PREVALENCE OF PATHOGENIC *Escherichia coli* VIRULENCE GENES IN RAW AND DOMESTICALLY TREATED WATERS IN DHAKA CITY: AN OVERLOOKED HEALTH HAZARD ANALYSIS

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A thesis submitted to the Department of Mathematics and Natural Sciences, Microbiology Program, in partial fulfillment of the requirements for the degree of Bachelors of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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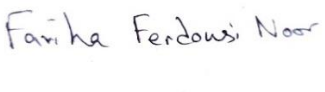

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Abstract

Diarrhea is one of the leading causes of infant mortality in developing countries like Bangladesh. This study aims to detect the presence of fecal coliform in the supplied water and treated water in the households of Dhaka city. The samples were collected from October 2018 till August 2019. The samples were further subjected to PCR to detect the presence of diarrheagenic *E.coli.* Nine primer pairs were used detect the five diarrheagenic strains of *E.coli* (EPEC, EHEC, ETEC, EAEC and EIEC). Fecal coliform counts produced by supplied water samples and treated water samples were categorized into the following: 0 count, 1-5 count, 6-30 count, 31-100 count, >100 count. Fecal coliform was not detected in 29.46% of the supplied water samples. On the other hand, 18.26% of the supplied water samples produced a fecal coliform count of greater than 100. 12.86% of supplied water samples have a count varying between 1 to 5, 27.81% of the samples have a 6 to 30 fecal coliform count and 11.62% of the samples have 31 to 100 fecal coliform count. ETEC was detected in 14.94% of the samples while EHEC was detected in 12.24% of the supplied water samples. 3.94% of the supplied water samples had EAEC. EPEC and EIEC were detected in 1.45% of the supplied water samples only. 52.62% of the treated water samples did not produce any fecal coliform count, while 1.65% of the treated water samples had a fecal coliform count greater than 100. 14.88%, 21.49% and 9.37% of the treated water samples had a fecal coliform count of 1-5, 6-30 and 31-100 respectively. ETEC, EHEC and EAEC were detected in 3.03%, 2.75% and 0.83% of the treated water samples respectively. EPEC and EIEC were not detected in the treated water samples.

Keywords: Diarrheagenic *Escherichia coli*: EPEC, ETEC, EHEC, EIEC and EAEC: Dhaka city: Household water: Supplied water: Treated water

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Chapter 1

Introduction

E.2. Significance of clean and safe water:

The significance of pursuing the development of a safe water supply for mankind all over the globe can't be overemphasized. Safe water is important for a person's well-being and is a fundamental human right. In developed countries, practically all illnesses, for example, Cholera, Polio, and Typhoid fever were abolished by providing adequate amounts of safe and clean drinking water. If a greater quantity of safe and pure water supply in developing countries could be ensured then an equivalent situation would have prevailed. No measure of clinical supplies or healthcare facilities can accomplish such an objective at such a low cost. Creating water systems that permit more than six billion individuals to have safe and clean water is no little accomplishment. One billion individuals are getting access to clean water due to the combined effort of government and global organizations, like, **UNICEF** and the **World Health Organization (WHO)**. However, more than one billion individuals still do not have access to safe water supply. According to the 2008 UNICEF **''Handbook on Water Quality,"** lacking water supplies combined with inefficient sterilization causes **3.4 million deaths every year**, which converts into somebody biting the dust **every 10 seconds**. Most of these deaths happen to kids due to their underdeveloped immune system and their higher susceptibility to catching maladies. These are some of the reasons why drinking clean and safe water is of the utmost importance.

1.2. Factors determining the safety of municipal water:

The condition and the purity of water mainly depend on the quality of the source from where the water was supplied, the treatment that is used in municipal water treatment plants, the amount of leftover disinfectant staying in the water and the condition in the conveyance network(pipes and overhead/underground reservoirs).
Length and duration of the treatment given to the water sources, materials, and length of the distribution pipes, total carbon, iron, lead, phosphate, and sulfate contents of the water, physical parameters of the water (pH, alkalinity, turbidity, hardness, conductivity), biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), loose deposit accumulation inside the pipes, and the overhead tanks and microbes remaining in the supply water are all factors that might contribute to the quality deterioration of the supply water.

1.3. Possibilities of fecal contamination

Diarrheal disease is one of the main reasons for high morbidity and mortality in a developing country like Bangladesh. Diarrhea, dysentery, typhoid, parasitic worm infectionsare often caused by the consumption of impure water and food. Various diseases are caused by several strains of *Escherichia coli*, which are found in drinking water. These diseases include diarrhea, dysentery, hemolytic uremia syndrome (kidney failure), bladder infections, septicemia, pneumonia and meningitis (Acharjee et al., 2011). Shiga toxins (Stx) also known as verotoxins (Vtx), include two major subtypes, shiga toxin 1 (Stx1) and shiga toxin 2 (Stx2). They produce organic acid and gas since they are facultative aerobes and are capable of sugar fermentation. These genera create different acids after fermentation and hence are known as “mixed acid fermenters”. They have different physiological characteristics. According to the World Health Organization (WHO), more than 45,000 children under 5 die each year due to diarrhea caused by drinking contaminated water. Having *E.coli* in water is a solid signal of recent sewage or animal waste contamination. It also shows the microbiological quality of water. The reasons behind drinking impure water might be poverty, lack of awareness and enough pure water. Some of the most important waterborne pathogens are listed in the following table.

Table 1.3.1 Waterborne pathogens of concern

Name of micro-organism	**Major associated diseases**	**Major reservoirs and primary sources**
Enteropathogen E. coli	Gastroenteritis	Human feces
*Shigella*spp.	Bacillary dysentery	Human feces
Vibrio cholera	Cholera	Human feces and freshwater zooplankton
Yersinia enterocolitica	Gastroenteritis	Human and animal feces
Campylobacter jejuni	Gastroenteritis	Human and animal feces
Salmonella typhi	Typhoid fever	Human feces
Salmonella paratyphi	Paratyphoid fever	Human feces
Other *Salmonella*	Salmonellosis	Human and animal feces
*Legionella pneumophila*andrelated bacteria	Acute respiratory illness (legionellosis)	Thermally enriched water
*Leptospira*spp.	Leptospirosis	Animal and human urine
Various mycobacteria	Pulmonary illness	Soil and water
Opportunistic bacteria	Variable	Natural waters

According to World Health Organization (WHO), almost 1.1 billion people around the world drink contaminated water (Kindhauser, 2003), and 88% of diarrheal diseasesare caused by the consumption of this contaminated water. Furthermore, social and environmental changes keep on bringing about new or re-emerging waterborne pathogen issues. 2.4% of world-wide diarrhea, 6% of malaria in a few developing countries and 7% of dengue fever in some developed countries in 2000 can be seen as an example of this. In total, the probable mortality was 1,54,000 (0.3%) deaths and the imputable burden was 5.5 million (0.4%) (Ashbolt, 2004). Climate change causes flooding and droughts, which in turn have a huge impact on drinking water and sanitation infrastructure. In addition to this, it can cause a variety of health-related issues. For instance, fecal contaminants are spread with the floodwater, which increases the risk of an outbreak of waterborne diseases like Cholera.

1.4. Possibilities of infection via drinking water

Safe water is fundamental for healthy living. However, large groups of people do not have access to safe water for consumption and other uses (WHO, 2008). Illness arises due to washing drinking and preparing food with contaminated water. The term waterborne illness is held by a large groupof diseases that are mainly transmitted by contact with or utilization of tainted water. Contaminants, such as microorganisms or parasites may inadvertently enter the drinking water supply and cause a number of infections, despite maintaining pristine conditions. However, such infections are quite rare and classifying these microorganisms or parasites or the diseases they may cause as “waterborne” would be inaccurate.

Drinking contaminated water can be a vector for the transmission of transferable illnesses (Andersson and Stenstrøm, 1986; Galbraith et al., 1987; Benton et al., 1989; Craun, 1991). Despite the fact that centralizations of pathogenic microorganisms may be exceptionally low, drinking water shows up at basically every person from a mass. Infections may prompt gastro-enteritis with watery loose bowels, vomiting, and stomach torment. (P.F.M Teunis, 1998)

1.5. Water-borne diseases in Dhaka

A pathogen is a microorganism equipped for causing ailment in a host. Compared to the microbial populace on earth, just a modest number are capable of causing sickness in people. Waterborne pathogens are discharged with the feces of people and transmitted through ingestion of substances that is contaminated with fecal material. Conversely, water-based pathogens happen normally in water and are typically not transmitted from individual to individual (e.g., *Legionella* spp.).

As of now, an excess of 140 known microorganisms are perceived as waterborne pathogens. Waterborne pathogens have been studied in detail for various reasons (Reynolds 2008).

Bangladesh is a country that is prone to flooding. Its capital Dhaka is often waterlogged during the monsoon season. Due to this reason, many water-borne diseases have been observed in Dhaka city. (Brian S. Schwartz, 2006). Urban poverty is largely due to the migration of the rural population to metropolitan cities. Sanitation is quite unhygienic in Dhaka city. In any case, Bangladesh produces 17 million metric tons of human feces and 57 million metric tons of urine every year. A significant segment of these excreta is dumped into water bodies and open spots. Hence, contamination of water sources and groundwater is a common phenomenon. A dominant part of the population in Dhaka experiences the ill effects of various sorts of water and excreta-borne diseases (PramanikBiplob, RUET).Amongst many of the water-borne diseases diarrhea, typhoid, cholera, and hepatitis A are the most common diseases in Dhaka city.

Escherichia coli (E. coli) microbes typically live in the digestion tracts of sound individuals and creatures. Most kinds of *E. coli* are innocuous or cause moderately concise looseness of the bowels. In any case, a couple of especially terrible strains, for example, *E. coli* O157:H7, can cause serious stomach cramps, bloody diarrhea, and vomiting.

One might be presented to *E. coli* from polluted water or food — particularly crude vegetables and half-cooked ground meat. Adults ordinarily recoup from contamination with *E. coli* O157:H7 inside seven days, however, young children and the older generation have a higher risk of building up a perilous type of kidney failure called the hemolytic-uremic syndrome.Hemolytic–uremic syndrome (HUS) is a gathering of blood issues described by low red platelets, intense kidney failure, and low platelets. Side effects ordinarily incorporate bloody diarrhea, fever, and vomiting. Kidney issues and low platelets at that point happen as the diarrhea is improving.

1.6. Five Diarrheagenic E .Coli Types

Of all the bacterial species, *Escherichia coli*, referred to as fecal coliforms or thermotolerant coliforms alongside some other species, has been one of the focal points of water bacteriology. The species was brought into water bacteriology, not due to its characteristic pathogenicity, but since it was a valuable marker of fecal contamination. The hypothesis was that if *E. coli* was available at that water resource, so could be pathogenic enteric microscopic organisms, for example, *Shigella* and *Salmonella* spp. Despite the realization that *E. coli* can cause ailment in people, its job as an enteric microbe in its own right has been recently reinforced with the appearance of *E. coli* O157:H7 and its relationship with hemorrhagic enteritis and hemolytic uremic syndrome. *Escherichia coli* are motile, non-spore forming, gram-negative bacilli. Usually, they are lactose fermenters and give a positive o-nitrophenyl-beta-d-galactopyranoside (ONPG) response. The repository for *E. coli* is in the digestive tracts of man and other warm-blooded creatures. In spite of the fact that it will get by in nature, it does not reproduce in nature and eventually ceases to exist (Feachem et al. 1983). Thus, when *E. coli*is found in nature, it is taken as a sign of fecal contamination. There has been some proof that *E. coli* can endure and multiply in tropical conditions thus its use as a marker of fecal contamination in the tropics is currently dubious (Rivera et al. 1988). *Escherichia coli* can cause an assortment of diseases in people. More often than not, this is because of the spread from the intestinal flora when the patient has some other deficiency or issue. Be that as it may, certain strains of *E.coli* can likewise cause the runs. In these cases, contamination follows immediate or backhanded fecal-oral spread from different people or creatures. This paper considers diarrheal diseases because of the different pathogenic kinds of *E. coli*. (Nataro and Kaper 1998).

***E. coli* infections are typically classified into five categories:**

- Enterotoxigenic (ETEC)
- Enteropathogenic (EPEC)
- Enteroinvasive (EIEC)
- Enterohemorrhagic (EHEC)
- Enteroaggregative (EAEC)

ETEC

ETEC strains produce a heat-stable (ST) and/or a heat-labile (LT) enterotoxin (Sears &Kaper 1996). ST (Sta and STb) and LT (LT-I and LT-II) are the two types of antigenic. STs are short peptides and LTs are large oligomeric toxins. Cholera toxins are relatable to LTs.

Watery diarrhea and abdominal cramping can be caused by ETEC. Chills, loss of appetite, fever, nausea, and bloating can occur as well but are not very common. These infections caused by ETEC can occur due to the consumption of contaminated water or food (from CDC).

EPEC

EPEC is a non-shiga toxin producing, gram-negative pathogen, serotype of *E. coli*. Eae+bfp=stx are classified as the strain of EPEC which belongs to classic *E.coli* serotypes and localized-like (LAL), diffuse (DA), aggregative adherence (AA) patterns are showed by these strains. (Theresa J. Ochoa)

EPEC fundamentally causes infection in kids under 2 years old and especially in infants under the age of 6 months. EPEC is a significant reason for diarrhea in infants. It is assumed that the disease is spread mainly from individual to individual.

EHEC

EHEC joins the harmfulness component of the EPEC with the creation of a toxin, the Shiga toxin. Stx 1 is identical to the Shigatoxin of *S.dysenteriae* type1.Stx2 is immunologically unmistakable and is just some 55–57% homologous. Inside the cytoplasm, the toxin disturbs protein synthesis and causes cell death. Looseness of the bowels is most likely brought about by the death of the intestinal absorptive cells while leaving the secretary cells flawless. The advancement of the hemolytic uraemic syndrome (HUS) is thought to follow after this toxin reaches the kidneys by traveling through blood. HUS is a mix of hemolytic weakness, thrombocytopenic purpura, and severe renal failure. The most frequently reported EHEC strain is Serotype O157:H7. (Paul R. Hunter,2003)

EHEC can cause bloody diarrhea and that can lead to hemolytic-uremic syndrome(Larry M. Bush). The outbreak can occur by consumption of contaminated drinking water. (Paul R. Hunter,2003)

EIEC

EIEC are closely related to *Shigella.* They use adhesin proteins to bind to and enter the intestinal cells and are highly invasive. It does not produce enterotoxins.

Abdominal cramps, malaise, tenesmus are caused by this but dysentery is an uncommon outcome. Most diseases caused by EIEC are believed to be food-or waterborne. (Lanyi et al. 1959)

EAEC

Enteroaggregative*Escherichia coli* strains (EAEC) are responsible for diarrhea. And have been involved in flare-ups of this illness. Strains of this classification are portrayed by the capacity to display an ordinary 'stacked block-like' example of aggregative adherence (AA) on the surfaces of Hep-2 and HeLa cells, just as the interceding development surfaces (Sergio Suzart, 2001). EAEC's strain 104: H4 is responsible for causing diseases. (Paul R. Hunter,2003)

1.7. Importance of one-step multiplex PCR (MPCR)

Traditional techniques for determining the presence of specific species are tedious and work concentrated thus, molecular methods, which offer speed, sensitivity and explicitness, have been created to address this issue. Multiplex PCR (MPCR) is generally applied in the different fields of microbiology for the rapid differentiation of microbial species without trading off accuracy (Settanni&Corsetti, 2007). Routine identification and differentiation of diarrheagenic*E. Coli*are normally founded, based on a mix of biochemical tests, serotyping, phenotypic assay dependent on virulence characteristics and molecular detection methods (Müller et al., 2007). Five sorts of diarrheagenic*E. Coli*have been detected from the water samples. EHEC, ETEC, EPEC, EAEC, and EIEC are the strains that have been identified. To recognize these five diarrheagenic types quickly, we developed a one-step multiplex PCR (MPPCR) assay utilizing nine primer pairs to amplify nine virulence genes explicit to the distinctive virotypes, with each gathering being represented (i.e., *stx*1 and *stx*2 for EHEC, *lt*, *st*h, and *st*p for ETEC, *eae*A and *bfp*A for EPEC, *agg*R for EAEC, and *ipa*H for EIEC) (Oh et al., 2014).

1.8. Selected zones of this study

Dhaka is one of the quickest developing megacity in the world. The total area of Dhaka city is 300 square kilometers where 18.237 million individuals are living. It is one of the most densely populated areas on the planet, with a density of 23,234 individuals for each square kilometer. Dhaka city is partitioned into various zones. The samples were collected and examined from Gulshan, Banani, Niketon, Baridhara, Badda, Khilgaon, Mohammadpur, Lalmatia, Uttara, Mirpur, Mugdapara, Tolarbagh, Moghbazar, Shanti Nagar, Jatrabari, Bangshal, Wari, Dhanmondi, Farmgate, Tejgaon, Mohakhali, Rampura, Bashundhara and Korail slum. Mirpur and Mohammadpur are the most populated area of Dhaka city. The total area of Mirpur is 58.66 km², where, 1074,232 people are living. In an area of 11.65 km² of Mohammadpur, there are 355,843 people residents. Gulshan and Uttara with population density, 29,086/ km² and 25,701/ km², are the most reputed area in Dhaka city.

E.2. Aim of the study

The goal of the study was to appraise the microbiological quality of treated water sources in Dhaka city by detecting the prevalence of diarrheagenic*E. Coli* in treated water system. Presence of *E. coli* indicates that fecal material is present in the water sample. This was used as evidence that sewage material had leaked into the water distribution system at some point. Samples were collected from the areas mentioned above because the aim was to get an idea of thesupplied water quality of the entire city. Alongside evaluating the water qualities, this study also identifies the potential risk of consumption of such waters. The persistence of *E. coli* in the water samples after subjecting it to boiling or passing through some kind of home water filtration has been tested as well. Molecular methods were used to detect the pathogen as traditional methods consume much time.

Chapter 2

Materials and Methods

2.1. Sample Collection Site

845 water samples were collected from 174 different households present in many different areas of Dhaka city. The areas were grouped to form 11 larger Areas.

Area 1 (Gulshan, Banani, Niketon, Baridhara, Badda)

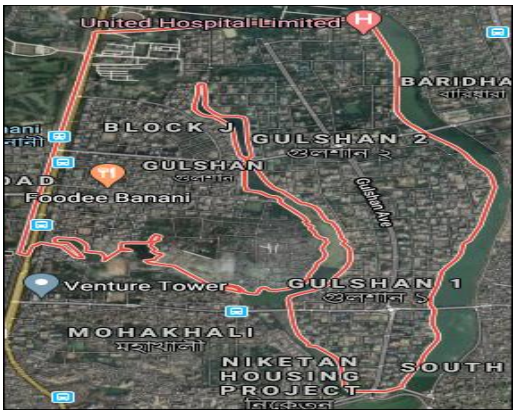

Figure 2.1.1.1: Sampling Site of Gulshan

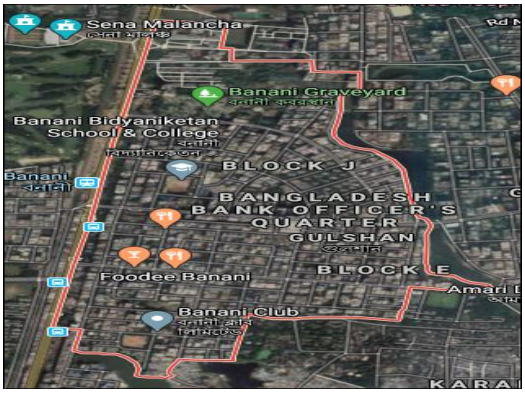

Figure 2.1.1.2: Sampling Site of Banani

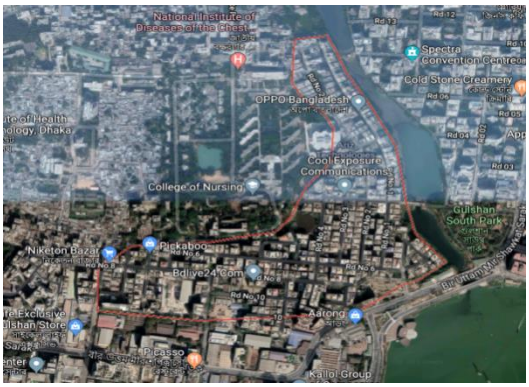

Figure 2.1.1.3: Sampling Site of Niketon

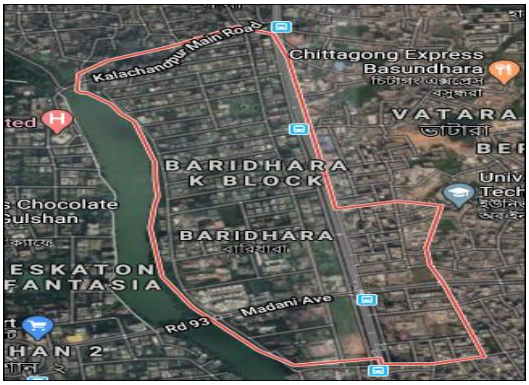

Figure 2.1.1.4: Sampling Site of Baridhara

Figure 2.1.1.5: Sampling Site of Badda

Area 2 (Khilgaon)

Figure 2.1.2.1: Sampling Site of Khilgaon

Area 3 (Mohammadpur, Lalmatia)

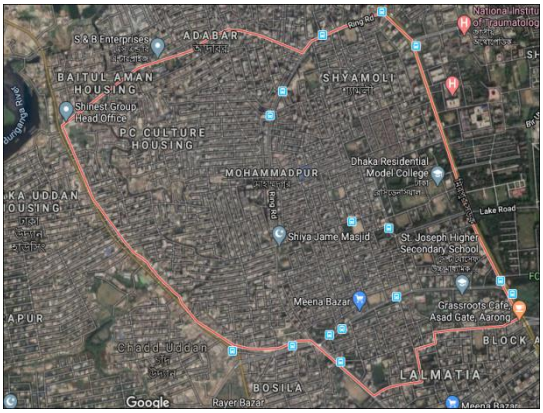

Figure 2.1.3.1: Sampling Site of Mohammadpur

Figure 2.1.3.2: Sampling Site of Lalmatia

Area 4 (Uttara)

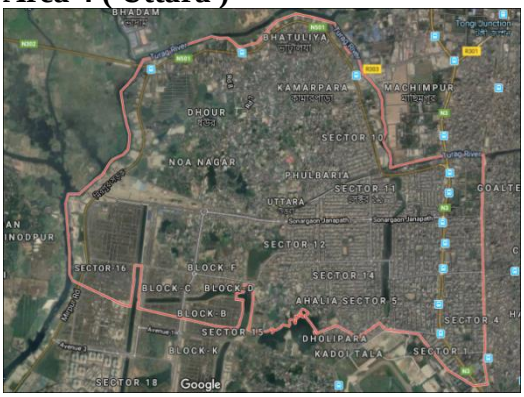

Figure 2.1.9: Sampling Site of Uttara

Area 5 (Mirpur, Mugdapara, Tolarbagh)

Figure 2.1.5.1: Sampling Site of Mirpur

Figure 2.1.5.2: Sampling Site of Mughdapara

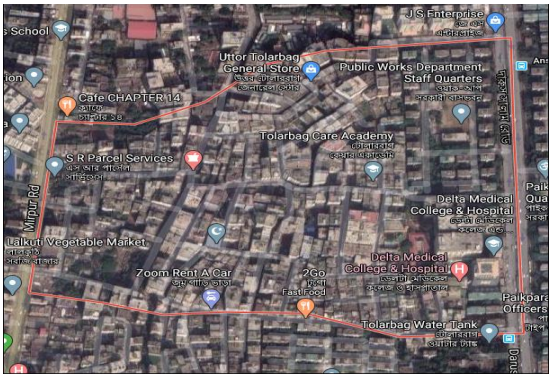

Figure 2.1.5.3: Sampling Site of Tolabagh

Area 6 (Moghbazar, Shanti Nagar)

