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DETECTION OF VIRULENCE POTENTIAL OF DIARRHOEAGENIC *ESCHERICHIA COLI* ISOLATED FROM SURFACE WATER OF RIVERS SURROUNDING DHAKA CITY

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ABSTRACT

A total of 21 isolates were included randomly on the basis of their initial identification by the appearance of metallic sheen and from the biochemical traits to detect virulent markers of diarrhoeagenic *E. Coli* isolated from surface water. The antibiotic susceptibility pattern of the isolates were determined against six most commonly used antibiotics. About 52% of the isolates showed resistance to at least one of the antibiotics. About 38% of the isolates were resistant to more than one antibiotics. However, all the isolates were sensitive to gentamicin (CN). Among the 21 isolates, a total of 5 randomly selected isolates were examined for the detection of virulent genes (*elt, est* and *stx*) by PCR based method. Among the 5 tested isolates, 3 harbored the gene *stx* encoding Shiga toxin (Stx) and 2 to *elt* encoding heat-labile enterotoxin (LT). None of the isolates contained *est* encoding heat-stable enterotoxin (ST). Data revealed that as the virulent marker genes were detected, diarrhoeagenic *E. coli* strains present in the surface water would become a major public health concern.

Key words: Diarrhoeagenic, *Escherichia coli*, Virulence potential, Surface water

INTRODUCTION

Diarrhoea is one of the world's leading causes of morbidity and mortality, resulting in about two million deaths per year (Alikhani *et al.* 2006). In addition, diarrhoeal illnesses account for an estimated 12,600 deaths each day in children under 5 years of age in Asia, Africa, and Latin America, especially in developing countries (Alikhani *et al.* 2006, Ngyuyen *et al.* 2005). The causes of diarrhoea include a wide range of viruses, bacteria, and parasites. Among the bacterial pathogens, *Escherichia coli* plays an important role (Ngyuyen *et al.* 2005).

E. coli, a predominant member of the human intestinal flora (Yang *et al.* 2007), is the type species of the genus *Escherichia* that contains mostly motile Gram-negative bacilli, family Enterobacteriaceae. The organism typically colonizes the infant gastrointestinal tract within hours of life, and thereafter *E. coli* and the host derived mutual benefit for decades. However, there are several highly adapted *E. coli* clones that

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have acquired specific virulence factors, which increase their ability to adapt to new niches and allow them to cause diseases. Three general clinical syndromes can result from infection with pathogenic *E. coli* strains: enteric/diarrhoeal disease, urinary tract infection and sepsis/meningitis (Weintraub 2007). Some strains are rendered pathogenic by their ability to possess specific virulence factors, such as enterotoxin or adherent fimbriae, that are genetically encoded by plasmid DNA, chromosomal DNA, and bacteriophage DNA (Yang *et al*. 2007). As long as these bacteria do not acquire genetic elements encoding virulence factors, they remain benign commensals (Weintraub 2007).

Strains that cause diarrhoea, acute gastroenteritis or colitis in humans are referred to as diarrhoeagenic or enterovirulent *E. coli.* These strains have been characterized by the clinical symptoms of the diseases they present as and the virulence mechanisms they express (Sooka *et al*. 2004). Four outbreaks related to diarrhoeagenic *E. coli* were reported in the United States during the period 1961 to 1970, with 188 cases of illness. One outbreak with 1,000 cases was reported during 1971 to 1988 (Martins *et al*. 1992).

Among the *E. coli* causing intestinal diseases, there are six well-described categories: enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC). Other diarrhoeagenic *E. coli* pathotypes have been proposed, such as cell detaching *E. coli* (CDEC) (Huang *et al.* 2006). These categories have virulence attributes that help bacteria to cause diseases by different mechanisms (Weintraub 2007). Enterotoxigenic *E. coli* (ETEC) also referred to as Shiga toxin-producing *E. coli* (STEC) or verocytotoxin producing *E. coli* (VTEC) (Bell 2002). These pathotypes (or virotypes) are classified according to their specific virulence determinants. These virulence determinants give each pathotype the capacity to cause a clinical syndrome with distinctive epidemiologic and pathologic characteristics (Robins-Browne *et al.* 2004).

