ARE THE LAKES AND RIVERS IN DHAKA CITY SAFE FOR RECREATIONAL WATER ACTIVITIES?

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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Declaration

I hereby declare that the thesis project titled **"Are the lakes and rivers in Dhaka City safe for Recreational water activities?"** has been written and submitted by me Urmi Nishat Nini and has been carried out under the supervision of Mahbubul Hasan Siddiqee, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Abstract:

Unplanned urbanization can lead to contamination of the surface waters with industrial and fecal pollutants. Fecal organisms can cause a number of diseases to humans in case of skin contact or accidental ingestion. The objective of this study was to determine the microbiological standards of Hatirjheel Lake and Buriganga River which are the two potential spots for recreational water activities. A total of 40 samples were tested; 30 from Hatrijheel and 10 from Buriganga. The samples were collected in an asceptic way from different points of Lake and River within duration of three months. The samples were processed and analyzed for quantification of fecal and total coliform. While at the same time, the samples were also processed for detection of *Shigella* spp. And *E.coli*O157:H7 after primary and secondary enrichment steps. Virulence genes *eaeA* and *IpaH* were checked for *E.coli*O157:H7 and *Shigella* spp. respectively. While 100% of the samples from both water-bodies were positive for coliforms (maximum count of $9x10^4$cfu./mL) whereas 43.3% and 80% of the samples were positive for fecal coliforms from Hatirjheel (with a maximum count of $3.2x\ 10^3$ cfu/mL) and Burigangal (with a maximum count of $6.2\ x10^3$ cfu/mL) respectively. The study reveals that both Hatirjheel Lake and Buriganga River can be endemically contaminated with fecal bacteria. Hence, strong precautions should be taken in case of direct contact with these waters while strong measures need to be taken to control pollution of these water-bodies by fecal organisms.

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Chapter 1

INTRODUCTION

1.0 Introduction

Water is an important part of human health and it is essential to have at least a minimum level of water in daily basis for survival. Microbiological level of water is needed to be maintained to avoid water disease outbreaks. Poor microbiological quality leads to disease outbreaks. Water borne diseases puts excessive burden on the population and health services of the country. (World Health Organization 1995). One of the major causes of death is diarrhea, which is mainly water based. 15% of 8.8 million death worldwide among children are estimated to be caused by diarrhea in 2008(Quadri et al. 2013). Other major diseases caused by waterborne diseases are typhoid, cholera, bloody diarrhea etc. Bangladesh is a developing country where acute dehydrating diarrhoea and cholera is a common disease and seen to break out about two times in a year(Chowdhury et al. 2011). The recent urbanization in Dhaka city is also responsible for diarrheal diseases as the water from different sources get mixed up with water of daily use. The poor urbanization causes the increase of waterborne diseases in the city.

Bacteria are introduced in water from many sources. Not all of them are harmful. But presence of these organisms may indicate the quality of the water. Coliform and fecal coliform are mainly prevalent in streams and the number increases during the rainy season. Total coliform group is less specific for fecal pollution and is mainly used as a parameter for contamination. (Grabow, Hilner, and Coubrough 1981)It includes various species like *Citrobacter, Enterobacter, Hafnia, Klebsiella, Escherichia etc*. Fecal Coliform are indicator for bacterial contamination from human and it mainly include many strains of *Escherichia (enteropathogenicE. coli,* enterotoxigenic *E. coli,* diarrheagenic *E. coli etc)*.The presence of even one fecal coliform in water indicates the water is not suitable for consumption and the presence of other pathogenic organisms(Ashbolt, Grabow, and Snozzi 2001). *Shigella* is also a member of *Enterobacteriaceae*and is responsible for *shigellosis*or bacillary dysentery. The species incudes -*Shigella dysenteriae*,*Shigella flexneri,Shigella sonnei and Shigella boydii.*(Cabral 2010). These bacteria enter in the surface water from wash from city, overflowed sewage system, drainage etc.(Schuettpelz 1969). The long history of illness outbreaks are focuses on the fact of accidental consumption of the organisms. The fate of the coliform indicates the survival rate of pathogenic bacteria. Globally, diarrhoea caused 0·801 million deaths among the children under 5(Liu et al. 2012). and

according to WHO, during 2003-2011, 2.8% of children under 5 died in diarhoeal diseases in Bangladesh(Rahman et al. 2014).Estimated 15% deaths worldwide per year is caused by ETEC (Enterotoxigenic *E.coli*), *Vibrio cholerae,Shigella spp*. and rotavirus (Qadri et al. 2005).

Surface water includes river, pond, lake water and reservoirs. Surface water is used for various public uses, Irrigation, transportation, cleaning, bathing etc. the surface water mostly gets mixed with the ground water when there is heavy rainfall or overflow or poor sewerage system. During rainy season, surface runoffs mainly get mixed with surface water sources. Wash-offs from roads, vegetative sites, sewage system, contaminated bottom sediments and many types of pollutants from industries get mixed with the water(Schuettpelz 1969). Urbanization or industrialization can also lead to surface water pollution. The wastages from industries, chemical wastescan get mixed with surface water and results as water quality degradation. It may even become non-recyclable if severe industrial wastes get mixed. In Bangladesh, the people around industrial area suffer from a variety of health problems due to unplanned industrialization(Ullah, A; Clemett, A;Chowdhury, N;Huq, T;Sultana, R;Chadwick 2001). Also due to excessive population pressure, unawareness and lack of law enforcement and also due to severe construction works, very few sources of surface water around the Dhaka city fulfills the criteria of good water quality(Islam et al. 2015).

A large part of the economy of Bangladesh depends on water bodies. The different types of surface water sources in Bangladesh are- ponds, rivers, canals, lakes etc. But there are differences in case of pollution type or rate among lake water, reservoirs and Rivers. Dhaka is situated just beside the Buriganga River. More than 700 rivers are situated in Bangladesh and many people, cities and towns are built surrounding these rivers. As a result of heavy population and industrialization, the rivers of Bangladesh are getting polluted. During past few years, there have been various types of industries are built beside these rivers. Tanneries, medical wastes, chemicals, dyes, nuclear and toxic materials, water from sewage systems, wastes from water based transports like unrefined oils, human wastes get mixed with River water(Islam et al. 2015)

As a result, the river water becomes heavily polluted, and unusable. About 700-1100 tons of municipal, industrial wastes , organic wastes of Dhaka city are flashed into Buriganga River daily(Chakraborty et al. 2013). On the other hand, the water quality of Lakes and reservoirs inside the city is different than that of River. It is mainly depended on population, drainage system and development works inside the city. The main lakes inside the Dhaka city includes Gulshan Lake, Banani Lake, Hatirjheel lake, Dhanmondi lake, Ramna lake ,crescent lake etc. the water of these artificial lakes are being abused by dumping of solid wastes, waste water, water from different adjoining areas etc.(Chakraborty et al. 2013). As a result, people who are involved in direct contract with these water bodies are getting Diarrhoea, skin disease, gastric ulcers, malnutrition etc.(Ullah, A; Clemett, A;Chowdhury, N;Huq, T;Sultana, R;Chadwick 2001) .

Due to different sources of pollution, the water quality of Hatirjheel and Buriganga River are different. Different physical parameters to determine water quality are- Dissolved oxygen (DO) biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, turbidity, conductivity, total dissolved solids (TDS), Total suspended solids (TSS),nitrate and phosphate(Ahammed et al. 2016).The water of Buriganga contains much more chemicals and heavy metals than that of Hatirjheel due to tannery and direct industrial wastages. DO is an important parameter but DO In Buriganga river was found far below than DOE(department of environment) standard (5 mg/L for sustaining aquatic life and 6 mg/L for using the river water as the source of drinking water) . In case of both BOD the standard DOE is 2mg/L and COD (acceptable level 4mg/L) were found to be exceeded to a greatest extent. Because of the manucipal waste water discharge, the level of NH_3-N was found to be higher than the acceptable level (0.5 mg/L) which is alarming. There were also heavy metals like lead (Pb) and chromium (Cr) which is thought to be cancer causing were found. Arsenic contamination was also present(Rahman and Al Bakri 2010).

Parameters	DOE standards to maintain the aquatic ecosystem	Compliance with standards (Yes/No)	
		Dry season	Wet season
Temperature	20 to 30°C	Y	Y
pH	6.5 to 8.5	Y	Y
EC	350 µs/cm	N	Y
DO	5 mg/L	N	N
BOD_5	2 mg/L	N	N
COD	4 mg/L	N	N
PO_4-P	6 mg/L	Y	Y
NH_3-N	0.5 mg/L	N	N
Pb	0.05 mg/L	Y	Y
Cr	0.05 mg/L	N	N

Figure 1:Compliance of Buriganga River water quality parameters with DOE guidelines(Rahman and Al Bakri 2010)

Hatirjheel on the other hand, has become one of the main source of drainage for surrounding area. Due to lack of proper maintenances and planning, the pollution is increasing. As Hatirjheel is being featured as a site of people's attraction, many attractions are built based on it. For example- restaurants, water boat, fountains etc. People all around the week come and visit this lake as visiting site. Because of visitation activity, the water quality of Hatirjheel is deteriorating(Islam et al. 2015) .Without even knowing how harmful the water can be, people are consuming or using it in many ways. This lake is situated beside housing estates, many business centers and roads. Several pipelines are seen to be discarding waste waters from these locations. As a result it is openly getting mixed with sewage water and organisms from different sources are getting mixed. During the rainy season, the water discharge rate increases and water from many other sources get mixed on it. High BOD and COD is being observed during rainy season, which gets lower during dry season. The BOD, Acidity, TDS in Hatirjheel was found to be respectively- 2.10 to 4.00 ppm, 0.83 to 9.50 ppm and 326.33 to 380.67 ppm, which is higher than that of two other major lakes (Ramna Lake and Crescent Lake). . Also the DO level is lower as the BOD level is higher(Islam et al. 2015). This also specifies that the water quality if Hatirjheel exceeds the drinking water quality according to DOE.

As there are differences in pollution level of Hatirjheel Lake and Buriganga River, the microorganisms are most likely to be affected in different way. These water may not be used directly for consumption, but people are very much depended on these water resources. Many sorts of activities are developing based on these water bodies. Buriganga River is being used as a source of livelihood for years for thousands of people. Several water transports for both long

distance and short distance are found in this River all the year around as it is one of the main sources of transportation of both human and products from remote areas and foreign. Also, people are using water from both the lake and river for washing purposes. Lot of people are seen to get contract with the lake water and river water to wash clothes and other daily used products. Even sometimes the local food vendors are seen to wash vegetables and utensils. Even as the lake has fountain now, the water get carried by air when the fountain is operating. By this, the water can be carried to the people visiting the area and also can get consumed by them. For these reasons, it has given rise to public health aspects and need to be analyzed if it can give rise to diseases like Diarrhoea, shigellosis etc.

