Plasmid Analysis of *Shigella dysenteriae* Type 1 Isolates Obtained from Widely Scattered Geographical Locations

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Plasmid profiles and antimicrobial susceptibility patterns of 343 strains of *Shigella dysenteriae* type 1, obtained from 18 different geographical locations, were analyzed. Three plasmids, with molecular sizes of 140, 6, and 2 megadaltons (MDa), were present in 94, 98, and 96%, respectively, of the 343 strains isolated during either epidemic or nonepidemic periods from 1965 to 1987. In addition to these plasmids, 83% of the strains harbored a 4-MDa plasmid and 25% harbored a 20-MDa plasmid. Various plasmid profiles were observed in which the 140-, 6-, and 2-MDa plasmids occurred commonly, irrespective of the place of isolation and drug resistance pattern of the strains. Certain profiles showed significant association with drug resistance patterns. These findings suggest that three plasmids, of molecular sizes 140, 6, and 2 MDa, are unique to *S. dysenteriae* type 1 strains and may indicate the global spread of a pathogenic bacterial clone. Additionally, these two ethidium bromide plasmids, plus plasmids of various other sizes, could be used to identify emerging subclones which are causing both epidemic and sporadic disease. Thus, plasmid profiles of *S. dysenteriae* type 1 strains can be used to monitor possible pandemic strains as well as individual epidemic strains.

Electrophoresis of plasmid DNA in agarose gels can be used to identify an epidemic strain as well as to identify the plasmids that may spread from one bacterial species to another (18). Analysis of plasmid profiles has been shown to be a powerful tool in epidemiological studies of infectious diseases caused by various microorganisms (3, 7, 13) and has now become a part of the routine system for surveillance of nosocomial infections in many countries (4). Extensive epidemiological studies have been performed on *Salmonella* isolates, but little work has been carried out on *Shigella* isolates.

In the past 18 years, a number of dysentery outbreaks caused by drug-resistant *Shigella dysenteriae* type 1 strains have occurred in developing countries around the world (10, 20; M. M. Rahman, I. Huq, C. R. Dey, A. K. M. G. Kibriya, and G. Curlin, Letter, Lancet i:406–407, 1974). The presence of identical plasmids in *S. dysenteriae* type 1 strains isolated from both Bangladesh and West Bengal was reported previously (14). We have also observed four plasmids in 94% of 50 *S. dysenteriae* type 1 strains isolated during April to October 1983 at the International Centre for Diarrhoeal Disease Research, Bangladesh (K. Haider et al., unpublished observation). These preliminary findings indicate that there may be one genetically related group of strains of *S. dysenteriae* type 1 which has persisted over several years and in separate geographical areas.

It has been reported that a 120-megadalton (MDa) plasmid is universally present in *S. sonnei* (16) and that a 120- to 140-MDa plasmid is present in other *Shigella* species (21). Prado et al. (15) have recently reported that a small plasmid (3.4 MDa) is also present in strains of *S. sonnei* isolated in different parts of the world. The presence of these two *S. sonnei* plasmids should be considered when using the plasmid patterns as an epidemiological marker. No such report on the presence of common plasmids, with the possible exception of the 140-MDa plasmid in *S. dysenteriae* type 1 strains, is available. This study was therefore undertaken to isolate and compare the total plasmid DNA in *S. dysenteriae* type 1 strains isolated in several geographical locations in Asia, the United States, and Costa Rica and hence to define a potentially pandemic clone and to look for common plasmids which may be associated with the O1 serotype, antibiotic resistance, or virulence properties.

**MATERIALS AND METHODS**

**Bacterial strains.** A total of 343 *S. dysenteriae* type 1 strains were obtained from 10 different countries: Bangladesh, the United States, Costa Rica, India, Burma, Nepal, Sri Lanka, Thailand, Singapore, and Saudi Arabia. These isolates were tested for their susceptibility to various antimicrobial agents and for the presence of plasmid DNA. The strains were isolated during outbreaks of dysentery or from isolated cases and were sent to us from the laboratories where the strains had been isolated or sent for confirmation.