The virulence mechanisms of the diarrhoeagenic *E. coli* and the genetic mechanisms underlying them are complex, and our understanding of them is changing rapidly (Hunter 2003). Numerous virulence factors including adhesins, host cell surface-modifying factors, invasins, toxins, and secretion systems are involved in *E. coli* pathogenic mechanisms. Strains of the same pathotype are genetically similar and carry the same virulence determinants involved in the infection. These virulence genes are ideal targets for the determination of the pathogenic potential of any given *E. coli* isolate (Bekel *et al*. 2003).

Some assays for the detection of diarrhoeagenic *E. coli* are available, such as biochemical reactions, serotyping, phenotypic assays based on virulence characteristics, and molecular detection methods (Ngyuyen *et al.* 2005). Since several virulence factors

have been identified in diarrhoeagenic *E. coli* strains, modern molecular detection methods, including PCR, multiplex PCR and DNA hybridization, have been also developed (Yang *et al.* 2007, Bekel *et al.* 2003). These methods are widely used for the detection of genes for specific virulence factors which aid in pathogenesis. The presence of these genes is the evidence that renders the virulence and that can be used to categorize diarrhoeagenic *E. coli* strains (Yang *et al.* 2007).

Although water is reported as one of the routes of dissemination of pathogenic *E. coli* strains, research on their distribution in the aquatic environment has not been extensive and a few studies using molecular techniques for pathogenic *E. coli* detection in water samples have been reported (Martins *et al.* 1992). So, the present study was conducted to assess the genetic occurrence of virulence-producing factors in *E. coli* isolates from surface water by detecting the presence of major virulence genes by PCR. Antibiotic sensitivity patterns of the isolates were also determined. Overall, the results revealed: (i) all the isolates were identified as *E. coli* based on the typical cultural and biochemical properties; (ii) about 48% of the isolates were sensitive to all antibiotics used; (iii) all the isolates showed sensitivity to gentamicin (CN); (iv) around 38% of the isolates were resistant to more than one antibiotics and (v) three of the isolates contained the gene *stx* encoding Shiga toxin (Stx) and two isolates contained the gene *elt* encoding heat-labile toxin (LT).

MATERIALS AND METHODS

A total of 10 surface water samples were included in this study. All the samples were collected from rivers (Buriganga, Turag and Sitalakya) and sampling sites were purposively selected from Dhaka city and its surrounding areas based on the location of the river. Rivers from which samples were collected and the sampling site for each sample are listed in Table 1. All the samples were collected during of July, 2008 to August, 2008.

Samples were collected aseptically in sterile polyethylene terephthalate (PET) bottles. They were transported to the laboratory as quick as possible and were preserved in ambient temperature. In general, the samples were analyzed soon after arrival.

Fifty µl of each sample was inoculated onto McConkey agar medium by a sterile glass rod through spread plate method. Two or three loopfuls of the samples were also inoculated onto McConkey agar medium by sterile inoculating loop through streak plate method. The culture plates were then incubated overnight at 37^{o}C. After incubation, presumptive *E. coli* colonies were selected and further inoculated onto McConkey agar medium to obtain pure cultures for further analysis.

The pure colonies of isolates were again grown on eosin methylene blue (EMB) agar to examine them for the growth of colonies with metallic sheen. Microscopy further confirmed the growth as *E. coli* (Pelczar *et al.* 1993). Xylose lysine deoxycholate (XLD) agar medium was also used to observe the characteristic yellow colonies of *E. coli* on this medium.

Table 1. Sampling sites of the samples.

Sample No.	River	Sampling sites
1	Sitalakya	Hajiganj, Narayanganj
2	Turag	Tongibazar, Tongi, Gazipur
3	Turag	Abdullahpur, Dhaka
4	Buriganga	Imamganj Berri Badh, Dhaka
5	Buriganga	Mitford, Dhaka
6	Sitalakya	Godnail, Narayanganj
7	Buriganga	Backland Badh Ghat, Wiseghat, Dhaka
8	Buriganga	Babubazar Bridge Ghat, Dhaka
9	Turag	Aminbazar, Dhaka
10	Turag	Gabtoli, Dhaka

According to 'Microbiological Laboratory Manual' by Cappuccino, G., and N. Fhernan (1996), the biochemical tests (sugar and citrate utilization, motility indole urea and oxidase test) were performed to identify the bacteria of interest.

