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Use of Pulsed-Field Gel Electrophoresis To Investigate an Outbreak of *Serratia marcescens*

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Pulsed-field gel electrophoresis (PFGE) typing was applied to the epidemiological investigation of 20 *Serratia marcescens* isolates collected from urine specimens of 17 patients and three urinals over a 2-month period. Twenty-five epidemiologically unrelated strains were also tested to determine the discriminatory power of PFGE. The PFGE fingerprints of each isolate were consistent in three different tests. The 20 outbreak isolates had an identical PFGE fingerprint pattern, while the epidemiologically unrelated strains demonstrated unique PFGE fingerprint patterns. The source of the outbreak was inadequately disinfected urinals. We conclude that PFGE served as a highly discriminatory and reproducible method for the epidemiological investigation of the outbreak of *S. marcescens* infection addressed by this study.

Serratia marcescens is an important pathogen of nosocomial infections. Outbreaks of nosocomial *S. marcescens* infections, including urinary tract infections, wound infections, pneumonia, and bacteremia, have been frequently reported (14, 17, 22). Traditional methods used for the typing of *S. marcescens* are often based on phenotypic characteristics and include biotyping, serotyping (1, 7), antibiogram analysis, bacteriocin typing (18), phage typing (8), and plasmid typing (6). Most of these techniques are not sufficiently sensitive to distinguish different strains or are affected by physiological factors (15). In recent reports, ribotyping (1, 3, 4, 10) and PCR (5, 10) have been used for typing *S. marcescens* and have shown a high degree of discriminatory potential and reproducibility. However, the evolution of DNA-regulating rRNA is very slow and sometimes the genetic mutations among clonally related strains cannot be detected by ribotyping (2, 9, 20). The reproducibility of PCR may be altered by operating conditions, such as the use of different thermal cyclers, the annealing temperature, and the concentrations of *Taq* polymerase, magnesium, template, and primer (12, 21). Pulsed-field gel electrophoresis (PFGE) typing is highly effective in molecular epidemiologic studies of bacterial isolates and is superior to ribotyping techniques in discriminating among isolates of *Escherichia coli*, *Staphylococcus aureus*, and many other species (13). Hence, PFGE typing can be used to evaluate the clonal relatedness among bacterial isolates and to investigate outbreaks (13, 19). To our knowledge, an investigation of a nosocomial *S. marcescens* outbreak by PFGE typing has never been reported before. We used PFGE typing in this study to investigate an outbreak of *S. marcescens*.

Twenty *S. marcescens* isolates (isolates 1 to 20) were collected from urine specimens of 17 patients and three urinals in the neurosurgical ward, the neurosurgical care unit (NCU), and other wards at Taichung Veterans General Hospital, Taiwan, between March 1996 and May 1996. Twenty-five epidemiologically unrelated isolates (isolates S1 to S25) were collected from other wards at Taichung Veterans General Hospital. Two epidemic strains (isolates S1 and S2 and isolates S3 to S6), proven by both ribotyping and PCR typing in a previous report (10), were included among the epidemiologically unrelated isolates. The bacteria were identified by the API 20E system (API-BioMerieux, La Balme les Grottes, France).

Genomic DNA was prepared as described previously (11). The bacterial suspension was prepared by the harvesting of bacterial colonies directly from overnight incubated culture on nutrient agar and was adjusted to a concentration of 10^9 CFU/ml in saline-EDTA buffer (75 mM NaCl and 25 mM EDTA [pH 7.5]) with a VITEK colorimeter (Hach Company, Loveland, Colo.). This bacterial suspension was then mixed with an equal volume of 2% low-melting agarose (Bio-Rad Laboratories, Richmond, Calif.) and was allowed to solidify in a 100-μl plug mold (Bio-Rad Laboratories). The DNA block was incubated overnight at 37°C in 2 ml of lysis buffer (10 mM Tris-HCl [pH 7.6], 100 mM EDTA, 100 mM NaCl, 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% sodium lauryl sarcosine, lysozyme [0.5 mg/ml]). Following this step, the lysis buffer was replaced by 2 ml of proteolysis buffer (1% sodium lauryl sarcosine, 0.5 M EDTA [pH 9.5], proteinase K [500 μg/ml; Sigma]) and this solution was incubated with gentle shaking at 56°C for 2 days, the proteolysis buffer being changed after 24 h. To eliminate the lysed bacterial material and inactivate proteinase K activity, the DNA blocks were washed once for 1 h at room temperature in 10 ml of Tris-EDTA buffer (10 mM Tris-HCl [pH 7.5], 10 mM EDTA) and once for 1 h at 37°C in Tris-EDTA buffer containing 1 mM phenylmethylsulfonyl fluoride (Sigma). To remove phenylmethylsulfonyl fluoride, the DNA blocks were washed once in 2 ml of Tris-EDTA buffer at 4°C for 1 h. A slice of each plug (1 by 3.5 by 5.0 mm) was cut and incubated for 24 h with 25 U of *Spe*I (Advanced Biotechnologies Ltd., London, United Kingdom); the use of the buffers and the reaction conditions were as recommended by the manufacturer. Other restriction endonucleases, such as *Apa*I, *Dra*I, and *Ssp*I (each from GIBCO BRL Life Technologies, Grand Island, N.Y.) and *Sma*I, *Sfi*I, and *Xba*I (each from Advanced Biotechnologies Ltd.), were also used to compare the effects of DNA digestion in the preliminary study. Restriction fragments of DNA were separated by PFGE with a CHEF-DRII apparatus (Bio-Rad Laboratories) through 1.2% SeaKem GTG agarose (FMC Bioproducts, Rockland, Maine). Electrophoresis was performed at 6 V/cm and 14°C. The run

