Temporal Shifts in the Dominance of Serotypes of *Shigella dysenteriae* from 1999 to 2002 in Dhaka, Bangladesh

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A total of 358 *Shigella dysenteriae* strains isolated from patients attending the Dhaka treatment center of the International Centre for Diarrheal Disease Research, Bangladesh, between the years 1999 and 2002 were included in this study. *S. dysenteriae* type 1, the dominant serotype in 1999 (76.4%), declined to 6.5% in 2002. On the other hand, *S. dysenteriae* types 2 to 12 were isolated with increasing frequencies of 19, 67, 73.5, and 87% in 1999, 2000, 2001, and 2002, respectively. Of these, types 2 and 4 were the most dominant serotypes, accounting for more than 18.7 and 28.5% of the total isolates, respectively. There was no isolation of serotypes 5, 7, 8, and 13 during this period. Twenty-eight (7.8%) of the isolates were atypical and agglutinated only with the polyvalent antiserum of *S. dysenteriae*. More than 98% of type 1 strains isolated between 1999 and 2001 were resistant to ampicillin, sulfamethoxazole-trimethoprim, and nalidixic acid. Among other serotypes of *S. dysenteriae*, Nal^r type 2 strains were isolated in 2001 and 2002. Although heterogeneous plasmid profiles were obtained depending on the presence or absence of a single plasmid, core plasmids were defined for particular serotypes. On the other hand, the same plasmid profile was found to be shared by different serotypes. Interestingly, plasmid patterns of types 2 and 4 were almost identical except that a middle-range plasmid of 70 to 60 MDa was present in type 4 in addition to the core plasmids. All the strains harboring the 140-MDa plasmid were positive for the *ipaH* gene, had Congo red binding abilities, and were positive by the Sereny test, demonstrating their invasive properties.

Shigellosis is one of the major diarrheal diseases afflicting humans in the developing and underdeveloped parts of the world, especially Bangladesh. Shigellosis produces inflammatory reactions and ulceration on the intestinal epithelium followed by bloody diarrhea and mucus in the stool. Shigellosis is endemic in Bangladesh, and it is estimated that dysentery accounts for 20% of deaths related to diarrhea among children (32). The genus *Shigella*, the causative agent of shigellosis, is comprised of four species, namely, *S. flexneri*, *S. dysenteriae*, *S. boydii*, and *S. sonnei*, and each of these species is further classified into 15 (including subtypes), 13, 18, and 1 serotypes, respectively, based on the O antigen component of lipopolysaccharide present on the outer membrane of the cell wall. *S. flexneri* and *S. dysenteriae* are the major concerns for developing countries. The manifestation of *S. dysenteriae* type 1 infection is more severe because of its capacity to produce Shiga toxin, which is exclusively produced by this type. Clinical infection can be transmitted by as few as 10 *Shigella* organisms (3), even without neutralization of gastric acid.

At least three periods of epidemic outbreaks of dysentery due to *S. dysenteriae* 1 have been recorded previously in the Indian subcontinent, in 1972–1973, 1983–1984, and 1993–1994 (1, 9, 18, 24). Despite improvement of municipal water supplies and sanitation, shigellosis still occurs frequently. This raises important questions about the causes of shigellosis, its transmission, epidemiology, and the effectiveness of public

health measures in overcoming this illness. Indiscriminate use of antibiotics in this region has resulted in the *Shigella* strains becoming resistant to multiple antibiotics. At present, most of the *Shigella* strains isolated from patients are resistant to ampicillin (AMP), sulfamethoxazole-trimethoprim (SXT), and nalidixic acid (2, 9, 15).

The prevalence of the serotypes of *S. flexneri* in Bangladesh was described in a previous report (27), which showed that there was a temporal variation in the dominance of different subserotypes. The emergence of some atypical serotypes of *S. flexneri* has also been reported (27). There are no reports of outbreaks caused by the serotypes of *S. dysenteriae* other than serotype 1 in Bangladesh or any other part of the world. In the present study, a detailed epidemiological study of the prevalence of different *S. dysenteriae* serotypes and their susceptibility to commonly used antibiotics among hospital patients was conducted over the last 4 years to evaluate the present status of shigellosis caused by *S. dysenteriae* serotypes in Bangladesh.

