Isolation of *Shigella dysenteriae* Type 1 and *S. flexneri* Strains from Surface Waters in Bangladesh: Comparative Molecular Analysis of Environmental *Shigella* Isolates versus Clinical Strains

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Bacillary dysentery caused by *Shigella* species is a public health problem in developing countries including Bangladesh. Although, shigellae-contaminated food and drinks are often the source of the epidemic's spread, the possible presence of the pathogen and transmission of it through environmental waters have not been adequately examined. We analyzed surface waters collected in Dhaka, Bangladesh, for the presence of shigellae by a combination of PCR assays followed by concentration and culturing of PCR-positive samples. Analysis of 128 water samples by PCR assays for *Shigella*-specific virulence genes including *ipaBCD*, *ipaH*, and *stx1* identified 14 (10.9%) samples which were positive for one or more of these virulence genes. Concentration of the PCR-positive samples by filtration followed by culturing identified live *Shigella* species in 11 of the 14 PCR-positive samples. Analysis of rRNA gene restriction patterns (ribotype) showed that the environmental isolates shared ribotypes with a collection of clinical isolates, but in contrast to the clinical isolates, 10 of the 11 environmental isolates were either negative or carried deletions in the plasmid-encoded invasion-associated genes *ipaB*, *ipaC*, and *ipaD*. However, all environmental *Shigella* isolates were positive for the chromosomal multicopy invasion-associated gene *ipaH* and all *Shigella dysenteriae* type 1 isolates were positive for the *stx1* gene in addition to *ipaH*. This study demonstrated the presence of *Shigella* in the aquatic environment and dispersion of different virulence genes among these isolates which appear to constitute an environmental reservoir of *Shigella*-specific virulence genes. Since critical virulence genes in *Shigella* are carried by plasmids or mobile genetic elements, the environmental gene pool may contribute to an optimum combination of genes, causing the emergence of virulent *Shigella* strains which is facilitated in particular by close contact of the population with surface waters in Bangladesh.

Shigellosis occurs as a disease endemic in Bangladesh, and at least three large epidemics caused by *Shigella dysenteriae* type 1 have occurred between 1972 and 1994, causing high morbidity and mortality, particularly in children (6, 16, 23). The most common underlying cause of death in fatal childhood shigellosis is severe colitis combined with septicemia and pneumonia (26). In Bangladesh, the predominant species of the genus *Shigella* are *S. flexneri* and *S. dysenteriae* type 1; infections due to *S. dysenteriae* 1 usually progress to the most severe stages of dysentery and life-threatening complications (6, 25). Factors affecting the emergence or decline of epidemic shigellosis are not clear, and shigellae are generally believed to have only a human or primate host. Shigellae-contaminated food and drinks are often the source of epidemic spread, and very little is known about its presence and possible spread through environmental waters. In many developing countries with inadequate sanitation, fecal contamination of environmental waters by enteric pathogens is very common. It is therefore important to understand whether *Shigella* can survive and persist in environmental waters in the absence of a primate host and the virulence characteristics of such environmental strains.

Identification of *Shigella* in environmental samples, where the number of organisms is likely to be small, is limited mainly by the lack of a suitable enrichment technique. Although DNA probes or PCR assays directed against the large invasion plasmid or genes encoding Shiga toxins (13, 20, 21, 29) can be used to detect the presence of the organism, isolation of the live bacteria is essential to characterize their pathogenic potential as well as their sensitivity to antimicrobial agents. Detailed analysis of a large number of water samples for the presence of *Shigella* by conventional culture methods is impractical, particularly because the number of non-lactose-fermenting colonies to be further analyzed may be too high (13). The present study was designed to isolate *Shigella* strains from the environment by a combination of PCR and culture methods and characterize them by appropriate biochemical and serological tests. Furthermore, molecular techniques were used to genetically characterize such environmental *Shigella* isolates and compare them with representative clinical isolates to understand the origin and pathogenic potential of the environmental *Shigella* isolates.