Figure 2.1.6.1: Sampling Site of Moghbazar

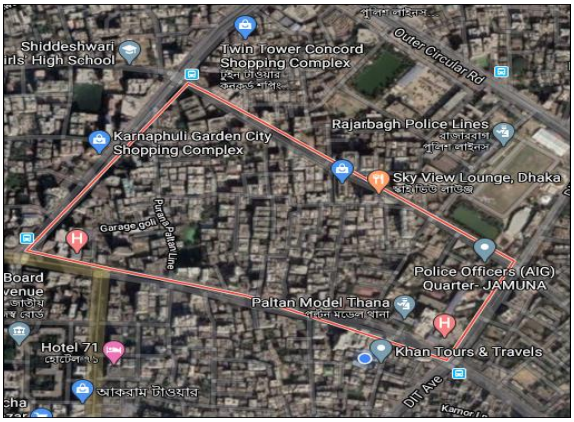

Figure 2.1.6.2: Sampling Site of Shanti Nagar

Area 7 (Jatrabari, Bangshal, Wari)

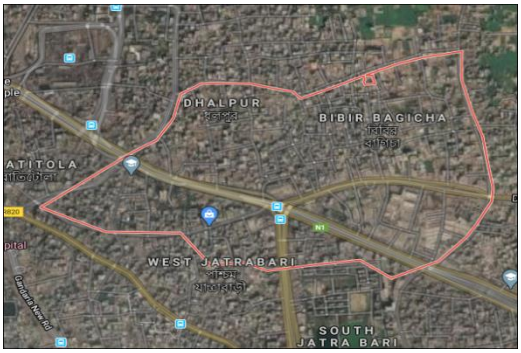

Figure 2.1.7.1: Sampling Site of Jatarabari

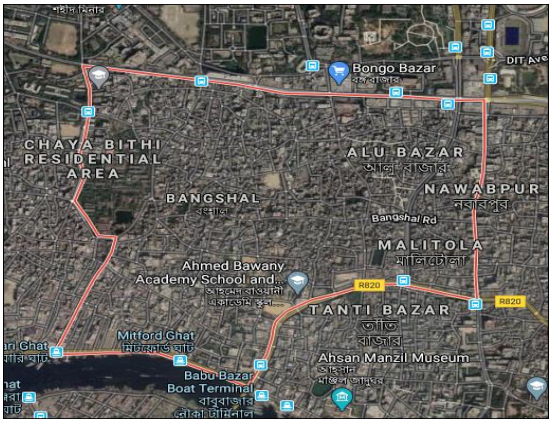

Figure 2.1.7.2: Sampling Site of Bangshal

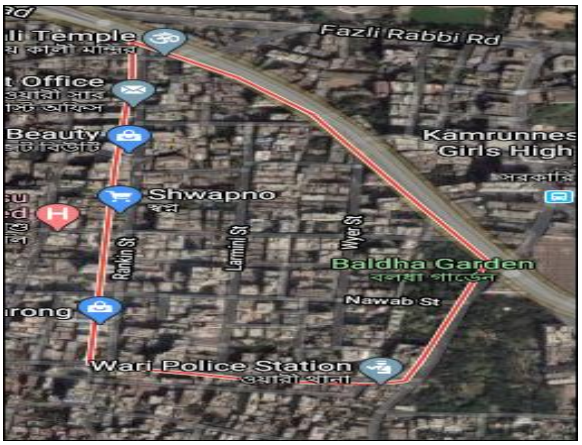

Figure 2.1.7.3: Sampling Site of Wari

Area 8 (Dhanmondi, Farmgate, Tejgaon)

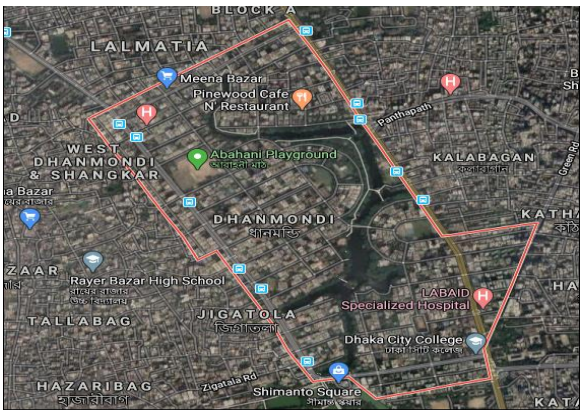

Figure 2.1.8.1: Sampling Site of Dhanmondi

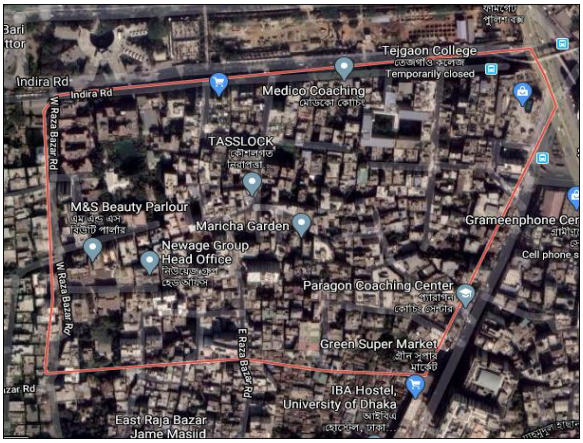

Figure 2.1.8.2: Sampling Site of Farmgate

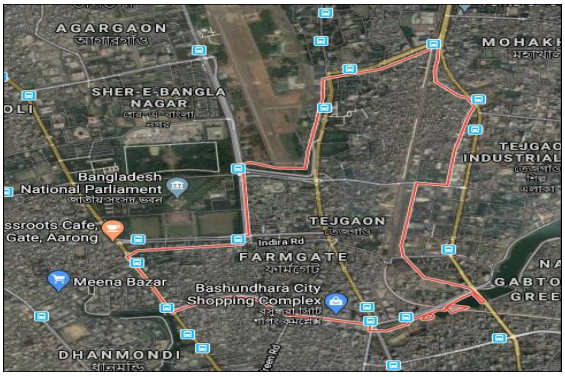

Figure 2.1.8.3: Sampling Site of Tejgaon

Area 9 (Mohakhali, Rampura)

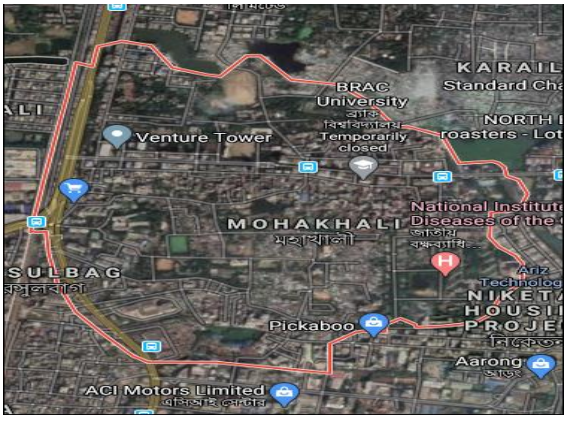

Figure 2.1.9.1: Sampling Site of Mohakhali

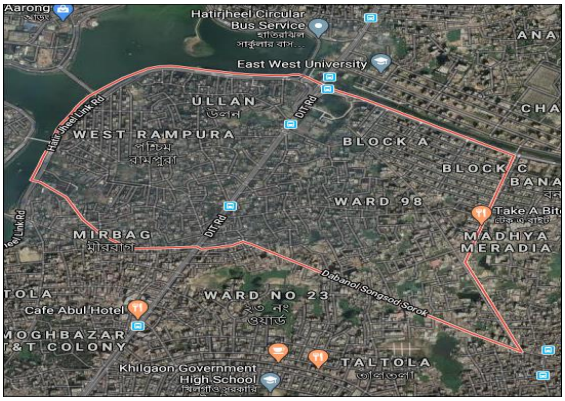

Figure 2.1.9.2: Sampling Site of Rampura

Area 10 (Bashundhara)

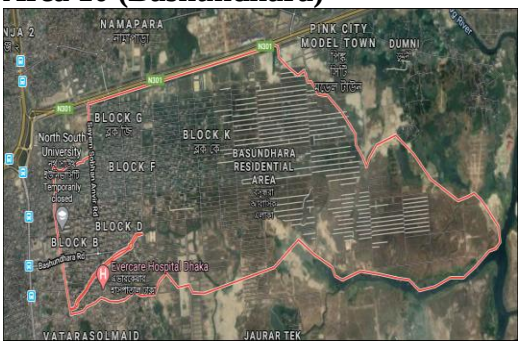

Figure 2.1.11.1: Sampling Site of Bashundhara

Area 11 (Korail slum)

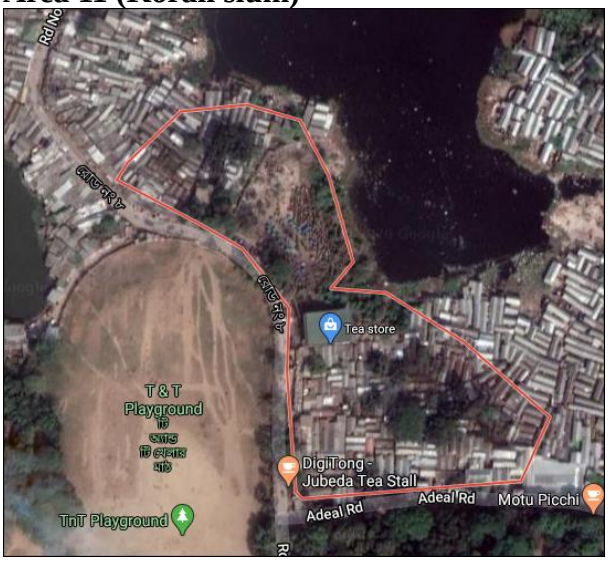

Fig 2.1.11.1 Sampling site of Korail slum

2.2. Sample Collection:

From September 2018 to August 2019, water samples from designated areas were collectedregularly. Sample collecting bottles were autoclaved before the time of collection.

2.3. Sample Processing

2.3.1. Filtration

After collecting 100 ml of samples, the samples were filtered by using membrane filter papers and were placed on m-FC agar and incubated for 24 hours at 44.5°C.Colonies made by fecal coliforms can appear in several shades of blue-colored colonies above the membrane filter paper. By sub-culturing the colonies on EMB they were verified as fecal coliform. Just the colonies that had shown up as metallic green sheen were *E. coli*. Again 100ml of water from the same samples was filtered. Membrane filter papers were set in conical flasks containing 50ml of TSB enhanced with 2.5% NaCl and the flasks were incubated at 37°C for 12 hours. 300µl of autoclaved glycerol were set in autoclaved microcentrifuge tubes. After incubation, TSB were moved into microcentrifuge tubes and vortexed. Micro-centrifuge tubes were stored at – 20◦C. 1.5ml of incubated TSB were moved to autoclaved microcentrifuge tubes and centrifuged at 14,000 rpm for 10 minutes. After centrifugation, the supernatants were disposed of. The pellets were stored at – 20℃ after the mouth of the micro-centrifuge tubes had been wrapped with parafilmfor DNA extraction.

2.3.2. DNA extraction protocol

Following 12 hours of incubation 1.7ml of TSB were moved to 2ml small micro-centrifuge tubes and loaded with 0.3ml of glycerol. TSB containing microscopic organisms were moved to small micro-

centrifuge tubes about 1.5ml and that centrifuged for 10 minutes at 14,000 rpm (Kobayashi et al., 2009). The pellets were collected and included 400µl of distilled water that was stored at room temperature and inverted the mixture for washing. The mixtures were centrifuged for 5 minutes at 13,000 rpm and afterward discarded supernatant. At that point, the pellets were re-suspended with 400µl of distilled water. The cells were lysed at 100◦C for 7 minutes. After heat shock, micro-centrifuge tubes were moved in ice for 10 minutes for a cold shock. After 10 minutes, centrifuges were performed for 5 minutes at 13,000 rpm. The supernatant contained with DNA were moved into new micro-centrifuge tubes and wrapped with parafilmand stored at – 20◦C. The supernatant were exposed to gel run to check for the presence of raw DNA.

2.4. Multiplex PCR assay for the discovery of five pathogenic Escherichia coli (E.coli) *types causing Diarrhea*

Table 2.4.1. Reference strains used as positive and negative controls in this study

Strain	**Genes**	**Control**	**Reference strain**
ETEC	*lt* *st*h *st*p	(+)ve	Isolated from the laboratory
EAEC	*agg*R	(+)ve	
EPEC	*eae*A *bfp*A	(+)ve	
EIEC	*ipa*H	(+)ve	
EHEC	*stx*1 *stx*2	(+)ve	
Salmonella typhimurium	*inv*A	(-)ve	

2.4.1. Construction of controls for PCR

In order to construct the positive control for PCR, reference bacterial strains of *Escherichia coli* (EPEC, EHEC, EIEC, EAEC and ETEC) were streaked onto nutrient agar (NA) and incubated for 24 hours at 37ºC. The strains were then again streaked onto EMB agar and incubated for 24 hours at 37ºC. After incubation, single colonies were picked and incubated in nutrient broth (NB).After incubation NB containing micro-centrifuge tubes were centrifuged for 10 minutes at 14,000 rpm. These pellets were utilized for DNA extraction. After DNA extraction, gel electrophoresis was performed to observe the presence of raw DNA. After confirming, these raw DNA were subjected to virulence genes specific PCR and confirmed the presence of those genes by observing expected band sizes in the gel.
Construction of the negative control for PCR were prepared by streaking *S. typhimurium*(Table 2.4.1) onto nutrient agar (NA) and incubated for 24 hours at 37ºC. After incubation, single colonies were picked and incubated in nutrient broth (NB). DNA extraction and gel electrophoresis was achieved following the same methods as positive control.
To run the multiplex PCR, we used DNA of only fecal coliform (+) ve samples, gathered from different zones in Dhaka city, to determine the presence of pathogenic *E.coli* virulence genes in these samples.

2.4.2. PCR conditions

PCR assay was performed in tubes with a total volume of 20µl. The reaction mixtures commonly contained nuclease-free water 4µl, forward primer 2µl, reverse primer 2µl, template 2µl. And master mix 10µl.
Primer pairs utilized for identification of marker virulence genes indicative of pathogenic *Escherichia coli* typesare shown in table 2.4.2.

Table 2.4.2: Primers used for the identification of marker virulence genes indicative of pathogenic Escherichia coli

Strain	**Target gene**	**Primer name**	**Sequence 5'-3'**	**Product size (bp)**	**Concentration of primer (nM)**	**Reference**
EHEC	*stx*2	EC-vt2_2-F	TAC CAC TCT GCA ACG TGT CG CGA TAC TCC GGA AGC ACA TT	297	75 75	(Oh et al., 2014)

		EC-vt2_2-R			
	*stx*1	EC-vt1_2-F EC-vt1_2-R	CGT CTT TAC TGA TGA TTG ATA GTG GC CGC GAT GCA TGA TGATGA C	637	120 120
ETEC	*st*h	ET-ST_(C)-F ST-148R	TTC GCT CAG GAT GCT AAA CCA TTA ATA GCA CCC GGT ACA AGC AGG	167	120 120
	lt	ET-LT-F ET-LT-R	GTA CTT CGA TAG AGG AAC TCA AAT GAA TAT ATT CTG GGT CTC CTC ATT ACA AGT ATC	530	240 240
EPEC	*eae*A	eaeA 220F eaeA 220R	CGG CGA TTA CGC GAA AGA CCT AAA TTT GCC GTA AAG CGG	248	120 120
	*bfp*A	EP-bfpA(400)-F3 EC-bfpA-R	AGA ATG CTA TTT CAG AAG TAA TGA GCG TTA CAT GCA GTT GCC GCT TC	400	240 240
EAEC	*agg*R	EC-aggR-F3 EC-aggR-r3	TTA AAA TAA GTC AAR AAT TGT TTT GGT GTT A ATT ATA AAA ATT AAC AAT ATC AGA ATA CAT CAG TAC AC	715	480 480
EIEC	*ipa*H	Sh-ipF1 Sh-ipR3	CCT TTT CCG CGT TCC TTG A CAG CAG CAA CAG CGA AAG AC	104	140 140

After the initial preparation was taken, PCR was performed under the following conditions: 35 cycles with an initial denaturation at 94˚C for 15 minutes, heat denaturation at 95°C for 30 seconds, primer annealing at 58°C for 60 seconds, followed by DNA extension at 72°C for 60 seconds and a final extension at 72˚C for 10 minutes in micro-centrifuge tubes gradient master cycler (Oh et al., 2014).

2.5 Gel electrophoresis

Traditional agarose gel electrophoresis was performed to confirm the PCR reaction amplified the correct target genes. The amplified DNA was separated by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized by UV trans-illuminator. A 1500 bp DNA ladder was used.

2.6 Statistical Analysis

Statistical analyses were done using SPSS (*Statistical Package for the Social Sciences) version 20.0 and Microsoft Excel.* Binary Logistic Regressions by SPSS were performed to determined the presence of *E.coli* strains and fecal coliform count in both raw and treated water and *P*-value <0.05 was considered statistically significant.

Chapter: 3

RESULTS

3.1.1 Average fecal count

Raw and treated water samples had been collected from 11 different areas in Dhaka city from October 2018 to August 2019. The average fecal count was calculated for the different areas for the duration mentioned (Table 3.1.1).

Table 3.1.1 Average fecal count for raw and treated water.

Area	**Average fecal coliform count**	
	Raw	Treated
1	43.48	6.12
2	26.46	7.71
3	25.1	6.37
4	16.38	13.64
5	38.35	30.24
6	70.33	8.02

7	55.44	0.09
8	45.91	8.5
9	87.9	16.48
10	51.9	6.75
11	265.37	48

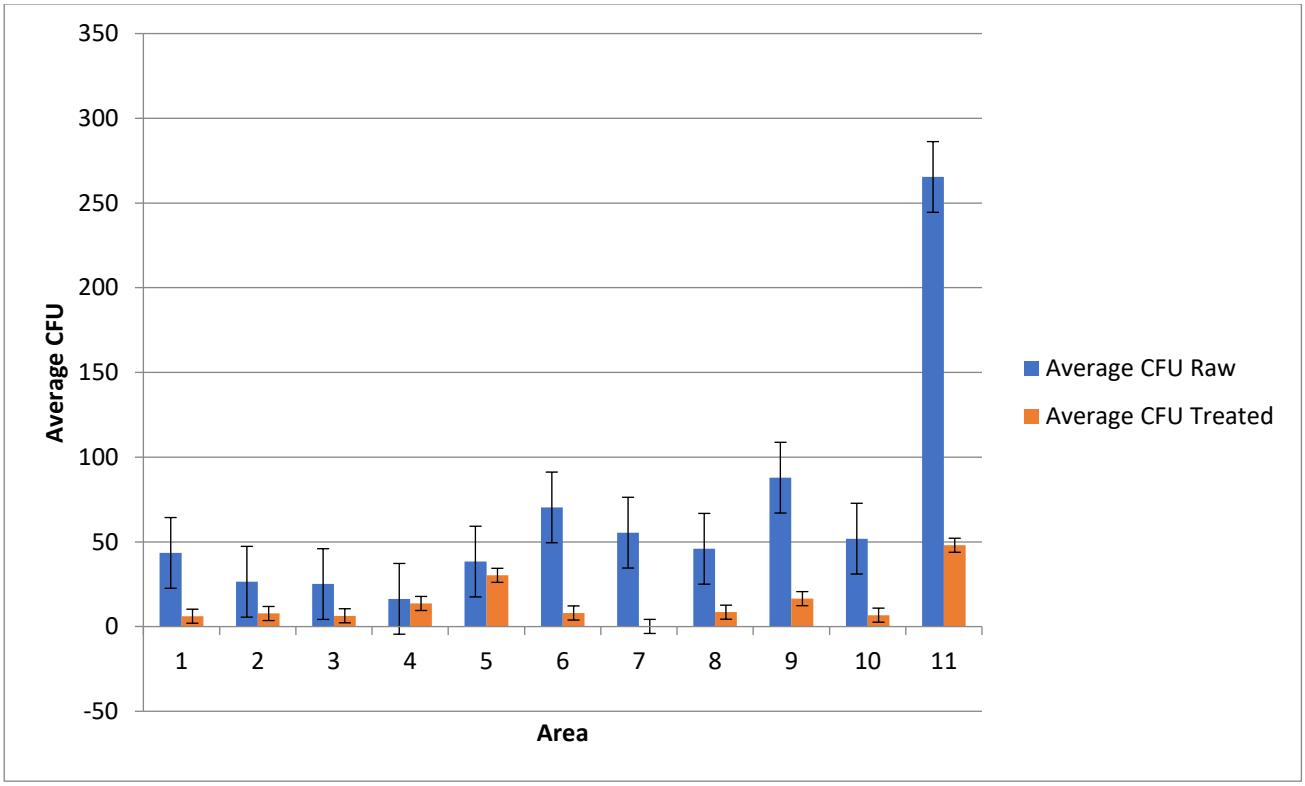

Fig 3.1.1 Bar chart depicting average fecal coliform count

Raw Water

When raw water is taken into consideration, Area 11 is the only area that has an average fecal count greater than 100. Area 6, 7, 9 and 10 have average fecal count between 50 and 100. Area 9 has the largest fecal coliform count in this range. Average fecal coliform count for Area 9 is 87.9. Area 6 follows, with an average fecal coliform count of 70.33. Area 7 has an average fecal coliform count of 55.44. This is followed by Area 10, which has an average fecal coliform count of 51.9. Area 10 has the smallest average fecal coliform count in the range of 50 to 100. Area 1, 2, 3, 4, 5 and 8 have average

fecal coliform count that ranges between 0 and 50. Area 8 has an average fecal coliform count of 45.91, which is the largest in this range. Area 1 closely follows with an average fecal coliform count of 43.48. Area 5 has an average fecal coliform count of 38.35. Area 2 has an average fecal coliform count of 26.46, closely followed by Area 3, which has an average fecal coliform count of 25.1. Area 4 has the smallest average fecal coliform count in the range 0-30, with an average fecal coliform count of 16.38.

Treated Water

Average fecal coliform count for treated water is much lower than the average fecal coliform count of raw water for all of the 11 areas. Areas 11, 5, 9 and 4 all have an average fecal coliform count that is greater than 10. Area 11 has the largest average fecal coliform count, with a value of 48. Area 5 has an average fecal coliform count of 30.24. There is a significant decrease in fecal coliform count between Area 5 and Area 9. Area 9 has an average fecal coliform count of 16.48. Area 4 has the smallest average fecal coliform count in this category. Average fecal coliform count for Area 4 is 13.64. Average fecal coliform count for Area 8, 6, 2, 10, 3, 1 and 7 is below 10. The values for average fecal coliform for Area 8, 6, 2, 10, 3 and 1 are quiet close to one another. Area 8 has an average fecal coliform count of 8.5, which is the largest value in this category. Average fecal coliform count for Area 2 is 8.02 and the average fecal coliform count for Area 2 is 7.71. Average fecal coliform count for Area 10, 3 and 1 closely follow one another with values of 6.75, 6.37, and 6.12 respectively. Area 7 shows the smallest average fecal coliform count with a value of 0.09.

Standard deviation

Standard deviation for raw water is 69.232 while the standard deviation for treated water is 13.775. This indicates that the average fecal coliform counts for raw water varies more from the average than the average fecal coliform count for treated water does.

3.1.2 Organisation of samples into different categories based on CFU.

Samples from all of the 11 areas were organized into different categories (Table 3.1.2.1), based on their CFU. Percentages of samples that fall under each of the categories were calculated for the 11 different areas.

