Use of Multiple Markers for Investigation of an Epidemic of *Shigella sonnei* Infections in Monroe County, New York

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Antibiotic susceptibility patterns, plasmid profiles, and endonuclease restriction analysis of plasmid DNA were used in the investigation of an epidemic of *Shigella sonnei* infections in Monroe County, New York, in 1988 and 1989. The epidemic peaked during the winter, included the simultaneous transmission of the disease from person to person and from common food sources, and especially affected inhabitants of the poor, inner-city neighborhoods, young children of both sexes, and women. Resistance to ampicillin, tetracycline, or trimethoprim-sulfamethoxazole, encoded in a 70-MDa plasmid, was found in most of the examined isolates. Unexpectedly, isolates from patients involved in a food-borne outbreak exhibited three different antibiotic susceptibility patterns, suggesting deletion of antibiotic resistance determinants in some strains. Antibiograms clearly separated food-borne outbreak-related and non-foodborne outbreak-related strains, distinguished more strains than did the plasmid profiles, and were useful in tracing the dissemination of individual isolates in the community. Restriction endonuclease analysis substantially increased the discriminatory value of plasmid profiles and validated the antibiogram results. The present study illustrates the complexity of epidemics of *S. sonnei* infections and shows the value of combining different biological markers in the investigation.

Because of improved sanitary conditions, the annual incidence of shigellosis in the United States has declined in the last 40 years (4). Nevertheless, epidemics of shigellosis continue to occur when crowding and poor sanitary conditions prevail, as in day-care centers, or when water or food is contaminated by the organism (3, 4, 11, 29).

Recently, we studied a prolonged epidemic of *Shigella sonnei* infections that occurred among residents of Monroe County, New York. A variety of markers, including antibiotic susceptibility patterns, plasmid profiles, and restriction endonuclease analysis of plasmid DNA, were used to characterize the organisms recovered. A complex epidemiological picture emerged which included a prolonged epidemic caused by a single strain and two intercurrent food-borne outbreaks, each seemingly caused by a different strain. The present report describes the epidemiological features of the outbreak and the contribution of the different techniques to the investigation.

**MATERIALS AND METHODS**

**Background.** Monroe County is located on the southern shore of Lake Ontario in the western part of New York State; it has a land area of 663 mi² (ca. 1,720 km²) and a population of 702,600 (1986 estimate). Approximately one-third of the county’s inhabitants (236,000) reside in the city of Rochester (28). In recent years, the total number of cases of *S. sonnei* infection reported to the Monroe County Health Department (MCHD) has been stable: 15 cases were reported in 1986, 20 cases were reported in 1987, and 12 cases were reported in the first half of 1988.

**Epidemiological investigation.** Culture-confirmed cases of shigellosis are routinely reported to the MCHD by the clinical microbiology laboratories of the different area hospitals, commercial laboratories, and private physicians. Infected individuals reported are interviewed within a week, and demographic data and information regarding date of onset of symptoms, contact with ill persons, travel, and consumption of food during the week prior to the onset of the disease are collected.

For the purposes of the study, cases of culture-confirmed infection with *S. sonnei* that occurred among residents of Monroe County since 3 July 1988 were identified by review of the records of the MCHD, and efforts were made to retrieve strains of *S. sonnei* isolated at four large hospitals in the city of Rochester during the previous months, as well as to prospectively collect fresh isolates. Collectively, the four hospitals detect most cases of *S. sonnei* infection reported to the MCHD. The surveillance was not intensified during the outbreak and was discontinued on 1 July 1989, when the number of cases declined to the preepidemic levels.

**Bacteriological investigation.** (i) **Antibiotic susceptibility testing.** Antibiotic susceptibility of isolates to ampicillin (AMP), tetracycline (TET), trimethoprim-sulfamethoxazole (TMP-SMX), and chloramphenicol was determined on Mueller-Hinton agar by the disk diffusion method of Bauer et al. (1).