The Diarrhea genic*E.coli* (DEC) along with other Enterotoxigenic *E.coli*are known to be endemic in developing countries. Many of them are known to produce heat stable enterotoxins along with colonization in small intestine(Fakhr, Gohar, and Atta 2016). The strain E. coli serotype O157:H7 causes abdominal pain, bloody diarrhea, hemolytic uremic syndrome, renal failure and Shiga like toxins (Cabral 2010). The drinking water is seen to be contaminated by these open water sources very frequently and cause disease outbreak. Antibiotic resistance are seen to be grown as a notable problem due to water pollution. In a study, the virulence gene for EPEC and ETEC were found in household water(Fakhr et al. 2016). *Shigella* virulence factor includesadherence of bacteria onto the surface of target epithelial cells, production of *IpaH* (Invasion plasmid antigen) gene which has direct role *Shigella* invasion process. In Bangladesh severe Shigellosis affected people are found and it is related to both environmental and hygiene conditions(Cabral 2010).

In Bangladesh, The prevalence of STEC(Shiga like toxin producing *E.coli*)was 0.5% among the hospitalized patients, which was 1.9% of the cases of community patients(Islam et al. 2008). It is found that the most occurrence of *E.coli*is found in wet warm months of August and for pathogenic *E.coli*strain, the peak season is from February to May(Albert et al. 1995). STEC is a serologically diverse group of foodborne, zoonotic pathogens. The serology group O157:H7 is a part of this Shiga like toxin producing *E.coli*(STEC) group. STEC is the causative agent of haemorrhagic colitis and diarrhea-associated haemolytic–uraemic syndrome (HUS) with or

without neurological complications(Aminul Islam et al. 2007). This HUS mostly affects kidney and causes bloody diarrhoea. There are not much study about this strain done in Bangladesh and it is not commonly found(Aminul Islam et al. 2007). This is why it is important to determine if the water of Hatirjheel lake and Buriganga River is contaminated with *E.coli* O157:H7 or not.

Like other prevalent countries, Bangladesh is also considered as endemic Zone of Shigellosis. There is changing pattern of Shigellosis observed in hospitals of Bangladesh which is 8–12% in the 1980s to 3% in 2008. *S. sonnei* is one of the primary causes of dysentery and now it is replacing *S. flexinary* and causing major problems in developing countries. In Dhaka, the overall *Shigella* isolates were found more than that of urban area(Das et al. 2013).. It is estimated that 1.1 million people die annually from Shigella infection and nearly 580,000 cases of shigellosis are reported among travelers from industrialized countries(Sharma, Singh, and Bajpai 2010).This is the purpose of this study to determine the presence of *Shigella spp.* in Hatirjheel and Buriganga River as many people are dependent on these water bodies.

According to the literature review, it is established that the water of Hatirjheel Lake and Buriganga River is contaminated with various kinds of pollutants. The microbiological parameter also indicates the presence of coliforms and other microbes in this water sources. This study mainly focuses on If the water from Hatirjheel lake and Buriganga River meets standard water quality or not and Characterizing microbial hazards in two main polluted water bodies (Hatirjheel lake and Buriganga River The quantitative analysis of Total and fecal coliform indicates how unusable the water is and it also indicates the presence of other organisms. The effect of pollutants on this organisms are not being studied commonly before. This study focuses on the pathogen *E.coli* O157:H7 and *Shigella spp*, the presence of these organisms on both Hatirjheel Lake and Buriganga River and comparison of their characteristics from these 2 sources. The common studies like antibiotic resistance pattern was checked, but it is not very much done on the organisms which are affected by environmental perspectives. The change can effect directly or indirectly on human health. If the resistance is observed, it can be harmful for human health. Also it is observed if the resistance is due to plasmid or not. The main focus is to observe if the pollution of these water bodies are affecting the pathogenicity or not.

Our aim of the study is to detect the level of pollution in the Hatirjheel Lake as well as Buriganga River and whether their occurrence varies or not. According to our hypothesis, the waters will be contaminated with E.coliO157:H7 and *Shigella spp*. and there will be a huge presence of total and fecal coliform. The contaminants will have different characteristics between the Lake and River due to the difference between pollutants. Also to observe the antibiotic resistance pattern difference between these two water sources and if there is any, is it due to the pollution level difference or not.

Chapter 2

MATERIALS AND METHODS

2.0 Materials and method:

2.1 Water Sampling:

Water samples were collected by using autoclaved bottles from different points of Hatirjheel and Buriganga River. To reach the points for water collection, small water buses and water ferry was used. The water was collected in asceptic way. The sample collection started from October 2017 and lasted at the end of January 2018. The total of 40 samples was collected from Hatirjheel Lake and 10 samples were collected from Buriganga River. The samples were collected weekly and bought to the lab within 2 hours of the collection.

2.2 Processing of water sample:

Specifically identify Total coliform and fecal coliform. Total coliform colonies were counted using MacConkey agar by spread plate method. The sample was diluted using saline until 10^{-3} and then 100 µl was spreaded in the agar media. One Raw sample was also spreaded. For Fecal coliform, using the same method the plates were spreaded. Then the plates containing MacConkey agar were incubated at 37°C for 24 hours. For MFC agar, the plates were incubated at 45°C for 48 hours. The suspected colonies of total coliform in MacConkey agar gave matte pink or pink colonies. The colonies giving matte pink are thought to be *E.coli* .The selected colonies were then spotted in CT-SMAC agar by picking up the colonies using toothpick. Then it was incubated in 37°C for 24 hours. The colonies which were visibly colorless were suspected as *E.coli* 0157:H7 stains were grown in Nutrient broth and stored at -20°C by using glycerol. . On the other hand, the colonies of dark blue color in MFC agar were thought to be fecal coliform. All the suspected colonies from both MacConkey agar and MFC agar were counted and cfu/ml was calculated and noted to see quantitative analysis. The equation followed to count cfu/ml was:

$$\text{Colony forming unit/ml} = \frac{\text{Number of colonies X Dilution Factor}}{\text{Volume Plated}}$$

For *Shigella spp.* Identification, 50ml water from sample was filtered at 1st using a 0.45µm filter paper and then 5ml peptone broth was added for enrichment. After adding peptone broth 1st was incubated for 24 hours at 37℃ then streaking was done in XLD agar and SS agar (Salmonella-

Shigella agar). The suspected colony gave red colored colony in XLD agar and colorless colony in (SS agar). Then the selected colonies were stored in T_1N_1 agar by the help of paraffin oil.

2.3Description of sampling sites:

Hatirjheel is one of the important lakes inside Dhaka city. It is being used as one of the main water sources inside the city for transportation and used as a drainage system. It is also connected with Gulshan Lake which is also a significant lake around that area(Journal and Technology 2014). Another source for sample collection is Buriganga River, which is situated beside the Dhaka city and being playing a vital role for many peoples life. Untreated waste products from water transport and numerous industries is being dumped in the river for years and so it has become polluted(Chakraborty et al. 2013). These two sites are being used as main source of sampling.

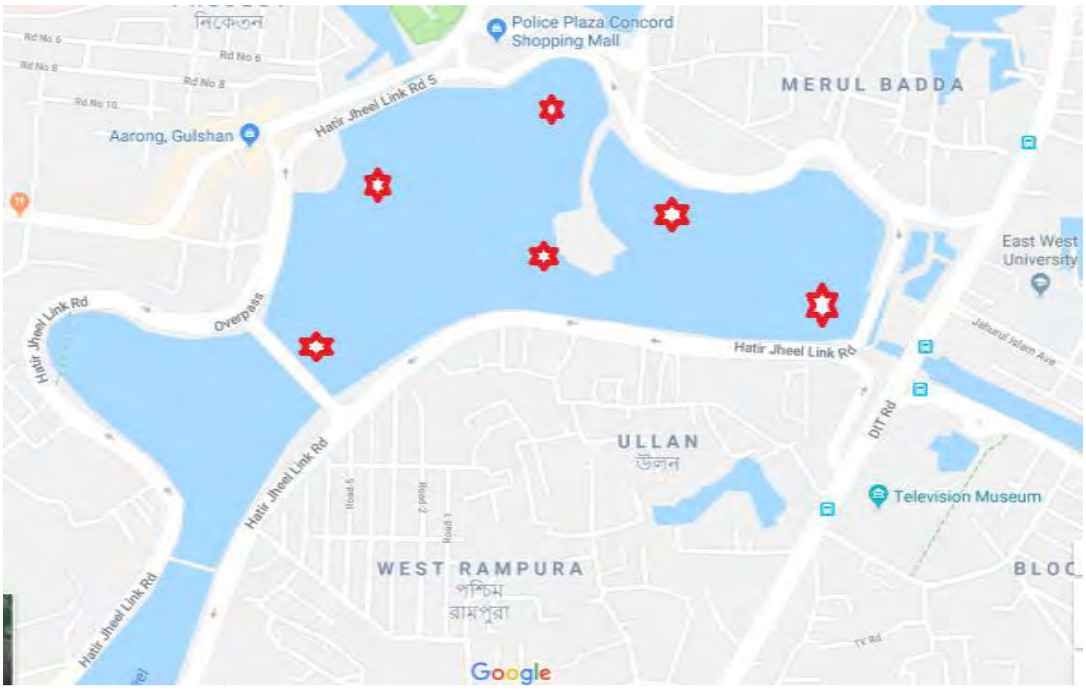

Figure 2: Hatirjheel sampling site

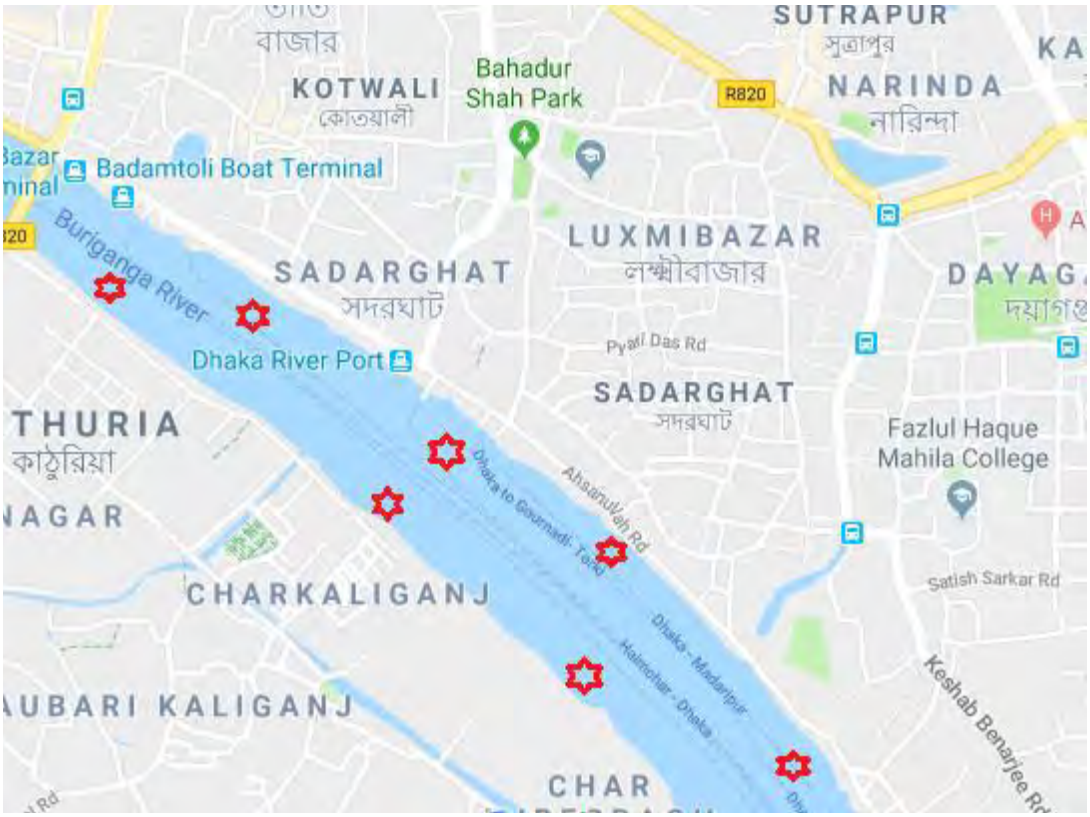

Figure 3: Buriganga River sampling site

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2.4Identification:

For presumptive selection, the samples were at 1st selected by streaking it selectively on EMB agar. The isolates which gave green metallic sheen were then selected for biochemical identification.