MATERIALS AND METHODS

Bacterial strains. From 1 January 1999 to 31 December 2002, 5,112 *Shigella* strains were isolated from patients attending the Dhaka treatment center operated by the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B), in Dhaka, Bangladesh. These strains were isolated and identified in the clinical microbiology laboratory by standard microbiological and biochemical methods (34). Among these, 358 isolates were collected and confirmed as *S. dysenteriae* by using polyvalent commercial antisera (Denka Seiken, Tokyo, Japan). The strains were grown in Trypticase soy broth (Difco Becton Dickinson and Company, Sparks, Md.) containing 0.3% yeast extract (TSBY) and stored at –70°C after addition of 15% glycerol and were used for further studies. *S. flexneri* 2a strain YSH6000 (22) and an *Escherichia coli* strain (ATCC 25922) that lacked the 140-MDa invasive plasmid and was sensitive to all antibiotics were used as positive and negative controls, respectively, in the Sereny test, the test for Congo red binding ability, and the PCR assay.

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FIG. 1. Prevalence of different species of *Shigella* in Dhaka, Bangladesh, from 1999 to 2002.

Serotyping. Serotyping of the 358 *S. dysenteriae* strains was confirmed by using the commercially available antiserum kit (Denka Seiken) specific for all type-factor antigens. The strains were subcultured on MacConkey agar (Difco Becton Dickinson and Company) plates, and after about 18 h of incubation, serological reactions were performed by the slide agglutination test as described previously (27). Strains which showed conflicting agglutination were suspended in normal saline, boiled for 2 h, and centrifuged at $1,200 \times g$ for 10 min to allow the pellet to settle, after which serotyping was done according to previously described procedures (27).

Antimicrobial susceptibility. The susceptibilities of the strains to the antimicrobial agents tested were determined by the disk diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (17), with commercial antimicrobial disks (Oxoid, Basingstoke, United Kingdom). The antibiotic disks used in this study were AMP (10 μ g), mecillinam (25 μ g), nalidixic acid (30 μ g), SXT (25 μ g), and ciprofloxacin (5 μ g). *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains for susceptibility studies.

Detection of *ipaH* by PCR. Detection of the *ipaH* gene was performed by amplification by PCR with primers according to previously described procedures (30). Primers (forward, 5'-TGGAAAACTCAGTGCCTCT-3'; reverse, 5'-CCAGTCCGTAAATTCATTCT-3') were synthesized using an Oligo 1000 DNA synthesizer (Beckman), available in our laboratory at ICDDR, B.

Keratoconjunctivitis assay (Sereny test). The Sereny test was performed by a procedure described elsewhere (14, 23). Briefly, an overnight culture of bacteria, suspended to a density of approximately 10^{10} viable cells in 20 μ l of phosphate-buffered saline, was dropped into the conjunctival sacs of guinea pigs. One eye served as the control. The guinea pigs were observed daily for 72 h, and their inflammatory responses were graded.

Determination of Congo red binding ability. TSBY with 1.5% agar and 0.01% Congo red (Sigma Chemical Co. Ltd.) was used to study the pigment binding abilities of the test strains as described previously (19, 22).

Isolation of plasmid DNA. Plasmid DNA was prepared by the alkaline lysis method of Kado and Liu (10), with some modifications as described previously (28). The molecular weight of the unknown plasmid DNA was determined by comparison with the mobilities of the plasmids of known molecular weights (7). The plasmids present in previously described strains *E. coli* PDK-9, R-1, RP₄, Sa, and V-517 (28) were used as molecular weight standards.

RESULTS

Serotyping and prevalence of serotypes. Of 5,112 *Shigella* isolates identified during the period 1999 to 2002, *S. flexneri* was the most predominant, with isolation rates of 57.4% ($n = 564$) in 1999, 53.6% ($n = 841$) in 2000, 65.7% ($n = 823$) in 2001, and 62.5% ($n = 821$) in 2002, followed by *S. boydii*, *S. dysenteriae*, and *S. sonnei*. The isolation rate of *S. boydii* was 18.3% ($n = 179$), 17.1% ($n = 268$), 14.8% ($n = 186$), and 19.6% ($n = 258$) in 1999, 2000, 2001, and 2002, respectively. *S. dysenteriae* accounted for 11.5% ($n = 113$) of the cases in 1999 followed by 16.1% ($n = 252$) in 2000, 10.7% ($n = 134$) in 2001, and 9.0% ($n = 118$) in 2002. *S. sonnei* was found to be the least prevalent during the period, with an isolation rate of 9.3% ($n = 91$) in 1999, 8.7% ($n = 136$) in 2000, 7.2% ($n = 90$) in 2001, and 6.5% ($n = 85$) (Fig. 1). Each year a number of strains were isolated which had characteristics typical of *Shigella* but could not be identified to the species level and were therefore designated as *Shigella*-like organisms (Fig. 1). Of the 358 *S. dys-*