(ii) **Plasmid isolation and restriction endonuclease analysis.** Plasmid DNA from 36 randomly selected isolates was extracted by the alkaline precipitation method of Birnboim and Doly (2) and resolved on a 0.7% agarose gel by electrophoresis at 100 V for 3 to 5 h. DNA was visualized by staining with ethidium bromide during electrophoresis and photographed under UV illumination. Although this technique does not allow clear visualization of large plasmids, including the constant 235-kb invasion plasmid, it is useful for the demonstration of small variable plasmids which can be used as epidemiological markers. *EcoRI* digestion of plasmid DNA was performed according to the manufacturer’s instructions. The products of the digestion were resolved by electrophoresis under conditions identical to those described for undigested plasmid DNA.

(iii) **Transfer of antibiotic resistance.** Transformation ex-
experiments were conducted as described by Maniatis et al. (15). *Escherichia coli* RR1 was transformed with isolated plasmid DNA from *S. sonnei* strains representing the following antibiotic resistance patterns: SRR (AMP sensitive, TET resistant, and TMP-SMX resistant) and RSS (AMP resistant, TET sensitive, and TMP-SMX sensitive). Transformants were selected on L agar (15) containing either AMP (100 μg/ml), TET (10 μg/ml), or TMP-SMX (15 μg of TMP per ml, 300 μg of SMX per ml). Plasmid DNA was extracted from transformants as described above.

(iv) Hybridization of 70-MDa plasmid with a TEM β-lactamase probe. DNA was transferred from agarose gels to nitrocellulose by the method of Smith and Summers (25). A radiolabeled, TEM-1-type β-lactamase probe was prepared from an *Eco*RI-*Hin*II restriction digest of plasmid pBR322 (7). A 1-kb restriction fragment containing the AMP resistance gene was purified by electrophoresis, and approximately 200 ng of the purified fragment was labeled with [³²P]dCTP by nick translation (15). Plasmid DNA was hybridized with the β-lactamase probe as follows. The nitrocellulose filter (ca. 9 by 12 cm) containing the target plasmid DNA was treated with 5 ml of prehybridization solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.5% sodium dodecyl sulfate [SDS], 4 mM NaPO₄, 0.1 mg of calf thymus DNA per ml) for 5 h at 37°C in a sealed plastic bag. The prehybridization solution was replaced with 5 ml of hybridization solution (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.5% SDS) containing 1 × 10⁶ cpm of probe per ml, and the contents were hybridized overnight at 37°C. The filter was then washed three times for 15 min each time at 37°C and once for 30 min at 60°C with 150-ml volumes of wash solution containing 6× SSC and 0.1% SDS. The filter was exposed to X-ray film with an intensifying screen for 2 to 4 days at −70°C and developed with an automatic X-ray developer.

RESULTS

Between 3 July 1988 and 1 July 1989, 304 cases of enteric disease caused by *S. sonnei* were reported to the MCHD (incidence of 43.2 cases per 100,000 population). Overall, 133 patients (43.7%) were males and 171 patients (56.3%) were females. The biweekly occurrence of cases is shown in Fig. 1. Correlation between antibiograms, plasmid profiles, and electrophoretic patterns of *Eco*RI digests of plasmid DNA is summarized in Table 1.

**Common source outbreaks.** During the study period, two distinct food-borne outbreaks of *S. sonnei* were observed. The first included 10 female residents of Monroe County who acquired shigellosis at a music festival in Michigan in August 1988 (Michigan outbreak). Antibiotic susceptibility testing was performed in two cases, and the isolates were found to be resistant to AMP and TET and sensitive to TMP-SMX. This pattern is referred to as RSS. In addition,

![FIG. 1. Occurrence of enteric disease due to *S. sonnei* in Monroe County by biweekly periods and susceptibility of isolates to AMP, TET, and TMP-SMX.](http://jcm.asm.org/)

### TABLE 1. Antibiograms, plasmid profiles, and restriction endonuclease analysis of plasmid DNA of *S. sonnei* isolated in Monroe County

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Antibiogram pattern (AMP, TET, TMP-SMX)</th>
<th>Plasmid profile</th>
<th><em>Eco</em>RI digestion pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michigan outbreak</td>
<td>RRR</td>
<td>ND³</td>
<td>ND</td>
</tr>
<tr>
<td>FPO</td>
<td>SRR</td>
<td>A</td>
<td>A₂</td>
</tr>
<tr>
<td></td>
<td>SRD</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SSS</td>
<td>A</td>
<td>A₂</td>
</tr>
<tr>
<td></td>
<td>RSS</td>
<td>B</td>
<td>B₁</td>
</tr>
<tr>
<td>Non-food-borne cases</td>
<td>SRR</td>
<td>A</td>
<td>A₂</td>
</tr>
<tr>
<td></td>
<td>SSS</td>
<td>A</td>
<td>A₂</td>
</tr>
</tbody>
</table>

