

## Evaluation of the Molecular Epidemiology of an Outbreak of Multiply Resistant *Shigella sonnei* in a Day-Care Center by Using Pulsed-Field Gel Electrophoresis and Plasmid DNA Analysis

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Outbreaks of diarrhea in child day-care centers (DCC) are common. This study was undertaken to evaluate the molecular epidemiology of an outbreak of diarrhea due to *Shigella sonnei*. This outbreak involved 25 of 52 (48%) DCC children and 14 of 132 (11%) teachers and household contacts. *S. sonnei* isolates from nine children and five contacts were characterized by antimicrobial susceptibility, plasmid content, plasmid DNA restriction fragment pattern, and pulsed-field gel electrophoresis (PFGE) of total genomic DNA; 33 isolates from Houston, Tex., Chicago, Ill., and Mexico City, Mexico, also were studied. All outbreak isolates were resistant to ampicillin and trimethoprim-sulfamethoxazole and shared five to six plasmids ranging from 3.3 to 70 MDa. A total of 8 of 12 temporally associated nonoutbreak Houston isolates had plasmid profiles and restriction fragment patterns similar to those of the outbreak strain, despite possessing different antibiotic susceptibility patterns. PFGE demonstrated identical DNA patterns among outbreak isolates and similar or identical patterns among temporally associated sporadic Houston isolates with plasmid profiles similar to that of the outbreak strain. All other nonoutbreak strains from Houston, Chicago, and Mexico had plasmid profiles, restriction fragment patterns, and PFGE patterns different from those of the outbreak strain. DCC outbreak isolates could be distinguished from most sporadic isolates by antimicrobial susceptibility testing, but plasmid analysis and PFGE could not differentiate common-source isolates from sporadic isolates in the same location during the same time period, indicating that isolates present in the community were genetically similar to those producing outbreaks in the DCC.

Children in day-care centers (DCCs) are at increased risk of diarrhea due to infection with many enteropathogens including *Shigella* spp. (3). Secondary spread of *Shigella* spp. from children in DCCs into their families and the community at large is common (3, 15, 16, 22, 24). Outbreaks of infection with *Shigella* spp. are difficult to control because of the low infectious inoculum, ease of transmission in the day-care setting, and resistance to multiple antibiotics (2, 11, 21, 23). Strains of *Shigella sonnei* resistant to multiple antibiotics are still relatively uncommon in the United States but are common in certain areas of the world (11, 23). This study evaluated an outbreak of diarrhea due to multiply resistant *S. sonnei* in a Houston, Tex., DCC with spread to family members. This study had three objectives: (i) to confirm the isolation of a single strain of *S. sonnei* during the outbreak; (ii) to define the association between the outbreak strain and independent isolates of *S. sonnei* from various locations; and (iii) to compare results of plasmid content, restriction endonuclease digestion of plasmids, and pulsed-field gel electrophoresis (PFGE) as applied to study the molecular epidemiology of this outbreak.

### MATERIALS AND METHODS

**Epidemiologic investigations.** The outbreak occurred in one of four DCCs monitored prospectively for diarrhea in 1990. Routine monitoring consisted of daily interviews of DCC personnel and of parents of absent children by a research nurse. Stool samples were collected weekly from each child and when any child developed diarrhea. Stool samples from children with diarrhea were tested for bacterial, viral, and parasitic enteropathogens by culture and enzyme-linked immunosorbent assays as previously described (20). When the outbreak of *S. sonnei* was recognized, stool specimens from teachers and asymptomatic children were cultured weekly. A questionnaire regarding diarrhea was completed by family members of DCC children. Stool specimens from symptomatic household contacts were cultured. The monitored population consisted of 52 DCC children (all children in three rooms) and all 132 of their teachers and household contacts. The age range of the DCC children was 3 to 36 months.