Table 3.1.2.1 Organisation of raw water samples based on CFU

Area	**Fecal coliform count**				
	0	**1 to 5**	**6 to 30**	**31 to 100**	**>100**
1	30.77%	9.61%	36.54%	5.77%	17.31%
2	19.23%	7.69%	42.31%	26.92%	3.85%
3	49.28%	15.94%	17.39%	13.04%	4.35%
4	47.62%	14.28%	19.05%	16.67%	2.38%
5	20.27%	13.51%	40.54%	17.57%	8.11%
6	25%	8.33%	25%	10.42%	31.25%
7	48.15%	7.42%	14.81%	14.81%	14.81%
8	38.30%	25.53%	12.76%	12.76%	10.65%
9	15.39%	9.62%	40.38%	1.92%	32.69%
10	9.52%	9.52%	52.38%	0%	28.58%
11	0.00%	12.50%	0.00%	4.17%	83.33%
SD	0.166911	0.052585	0.16032	0.079213	0.232891

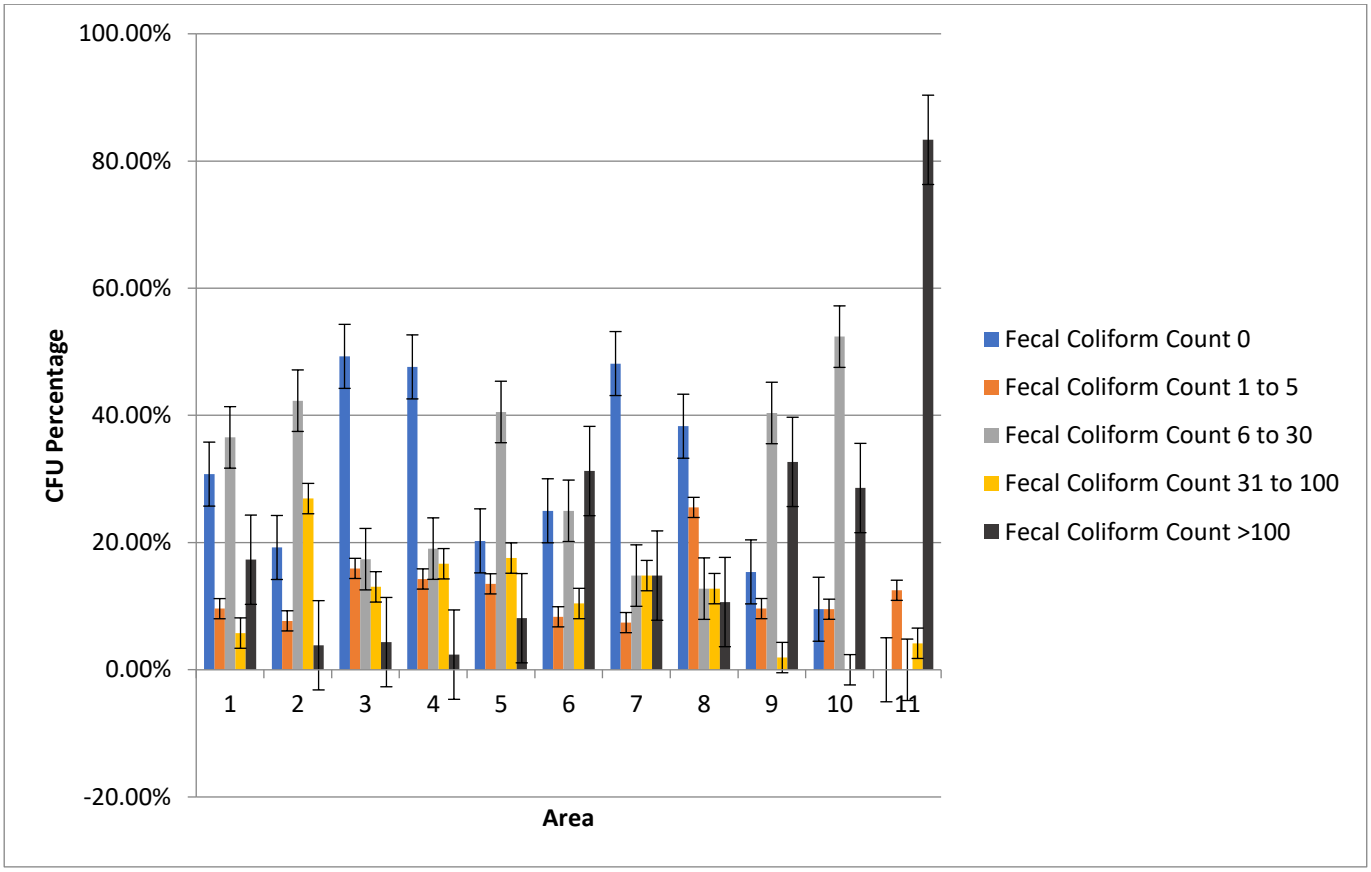

Fig 3.1.2.1 Percentage of sample in each fecal coliform count category for raw water samples

49.28% of samples in Area 3, 47.62% of samples in Area 4, 48.15% of samples in Area 7 and 38.30% of samples in Area 8 do not show any fecal coliform count. Majority of the samples from Area 1, 2, 5 and 9 have fecal coliform count that ranges from 6 to 30. 36.54% of samples in Area 1, 42.31% of samples in Area 2, 40.54% of samples in Area 5, 40.38% of samples in Area 9, 52.38% of samples in Area 10 have fecal coliform count that ranges between 6 and 30. 31.25% of samples in Area 6 and 83.33% of samples in Area 11 have a fecal coliform count that is greater than 100.

A very small percentage of the samples from Area 6 and 7 have fecal coliform counts that range between 1 and 5. 8.33% of samples in Area 6 and 7.42% of the samples in Area 7 belong in this category. Area 10 does not have any sample whose fecal coliform count ranges from 31 to 100. Only 1.92% of the samples from Area 9 and 5.77% of the samples from Area 1 belong in this category. 3.85% of the samples in Area 2, 4.35% of the samples in Area 3, 2.38% of the samples in Area 4, 8.11% of the

samples in Area 5 and 10.65% of the samples in Area 8 have fecal coliform counts greater than 100. Area 11 does not have any samples with a fecal coliform count ranging between 0 and 5.

Standard deviation for fecal coliform count greater than 100 is 0.232891. Standard deviationfor this particular category is the largest indicating that the percentages in this category varies the most from the average value. Standard deviation for fecal coliform count ranging between 1 and 5 is 0.052585. This value for standard deviation is the smallest amongst all of the categories. This indicates that the percentages in this category vary the least from the average.

Table 3.1.2.2 Organisation of treated water samples based on CFU

Area	**Fecal coliform count**				
	0	**1 to 5**	**6 to 30**	**31 to 100**	**>100**
1	53.12%	18.75%	28.13%	0.00%	0.00%
2	38.10%	14.28%	38.10%	9.52%	0.00%
3	65.67%	17.91%	4.48%	11.94%	0.00%
4	60%	8%	16%	16%	0.00%
5	41.79%	10.45%	29.85%	11.94%	5.97%
6	40.91%	27.27%	25%	6.82%	0.00%
7	95.24%	4.76%	0.00%	0.00%	0.00%
8	66.67%	5.55%	16.67%	11.11%	0.00%
9	42.42%	12.12%	27.27%	15.16%	3.03%
10	0.00%	41.67%	58.33%	0.00%	0.00%
11	40.00%	0.00%	20.00%	20.00%	20.00%
SD	0.2371828	0.117491	0.159094	0.069036	0.060693

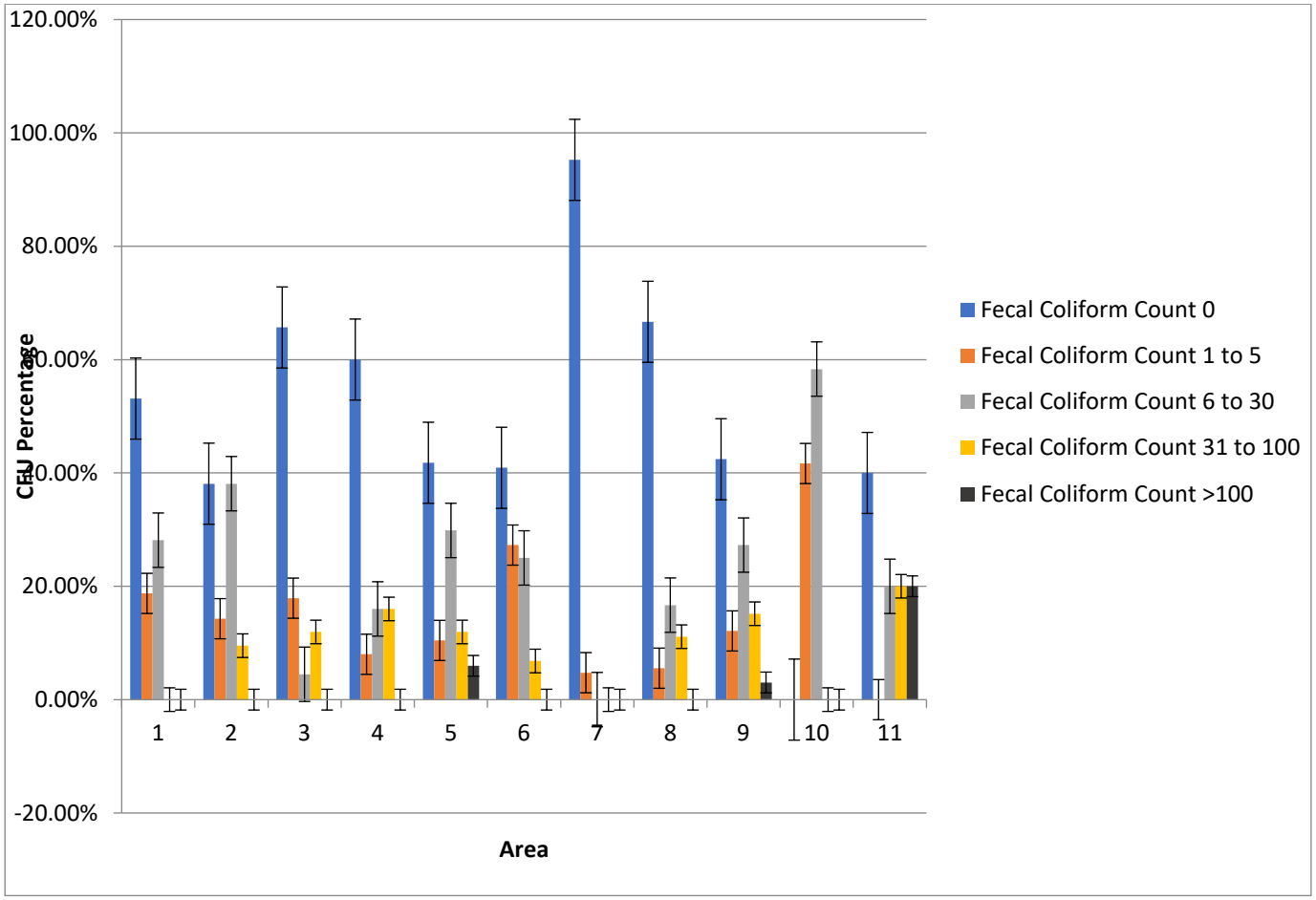

Fig 3.1.2.2 Percentage of sample in each fecal coliform count category for treated water samples

Apart from Area 10, majority of treated water samples from all of the areas do not have fecal coliform. 53.12% of samples from Area 1, 65.67% of samples from Area 3, 60% samples from Area 4, 41.79% of sample from Area 5, 40.91% of sample from Area 6, 95.24% of sample from Area 7, 66.67% of sample from Area 8, 42.42% of sample from Area 9 and 40% of sample from Area 11 do not have fecal coliform. Area 2 shows 38.10% of its sample in the 0 FC count category and 6 to 30 FC count category. Majority of the treated water sample, that is 58.33% of the treated water samples from Area 10 have a fecal coliform count ranging from 6 to 30.

Areas 1 through 10 do not have any treated water samples that have fecal coliform count exceeding 100.

In fact, fecal coliform count does not even exceed 30 for treated water samples in Area 1 and 10. Area 11 does not have any treated water sample with a fecal coliform count ranging from 1 to 5.

Fecal coliform count exceeding 100 category has a standard deviation of 0.060693. Percentages in this category vary the least as this category has the smallest standard deviation. 0 FC count category has a standard deviation 0.2371828 indicating that the values in this category varies the most. Standard deviation for the 0 FC count is the larger than those of the other categories when treated water is being considered.

3.2 PCR Results

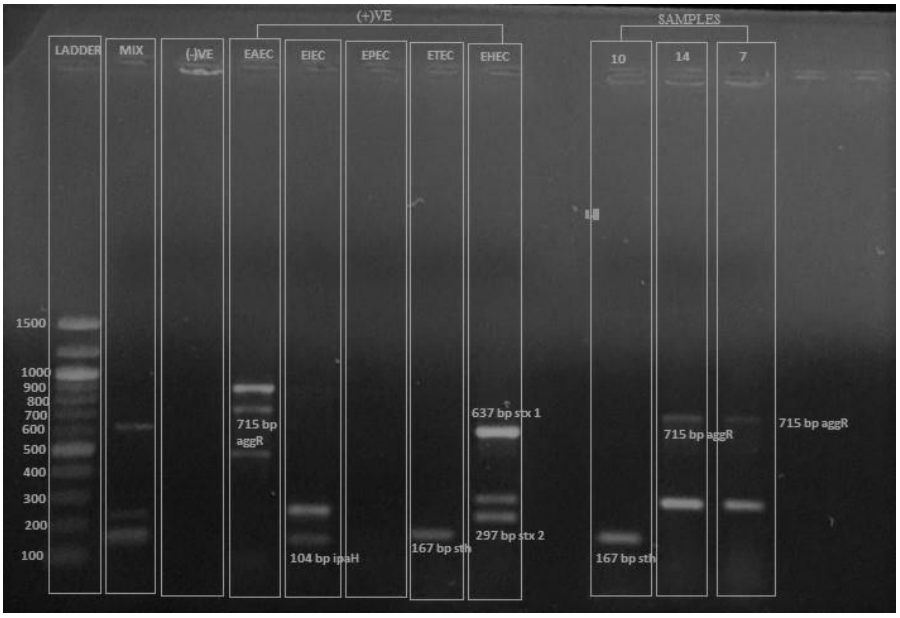

Fig 3.2.0.1 Image of a gel run after multiplex PCR was carried out. The ladder has been placed on the extreme left. This is followed by positive controls for the five different strains. Sample 10 was identified as ETEC while sample 14 and 7 was identified as EAEC

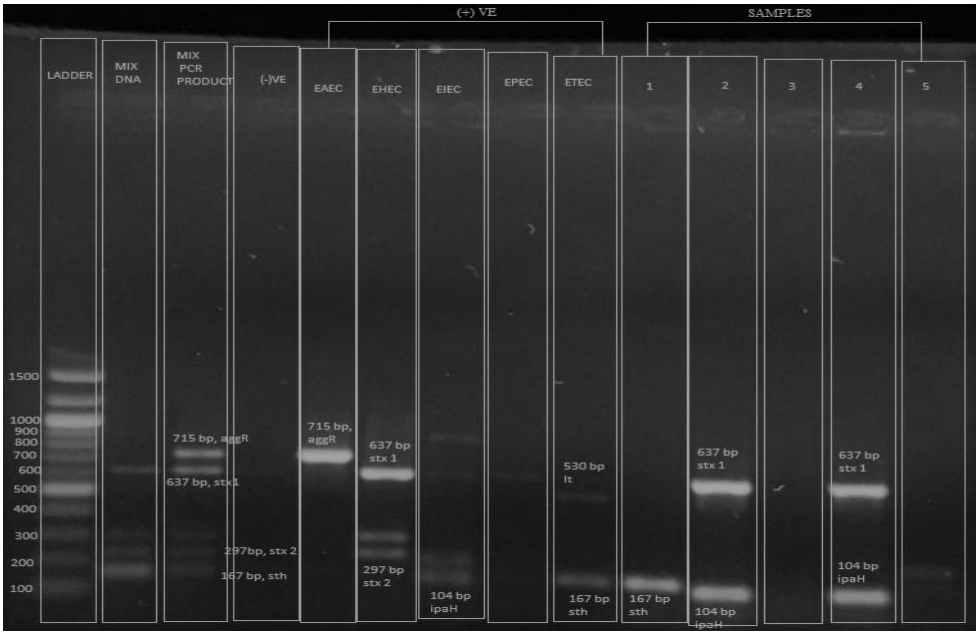

Fig.3.2.0.2 Image of gel run after multiplex PCR was carried out. The ladder is on the extreme left column, followed by the positive control. Sample 1 was identified as ETEC. Sample 2 and 4 had marker virulence genes both EHEC and EIEC. Sample 3 and 5 did not test positive for any of the strains.

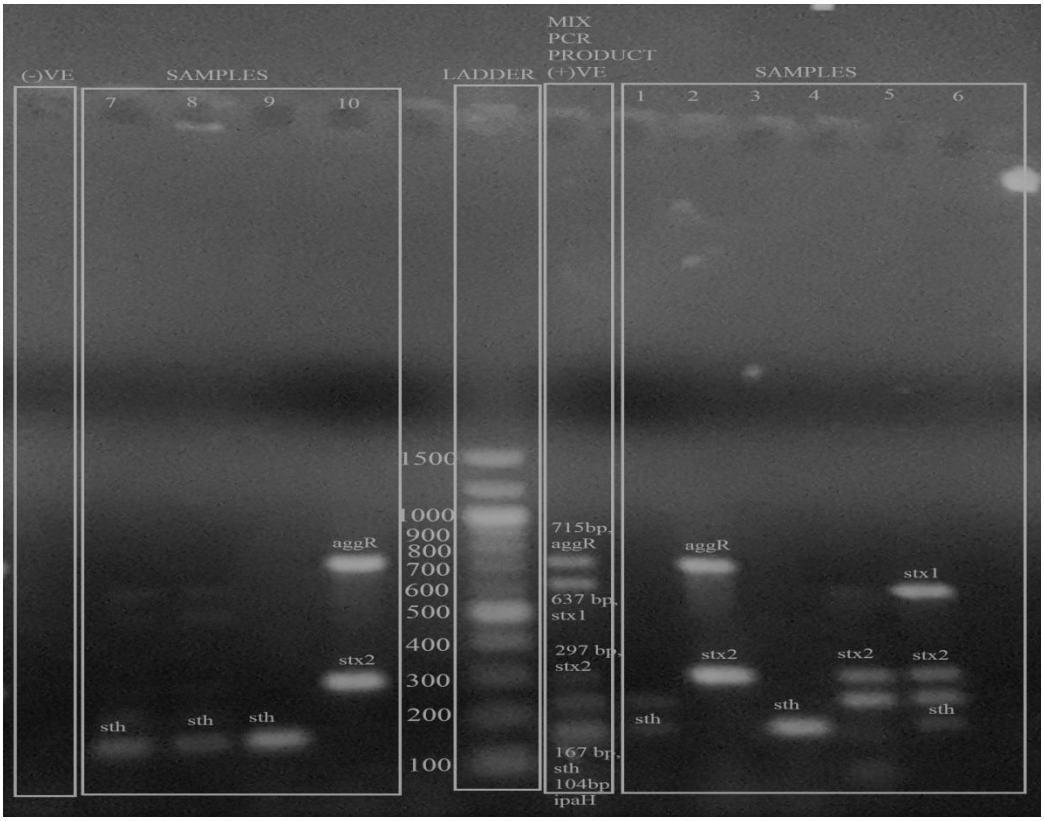

Fig.3.2.0.3 Image of a gel run after multiplex PCR was carried out. Ladder was placed at the center of the gel. Sample 1, 3, 7, 8 and 9 were identified as ETEC. Sample 2 and 10 had markers for both

EAEC and EHEC. Sample 5 had markers for EHEC and EIEC. Sample 4 was identified as EHEC.

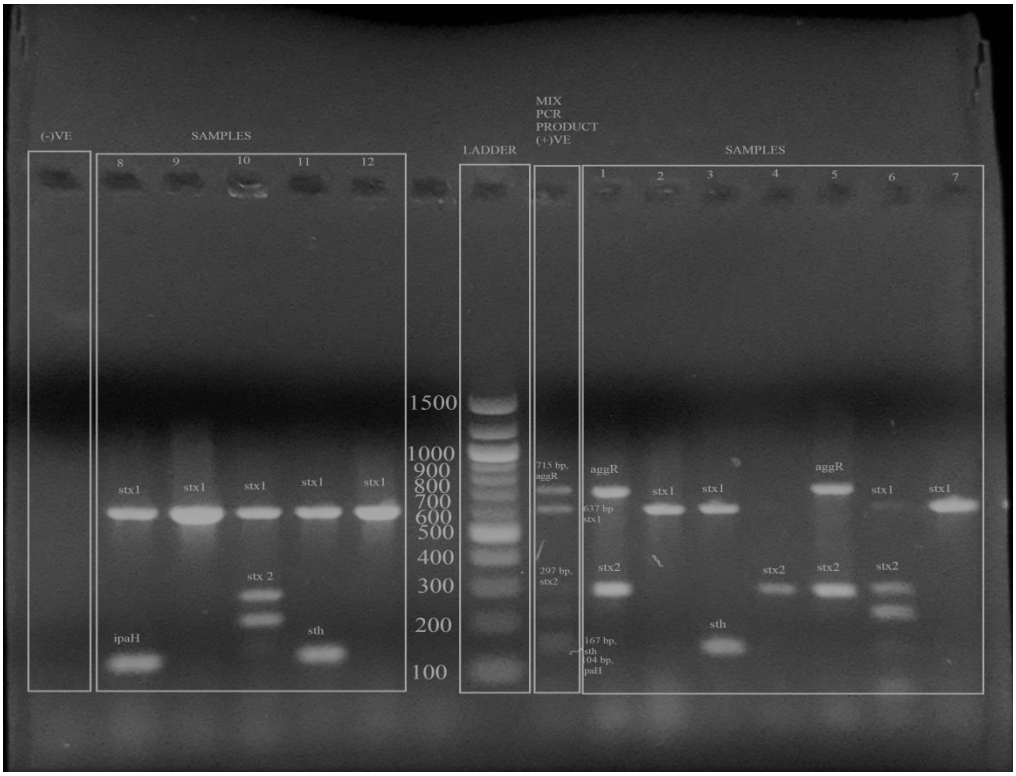

Fig.3.2.0.4 Image of a gel run after multiplex PCR was carried out. The ladder was placed at the center of the gel. Sample 2, 4, 6, 7, 9, 10 and 12 were identified as EHEC. Sample 1 and 5 had markers for both EHEC and EAEC. Sample 11 and 3 had markers for both EHEC and ETEC. Sample 8 had markers for EHEC and EIEC.

