

Pathogenic Escherichia Coli Isolated from Sewage Treatment Plant in Dhaka City

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ABSTRACT

Though the bacterium inhabited in lower intestinal tract often discharged into environment through faeces or wastewater effluent. The presence of *E. coli* in surface water is a sign of faecal contamination. Main focus of this study was to isolation and identification of pathogenic strains of *E. coli* in the pagla sewerage water treatment plant of Dhaka city, to understand the effectiveness of the sewerage treatment plant. Water samples were collected and total 24 number collected water samples 480 *E. coli* strains were isolated. Various cultural and biochemical tests were performed to isolate *E. coli*. An important aspect of this study was to examine the use of specific fluorescent dye 4-methyl-umbelliferone (MUG). 40 *E. coli* isolates were selected for MUG test among them 37 isolates were positive, which means 92.50% *E. coli* isolates were MUG test positive. Another important feature of this study was to examine *E. coli* isolates in CR-SMAC (Sorbitol- MacConkey agar containing cefixime and rhamnose) plate. CR-SMAC is the world recognized selective medium for *E. coli* 0157:H7. In SMAC, lactose has been replaced by sorbitol. *E. coli* 0157:H7 do not ferment sorbitol and produce colorless colony; on the other hand most other *E. coli* ferment sorbitol and thus produces pink colony. Among 480 *E. coli* isolates 80 *E. coli* isolates were isolated from raw water point, were selected and after screening was done by plate technique and after biochemical test 40 *E. coli* strains were selected for multiplex PCR. The results of multiplex PCR showed that, two pathogenic strains (376 bp and 322 bp) were present in the raw water point. Data presented that as virulent marker genes were absent at the final water point so the treatment plant works effectively.

Keywords: Sewage Treatment Plant; Pathogenic Strain; Surface Water; Escherichia Coli 0157:H7

Abbreviations: MUG: Methyl-Umbelliferone; CR-SMAC: Sorbitol- Macconkey Agar Containing Cefixime And Rhamnose; ETEC: Enterotoxigenic *E. coli*; WHO: World Health Organization; EPEC: Enteropathogenic *E. coli*; EIEC: Enteroinvasive *E. coli*; EA-ggEC: Enteroaggregative Escherichia coli; CDEC: Cell Detaching *E. coli*; EHEC: Enterohaemorrhagic Escherichia Coli; PCR: Polymerase Chain Reaction; PET: Polyethylene Terephthalate; PCR: Polymerase Chain Reaction; EML: Environmental Microbiology Laboratory; TC: Total Coliform; FC: Faecal Coliform; FS: Faecal Streptococci; MFC: Membrane Faecal Coliform; CFU: Colony Forming Unit; LT: Heat-Labile; HUS: Haemorrhagic Uremic Syndrome; UTI: Urinary Tract Infection

Introduction

E. coli is a rod shaped, Gram-negative bacterium in the Family Enterobacteriaceae is the commonly inhabits the lower intestinal tract of humans and animals as well as one of the significant pathogen (Lorenz, et al. [1-3]). The organism usually inhabits the infant gastrointestinal tract within hours of birth and afterward *E. coli* and the host descend mutual assistance (Nataro, et al. [4,5]). Though *E. coli* usually confined to the intestinal lumen but in case of immune suppressed patients can be infected by nonpathogenic strains of *E.coli*. Most *E. coli* strains live offensively in the human intestine as intestinal flora. However pathogenic strains can cause severe diarrheal diseases in healthy individuals. Pathogenic *E.coli* generally caused (Allocati, et al. [3,6]).

- i) Urinary tract infection
- ii) Meningitis and
- iii) Enteric or diarrheal disease

Diarrheal diseases are one of the major public health problems due to high rate of morbidity in infants in our country; however, it is the predominant reasons of mortality, ensuing in about two million deaths worldwide each year (Alikhani, et al. [7]). World Health Organization (WHO) estimated that 80% diseases are caused by inadequate sanitation, contaminated water or unavailability of safe water in our country. *E. coli* strains can survive on these sources executes direct relationship to the incidence of the disease. Highly adaptive *E.coli* clones have the capacity to cause an extensive range of diseases. Survival is related with maintenance of viability under unfavorable environment (Pickup [8]). When *E. coli* is released to the environment it encounters nutritional and physicochemical shocks or stresses affecting short duration of survival. (Savageau, et al. [9-11]). Recent research discovers that *E. coli* can survive in the environment, soil, water for almost a month's (Devane, et al. [12]). Not only *E.coli*, other species like *Klebsiella* spp, *Vivrio* spp and *Candida albicans* also able to survive in tropical waters and may multiply (Lopez- Torres, et al. [13,14]).