FIG. 2. Distribution of different serotypes of *S. dysenteriae* from 1999 to 2002 in Dhaka, Bangladesh.

enteriae isolates, 122 were identified as serotype 1, of which 68 strains (76.4%) were isolated in the year 1999, but in the following 3 years the isolation of type 1 strains decreased drastically from 27.1% ($n = 46$) in 2000 to 8.8% ($n = 6$) in 2001 and 6.5% ($n = 2$) in 2002. On the other hand, isolation of *S. dysenteriae* types 2 to 13 increased gradually during the study period at a rate of 19% in 1999, 67% in 2000, 73.5% in 2001, and 87% in 2002. Among the other serotypes, type 2 increased from 15.7% ($n = 14$) in 1999 to 17.6% ($n = 30$) in 2000, 14.7% ($n = 10$) in 2001, and 41.9% ($n = 13$) in 2002. Type 4 increased from 1% ($n = 1$) in 1999 to 41.8% ($n = 71$) in 2000, 35.3% ($n = 24$) in 2001, and 19.4% ($n = 6$) in 2002 (Fig. 2). *S. dysenteriae* serotypes 5, 7, 8, and 13 were not isolated during this period. Interestingly, a higher prevalence of atypical strains was observed which were identified as *S. dysenteriae* by using the polyvalent antisera but which did not agglutinate with the type-specific antisera. A total of 28 (7.8%) atypical strains were identified over the 4-year period, of which 4.5% ($n = 4$) was in 1999, 5.9% ($n = 10$) was in 2000, 17.6% ($n = 12$) was in 2001, and 6.5% ($n = 2$) was in 2002.

Antimicrobial susceptibility test. The antibiotic susceptibility test showed that more than 98% of the *S. dysenteriae* type 1 strains were resistant to AMP, SXT, and nalidixic acid from 1999 to 2001. Although the isolation rate of type 1 strains was very low in 2002, all the strains were resistant to all the anti-

biotics tested. None of the *S. dysenteriae* 2 strains isolated between 1999 and 2000 were resistant to nalidixic acid, but 20 and 8% of strains of this serotype resistant to nalidixic acid were isolated in 2001 and 2002, respectively. Resistance to AMP and SXT within serotype 2 increased during the period of study. All the strains of serotype 4 were resistant to SXT, and resistance to nalidixic acid increased from 0% in 1999 to 33% in 2002. The isolation rate of AMP-resistant strains of type 4 was 15 and 5.5% in 2000 and 2001, respectively, while none of the strains were found to be resistant in 1999 and 2002. None of the strains of any serotype were resistant to ciprofloxacin or mecillinam.

Test for invasiveness. Plasmid analysis showed that all the strains of *S. dysenteriae* irrespective of serotype (including the atypical strains) harbored the 140-MDa invasive plasmid, and all the strains were positive for the *ipaH* gene when examined by PCR, showing an amplicon of 423 bp. Representative strains of each serotype were selected for the Sereny test, in which keratoconjunctivitis was observed within 24 h for all strains having the 140-MDa plasmid. These strains were also positive for Congo red binding ability, forming pigmented colonies.