The suspected colonies were then tested for following biochemical tests to detect *Escherichia coli.*

Gram stain:

Gram staining was done to differentiate between two principal groups of bacteria: gram positive and gram negative.

Biochemical characterization of the bacteria:

Several biochemical tests were carried out in order to have a presumptive identification of the potential bacteria chosen before. Most of the methods were done according to the microbiology laboratory manual (Cappuccino. and Sherman 2014). The biochemical tests performed were Triple sugar iron agar test, IMViC test (Indole production test, Methyl red test, Voges- Proskauer test, MIU test (Motility test, Indole test and Urease test), and Catalase test.

Triple sugar Iron agar Test:

Triple sugar iron test was done to differentiate among the different groups or genera of the Enterobacteriaceae based on the ability to reduce sulfur and ferment carbohydrates. Triple sugar iron slants were prepared in the test tubes by autoclaving at 15 psi 121^0C. Using sterile technique; small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes by means of a stab and streak inoculation method with an inoculating needle. The screw caps were not fully tightened and the tubes were incubated for 24 hours at 37^0C (Cappuccino. and Sherman 2014)

IMVIC test

Indole Production test:

Indole production test was done to determine the ability of the bacteria to degrade the amino acid tryptophan by the enzyme tryptophanase. Tryptophan broth of 5 ml in each test tube was prepared by autoclaving at 15 psi 121◦C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes by means of a loop inoculation method with an inoculating loop and the tubes were incubated for 48 hours at 37◦C. In order to test for indole production, 5 drops of Kovac's reagent was added directly into the tubes(Macwilliams 2013).

Methyl red test:

Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of high concentration of acid end products. MR-VP broth of 7 ml in each test tubes were prepared by autoclaving at 15 psi 121^0C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes

by means of a loop inoculation method with an inoculating loop and the tubes were incubated for 24 hours at 37◦C. After 24 hours 3.5 ml from the culture tubes were transferred to clean test tubes for Voges- Proskauer test and the remaining broth were re-incubated for additional 24 hours. After 48-hour incubation 5 drops of methyl red indicator was added directly into the remaining aliquot of the culture tubes to observe the immediate development of a red color(Cappuccino. and Sherman 2014).

Vogues Proskauer test:

Voges Proskauer test was done to determine the capability of the organism to produce non acidic or neutral end products such as acetylmethylcarbinol. To the aliquot of MR-VP broth after 24 hour incubation, 0.6 ml (12 drops) of 5% alpha naphthol (reagent A) was added followed by 0.2 ml (4 drops) of 40% KOH (reagent B). The tube was gently moved to expose the medium to atmospheric oxygen (30 seconds-1 minute) and the medium was allowed to remain undisturbed for 10-15 minutes. The test was read, but not beyond, one hour following the addition of the reagents (Sylvia 2009)

MIU test (motility-Indole-Urease test):

MIU test was done to simultaneously determine the ability of the bacteria to produce indole, check motility and degrade urea by means of the enzyme urease. MIU media was prepared by 12 autoclaving at 15 psi 121^0C. The media was cooled to about 50-55^0C and 100 ml of urea glucose solution was added aseptically to 900 ml base medium. After that, 6 ml solution was transferred to each sterile test tube and allowed to form a semi solid medium. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes by means of a stab inoculation method with an inoculating needle and the tubes were then incubated for 24 hours at 37^0C(Shields and Laura Cathcart 2011).

Catalase test:

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase. A microscopic slide was placed inside a petri dish. Using a sterile inoculating loop, a small amount of bacteria from 24-hour pure culture was placed onto

the microscopic slide. 1 drop of 3% H2O2 was placed onto the organism on the microscopic slide using a dropper and observed for immediate bubble formation(Reiner 2016).

2.5Determination of virulence gene:

For determination of Shigella virulence gene, the *IpaH* gene locus was targeted. *IpaH* gene whose protein product is necessaryforinvasionofcolonicepithelial cells and also forthedetection of Shigella in environment(Sharma et al. 2010).

For *E.coli* O157:H7 strain detection, the virulence gene *eae*locuswas targeted.These gene *eae*is common among Shiga-like toxin producing *E.coli*(SLT-EC), which is responsible for enteric diseases and attachment of bacterium to colonic epithelial cell.

2.5.1DNA extraction by boiling method:

The selected isolates were inoculated in nutrient broth and incubated at 37^0C for 24h. The next day, 1.5ml of the broth was transferred to Eppendorf and centrifuged at 15,000g for 15 min. The supernatant was eliminated, and the pellet was resuspended in molecular biology grade water (Eppendorf, Hamburg, Germany) and centrifuged at 15,000g for 10 min. The supernatant was discarded and the pellet was resuspended in 40µl of autoclaved distilled water, subjected to boiling at 100°C in a water bath for 10min, cooled on ice foe further 13 10minutes and centrifuged at 15,000g for 10s before it was stored at -20°C. Aliquots of 2 µl of template DNA were used for PCR. (Queipo-Ortuño et al.)

2.5.2 PCR:

Polymerase Chain Reaction (PCR) is an in vitro technique based on the principle of DNA polymerization reaction by which a particular DNA sequence can be amplified and made into multiple copies. It relies on thermal cycling consisting of repeated cycles of heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA using thermostable DNA polymerase, primer sequence (complementary to target region) and dNTPs. Aliquots of extracted DNA by boiling method were used as template for the PCR encoding virulent gene *IpaH*.

Shigella specific *IpaH* primers (5'-GCTGGAAAAACTCAGTGCCT-3' and 5'-CCAGTCCGTAAATTCATTCT-3') was used to detect *Shigella*(Sharma et al. 2010).

Table 1. Primer used for gene specific PCR (Shigella)

Target gene	primers	Sequence 5'-3'	Amplicon size (bp)	Annealing temp	reference
IpaH	Forward primer	5'-GCTGGAAAAACTCAGTGCCT-3'	427	36˚C	(Sharma et al. 2010)
	Reverse primer	5'-CCAGTCCGTAAATTCATTCT-3'			

25µl of PCR mixture contained 12.5µl of Master Mix, 2µl of both primers, 6µl of nuclease free water and finally 2.5µl of the template DNA. The amplification was carried out with the reaction condition: initial denaturation at 94˚C for 3 minutes, followed by 45 cycles of 94˚C for 1 minute, 36˚C for 1 minute, 72˚C for 2 minute and final extension was at 72˚C for 10 minutes (Sharma et al. 2010).

By using the DNA boiling method, the DNA was extracted for *E.coli* O157:H7 strain detection and it was used as PCR encoding target gene *eae. E.coli* 0157:H7 specific targeted primers (5'-CAG GTC GTC GTG TCT GOCT AAA3-3' and 5'-TCA GCG TGG TTG GAT CAA CCT-3') was used to detect *E.coli* O157:H7.

Table 2: Primer used for gene specific PCR (E.coli 0157:H7)

Target gene	primers	Sequence 5'-3'	Amplicon size (bp)	Annealing temp	reference
eae	Forward primer	5'-CAG GTC GTC GTG TCT GOCT AAA3-3'	1,087	55-60˚C	(Gannon et al. 1993)
	Reverse primer	5'-TCA GCG TGG TTG GAT CAA CCT-3'			

12.5µl of PCR mixture contained 6.25 µl of Master Mix, 1µl of both primers, 1.75µl of nuclease free water and 2.5µl of template DNA. . The amplification was carried out with the reaction condition: the initial temperature was 94˚C for 2 minutes, carried out by 35 cycles of 94˚C for 1 m minute, 55˚C for 1 minute, 72˚C for 2 minute and final extension at 72˚C for 5 minutes(Gannon et al. 1993).

2.5.3 Gel conditions

1.5% agarose gel was made using 1X TE Buffer and run with 1X TBE buffer. For staining Ethidium bromide was added to the gel after it cooled down slightly. 2µl dye and 6µl of the PCR product were mixed well and then added to each well. The gel was run for 1 hour at 70V. The product size (427 bp) was determined with 1000 bp DNA molecular weight ladder(Sharma et al. 2010). Finally, the gel image was viewed under UV light. For each PCR test,two positive (*Shigella flexneri*and*Shigella dysenteriae*) and negative control (sterile dH2O) were used separately along with water sample.

And for *E.coli* o157:H7 strain, a positive control (E.coli o157:H7 stain from lab stalk) and a negative control (sterile dH2O) was used. And the gel was 1% agarose gel , made by using 1X TE Buffer and run with TBE Buffer. For staining also Ethidium bromide was added to the gel after cooling 2µl dye and 6µl of the PCR product were mixed well and then added to each well.

The gel was run for 45 minutes at 80V. The product size (1087 bp) was determined with 1000bp DNA molecular weight ladder(Gannon et al. 1993).

2.6 Determination of antibiotic resistant pattern of the target:

The 16 isolates were selected from lake and River to observe antibiotic resistant pattern. 13 from Hatirjheel Lake and 3 from Buriganga River in case of *Escherichia coli* strains. And in case of Shigella species, only one sample was taken from Buriganga River which was confirmed to be Shigella. The method used to compare the antibiotic susceptibility and resistance pattern was by Kirby-Bauer disc diffusion method. Antibiogram was done using the antibiotics which are commonly used against *Escherichia coli* and *Shigella spp.*

2.6.1 Kirby-Bauer disc diffusion:

The Kirby-Bauer disc diffusion method was done by following CLSI (clinical laboratory standard institute) For *Shigella* spp recommendations for. Commercial antibiotics discs were used following the amount recommended by CLSI.: . The antibiotics taken were – Imipenem (10µg), Nalidixic acid (30 µg), Tetracycline (30µg), Chloramphenicol (30 µg),Azithromycin (15µg), Doxycycline(30µg), Ciprofloxacin (5µg) and Norfloxacin(10µg).