**Bacterial strains.** *Shigella* infections were diagnosed in 12 children and 9 household contacts; 8 of 9 contacts belonged to 4 families with an infected DCC child. A total of 14 of the 21 infections were diagnosed in our laboratory. This study evaluated *S. sonnei* isolates from 26 stool specimens obtained from these 14 individuals (9 DCC children and 5 contacts from 3 of the 9 families). The 33 reference strains used for comparison are listed in Table 1. The seven Houston City Health Department isolates obtained in 1985 and 1986 were included in a previous study (17). All comparison strains were not evaluated by each technique; only plasmid profile analysis was performed on all 26 *Shigella* isolates.

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TABLE 1. Sources of reference strains of *S. sonnei*

Source of strains	Year(s) of isolation	No. of strains
Houston		
City Health Department	1985-1986	7
	1991	9
Texas Children's Hospital	1991	3
Other Houston laboratories	1980-1985	4
	1992	1
Chicago	1990	2
Mexico City	1990	7

**Antimicrobial susceptibility.** Disk diffusion tests were performed with antibiotic-containing disks obtained from BBL Microbiology Systems, Cockeysville, Md. The MICs of trimethoprim for *Shigella* strains were determined with agar dilution. Both methods were performed as recommended by the National Committee for Clinical Laboratory Standards (13, 14).

**Plasmid profile analysis.** Plasmid profile analysis was performed by the method of Kado and Liu (7). Plasmid size above 10.5 MDa was determined by reference to strains containing plasmids of known size as described previously (1). Plasmid size below 10.5 MDa was determined with a supercoiled DNA ladder (GIBCO-Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

**Mating experiments.** *Escherichia coli* C600 (nalidixic acid and rifampin resistant) was the recipient strain. Transconjugants were selected on Mueller-Hinton agar (Difco, Detroit, Mich.) supplemented with trimethoprim (20 µg/ml) and nalidixic acid (50 µg/ml) and were tested for growth on rifampin (100 µg/ml) and trimethoprim (1,000 µg/ml). Antibiotics were obtained from Sigma (St. Louis, Mo.).

**Plasmid DNA isolation and restriction enzyme digestion.** Bacteria were grown in brain heart infusion broth (Difco) or Luria-Bertani medium at 37°C for 16 h. Plasmid DNA was prepared by hydrolysis of chromosomal DNA with 1% sodium dodecyl sulfate in 0.2 N NaOH as described by Sambrook et al. (18). Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, Ind.), and digestion was performed according to the manufacturer's instructions.

**PFGE of total genomic DNA.** PFGE was performed with the contour-clamped homogeneous electric field (CHEF-DRII) apparatus from Bio-Rad Laboratories (Richmond, Calif.) as described previously (12). Genomic DNA in 0.8% agarose plugs (InCert Agarose; FMC Bioproducts, Rockland, Maine) was digested with *Xba*I (recognition site, T/CTAGA; GIBCO-Bethesda Research Laboratories). Plugs were incubated at 37°C overnight in React 2 buffer (GIBCO-Bethesda Research Laboratories). DNA was electrophoresed in 1.2% SeaPlaque GTG agarose (FMC Bioproducts) at 200 V for 30 h; the pulse time was increased from 5 to 35 s. A ladder of bacteriophage lambda concatemers was used to determine the sizes of *Xba*I restriction fragments (New England Biolabs, Beverly, Mass.).

## RESULTS

The outbreak lasted 15 weeks from July to November 1990. Diarrhea was reported in 25 of 52 children and 14 of 132 contacts, with attack rates of 48 and 11%, respectively. *S. sonnei* was isolated from 21 individuals (12 children and 9 contacts). This study evaluated 26 *S. sonnei* isolates ob-