Plasmid profile analysis. Analysis of plasmid DNA of *S. dysenteriae* revealed that all the strains irrespective of serotype contained a heterogeneous population of plasmids ranging be-

FIG. 3. Agarose gel electrophoresis of plasmid DNA showing representative patterns of the different serotypes of *S. dysenteriae*. Lanes: A, *E. coli* PDK-9 and R-1 (marker); B, *S. dysenteriae* 1; C, *S. dysenteriae* 2; D, *S. dysenteriae* 3; E, *S. dysenteriae* 4; F, *S. dysenteriae* 6; G, *S. dysenteriae* 9; H, *S. dysenteriae* 11; I, *S. dysenteriae* 12; J, *S. dysenteriae* 13; K, *S. dysenteriae* 14; L, *S. dysenteriae* 15; M, *E. coli* V-517. CHR indicates the banding position of the chromosomal DNA.

tween 140 and 0.8 MDa. The 140-MDa plasmid carrying the virulence marker was present in all the strains. Based on molecular weight, the pattern of different plasmids at the serotype level was also very diverse (Fig. 3). Depending on the number of plasmids, individual serotypes were grouped into different patterns. Serotype 1 was divided into 4 patterns, type 2 was divided into 10, type 3 was divided into 2, type 4 was divided into 10, type 6 was divided into 3, type 9 was divided into 2, type 11 was divided into 3, and type 12 was divided into 3. Although plasmid pattern was determined by the presence or absence of a single plasmid within a group of strains, a number of small plasmids were also found to be present universally in all the strains of a particular serotype (Table 1).

DISCUSSION

Of the 13 serotypes of *S. dysenteriae*, type 1 strains are of foremost importance because of this type's higher prevalence, epidemic potential, and severity of clinical manifestations. In zones where shigellosis is endemic, frequent epidemics of shigellosis have occurred previously at distinct intervals mainly due to *S. dysenteriae* type 1. At least three large epidemics caused by *S. dysenteriae* type 1 occurred between 1972 and 1994, caus-

ing high morbidity and mortality, particularly among children (1, 9, 18, 24). Apart from the dysenteric manifestation, type 1 infections are also associated with other complications like hemolytic-uremic syndrome, hemorrhagic colitis, sepsis, and purpura (11). Moreover, the low infectious dose (3) of the bacteria facilitates the rapid spread of the epidemic especially in a developing-country setting. According to previous studies, *S. dysenteriae* 1 was prevalent in rural and urban areas of Bangladesh during the period 1994 to 1996 (9). At the same time it was also noted that the isolation rate of other *S. dysenteriae* serotypes (types 2 to 13) was much lower than that of *S. dysenteriae* 1. However, in the present study, analysis of the serotyping results for the *S. dysenteriae* strains isolated between 1999 and 2002 has shown that the incidence of serotype 1 declined abruptly from 76.4 to 6.5%, while the isolation rate of other *S. dysenteriae* types (types 2 to 12) has increased from 19 to 87% (Fig. 2). From the data in this study, *S. dysenteriae* 2 and 4 were observed as predominant serotypes.

According to the history of this genus, *Shigella* spp. can easily become resistant to antibiotics (35). Over the years, *Shigella* spp. in many parts of the world have acquired resistance to commonly used antimicrobials, resulting in treatment failure and increased mortality (13). In the early 1970s, almost all *Shigella* isolates in Bangladesh were susceptible to sulfathiazole, streptomycin, tetracycline, and chloramphenicol. Other drugs for treating shigellosis, namely, AMP, SXT, and nalidixic acid, were introduced into Bangladesh in 1972, 1982, and 1985, respectively (8). In a previous study, it was observed that more than 90% of strains of *S. dysenteriae* 1 isolated during 1991 to 1996 from Matlab and Dhaka were resistant to AMP, SXT, and nalidixic acid (9). In the present study, 159 (44.4%) strains of *S. dysenteriae* were resistant to more than one antibiotic and 248 (69%) strains were resistant to SXT. It was noted that more than 98% strains of type 1 isolated from 1999 to 2001 were resistant to AMP, SXT, and nalidixic acid while all the strains in 2002 were resistant to these antibiotics. In the case of types 2 and 4, resistance to nalidixic acid has been increasing from 1999 to 2002. However, resistance to AMP in type 4 strains declined consecutively over the years while resistance in type 2 strains increased gradually. More importantly, all the 102 strains of type 4 were resistant to SXT during the study period. This important trend of antibiotic resistance indicates that the recently prevalent serotypes of *S. dysenteriae* are becoming resistant to newer drugs like nalidixic acid. Multiple antibiotic resistance and similar resistance patterns were found in more than one serotype of *S. dysenteriae*, which indicated that it was not a characteristic of any particular serotype. However, it is notable that none of the strains of *S. dysenteriae* resistant to ciprofloxacin or mecillinam were isolated in Bangladesh.