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TABLE 1. Characteristics of outbreak-related *S. marcescens* isolates in this study

Isolate no.	Patient no.	Date of isolation (mo/day/yr)	Source	Ward	PFGE[a] genotype
1	1	3/9/96	Urine	NS[b]	A
2	2	3/14/96	Urine	NS	A
3	3	3/16/96	Urine	NS	A
4	4	3/22/96	Urine	NS	A
5	5	4/1/96	Urine	NS	A
6	6	4/8/96	Urine	NS	A
7	7	4/18/96	Urine	NS	A
8	8	4/20/96	Urine	NS	A
9	9	4/28/96	Urine	NS	A
10	10	5/6/96	Urine	NS	A
11	11	4/20/96	Urine	NCU	A
12		4/20/96	Urinal 1	NCU	A
13		4/20/96	Urinal 2	NCU	A
14		4/20/96	Urinal 3	NCU	A
15	12	5/10/96	Urine	NCU	A
16	13	3/14/96	Urine	NEU[c]	A
17	14	4/9/96	Urine	NEU	A
18	15	4/12/96	Urine	CVS[d]	A
19	16	4/15/96	Urine	Burn unit	A
20	17	4/26/96	Urine	General ward	A

[a] PFGE of DNA after digestion by *Spe*I.
[b] NS, neurosurgical.
[c] NEU, neurology.
[d] CVS, cardiovascular surgery.

time was 22 h, with pulse time ramping from 5 to 25 s. A lambda ladder (Bio-Rad Laboratories) was used as the molecular size marker. Reproducibility was checked by three different tests. Isolates were considered to be genetically indistinguishable if their restriction patterns had the same numbers of bands and the corresponding bands were the same apparent size. Isolates were considered to be closely related if their PFGE patterns showed two to three band differences, consistent with a single genetic event. Isolates were considered to be possibly related if their PFGE patterns showed four to six band differences, consistent with two independent genetic events. Isolates were considered to be unrelated if their PFGE patterns showed seven or more band differences, consistent with three or more independent genetic events (19).

Minimal cutting of *S. marcescens* DNA by *Xba*I and overactive cutting of *S. marcescens* DNA by *Dra*I, *Ssp*I, *Apa*I, *Sma*I, and *Sfi*I were found (data not shown). *Spe*I was found to be the most suitable restriction endonuclease for digestion of *S. marcescens* DNA in PFGE typing in this study. *Spe*I has also been used for PFGE typing of *Serratia odorifera* in a previous report (16). The pulse time in that study ramped from 5 to 60 s over a 22-h period. The larger DNA fragments were widely separated; thus, smaller DNA fragments did not show clear bands in that study. Therefore, we reduced the terminal switch time to 25 s to shorten the distance among larger fragments and to make smaller fragments more resolvable in our study. However, the resolution of the smaller genomic fragments (<145 kb) is still less than optimal in some strains. This may be due to frequent cutting of the DNA in the smaller fragments. This poor resolution in the smaller fragments may lead to difficulty of interpretation. Some strains occasionally demonstrated autodegradation in this study. The autodegradation of DNA fragmented spontaneously and randomly by endogenous nucleases usually results from the failure to chill and wash the bacteria promptly when preparing the DNA agarose gel blocks (13). However, this was not the cause of autodegradation in our study, because the bacteria had been carefully kept in ice during preparation of the DNA gel blocks. The autodegradation in our study might be due to nonspecific degradation by exogenous endonucleases during digestion.