³ ND, not done.
TABLE 2. Distribution of 251 cases of shigellosis without identified common food or water source of infection by sex and age

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of cases in age group (in yrs):</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-9</td>
<td>10-19</td>
<td>20-29</td>
<td>30-39</td>
<td>40-49</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>7</td>
<td>13</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>74</td>
<td>12</td>
<td>23</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

the strain was susceptible to chloramphenicol. The strain was unavailable for further study.

The second food-borne outbreak occurred among the ~200 attendees of a private funeral party that took place in Rochester on 28 February 1989 (funeral party outbreak [FPO]). About 80 attendees suffered from acute gastrointestinal symptoms and fever. A case-control study and culture of the food served incriminated a potato salad. The food handler had diarrhea when she prepared the food but refused to submit a specimen for culture. She denied recent travels or use of antimicrobial drugs during the 2 weeks prior to the onset of the disease. Stool or rectal swab cultures were positive for S. sonnei in 44 patients, all but one of whom were residents of Monroe County. Of the 43 Monroe County cases, 18 occurred in males and 25 occurred in females. Nineteen culture-confirmed cases occurred among children <10 years of age. The onset of symptoms occurred within 24 h in 2 cases, within 48 h in 35 cases, within 72 h in 41 cases, and within 96 h in all 43 culture-proven cases. Isolates from 32 cases were available for antibiotic susceptibility testing, and all were susceptible to chloramphenicol. Of these 32 isolates, 19 exhibited the SSS pattern (susceptible to all three antibiotics), 12 were SRR, and a single isolate was SRS (susceptible to AMP, resistant to TET, and susceptible to TMP-SMX). Unfortunately, the unique SRS strain was lost. Only the SSS phenotype was recovered from the potato salad.

Cases without a common food or water source. For 251 cases, no common food or water source was identified. One hundred fifteen cases (45.8%) occurred in males, and 136 cases (54.2%) occurred in females. The disease was most common among young children. Of the 251 patients, 105 (41.8%) were under 5 years of age and 157 (62.5%) were under 10 years of age. The distribution of cases by sex and age group is shown in Table 2. Female predominance among patients older than 10 years was found to be statistically significant (0.01 > P > 0.001 by the chi-square test). Contact with individuals who had diarrhea during the week before onset of symptoms and simultaneous or successive occurrence of other cases of dysentery among family members were common. Two hundred fifteen cases occurred among individuals who lived in the city of Rochester (12-month incidence of 91.1 cases per 100,000 population); most lived in the less affluent neighborhoods. Only 36 cases occurred among residents of the economically more advantaged suburbs (12-month incidence of 7.7 per 100,000 population). All of the 147 isolates tested for antibiotic susceptibility were found to be susceptible to chloramphenicol, and 137 were RSS. The 10 isolates that exhibited an antibiotic susceptibility pattern different from the predominant RSS pattern were identified in the 9 weeks that followed the large FPO. Eight isolates were SSS, and two were SRR. Contact with a person involved in the FPO was established in only one of these cases. Plasmid profile and endonuclease restriction analysis of these strains were identical to those observed among attendees of the funeral party.

Plasmid isolation and restriction endonuclease assay results. Electrophoresis of isolated plasmid DNA showed the plasmid contents of large FPO and non-FPO (NFPO) isolates to be quite similar to each other but considerably different from that of control strains isolated in California (Fig. 2). However, all 26 NFPO strains tested lacked two additional bands noted for the 18 FPO isolates examined (Fig. 2, lanes A and B, respectively). The plasmid profiles of all FPO isolates appeared to be identical, regardless of their antibiotic susceptibility patterns. EcoRI restriction endonuclease analysis of isolated plasmid DNA showed that each antibiogram was associated with a unique pattern (Fig. 3).