The essential step in the pathogenesis of shigellosis is invasion of human colonic mucosa (12). A number of studies (16, 20, 21, 33) have suggested that the genetic locus for the invasiveness of *S. flexneri* is located on the 140-MDa plasmid. The invasion plasmid antigen H (*ipaH*) gene, which is present as multiple copies in both the large plasmid and the chromosome (31), contributes to the invasive property of *Shigella* spp. All the strains in this study contained the *ipaH* gene, which was detected by PCR analysis. Further, additional experiments on virulence involving Congo red binding ability and the Sereny

TABLE 1. Presence of common plasmids (core plasmid) in different serotypes of *S. dysenteriae*

<i>S. dysenteriae</i> serotype (no. of isolates)	Core plasmids (MDa)
1 (n = 122)	140, 6, 2
2 (n = 67)	140, 4, 1.6
3 (n = 4)	140, 2.7, 1.8, 1.4
4 (n = 102)	140, 70–60, 4, 1.6
6 (n = 7)	140, 2.7, 1.8, 1.4
9 (n = 15)	140, 2.7, 1.8, 1.4, 1.2
11 (n = 6)	140, 4, 2.7, 1.8, 1.4, 1.0
12 (n = 7)	140, 2.7, 1.8, 1.4

test were carried out in order to confirm the plasmid-mediated invasiveness of representative strains.

Plasmid profile analysis is well documented as meaningful in epidemiological studies of enteric pathogens, particularly if there is a broad spectrum of plasmid patterns in the bacterial population (25). *Shigella* species usually harbor a heterogeneous population of plasmids ranging in number from 2 to as many as 10 (25). When used as a fingerprint for strains, the plasmid profile may aid in differentiating strains, identifying a source of infection, or evaluating the efficacy of control measures (26; L. W. Riley and M. L. Cohen, Letter, *Lancet* **i**:573, 1982). Analysis of the plasmid DNA of *S. dysenteriae* strains showed that all strains contained multiple plasmids ranging between 140 and 0.8 MDa. Plasmid patterns of different serotypes were found to be different. Within a single serotype multiple patterns were observed, indicating their clonal heterogeneity. In a previous study, numerous plasmid patterns were found in *S. dysenteriae* type 1 isolated from scattered as well as defined geographical origins (6). It was also shown elsewhere that the core plasmids (140, 6, and 2 MDa) were present in both the epidemic and nonepidemic isolates of *S. dysenteriae* type 1, which correlated with the results of our study. Other than type 1, no study has yet been performed on plasmid profiles of other *S. dysenteriae* serotypes, except for a study in Ethiopia which focused on plasmid-mediated antibiotic resistance (4, 5). This is the first report on the diversity of plasmid profiles of different serotypes of *S. dysenteriae*, which we believe will provide useful insights into the epidemiology of this organism in Bangladesh. In our study, we have found a number of core plasmids associated with the specificity of individual serotypes irrespective of drug resistance patterns. Within serotype 4, a plasmid of approximately 70 to 60 MDa in size was commonly found in all strains, showing their identity. From the previous studies, it has been demonstrated that the middle-range plasmids are more likely to carry the antibiotic resistance factor (28, 29). In our study, we have found that the prevalence of type 4 strains is increasing and also that these strains are acquiring resistance to multiple antibiotics including nalidixic acid. Since all the recent isolates of type 1 were resistant to multiple drugs, despite their decreasing prevalence, a resistance gene might be transferred from one serotype to another in mutual interactions within the host environment or outside the host.

Overall there appears to be a changing trend in the dominance of serotypes of *S. dysenteriae* during the period between 1999 and 2002 in Bangladesh, in which type 1 was replaced by type 4 and then type 2. The antibiotic resistance patterns and the plasmid profiles of types 1, 2, and 4 have little diversity, which underscores the necessity of extensive study of these strains at the molecular level to get detailed insight into the shifting trends of prevalence.