MATERIALS AND METHODS

Bacterial strains and water samples. Water samples for the study were collected once every 2 weeks during a period of 6 months between June and

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TABLE 1. PCR primers used in this study to detect
Shigella-specific virulence genes

Primer	Description	Sequence
EVT-1	Sense primer for *stx1*	5′-CAACACTGGATGATCTCAG
EVT-2	Antisense primer for *stx1*	5′-CCCCCTCAACTGCTAATA
IpaBCD-u	Upstream primer for *ipaBCD*	5′-GCTATAGCAGTGACATGG
IpaBCD-d	Downstream primer for *ipaBCD*	5′-ACGAGTTCGAAGCACTC
IpaH-u	Upstream primer for *ipaH*	5′-GCTGGAAAAACTCAGTGCCT
IpaH-d	Downstream primer for *ipaH*	5′-CCAGTCCGTAAATTCATTCT

November 2001 in different sampling sites in Dhaka. A total of 128 water samples, which included samples from different sites along two major rivers and a lake in Dhaka City, were analyzed in the study. All water samples were collected in sterile containers and transported to the laboratory for processing within 2 h of collection. Initially, all samples were immediately subjected to multiplex PCR assays as well as culture as described later in this paper. An environmental water sample mixed with different dilutions of a control, *S. dysenteriae* type 1 strain 33891 (15), was also included as a positive control to determine the detection limit of these assays. Non-lactose-fermenting colonies were picked from culture plates and were subjected to further analysis for the identification and isolation of possible *Shigella* colonies. After a review of these preliminary PCR and culture results, samples taken after August 2001 which were positive in an initial round of screening by PCR only were further analyzed by culture methods. Clinical *Shigella* strains used as controls in this study were either obtained from patients who reported to the treatment facilities of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) or obtained from the culture collection of ICDDR,B.

PCR assays. PCR reagents and kits were obtained from either the Perkin-Elmer Corporation (Norwalk, Conn.) or Bethesda Research Laboratories (Gaithersburg, Md.). Other enzymes and chemicals used in this assay were from Sigma Chemical Company (St. Louis, Mo.). Water samples (1.5 ml) were centrifuged initially for 2 min at low speed (1,000 rpm) in an Eppendorf microcentrifuge tube to precipitate debris. The supernatant was transferred into a fresh tube and centrifuged at high speed (14,000 rpm) to collect the suspended microorganisms. The pellet was resuspended in 100 μl of 50 mM Tris-HCl buffer (pH 7.5) containing 20% (wt/vol) sucrose, 50 mM EDTA, and 100 μg of lysozyme/ml. The suspension was incubated at 37°C for 30 min, and then 300 μl of a solution containing 50 mM NaCl, 1% (wt/vol) sodium dodecyl sulfate (SDS), and 200 μg of proteinase K/ml was added. The sample was incubated for an additional 60 min at 37°C, and then extraction was performed twice with an equal volume of phenol-chloroform (1:1, vol/vol) containing 2% isoamyl alcohol. The nucleic acids were precipitated by adding 2.5 volumes of absolute ethanol, harvested by centrifugation, and resuspended in 15 μl of TE buffer (10 mM Tris-Cl [pH 7.5], 1 mM EDTA). One to five microliters of the extracted nucleic acids was

used in a multiplex PCR assay for the genes encoding invasive plasmid antigens (*ipaB*, *ipaC*, and *ipaD*) and Shiga toxin (*stx1*), as well as a second PCR assay for *ipaH*. For analyzing *Shigella* isolates by PCR, colonies were suspended in sterile distilled water and disrupted by boiling for 3 min, and aliquots of the suspension were directly used for PCR assays. Dilutions of a known *Shigella* strain were included with each batch of PCR assays as a positive control to determine the detection limit of the assay. PCR primers used in this study were either previously reported (22) or synthesized based on published sequences of the relevant genes (27, 28). Descriptions and sequences of the PCR primers used in this study are given in Table 1. Thermal cycling parameters for the PCR assays consisted of denaturation at 94°C for 2 min, annealing of primers at 55°C for 2 min, and primer extension at 72°C for 3 min. Amplification was performed for 35 cycles, and the expected sizes of the amplicons were ascertained by electrophoresis in 1.5% agarose gels with an appropriate molecular size marker (1-kb DNA ladder; Gibco BRL). The identities of all PCR amplicons were further confirmed by Southern blot hybridization with specific probes.