The Kirby-Bauer method was followed in antimicrobial susceptibility test(Hudzicki 2009). The selected isolates were grown on Nutrient agar (overnight growth). Inoculated into 1 ml of Muller-Hinton Broth (MHB). The culture were then incubated in a shaker at 37°C for 4 hours to obtain the actively growing culture, equivalent to 0.5 McFarland standard ($1.5x\ 10^8$ CFU/mL). A sterile cotton swab was dipped into the standard suspension, excess broth was purged by pressing and rotating the swab firmly against the inside wall of the tube above the fluid. The swab was then streaked evenly in three directions over the entire surface of the agar plate to obtain a uniform inoculum. A final sweep was made of the agar rim with the cotton swab. This plate was then allowed to dry for three to five minutes before the disks were applied. Antibiotic impregnated disks were then applied to the surface of the inoculated plates with sterile syringe needle. All disks were gently pressed down onto the agar with sterile forceps to ensure complete contact with the agar surface. Within 15 minutes after the disks were applied, the plates were inverted and placed in an incubator at 37°C. After overnight incubation, the plates were examined for zone of inhibition and the diameter of the zone of inhibition was measured to the

nearest whole millimeter by a ruler. The zone diameters for individual antimicrobial agents were then translated into susceptible, intermediate, or resistant categories according to the CLSI guidelines (CLSI 2017).

2.6.2Plasmid Profiling

After the antibiogram, plasmid profiling was further performed with the isolates used there in order to determine whether the antibiotic resistance gene is present in the plasmid or not. Hence, plasmid extraction was performed only on the isolates showing antibiotic resistance. Alkaline lysis method was used to extract the bacterial plasmid (Plasmid DNA Extraction From)

Procedure:

- The bacterial cells were grown overnight in LB broth overnight.
- 1.5ml of the broth was transferred to an Eppendorf in order to harvest by centrifugation at 10000 rpm for 2 min (repeated this step 3 times).
- The pellet was resuspended in Solution 1 (200 µl) and then freshly prepared solution 2 (400 µl) was added; mixed by gentle inversion.
- The cells were incubated at room temperature for 5 mins for cell lysis.
- To this, ice cold solution 3 (300 µl) was added and mixed by inversion and incubated on ice for 10 mins.
- The mixture was then centrifuged at 12000 rpm for 15 mins.
- The supernatant was transferred in a fresh tube.
- To this, equal volume of phenol: chloroform: IAA (25:24:1) was added, mixed by vortexing and centrifuged at 12000 rpm for 2 min.
- The supernatant was carefully collected an equal volume of choloroform : IAA (24:1) was added to it, followed by mixed by vortexing and centrifuged at 12000 rpm for 5 mins.
- The supernatant was collected and then 0.6 volume of isopropanol was added to this, then mixed by inversion and centrifuged at 12000 rpm for 15 minutes.
- The DNA pellet was washed in 70% ethanol, air-dried and resuspend in 20 µl of TE buffer.

2.6.3 Separation of plasmid DNA by Gel Electrophoresis:

Plasmid DNA was separated by horizontal electrophoresis in 0.7% agarose slab gels in TBE buffer at room temperature at 70 volts for 60 minutes. 8µl of plasmid DNA solution was mixed with 2µl of tracking dye and was loaded into individual well of the gel. The DNA bands were seen under UV light. A positive control was used as known size marker and 10 kbp DNA ladder was used in order to determine the size of the unknown plasmid.

Chapter 3

RESULTS

3.0Results

3.1 Quantitative analysis:

The cfu/ml of each sample was counted for quantitative result in both MacConkey and MFC agar separately for Hatirjheel and Buriganga River. The result showed that there were presence of Total coliform in every sample collected from Buriganga River and Hatirjheel. In case of Fecal coliform, there was presence in Hatirjheel about 43.3% and In Buriganga River 80%.

Table 3: Hatirjheel sample total coliform agar Average count

sample number	Average cfu/ml
H1	TNTC
H2	16.5 $X10^2$
H3	TNTC
H4	18.05 X 10^3
H5	8.2 X 10^3
H6	TNTC
H7	3.1 X 10^3
H8	8.2 X 10^3
H9	2.8 X 10^3
H10	4.35 X 10^3
H11	4 X 10^3
H12	4.15 X 10^3
H13	6.4 X 10^3
H14	7.9 X 10^3
H15	2.2 X 10^3

Sample no.	Average cfu/ml
H16	4×10^3
H17	7.3×10^3
H18	TNTC
H19	TNTC
H20	TNTC
H21	TNTC
H22	3.7×10^3
H23	7.45×10^3
H24	TNTC
H25	TNTC
H26	16.85×10^3
H27	7.55×10^3
H28	TNTC
H29	3.2×10^3
H30	TNTC

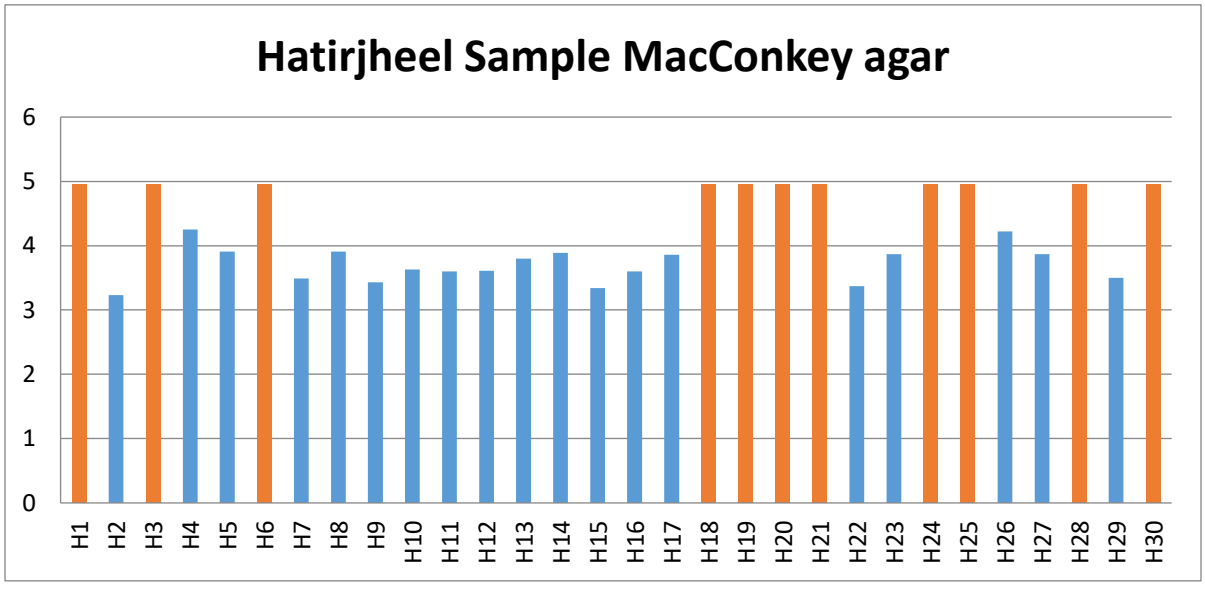

Figure 4: Cfu/ml count of Hatirjheel sample at MacConkey Agar. (Red color indicates TNTC)

Table 4: MacConkey agar Buriganga river total coliform Average count

Sample no.	Average cfu/ml
BG 31	TNTC
BG32	10.55×10^3
BG33	21.6×10^3
BG34	8×10^3
BG 35	13.5×10^3
BG 36	TNTC
BG 37	TNTC
BG 38	4.5×10^3
BG 39	4.73×10^3
BG 40	4.95×10^3

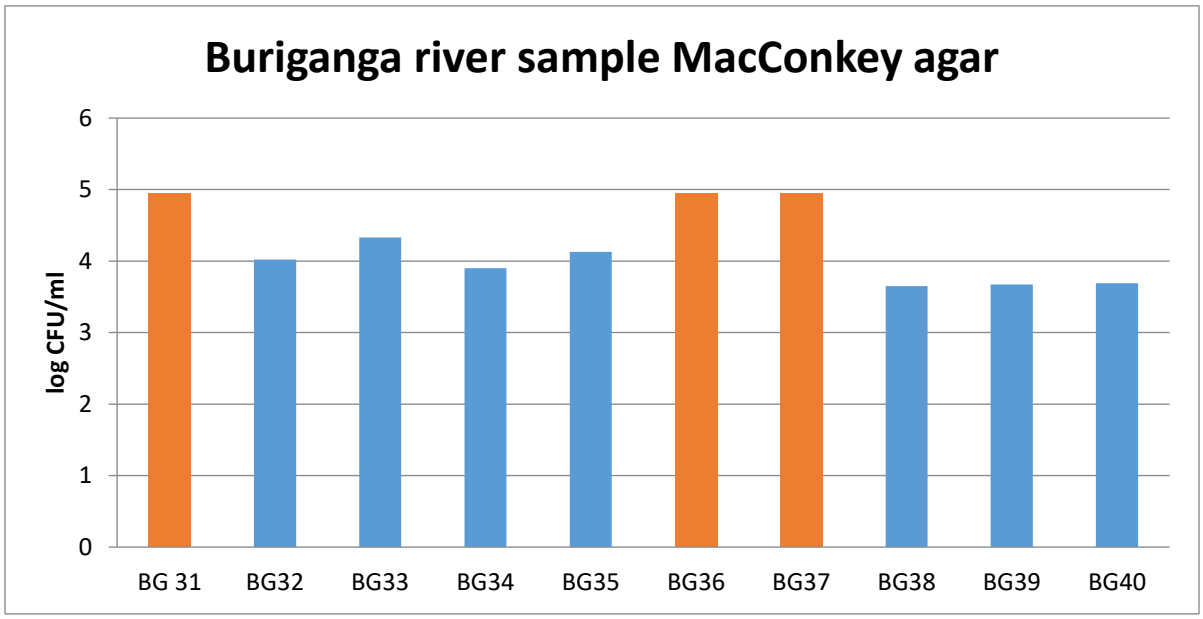

Figure 5: Cfu/ml count of Buriganga River sample at MacConkey Agar. (Red color indicates TNTC)

Table 5: MFC agar Hatirjheel fecal coliform Average count

Sample no.	Average cfu/ml
H 1	0
H2	0
H3	0
H4	1×10^2
H5	0
H6	0
H7	0
H8	8×10^2
H9	2×10^2
H10	0
H11	0
H12	0
H13	0
H14	$1X\ 10^2$
H15	0
H16	0
H17	0
H18	7×10^2
H19	3.2×10^3
H20	5×10^2
H21	5×10^2
H22	1×10^2
H23	3.7×10^3
H24	0
H25	0
H26	3×10^2
H27	0