Susceptibility of environmental isolates against different antimicrobial agents was determined *in vitro* by employing the standardized agar disc-diffusion method, more commonly known as the 'Kirby-Bauer Method' (Barry and Thorsberry 1985). A suspension of the test organism was prepared by adjusting the turbidity of the broth in phosphate buffered saline by comparing with that of the McFarland standard solution. With the help of a sterile cotton swab, a uniform lawn of bacterial growth was prepared on Muller-Hinton agar plates (pH 7.0). Before streaking, the swab was passed against the wall of the tube containing the suspension to drain out the excess fluid. Commercially available antimicrobial discs (Oxoid, Hampshire, UK) were used for the test. Ampicillin (AMP), gentamicin (CN), trimethoprim-sulphamethoxazole (SXT), nalidixic acid (NA), ciprofloxacin (CIP) and ceptriaxon (CRO) were tested against the isolates.

Antibiotic discs were applied aseptically to the surface of the inoculated plates at appropriate spatial arrangement by means of a pair of sterile forcep. The plates were then inverted and incubated at 37^{o}C for 24 hours. Susceptibility to the specific antibiotic was interpreted by the presence of clear zone around the disc. The zone diameters for

individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring to an interpreting table (Barry and Thorsberry 1985).

A 3 ml of Luria-Bertani (LB) broth was first inoculated by a single colony from McConkey agar plate. After overnight incubation at 37°C with agitation (120 rpm), 1 ml sample was taken from the broth midium into an Eppendorf tube. The sample was then centrifuged at 13000 rpm for 10 minutes. After discarding supernatant, 1 ml of sample was taken in the Eppendorf tube containing pellet. The sample was centrifuged at 13000 rpm for 10 minutes and the supernatant was completely discarded. After that, the pellet was dissolved in 200 µl phosphate buffer saline (PBS) and the mixture was properly mixed. Then the mixture was centrifuged at 13000 rpm for 10 minutes. After discarding supernatant, the pellet was dissolved in 200 µl PBS and mixed. The sample was boiled for 10 minutes and cooled in ice for 30 minutes, consecutively. Then the sample was centrifuged at 13000 rpm for 10 minutes. The supernatant was transferred to a fresh Eppendorf tube and stored at -20^{o}C for further use as template DNA for PCR.

PCR was performed to detect *elt*, *est*, and *stx* genes, encoding LT, ST, and STx, respectively. The primer sets used are listed in Table 2. Among the 21 isolates, a total of 5 were examined. Initial denaturation of DNA was carried out at 94^{o}C for 5 minutes followed by PCR consisting of denaturation at 94^{o}C for 1 minute, annealing at 64°C for 1 minute and extension at 72^{o}C for 2 minutes for each cycle in a DNA RoboCycler gradient tempeture cycler (Stratagene, La Jolla, Calif.). After completion of 35 cycles, PCR products were resolved by 1% agarose gel electrophoresis at 50 mv for 1 hour and the gel was stained with ethidium bromide for 20 minutes. The gel was visualized through a UV transilluminator (Bio-Rad).

Table 2. List of primers.

Gene	Primer sequence
elt (250 bp)	5′-TCTCTATGTGCATACGGAGC-3′
	3′-CCATACTGATTGCCGCAAT-5′
est (300 bp)	5′-TTAATAGCACCCGGTACAAGCAGG-3′
	3′-CCTGACTCTTCAAAAGAGAAAATTAC-5′
stx (550 bp)	5′-TTTAGGATAGACTTCTCGAC-3′
	3′-CACATATAAATTATTTCGCTC-5′

RESULTS

After inoculation of all the 10 samples, typical colonies having the following characteristics on MacConkey agar, EMB agar and XLD agar media (Table 3) were considered as *E. coli* strains.

Table 3. Colony characteristics of *E. coli* on MacConkey agar, EMB agar and XLD agar media.