Culturing of environmental samples. Environmental water samples were analyzed for the presence of *Shigella* by modification of a previously described method (1). Briefly, 50 ml of water was centrifuged at low speed (3,000 rpm in a Sorval SS34 rotor) for 5 min to precipitate debris, the supernatant was filtered through a 0.22-μm-pore-size filter (Millipore Corporation, Bedford, Mass.), and the filters were removed and incubated in nutrient broth for 4 h at 37°C with shaking. Aliquots of this suspension were streaked on MacConkey agar plates (Difco; Becton Dickinson and Company) and xylose lysine desoxycholate agar plates (Plasmatec Laboratory Products Ltd., Dorset, United Kingdom) and incubated overnight at 37°C. Suspected colonies were picked and subjected to biochemical and serological tests to identify *Shigella*. Depending on the number of non-lactose-fermenting colonies appearing on each plate, between 5 and 15 colonies were tested by biochemical methods. Eventually, culture-confirmed *Shigella* isolates were further analyzed by PCR and probes for the presence of virulence genes.

Probes and hybridization. The gene probe used in this study to detect the *ipaB*, *ipaC*, and *ipaD* genes was a 3.4-kb *Hin*dIII fragment of pWR1002 (28, 29). Probes for the *ipaH* and *stx1* genes were PCR-generated amplicons derived from a control, *S. dysenteriae* type 1 strain 33891 (15), with specific primers (Table 1). The rRNA gene probe was a 7.5-kb *Bam*HI fragment of the *Escherichia coli* rRNA clone pKK3535 described previously (4, 8). Southern blots were prepared using nylon filters (Hybond; Amersham International Plc., Ayelesbury, United Kingdom) and processed by standard methods (17). Probes were labeled by random priming (11) with a random primers DNA labeling kit (Gibco BRL) and [α-³²P]dCTP (3,000 Ci/mmol; Amersham).

Restriction fragment length polymorphisms (RFLP) in different genes were analyzed as described previously (8, 9). Briefly, total DNA was isolated from strains grown overnight, and approximately 5 μg of the whole-cell DNA was digested with the appropriate restriction enzymes (Bethesda Research Laboratories) by using 5 U of enzyme per μg of DNA. The digested DNAs were electrophoresed in 0.8% agarose gels, and DNA fragments from the gels were blotted onto nylon membranes (Hybond-N; Amersham). Southern blots were prehybridized for 2 h and then hybridized with freshly denatured radioactively
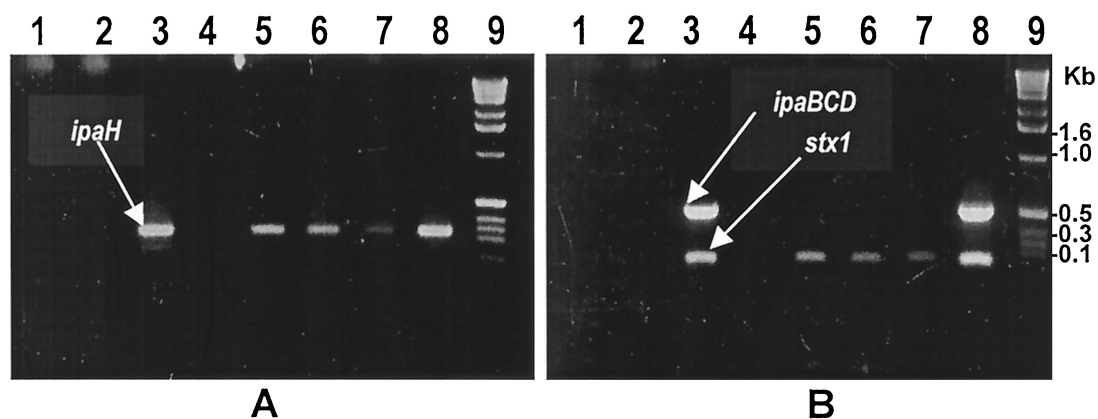

FIG. 1. PCR analysis of environmental surface water samples for *Shigella*-specific virulence genes. Products of a PCR assay for the *ipaH* gene (A) and a multiplex PCR assay for the *ipaBCD* and *stx1* genes (B) are shown. Lanes 1 through 7 contain PCR products of environmental water samples, and lane 8 contains a positive control strain. Lane 9 shows the 1-kb DNA ladder (Bethesda Research Laboratories) used as molecular size markers.

TABLE 2. Screening of surface water samples by PCR for *Shigella*-specific genes and by culture for *Shigella* spp.