According to distinct epidemiological and clinical features five specific virulence serotypes identified which are Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteraggregative *Escherichia coli* (EAggEC) (Nguyen, et al. [15,3]). Other diarrhoeagenic *E. coli* pathotypes have been proposed, for example diffusely adherent *E. coli* (DAEC), Cell Detaching *E. coli* (CDEC) (Huang, et al. [16]). Different diagnostic methods are developed for identify *E. coli* such as culture-based enumeration technique and molecular methods remain the reliable techniques like Polymerase Chain Reaction (PCR). *E. coli* identification in water samples previously described (Martins, et al. [17]). So, the present study was carried out to sort out the genetic occurrence of virulence-producing

factors *E. coli* isolates from water sample by discovering the presence of virulence genes by PCR.

Identification of Virulence Prospective of *E. coli*

E. coli is a significant indicator of entero-pathogenic contamination and well-studied bacterium as standardized data exist. *E. coli* is an organism whose presence is thought to be related with pollution and presence of pathogens. But their abundance and genetic diversity in association yet to be discovered, so the focus of this study was lead with the aim of isolating, studying abundance of *E. coli* and their genetic diversity in sewage water treatment plant using classical microbiology and molecular biology technique. Approach was made to identify five major pathogenic strains of *E. coli* by multiplex PCR.

Materials and Method

Samples were collected from sewage water and sample sites are selected at four different point of pagla sewage treatment plant. Samples were collected aseptically in sterile polyethylene terephthalate (PET) bottles. They were conveyed to the laboratory as fast as possible and were preserved in appropriate temperature. Soon after arrival samples were analyzed. All the studies were carried out in the Environmental Microbiology Laboratory (EML) under Laboratory Sciences Div. Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC) and Enteraggregative *E. coli* (EAggEC) were used in this study as positive control which were collected from lab stock.

Reconstitution and Confirmation of the Strain

The strain was streaked on NA plate and incubated at 37°C for 24 Hours. Gram staining, 2/3 biochemical test according to the standard procedures reconfirmed the strain. The organism were then stocked and preserved in 30% glycerol broth and kept at -70°C for further study.

Processing of the Sample

Before making any dilution, each of the water samples was shaken vigorously. 100µl of water sample was added to 900µl of sterile normal saline to give a 10⁻¹ dilution. Then 100µl of the sample from 10⁻¹ dilution was transferred to 900µl of sterile normal saline to prepare a 10⁻² dilution. In this way, ten-fold serial dilution of water sample was made from 10⁻¹ to 10⁻⁵ by using sterile normal saline. Each dilution was thoroughly mixed using vortex mixture.

Short-Term Preservation

The pure culture was streaked on NA plates and grow overnight at 37°C. From the heavy growth area, cells were accumulated scratching with a sterile needle and stabbed in T1 N1 soft Agar in

a cryotube or eppendorf tube. Then the samples were stocked up at room temperature for short time (within 1 year).

Molecular Techniques Used for Identification of *E. coli*

Polymerase Chain Reaction (PCR): Polymerase Chain Reaction (PCR) is a *in vitro* method for synthesis of nucleic acid in which a particular segment of DNA can be specifically amplified. Primers hybridize with complementary strands of the target sequence and are oriented so that DNA synthesized by the polymerase proceeds across the region between the primers. Since the extension products themselves are also complementary and capable of binding primers, consecutive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycles. The result is an exponential accumulation of specific target DNA fragments approximately designated by 2^n where n is the number of cycles of amplification performed.

Enumeration of Total Coliform (TC), Faecal Coliform (FC), Faecal Streptococci (FS) from water sample: Total Coliforms are *E. coli*, *Klebsiella*, *Citrobacter* and *Enterobacter*. Coliforms are gram negative, rod shaped, non-spore forming bacteria capable of growth in the presence of bile salt or surface active agents with similar growth inhibiting properties, which are cytochrome α -oxidase negative and able to ferment lactose at either 35°C or 37°C with the production of acid, gas and aldehyde within 24 to 48 hours. Membrane faecal co-

liform (mFC) agar plate was used for the enumeration of coliforms. Then incubated at 37°C for 18 to 24 hours. Blue colonies were considered as total coliform (Rashid [18]). Finally, Colony Forming Unit (CFU) was calculated. For the enumeration of fecal coliform same procedure was followed that described for the enumeration of total coliform except the incubation temperature. In case of faecal coliform plates were incubated at 44.5°C for overnight and enumerated accordingly. The 0.1ml of samples was spreaded on MacConkey agar plate and incubated at 37°C for 24 hours. After 24 hours incubation pink or red color were enumerated as *E. coli*.