Medium	Colony Morphology
MacConkey agar	Bright pink or red, flat, convex, dry
EMB agar	Metallic sheen
XLD agar	Yellow, circular

Thus from 10 surface water samples, 21 *E. coli* isolates were selected for further analysis which included the morphological and biochemical identification, testing of antibiotic susceptibility and PCR-based analysis for the detection of *est*, *elt* and *stx* genes.

Table 4. Biochemical characteristics of presumptive *E. coli* isolates.

Strain ID	TSI	MIU				Urea	Citrate	Oxidase
	Slant	Butt	H_2S	Mot	Ind			
Control strain	A	AG	-	+	+	-	-	-
SWD-01	A	AG		+	+	-	-	-
SWD-02	A	AG	-	+	+	-	-	-
SWD-03	A	AG	-	+	+	-	-	-
SWD-04	A	A	-	+	+	-	-	-
SWD-05	A	AG	-	+	+	-	-	-
SWD-06	A	AG	-	+	+	-	-	-
SWD-07	A	AG	-	+	+	-	-	-
SWD-08	A	AG	-	+	+	-	-	-
SWD-09	A	A	-	+	+	-	-	-
SWD-10	A	AG	-	+	+	-	-	-
SWD-11	A	AG	-	+	+	-	-	-
SWD-12	A	A	-	+	-	-	-	-
SWD-13	A	AG	-	+	+	-	-	-
SWD-14	A	AG	-	+	+	-	-	-
SWD-15	A	A	-	+	-	-	-	-
SWD-16	A	AG	-	+	+	-	-	-
SWD-17	A	AG	-	+	+	-	-	-
SWD-18	A	AG	-	+	+	-	-	-
SWD-19	A	AG	-	+	+	-	-	-
SWD-20	A	AG	-	+	+	-	-	-
SWD-21	A	AG	-	+	+	-	-	-

TSI : Triple sugar iron test, MIU : Motility indole urea test, Mot : Motility, Ind : Indole, K : Alkaline reaction, A : Acidic reaction AG : Acidic reaction and gas, + : Positive, - : Negative.

All the isolates exhibited similar staining patterns. The isolates were found to be short Gram-negative rods that was characteristic of *E. coli*, and endosporeless. Detailed

biochemical study revealed that all the strains had the biochemical characteristics typical of *E. coli* although some showed some variation in biochemical results. All the isolates fermented glucose with acidic reactions and some produced gas in the tubes of TSI agar. All the strains exibited motility and showed citrate and urea negative reactions. All the strains showed indole positive reaction except two isolates that showed negative reactions (Table 4).

All the strains were tested for their antibiotic susceptibility against 6 commonly used antibiotics. All the isolates showed susceptibility to gentamicin (CN). The isolates showed variation in susceptibility pattern (Fig. 1, Tables 5 and 6) against the other antibiotics used in this study. The resistance rate (42.9%) among the isolates was higher against both ampicillin and nalidixic acid. Most of the isolates (85.7%) showed to be sensitive against the other 3 antibiotics. 38.1% of the isolates were found to be multi-drug resistant.

Table 5. Susceptibility pattern of *Escherichia coli* isolates against different antibiotic discs.

Strain ID	Antibiotic Susceptibility					
	AMP	CN	SXT	NA	CIP	CRO
SWD-01	R	S	R	R	S	R
SWD-02	S	S	S	S	S	S
SWD-03	S	S	S	S	S	S
SWD-04	S	S	S	S	S	S
SWD-05	S	S	S	R	S	S
SWD-06	R	S	R	S	S	S
SWD-07	S	S	S	S	S	S
SWD-08	R	S	R	R	R	R
SWD-09	R	S	S	R	R	R
SWD-10	R	S	S	R	R	S
SWD-11	S	S	S	R	S	S
SWD-12	R	S	S	R	S	S
SWD-13	R	S	S	R	S	S
SWD-14	R	S	S	R	S	S
SWD-15	S	S	S	S	S	S
SWD-16	S	S	S	S	S	S
SWD-17	S	S	S	S	S	S
SWD-18	S	S	S	S	S	S
SWD-19	S	S	S	S	S	S
SWD-20	S	S	S	S	S	S
SWD-21	R	S	S	S	S	S

AMP : Ampicillin, CN : Gentamicin, SXT : Trimethoprim-sulphamethoxazole, NA : Nalidixic acid, CIP : Ciprofloxacin, CRO : Ceptriaxon, S : Sensitive, R : Resistant.