Source	No. of samples	No. positive by PCR (%)	No. confirmed by culture (%)	*Shigella* species isolated	
				S. dysenteriae 1	*S. flexneri*
River	86	10 (11.6)	7 (8.1)	3	4
Lake	42	4 (9.5)	4 (9.5)	1	3

labeled probes at 68°C for 18 h in a solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution (1× Denhardt solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, and 0.02% bovine serum albumin), 0.5% SDS, and 100 μg of freshly denatured sheared salmon sperm DNA per ml. Hybridized blots were washed once in 2× SSC for 5 min at room temperature, two times in 2× SSC–0.1% SDS for 10 min at 65°C, and once in 0.2× SSC–0.1% SDS for 15 min at 65°C. Autoradiographs were developed from the hybridized filters with either Kodak (Rochester, N.Y.) X-Omat AR film or Fuji X-ray film at −70°C as described previously (9). RFLP in different genes and their flanking chromosomal sequences were studied from the bands that appeared in the corresponding autoradiographs. Banding profiles derived from different strains were compared based on the absence or presence of bands in different loci.

Antimicrobial susceptibility analysis. All isolates were tested for antimicrobial resistance by the method of Bauer et al. (2) on standard antibiotic disks (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) at the following antibiotic concentrations (micrograms/disk): ampicillin, 10; chloramphenicol, 30; streptomycin, 10; tetracycline, 30; trimethoprim-sulfamethoxazole, 1.25 and 23.75, respectively; kanamycin, 30; erythromycin, 15; gentamicin, 10; ciprofloxacin, 5; norfloxacin, 10; rifampin, 5; and nalidixic acid, 30.

RESULTS AND DISCUSSION

Detection of *Shigella* in environmental water. Understanding the ecology of *Shigella* had been limited mainly by the lack of suitable techniques to detect the presence of *Shigella* in environmental samples. In the present study, we used a combination of PCR and culture techniques to detect *Shigella* in surface waters. Of a total of 128 water samples tested, 14 (10.9%) were positive by PCR for one or more *Shigella*-specific virulence-associated genes (Fig. 1). Culturing of these 14 samples allowed isolation of live *Shigella* species from 11 samples (Table 2). Initially, we standardized the assays by simultaneously conducting PCR tests as well as culturing of environmental water samples and water samples mixed with different dilutions of control *S. dysenteriae* type 1 strain 33891. As expected, water samples which yielded live colonies on culture were invariably positive by PCR and none of the PCR-negative samples were positive by culturing. PCR assays were able to distinctly identify as few as 5 CFU of the bacterium/ml in water samples. Subsequently, for the environmental samples, we cultured only those samples which were found to be positive in an initial round of screening by PCR assays. However, in the present study, 3 of 14 PCR-positive samples did not yield any *Shigella* colonies on culture. These three samples were distinctly positive for the *ipaH* gene in repeated PCR assays. It is not clear whether the inability to culture *Shigella* from these three PCR-positive samples was due to the absence of live cells, since dead cells or their nucleic acids may also yield a positive PCR. An alternative explanation could be the possible presence of the *ipaH* gene in other species of environmental bacteria which escaped detection in our culture assay. Nevertheless, these results showed that PCR-based detection of *Shigella*-specific virulence genes can be used as an indicator for the possible presence of live *Shigella* strains in environmental waters. It is
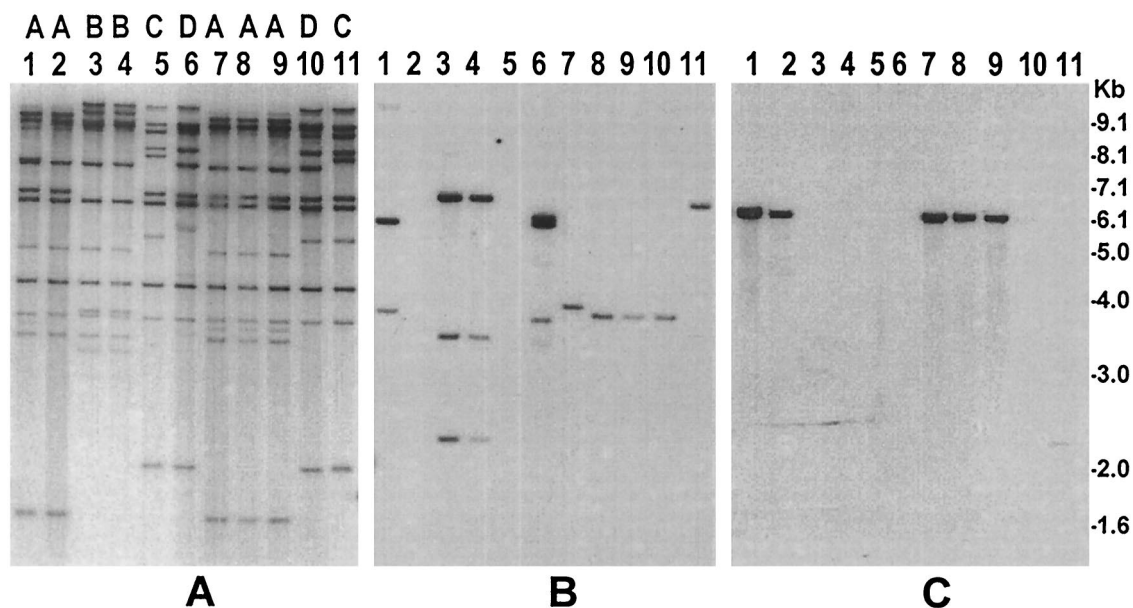