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REFERENCES

- Bennish, M. L., and B. J. Wojtyniak. 1991. Mortality due to shigellosis: community and hospital data. *Rev. Infect. Dis.* **13**:S245–S251.
- Bennish, M. L., M. A. Salam, M. A. Hossain, J. Myaux, E. H. Khan, J. Chakraborty, F. Henry, and C. Ronsmans. 1992. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983–1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Clin. Infect. Dis.* **14**:1055–1060.
- Dupont, L. L., M. M. Levine, R. B. Hornick, and S. B. Formal. 1989. Inoculum size in shigellosis and implications for expected mode of transmission. *J. Infect. Dis.* **159**:1126–1128.
- Gebre-Yohannes, A., and B. S. Drasar. 1988. Transferable or mobilisable antibiotic resistance in *Shigella dysenteriae* types 1, 2, 3, 4, 6 and 7 isolated in Ethiopia during 1974–85. *J. Med. Microbiol.* **27**:285–289.
- Gebre-Yohannes, A., and B. S. Drasar. 1990. Plasmid profiles of antibiotic-resistant *Shigella dysenteriae* types 2, 3, 6 and 7 isolated in Ethiopia during 1976–85. *Epidemiol. Infect.* **105**:65–72.
- Haider, K., A. K. Bradford, K. A. Talukder, and M. I. Huq. 1988. Plasmid analysis of *Shigella dysenteriae* type 1 isolates obtained from widely scattered geographical locations. *J. Clin. Microbiol.* **26**:2083–2086.
- Haider, K., M. I. Huq, K. A. Talukder, and Q. S. Ahmed. 1989. Electrophoretotyping of plasmid DNA of different serotypes of *Shigella flexneri* isolated in Bangladesh. *Epidemiol. Infect.* **102**:421–428.
- Hossain, M. A., M. J. Albert, and K. Z. Hasan. 1990. Epidemiology of shigellosis in Teknaf, a coastal area of Bangladesh; a ten years survey. *Epidemiol. Infect.* **105**:41–49.
- Hossain, M. A., M. Rahman, Q. S. Ahmed, M. A. Malek, R. B. Sack, and M. J. Albert. 1998. Increasing frequency of mecillinam-resistant *Shigella* isolates in urban Dhaka and rural Matlab, Bangladesh: a 6-year observation. *J. Antimicrob. Chemother.* **42**:99–102.
- Kado, C. L., and S. T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* **145**:1365–1373.
- Klee, S. R., B. D. Tzschaschel, I. Falt, A. Karnell, A. A. Lindberg, K. N. Timmis, and C. A. Guzman. 1997. Construction and characterization of live attenuated vaccine candidate against *Shigella dysenteriae* type 1. *Infect. Immun.* **65**:2112–2118.
- LaBrec, E. H., and H. Schneider. 1964. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. *J. Bacteriol.* **88**:1503–1518.
- Levine, M. M. 1986. Antimicrobial therapy for infectious diarrhoea. *Rev. Infect. Dis.* **8**:S207–S216.
- Mackel, D. C., L. F. Langley, and L. A. Venice. 1961. The use of guinea-pig conjunctivae as an experimental model for the study of virulence of *Shigella* organisms. *Am. J. Hyg.* **73**:219–223.
- Mamun, K. Z., S. Tabassum, M. A. Hossain, and P. Shears. 1997. Antimicrobial susceptibility of *Shigella* from a rural community in Bangladesh. *Ann. Trop. Med. Parasitol.* **91**:643–647.
- Maurelli, A. T., B. Blackmon, and R. Curtiss III. 1984. Loss of pigmentation in *Shigella flexneri* is correlated with loss of virulence and virulence-associated plasmid. *Infect. Immun.* **43**:397–401.
- National Committee for Clinical Laboratory Standards. 1990. Performance standards for antimicrobial disc susceptibility tests, 4th ed. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Rahaman, M. M., M. M. Khan, K. M. S. Aziz, M. S. Islam, and A. K. M. G. Kibria. 1975. An outbreak of dysentery caused by *Shigella dysenteriae* type 1 on a coral island in the Bay of Bengal. *J. Infect. Dis.* **132**:15–19.
- Sakai, T., C. Sasakawa, S. Makino, K. Kamata, and M. Yoshikawa. 1986. Molecular cloning of a genetic determinant for Congo red binding ability which is essential for the virulence of *Shigella flexneri*. *Infect. Immun.* **51**:476–482.
- Sansonetti, P. J., D. J. Kopecko, and S. B. Formal. 1982. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect. Immun.* **35**:852–860.
- Sansonetti, P. J., T. L. Hale, G. J. Dammin, C. Kapfer, H. Collins, and S. B. Formal. 1983. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. *Infect. Immun.* **39**:1392–1402.
- Sasakawa, C., K. Kamata, T. Sakai, S. Y. Murayama, S. Makino, and M. Yoshikawa. 1986. Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and Congo red binding activity in *Shigella flexneri*. *Infect. Immun.* **51**:470–475.
- Sereny, B. 1957. Experimental keratoconjunctivitis Shigellosa. *Acta Microbiol. Acad. Sci. Hung.* **2**:293–296.
- Shahid, N. S., M. M. Rahaman, K. Haider, H. Banu, and N. Rahman. 1985. Changing pattern of resistant Shiga bacillus (*Shigella dysenteriae* type 1) and *Shigella flexneri* in Bangladesh. *J. Infect. Dis.* **152**:1114–1119.
- Surdeanu, M., E. Pencu, M. Tonciu, I. Mihai, and L. Ciudin. 2000. Differentiation of *Shigella* strains by plasmid profile analysis, serotyping and phage typing. *Rom. Arch. Microbiol. Immunol.* **59**:103–117.