Sample No.	Average cfu/ml
H28	0
H29	1 X 10^2
H30	1 X 10^2

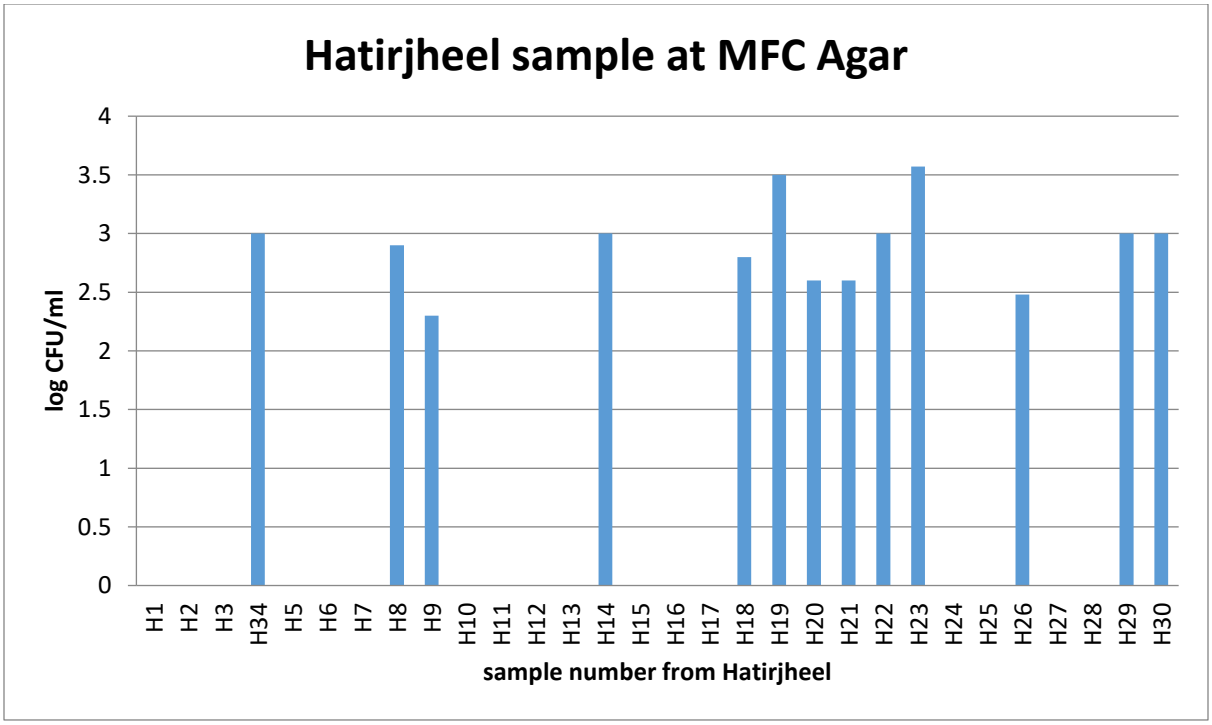

Figure 6: CFU/ml count of Hatirjheel sample at MFC Agar

Table 6: MFC agar fecal coliform Buriganga river average count

Sample no.	Average cfu/ml
BG 31	0
BG 32	6.2 X 10^3
BG 33	1.6 X 10^3
BG 34	1.6 X 10^3
BG 35	3.3 X 10^3
BG 36	0

Sample no.	Average cfu/ml
BG 37	2X 10^2
BG 38	2 X 10^3
BG 39	9 X 10^2
BG 40	5 X 10^2

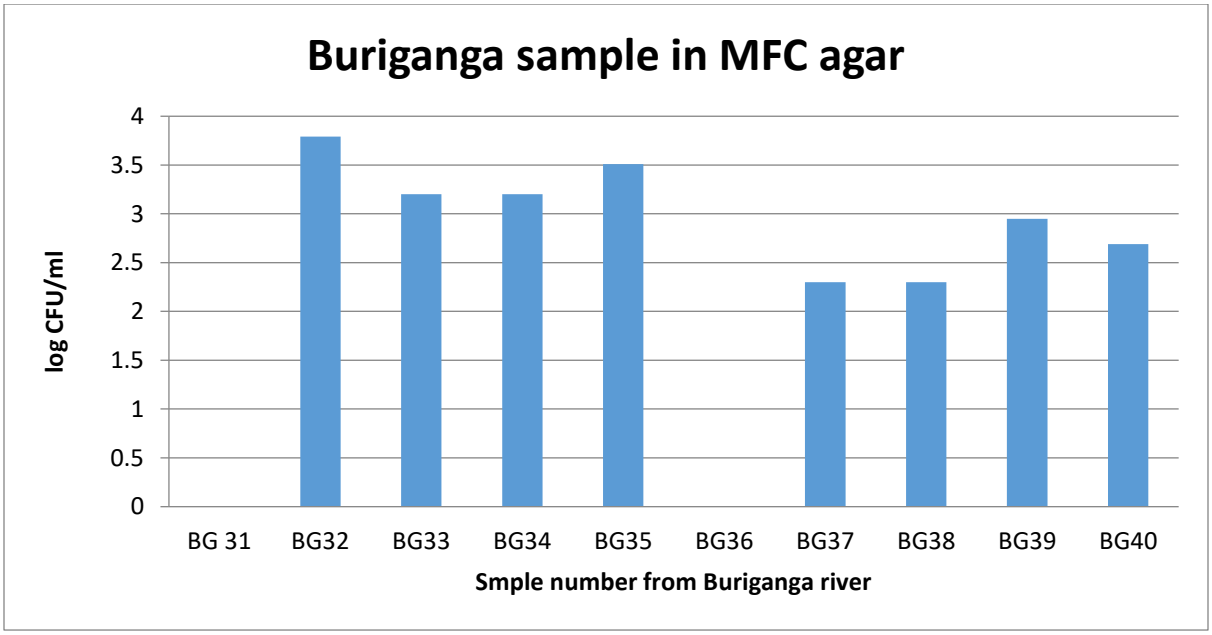

Figure 7: Cfu/ml count of sample from Buriganga River at MFC agar.

The average count of Total and Fecal coliform per Day was counted to correlate with the temperature of the day before sampling.

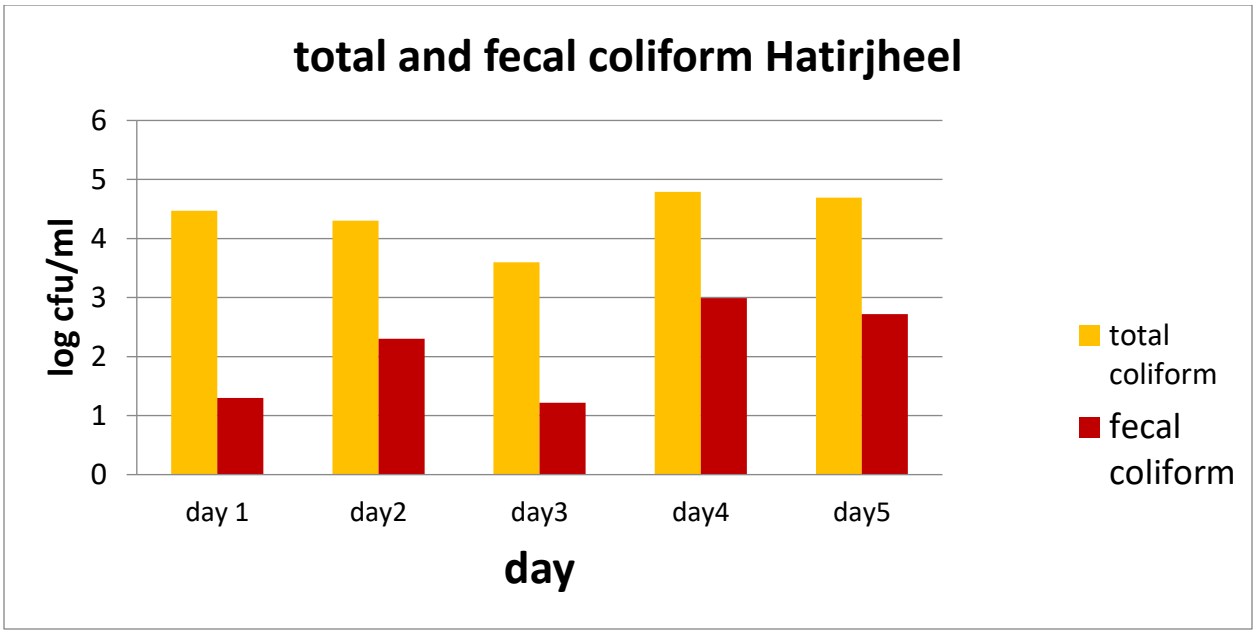

Figure 8: Average count of Total and Fecal coliform from Hatirjheel /day.

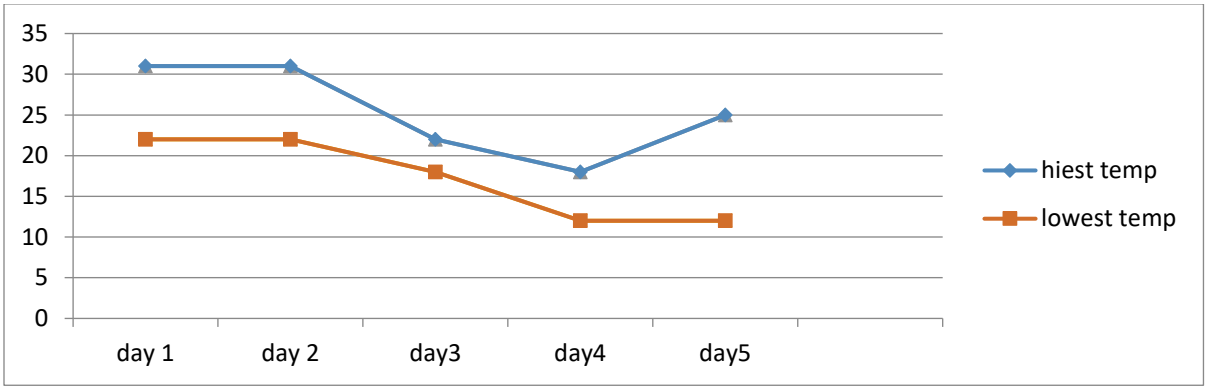

Figure 9: Temperature of the day before sampling

Table 7 : Correlation of per day average count from Hatirjheel with temperature

Lowest temperature of the day	Highest temperature of the day
-0.70227	-0.39065

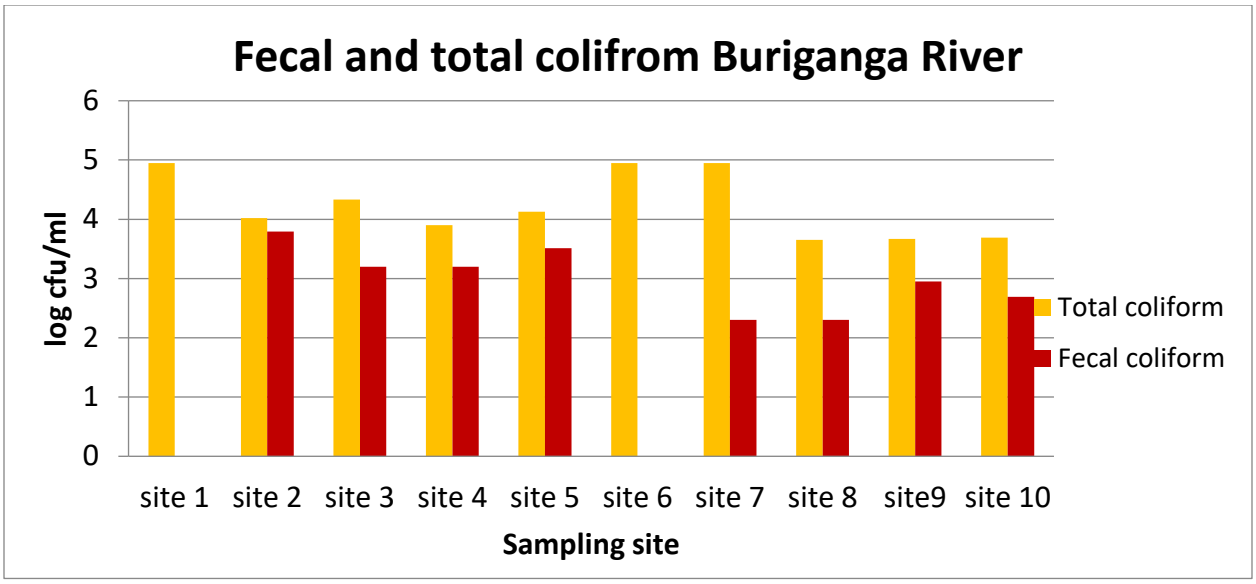

Figure 10: Fecal and total coliform average / site

3.2 Identification:

The following table describes the result of identification test for the samples by biochemical tests. Few of the samples gave slightly different results in case of some biochemical tests. But mostly the samples gave desired results. Of all the 11 sets of biochemical tests, if the samples gave 80% of the tests desired results, it is regarded as presumptively positive. Here we can see that >80% of the results are positive, so we can say that the samples were presumptively *Escherichia coli*