3.2.1 PCR results for Area 1 (Gulshan, Banani, Niketon, Baridhara and Badda)

Table 3.2.1.1 PCR results for raw water samples of Area 1

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	3	0	1	1	0
Nov-18	1	0	0	1	0
Dec-18	1	1	1	0	0
Jan-19	2	1	0	2	1
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0

May-19	0	1	0	1	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	1.026911	0.467099	0.404519917	0.687552	0.301511

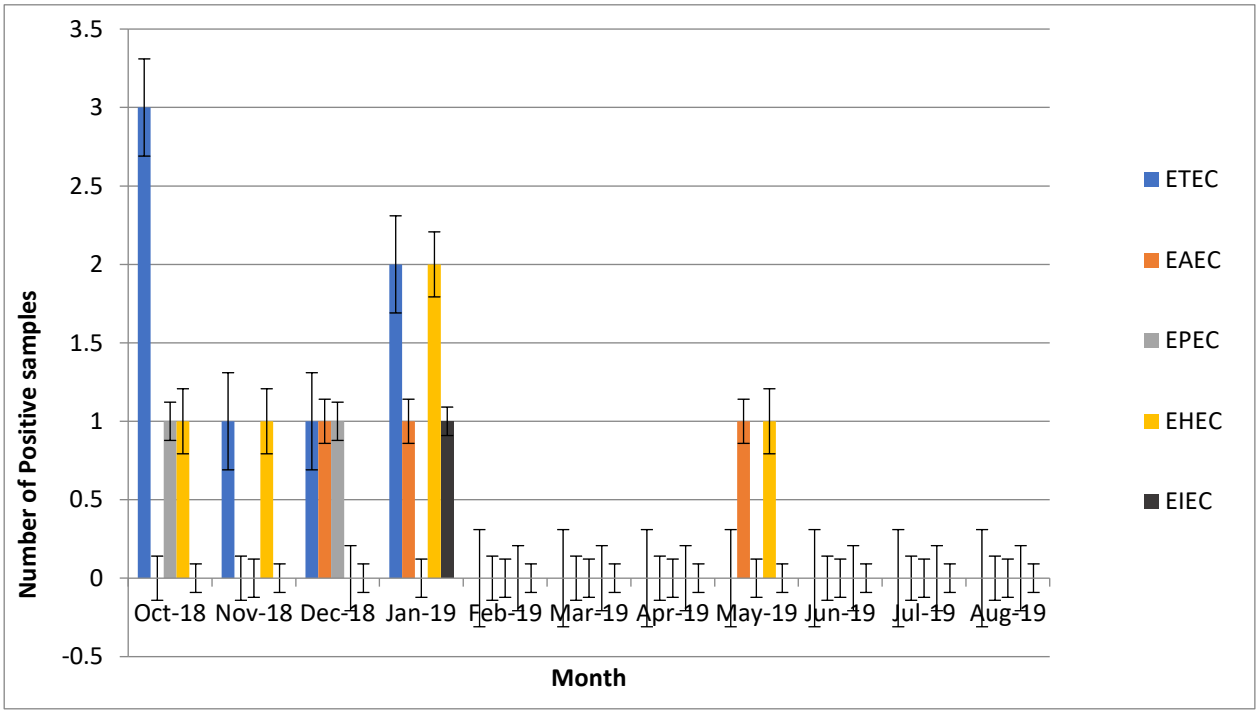

Fig 3.2.1.1 PCR results for raw water samples of Area 1

Monthly variation

5*E.coli* positive samples were detected in the month of October 2018. 3 of these samples were identified as ETEC. 1 sample was identified as EPEC and the other was identified as EHEC. 2 positive samples were detected the following month, 1 of which was identified as ETEC, while the other was identified as EHEC. A total of 3 positive *E.coli* samples were detected in the month of December 2018. The samples were identified as ETEC, EAEC and EPEC. The largest number of positive samples was detected in January 2019. 6*E.coli* positive samples were detected this month. 4 different strains were detected in this month as well. This month showed the most variation in term of strains detected. 2 of the samples were identified as ETEC. 2 EHEC samples were detected this month. 1 EAEC and 1 EIEC

sample were also detected in the month of January 2019. 1 EHEC and 1 EAEC samples were detected in May 2019. No positive samples were detected from February to April and June to August of 2019.

Seasonal variation

2 samples were detected in the pre-monsoon season. 1 of the samples were EAEC while the other was EHEC. The smallest numbers of *E.coli* samples were detected in the pre-monsoon season. 5*E.coli* samples were detected in the monsoon season, 3 of which were ETEC. 1 EPEC and 1 EHEC sample were also identified during this season. 11 positive samples were detected during the winter season. Largest portion of the samples detected in winter were ETEC. 4 samples were identified as ETEC, 3 were identified as EHEC and 2 were identified as EAEC. Only 1 EPEC and EIEC strain were detected in winter. All of the 5 strains were detected in winter.

Variation in Strain

7 ETEC positive samples and 2 EPEC positive samples were detected for Area 1. These samples were mainly found during the monsoon and winter season, from October 2018 to January 2019. When ETEC positive samples are taken into consideration, majority of the samples were detected in October of 2018. 5 samples were identified as EHEC. EHEC samples were found in all seasons. Majority of the EHEC samples were detected in January of 2019. 3 samples were identified as EAEC during the entire time period. These samples were found during the winter and the pre-monsoon season. Only 1 EIEC sample was detected for Area 1, during winter season, in January 2019.

Standard deviation

Standard deviation calculated for the ETEC versus Months column has a value of 1.026911, indicating that the number of ETEC positive samples found through the months varies the most from the average number of ETEC calculated. Standard deviation for EIEC is 0.301511, indicating that the number of EIEC samples detected from October 2018 to August 2019 varies the least from the average. Standard deviation for EPEC and EAEC are very close to each other. Standard deviation for EAEC is 0.467099 and the standard deviation for EPEC is 0.40451997. Standard deviation for EHEC is 0.687552.

Table 3.2.1.2 Percentage of raw water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	5.77
	*stx*1	5.77
ETEC	*st*h	13.46
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	3.85
EAEC	*agg*R	5.77
EIEC	*ipa*H	1.92

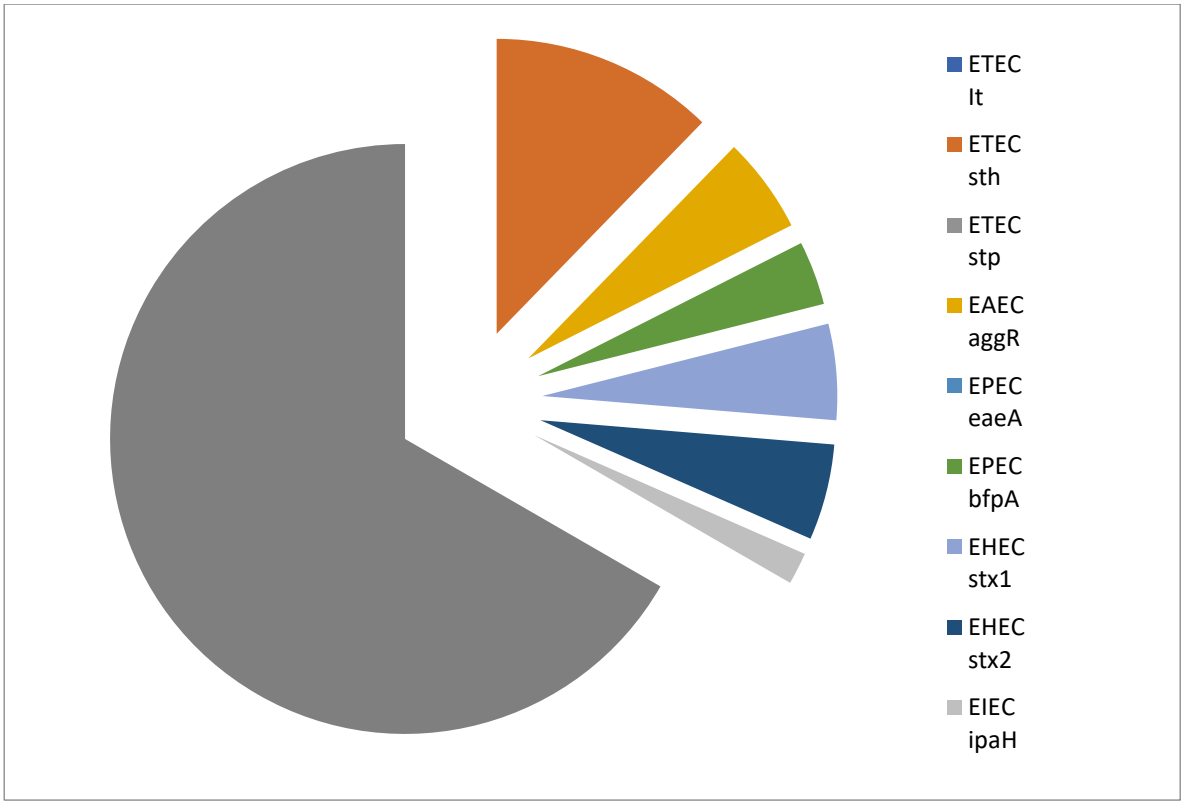

Fig 3.2.1.2 Pie chart representing the results of Table 3.2.1.2

Variation in the genes detected

73.08% of the samples from Area 1 did not have any of the marker virulence genes. 13.46% of the samples had *st*h gene, 3.85% had *bfp*A gene and 1.925 of the samples had *ipa*H gene. Marker virulence gene *stx*1, *stx*2 and *agg*R each had 5.77% positive samples. None of the samples had *st*p, *lt* and *eae*A gene. Some samples have more than one virulence marker gene.

Table 3.2.1.3 PCR results for treated water samples of Area 1

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0

None of the treated water samples from Area 1 tested positive for any of the marker virulence gene.

3.2.2 PCR results for Area 2 (Khilgaon)

Table 3.2.2.1 PCR results for raw water samples of Area 2

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	2	0	0	1	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	1	0	0	2	0
Feb-19	0	0	0	1	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	1	0
Aug-19	0	0	0	0	0

SD	0.674949	0	0	0.687552	0

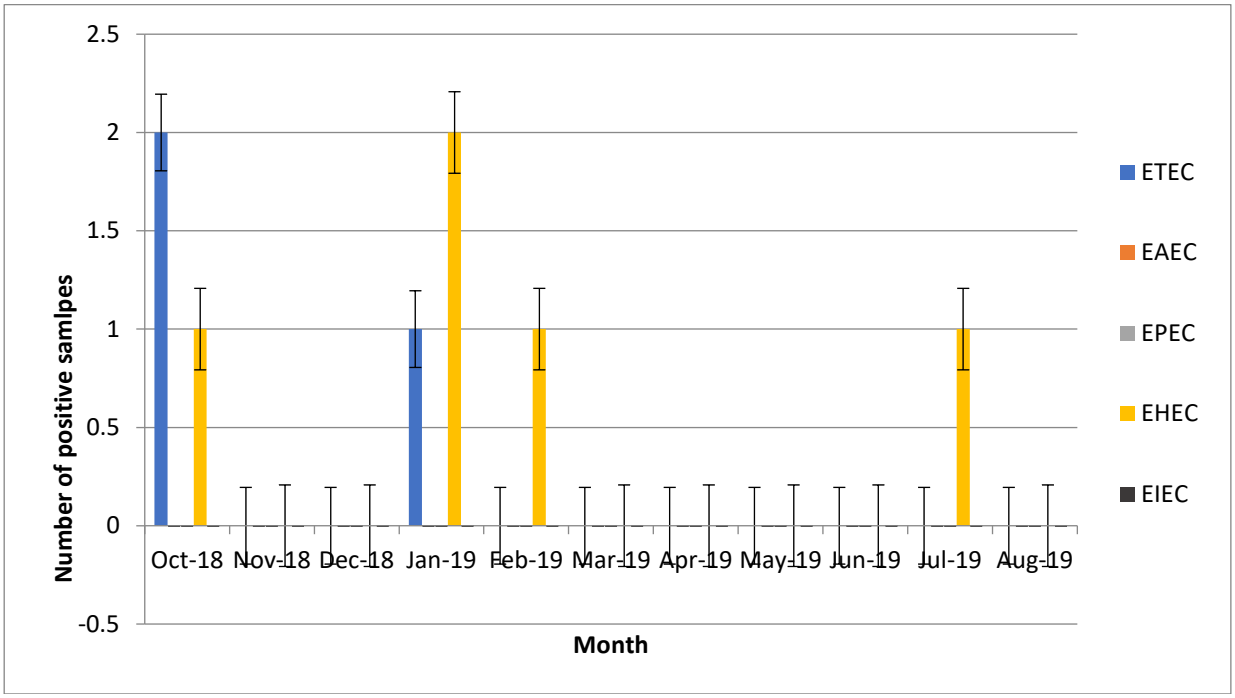

Fig 3.2.2.1 PCR results for raw water samples of Area 2

Monthly variation

2 samples were identified as ETEC and 1 sample was identified as EHEC in October 2018. No *E.coli* samples were detected for the rest of 2018. 1 ETEC sample and 2 EHEC sample were detected in January 2019. 1 EHEC sample was detected in the month of February 2019 and July 2019 each. No positive samples were detected from March 2019 to June 2019. There were no positive samples in the month of August 2019 as well.

Seasonal variation

E.coli positive samples were not found during the monsoon season. 4 positive samples were detected in winter. 1 of the samples were ETEC while the remaining 3 were EHEC. 4 positive samples were detected in the pre-monsoon season as well. 2 of these were ETEC, while the other 2 were EHEC.

Variation in strain

Only ETEC and EHEC strains were identified for raw water samples in Area 2. 3 ETEC samples were identified between October 2018 and January 2019, at the end of the monsoon season and in winter

season. 5 raw water samples of Area 2 were also identified as EHEC. EHEC samples were found during the monsoon and winter season. Greatest numbers of EHEC samples were found in January. EAEC, EPEC and EIEC were not detected in raw water samples of Area 2.

Standard deviation

Standard deviation for ETEC and EHEC are quiet close to one another. Standard deviation for EHEC is slightly higher indicating that the number of EHEC detected for the 11 months varies slightly more from the average than the number of ETEC detected. Standard deviation for ETEC is 0.674949 while the standard deviation for EHEC is 0.687552.

Table 3.2.2.2 Percentage of raw water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	15.38
	*stx*1	11.54
ETEC	*st*h	11.54
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

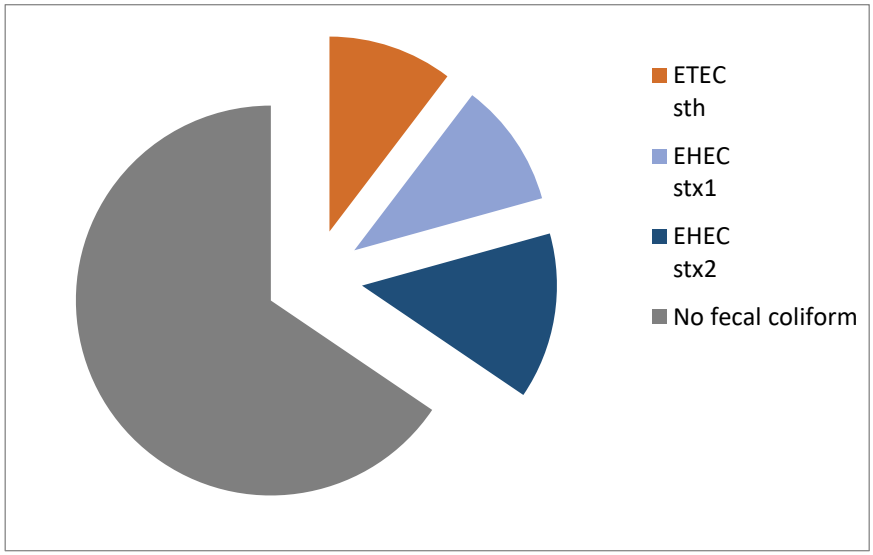

Fig 3.2.2.2 Pie chart representing the results of Table 3.2.2.2

Variation in the genes detected

73.08% of the samples did not have any of the marker virulence genes. 11.54% of the samples have ETEC's *st*h gene. The same is seen with EHEC's *stx*1 gene. 15.38% of the samples have *stx*2 gene. More than one virulence gene has been detected in some positive samples.

Table 3.2.2.3 PCR results for treated water samples of Area 2

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	1	0	0	0	0
Nov-18	0	0	0	1	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.301511	0	0	0.301511	0

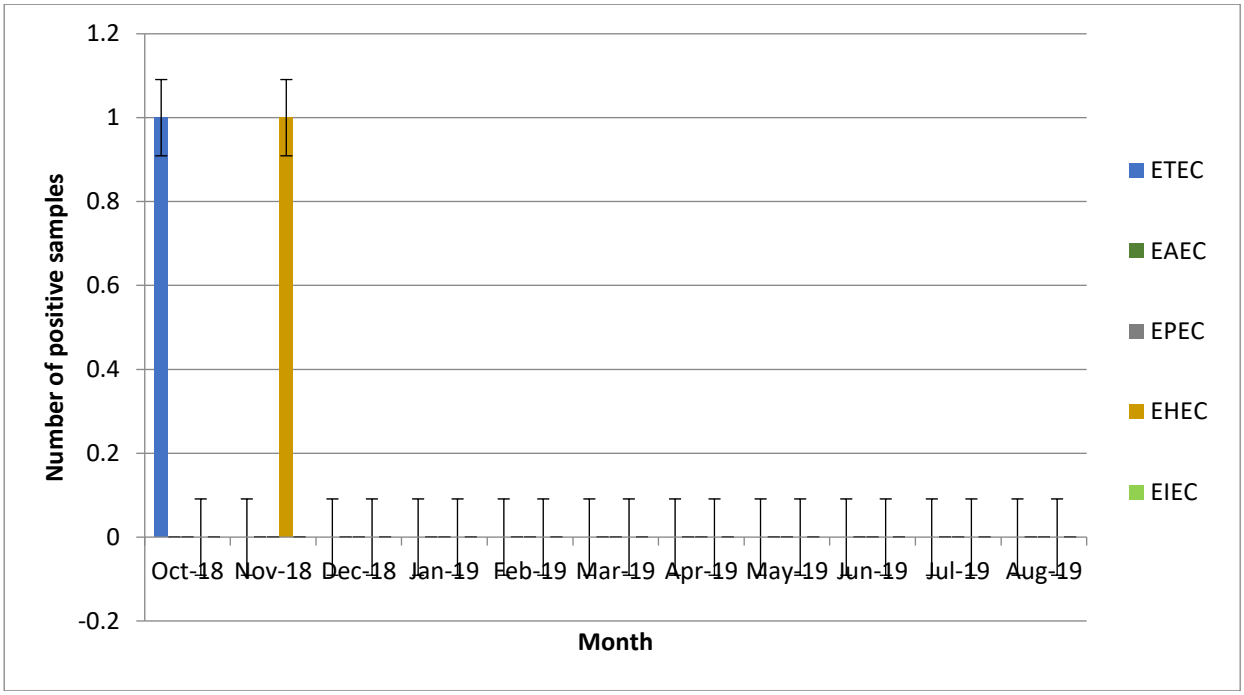

Fig 3.2.2.3 PCR results for treated water samples of Area 2

Monthly variation

When treated water is being considered, only 2 positive *E.coli* samples were detected in Area 2. Positive samples were detected in October and November of 2018. No positive samples were found for the remaining of the sampling duration. The sample detected in October of 2018 was identified as ETEC, while the sample detected in November was identified as EHEC.

Seasonal variation

Positive samples were detected at the end of monsoon season and the beginning of winter season.

Variation in strain

1 ETEC and 1 EHEC positive sample were detected amongst the treated water samples collected from Area 2. ETEC was detected at the end of the monsoon season, in October. EHEC was detected in

January, at the beginning of winter. None of the other strains were found.

Standard deviation

Standard deviation for both ETEC and EHEC is 0.301511.

Table 3.2.2.4 Percentage of treated water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	0
	*stx*1	4.76
ETEC	*st*h	4.76
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

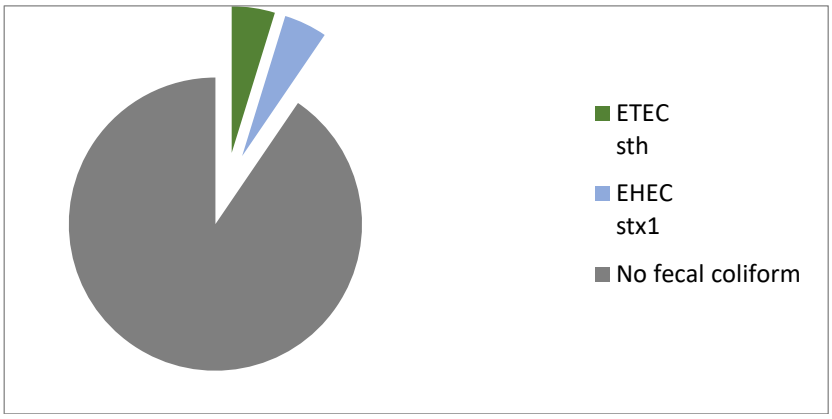

Fig 3.2.2.4 Pie chart representing the results of Table 3.2.2.4

Variation in genes detected

90.48% of the samples did not have any virulence gene. 4.76% of the samples had *st*h gene. 4.76% of the samples also had EHEC's *stx*1 gene.

3.2.3 PCR results for Area 3 (Mohammadpur and Lalmatia)

Table 3.2.3.1 PCR results for raw water samples of Area 3

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	2	0	0	0	0
Nov-18	1	1	1	0	0
Dec-18	2	0	0	2	0
Jan-19	3	0	0	3	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	1.103713	0.301511	0.301511	1.035725	0

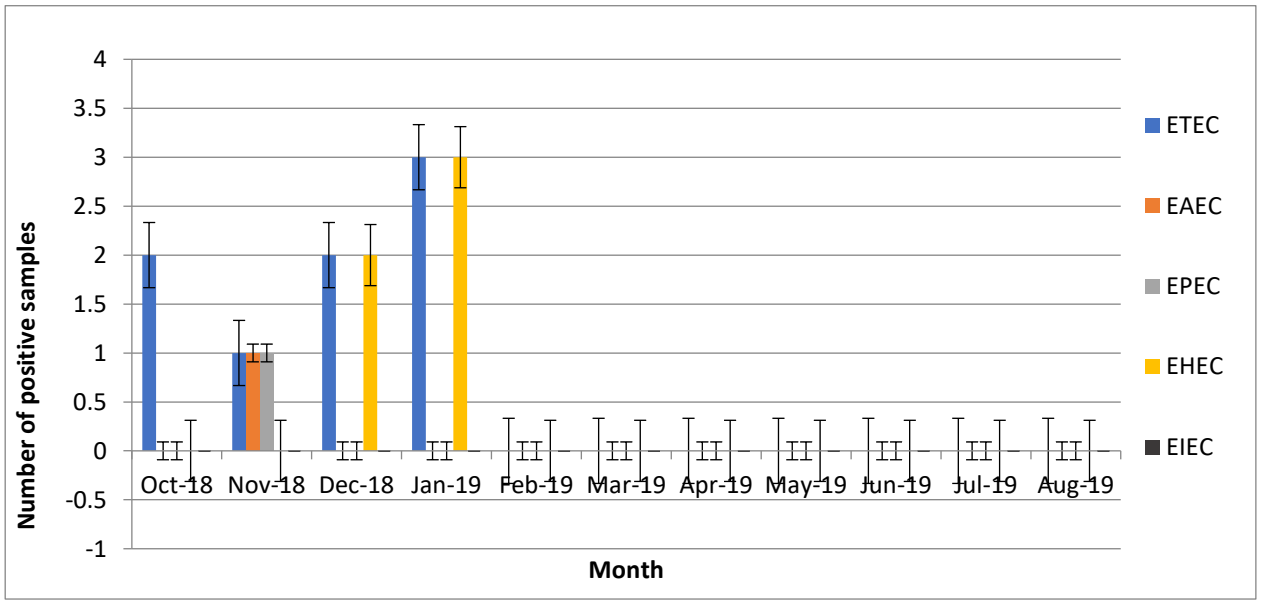

Fig 3.2.3.1 PCR results for raw water samples of Area 3

<u>Monthly variation</u>

E.coli positive samples were detected from October 2018 to January 2019. Positive samples were not found for the rest of the sampling duration. 2 ETEC samples were detected in October. 3*E.coli* positive

samples were detected in November. These were identified as ETEC, EPEC and EAEC. 4 samples were detected in December, 2 of which were ETEC while the other 2 were EHEC. Out of the 6 samples detected in January, 3 were identified as EHEC while the other 3 were identified as ETEC.

Seasonal variation

E.coli positive samples were detected at the end of monsoon and in winter. 2 samples were detected at the end of the monsoon season, which were ETEC in nature. 13 positive samples were detected in winter.

Variation in strain

8 of the samples detected were identified as ETEC. 3 ETEC samples were detected in January 2019. The highest number of ETEC samples was detected in this month. ETEC samples were detected at the end of monsoon and in winter. 5 EHEC samples were detected during winter. The highest number of EHEC samples was also detected in January. A single EAEC and EPEC sample was also detected In November 2018, at the beginning of winter. None of the samples were identified as EIEC.

Standard deviation

Standard deviation for both EAEC and EPEC is 0.301511. Standard deviation for EHEC is 1.035725 while the standard deviation for ETEC is 1.103713. Standard deviation for ETEC is the largest, indicating that the number of positive samples detected through the months varies the most from the average number of the particular strain (ETEC) detected. Standard deviation for EIEC is 0.

