoradiography by using X-Omatic AR film with intensifying screens (Dupont, Boston, Mass.) at −70°C for a maximum of 24 h. Ribotype patterns are designated by Roman numerals.

We observed an increase in the number of cultures positive for *P. cepacia* during the summer of 1987 (Fig. 1). In 1987, at least one culture of specimens from 38 patients was positive for *P. cepacia*, including those from 13 new patients with *P. cepacia* during the second quarter alone. In all of 1985, 1986, and 1988, specimens from 34 patients, or an average of 2.8 patients per quarter, were culture positive for *P. cepacia*. During the study period there were no changes in the method of isolation of *P. cepacia* in the clinical labora-

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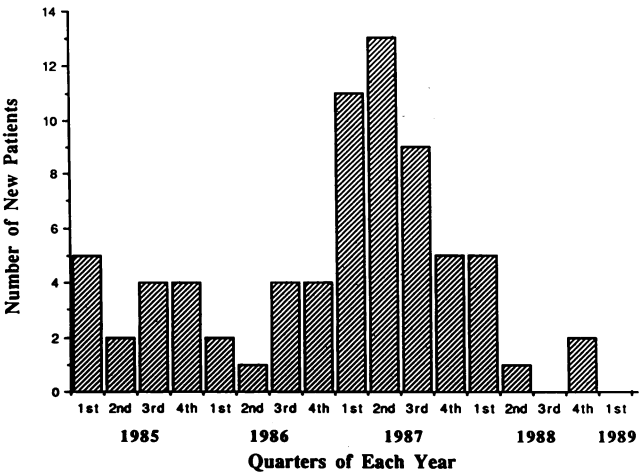

FIG. 1. Numbers of new patients with *P. cepacia* isolated from any site by quarters of each year at the Minneapolis VAMC between January 1985 and March 1989. Each patient was counted only once, at the time of isolation of the first isolate.

tory, nor were there any attempts to use cultures to screen patients for infection with *P. cepacia*.

Both DNA fingerprinting by pulsed field electrophoresis (Fig. 2A) and ribotyping (Fig. 2B) revealed a single pattern for all 36 isolates from 16 of 18 (89%) patients (Fig. 2A, pattern A, lanes 3 and 4, and Fig. 2B, pattern I, lanes 2 and 3). A second, distinct pattern distinguished the four isolates from the remaining two patients (Fig. 2A, pattern B, lanes 5 and 6, and Fig. 2B, pattern II, lanes 4 and 5). None of 25 control isolates unrelated to the outbreak had identical patterns (data not shown).