Species-Specific PCR of *E. coli*

For species specific detection of *E. coli* a recently developed primer set is used. The target gene was 60-Mda plasmid. PCR amplification of the target DNA was carried out in a thermal cycler (BIO RAD) using 0.5 ml PCR tubes with a reaction mixture volume of 25 μ l. Each of the reaction mixtures contained 1 μ l template DNA (lysate), 10 pmol of each primer, 1 μ l of 10 pmol deoxynucleoside triphosphates (dNTPs, Invitrogen, USA), 0.25 μ l of (5U/ μ l, Germany) of Taq DNA polymerase, 2.5 μ l of 10x PCR reaction buffer (Invitrogen, USA), 0.75 μ l of MgCl₂ (50mM, Invitrogen, USA) and nuclease free water to a final volume of 25 μ l. The reaction mixture was subjected to an amplification of 35 cycles, according to the following PCR process specification mentioned in the (Tables 1 & 2).

Table 1: Primers used for species-specific identification of *E. coli*.

Target gene	Primer sequence	Size (bp)	Annealing temperature
60-Mda plasmid	F5'-ACGATGTGGTTTATTCTGGA R3'-CTTCACGTCACCATACATAT	224	55°C

Table 2: PCR conditions applied for amplification with MFS1F and MFSIR primer sets.

Gene	Template denaturation	Denaturation	Annealing	Extension	Final
60-Mda plasmid	950C for 5 min	950C for 1 min	550C for 1 min	720C for 1 min	720C for 10 min

Agarose Gel Electrophoresis of PCR Product

Electrophoresis was carried out in a horizontal gel apparatus. The method followed for agarose gel electrophoresis was described by (Maniatis, et al. [19]). Electrophoresis was conducted in horizontal agarose gels containing 1.0 (Fisons). Agarose (1.0%) was dissolved in TBE (Tris-Borate EDTA) electrophoresis buffer (1x TBE, appendix) by boiling in a micro oven for 5 minutes. Then it was allowed to cool to 55 °C-60 °C and poured into a plastic chamber. Wells were prepared in the gel with combs containing either 8 or 16 teeth. Gel was allowed to solidify for half an hour at room temperature and placed in the electrophoresis tank PCR product (15 μ l) and tracking dye (3 μ l) were mixed with the help of a micropipette and loaded into the wells accordingly. The gel was covered

with sufficient amount of 1x TBE buffer before electrophoresis. The power pack supplied regular current. Electrophoresis was carried out at 55 volts until the dye front moved to the end of the gel.

Staining of PCR Product

The gel was stained after run by soaking the gel in 30 μ l of ethidium bromide in 150ml of TAE buffer, for half an hour. The gel was destained by washing with 100ml distilled water and DNA bands were viewed on an Ultraviolet Trans illuminator. Gels were photographed by using a Gel Documentation unit. A multiplex PCR for virulence gene of *E. coli*: A multiplex PCR was performed with 6 sets of primer of which 3 sets identify the presence of virulence factor coding genes present in the core region. Another one is cod-

ing for an outer membrane protein which might have some role in adhesion of in human gut. List of primers and their amplicon size is mentioned in the table. Chromosomal DNA extracted by the above mentioned procedure was used as template for doing PCR in a thermal cycler. Multiplex PCR was performed using 0.2 ml PCR tubes with a reaction mixture volume of 25 μ l. Each of the reaction mixture contained 1 μ l template DNA (lysate), 10 pmol of each primer, 1 μ l of 10 Mm Deoxynucleoside Triphosphates (dNTPs, Invitrogen,

USA) 0.25 μ l (5U/ μ l, Invitrogen, USA) of Taq DNA polymerase, 2.5 μ l of 10X PCR reaction buffer (Invitrogen, USA), 0.75 μ l of MgCl₂ (50mM, Invitrogen, USA) and nuclease free water to a final volume of 25 μ l. The thermal cycler was programmed as follows: initial denaturation a 95°C for 5 min, followed by 30 cycles consisting of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min and a final extension step of 72°C for 10 min (Table 3).