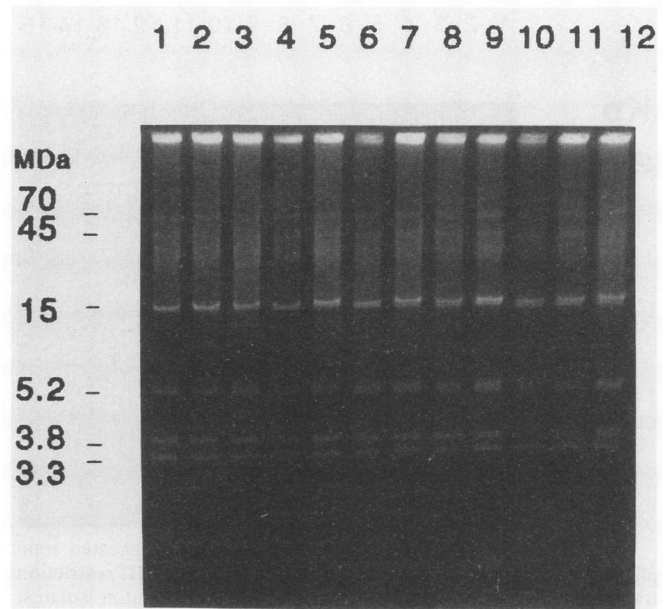


FIG. 1. Agarose gel electrophoresis of total plasmid contents of 12 *S. sonnei* isolates from the Houston DCC outbreak. The molecular sizes of six shared plasmids are shown in megadaltons.

tained from 14 individuals (9 children and 5 contacts) on different days during the outbreak. *Shigella* infections in the remaining seven individuals were diagnosed in other laboratories, and the isolates were not available for study. The documented duration of excretion of *S. sonnei* by individual patients varied between 1 and 37 days.

**Antibiotic susceptibility testing.** All outbreak isolates were resistant to ampicillin, trimethoprim, and trimethoprim-sulfamethoxazole; moderately susceptible to cephalothin and amoxicillin-clavulanate; and susceptible to nalidixic acid, tetracycline, furazolidone, ciprofloxacin, and norfloxacin. The outbreak isolates possessed high-level resistance to trimethoprim (6); the MIC of trimethoprim for the outbreak strain was between 1,024 and 2,048 µg/ml (12 isolates tested). Among the 26 sporadic U.S. isolates tested, 2 were resistant to trimethoprim and 11 were resistant to ampicillin.

**Plasmid profile analysis.** All but 2 of 26 outbreak isolates shared six plasmids. The sizes of these plasmids were 70, 45, 15, 5.2, 3.8, and 3.3 MDa (Fig. 1). One isolate lacked the 3.3-MDa plasmid, and another possessed an extra 38-MDa plasmid. The 120-MDa invasiveness plasmid had been lost during storage from all *S. sonnei* isolates studied.

A single isolate from each of 14 culture-positive individuals involved in the outbreak was mated with *E. coli* C600. The recipient strain lacked any plasmids and was resistant to nalidixic acid but was susceptible to trimethoprim. Transconjugants receiving the 45-MDa plasmid from the outbreak isolates were resistant to both trimethoprim and ampicillin.

The outbreak isolates were compared with 13 temporally associated (1991 to 1992) sporadic *S. sonnei* isolates from Houston residents. Plasmid profile analysis distinguished only 4 of these 13 isolates from the outbreak isolates. Plasmid profiles of the other nine isolates were similar to the outbreak profile.

The similarity in plasmid content of most temporally associated sporadic Houston isolates and outbreak isolates