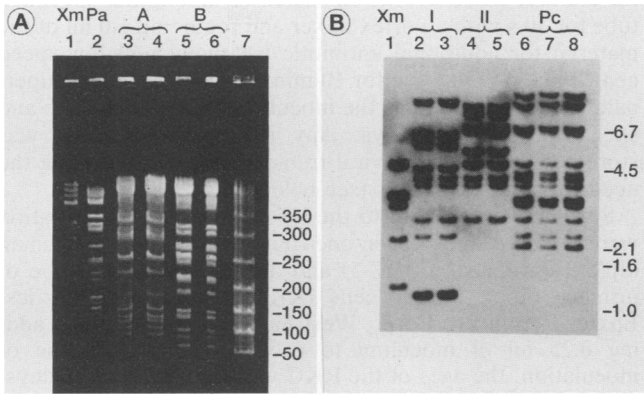

FIG. 2. (A) DNA fingerprint analysis using transverse alternating pulsed field gel electrophoresis. An ethidium bromide-stained agarose gel of chromosomal DNA was digested with *Spe*I restriction endonuclease and separated by pulsed field gel electrophoresis. Molecular masses, in kilobases, are indicated. Lane 1, Sample of DNA from an isolate of *Xanthomonas maltophilia*; lane 2, a sample of DNA from an isolate of *Pseudomonas aeruginosa*; lanes 3 and 4, representative samples of DNA from two isolates (from different patients) with typing pattern A; lanes 5 and 6, representative samples of DNA from two isolates (from different patients) with typing pattern B; lane 7, molecular mass markers. (B) Ribotype analysis. Autoradiograph of southern blot of *Eco*RI endonuclease digestion of bacterial DNA hybridized with radiolabeled *E. coli* rRNA. Molecular masses, in kilobases, are indicated. Lane 1, Sample of DNA from an isolate of *X. maltophilia*; lanes 2 and 3, representative samples of DNA from two isolates (from different patients) with typing pattern I; lanes 4 and 5, representative samples of DNA from two isolates with typing pattern II; lanes 6 to 8, samples of DNA from isolates of *P. cepacia* from a source unrelated to the outbreak.

DNA fingerprinting by pulsed field gel electrophoresis and ribotyping successfully classified *P. cepacia* isolates from a nosocomial outbreak at the Minneapolis VAMC. Both techniques separated the isolates into the same two distinct groups. A majority of the patients studied, 16 of 18 (89%), had the same strain. All isolates from any patient, either from multiple sites or multiple points in time, had the same pattern by both methods.

Newer methods for typing bacteria include plasmid analysis and comparison of DNA banding patterns by DNA fingerprinting, pulsed field gel electrophoresis, or ribotyping (7, 8). As first reported by Stull et al. (7), ribotyping is more stable and uniformly applicable than is plasmid analysis to all strains of *P. cepacia*. LiPuma and colleagues (2) used ribotyping to successfully and reproducibly classify *P. cepacia* isolates from patients at cystic fibrosis treatment centers. Rabkin et al. (4) compared ribotyping with many other methods of typing *P. cepacia* to determine the relatedness of isolates involved in an outbreak. They concluded that ribotyping, serotyping, and biochemical testing are all useful.

This study, involving more isolates from more patients than previous studies (2, 4), confirms the clinical utility of DNA fingerprinting with TAFE and by ribotyping in classifying isolates of *P. cepacia* involved in an outbreak. These newer methods of typing *P. cepacia* may help to clarify the epidemiology of *P. cepacia* infections.

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REFERENCES

1. **Isles, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming, and H. Levison.** 1984. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J. Pediatr. **104**:206–210.
2. **LiPuma, J. J., J. E. Mortensen, S. E. Dasen, T. D. Edlind, D. V. Schidlow, J. L. Burns, and T. L. Stull.** 1988. Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centers. J. Pediatr. **113**:859–863.
3. **O'Neil, K. M., J. H. Herman, J. F. Modlin, E. R. Moxon, B. Chir, and J. A. Winkelstein.** 1986. *Pseudomonas cepacia*: an emerging pathogen in chronic granulomatous disease. J. Pediatr. **108**:940–942.
4. **Rabkin, C. S., W. R. Jarvis, R. L. Anderson, J. Govan, J. Klinger, J. LiPuma, W. J. Martone, H. Monteil, C. Richard, S. Shigeta, A. Sosa, T. Stull, J. Swenson, and D. Woods.** 1989. *Pseudomonas cepacia* typing systems: collaborative study to assess their potential in epidemiologic investigations. Rev. Infect. Dis. **11**:600–607.
5. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503.
6. **Steere, A. C., J. H. Tenney, D. C. Mackel, M. J. Snyder, S. Polakavetz, M. E. Dunne, and R. E. Dixon.** 1977. Pseudomonas species bacteremia caused by contaminated normal human serum albumin. J. Infect. Dis. **135**:729–735.
7. **Stull, T. L., J. J. LiPuma, and T. D. Edlind.** 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. **157**:280–286.
8. **Vasil, M. L., D. P. Krieg, J. S. Kuhns, J. W. Ogle, V. D. Shortridge, R. M. Ostroff, and A. I. Vasil.** 1990. Molecular analysis of hemolytic and phospholipase C activities of *Pseudomonas cepacia*. Infect. Immun. **58**:4020–4029.