Table 3.2.3.2 Percentage of raw water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	4.35
	*stx*1	5.80
ETEC	*st*h	10.14
	*st*p	0
	lt	4.35
EPEC	*eae*A	0
	*bfp*A	1.45
EAEC	*agg*R	1.45

EIEC	*ipa*H	0

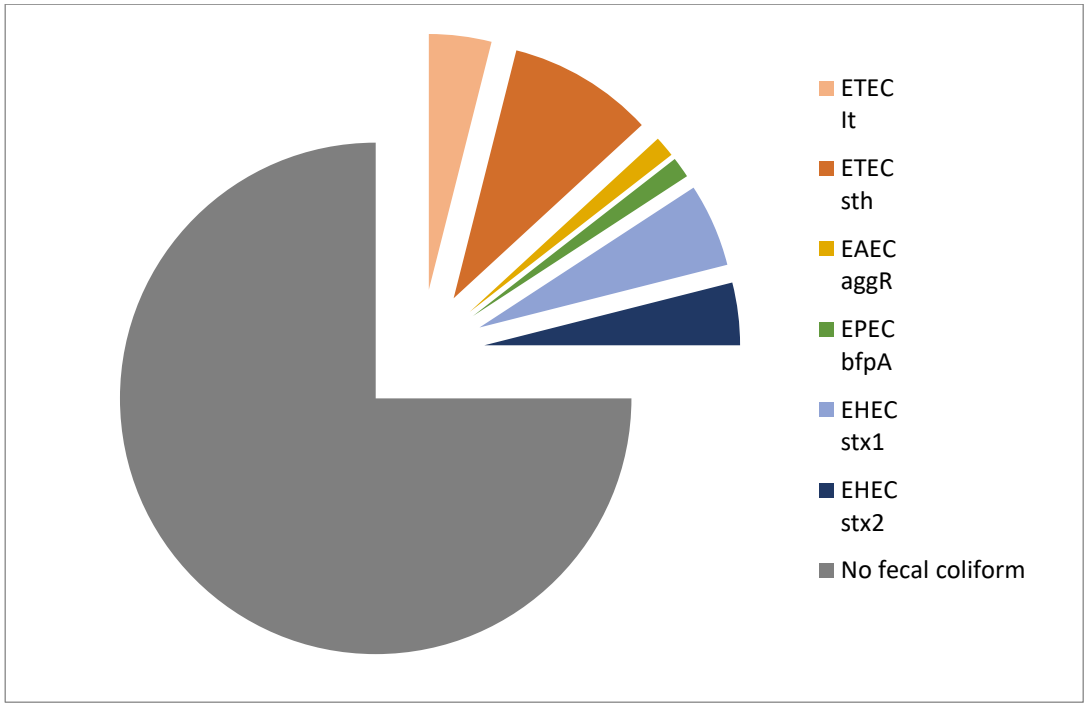

Fig 3.2.3.2 Pie chart representing the results of Table 3.2.3.2

Variation in genes detected

82.61% of the samples did not have any of the marker virulence genes. The most commonly found marker gene is *st*h, with 10.14% of the samples possessing this gene. 4.35% of the samples have *lt* gene. Majority of the EHEC sample, that is 5.80% of the samples, have *stx*1 gene. EHEC's other marker virulence gene *stx*2, is present in 4.35% of the sample. 1.45% of the samples have the *agg*R gene. The same is seen with *bfp*A gene. None of the samples had *ipa*H gene, *st*p gene or *eae*A gene. More than one virulence marker gene was detected and identified in certain samples.

Table 3.2.3.3 PCR results for treated water samples of Area 3

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	0	0	0	1	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0	0	0	0.301511	0

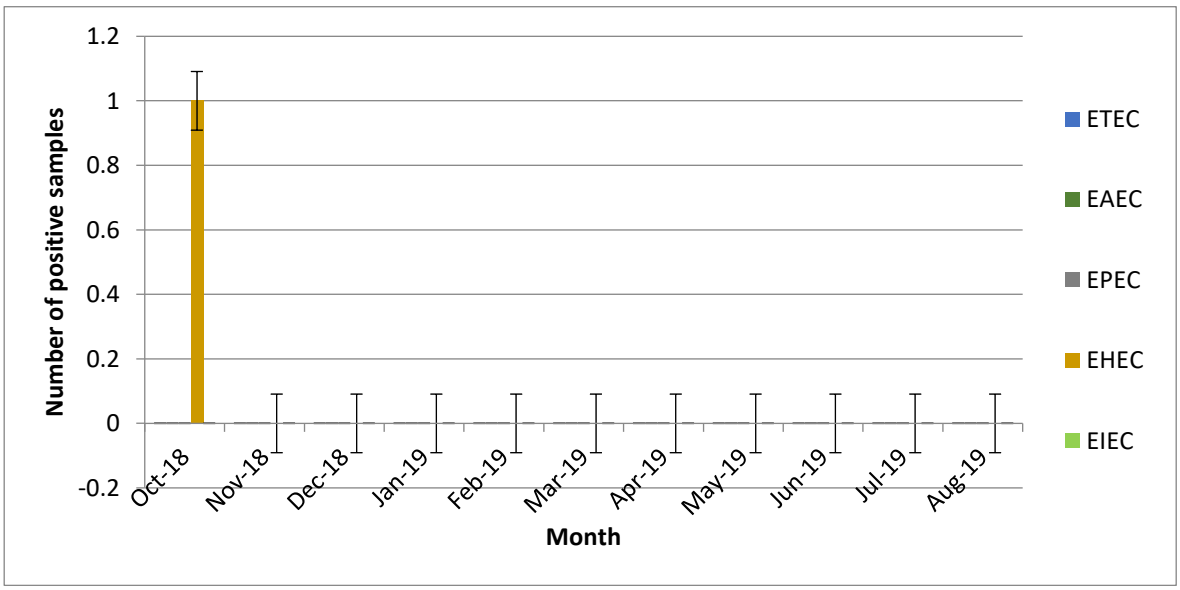

Fig 3.2.3.3 PCR results for treated water samples of Area 3

Monthly variation

1 EHEC positive sample was detected in October 2018. No other positive samples were detected for the rest of the sampling period.

Seasonal variation

1 EHEC sample was detected at the end of monsoon season. No positive samples were detected during the other seasons.

Variation in strain

The positive sample detected was identified as EHEC. The sample was detected in October, at the end of the monsoon season. No other strain was detected.

Standard deviation

Standard deviation for EHEC is 0.301511.

Table 3.2.3.4 Percentage of treated water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	1.49
	*stx*1	0
ETEC	*st*h	0
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

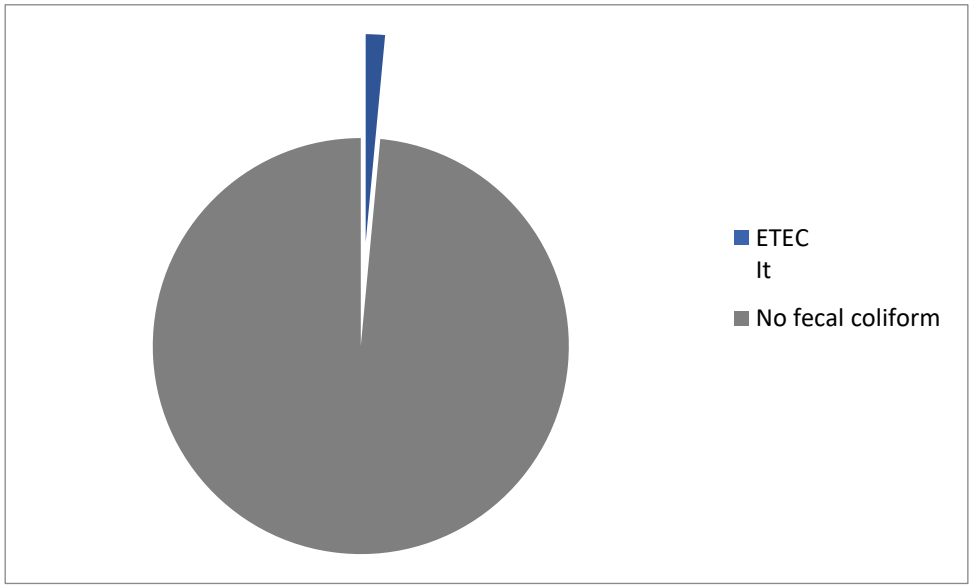

Fig 3.2.3.4 Pie chart representing results of Table 3.2.3.4

Variation in gene detected

98.51% of the samples do not have any marker virulence gene. The remaining 1.49% of the samples have *stx*2 gene.

3.2.4 PCR results for Area 4 (Uttara)

Table 3.2.4.1 PCR results for raw water samples of Area 4

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	1	0	0	1	0
Nov-18	1	0	0	1	0
Dec-18	1	0	0	1	0
Jan-19	1	0	0	1	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.504525	0	0	0.504525	0

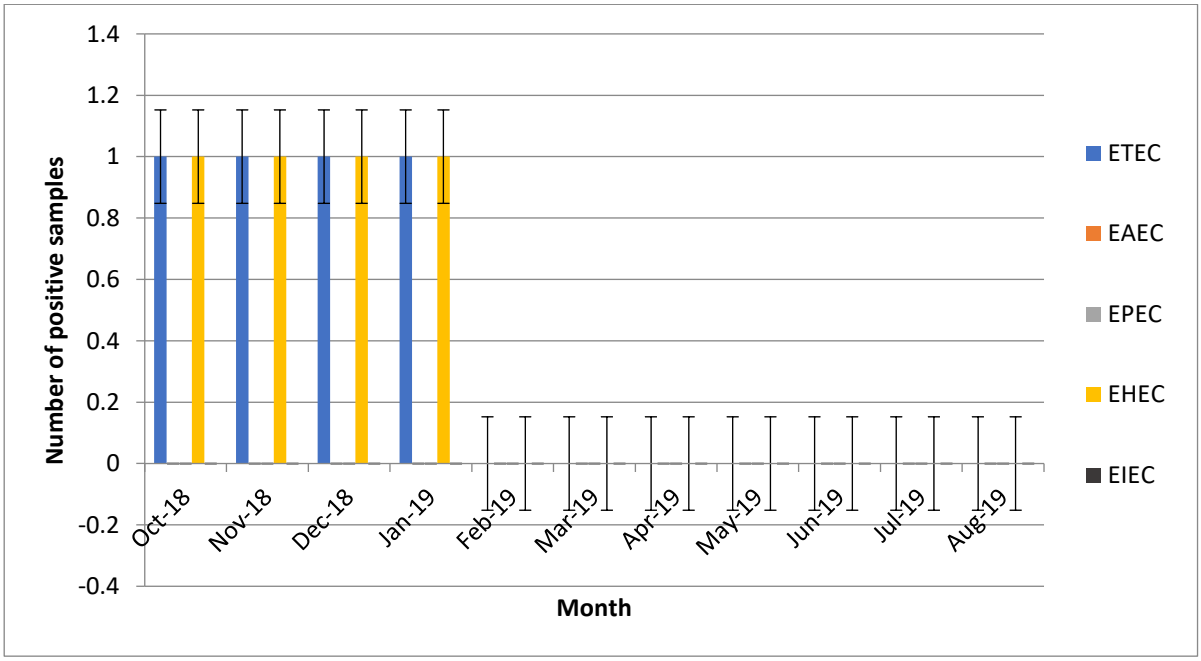

Fig 3.2.4.1 PCR results for raw water samples of Area 4

Monthly variation

2*E.coli* positive samples were detected in October of 2018. One of these samples was ETEC while the other was EHEC. The same is seen in November and December of 2018 and January of 2019. Positive samples were not detected from February of 2019 to August of 2019.

Seasonal variation

2*E.coli* positive samples were detected at the end of the monsoon season. One of the samples detected was identified as ETEC, while the other was identified as EHEC. 6*E.coli* positive sample was detected in the winter season. 3 of these were ETEC, while the other 3 were EHEC. Positive samples were not detected during the pre-monsoon season.

Variation in strain

4 ETEC samples were detected during the entire sampling time period. 1 ETEC sample was detected each month, from October 2018 to January 2019. 4 EHEC samples were detected. EHEC follows the same pattern as ETEC. The samples were detected at the end of monsoon and in the winter season. Other strains were not detected.

Standard deviation

Standard deviation for both ETEC and EHEC is 0.504525.

Table 3.2.4.2 Percentage of raw water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	7.14
	*stx*1	2.38
ETEC	*st*h	7.14
	*st*p	0
	lt	2.38
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

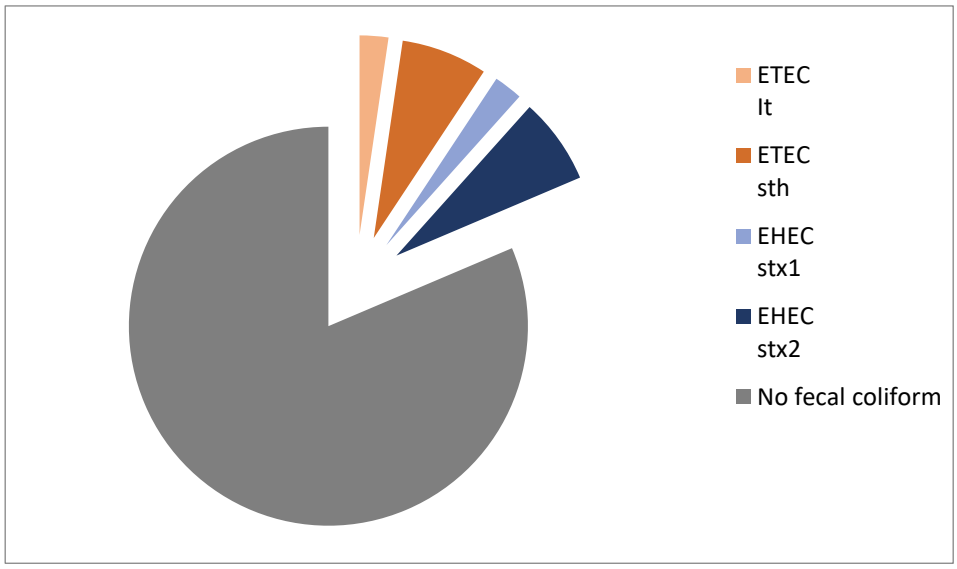

Fig 3.2.4.2 Pie chart representing the results of Table 3.2.4.2

Variation in the genes detected

83.33% of the samples do not have any of the marker genes. None of the samples have*st*p, *eae*A, *bfp*A, *agg*R or *ipa*H gene. Majority of the ETEC positive sample, 7.14% of the total sample have *st*h gene. 2.38% of the samples have ETEC's other marker virulence gene, *lt* gene. Majority of the EHEC samples have *stx*2 gene while the minority have the *stx*1 gene. 7.14% of the samples have *stx*2 gene while 2.38% of the samples have *stx*1 gene. A small number of positive samples have more than one virulence marker gene.

Table 3.2.4.3 PCR results for treated water samples of Area 4

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	0	0
Nov-18	1	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0

Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	1	0	0	1	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.40452	0	0	0.301511	0

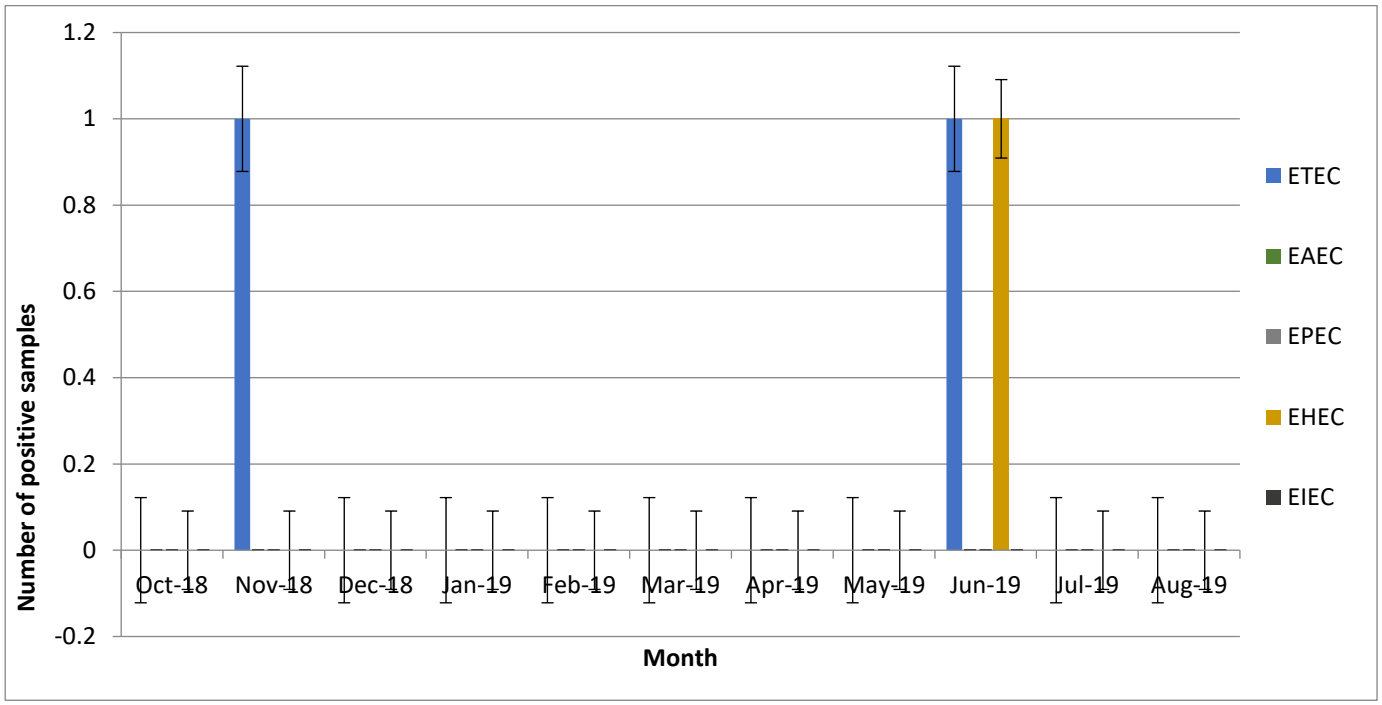

Fig 3.2.4.3 PCR results for treated water samples in Area 4

Monthly variation

A single ETEC positive sample was detected in the month of November in 2018. 2 samples were detected in June 2019. One of the samples was ETEC while the other was EHEC. No positive samples were detected from December of 2018 to May of 2019. Positive samples were not detected in October of 2018 and August of 2019 as well.

Seasonal variation

1 ETEC positive sample was detected in at the beginning of winter. 2*E.coli* positive samples were detected at the beginning of the monsoon season as well. These samples were of the ETEC and EHEC

strain. No positive samples were detected during the pre-monsoon season.

Variation in strain

2 ETEC positive samples were detected throughout the sampling period, once in October 2018 and again in June 2019. 1 EHEC sample was also detected in June 2019, in the monsoon season. No one other strains were detected in the treated water samples of Area 4.

Standard deviation

Standard deviation for ETEC is 0.40452 while the standard deviation for EHEC is 0.301511. This indicates that the number of ETEC samples detected through the months varies more from the average number of ETEC detectedthan EHEC does from the average number of EHEC detected..

Table 3.2.4.4 Percentage of treated water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	4
	*stx*1	0
ETEC	*st*h	8
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

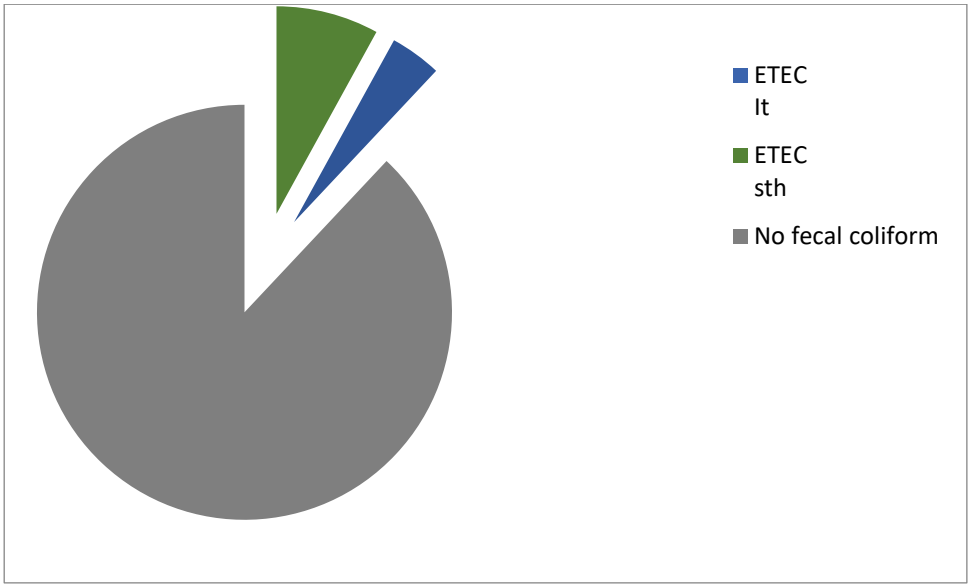

Fig 3.2.4.4 Pie chart representing the results of Table 3.2.4.4

Variation in the gene detected

88% of the samples do not have any of the marker virulence genes. The samples in which *E.coli*was detected have either *st*h gene or *stx*2 gene. 8% of the treated water sample of Area 4 have *st*h gene. 4% of the samples have *stx*2 gene.

3.2.5 PCR results for Area 5 (Mirpur, Mugdapara and Tolarbagh)

Table 3.2.5.1 PCR results for raw water samples of Area 5

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	8	0	0	6	2
Nov-18	5	1	0	0	0
Dec-18	2	0	0	3	0
Jan-19	5	1	0	2	0
Feb-19	0	0	0	0	0
Mar-19	1	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0

Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	2.80908	0.40452	0	1.94936	0.60302

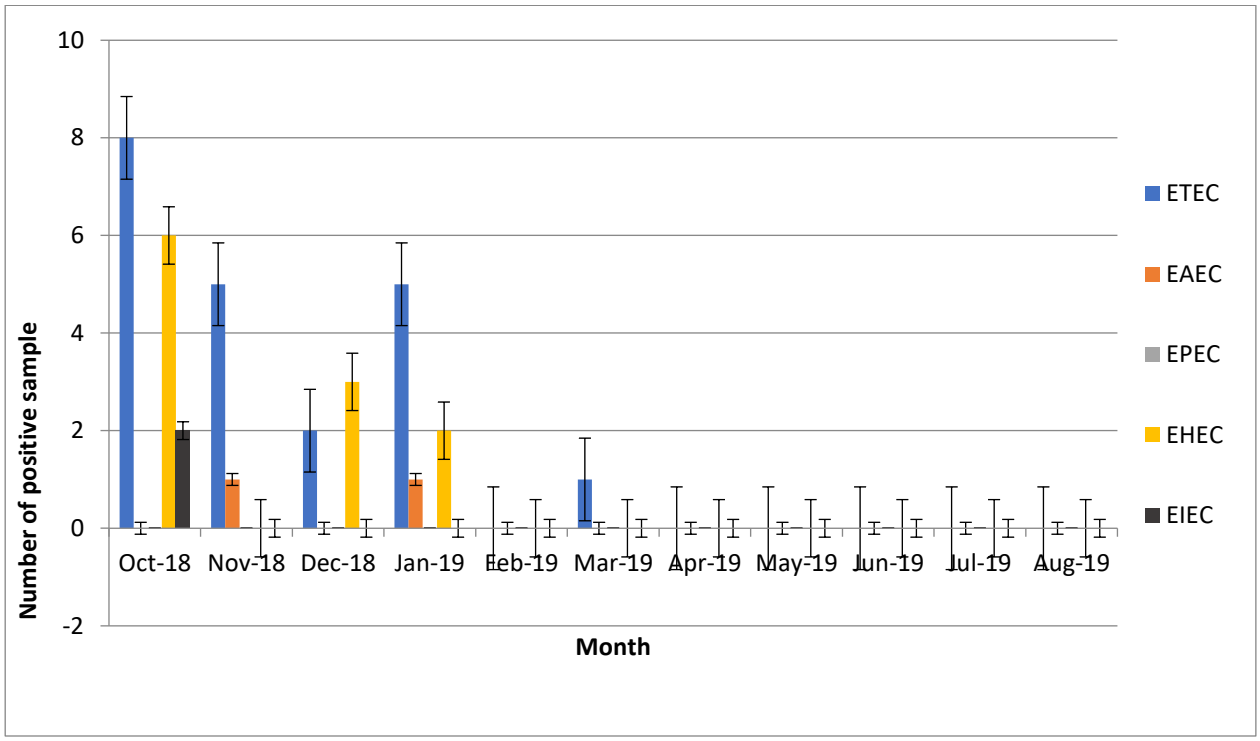

Fig 3.2.5.1 PCR results for raw water samples of Area 5

Monthly variation

16*E.coli* positive samples were detected in October 2018. 8 of these were ETEC, 6 were EHEC and 2 were EIEC. Number of positive samples detected in November was less than half of the positive samples detected in October. 6 positive samples were detected in November 2018, 5 of which were identified as ETEC. The one remaining positive sample was identified as EAEC. 3 EHEC and 2 ETEC samples were detected the following month. 8*E.coli* positive samples were detected in January 2019. 5 of these were identified as ETEC, 2 were identified as EHEC and only one was identified as EAEC. Positive samples were not detected from April to August of 2019. Positive samples were also not detected in February of 2019.