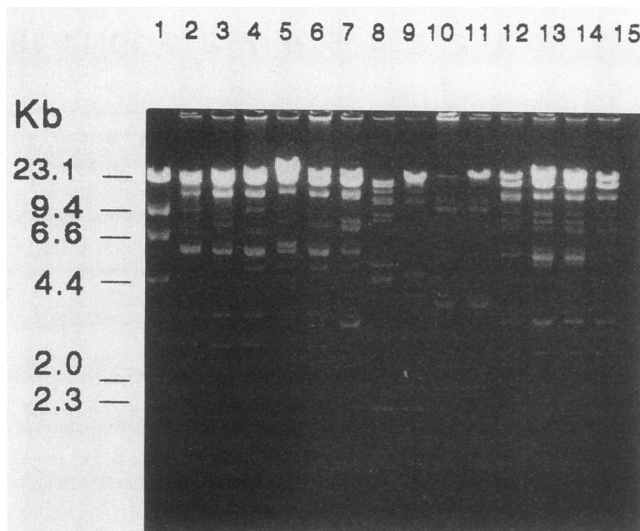


FIG. 2. Restriction fragment patterns of *Bam*HI-digested total plasmid DNA from *S. sonnei* isolates. Lanes: 1, *Hind*III restriction fragments of phage lambda; 2 to 9, 1991 sporadic Houston isolates; 10 and 11, 1990 Chicago isolates; 12 to 15, Houston DCC outbreak isolates. The numbers indicate the sizes of *Hind*III restriction fragments of phage lambda in kilobases.

contrasted with differences between antibiotic susceptibility patterns of these two groups of isolates. All outbreak isolates had the same antibiotic susceptibility pattern. All nine temporally associated Houston isolates with plasmid profiles similar to the outbreak profile had different antibiotic susceptibility patterns. All nine isolates were trimethoprim susceptible, and only one isolate was ampicillin susceptible.

In contrast to the similarity of plasmid profiles of most temporally associated Houston isolates, the Chicago, Ill., isolates, the 1980 to 1986 Houston isolates, and the Mexico isolates were easily distinguished from the outbreak strain by plasmid profile analysis. Both plasmid profile analysis and antimicrobial susceptibility testing distinguished the 1990 to 1992 Houston isolates of *S. sonnei* from those isolated in Houston between 1980 and 1986.

**Restriction fragment analysis of plasmid DNA.** Plasmid DNA was isolated and digested with *Eco*RI and *Bam*HI. We evaluated 14 outbreak isolates (14 cases), 12 temporally associated (1991) sporadic Houston isolates, and 2 1990 Chicago isolates. *Eco*RI and *Bam*HI digestion of outbreak strain plasmid DNA produced 22 and 13 fragments, respectively. Representative *Bam*HI patterns, the easier patterns to compare, are shown in Fig. 2. Restriction fragment patterns of plasmid DNA from outbreak isolates were similar (Fig. 2, lanes 12 to 15). There was some variation among *Eco*RI and *Bam*HI restriction fragment patterns of plasmid DNA from outbreak isolates that involved one to three bands, consistent with a small number of changes occurring among plasmids of the outbreak strain.

Restriction fragment pattern analysis confirmed the plasmid profile data regarding similarity of 8 of 12 temporally associated sporadic Houston isolates to the outbreak isolate (Fig. 2, lanes 2 to 7), although minor differences can be noted in bands above the 4.4- to 6.6-kb range. Plasmid DNA of the other four 1991 Houston isolates and two 1990 Chicago isolates with plasmid profiles easily distinguishable from the outbreak plasmid profile had restriction fragment patterns

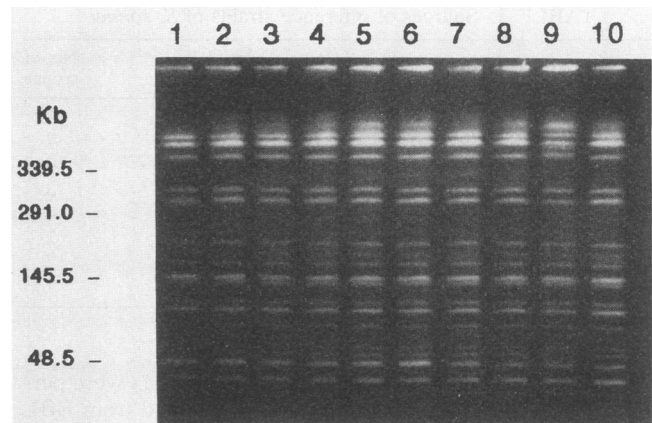


FIG. 3. PFGE of *Xba*I-digested genomic DNA of 10 *S. sonnei* isolates from the Houston DCC outbreak. The relative positions of molecular size markers are shown. The numbers indicate the sizes of selected lambda concatemers in kilobases.

that were clearly different from the outbreak restriction fragment pattern (Fig. 2, lanes 8 to 11).