The isolates were grown on Trypticase soy agar or broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.3% yeast extract. The identity of all isolates was confirmed biochemically (5) and serologically by using commercially prepared antisera (Burroughs Wellcome Co., London, England) on glass slides (6). All strains were routinely checked for roughness by autoagglutination in physiological saline (0.85% NaCl).

**Isolation and analysis of plasmid DNA.** Plasmid DNA was extracted and separated by vertical electrophoresis in 0.7% agarose (Sigma Chemical Co., St. Louis, Mo.) slab gels (15) by 12 by 0.3 cm) (2, 11). The gels were stained in ethidium bromide solution (0.5 μg/ml) for 30 min and destained overnight at 4°C. Photographs were taken with an MP4 Land camera with type 55 P/N film and a no. 22 Wratten gelatin filter (Eastman Kodak Co., Rochester, N.Y.) with UV transillumination. Plasmids of known molecular size were used as controls in each gel. The molecular size of each plasmid in each *S. dysenteriae* type 1 strain was estimated by comparison with standards: pDK-9 (140 and 105 MDa), R1 (62 MDa), RP4 (36 MDa), and Sa (23 MDa).

**Sereny test.** The Sereny test (19) was performed by inoculating 20 μl of each *S. dysenteriae* type 1 strain (3 × 10⁸ cells per ml) into the eye of an adult guinea pig. Strains that...
produced keratoconjunctivitis within 72 h were considered invasive.

**Antimicrobial susceptibility tests.** The antimicrobial susceptibility tests (1) were performed with commercially available disks (BBL Microbiology Systems) at the following antibiotic concentrations (in micrograms per disk): ampicillin, 10; chloramphenicol, 30; streptomycin, 10; tetracycline, 30; trimethoprim-sulfamethoxazole (TMP-SMX), 1.25 and 23.75, respectively; kanamycin, 30; gentamicin, 10; and nalidixic acid, 30.

**Tests for statistical analysis.** The association between the plasmid profiles and drug resistance patterns was assessed by using the odds ratio, and significance was tested by using the chi-square distribution with one degree of freedom.

**RESULTS**

Since *S. dysenteriae* type 1 is the most virulent *Shigella* serotype which has been associated with epidemics, we concentrated our investigation on this serotype only. We used 343 strains obtained from 18 different laboratories in 10 countries. These strains were collected from geographically diverse areas (Table 1) and were all confirmed as shigelae. Except for six strains that agglutinated in physiological saline and were rough, all others were smooth and were serologically identified as *S. dysenteriae* type 1.

Each plasmid profile pattern (P1 to P7) is shown in Fig. 1. The molecular sizes of the plasmid species were determined from such gels. Distribution analysis of each of the predominant plasmids showed that the 140-, 6-, and 2-MDa plasmids were the most frequently encountered (94, 98 and 96%, respectively) in *S. dysenteriae* type 1, irrespective of the country of isolation and of drug resistance patterns. Therefore, we considered these plasmids to be the serotype-specific plasmids, or core plasmids, of this species. Although the 4- and 20-MDa plasmids were also present in a large number of strains, they were present only in strains that had multiple antibiotic resistances; therefore, they were not consistently encountered. Other plasmids of various sizes were seen; however, each had a low frequency of detection. Six plasmid profiles (P1 to P6) were found in the 343 strains with a frequency ranging from 1 to 51%. There were also 66 isolates with various plasmid profiles that occurred at a frequency of <1%. The most frequently encountered plasmid profiles (P1: 140, 6, and 2 MDa; P2: 140, 6, 4, and 2 MDa; P3: 140, 20, 6, 4, and 2 MDa; P4: 140, 80, 6, and 2 MDa; P5: 140, 20, 6, 4, 2, and 0.5 MDa; P6: 140, 65, 35, 6, 2, and 0.5 MDa; P7: other various profiles).

**DISCUSSION**

An analysis of the plasmid DNA of *S. dysenteriae* type 1 strains has shown that three plasmids, of 140, 6, and 2 MDa, were present in strains obtained from diverse geographical locations during both epidemic and nonepidemic periods.