Seasonal variation

Majority of the *E.coli* positive samples were detected in winter. 19 samples were detected during this season. Most of the positive samples were identified as ETEC. 12 ETEC, 5 EHEC and 2 EAEC samples were detected in winter. 16 positive samples were detected at the end of the monsoon season. Half of the positive samples detected in the monsoon season was identified as ETEC. For the remaining 8 positive samples, 6 were identified as EHEC and 2 were identified as EIEC. 1 ETEC sample was detected in the pre-monsoon season.

Variation in strain

Majority of the positive samples were identified as ETEC. 21 ETEC samples were detected mainly at the end of the monsoon season and in the winter season, from October of 2018 to March of 2019. Majority of the ETEC samples were identified in October. 11 EHEC samples were identified in winter ant at the of monsoon, from October 2018 to January 2019. Greatest number of EHEC samples was detected in October. 2 EAEC and 2 EIEC samples were also detected throughout the entire sampling period. EAEC samples were detected in winter, from November to January, while EIEC samples were detected at the end of the monsoon season in October.

Standard deviation

ETEC has the largest standard deviation value amongst the standard deviation of all of the strains, indicating that the number of ETEC samples detected throughout the sampling period varies the most from the average. Standard deviation for ETEC is 2.80908. Standard deviation for EAEC is 0.40452. This is the smallest standard deviation value, indicating that the number of EAEC detected throughout the months varies the least from the average number of EAEC detected. Standard deviation for EHEC is 1.94936 and the standard deviation for EIEC is 0.60302.

Table 3.2.5.2 Percentage of raw water samples detected for marked virulence gene.

Strain	**Target gene**	**Percentage (%)**
EHEC	*stx*2	6.76

	*stx*1	13.51
ETEC	*st*h	21.62
	*st*p	0
	lt	8.11
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	2.70
EIEC	*ipa*H	2.70

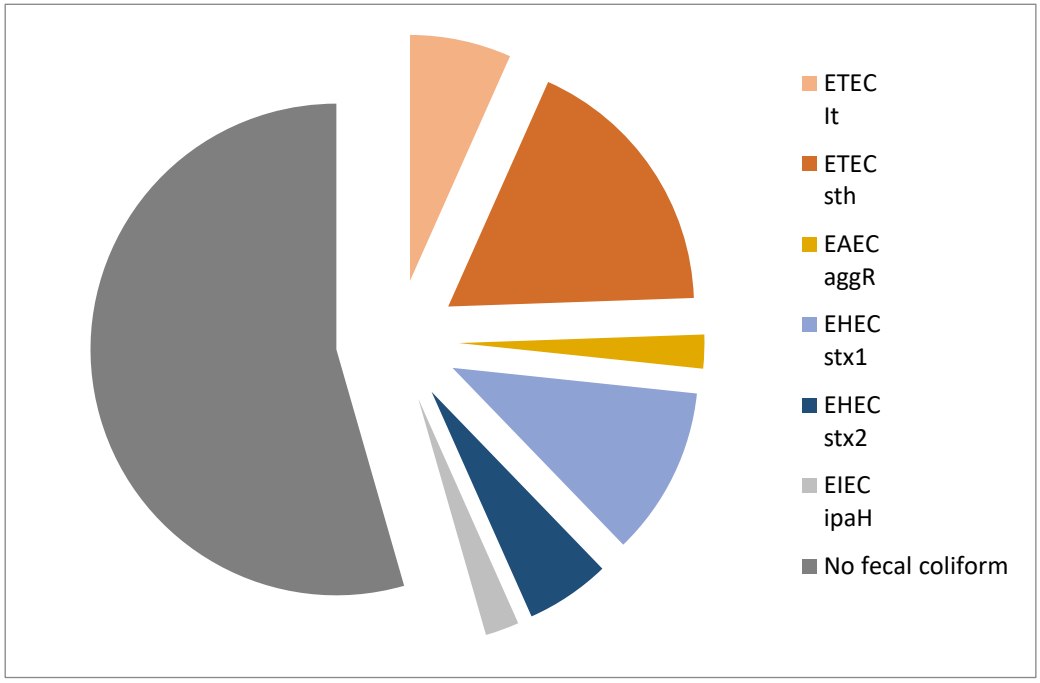

Fig 3.2.5.2 Pie chart representing results of Table 3.2.5.2

Variation in the genes detected

66.26% of the raw water samples of Area 5 do not have any of the marker virulence genes. Majority of *E.coli* positive samples have ETEC's *st*h gene. 21.62% of the entire raw water samples have this gene. 8.11% of the sample have *lt* gene, ETEC's other marker gene. Most of the samples that were identified as EHEC, or 13.51% of the raw water samples from Area 5, have *stx*1 gene. 6.76% of the samples have *stx*2 gene. *agg*R gene and the *ipa*H gene, each, makes up 2.70% of the samples . None of the samples have *st*p, *eae*A or *bfp*A gene. More than one marker virulence gene has been detected and identified in some positive samples.

Table 3.2.5.3 PCR results for treated water samples of Area 5

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	2	2	0	2	0
Jan-19	3	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	1	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	1.035725	0.603023	0	0.603023	0

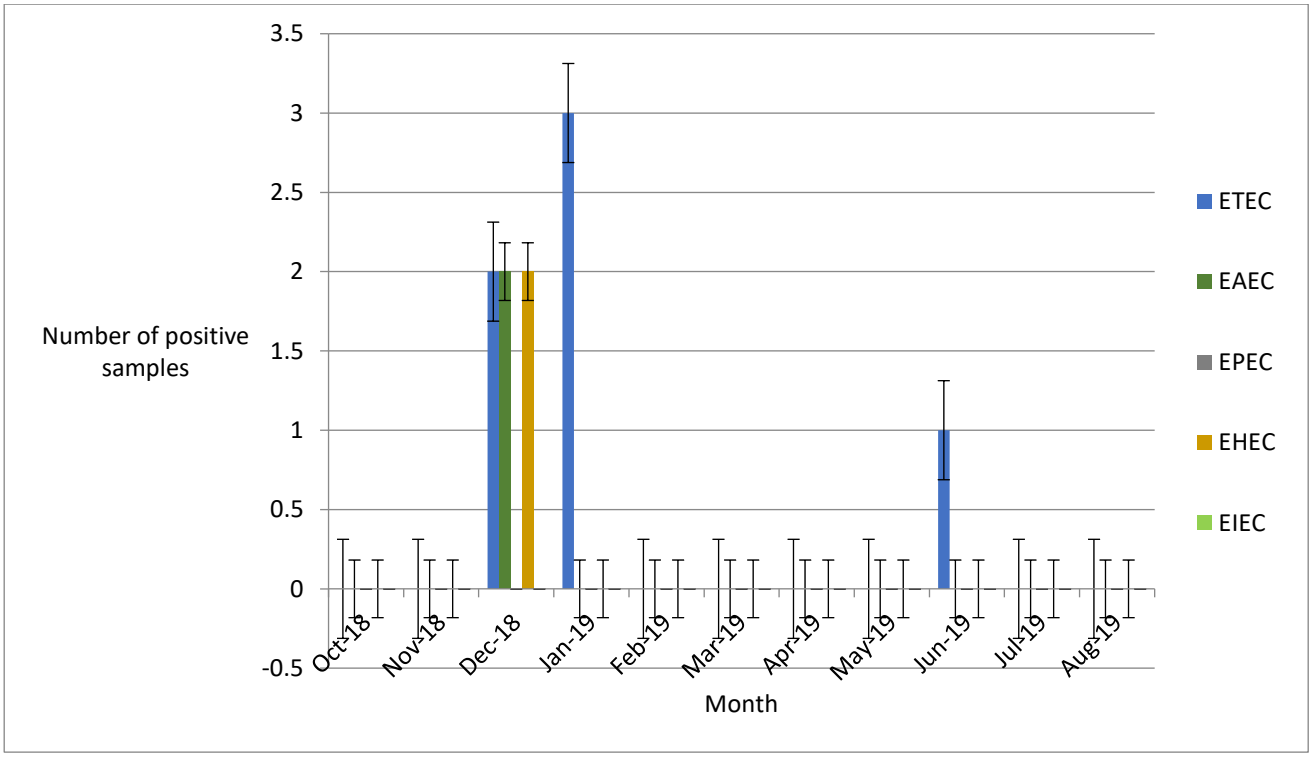

Fig 3.2.5.3 PCR results for treated water samples in Area 5

Monthly variation

In case of treated water samples of Area 5, *E.coli* positive samples were only detected in December 2018, January 2019 and June 2019. Majority of the samples were detected in December of 2018. 6 positive samples were detected this month. The samples were identified as ETEC, EHEC and EAEC,

with 2 positive samples belonging to each of the strains. In the following month, 3 ETEC samples were detected. The sample detected in June 2019 was identified as ETEC.

Seasonal variation

Majority of the positive samples were detected during the winter season. 9*E.coli* positive samples were detected during this season, 5 of which were ETEC, 2 were EAEC and the remaining 2 were EHEC. The sample that was detected during the monsoon season was identified as ETEC. *E.coli* positive samples were not detected in the pre-monsoon season.

Variation in strain

6 ETEC samples were detected during the entire sampling period. These were detected in the winter and monsoon season, with the greatest number of ETEC being detected in January 2019. 2 EAEC samples were detected in December 2018, during winter. The same was seen with the 2 samples identified as EHEC. EPEC and EIEC were not detected.

Standard deviation

Standard deviation for ETEC is 1.035725. This value of standard deviation is the largest amongst all of the other. Standard deviation for EAEC and EHEC is 0.603023. These values are smaller than the standard deviation of ETEC indicating that the number of EAEC or EHEC detected through the months varies much less from their averages than ETEC does from its average.

Table 3.2.5.4 Percentage of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	2.99
	*stx*1	0
ETEC	*st*h	7.46
	*st*p	0
	lt	1.49
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	2.99
EIEC	*ipa*H	0

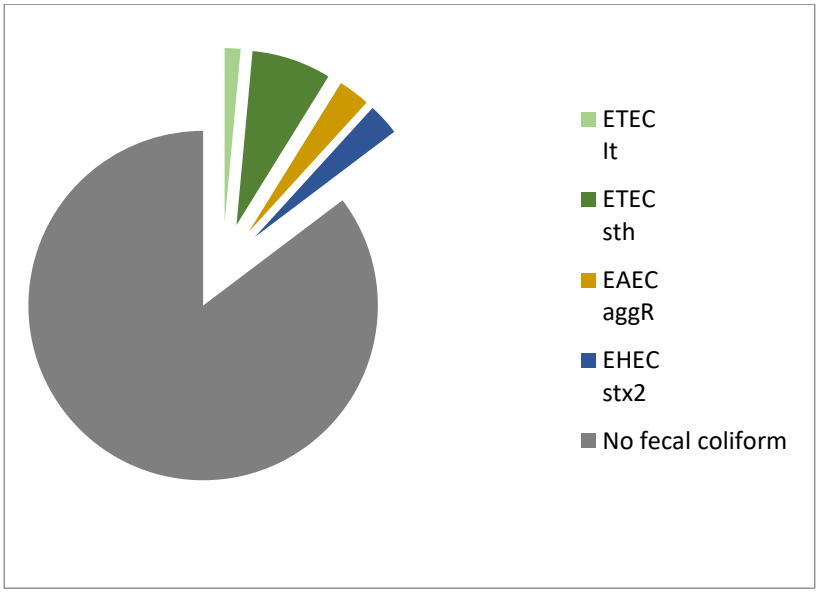

Fig 3.2.5.4 Pie chart representing the results of Table 3.2.5.4

Variation in the genes detected

86.57% of the treated water sample does not have any marker virulence gene. Majority of the *E.coli* positive samples have *st*h gene. 7.46% of the treated water samples have this gene. 1.49% of treated water samples have *lt* gene. *agg*R and the *ipa*H gene, each, makes 2.99% of the treated water samples of this area. EPEC strain was not detected. Hence, none of the samples had *eae*A or *bfp*A gene. Treated water samples of Area 5 did not have *st*p or *stx*1 gene either. More than one virulence marker gene is present in some positive samples.

3.2.6 PCR results of Area 6 (Moghbazar, Shanti Nagar, Malibagh and Jatrabari)

Table 3.2.6.1 PCR result for raw water samples of Area 6

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	2	2	0	0	0
Nov-18	1	1	1	1	1
Dec-18	1	1	0	2	0
Jan-19	2	0	0	1	1
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0

May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.8202	0.6742	0.301511	0.6742	0.40452

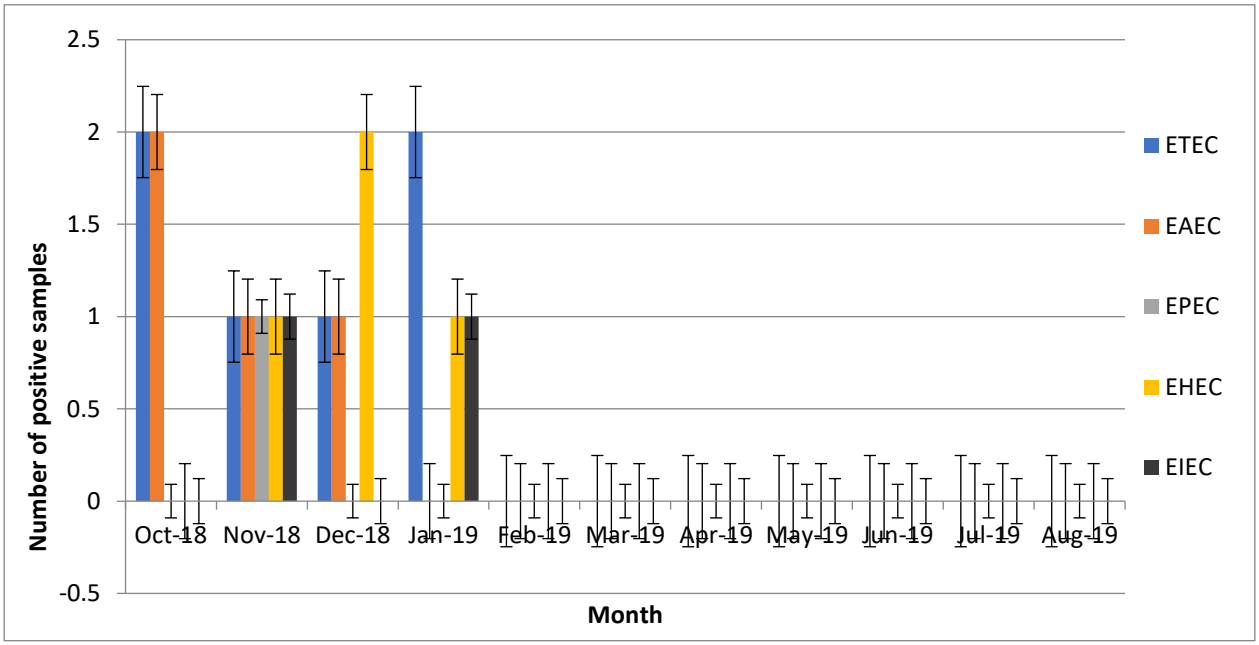

Fig 3.2.6.1 PCR results for raw water samples of Area 6

Monthly variation

E.coli positive samples were detected from October of 2018 until January of 2019. No *E.coli* positive samples were found from February of 2019 to August of 2019. 2 ETEC and 2 EAEC samples were detected in October 2018. All of the 5 different strains were detected in the following month. 4*E.coli* positive samples were detected in December. 2 of these samples were identified as EHEC, 1 was identified as ETEC, while the remaining 1 was identified as EAEC. 4 samples were detected in January of 2019 as well, 2 of which were ETEC, 1 was EHEC and 1 was EIEC.

Seasonal variation

Majority of the *E.coli* positive samples were detected during the winter season. 13 samples were

detected in this season, 4 of which were ETEC. The same number of EHEC samples was also detected in winter. 2 EAEC and 2 EIEC samples between November and January. Only a single EPEC sample was detected in this season. 4*E.coli* positive samples were detected during the monsoon season. 2 of the samples were identified as ETEC while the other 2 were identified as EAEC. No positive samples were detected in the pre-monsoon season.

Variation in strain

All 5 strains were detected in the raw water samples of Area 6. The most commonly found strain in the raw water samples of Area 6 is ETEC. 6 ETEC samples were found during the entire sampling period, mainly during winter and some even at the end of the monsoon season. 4 of the *E.coli* positive samples were identified as EAEC. This strain was detected at the end of monsoon and in the winter season. Greatest numbers of EAEC samples were detected in October of 2018. 4 EHEC, 2 EIEC and 1 EPEC sample were found during throughout the entire sampling period. All of these were detected during the winter season. Greatest numbers of EHEC samples were found in December of 2018.

Standard deviation

Standard deviation for ETEC is 0.8202, which is the largest standard deviation value calculated for the raw water samples of Area 6. This indicates that the number of ETEC samples detected through the months varies the most from its average than the five strains do from their average. On the other hand, the smallest standard deviation calculated for the raw water samples of Area 6 is for EPEC. This indicates that the number of EPEC samples detected through the months varies the least from its average value. Standard deviation for both EAEC and EHEC is 0.6742, while the standard deviation of EIEC is 0.40452.

Table 3.2.6.2 Percentage of raw water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	6.25
	*stx*1	4.17

ETEC	*st*h	12.50
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	2.08
EAEC	*agg*R	8.33
EIEC	*ipa*H	4.17

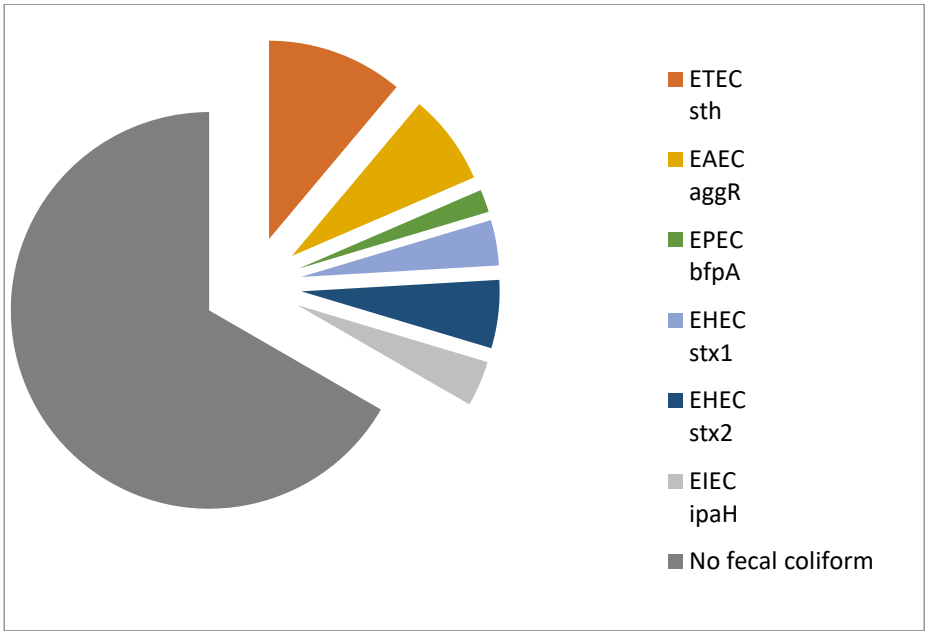

Fig 3.2.5.2 Pie chart representing results of Table 3.2.6.2

Variation in the genes detected

75% of the raw water samples of Area 6 do not have any of the marker virulence genes. The samples that were identified as ETEC, or 12.5% of the raw water samples have *st*h gene. 8.33% of samples have *agg*R gene, 2.08% have *bfp*A gene and 6.26% have *stx*2 gene. The *stx*1 gene and the *ipa*H gene, each, make up 4.17% of the raw water samples of Area 6. None of the positive samples have *lt*, *st*p or *eae*A gene. Some positive sample have more than one virulence gene.

Table 3.2.6.3 PCR results for treated water samples of Area 6

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0

Jan-19	0	0	0	1	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0	0	0	0.301511	0

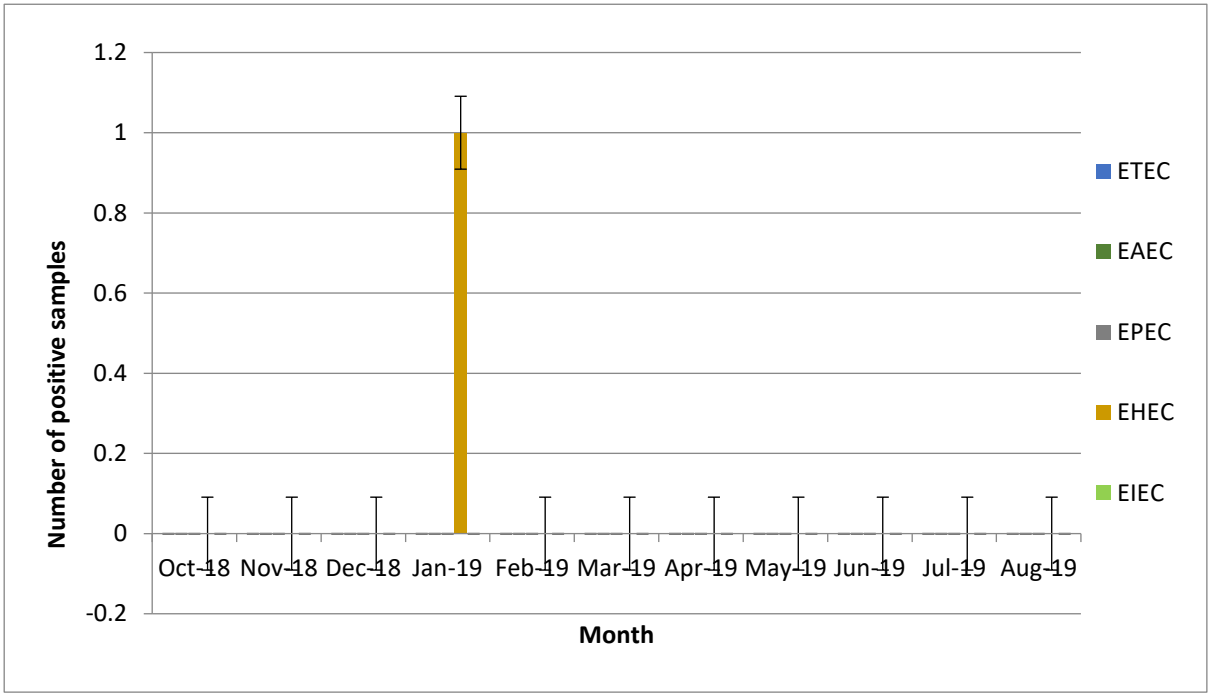

Fig 3.2.6.3 PCR results for treated water samples in Area 6

Monthly variation

A single *E.coli* positive sample was detected in January of 2019. The sample was identified as EHEC. Apart from this, no other positive sample was detected throughout the sampling period.

