Shigella sonnei Strains Isolated from U.S. Summer Students in Guadalajara, Mexico, from 1986 to 1992

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Received 22 December 1993/Returned for modification 17 February 1994/Accepted 26 July 1994

Plasmid DNA analysis and antibiotic susceptibilities were used to study strains of Shigella sonnei isolated from U.S. travelers to Guadalajara, Mexico, over a period of seven years (1986 to 1992). One hundred sixty-one isolates were analyzed. By the use of cluster analysis, eight different plasmid profiles were identified during this interval. At any point in time, three to seven different plasmid profiles were present in this population. The introduction of strains that carried a new plasmid with a molecular mass of 5.1 Mda was coincidental with an increase in isolation of S. sonnei in 1988. This new plasmid was present in 87.5% of the isolates that were resistant to chloramphenicol. Shigellosis in Guadalajara follows a pattern of hyperendemic transmission with transient peaks of high-frequency isolation of S. sonnei. This pattern results from the concurrent presence of a heterogeneous group of strains as opposed to the widespread transmission of one or a few clones.

It is estimated that as many as 1,000,000 people traveling to Mexico develop diarrhea every year. Shigella species account for as many as 20% of the cases of traveler's diarrhea in Mexico, and Shigella sonnei is the most common species isolated (3). S. sonnei is also the most common species found in the United States, accounting for about 60% of all cases of shigellosis reported to the Centers for Disease Control and Prevention every year (2).

Two epidemiologic patterns for shigellosis have been described. Cyclic epidemics with major shifts in serogroups, Shigella dysenteriae O1 strains initially predominating and then being subsequently replaced by Shigella flexneri, which is then replaced by S. sonnei, each cycle lasting 20 to 40 years (7), have been observed since the turn of the century. These cycles are considered to reflect changes in herd immunity to one species that is present for a long period of time until immunity in the population reaches a critical level. More recently a second pattern, characterized by continuous low levels of transmission alternated with periodic, transient nationwide increases in isolation of Shigella organisms (9), has been recognized. The explanation for these transient peaks in the frequency of Shigella infection remains unclear. The most recent peak in the United States was seen in 1988, and S. sonnei accounted for most of the increase in isolates (9).

The existence of only one serotype of S. sonnei has complicated the study of the epidemiology and transmission of this organism. Plasmid DNA analysis has been employed to differentiate strains (1, 10), to characterize antibiotic resistance (5), and to investigate outbreaks caused by this organism (8, 16). However, very few studies of shigellosis have extended their observations over prolonged periods of time. Through our studies of traveler's diarrhea in Mexico we have had the opportunity to examine over the course of several years a well-defined population of U.S. summer students in an area where S. sonnei is hyperendemic. In this study, we used plasmid DNA analysis and antibiotic susceptibilities for differentiation of S. sonnei strains isolated from U.S. summer students in Guadalajara, Mexico, from 1986 to 1992 and to determine the occurrence of changes among the strains in relation to changes in the rate of S. sonnei isolation during this period.

(Received in revised form 26 July 1994.)

MATERIALS AND METHODS

Subjects and specimens. S. sonnei isolates obtained from fecal specimens from patients with acute diarrhea who reported to our clinic in Guadalajara between the summers of 1986 and 1992 were used in this study. All the subjects were U.S. adults attending either the University of San Diego (San Diego, Calif.) or the University of Arizona (Tucson, Ariz.) summer program in Guadalajara, Mexico, held during July and August in each year of the study. Written informed consent was obtained from all patients, and the human experimentation guidelines of our institution were followed in the conduct of this clinical research. Diarrhea was defined as the occurrence of four or more unformed stools in a 24-h period (or three unformed stools in an 8-h period) plus the presence of an additional symptom of enteric disease such as nausea, vomiting, abdominal pain, fever, fecal urgency, or tenesmus. Isolates were identified as S. sonnei by methods previously described (11). Briefly, fecal specimens were inoculated onto MacConkey, salmonella-shigella, Tergitol 7, and DNase plates (Difco Laboratories, Detroit, Mich.). Suspicious colonies growing on the plate media were identified by using standard biochemical tests and by using commercial antisera (Difco). Strains isolated were stored in peptone stabs.

Plasmid analysis. Plasmid DNA analysis for all the isolates was performed in the laboratories of the Center for Infectious Diseases of the University of Texas Health Science Center in Houston at the end of the study period (summer 1992). Each strain selected for study was subcultured from peptone stabs before being tested to check for purity. Extraction of plasmid DNA from S. sonnei isolates was performed according to the method of Kado and Liu (6). Electrophoresis was carried out in 1.0% horizontal agarose gels for 2 h at 75 V.