Table 3: Primer for pathogenic strain of *E. coli*.

Category	Primer	Target gene	Primer Sequence	Amplimr Size (bp)
EAEC	EA	pCVD	5'-CTGGCGAAAGACTGTATCAT-3' 5'-CAATGTATAGAAATCCGCTGTT-3'	630
EIEC	SHIG	ial	5'-CTGGTAGGTATGGTGAGG-3' 5'-CCCAGGCCAACAAAT'TAT'TTCC-3'	320
EHEC	MK1 MK2	SLT-I And SLT-II	TTTACGATAGACTTCTCGAC CACATATAAAT'TAT'TTCGCTC	227
EPEC	bfpA	bfpA	5'-TTCTTGGTGTGCTTGCCTGTCTTTT-3' 5'-TTTTGTTTGTTGTATCTTTTGTA-3'	367
ETEC	LT ST	eltB estA	5'-TCTCTATGTGCATACGGAGC-3' 5'-CCATACTGATTGCCGCAAT-3'	322
EHEC /EPEC	eae	eaeA	5'-CACACGAATAAACTGACTAAAATG-3' 5'-AAAAACGCTGACCCGCACCTAAAT-3'	376

Post-Amplification Detection

Agarose- gel-electrophoresis: The post- amplification detection of PCR is performed by agarose- gel- electrophoresis. The successful amplification of virulent gene is examined by resolving the PCR product in 1.2 % agarose gel. After migration for 4h at 60 volts, the gel bromide and. Photographed in an UV- transilluminator). During migration, a 100bp molecular weight marker is always run along with the test sample for measuring the size of DNA band (s) in PCR product. Following migration, DNA band of above mentioned amplicon size was considered as successful amplification of the specific gene.

Result

Abundance of *E. coli* in raw, inlet, middle and outlet water: The *E. coli* count was in the range of 4-5.32 log₁₀ cfu/ml in the 24 different studied raw water point while in the inlet water point it was log₁₀ cfu/ml ranged from 3.30-4.54 log₁₀ cfu/ml. The variation of *E. coli* count was found in the range of 1.54-3.31 log₁₀ cfu/ml in the middle water point and the range was 1.47-2.47 log₁₀ cfu/ml in the outlet water point. The highest count of *E. coli* was re-

corded 5.32 log₁₀ cfu/ml in the month of May in raw water and the lowest value was recorded in the month of December and the value was 1.47 log₁₀ cfu/ml in outlet water. (Tables 4 & 5). In this study, 24 number water samples were collected and from collected water samples 480 *E. coli* strains were isolated. An important aspect of this study was to examine the use of specific fluorescent dye 4-methyl-umbelliferone (MUG) that has been available recently for detection of *E. coli*. *E. coli* isolates possessing the specific enzyme β -glucuronidase produced fluorescence with MUG under long wave length (365nm) of UV light were selected and differentiated from other microorganisms. In this study, 40 *E. coli* isolates were selected for MUG test among them 37 isolates were positive, which means 92.50% *E. coli* isolates were MUG test positive. The other important aspect of this study was to examine *E. coli* isolates in CR-SMAC (Sorbitol- MacConkey agar containing cefixime and rhamnose) plate. CR-SMAC is the world recognized selective medium for *E. coli* O157:H7. In SMAC, lactose has been replaced by sorbitol. *E. coli* O157:H7 do not ferment sorbitol and produce colorless colony; on the other hand, most other *E. coli* ferment sorbitol and thus produces pink colony (Table 6).

Table 4: Result of the MUG test.

Sample	No. of sample tested (MUG test)	No. of positive isolates	No of sample tested(CR-SMAC test)	No of positive isolates
E.coli isolates from raw water point	20	18	20	17
E.coli isolates from outlet water point	20	19	20	13

Table 5: Percentage of positive isolates from MUG and CR-SMAC test.

Total no of sample tested	Number of positive isolates	Percentage of isolation
40(MUG test)	37	92.5%
40(CR-SMAC test)	30	75%

Table 6: Abundance of *E. coli*.