Seasonal variation

A single EHEC sample was detected in the winter season. No positive sample was detected during the pre-monsoon or monsoon season.

Variation in strain

Only EHEC strain was detected amongst the treated water samples of Area 6. None of the other strains were detected. The EHEC sample was detected in January 2019.

Standard deviation

Standard deviation for EHEC is 0.301511.

Table 3.2.6.4 Percentage of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	2.27
	*stx*1	0
ETEC	*st*h	0
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

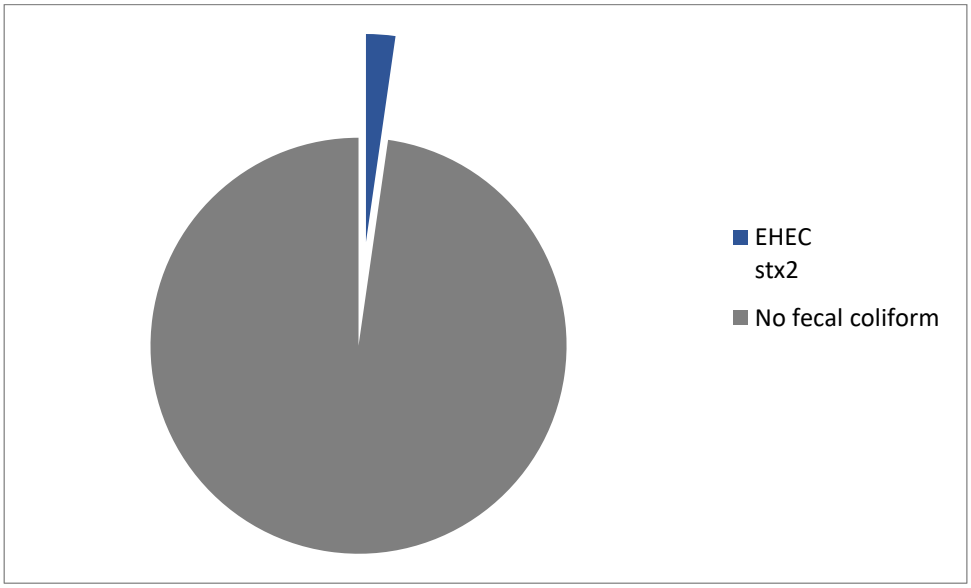

Fig 3.2.6.4 Pie chart representing the results of Table 3.2.6.4

Variation in the genes detected

97.73% of the treated water samples did not have any marker virulence genes. The remaining 2.27% of

the samples have *stx*2 gene.

3.2.7 PCR results of Area 7 (Bangshal and Wari)

Table 3.2.7.1 PCR results of raw water samples of Area 7

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	1	0	0	1	0
Jan-19	1	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.40452	0	0	0.301511	0

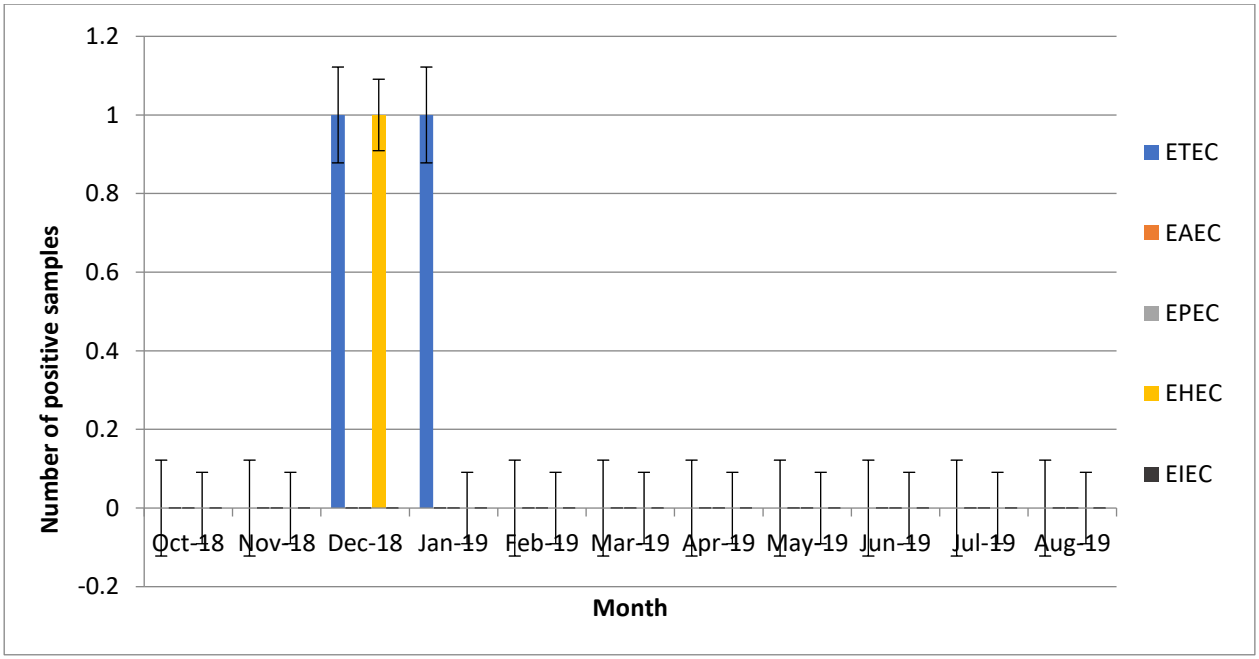

Fig 3.2.7.1 PCR results of raw water samples of Area 7

Monthly variation

Positive samples were only detected in December 2018 and January 2019. No other positive samples were detected throughout the sampling period. 2*E.coli* positive samples were detected in December of 2018, one of the samples being identified as ETEC while the other was identified as ETEC. A single ETEC sample was identified in January of 2019.

Seasonal variation

3*E.coli* positive samples were detected during the winter season. 2 of these samples were ETEC, while the remaining 1 was EHEC. Positive samples were not detected in the monsoon or the pre-monsoon season.

Variation in strain

2 ETEC samples were detected during winter in December of 2018 and January of 2019. A single EHEC sample was also detected in December of 2018. No other strain were detected in the raw water samples of Area 7.

Standard deviation

Standard deviation calculated for ETEC is 0.40452, while the standard deviation for EHEC is 0.301511. This indicates that the number of ETEC samples detected through the months varies more from the average number of ETEC detectedthan EHEC does from the average number of EHEC detected.

Table 3.2.7.2 Percentage of raw water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	3.70
	*stx*1	0
ETEC	*st*h	3.70
	*st*p	0
	lt	3.70
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

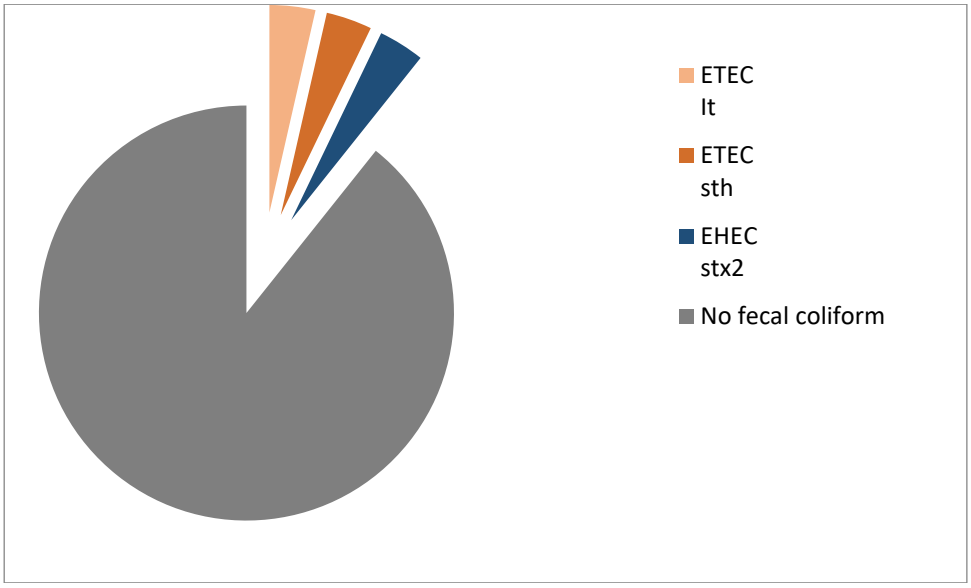

Fig 3.2.7.2Pie chart representing results of Table 3.2.7.2

Variation in the genes detected

92.59% of the raw water samples of Area 7 do not have any marker virulence gene. 3.70% of the samples have *stx*2 gene. The *lt* gene and *st*h gene, each, makes up 3.70% of the samples. None of the other marker virulence genes was present on the raw water samples of Area 7. Some samples have more than one marker virulence gene.

Table 3.2.7.3 PCR results of treated water samples of Area 7

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0

None of the treated water samples of Area 7 tested positive for *E.coli*.

3.2.8 PCR results of Area 8 (Dhanmondi, Farmgate and Tejgaon)

Table 3.2.8.1 PCR results for raw water samples of Area 8

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	2	2	1	1	0
Nov-18	1	1	0	1	0
Dec-18	0	0	0	2	0
Jan-19	1	0	0	2	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	1	0
Apr-19	0	0	0	0	0
May-19	0	1	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.6742	0.6742	0.301511	0.80904	0

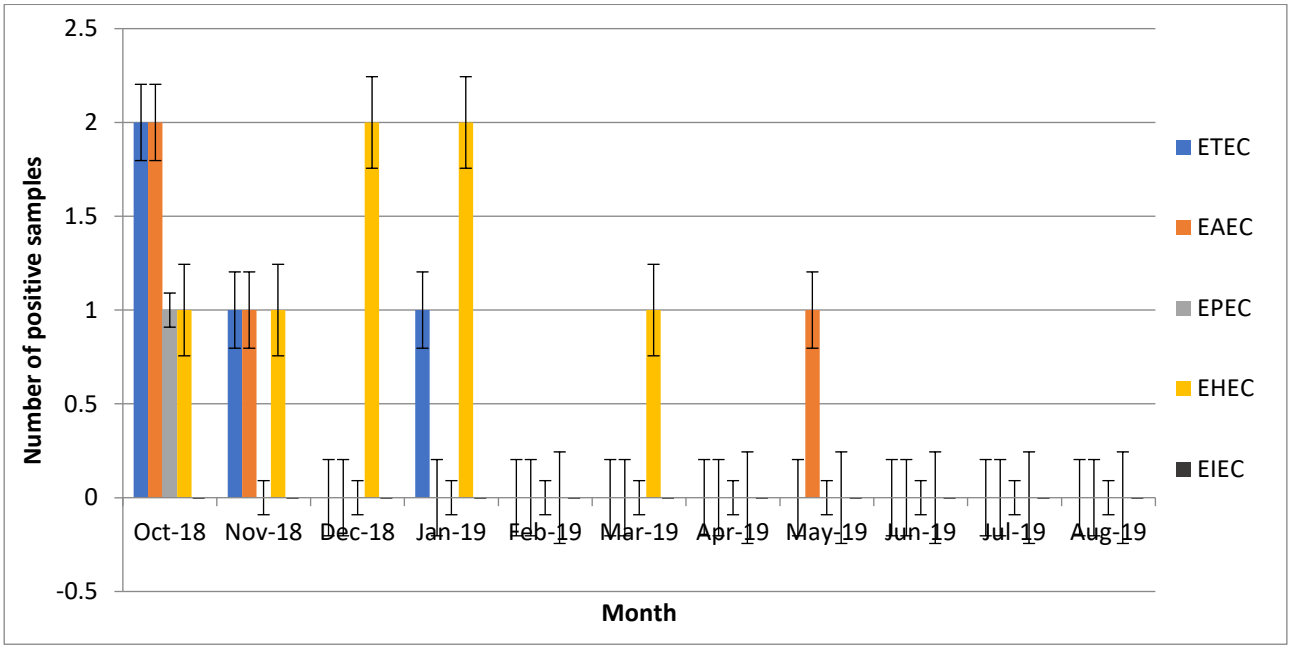

Fig 3.2.8.1 PCR results for raw water samples of Area 8

Monthly variation

In October 2018, 6 *E.coli* positive samples were detected. 2 of these samples were ETEC, 2 were EAEC, 1 was EPEC while the remaining 1 was EHEC. In the following month, 3 positive samples were detected. These were identified as ETEC, EAEC and EHEC. In December of 2018, 2 EHEC samples were detected. A total of 3 positive samples were detected in January of 2019. 1 of the positive samples were identified as ETEC, while the other 2 were identified as EHEC. A single positive sample was detected in March and May of 2019. The sample detected in March was identified as EHEC, while the sample detected in May was identified as EAEC. Positive samples were not detected in February, April, June, July and August of 2019.

Seasonal variation

Positive samples were detected in all the three seasons. Greatest numbers of positive samples were detected in winter, with 8*E.coli* positive samples being detected this season. Majority of these samples are EHEC in nature. 5 EHEC samples detected in the winter season. 2 ETEC and 1 EAEC were also detected in the winter. 6*E.coli* positive samples were detected in the monsoon season. 2 of these were ETEC and 2 were EAEC. A single EPEC and EHEC was also detected in this season. 2 samples were detected in the pre-monsoon season, 1 of which was EAEC while the other 1 was EHEC.

Variation in strain

A large portion of the positive samples was identified as EHEC. 7 EHEC samples were identified all throughout the sampling time-period. 4 EAEC and 4 ETEC samples were also identified. Like EHEC, EAEC samples were detected in all of the three seasons, with the greatest number of EAEC samples being identified in October of 2018. The ETEC samples were identified at the end of the monsoon season and in the winter season. The greatest numbers of ETEC samples were identified in October 2018. A single EPEC sample was identified at the end of the monsoon season, in October 2018. Raw water samples of Area 8 did not have any EIEC.

Standard deviation

Standard deviation for EHEC is 0.80904. This the largest value calculated for standard deviation, for the raw water samples of Area 8. Standard deviation of EAEC and ETEC is 0.6742. Standard deviation of EPEC is 0.3015.

Table 3.2.8.2 Percentage of raw water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	4.26
	*stx*1	10.64
ETEC	*st*h	8.51
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	2.13
EAEC	*agg*R	8.51
EIEC	*ipa*H	0

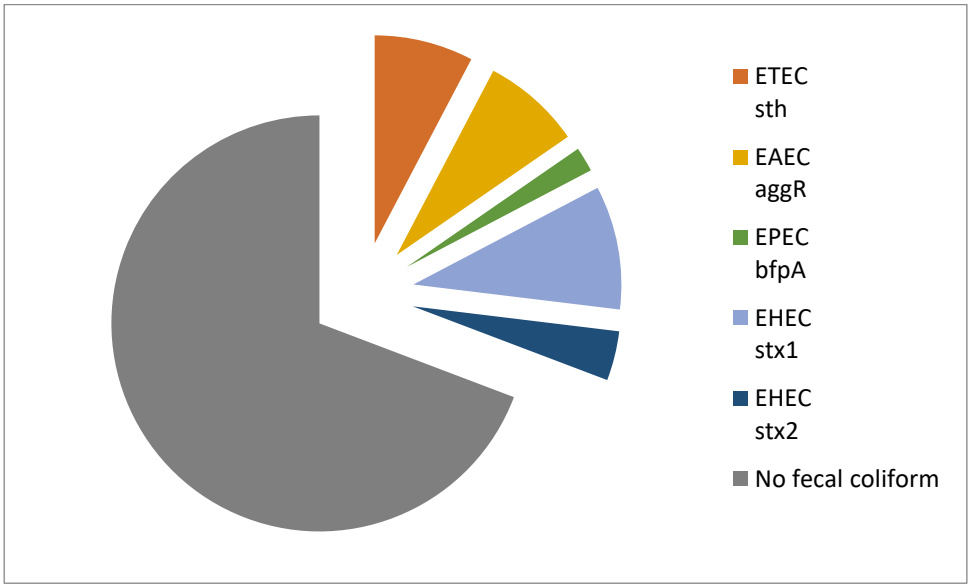

Fig 3.2.8.2 Pie chart representing results of Table 3.2.8.2

Variation in the genes detected

76.60% of the raw water samples do not have any of the marker virulence genes. Majority of the samples that were identified as EHEC, 10.64% of the raw water samples of Area 8 have *stx*1 gene. 4.26% of the samples have *stx*2 gene. *st*h gene and *agg*R gene, each, makes up 8.51% of the samples. 2.13%

of the samples have *bfp*A gene. None of the raw water samples of Area 8 have *lt*, *st*p, *eae*A or *ipa*H gene. More than one virulence marker gene is present in some samples.

Table 3.2.8.3 PCR results for treated water samples of Area 8

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	1	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.301511	0	0	0	0

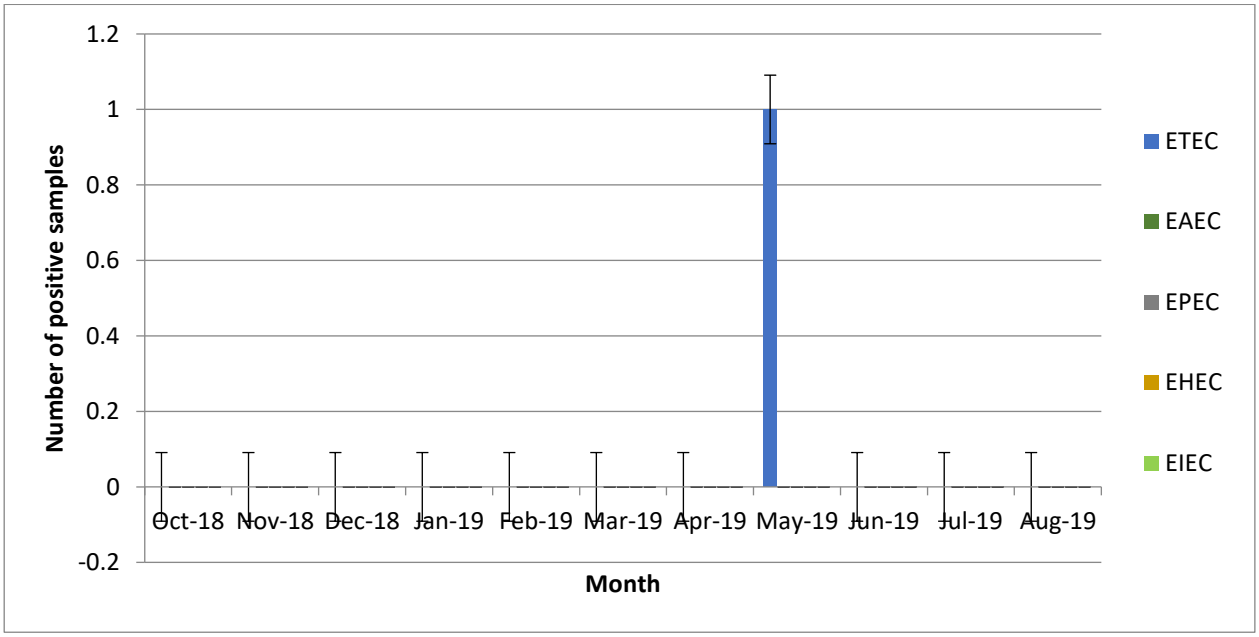

Fig 3.2.8.3 PCR results for treated water samples of Area 8

<u>Monthly variation</u>

A single ETEC sample was detected in May of 2019. Positive samples were not detected in any of the

other months.

Seasonal variation

A single ETEC sample was detected in the pre-monsoon season. Positive samples were not detected in winter or monsoon season.

Variation in strain

Only 1 treated water sample tested positive for *E.coli*. The sample was identified as ETEC. None of the other strains were detected.

Standard deviation

Standard deviation for ETEC is 0.301511.

Table 3.2.8.4 Percentage of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	0
	*stx*1	0
ETEC	*st*h	2.78
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

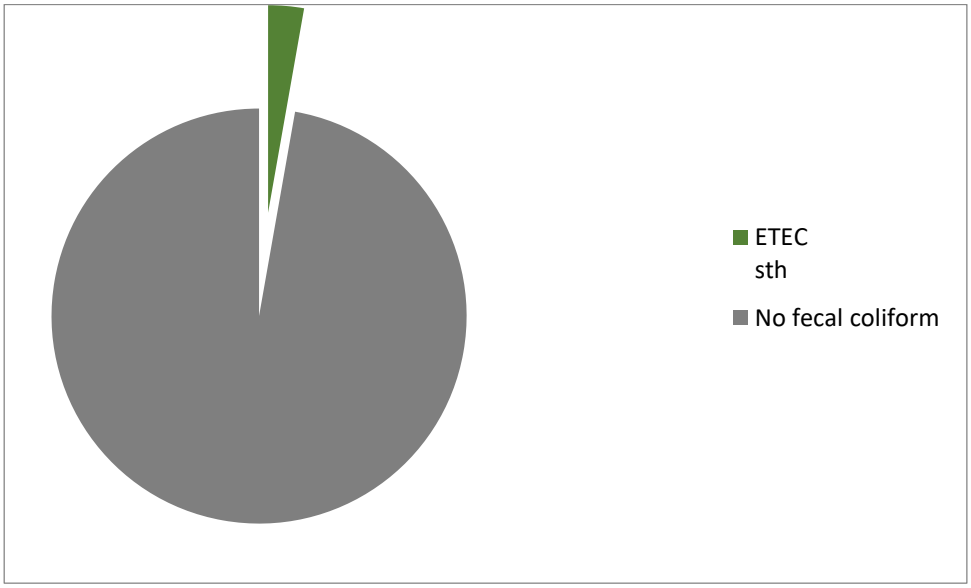

Fig 3.2.8.4 Pie chart representing results of Table 3.2.8.4

Variation in the genes detected

97.22% of the samples do not have any of the marker virulence genes. The samples that was identified as ETEC has the *st*h gene. This means that 2.78% of the samples have s*t*h gene. None of the other marker virulence genewere detected.

3.2.9 PCR results of Area 9 (Mohakhali and Rampura)

Table 3.2.9.1 PCR results for raw water samples of Area 9

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	1	0	0	0	0
Nov-18	2	1	0	4	1
Dec-18	3	0	0	1	0
Jan-19	1	0	0	2	1
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	1.026911	0.301511	0	1.2862914	0.40452

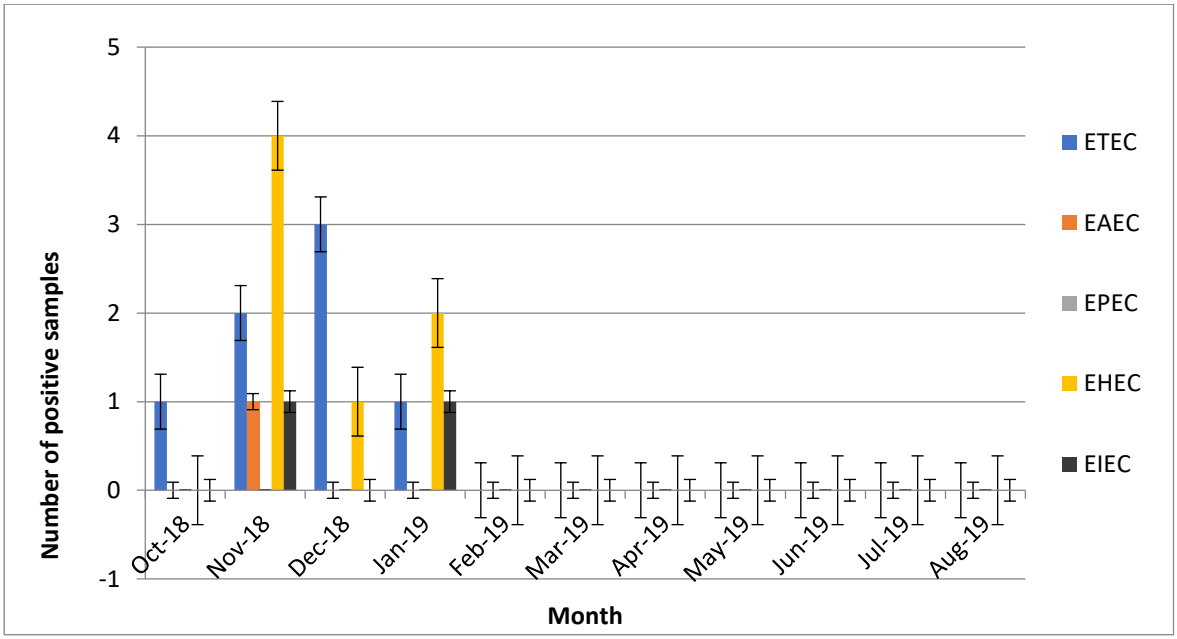

Fig 3.2.9.1 PCR results for raw water samples of Area 9

Monthly variation

E.coli positive samples were detected in the raw water samples of Area 9 from October 2018 until January 2019. *E.coli* positive samples were not detected in any of the other months. In October 2018, a single ETEC sample was detected. When raw water samples of Area 9 is considered, the largest number of *E.coli* positive samples were detected in November of 2018. 8*E.coli* positive samples were detected in November. 4 of these samples were identified as EHEC, while 2 were identified as ETEC. 1 EAEC and 1 EIEC were also identified this month. In both December 2018 and January 2019, 4 positive samples were identified. In December 2018, 3 ETEC and 1 EHEC samples were identified. In January 2019, 2 EHEC, 1 ETEC and 1 EIEC sample was identified.