Antibiotic susceptibility testing. Antibiotic susceptibilities
were determined by disk diffusion. The antimicrobial agents tested included ampicillin (10 μg), aztreonam (30 μg), chloramphenicol (30 μg), furazolidone (100 μg), gentamicin (10 μg), ofloxacin (5 μg), sulfisoxazole (0.25 mg), trimethoprim (1.25 μg)-sulfamethoxazole (23.75 μg), and tetracycline (30 μg).

Statistical analysis. The presence or absence of the different plasmids was recorded for each isolate. The percent similarity of isolates was estimated by using the simple matching coefficient method. Cluster analysis was used to group isolates into plasmid profiles, and a dendrogram showing these profiles was generated by the unweighted pair group method for arithmetic averages (4).

RESULTS
Two hundred and two cases of acute diarrhea caused by S. sonnei occurred during the study period. Table 1 shows the distribution of cases according to years of isolation. Interestingly, a marked increase in the number of isolates occurred in 1988, when S. sonnei accounted for almost 34% of all cases of diarrhea in our population. One hundred sixty-one (79.7%) of the 202 original isolates were recovered by subculture from peptone stabs and used for the rest of this study.

Electrophoresis of the total plasmid content of S. sonnei isolates demonstrated that there were up to 11 distinct plasmids, with molecular masses ranging from 0.7 to 140 MDa. Because of the documented instability of the large plasmids of S. sonnei when subcultured (13), only plasmids with molecular masses of less than 15 MDa were used for cluster analysis. Figure 1 is a dendrogram representing cluster analysis results calculated from the percent similarity values of clinical isolates. Clusters were defined as groups of strains with at least 75% similarity (the lowest level at which significant homogeneity is expected) in order to enable the maximum number of strains to be grouped. Cluster analysis of the 161 Guadalajara isolates was used to define eight different plasmid profiles, shown in Table 2. Each different plasmid profile contained from one to six plasmids.

At any point in time, strains representing three to seven different plasmid profiles were present in our patient population (Table 2). Plasmid profiles 1 and 2 were mainly seen from 1986 to 1988, and then they declined in prevalence. The increased rate of S. sonnei isolation observed in 1988 was coincidental with the emergence of new plasmid profiles (profiles 4, 5, and 6) in addition to a marked increase in the frequencies of the profiles previously present (profiles 1, 2, and 3). Some of these, including profiles 3, 5, and 6, and also profile 7, which appeared after 1988, carried a new plasmid band with

![Dendrogram showing cluster analysis of the electrophoretic plasmid patterns of 161 strains of S. sonnei. The percent similarity values are indicated on the horizontal axis. Profiles were defined as clusters of strains possessing ≥75% similarity.](http://jcm.asm.org/Downloaded from http://jcm.asm.org on August 27, 2017 by guest)
Antimicrobial susceptibility testing was completed for 143 of the S. sonnei isolates from Guadalajara, Mexico (Table 3). No resistance to aztreonam, gentamicin, or ofloxacin was found among S. sonnei strains during the study period. Resistance to chloramphenicol increased markedly after 1988, coincidently with the emergence of the new plasmid profiles. Interestingly, 28 (87.5%) of the 32 isolates resistant to chloramphenicol possessed the new 5.1-MDa plasmid (P < 0.001; chi-square test). Resistance of S. sonnei to ampicillin, sulfisoxazole, and tetracycline remained high during the study period. No particular plasmid profile was predictive of a particular pattern of antibiotic susceptibility.

**DISCUSSION**

We have some knowledge concerning a large plasmid that encodes properties essential for virulence and concerning plasmids which confer drug resistance, but little is known about the small plasmids of S. sonnei. However, the reported stability of these small plasmids when subcultured allows their utilization as epidemiological markers (10, 12). The total number of plasmids present in any given bacterial population can affect the results of the epidemiologic analysis. A small total number can compromise our ability to discriminate among strains. In contrast, the more plasmids an organism contains, the more specific is the plasmid profile as a marker for a single strain, although it may sometimes complicate the interpretation of the results when large, diverse populations are studied (15).