FIG. 2. Molecular analysis of *S. dysenteriae* type 1 and *S. flexneri* strains isolated from environmental waters in Bangladesh and comparison with representative clinical *Shigella* strains. Genomic DNA was digested with *Hind*III, and corresponding Southern blots were hybridized with the rRNA gene probe (A), *ipaBCD* probe (B), and Shiga toxin gene probe (C). Ribotype designations A through D, which correspond to the rRNA gene restriction patterns of different strains, are shown on top of the corresponding lanes. Lanes 1, clinical *S. dysenteriae* type 1 strain (SD-16); lanes 2 and 7 through 9, *S. dysenteriae* type 1 strains (SD-477, SD-461, SD462, and SD-469) isolated from environmental waters; lanes 3 and 4, clinical *S. dysenteriae* type 2 strains (AF-8724 and AF-979); lanes 5, 10, and 11, *S. flexneri* strains (SF-525, SF-455, SF-526) isolated from environmental waters; lanes 6, clinical *S. flexneri* strain (SF-12163). Numbers indicating molecular sizes of bands correspond to the 1-kb DNA ladder (Gibco BRL) used as molecular size markers.

worth mentioning that it is impractical to test a large number of samples by culture followed by a detailed analysis of non-lactose-fermenting colonies. Hence, an initial screening by PCR may reduce the number of samples to be cultured and practically enhance the detection of *Shigella* in environmental samples.

Molecular analysis of environmental *Shigella* strains. To understand the possible origin of the environmental *Shigella* strains and their potential to cause disease, we compared the environmental strains with virulent *Shigella* strains isolated from hospitalized patients. The most important components of virulence in *Shigella* infection include adherence, invasiveness, and toxigenicity (24, 25). The genes for these virulence factors reside on either plasmids or the bacterial chromosome or on lysogenic bacteriophages. *Shigella* strains have large plasmids that encode many virulence genes (5, 24), especially genes involved in invasion and intracellular movement, and these products are known as invasion plasmid-associated antigens. We examined the environmental strains for the presence of different virulence genes and compared them with the clinical strains by restriction analysis of conserved rRNA genes (ribotype). Ribotyping was used previously to analyze *Shigella* (8) and toxigenic *Vibrio cholerae* (9) strains and demonstrated that restriction patterns of conserved rRNA genes may be considered fairly stable markers for identifying different clones of enteric bacteria. In the present study, analysis of RFLP in the rRNA genes of the 11 environmental strains and 10 clinical strains showed a total of four different ribotype patterns, designated A through D (Fig. 2). While all *S. dysenteriae* type 1 isolates belonged to ribotype pattern A, *S. flexneri* strains produced two ribotypes, C and D, and the environmental strains shared ribotypes with the clinical strains, suggesting a clonal relationship. However, when analyzed for the presence of virulence genes by PCR, 10 out of the 11 environmental strains were found to be negative for the *ipaB*, *ipaC*, and *ipaD* genes, which encode factors involved in epithelial cell invasion by *Shigella* species (5). These strains were further analyzed by Southern blot hybridization using a DNA probe for the *ipaB*, *ipaC*, and *ipaD* genes. Strains which were negative in the PCR assay for *ipaBCD* produced a small-sized *Hin*dIII restriction fragment (~7.0 to 2.5 kb) of the *ipaB*, *ipaC*, and *ipaD* genes and flanking sequences compared to the clinical strains, which produced multiple bands between 7.5 and 2.2 kb (Fig. 2). This suggested that these environmental strains carried a deletion in the *ipaBCD* gene cluster.