Table 1 lists the epidemiological data and the PFGE patterns of the outbreak isolates. The PFGE fingerprints of each isolate were consistent on three different tests. Hence, the reproducibility of PFGE analysis for *S. marcescens* is good. Twenty outbreak-related isolates (isolates 1 to 20) had an identical PFGE fingerprint pattern (Fig. 1). These outbreak-related isolates also showed an identical ERIC1 primed PCR fingerprint pattern (data not shown). PFGE fingerprints of the 25 epidemiologically unrelated strains (isolates S1 to S25) demonstrated 21 distinct patterns (Fig. 2). The isolates of two epidemic strains (S1 and S2 and S3 to S6), proven by both ribotyping and PCR typing in a previous report (10), presented identical PFGE fingerprint patterns. The PFGE patterns of isolates S19 and S21 showed a one-fragment difference, consistent with the deletion of a DNA fragment (the deleted DNA did not contain a restriction site). Hence, they were considered closely related. The PFGE patterns of isolates S16 and S20 showed a four-band difference, consistent with two genetic events. Hence, they were considered possibly related. The other epidemiologically unrelated strains demonstrated unique PFGE patterns. Hence, the discriminatory power of PFGE typing for *S. marcescens* is satisfactory.

This outbreak of nosocomial *S. marcescens* urinary tract infections was due to a single epidemic strain colonizing the urinals and spreading among patients. According to our investigation, these urinals were not adequately disinfected and therefore were colonized by this epidemic *S. marcescens* strain. The urinals served as reservoirs. The epidemic strain was transmitted when nursing staff members emptied drainage bags into the urinals. To our knowledge, this is the first report of using a molecular biology method to prove that urinals can be the source of an outbreak of nosocomial *S. marcescens* urinary tract infection. Isolates 16 to 20, collected from unrelated wards, were, incidentally, found to have a PFGE pattern identical to that of isolates collected from the neurosurgical ward and the NCU. Although we have no evidence of the mode of intrahospital transmission, it can be speculated that these epidemic strains were transmitted via transient carriage on the hands of hospital personnel (17).

In conclusion, PFGE typing was found by this study to be a highly discriminatory and reproducible method for the epidemiological investigation of *S. marcescens* infection. This typing method can facilitate the reliable evaluation of the clonal re-

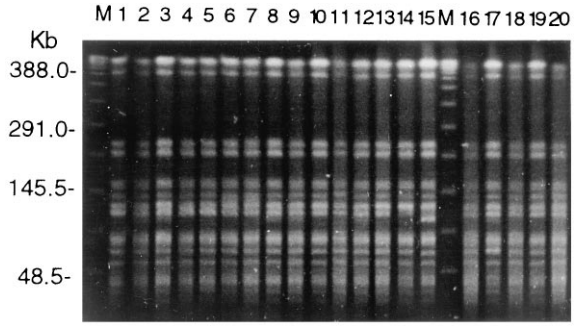

FIG. 1. PFGE fingerprints of *S. marcescens* isolates from the neurosurgical ward and the NCU after digestion with *Spe*I. Lane M, lambda ladder (Bio-Rad; molecular size marker); lanes 1 to 20, PFGE fingerprints of isolates 1 to 20, respectively.

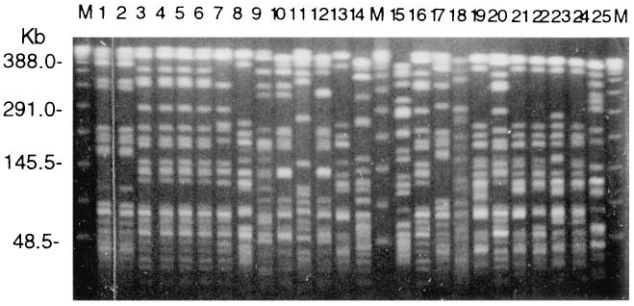

FIG. 2. PFGE fingerprints of 25 epidemiologically unrelated isolates. Lane M, lambda ladder (Bio-Rad; molecular size marker); lanes 1 to 25, PFGE fingerprints of epidemiologically unrelated isolates S1 to S25, respectively.

lationship of *S. marcescens* isolates and the identification of the common sources of outbreaks. However, since the results of the present study are based on an analysis of only 45 isolates, further studies involving much larger numbers of strains and additional restriction enzymes will be required in order to validate the discriminatory power and reproducibility of PFGE.

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