Enteric bacteria can exchange genetic information readily, and plasmid profiles and antibiotic susceptibility results are particularly sensitive to environmental pressures. Occasionally, isolates that are considered part of an outbreak may exhibit different, but related, plasmid profiles and antibiotic susceptibility patterns (4). Cluster analysis is used to establish the degree of relatedness among strains, information that may be useful in epidemiological studies (4, 14). In the present study, the use of cluster analysis allowed us to classify all S. sonnei isolates into eight different groups or profiles, and we related this classification to the changes in the isolation of S. sonnei observed between 1986 and 1992. We observed that the reservoir of S. sonnei for U.S. travelers in this large urban area of Mexico where shigellosis is hyperendemic is made up of a heterogeneous group of strains from different plasmid profiles, which persist for a few years before their apparent replacement by strains that differ from the ones initially present. The observation of new plasmid profiles was coincidental with an increase in the isolation of S. sonnei in our population in 1988 to levels well above the ones seen in the preceding years. Interestingly, this peak in frequency was transient, and the levels of isolation promptly returned to their baseline range.

The pattern we describe parallels the trends reported in the United States (9). In both cases transient peaks in isolation of S. sonnei occurred in 1988, with rapid returns to baseline levels in the following year. While in the case of the United States widespread transmission of one or a few S. sonnei strains has been proposed as the likely cause of these events (9), our results obtained by plasmid DNA analysis suggest that in the case of S. sonnei in Guadalajara the presence of a heterogeneous group of strains may help to explain the hyperendemicity of this organism, and we note that the observation of several new strains adding to this already mixed pool was coincidental with the 1988 peak.

The marked increase in resistance to chloramphenicol observed after 1988 was concomitant with the observation of new plasmid profiles in our population. Without the plasmid studies one could have thought that all these chloramphenicol-resistant strains were derived from the same ancestral strain. In this case plasmid analysis helped us to recognize the presence of more than a single clone as the carrier of the new characteristic.

These laboratory observations, based on differences in plasmid content and antibiotic susceptibility, suggest how complex the epidemiology of S. sonnei may be in this area. The uniqueness of a well-defined population and the length of the surveillance period allowed us to recognize that the hyperendemic situation and the transient peaks in isolation of S. sonnei in Guadalajara are the results of the concurrent action of

### TABLE 2. Plasmid profiles of 161 isolates of S. sonnei from Guadalajara

<table>
<thead>
<tr>
<th>Plasmid profile</th>
<th>Total no. of strains isolated</th>
<th>Presence of plasmid of the following size (MDa)*</th>
<th>5.1</th>
<th>5.0</th>
<th>4.2</th>
<th>3.3</th>
<th>1.0</th>
<th>0.9</th>
<th>0.7</th>
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<tr>
<td>1</td>
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<td>+</td>
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<td>+</td>
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<td>2</td>
<td>15</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>+</td>
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<td>5</td>
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<tr>
<td>6</td>
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</tr>
</tbody>
</table>

*+, plasmid was present in all isolates; +/-, differs among strains.

### TABLE 3. Antibiotic susceptibilities of 143 S. sonnei isolates from Guadalajara, Mexico

<table>
<thead>
<tr>
<th>Year of study</th>
<th>No. of strains tested</th>
<th>% of strains resistant to the following antimicrobial agent*</th>
</tr>
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<tr>
<td></td>
<td>AM ATM CL GM OFX SU SXT TE</td>
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<tr>
<td>1986</td>
<td>17 35 0 6 0 0 88 18 94</td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td>11 64 0 9 0 0 100 0 82</td>
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<tr>
<td>1988</td>
<td>71 74 0 13 0 0 88 12 86</td>
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</tr>
<tr>
<td>1989</td>
<td>6 67 0 33 0 0 83 13 92</td>
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</tr>
<tr>
<td>1990</td>
<td>12 93 0 57 0 0 93 14 100</td>
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<tr>
<td>1991</td>
<td>5 60 0 20 0 0 100 20 100</td>
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</tr>
<tr>
<td>1992</td>
<td>21 81 0 55 0 0 84 16 84</td>
<td></td>
</tr>
</tbody>
</table>

* Antimicrobial agents: AM, ampicillin; ATM, aztreonam; CL, chloramphenicol; GM, gentamicin; OFX, ofloxacin; SU, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline.
several strains as opposed to the widespread transmission of a single type. This heterogeneity may have implications for our understanding of the epidemiology and transmission of this organism.

ACKNOWLEDGMENTS

We thank Melinda A. Smith and Zhi D. Jiang for technical assistance.

REFERENCES