Transfer of antibiotic resistance and plasmid analysis of transformants. Transfer of plasmid DNA from an S. sonnei isolate resistant only to AMP (RSS pattern) into competent E. coli RR1 yielded transformants resistant to AMP and sensitive to TET and TMP-SMX. Transformation with DNA from an SRR S. sonnei isolate produced organisms sensitive to AMP and resistant to both TET and TMP-SMX. TET and TMP-SMX resistance always cotransferred; no transfor-
mants resistant only to TET or TMP-SMX were detected. A 70-MDa plasmid was common to all RSS and SRR transformants, and in some cases it was apparently the only plasmid present. Thus, the 70-MDa plasmid appeared to confer resistance to AMP, TET, and TMP-SMX, although never to all three antibiotics within a single strain. Restriction endonuclease analysis of the 70-MDa plasmid isolated from transformants showed differences between the plasmid conferring resistance to AMP only and the plasmid conferring resistance to TET and TMP-SMX (Fig. 4).

Hybridization with a TEM-1-type β-lactamase probe. Hybridization of the isolated 70-MDa plasmid with a β-lactamase probe showed conclusively that the determinant for AMP resistance was located on this plasmid as suggested. The β-lactamase probe did not hybridize with the 70-MDa plasmid conferring resistance to TET and TMP-SMX. Hybridization of the β-lactamase probe to an EcoRI digest of the 70-MDa plasmid conferring AMP resistance showed that the AMP resistance gene was located on an 8-kb restriction fragment (data not shown).

DISCUSSION

The incidence of S. sonnei infections is usually low during the cooler part of the year, and epidemics generally occur during the warm months, related perhaps to the proliferation of fly vectors (13). The Monroe County epidemic was unusual in that, although it started in July, it reached its peak in midwinter and declined during the spring. Similar clustering of cases of shigellosis during the winter has been observed in England (6) and in the coldest regions in Poland (12).

The epidemic curve observed in the first 8 months of the study is consistent with progressive, person-to-person transmission of the disease (19). S. sonnei was most common among urban, lower socioeconomic groups, a well-known trend of bacillary dysentery in the United States (19, 20). Reported cases frequently occurred among children younger than 5 years and adults in the 20- to 29-year age group, suggesting intrafamily 20- to 29-year transmission of the disease from young children to their parents. The high attack rate of bacillary dysentery in children has been explained by the poor hygienic habits and higher clinical case-to-asymptomatic excreter ratio among young children (24). However, it is also possible that adults are more likely to bring children with gastroenteritis for medical attention than to seek medical assistance for themselves for a similar illness and that stool cultures are performed more frequently in pediatric cases than in adult cases of diarrhea. Cases of shigellosis among children younger than 10 years showed a nonsignificant predominance of males. For all other age groups combined, a 1.94:1 female-to-male ratio was observed. Assuming equal susceptibility to S. sonnei infections and equal symptomatic-to-asymptomatic ratio in both sexes, these data suggest that adult women are more prone to acquire the disease because of a closer contact with young children (22, 23).

The antibiotic susceptibility pattern and plasmid profile demonstrated that, until March 1989, a single strain was responsible for all cases with no common food or water source of infection. Circulation of a single S. sonnei strain or a limited number of S. sonnei strains has also been noted in epidemics occurring in the United States and Europe (16, 26), in contrast to the coexistence of multiple strains within the community and even among family members in underdeveloped countries (10, 27). The persistence of the strain in a stable form over a prolonged period of time is noteworthy and has been also observed in the S. sonnei strain that caused bacillary dysentery in Guadalajara, Mexico, during the years 1981 to 1985 (17).

The outbreak of shigellosis that took place in Michigan in August 1988 contributed to a small number of cases in the Monroe County epidemic and has been extensively described in a recent report (14). As was the case during the music festival, secondary transmission of the AMP- and TET-resistant (RRS) strain apparently did not occur, probably because young children were not involved in the original outbreak.

The large food-borne FPO that occurred at the end of February 1989 had many of the features described for food-borne shigellosis, including preparation of the meals in a noncommercial kitchen by a diarrheic food handler, contamination of a salad rich in carbohydrates, a short incubation period, and a high attack rate (2, 8, 9, 21). Because of the large number of cases of shigellosis observed in the community during the previous months, the food-borne outbreak was thought to be the result of contamination of food by the S. sonnei strain circulating in Monroe County at that time. However, the antibiograms and plasmid fingerprints indicated that the FPO was not caused by the prevalent strain.