Different water point	N	Mean	Std.Deviatin	Std.Error	95% confidence interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Raw water	24	36240.63	41092.489	8387.969	18888.79	53592.46	10000	212000
Inlet water	24	18913.71	7246.988	1479.285	15853.57	21973.84	2000	35000
Middle water	24	445.54	479.763	97.931	242.96	648.13	35	2080
Outlet water	24	124.33	70.963	14.485	94.37	154.3	20	300
Total	96	13931.05	25449.983	2597.478	8774.41	19087.7	12055	212000

Result of Multiplex PCR

Determination of virulence genes by multiplex PCR: Template DNA from each strain was subjected to amplification by PCR to detect the presence of eaeA, bfpA and eltB, estA, bfpA, ial and pCVD genes using specific primers (Appendix). Samples that having the genes were given bands of expected size. At first 40 *E. coli* isolates from raw water point were subjected to a multiplex PCR targeting genes of *E. coli* core region namely eaeA, bfpA and eltB, estA. Sample no 26 was positive for eaeA gene. Sample 56 was positive for eltB and estA gene.

Detection of eaeA gene by multiplex PCR: Representative *E. coli* isolates were subjected to multiplex PCR for the detection of specific *E.coli* virulence genes. eaeA positive isolate was 26 number samples. Positive sample gave 376 bp bands for primer specific to

eaeA gene. The result has been shown in the (Figure 1). Detection of eltB and estA gene by multiplex PCR: Representative *E. coli* isolates were subjected to multiplex PCR for the detection of specific *E. coli* virulence genes. eltB and estA positive isolate was 56 number sample. Positive sample gave 322 bp bands for primer specific to eltB and estA gene. The result has been shown in the (Figure 2). Next 40 *E. coli* isolates from outlet water point were subjected to a multiplex PCR including 5 target genes of *E. coli* core region namely eaeA, bfpA and eltB, estA, bfpA, ial and pCVD. All samples were negative for eaeA, bfpA and eltB, estA, bfpA, ial and pCVD gene. The result has been shown in the (Figure 3). The prevalence of *E. coli* is the highest in raw water followed by inlet water. The prevalence is negligible in middle water and outlet water compared to raw and inlet water (Table 7).

Table 7: Anova F-test for the comparison of the prevalence of *E. coli* in different water points.

Abundance of <i>E. coli</i>	Sum of squares	df	Mean square	F	Sig.
Between groups	2.15E+10	3	7160227047	16.448	0
Within groups	4.01E+10	92	435336675.5		
Total	6.15E+10	95			