**PFGE.** High-molecular-weight genomic DNA was prepared from 14 outbreak isolates (14 cases) and digested with the restriction endonuclease *Xba*I. Restriction fragment patterns of chromosomal DNA were demonstrated by PFGE. *Xba*I restriction fragment patterns of DNA from outbreak isolates were virtually identical (Fig. 3). *Xba*I produced 21 fragments; their size ranged from 19 to 457 kb. We compared the outbreak restriction fragment pattern with *Xba*I restriction fragment patterns of DNA from 13 reference isolates from the United States: 7 1991 Houston isolates with plasmid profiles similar to the outbreak profile and 4 1991 Houston isolates and 2 1990 Chicago isolates with plasmid profiles different from the outbreak profile. DNA from three of the seven isolates with plasmid profiles similar to the outbreak profile had *Xba*I restriction fragment patterns similar to the outbreak pattern (Fig. 4, lanes 1, 2, and 4). Patterns of strains in lanes 3 and 6 resembled that of the outbreak strain,

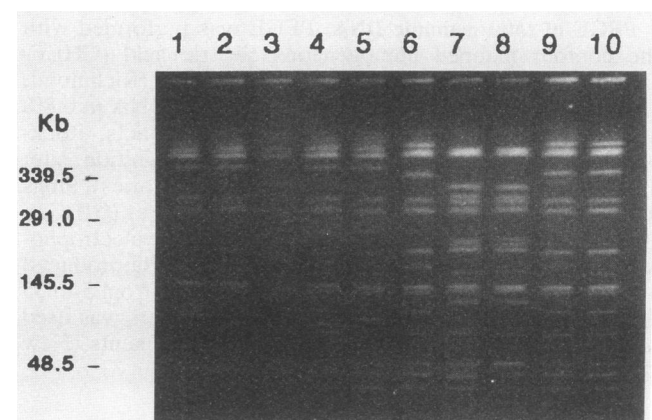


FIG. 4. PFGE of *Xba*I-digested genomic DNA of sporadic isolates of *S. sonnei*. Lanes: 1 to 8, isolates from Houston in 1991; 9 and 10, isolates from Chicago in 1990. The restriction fragment pattern in lane 5 is identical to the Houston DCC outbreak pattern. The relative positions of molecular size markers are shown. The numbers indicate the sizes of selected lambda concatemers in kilobases.

but because of the larger number of different bands, we did not feel comfortable in calling them related. Another four isolates with plasmid profiles similar to the outbreak profile had *Xba*I restriction fragment patterns identical to the outbreak pattern (i.e., Fig. 4, lane 5). The other six isolates were readily distinguished from the outbreak strain by both plasmid profile analysis and PFGE (Fig. 4, lanes 7 to 10). We also studied genomic DNA from seven 1990 Mexico City, Mexico, isolates of *S. sonnei* and demonstrated five *Xba*I restriction fragment patterns, one of which was identical to the pattern associated with the 1990 Houston DCC outbreak.

## DISCUSSION

We studied a DCC outbreak of diarrhea due to a strain of *S. sonnei* resistant to trimethoprim-sulfamethoxazole and ampicillin by plasmid profile analysis, restriction endonuclease digestion of plasmid DNA, and field-inversion gel electrophoresis of total genomic DNA. Several molecular methods have been applied previously to study sporadic and epidemic isolates of *S. sonnei* (5, 8, 17, 19, 21). Experience in our laboratory with PFGE of total genomic DNA for epidemiologic studies of *Enterococcus* species and enteroinvasive *E. coli* has been encouraging (4, 10). In this study, all outbreak isolates were shown to be identical with antimicrobial susceptibility testing, demonstration of total plasmid content, analysis of restriction enzyme fragment pattern of plasmid DNA, and PFGE of total genomic DNA. Tacket and Cohen (21) used plasmid analysis to study two outbreaks of *S. sonnei* infections in DCCs in two neighboring counties in Florida. All but 1 of 15 outbreak isolates evaluated had identical plasmid profiles. All 14 isolates with identical plasmid profiles were resistant to ampicillin. Resistance to ampicillin was transferable on a 60-MDa plasmid. The *Hind*III restriction enzyme patterns of the 60-MDa plasmid from isolates obtained in the two outbreaks were identical but differed from the pattern obtained after digesting the 52-MDa plasmid from the one ampicillin-susceptible isolate.