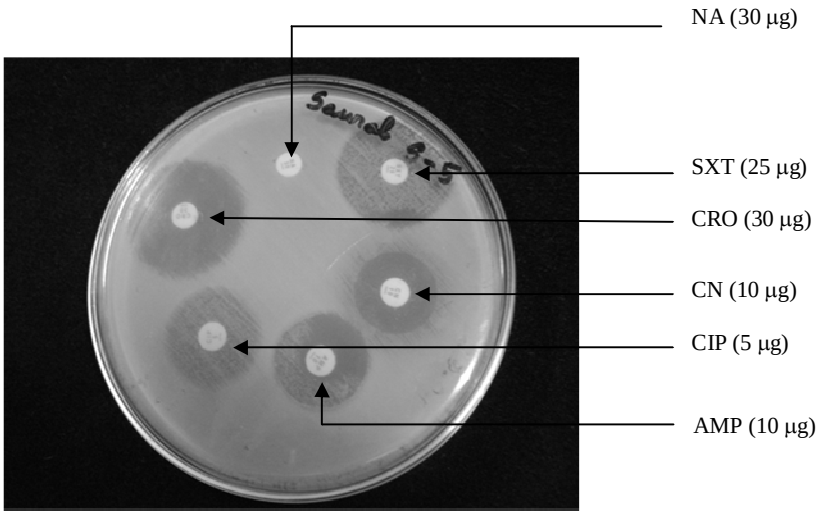

Fig. 1. Antibiotic susceptibility pattern of *E. coli* Isolate (SWD-05) by disc diffusion assay. The isolate was resistance to nalidixic acid and showed to be sensitive to other antibiotics.

Table 6. Frequency of antibiotic resistance/susceptibility of *E. coli* isolates.

Antibiotic	Sensitive (%)	Resistant (%)
Ampicillin	57.1	42.9
Gentamicin	100	0
Trimethoprim-Sulphamethoxazole	85.7	14.3
Nalidixic acid	57.1	42.9
Ciprofloxacin	85.7	14.3
Ceptriaxon	85.7	14.3

PCR assay was done for *in vitro* determination of the *stx*, *elt* and *est* genes encoding shiga-toxin (Stx), heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) to confirm the presence of diarrhoeagenic *E. coli* (especially EHEC and ETEC) strains in the surface water samples. A total of 5 randomly selected isolates were tested among the 21 isolates.

Among the 5 isolates, 3 showed bands for the *stx* gene and 2 showed bands for the *elt* gene (Fig. 2, Table 6). These results indicated the occurrences of diarrhoeagenic *E. coli* strains in the tested samples as the virulence marker genes were detected.