Table 8: Biochemical test chart of total coliform.

sample number	sample	Gram staining	TSI				MIU		indole	Methyl red	VogesProskae-ur	catalase
			slant	butt	gas	H_2S	motility	urease				
1.	H1	_	Y	Y	+	-	+	-	+	+	-	+
2.	H3	-	Y	Y	+	-	+	-	+	+	-	+
3.	H5	-	Y	Y	+	+	+	+	+	+	+	+
4.	H6	-	Y	Y	+	-	+	-	+	+	-	+
5.	H8	-	Y	Y	+	-	+	-	+	+	-	+
6.	H9	-	Y	Y	+	-	+	-	+	+	-	+
7.	H14	-	Y	Y	+	-	+	+	+	+	-	+
8.	H15	-	Y	Y	+	-	+	+	+	+	-	+
9.	H18	-	Y	Y	+	-	+	+	-	+	-	+
10.	H19	-	Y	Y	+	-	+	+	+	+	-	+
11.	H23	-	Y	Y	-	-	+	-	+	+	-	+
12.	H24	-	Y	Y	+	-	+	-	-	+	-	+
13.	H30	-	Y	Y	+	-	+	+	-	+	+	+
14.	BG32	-	Y	Y	-	-	+	+	-	+	-	+
15.	BG34	-	Y	Y	-	-	+	-	+	+	-	+
16.	BG40	-	Y	Y	-	-	+	+	+	+	-	+

Table 9: Sampling month, number of samples and percentage of positive sample.

Month of collection	Sampling site	Number of analysed samples	Positive sample (%)
October 2017	Hatirjheel Lake	3	66.7%
November , 2017	Hatirjheel Lake	5	100%
January ,2018	Hatirjheel lake	5	60%
	Buriganga River	3	66.7%

A total of 30 samples were collected from Hatirjheel Lake and 13 of them gave presumptively positive result for *E.coli* strain. And From Buriganga River, total 10 samples were collected and only 2 of them gave positive result. This concluded 37.5% of the total sample gave positive result.

3.3 Virulence Determination

After the presumptive identification of the samples, genetic amplification of the pathogenic gene *IpaH*, and *eae*was performed using PCR in order to determine whether the isolates present in the environment are carrying this gene and are capable of causing diseases if ingested. The primer used in the PCR mixture was specific for *IpaH*which was then run in 1.5% agarose gel. The bands are usually approximately 427 bp long DNA. Only one of the isolates gave positive bands for the specific gene which means that the isolates present the surface of the waterbodies might be pathogenic *Shigella* spp. And for the primer *eae*which was run at 1% agarose gel . The bands are approximately to show at 1087 bp, which means it must show at the top of the gel run. But there was no band seen after gel run.Which means there were probably no *E.coli* o157:H7 strain present in the water sample.

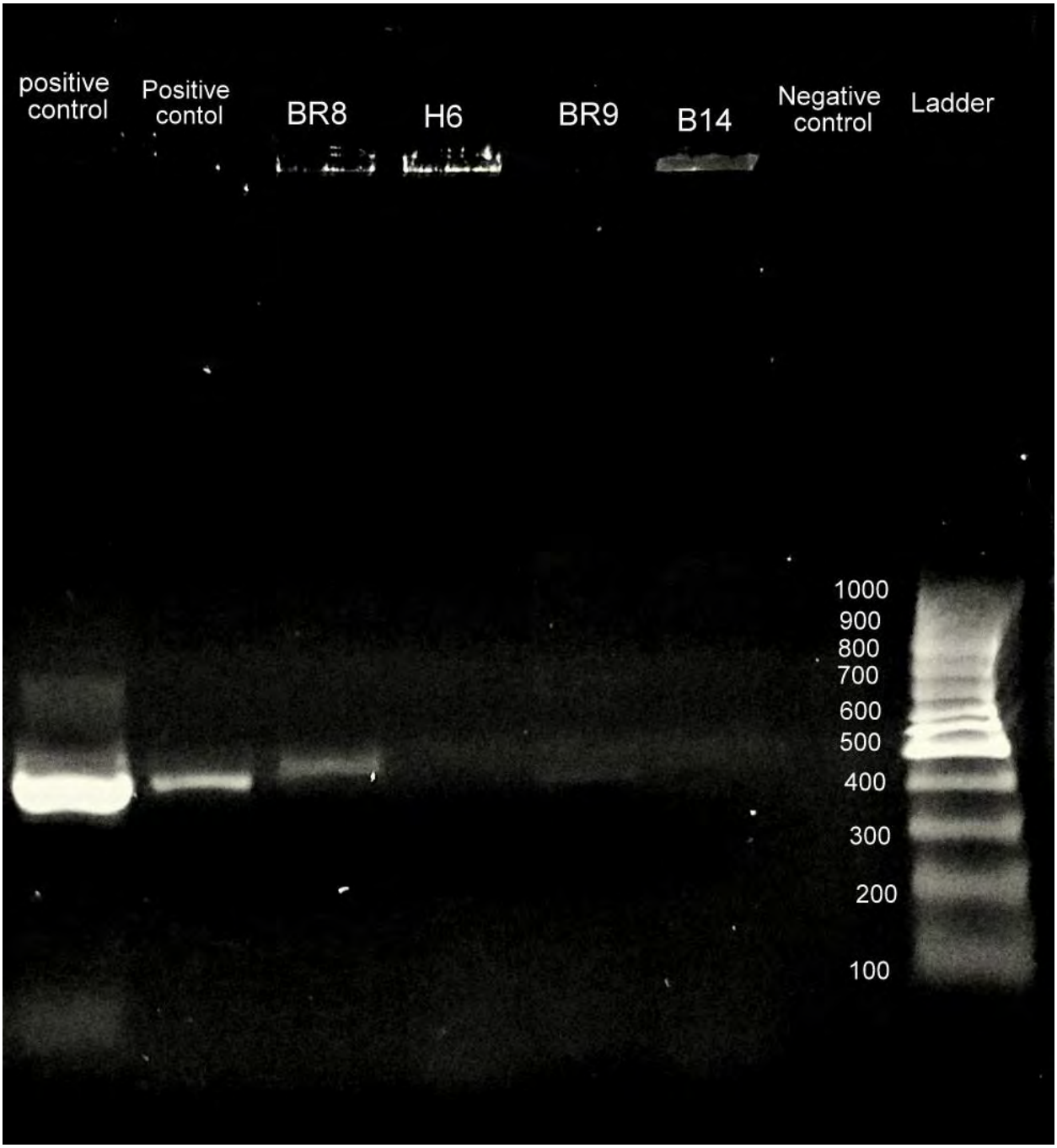

Figure 11: Agarose gel electrophoresis of isolates from Hatirjheel Lake and Buriganga River showing positive bands for primer specific *IpaH* gene.

3.4 Antibiogram:

Antibiogram was done with the confirmed PCR product of Shigella with 8 antibiotics according to CLSI.

Table 10: antibiotic susceptibility test of Shigella spp.

Sample	IMIPENEM	CHLORAMPHENICOL	TETRACYCLINE	NALIDIXIC ACID	AZITHROMYCIN	DOXYCYCLINE	NORFLOXACIN	CIPROFLOXACIN
BR8	S	S	S	S	S	S	S	S

Sensitive=S, Resistant = R , Intermediate= I

In case of *Shigella* spp. the isolate from Buriganga River showed complete sensitivity against all 8 antibiotics. There was no resistance observed against any antibiotics.

3.5 Plasmid profiling:

Plasmid profiling was done for both presumptive E.coli and *Shigella* isolates. There was no plasmid found in both cases. In case of Shigella isolate, the positive control gave band, but the isolate BR8 did not showed any band. So, we can say that the sensitivity this organism had was not due to presence of any plasmid.

As there was no plasmid found in case of *E.coli* too, we can say that the resistance pattern observed was also not for presence of any plasmid. It may be chromosomal.

Figure 12: Agarose gel electrophoresis band for plasmid extracted from *Shigella* isolate.

Chapter 4

DISCUSSION

4.0 Discussion:

The purpose of this study was to isolate and identify *E.coli* O157:H7 and *Shigella spp.* from water of both Hatirjheel and Buriganga River. *E.coli* O157:H7 is causative agent of food borne and water borne diarrhoeal diseases. *Shigella spp.*is causative agent for shigellosis, the cause of serious gastrointestinal diseases like dysentery. Due to water pollution in this open water sources severe waterborne diseases like diarrhoea and dysentery has increased(Reza and Yousuf 2016). The diarrhoea is one of the major causes of morbidity and mortality among children under 5. the mortality rate due to unsafe water in 2012 was 20.1% per 100000 population(Coverage and Protection 2017).This is why it is important to know if there is presence of *E.coli* O157:H7 and *Shigella spp.*in two major Water bodies of Dhaka.

For identification of *E.coli* strain, Biochemical tests were done. 37.5% of total 40 samples collected from lake and River gave positive result for *E.coli* strain. 43.3% in lake water and 20% in river water.

After isolation of presumptive *Shigella spp*. and *E.coli spp.*, the samples were further analysed to confirm by PCR. In case of *E.coli spp.* PCR was done to specifically identify the *E.coli* O157:H7 strain. The primer used for identifying *E.coli* O157:H7 contained *eae*. The *eae* locus is found AE gene and it detects specifically the presence of *E.coli* O157:H7 strain(Gannon et al. 1993). Out of 16 isolates, which were previously tested and presumptively thought to be *E.coli* O157:H7strain by biochemical tests were taken and tested for confirmation through PCR. None of the isolates gave positive bands on PCR so we can assume that there might be absence of pathogenic*E.coli* O157:H7 in the water of both Hatirjheel Lake and Buriganga River. This strain is very much pathogenic and not easily found in open water sources. It is also previously proved that, the attribute can be lost during storage or the homology was of *eae* gene was too low to be detected(Gannon et al. 1993).