In general, point-source outbreaks of infectious diarrhea are characterized by isolation of organisms that exhibit identical biological properties (8). The simultaneous recovery of isolates with three different antibiotic susceptibility patterns in the food-borne outbreak is noteworthy and has not been described in previous epidemiological studies of shigellosis originating from a common source. The reasons for this unusual finding remain speculative. The transformation and TEM β-lactamase probe experiments demonstrated that the determinants for antibiotic resistance of the NFPO and FPO strains were both carried exclusively on a 70-MDa conjugative plasmid. It may be postulated that the food was originally contaminated by a strain that had the determinants for TET and TMP-SMX resistance. While the complete set of determinants of resistance to TET and TMP-SMX was conserved in isolates with the SRR phenotype, deletions resulted in an SRS or SSS phenotype without significant alteration of the electrophoretic mobility of the large 70-MDa plasmid. Although a single phenotype (SSS) was isolated from the food and from individual patients (SSS, SRS, or SRR), it should be noted that only a small number of
bacterial colonies are used to set up an antibiotic susceptibility test; this small number of bacterial colonies might have resulted in the random selection of a single phenotype from a mixed bacterial population. Moreover, in patients with dual infection, only the most resistant phenotype would have been recognized by the antibiogram. It may be suggested that the differences observed in the restriction endonuclease analysis of plasmid DNA between the SSS and SRR strains are too large to be the result of the loss of a transposon encoding resistance to TET and TMP-SMX (Fig. 3). An alternative interpretation could be that Monroe County isolates possessed two comigrating 70-MDa plasmids, one of which encoded antibiotic resistance. Loss of this plasmid would have resulted in an SSS antibiotic susceptibility pattern without appreciable change in the plasmid profile.

The SRR and SSS strains disseminated in the community and caused secondary cases during a short period of time following the FPO. It is suggested that the large number of young children involved in the original outbreak contributed to the successful propagation of the FPO strains.

The complex epidemiology of the Monroe County epidemic allowed the evaluation of the ability of antibiograms, plasmid profiles, and restriction endonuclease analysis to define differences and similarities between epidemiologically associated and nonassociated strains. By using plasmid profiles, identity among strains isolated from patients involved in food-borne outbreaks or from children attending day-care centers and simultaneous circulation in the community of multiple strains with identical antibiotic susceptibility patterns have been shown (8, 18, 21, 26, 27). However, identical electrophoretic patterns of undigested plasmid DNA from S. sonnei strains isolated from wide geographic areas have been found (17). Under these circumstances, plasmid profiles cannot be used to demonstrate a common source of infection. In the present study, although plasmid fingerprints consistently discriminated between FPO and NFPO strains, the technique failed to demonstrate differences between the two FPO phenotypes studied. However, consistent differences were found between the EcoRI restriction fragments of the SSS and SRR phenotypes, confirming the importance of endonuclease cleavage patterns as a supplementary epidemiological tool (27).

Antibiotic susceptibility patterns have also been used to discriminate intraspecies strains. However, identity among antibiograms may be the result of antibiotic pressure and may not necessarily indicate epidemiological relatedness (5). Moreover, since routine antibiotic therapy is usually not recommended for mild cases of shigellosis, clinical microbiology laboratories frequently do not test antimicrobial susceptibilities of Shigella isolates. The results of the present study show that the antibiotic susceptibility patterns of S. sonnei may be unpredictable because of the prevalence of antibiotic-resistant and multiple-antibiotic-resistant strains and suggest that isolates should be routinely tested. In addition to the important clinical information provided to the physician, antibiograms clearly discriminated between FPO and NFPO isolates, distinguished more strains than did the plasmid profiles, and also provided an economical method to monitor the dissemination of the different strains in the community, while the combination of plasmid profiles and restriction endonuclease analysis confirmed the specificity of the antibiogram fingerprints.

The Monroe County epidemic shows that the combination of multiple markers such as antibiotic susceptibility patterns, plasmid profiles, and restriction endonuclease analysis of plasmid DNA can be useful in the investigation of complex epidemics of S. sonnei.

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