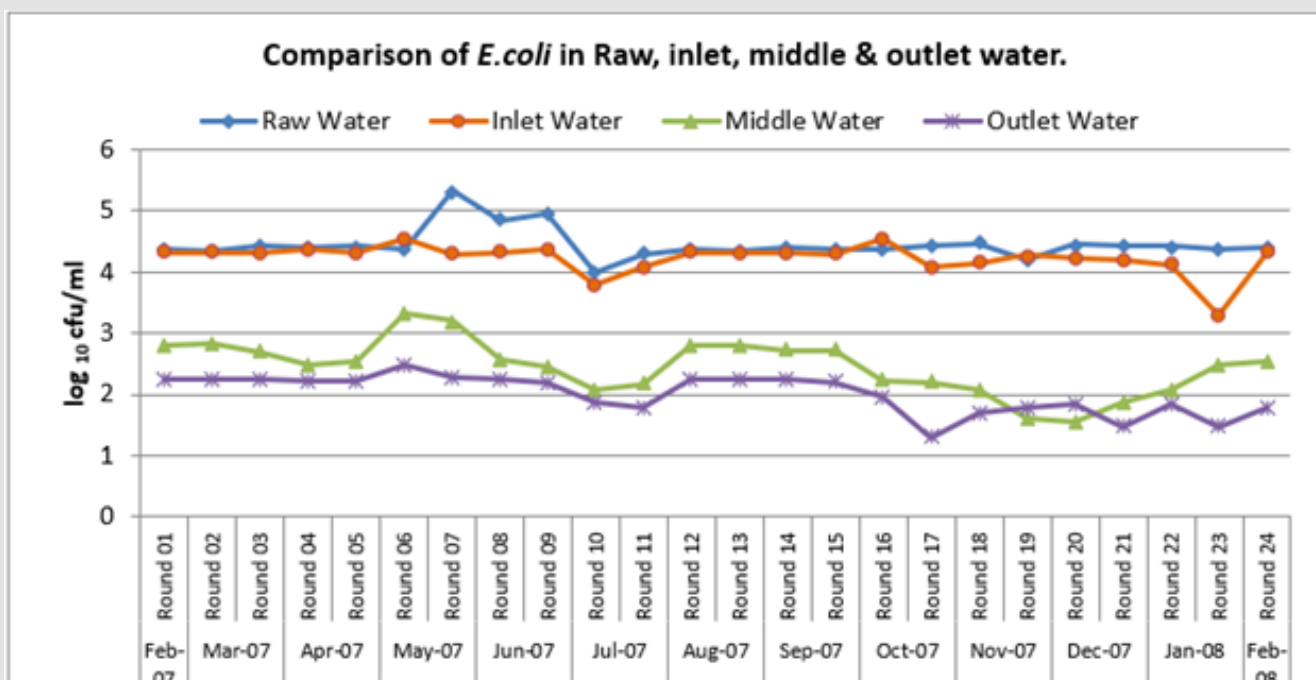
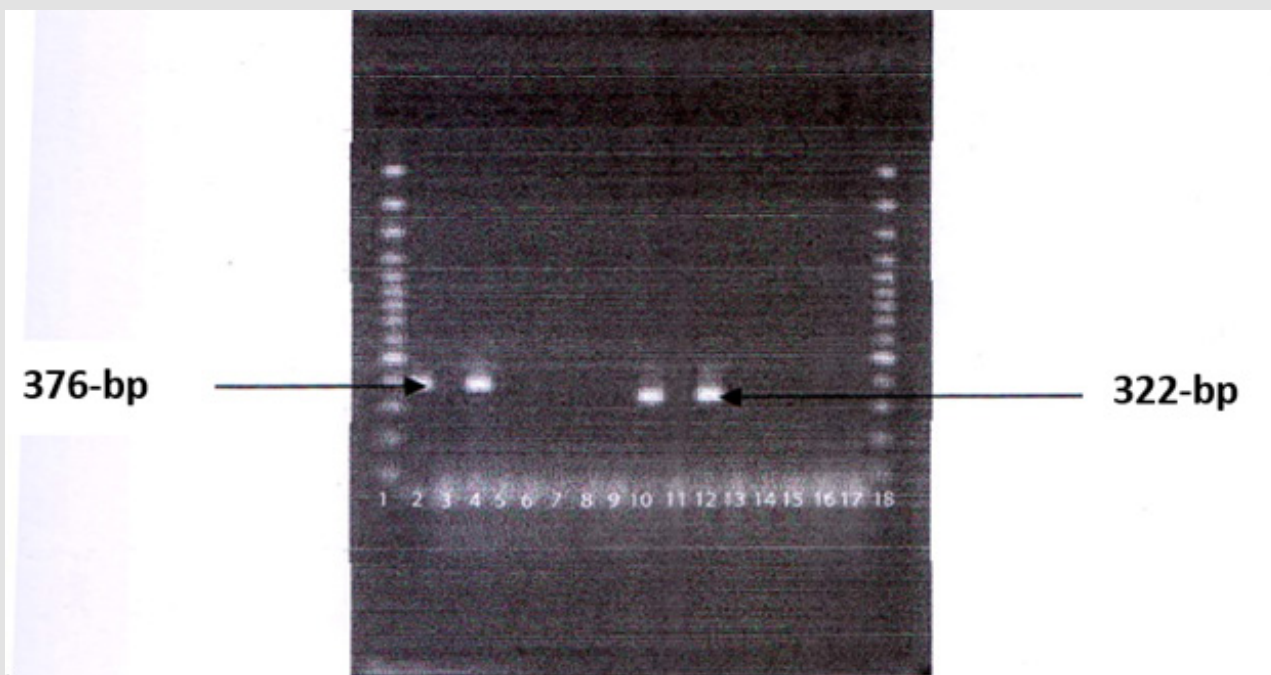


Figure 1.



Note: Lane 1 and 18- Molecular weight marker.

Lane2- Positive control (eaeA)

Lane4-Positive isolates (26)

Lane10- Positive isolates (56)