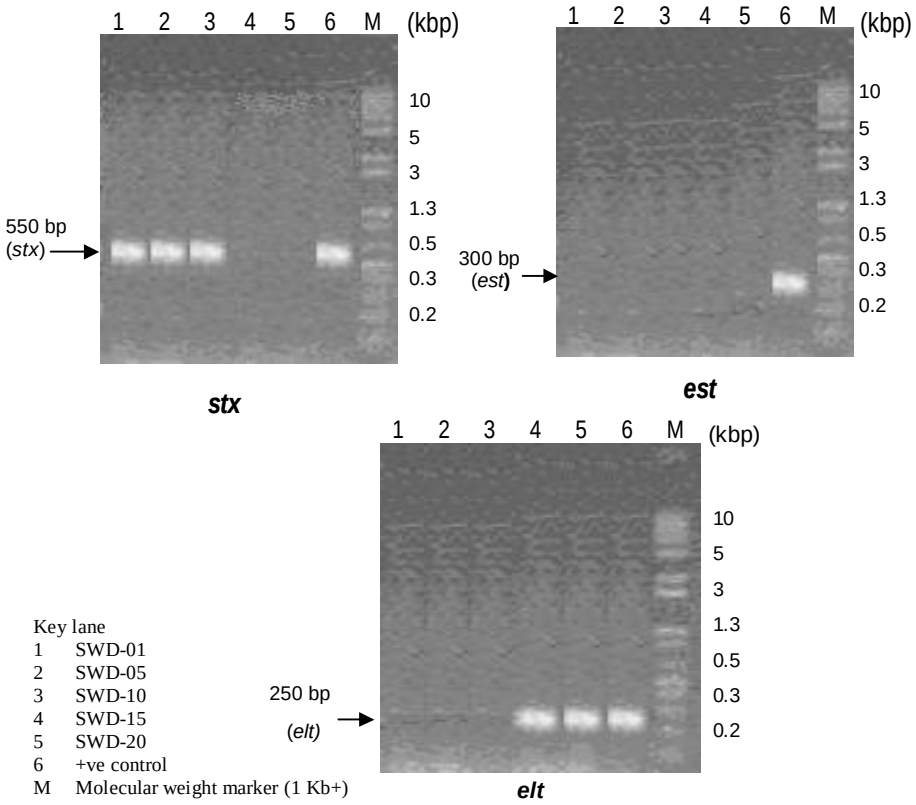

Fig. 2. One per cent agarose gel electrophoresis of PCR products of *stx*, *est* and *elt* genes of *E. coli* strains isolated from surface water. Among 5 isolates, 3 showed positive result for the *stx* gene and two contained the *elt* gene. No band was detected for the *est* gene.

Table 7. PCR result of virulence genes of selected *E. coli* isolates.

Strain ID	Lane No	*Stx*	*Est*	*Elt*
SWD-01	A	+	-	-
SWD-05	B	+	-	-
SWD-10	C	+	-	-
SWD-15	D	-	-	+
SWD-20	E	-	-	+

+ = Positive, – = Negative.

DISCUSSION

Diarrhoeal disease is a major problem throughout the world with attack rates ranging from 2 to 12 or more episodes per person per year, and is responsible for high morbidity

and mortality among children, especially in developing countries (Nguyen *et al*. 2005, Hien *et al.* 2007). In addition, diarrhoeal illnesses account for an estimated 12,600 deaths each day in children under 5 years of age in Asia, Africa, and Latin America. On a global scale, diarrhoeal illness due to *E. coli* is a major cause of morbidity and mortality, especially in children (Hunter 2003). Thousands of serotypes of *E. coli* species, in the *Escherichia* genus, within the family of *Enterobacteriaceae*, form the intestinal bacterial group described as gram negative, non-sporulating facultative anaerobic rod, usually motile by peritrichous flagella (Orskov and Orscov 1983). Pathogenic *E. coli* that do not belong to the normal microbiota, harbor virulence factors, such as adhesins, invasins, entero- and cyto-toxins encoded by extrachromosomal plasmids, chromosomal pathogenicity islands, or bacteriophage integrated virulence factors for defeating host defences in order to cause intestinal and extra-intestinal diseases and known as diarrhoeagenic *E. coli* (Kaper *et al.* 2004).

E. coli is found in aquatic ecosystems physically isolated from any source of fecal contamination. *E. coli* that co-exists in aquatic environment may influence genetic exchanges through lateral gene transfer (Alam *et al.* 2006). It is thought that increasing water temperatures could favor the growth of bacteria, including pathogenic *E. coli* that are adapted to higher temperatures (Vital *et al.* 2008). *E. coli* strains have acquired genetic determinants (virulence genes) rendering them pathogenic for both humans and animals. These virulence genes are ideal targets for the determination of the pathogenic potential of any given *E. coli* isolate. Numerous molecular methods have been used to detect and identify pathogenic *E. coli* strains, including DNA-DNA hybridization, PCR, and multiplex PCR (Bekal *et al.* 2003). The present study was designed to determine the presence of virulence markers of pathogenic *E. coli* from surface water in Bangladesh by PCR-based method. Recent studies showed that enterotoxigenic *E. coli* (ETEC) can be isolated relatively frequently from surface water samples in Bangladesh (Qadri *et al*. 2005, Aziz *et al.* 1986). Enteroinvasive *E. coli* (EIEC) has not been detected in Bangladesh. Clinical cases caused by enterohaemorrhagic *E. coli* (EHEC) in the Indian subcontinent remain unknown, although their occurrence in the aquatic ecosystem remains to be determined (Alam *et al*. 2006). Hence, the present study also had the aim to determine the occurrence of pathogenic *E. coli* strains in the aquatic environments of Bangladesh.