Fresh contamination of surface water by the fecal material of dysentery patients is a possibility in developing countries where sanitation is poor, resulting in the presence of shigellae in surface water. Several previous studies have also detected shigellae in surface water or sewage samples (7, 18) and have indicated that *Shigella* strains can possibly be transported by surface waters (18). Even a recreational spray fountain was found to be contaminated with *Shigella* and was associated with the spread of the pathogen (12). However, in the present study, the demonstration of the absence or deletion of crucial virulence genes in environmental *Shigella* isolates suggested that the presence of these strains was not possibly due to fresh fecal contamination of surface water, but instead, these strains may have survived and persisted in the aquatic environment. It appears that since in the environment the invasion-associated
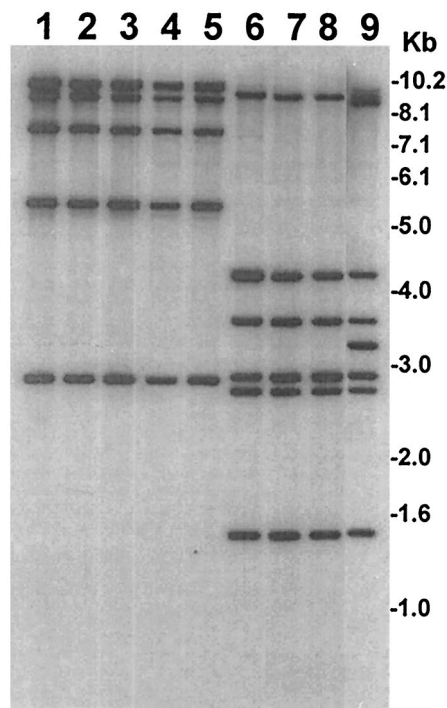

FIG. 3. Analysis of *S. dysenteriae* type 1 and *S. flexneri* strains isolated from environmental waters in Bangladesh for the presence of *ipaH* genes. Genomic DNAs were digested with *Hin*dIII, and corresponding Southern blots were hybridized with the *ipaH* probe. Identities of the environmental isolates (strain numbers) represented in different lanes are as follows. Lanes 1 and 9 represent clinical strains of *S. dysenteriae* 1 (SD-16) and *S. flexneri* (SF-12163), respectively, that were included in the study for comparison with environmental isolates. Lanes 2 through 5 represent *S. dysenteriae* 1 isolates (SD-461, SD-462, SD-469, and SD-479), and lanes 6 through 8 represent *S. flexneri* isolates (SF-455, SF-505, and SF-507). Numbers indicating molecular sizes of bands correspond to the 1-kb DNA ladder (Gibco BRL) used as molecular size markers.

genes did not have any known function, the bacteria apparently lost some of these plasmid-encoded genes. However, a chromosomally located multicopy virulence gene, *ipaH*, which is also known to have a role in producing invasive characteristics (5, 21), was found to be more stable and was present in all the environmental strains analyzed (Fig. 3). Therefore, PCR screening of environmental samples for the *ipaH* gene should provide a better indicator of the possible presence of shigellae than screening for the plasmid-carrying *ipaBCD* genes.

Taxonomically, the genus *Shigella* appears closely related to *E. coli*, which is a normal inhabitant of the human and animal intestine. It has been suggested that the four species of *Shigella* are so closely related to *E. coli* that all these bacteria could be considered members of a single species (14). They share greater than 90% homology by DNA-DNA reassociation analysis and display colinearity of their chromosomes such that gene transfer by conjugation and transduction and formation of recombinant between *Shigella* and *E. coli* occur with high efficiency (14, 19). The major virulence genes in *Shigella* are encoded by accessory genetic elements such as plasmids or by chromosomal genes of bacteriophage origin (20, 24, 27, 28). This is suggestive of the possibility that shigellae may lose or

TABLE 3. Characteristics of *Shigella* strains isolated from surface water samples and their comparison with clinical strains isolated from patients with shigellosis