On the other hand, the *Shigella* species was detected among the four isolates tested from Hatirjheel and Buriganga River. The primer used for detection of *Shigella*spp. was *IpaH*. The *IpaH* gene is specific for detection of *Shigella*spp. which is also responsible for colonic invasion of the organism(Sharma et al. 2010). Among four isolates selected after isolating in selective media (XLD), only one of the isolates showed band at the same position as the two positive controls (*S. flexneri*and *S.dysenteriae*).the isolate was from Buriganga River.So we can say that,

the pathogenic gene containing organism was present in the Buriganga River water and It can infect Human. If the water from the river inters our body, it has potential to cause *Shigella* mediated diseases.

In quantitative analysis, we can see the presence of total coliform and fecal coliform in both sources of water. The total coliform was seen to be present in every sample and in case of Hatirjheel lake 36.7% and Buriganga shoed 30% TNTC (too numerous to count) counts. The Fecal coliform presence was also observed, In Hatirjheel about 43.3% and In Buriganga River 80%. The presence of *E.coli* in water indicates the recent fiscal contamination and the water is unsafe for direct consumption. The objective is to obtain 0 *E.coli* per 100 ml of water(Gorchev and Ozolins 2011). The presence of fecal and total coliform at this high number if the water get mixed with drinking water, many enterogenic disease outbreak may occur. As For observing the pattern of count of fecal coliform / day of sampling, we can say that the fecal count increased. The count increased as the temperature got lower. We can see that, the highest count was found at day 4 , when the temperature was 18/20°C. the lowest among all other sampling day. And if we can see the correlation with the temperature, it was -0.7for lowest temperature. Which indicates strong Correlation. Also with the highest temperature of the dauy, it is -0.3, which is also moderate. So, we can say that, the colony count increased as the temperature lowered. . During this season, from November to January, the water condition is bad, or we can say the water quality is bad. In Hatirjheel and Buriganga River.

Further studies were done for both species to observe antibiotics resistance pattern. Multiple drug resistance of *E.coli* strain have been reported in Bangladesh in recent years(Reza 2009).Also a high rate of development of antimicrobial resistance in *Shigella*strains have been reported in Bangladesh(Rahman et al. 2007). After the identification, the antibiotic resistance pattern has been observed for both isolates.

Shigella isolates, there were no resistance pattern observed against all eight antibiotics. The. It may be due to contamination from different drainage system and sewerage system. The lake water being connected with direct discharge of waste water from many commercial buildings and slums can also be the reason(Islam et al. 2015).

For confirmed *Shigella* spp. isolate, the plasmid profiling was done. Plasmid extraction was conducted to observe if the antibiotic susceptibility was due plasmid mediated or chromosome

mediated. But, after performing gel electrophoresis, there was no presence of any plasmid in both of the organisms. So, we can conclude that the resistance and sensitivity was chromosomal in case of *Shigella spp.*

There was no resistance found in case of *Shigella spp.* so, we can say that these drugs can be effective against the disease caused by *Shigella spp.* From river water. Previously Bangladesh resistance pattern was observed against these drugs. Ampicillin resistance was found associated with *TEM β-lactamase* genes while in 22% of the isolates Resistance of Shigella spp. to chloramphenicol, tetracycline has mainly been attributedto the presence of catA1, and *tetB* genes. Resistance to trimethoprim is associated with *dhfrIa* or *dhfrIIIc* genes. A study conducted in Bangladesh revealed a rise in resistantstrains of Shigella towards ciprofloxacin from 0% in 2004 to 44%in 2010(Puzari, Sharma, and Chetia 2017). Maybe the change is due to exposure of industrial and metallic wastes in river water which made the organism sensitive against most common drugs which were previously ineffective.

Due to the difference between the pollutants in Lake Water and river water, it is hypothesized that the characteristics and antibiotic resistance pattern among these two major water source might be different. The result found that in case antibiotic resistance pattern, there was not much difference in River water and lake water for both *Shigella spp.* and *E.coli* strain. Resistance against same antibiotics were found, also the sensitivity against same antibiotics were found in almost similar percentages in both River and lake water. Presence of plasmid was not observed in both organisms in both sources. This leads to possible chromosomal resistance. It can also be the reason of storage problem, which lead the organisms to transfer the plasmids to other organisms or to the environment as it might not need it in laboratory environment. The count of presence of fecal coliform showed noticeable difference in Lake Water and River water. This may bedue to solidwaste and urban sewage discharge in the river water(Ahammed et al. 2016).

Conclusion and Future Direction:

In a World Health Organization report it has been suggested that there are 380,000 deaths annually in children less than 5 years of age that are caused by diarrhoea associated diseases. Although it is known that the surface water of Hatirjheel Lake and Buriganga River is contaminated, but still it was need to be observed how contaminated it is. The huge pollution occurring to the environment is a matter of concern for a developing country like Bangladesh. The population density is also the reason for surface water contamination and cannot be easily stopped. Emerging diseases at different seasons every year is also a major concern. As people's life is dependent on water. There has been regular dependence of these two water bodies being detected for years and in future it may increase, it is needed to acknowledge how harmful this water sources can be. How the pollutants are affecting in antimicrobial characteristics and indicating the presence of other harmful organisms. Study focused on *Shigella spp*. *E.coli* O157:H7 total and fecal coliform presence in Hatirjheel Lake and Buriganga River. *Shigella spp.* was proved by PCR to be present in river water. The absence of *E.coli* O157:H7 strain is normal as this organism is not easy to find in open water sources. However, the biochemical tests showed presence of *E.coli*strains. So, pathogenic strains are available in this water sources. For further addition, the antibiotic resistance pattern showed the occurrence of resistance may be due to horizontal gene transfer or mutation among chromosomes.

For Future , this work can be expended to observe what particular strains of *E.coli*is present in the water and to observe the survival rate for both *E.coli*and *Shigella spp.* in other water sources of daily use like pipe water or drinking water. Finally, the presence of antibiotic resistance genes in these isolates should be figured out.

Chapter 5

Reference

References:

1.Ahammed, Shaikh Sayed, Sadia Tasfina, K. Ayaz Rabbani, and Adbul Khaleque. 2016. "An Investigation Into The Water Quality Of Buriganga - A River Running Through Dhaka." (March).

2. Albert, M. J. et al. 1995. "Controlled Study of Escherichia Coli Diarrheal Infections in Bangladeshi Children . Controlled Study of Escherichia Coli Diarrheal Infections in Bangladeshi Children." 33(4):973–77.

3. Aminul Islam, M. et al. 2007. "Shiga Toxin-Producing Escherichia Coli Isolated from Patients with Diarrhoea in Bangladesh." *Journal of Medical Microbiology* 56(3):380–85.

4. Ashbolt, Nj, Wok Grabow, and Mario Snozzi. 2001. "Indicators of Microbial Water Quality." *Water Quality: Guidelines, Standards and Health* (Grabow 1996):289–316.

5.Cabral, João P. S. 2010. "Water Microbiology. Bacterial Pathogens and Water." *International Journal of Environmental Research and Public Health* 7(10):3657–3703.

6. Cappuccino., James G; and Natalie Sherman. 2014. *Microbiology A Laboratory Manual*.

7. Chakraborty, Chandan, Mazaharul Huq, Sobur Ahmed, Taslima Tabassum, and Rubel Miah. 2013. "Analysis Of The Causes And Impacts Of Water Pollution Of Buriganga River : A Critical Study." *International Journal of Scientific & Technology Research* 2(9):245–52.

8. Chowdhury, Fahima et al. 2011. "Impact of Rapid Urbanization on the Rates of Infection by Vibrio Cholerae O1 and Enterotoxigenic Escherichia Coli in Dhaka, Bangladesh." *PLoS Neglected Tropical Diseases* 5(4).

9. CLSI. 2017. *Performance Standards for Antimicrobial Susceptibility Testing. 27th Ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute*.

10. Coverage, Health Service and Financial Protection. 2017. "2017 Health SDG Profile: Bangladesh." 3:13–16.

11. Das, Sumon Kumar et al. 2013. "Changing Emergence of Shigella Sero-Groups in Bangladesh: Observation from Four Different Diarrheal Disease Hospitals." *PLoS ONE* 8(4).

12. Fakhr, Ahmed Elsadek, Maha Kamal Gohar, and Amal Hassan Atta. 2016. "Impact of Some Ecological Factors on Fecal Contamination of Drinking Water by Diarrheagenic Antibiotic-Resistant Escherichia Coli in Zagazig City, Egypt." *International Journal of Microbiology* 2016.

13. Gannon, Victor P. J., Michael Rashed, Robin K. King, and Elizabeth J. Golsteyn Thomas. 1993. "Detection and Characterization of the Eae Gene of Shiga-Like Toxin-Producing Escherichia Coli Using Polymerase Chain Reaction." 31(5):1268–74.

14. Gorchev, H. G. and G. Ozolins. 2011. "Guidelines for Drinking-Water Quality." *Who* 1:564.

15. Grabow, W. O. K., C. A. Hilner, and P. Coubrough. 1981. "Evaluation of Standard and Modified M-FC, MacConkey, and Teepol Media for Membrane Filtration Counting of Fecal Coliforms in Water." *Applied and Environmental Microbiology* 42(2):192–99.

16. Hudzicki, Jan. 2009. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol." *... -Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol.(...* (December 2009):1–14.

17. Islam, M. S., M. Rehnuma, S. S. Tithi, M. H. Kabir, and L. Sarkar. 2015. "ISSN 1999-7361 Investigation of Water Quality Parameters from Ramna , Crescent and Hatirjheel Lakes in Dhaka City ISSN 1999-7361." 8(1):1–5.

18. Islam, Mohammad A. et al. 2008. "Prevalence and Genetic Characterization of Shiga Toxin-Producing Escherichia Coli Isolates from Slaughtered Animals in Bangladesh." *Applied and Environmental Microbiology* 74(17):5414–21.

19. Journal, International and O. F. Technology. 2014. "Assessment Of Water Quality Of Hatirjheel Lake In Dhaka City." 2(6):97–100.

20. Kim, In Suk et al. 2007. "Diversity of Ampicillin Resistance Genes and Antimicrobial Susceptibility Patterns in Haemophilus Influenzae Strains Isolated in Korea." *Antimicrobial Agents and Chemotherapy* 51(2):453–60.

21. Liu, Li et al. 2012. "Global, Regional, and National Causes of Child Mortality: An Updated Systematic Analysis for 2010 with Time Trends since 2000." *The Lancet* 379(9832):2151–61.

22. Macwilliams, Maria P. 2013. “Indole Test Protocol Author Information.” (April):2–6.

23. Munita, Jose M., Cesar A. Arias, Antimicrobial Resistance Unit, and Alemana De Santiago. 2016. “HHS Public Access.” *Mechanisms of Antibiotic Resistance* 4(2):1–37.

24. Puzari, Minakshi, Mohan Sharma, and Pankaj Chetia. 2017. “Emergence of Antibiotic Resistant Shigella Species: A Matter of Concern.” *Journal of Infection and Public Health* 11(4):451–54.