A total of 10 surface water samples were collected in time periods between July 2008 and August 2008 from 3 rivers (Buriganga, Turag, and Sitalakya) surrounding Dhaka city. A total of 21 isolates were considered in this study randomly based on their primary identification by production of metallic sheen colonies on EMB agar and biochemical behaviors. However, all the strains showed the typical biochemical characteristics of *E. coli* as compared to the control strain.

Antimicrobial resistance in human pathogens has become a major public health issue (Roe *et al.* 2003). Some recent studies report presence of multi-antimicrobial resistant *E. coli* isolates positive for virulence determinants for enterohaemorrhagic *E. coli* (EHEC) from surface waters. Some earlier studies have also shown that clinical and surface water isolates of *E. coli* exhibiting resistance to ciprofloxacin were multiple antimicrobial resistant. The resistance to nalidixic acid was significantly associated to cephalothin and tetracycline (Ram *et al.* 2007). However, the resistance pattern of *E. coli* to several antibiotics is not always uniform as it depends on the source of isolation. From the the present study we found that the isolates were sensitive to gentamicin. 10 of the isolates (47.7%) were sensitive to all the antibiotics. The other isolates (52.3%) showed variation in their susceptibility pattern to the antibiotics used except gentamicin. A total of 8 isolates (38.1%) were resistant to more than one antibiotics i.e. showed multi-drug resistance. This revealed that the incidence of naturally acquired multi-drug resistant strains is increasing which is really alarming and also indicated the possibility for the presence of diarrhoeagenic *E. coli* strains in the environment.

In the earlier studies, *E. coli* strains isolated from diarrhoeal patients produced Stx but no significant proof of etiological significance of EHEC in diarrhoeal diseases or the possible pathogenetic significance of Stx was available (Karmali 1989). Most DNA probes and PCR techniques for EHEC have been directed toward the detection of genes encoding Stx (Natro and Kaper 1998). Enterotoxigenic *Escherichia coli* (ETEC) has been reported to be a major cause of diarrhoea in humans throughout the world, especially in developing countries. ETEC strains express well-defined enterotoxins, heat-stable toxin (ST) and/or heat-labile toxin (LT). The bacteria first colonize and multiply in the small intestine and produce these toxins (Ansaruzzaman *et al.* 2007). The genes encoding LT (*elt* or *etx*) reside on plasmids that also may contain genes (*est*) encoding ST. Detection of ETEC has long relied on detection of the enterotoxins LT and/or ST (Natro and Kaper 1998).

Based on these lines of informations, the authors determined the occurrence of diarrhoeagenic *E. coli* in the surface water of Dhaka, by the PCR based method to detect the presence of virulence genes as *stx* encoding Stx, *elt* encoding LT and *est* encoding ST, the major virulence potentials of diarrhoeagenic *E. coli* (especially of EHEC and ETEC). Among the 21 isolates that was included in this study, a total of 5 randomly selected isolates (SWD-01, SWD-05, SWD-10, SWD-15, and SWD-20) were examined by the PCR based method. The gene (*stx*) encoding Stx was amplified in three (3) isolates among the five (5) isolates tested. Two isolated was shown to harbor the gene (*elt*) encoding heat-labile enterotoxin (LT). None of the tested isolates were positive for the *est* gene encoding heat stable enterotoxin of *E. coli.* These results were the indication of the presence of diarrhoeagenic *E. coli* in the tested samples as *stx* and *elt* were detected. The

aquatic environment of the regions from where the samples were collected serve as a reservoir for *E. coli* strains with the genetic potential to produce Stx and LT. The results of antibiogram showed that the *elt* containing *E. coli* strains (SWD-15 and SWD-20) were sensitive to all the antibiotic used. On the other hand, the *stx* containing *E. coli* strains (SWD-01, SWD-05 and SWD-10) showed antimicrobial resistance. They were commonly resistant to nalidixic acid and two of them (SWD-01 and SWD-10) showed resistance to other antibiotics. These results also supported the previous findings that indicated the correlation of the occurrence of EHEC with the multidrug resistance in aquatic environment.

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