26. Tacket, C. O., and M. L. Cohen. 1983. Shigellosis in day care centers: use of plasmid analysis to assess control measures. *Pediatr. Infect. Dis. J.* **2**:127–130.
27. Talukder, K. A., D. K. Dutta, A. Safa, M. Ansaruzzaman, F. Hassan, K. Alam, K. M. N. Islam, N. I. A. Carlin, G. B. Nair, and D. A. Sack. 2001. Altering trends in the dominance of *Shigella flexneri* serotypes and emergence of serologically atypical *S. flexneri* strains in Dhaka, Bangladesh. *J. Clin. Microbiol.* **39**:3757–3759.
28. Talukder, K. A., M. A. Islam, D. K. Dutta, F. Hassan, A. Safa, G. B. Nair, and D. A. Sack. 2002. Phenotypic and genotypic characterization of serologically atypical strains of *Shigella flexneri* type 4 isolated in Dhaka, Bangladesh. *J. Clin. Microbiol.* **40**:2490–2497.
29. Talukder, K. A., Z. Islam, M. A. Islam, D. K. Dutta, A. Safa, M. Ansaruzzaman, A. S. G. Faruque, S. N. Shahed, G. B. Nair, and D. A. Sack. 2003. Phenotypic and genotypic characterization of provisional serotype *Shigella flexneri* 1c and clonal relationships with 1a and 1b strains isolated in Bangladesh. *J. Clin. Microbiol.* **41**:110–117.
30. Vargas, M., J. Gascon, M. T. Jimenez De Anta, and J. Vila. 1999. Prevalence of *Shigella* enterotoxin 1 and 2 among *Shigella* strains isolated from patients with traveler's diarrhea. *J. Clin. Microbiol.* **37**:3608–3611.
31. Venkatesan, M. M., J. M. Buysse, and D. J. Kopecko. 1989. Use of *Shigella flexneri* *ipaC* and *ipaH* gene sequences for the general identification of *Shigella* spp. and enteroinvasive *Escherichia coli*. *J. Clin. Microbiol.* **27**:2687–2691.
32. Victora, C. G., S. R. A. Huttly, S. C. Fuchs, F. C. Barros, M. Garenne, O. Leroy, O. Fontaine, J. P. Beau, V. Fauveau, H. R. Chowdhury, M. Yunus, J. Chakraborty, A. M. Sarder, S. K. Kapoor, M. K. Bhan, L. M. Nath, and J. C. Martines. 1993. International differences in clinical patterns of diarrhoeal deaths: a comparison of children from Brazil, Senegal, Bangladesh and India. *J. Diarrhoeal Dis. Res.* **11**:25–29.
33. Watanabe, H., and A. Nakamura. 1985. Large plasmids associated with virulence in *Shigella* species have a common function necessary for epithelial cell penetration. *Infect. Immun.* **48**:260–262.
34. World Health Organization. 1987. Programme for control of diarrhoeal disease, p. 9–20. In *Manual for laboratory investigation of acute enteric infections*. CDD/93.3, revision 1. World Health Organization, Geneva, Switzerland.
35. World Health Organization. 2001. Antimicrobial resistance in shigellosis, cholera and campylobacteriosis. WHO/CDS/CSR/DRS/2001.8. World Health Organization, Geneva, Switzerland.