25. Qadri, Firdausi, Ann-Mari Svennerholm, A. S. G. Faruque, and R. Bradley Sack. 2005. “Enterotoxigenic Escherichia Coli in Developing Countries: Epidemiology, Microbiology, Clinical Features, Treatment, and Prevention.” *Clin Microbiol Rev* 18(3):465–83.

26. Quadri, Farheen et al. 2013. “Health Care Use Patterns for Diarrhea in Children in Low-Income Periurban Communities of Karachi, Pakistan.” *American Journal of Tropical Medicine and Hygiene* 89(SUPPL.1):49–55.

27. Rahman, Ahmed Ehsanur et al. 2014. “Childhood Diarrhoeal Deaths in Seven Low- and Middle-Income Countries.” *Bulletin of the World Health Organization* 92(9):664–71.

28. Rahman, M. a. and D. Al Bakri. 2010. “A Study on Selected Water Quality Parameters along the River Buriganga, Bangladesh.” *Iranica Journal of Energy and Environment (IJEE)* 1(2):81–92.

29. Rahman, Mahbubur et al. 2007. “Increasing Spectrum in Antimicrobial Resistance of Shigella Isolates in Bangladesh : Resistance to Azithromycin and Ceftriaxone and Decreased Susceptibility to Ciprofloxacin.” 25(2):158–67.

30. Reiner, Karen. 2016. “Catalase Test Protocol.” (November 2010):1–9.

31. Reza. 2009. “Antibiotic Resistance of <I>Escherichia Coli</I> Isolated From Poultry and Poultry Environment of Bangladesh.” *American Journal of Environmental Sciences* 5(1):47–52.

32. Reza, Arif and Tariq Bin Yousuf. 2016. “Impacts of Waste Dumping on Surface Water Quality in the Buriganga River , Bangladesh and Possible Mitigation Measures.” (April).

33. Schuettpelz, Duane H. 1969. “Fecal and Total Coliform Tests in Water Quality Evaluation.”

1–25.

34. Sharma, Anjana, Susheel Kumar Singh, and Divya Bajpai. 2010. “Phenotypic and Genotypic Characterization of Shigella Spp. with Reference to Its Virulence Genes and Antibiogram Analysis from River Narmada.” *Microbiological Research* 165(1):33–42.

35. Shields, Patricia and Laura Cathcart. 2011. “Motility Test Medium Protocol.” *American Society of Microbiology* (November 2011):1–10.

36. Sylvia, McDevitt. 2009. “Methyl Red and Voges-Proskauer Test Protocols.” *American Society for Microbiology* (December 2009):6.

37. Ullah, A; Clemett, A;Chowdhury, N;Huq, T;Sultana, R;Chadwick, M. 2001. “Human Health and Industrial Pollution in Bangladesh Human Health and Industrial Pollution in Bangladesh.”

38. World Health Organization. 1995. “Water and Public Health Session Objectives.” *Water and Public Health* 1–19.

Appendix

Media compositions

The composition of all media used in the study is given below:

All the media used were from Himedia

MacConkey agar:

Composition	**Amount (g/L)**
Peptones (meat and casein)	3
Pancreatic digest of gelatin	17
Lactose monohydrate	10.0
Bile salts	1.50
Sodium chloride	5.0
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
pH after sterilization(at 25°C)	7.1±0.2

MFC Agar:

Composition	**Amount (g/L)**
Tryptose	10.000
Proteose peptone	5.000
Yeast extract	3.000
Lactose	12.500
Bile salts mixture	1.500
Sodium chloride	5.000
Aniline blue	0.100
Agar	15.000
Final pH (at 25°C)	7.4±0.2

XLD Agar:

Composition	Amount (g/L)
Yeast Extract	3.0
L-Lysine	5.0
Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.0
Sodium deoxycholate	2.5
Sodium thiosulfate	6.8
Ferric ammonium citrate	0.80
Phenol red	0.80
Agar	15.0
Final pH at 25^0C	7.4±0.2

SS Agar:

Composition	Amount (g/L)
Proteose Peptone	5.0
Lactose	10.0
Bile salts mixture	8.5
Sodium citrate	8.5
Sodium thiosulfate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	13.5
Final pH at 25^0C	7.0±0.2

Nutrient Agar:

Composition	Amount (g/L)
Peptone	5.0
Sodium Chloride	5.0
Beef extract	3.0
Agar	15.0
pH	7.0

MacConkey Sorbitol agar base:

Composition	Amount (g/L)
Casein enzymic hydrolysate	17.000
Meat peptone	3.000
D-Sorbitol	10.000
Bile salts mixture	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
Final pH (at 25°C)	7.1±0.2

Nutrient Broth:

Component	Amount (g/L)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH at 25^0C	7.4±0.2

Luria Bertani Broth:

Component	Amount (g/L)
Casein enzymic hydrolysate	10.0
Yeast extract	5.0
Sodium chloride	10.0
Final pH at 25^0C	7.5±0.2

Mueller- Hinton Agar:

Component	Amount (g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH at 25^0C	7.3± 0.1

Saline:

Component	Amount (g/L)
Sodium chloride	9.0

Motility, Indole, Urease Agar:

Component	Amount (g/L)
Peptone	3%
Sodium chloride	0.5%
Urea	2%
Mono Potassium Phosphate	0.2%
Phenol Red	0.0005%

Agar	0.4%
pH	7

Triple Sugar Iron (TSI):

Component	**Amount (g/L)**
Beef extract	3.0
Peptone	20.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose monohydrate	1.0
Ferrous sulfate	0.2
Sodium chloride	5.0
Sodium thiosulfate	0.3
Phenol red	0.024
Agar	12

Indole Broth:

Component	**Amount (g/L)**
Peptone	10.0
Sodium chloride	5.0

Methyl Red and Voges Proskauer Media (MR-VP):

Component	**Amount (g/L)**
Peptone	7.0
Dextrose	5.0
Dipotassium hydrogen phosphate	5.0
Final pH	7.0

Reagents and Buffer

Gram's iodine (300 ml)

To 300 ml distilled water, 1 g iodine and 2 g potassium iodide was added. The solution was mixed on a magnetic stirrer overnight and transferred to a reagent bottle and stored at room temperature.

Crystal Violet (100 ml)

To 29 ml 95% ethyl alcohol, 2 g crystal violet was dissolved. To 80 ml distilled water, 0.8 g ammonium oxalate was dissolved. The two solutions were mixed to make the stain and stored in a reagent bottle at room temperature.

Safranin (100ml)

To 10 ml 95% ethanol, 2.5 g safranin was dissolved. Distilled water was added to the solution to make a final volume of 100 ml. The final solution was stored in a reagent bottle at room temperature.

Kovac's Reagent (150 ml)

To a reagent bottle, 150 ml of reagent grade isoamyl alcohol, 10 g of pdimethylaminobenzaldehyde (DMAB) and 50 ml of HCl (concentrated) were added and mixed. The reagent bottle was then covered with an aluminum foil to prevent exposure of reagent to light and stored at 4°C.

Methyl Red (200 ml)

In a reagent bottle, 1 g of methyl red powder was completely dissolved in 300 ml of ethanol (95%). 200 ml of destilled water was added to make 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol and stored at 4°C.

Barrit's Reagent A (100 ml)

5% (wt/vol) a-naphthol was added to 100 ml absolute ethanol and stored in a reagent bottle at

4°C.

Barrit's Reagent B (100 ml)

40% (wt/vol) KOH was added to 100 ml distilled water and stored in a reagent bottle at 4°C.

Catalase Reagent (20 ml 3% hydrogen peroxide)

From a stock solution of 35 % hydrogen peroxide, 583 µl solution was added to 19.417 ml distilled water and stored at 4°C in a reagent bottle.

Urease Reagent (50 ml 40% urea solution)

To 50 ml distilled water, 20 g pure urea powder was added. The solution was filtered through a HEPA filter and collected into a reagent bottle. The solution was stored at room temperature.

1M Tris HCl:

In a McCartney bottle, 1.576g Tris HCl was added. Then 10 ml distilled water was added to prepare 10 ml 1M Tris HCl. After that pH was adjusted to 8. Then it was stored at 4ºC.

0.5M EDTA:

In a McCartney bottle, 1.861 g EDTA was added. Then 10 ml distilled water was added to prepare 10 ml 0.5M EDTA. After that pH was adjusted to 8. Then it was stored at room temperature.

1X TBE Buffer:

In a Durham bottle, 5.4 g of Tris base, 2.75 g of Boric Acid, 2ml of 0.5M EDTA were added. Then 500 ml distilled water was added to prepare 500 ml 1X TBE Buffer. After that pH of the buffer was adjusted to 8. Then it was autoclaved at 15psi 121ºC. After autoclave, it was stored at room temperature.

Supplementary

Supplementary

PCR mixture preparation:

component	Amount (µl) for a 25 µl PCR reaction volume
Eva Green Super Mix (X2) Bio Rad	**12.5**
Nuclease free water	**6**
Primer F (Forward) 100 pmol	**2**
Primer R (Reverse) 100 pmol	**2**
DNA template	**2.5**
MQ total volume	**25**

PCR Programs used for target DNA amplification:

Shigella spp

Temperature	**94˚C**	**94˚C**	**36˚C**	**72˚C**	**72˚C**
time	**3 min**	**1 min**	**1 min**	**2min**	**10 min**
Cycles 45					

***E.coli* O157:H7**

Temperature	**94˚C**	**94˚C**	**55˚C**	**72˚C**	**72˚C**
time	**2**	**1 min**	**1 min**	**2min**	**5 min**
Cycles 35					

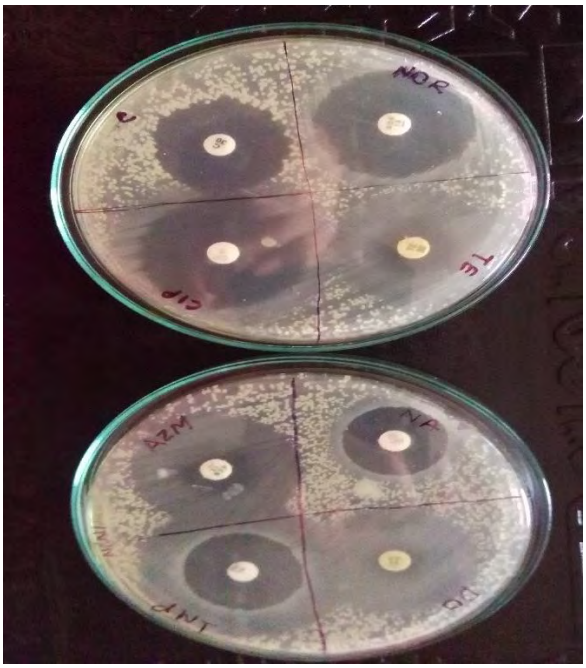

Figure: Antibiogram

Recipe of solutions used for Plasmid Extraction

Plasmid DNA extraction of the isolates were performed using the alkaline lysis method. For this technique, essentially 3 solutions were used which were added to the pellet after centrifugation.

1. Solution I:

- Tris
- EDTA
- Glucose

2. Solution II:

- NaOH
- SDS

3. Solution III: (ICE COLD)

- Sodium Acetate
- Acetic acid