Strain no.	Description	Source	Ribotype[a]	PCR analysis of virulence genes			Antibiotic resistance[b]
				ipaH	*ipaBCD*	*stx1*	
SD-16	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, Rf, S, SXT, C, E
SD-37	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, Rf, S, SXT, C, E
SD-39	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, Rf, S, SXT, C, E
SD-2078	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, Rf, S, SXT, C, E
SD-2146	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, S, SXT, C, E
SD-2499	*S. dysenteriae* type I	Patient	A	+	+	+	A, T, Na, S, SXT, C, E
AF-8724	*S. dysenteriae* type II	Patient	B	+	+	−	T, Rf, E
AF-979	*S. dysenteriae* type II	Patient	B	+	+	−	T, Rf, E
SF-5C592	*S. flexneri* 2a	Patient	C	+	+	−	S, Rf, E
SF-12163	*S. flexneri* 2b	Patient	D	+	+	−	T, S, Rf, C, E
SD-461	*S. dysenteriae* type I	Surface water	A	+	−	+	A, T, Na, Rf
SD-462	*S. dysenteriae* type I	Surface water	A	+	−	+	A, T, Na, Rf
SD-469	*S. dysenteriae* type I	Surface water	A	+	−	+	A, T, Na, Rf, S
SD-477	*S. dysenteriae* type I	Surface water	A	+	−	+	A, T, Na, Rf, S
SF-455	*S. flexneri* 3a	Surface water	D	+	−	−	Rf
SF-505	*S. flexneri* 3a	Surface water	D	+	−	−	Rf
SF-507	*S. flexneri* 3a	Surface water	D	+	−	−	S, Rf
SF-508	*S. flexneri* 3a	Surface water	D	+	−	−	Rf
SF-509	*S. flexneri* 3a	Surface water	D	+	−	−	Rf
SF-525	*S. flexneri* 2a	Surface water	C	+	−	−	Rf
SF-526	*S. flexneri* 2b	Surface water	C	+	+	−	A, T, S, Rf

[a] Ribotypes are based on the *Hin*dIII restriction patterns of the rRNA genes and their flanking chromosomal sequences.
[b] All strains were tested for resistance to ampicillin (A), tetracycline (T), chloramphenicol (C), erythromycin (E), gentamicin, ciprofloxacin, norfloxacin, nalidixic acid (Na), streptomycin (S), rifampin (Rf), and trimethoprim-sulfamethoxazole (SXT).

acquire clusters of genes encoding virulence as well as other accessory functions and thus the transit between virulent and possible nonvirulent forms which are indistinguishable from normal gut flora (3, 14, 19). Conversion from a nonpathogenic to pathogenic form has been demonstrated for another enteric pathogen, *V. cholerae*, which also produces a phage-encoded enterotoxin (10).

Epidemiological implications. *Shigella* epidemics spread through contaminated water and food, and transmission of the pathogen is believed to be facilitated by a very low infectious dose of *Shigella* (6, 23, 25). However, the mechanism associated with periodic outbreaks of shigellosis in areas of shigella endemicity is not clear. The presence of *Shigella* in surface waters, as demonstrated in the present study, may have public health implications. Although most of the environmental strains lacked one or more of the known virulence genes, the environmental strains shared a ribotype with the clinical strains (Table 3) and belonged to the two species which are associated with most cases of shigellosis in Bangladesh (6). Moreover, the serotypes of these environmental strains were the same as those of the clinical strains. The acquisition of clusters of virulence genes is recognized as an important element in the evolution of bacterial pathogens (14). Thus, plasmid- or bacteriophage-mediated horizontal transfer of genes may lead to the emergence of virulent *Shigella* strains from closely related avirulent precursors. The detection of *Shigella* strains in the surface water thus appears significant. It seems possible that these strains may participate in receiving or donating virulence genes, leading to the origination of virulent *Shigella* strains. Previously reported epidemics of shigellosis have always been preceded by the appearance of new antibiotic resistance among *Shigella* strains. The drug resistance patterns of the environmental and clinical *Shigella* strains are summarized in Table 3. It is important to note that most of the environmental strains were resistant to one or more antibiotics. These strains may thus also serve as reservoirs of drug resistance genes.

The environmental biology of *Shigella*, which is an almost exclusively human pathogen, is poorly understood, and animal or environmental reservoirs have not been identified. In the present study, we demonstrated the existence of *Shigella* in surface water in Bangladesh, where a large part of the rural and semiurban population consume surface water for daily needs. The present study also emphasizes the need to study the emergence of virulent *Shigella* strains from possible environmental nonvirulent progenitors by the acquisition of virulence genes and its relation to outbreaks of shigellosis.

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