Strains of *S. sonnei* genetically related to the Houston DCC outbreak strain predominated among temporally associated sporadic Houston isolates obtained in 1991 and 1992. The predominance of this group of closely related strains of *S. sonnei* was not observed among *S. sonnei* isolates obtained from Chicago in 1990, from Houston between 1980 and 1986, or from Mexico City in 1990. The genetic similarity of most temporally associated Houston sporadic isolates of *S. sonnei* was demonstrated by plasmid profile analysis, restriction enzyme fragment pattern analysis of plasmid DNA, and PFGE of total genomic DNA. The predominance of a limited number of related plasmid profiles among *S. sonnei* isolates in a geographic region has been reported previously. Prado et al. (17) performed plasmid profile analysis on isolates of *S. sonnei* from Mexico (Mexico City and Guadalajara) and Houston and found that the majority (51 of 57) of strains had identical or nearly identical plasmid profile patterns that differed from plasmid profiles of *S. sonnei* isolated in Guatemala, Egypt, and The Gambia. Predominance of one plasmid profile was maintained in Guadalajara over a 5-year period. Yagupsky et al. (25) used molecular techniques to study the epidemiology of *S. sonnei* in Monroe County, New York. They performed plasmid profile analysis on 26 of 251 sporadic isolates reported during a 12-month period. All isolates had identical plasmid profiles. Yagupsky et al. (25) also performed plasmid profile analysis on 18 of 43 isolates obtained from a large foodborne outbreak within the county and observed a pattern nearly

identical to that of sporadic isolates. Litwin et al. (8) evaluated isolates of *S. sonnei* obtained during a 12-month period in Pima County, Arizona. They examined plasmids less than 20 kb in length. Most isolates (70 of 79) shared three to six plasmids of the same size. A 5.1-kb plasmid was seen for 26 isolates and was associated with recent travel to Mexico (8, 9). In contrast, in the study of Tacket and Cohen (21) the outbreak plasmid profile was distinguished from the profile of three recent sporadic isolates from the same state.

Epidemiologic investigations have less frequently involved chromosomal DNA than plasmid DNA. Hinojosa-Ahumada et al. (5) probed chromosomal DNA restriction fragments with labeled RNA (ribotyping). These investigators selected 100 independent isolates of *S. sonnei* obtained in 16 states from 1985 to 1986 and isolates from four outbreaks in 3 states. With four restriction enzymes, nine patterns were produced from all isolates. The patterns of the four outbreaks were distinguished from each other and from those of the independent isolates. Soldati and Piffaretti (19) applied field-inversion gel electrophoresis to the typing of epidemic and sporadic strains of *Shigella* species. They performed field-inversion gel electrophoresis on *Nor*I-digested genomic DNA from nine independent isolates of *S. sonnei*. They reported seven patterns among the nine isolates. We included PFGE in our molecular techniques so that we could analyze chromosomal DNA as well as plasmid DNA. In our study, all of the independent, temporally associated Houston isolates with plasmid profiles similar to that of the outbreak strain exhibited PFGE patterns similar or identical to that of the outbreak strain. The Houston isolates with plasmid profiles different from those of the outbreak isolates and isolates from Chicago and Mexico had PFGE patterns different from that of the outbreak strain.

In this study, antimicrobial susceptibility testing distinguished DCC outbreak isolates from most sporadic isolates. However, plasmid profile analysis, restriction enzyme fragment analysis of plasmid DNA, and PFGE could not easily differentiate common-source isolates from sporadic isolates in the same city during a similar time period. Our study demonstrated that when closely related strains predominate during a defined period in a certain geographic area, it may be difficult to distinguish an outbreak strain from related sporadic isolates.

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