**TABLE 1. Presence of different plasmid profiles in 343 *S. dysenteriae* type 1 strains isolated from various geographical locations**

<table>
<thead>
<tr>
<th>Country of isolation</th>
<th>No. of strains isolated</th>
<th>Yr isolated</th>
<th>No. of strains with following profile*:</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>170</td>
<td>1984–1987</td>
<td>3</td>
<td>127</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td></td>
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<tr>
<td>India</td>
<td>11</td>
<td>1967–1985</td>
<td>11</td>
<td>39</td>
<td>22</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>4</td>
<td>1985</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td>1</td>
<td>1976</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>7</td>
<td>1965–1968</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nepal</td>
<td>20</td>
<td>1984–1985</td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>6</td>
<td>1986</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Burma</td>
<td>4</td>
<td>1984</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Costa Rica</td>
<td>11</td>
<td></td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>9</td>
<td>1972–1980</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>4</td>
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</table>

*Plasmid profiles are as follows: P1: 140, 6, and 2 MDa; P2: 140, 6, 4, and 2 MDa; P3: 140, 20, 6, 4, and 2 MDa; P4: 140, 80, 6, and 2 MDa; P5: 140, 20, 6, 4, 2, and 0.5 MDa; P6: 140, 65, 35, 6, 2, and 0.5 MDa; P7: other various profiles.
These three plasmids were present in both susceptible and resistant strains and appear to constitute a stable gene pool. Initially, our findings with a limited number of strains from Dhaka (20) suggested that a single plasmid pattern was present; thus, a single strain might be responsible for the epidemic of shigellosis. Our present findings with the strains obtained from other parts of the world suggest that these three plasmids are unique to this Shigella serotype, perhaps reflecting the global spread of one bacterial clone. That these strains indeed originate from a single clone must be substantiated by results of restriction enzyme digestion of plasmid and chromosomal DNA (23). We were unable to use these techniques in the present study, but we plan to use them in the future.

In the literature, we found no report documenting relationship between serotypes of S. dysenteriae and plasmid profiles. However, the presence of serotype-specific plasmids in S. sonnei has been shown (15). This contrasts with earlier findings showing that a variety of small plasmids (\(\leq 6\) MDa) were present in Shigella species (8, 9, 22).

It has been shown by other workers that there is a correlation between the presence of the 140- and 6-MDa plasmids in strains of S. dysenteriae type 1 and virulence (17, 24). In our study, we observed that 21 strains, all lacking the 140-MDa plasmid, were noninvasive in the Sereny test and that 5 strains without the 6-MDa plasmid autoagglutinated in normal saline, indicating incomplete O-antigen synthesis. These findings further confirm the significance of these two plasmids in virulence. The genes present in the 2-MDa plasmid must be evaluated and characterized. Other plasmids of S. dysenteriae type 1 strains have not been fully characterized and may code for a variety of functions.

In addition, analyses of plasmid profiles are useful tools with which to document the appearance of plasmids associated with important phenotypic characteristics. For example, we have reported that plasmids in the molecular size range of 20 to 44 MDa were correlated with drug resistances in S. dysenteriae type 1 strains (8, 12).

The strong associations we observed between plasmid profiles and drug resistance patterns suggest that plasmids other than the core plasmids may have epidemiological significance and should be evaluated carefully. To confirm that this is true, additional experiments involving conjugation studies and curing experiments must be carried out.

In summary, our results show that three core plasmids, of 140, 6, and 2 MDa, are present almost universally in S. dysenteriae type 1 strains, indicating the possible global spread of a single strain. Plasmid profiles were likewise found to be useful as markers when plasmids other than the core plasmids were present. However, plasmid profile are best to studying sudden outbreaks of disease due to a particular species, since strains can lose or gain plasmids over time. Chromosomal DNA analysis by restriction digestion gives more precise information regarding differences between strains. Therefore, plasmid profile analysis of S. dysenteriae type 1 strains can be used in the broad or more limited epidemiological setting, although there are certain limitations to its application.

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**LITERATURE CITED**


