Enterotoxigenic *Escherichia coli* Isolated from Surface Water in Urban and Rural Areas of Bangladesh

Enterotoxigenic *Escherichia coli* (ETEC) is very commonly a cause of acute watery diarrhea in infants and young children in Bangladesh (2). Although organisms of this type are known to be spread through food and water contaminated by feces, limited information is available on climatic factors or the presence of ETEC in surface waters (ponds, rivers, and lakes) that are used for drinking, washing, cooking, and bathing in developing countries (3). This is in contrast to the extensive efforts that have been put forth to characterize the presence of *Vibrio cholerae* in the aquatic environment (4).

We therefore carried out studies to isolate ETEC from surface water samples in sites close to field areas where cholera is endemic (4). From February to July 2001, water samples were collected every two weeks from sources around the city of Dhaka and from the Matlab rural field area in Bangladesh. The samples screened were from seven water sources (five ponds and two rivers [66 samples]) around the city of Dhaka and from four sources from Matlab (one river, one lake, and two ponds [41 samples]). Water from each site was tested for ETEC; on average, each site was sampled 10 times. To isolate ETEC, 150-ml aliquots of each water sample were filtered through a 0.22-μm Millipore membrane, the filter was then washed with 1 ml of MacConkey broth (Difco, Becton Dickinson, Sparks, Md.), 9-ml volumes of fresh MacConkey broth were inoculated with the resulting solution, and the inoculated broth was incubated for 4 h at 37°C. Subsequently, 100-μl volumes of these cultures were spread on MacConkey agar and grown overnight at 37°C. From each sample, 54 lactose-fermenting *E. coli* colonies were tested for heat-labile toxin (LT) and heat-stable toxin (ST) by enzyme-linked immunosorbent assay (2). All enterotoxin-positive *E. coli* colonies were tested for the expression of colonization factors (CFs), which are important virulence antigens of ETEC (over 21 have been described previously) (1). Thirteen CFs, including CFA/I, CS1-CS8, CS12, CS14, CS17, and CS21, for which specific monoclonal antibodies were available were used to test for ETEC by a dot blot immunoblot method (2). ETEC was isolated from 32% (34/107) of the samples; of those samples, 22 (65%) produced only ST, 3 (9%) produced only LT, and 9 (26%) produced both ST and LT (Table 1). Out of all the strains, 44% were positive for one of the following CFs or CF combinations: CFA/I, CS1+CS3, CS5+CS6, CS4+CS6, CS6, CS8, and CS14.

Antigenic serogrouping was carried out using commercial antisera (Denka Seiken, Japan). The predominant serogroups of the ETEC isolates were O6 (12%) and O25 (12%), followed by O78 (6%), O115 (6%), O167 (6%), and O126 (6%) (Table 1). H serogrouping was not carried out.

The ETEC strains were tested for their sensitivity to 15 antimicrobial drugs (5). Approximately 82% of the strains were resistant to one (21%) or more drugs (79%), which included erythromycin (74%), ampicillin (12%), cotrimoxazole (26.5%), doxycycline (15%), streptomycin (20.6%), tetracycline (15%), furazolidone (6%), and nalidixic acid (12%). For erythromycin, complete resistance was seen in 24% and intermediate resistance in 50% of the ETEC strains. None of the strains were resistant to chloramphenicol, ciprofloxacin, norfloxacin, neomycin, mecillinum, gentamicin, or ceftiraxone.

To fingerprint the ETEC strains, we used pulsed-field gel electrophoresis, with XbaI-digested chromosomal DNA and plasmid profiling (5). Neither the PFGE nor the plasmid profile showed any relationship with the CF type, toxin phenotype, or antibiotic resistance pattern.

In summary, our study shows that ETEC is highly prevalent in surface waters in both rural and urban areas in Bangladesh. Furthermore, these environmental strains of ETEC had characteristics similar to ETEC found in patients with diarrhea in Bangladesh (2). ETEC-contaminated surface water may be a frequent source of infection causing acute watery diarrhea and a possible reason for the endemicity of this pathogen in Bangladesh. Further studies are in progress to better understand the ecology and survival of ETEC in the environment.

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