Lane 3 and 11- Negative control

Figure 2: Agarose gel Electrophoresis of multiplex PCR

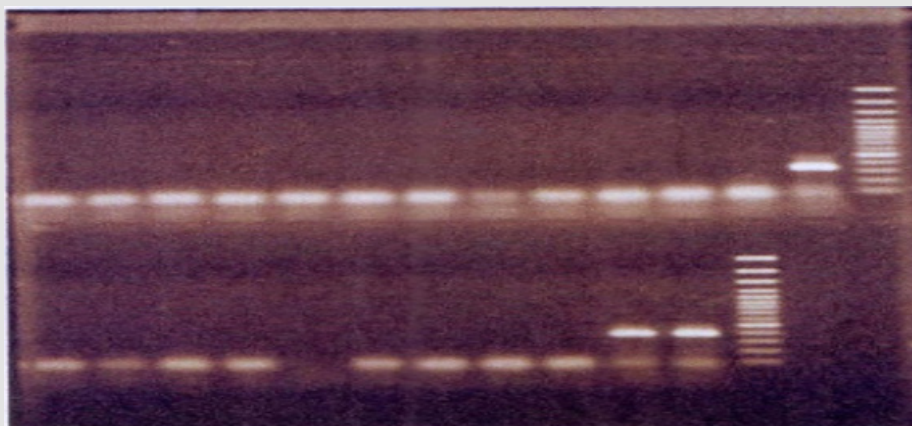
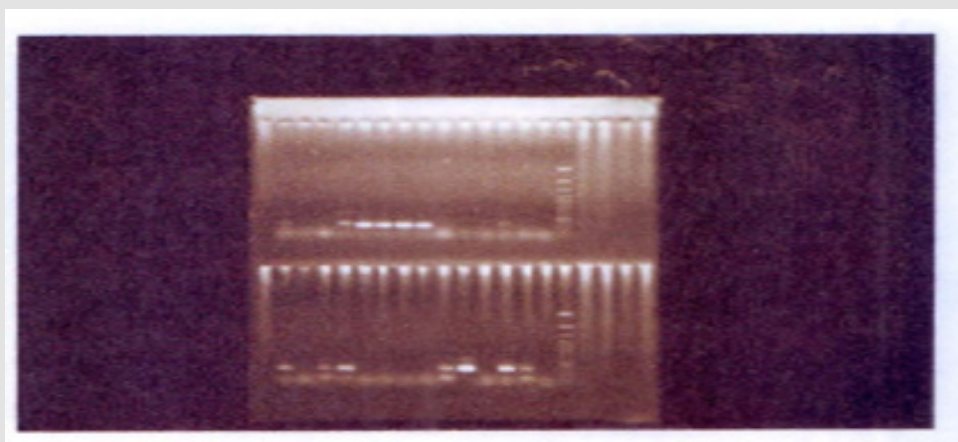


Figure 3: Agarose gel electrophoresis of PCR product. Lane 2, 24 and 25 contain +ve control, Lane 3, -ve control Lane 1 and 18- Molecular weight marker. Lane 3-23 shows the sample And 1, 26 shows molecular Weight marker.

Interpretation

P-value= 0.000 is less than 0.01. This indicates that Anova F-test rejects the null hypothesis of equal prevalence of *E. coli* in different type of water at 1% level of significance. So the prevalence of *E. coli* may not be equal in Raw, Inlet, Middle and Outlet water. Now it is

required to conduct a multiple comparison to test which pair of water type differ significantly in the prevalence of *E. coli*. Prevalence of *E. coli* significantly differs in Raw-Middle and Raw-Outlet type water at 1% significance level and all other pairs at 5% significance level except Middle-Outlet pair (Figure 4).



Note: Lane 5-8, 15, 18, 24, 26, shows +control, Lane 1-4 and 9-23 shows the sample Lane 14 and 29 shows molecular weight marker.
Figure 4: Agarose gel electrophoresis of PCR product.

Multiple Comparisons by Least Significant Different (LSD)

LSD: (Table 8).

Table 8: Dependent variable: Abundance of *E. coli*.

I)Types of water J) Types of water	Mean Difference (I-J)	Std.Error	Significance	95% confidence interval	
				Lower Bound	Upper Bound
Raw water Inlet Water	17326.92*	6023.127	0.005	5364.47	29289.37
Middle Water	35795.08*	6023.127	0	23832.63	47757.53

Outlet Water	36116.29*	6023.127	0	24153.84	48078.74
Inlet water Raw Water	-17326.92*	6023.127	0.005	-29289.37	-5364.47
Middle Water	18468.17*	6023.127	0.003	6505.72	30430.62
Outlet Water	18789.38*	6023.127	0.002	6826.93	30751.82
Middle water Raw Water	-35795.08*	6023.127	0	-47757.53	-23832.63
Inlet Water	-18468.17*	6023.127	0.003	-30430.62	-6505.72
Outlet Water	321.21	6023.127	0.958	-11641.24	12283.66
Outlet water Raw Water	-36116.29*	6023.127	0	-48078.74	-24153.84
Inlet Water	-18789.38*	6023.127	0.002	-30751.82	-6826.93
Middle Water	-321.21	6023.127	0.958	-12283.66	11641.24

Discussion

Diarrhoeal disease is a foremost cause throughout the world and is accountable for high morbidity and mortality in Asia, Africa and Latin America (Nguyen, et al. [20,21]). especially in children (Hunter [22]). Due to antimicrobial resistance of bacteria *E. coli* become a problem worldwide (Bayu Dharma S [23]). Among thousands of serotypes of *E. coli* species pathogenic *E. coli* harbor virulence factors, such as adhesins, invasins, entero- and cyto-toxins encoded by extra chromosomal plasmids, chromosomal pathogenicity islands, or bacteriophage united virulence factors for conquering host defences in order to cause intestinal and extra-intestinal diseases and known as diarrhoeagenic *E. coli* (Kaper, et al. [24]).

Multiplex Polymerase Chain Reaction (PCR) for Pathogenic Strain of *E. coli*

The results of multiplex PCR showed that, one isolate had band around 376 bp and another band was around 322 bp. Another study carried by (MUNSHI, et al. [25]). isolated diarrhoeagenic *E. coli* from surface water, among 21 isolates 5 isolates were randomly selected for PCR test and 3 contained the shiga toxin (stx) gene while 2 to elt encoding heat-labile enterotoxin (LT), which indicate virulent gene encoding *E. coli* present in surface water. The only genes detected were eae and stx2, which were carried by 37.69% (n_248) of the isolates. Only eae was harbored by 26.74% (n_176) of the isolates, representing potential a typical EPEC strains, while only stx2 was detected in 10.33% (n_68) of the isolates, indicating potential STEC strains (Chandran, et al. [14]).

Enterotoxigenic *E. coli* (ETEC) Isolates

Enterotoxigenic *E. coli* (ETEC) is a gram-negative diarrheal pathogen that infects almost 200 million people worldwide. (Gupta SK, et al. [26]). ETEC cause deaths almost 100,000 yearly and depend on a trio of ETEC virulence factors to cause disease (Hosangadi, et al. [27,11]). The prototypical ETEC strain H10407 contains

heat-labile (LT) and heat-stable (ST) enterotoxins along with the colonization factor antigen I (CFA/I) adhesin (Evans, et al. [28,29]). dwelt on diarrheal epidemic caused by waterborne ETEC. From Bangladesh, one ETEC out of 27 *E. coli*, was detected in pond water (Apha, et al. [30,19]). observed that out of 144 *E. coli* isolates 10 were identified as toxigenic. In this study the virulent gene of ETEC were eltB and estA. And the isolated ETEC strain was collected in the summer season. In previous studies on the seasonality of ETEC prevalence, maximum isolation was in the warm and humid seasons and minimum in the winter months (Albert, et al. [8]).

Enterohemorrhagic and Enteropathogenic *E. coli* Isolates

In the present study, also identified another pathogenic strain of *E. coli*, which contained eaeA gene and gave band around 322 bp. This virulent gene can be present both in EHEC and ETEC strain. EHEC cause Haemorrhagic Uremic Syndrom (HUS) and EPEC infection causes urinary tract infection (UTI) which is primarily a disease of infants younger than 2 years (Nataro, et al. [4,5]). According to Clinical reports published by World Health Organization (WHO), a number of person infected with HUS die within 24 hours of symptoms displayed. Karmali, et al. [31]). reported the association of sporadic cases of hemolytic uremic syndrome with faecal cytotoxin and cytotoxin producing *E. coli* in stool. Among 40 *E. coli* isolates, one was eaeA gene, so the probability of getting this pathogenic strain was 2.5% (Pitcher, et al. [32]).

Conclusion

Pathogenic *E. coli* 0157:H7 is a deadly pathogen that is widely present in our country. The most common 0157: H7 outbreaks includes multistate outbreaks at 2006 and in Japan 1996 (Hadjilouka, et al. [29]). The higher recovery of *E. coli* indicates that this pathogen can survive outside their natural habitat for a long time. The biochemical properties of all strains of *E. coli* were not similar because of the genetic variation of this pathogen. The result of the MUG and CR-SMAC test indicates that all of their colony character-

istics were not same also. The result of multiplex PCR indicates that pathogenic *E. coli* strain was also present. The virulent gene was *eaeA*, *eltB* and *estA* which represent the high incidence of diarrheal disease due to pathogenic *E. coli*. [Jalliffier-Verne, et al. [33]].

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