Seasonal variation

Majority of the positive samples were detected in the winter season. 16 *E.coli* positive samples were found this season, 7 of which were identified as EHEC, 6 were ETEC, 2 were EIEC and 1 was EAEC. A single ETEC sample was also detected at the end of the monsoon season. Positive samples were not detected in the pre-monsoon season.

Variation in strain

Majority of the *E.coli* positive samples found in the raw water samples of Area 9 were identified as either ETEC or EHEC. 7 ETEC samples were detected mainly in the winter season and at the end of the monsoon season. The greatest numbers of EHEC samples were detected in December of 2018. 7 EHEC samples were also detected in the winter season, with the largest number of EHEC being detected in November of 2018. 2 EIEC and 1 EAEC samples were detected in the winter season as well. EPEC samples were not detected throughout the sampling period.

Standard deviation

The number of EHEC samples detected throughout the sampling period varies the most from its average. This is indicated by the fact that the standard deviation calculated for EHEC is the largest. Standard deviation for EHEC is 1.2862914. The number of EAEC samples detected throughout the sampling period varies the least from the average. This is indicated by the fact that standard deviation for EAEC is the smallest. Standard deviation for EAEC is 0.301511. Standard deviation for EIEC is 0.40452 while the standard deviation for ETEC is 1.026911.

Table 3.2.9.2 Percentages of raw water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	9.61
	*stx*1	3.85
ETEC	*st*h	9.61
	*st*p	0
	lt	5.77
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	1.92

EIEC	*ipa*H	3.85

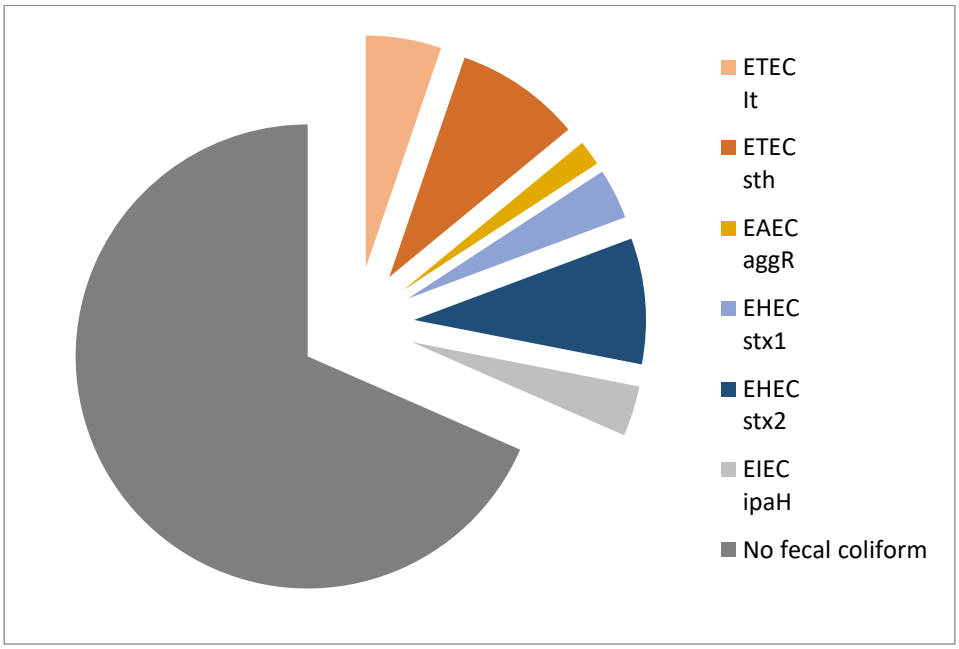

Fig 3.2.9.2 Pie chart representing results of Table 3.2.9.2

Variation in the genes detected

75% of the raw water samples of Area 9 do not have any marker virulence gene. Majority of the positive samples have either *stx*2 gene or *st*h gene, or both. *st*h gene and *stx*2 gene, each, makes up 9.61% of the raw water samples of Area 9. Similarly, *stx*1 gene and *ipa*H gene, each, makes up 3.85% of raw water samples of this area. 5.77% of the samples have*lt* gene and 1.92% of the samples have *agg*R gene. None of the samples have*st*p gene, *bfp*A gene or *eae*A gene. More than one marker virulence gene is present in some of the positive samples.

Table 3.2.9.3 PCR results for treated water samples of Area 9

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	1	0
Nov-18	0	1	0	0	0
Dec-18	0	0	0	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0

Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0	0.301511	0	0.301511	0

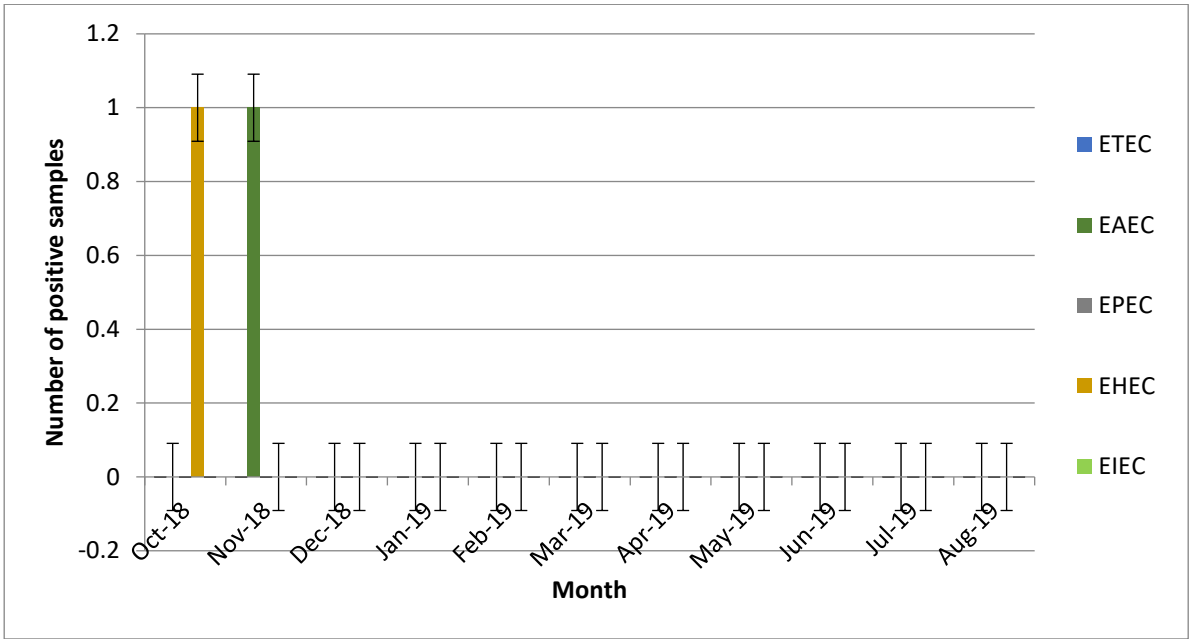

Fig 3.2.9.3 PCR results for treated water samples of Area 9

Monthly variation

An *E.coli* positive sample was detected in October of 2018, which was identified as EHEC. A positive sample was also detected in November 2018. This was identified as EAEC. Positive samples were not detected for the remainder of the sampling period.

Seasonal variation

An EHEC sample was detected at the end of the monsoon season. A single EAEC sample was also detected at the beginning of the winter season. Positive samples were not detected in the pre-monsoon season.

Variation in strain

Only EHEC and EAEC strain were detected in the treated water samples of Area 9. None of the other

strains were detected. The sample identified as EHEC was detected in the monsoon season, in October 2018. The sample identified as EAEC was detected in the winter season, in November 2018.

Standard deviation

Standard deviation for both EHEC and EAEC is 0.301511.

Table 3.2.9.4 Percentages of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	3.03
	*stx*1	0
ETEC	*st*h	0
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	3.03
EIEC	*ipa*H	0

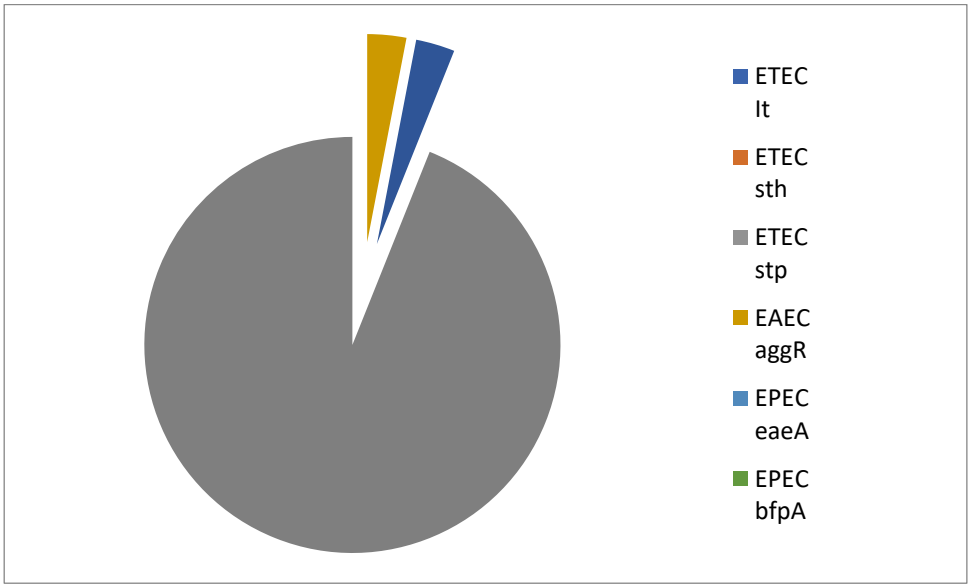

Fig 3.2.9.4 Pie chart representing results of Table 3.2.9.4

Variation on the genes detected

93.94% of the treated water samples do not have any marker virulence gene. 3.03% of the sample have *stx*2 gene. Another 3.03% of the samples have *agg*R gene. No other marker virulence gene was found in the treated water samples of Area 9.

3.2.10 PCR results of Area 10

Table 3.2.10.1 PCR results for raw water samples of Area 10

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	1	0	0	1	0
Nov-18	0	0	0	0	0
Dec-18	0	1	1	0	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0.301511	0.301511	0.301511	0.301511	0

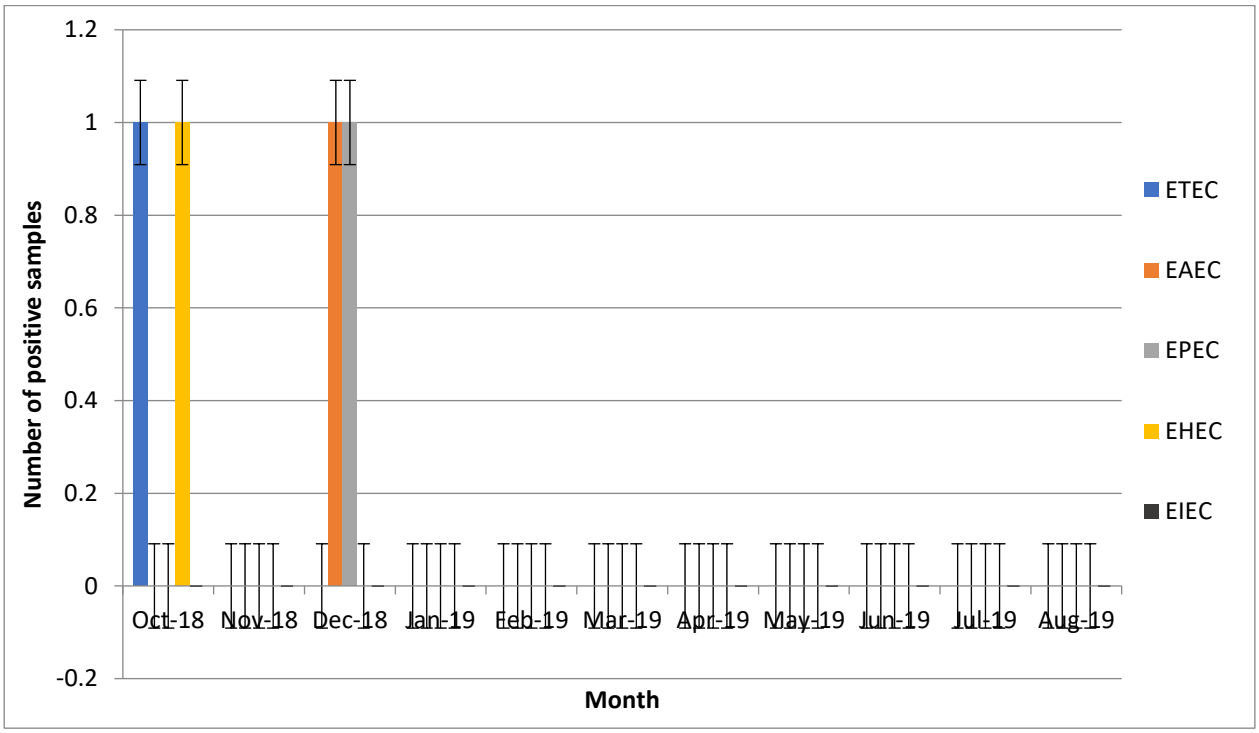

Fig 3.2.10.1 PCR results for raw water samples of Area 10

Monthly variation

In case of raw water samples of Area 10, positive *E.coli* samples were detected at the beginning of the sampling duration. In October 2018, 2 positive *E.coli* samples were detected. One of the samples were identified as ETEC, while the other was identified as EHEC. In the following month, no positive samples were detected. In December of 2018, 1 EAEC and 1EPEC was detected. Positive samples were not detected after this point of the sampling duration.

Seasonal variation

Samples that had *E.coli*were detected at the end of monsoon season and in the winter season. 1 ETEC and 1 EHEC sample was detected at the end of the monsoon season. 2 positive samples were also detected in the winter season. These were identified as EAEC and EPEC. Positive samples were not detected in the pre-monsoon season.

Variation in strain

EIEC is the only strain that was not detected in the raw water samples of Area 10. The remaining four strains were detected throughout the sampling duration. 1 ETEC and 1 EHEC sample were detected at the end of monsoon season, while 1 EAEC and 1 EPEC sample were detected in the winter season.

Standard deviation

Standard deviation for ETEC, EHEC, EPEC and EAEC is 0.301511.

Table 3.2.10.2 Percentage of raw water samples detected for marker virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	4.76
	*stx*1	4.76
ETEC	*st*h	4.76
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	4.76
EAEC	*agg*R	4.76

EIEC	*ipa*H	0

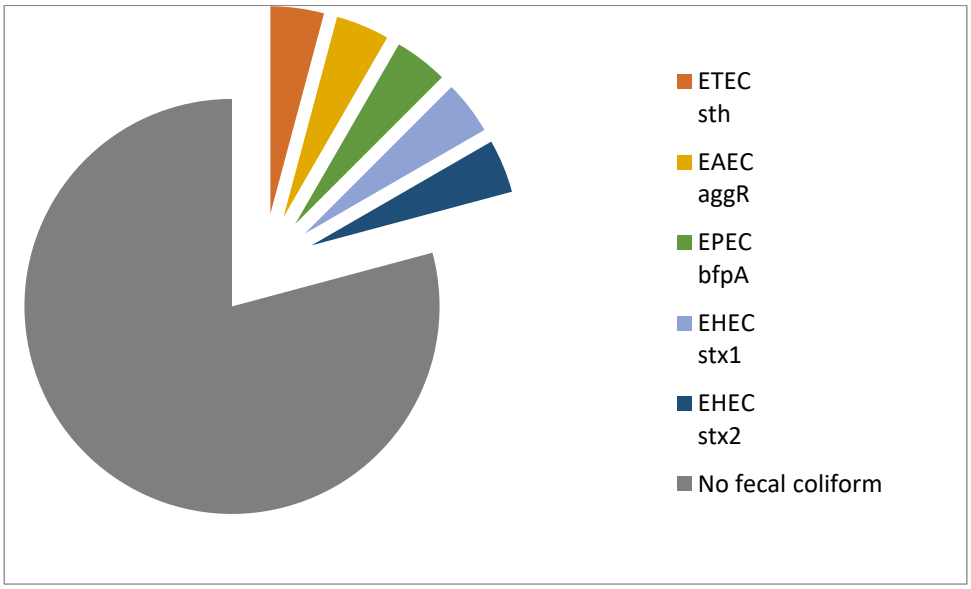

Fig 3.2.10.2 Pie chart representing results of Table 3.2.10.2

Variation in genes detected

90.48% of the samples do not have any virulence gene. None of the samples have*lt* gene, *st*p gene, *eae*A gene or *ipa*H gene. *st*h gene, *agg*R gene, *bfp*A gene, *stx*1 gene and *stx*2 gene, each, makes up 4.76% of raw water samples of Area 10. More than one virulent marker gene was detected in some positive sample.

Table 3.2.10.3 PCR results for treated water samples of Area 10

Month	**ETEC**	**EAEC**	**EPEC**	**EHEC**	**EIEC**
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	1	0
Jan-19	0	0	0	0	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0

Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0
SD	0	0	0	0.301511	0

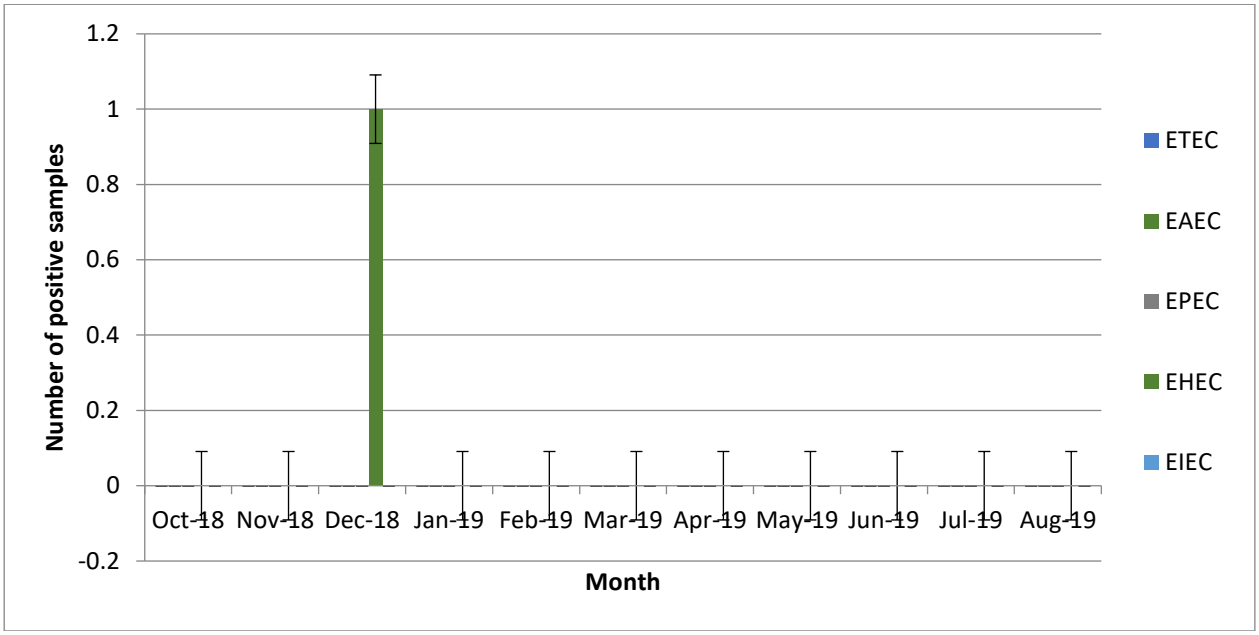

Fig 3.2.10.3 PCR results for treated water samples of Area 10

Monthly variation

A single *E.coli* positive sample was detected amongst the treated water samples of Area 10. The sample was detected in December 2018 and was identified as EHEC.

Seasonal variation

A single positive sample was detected in the winter season. Samples were not detected in the pre-monsoon or monsoon season.

Variation in strain

EHEC is the only strain that was detected in the treated water samples of Area 10. None of the other four strains were detected.

Standard deviation

Standard deviation for EHEC is 0.301511.

Table 3.2.10.4 Percentage of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	0
	*stx*1	8.33
ETEC	*st*h	0
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

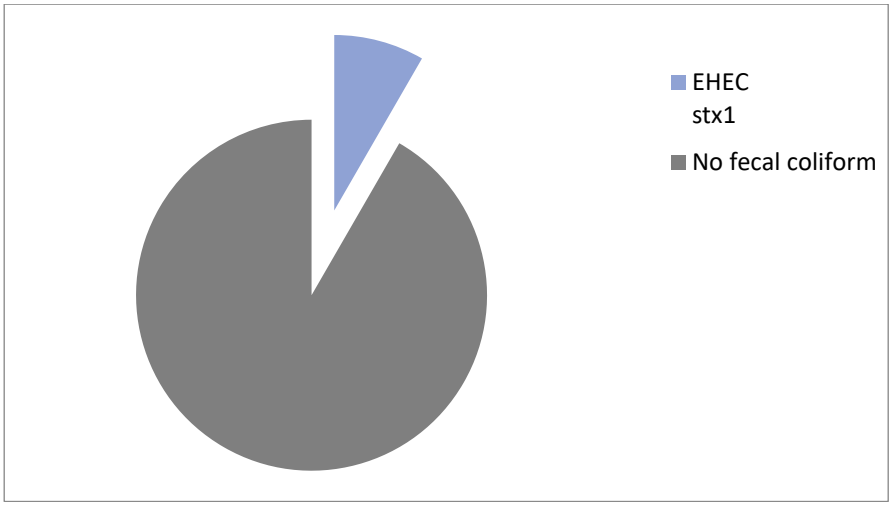

Fig 3.2.10.4 Pie chart representing results of Table 3.2.10.4

<u>Variation in the genes detected</u>

91.67% of the treated water samples of Area 10 do not have any virulence gene. The only marker virulence that was detected was the stx1 gene. 8.33% of the treated water samples of Area 10 have this gene.

3.2.11 PCR results of Area 11 (Korail slum)

Table 3.2.11.1 PCR results for raw water samples of Area 11

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	1	1	0	2	0
Nov-18	1	1	1	0	0
Dec-18	5	0	0	2	0
Jan-19	1	0	0	3	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	1	0	0	1	0
Jun-19	0	0	0	0	0
Jul-19	0	1	0	1	0
Aug-19	0	0	0	0	0
SD	1.47093	0.467099	0.301511	1.07872	0

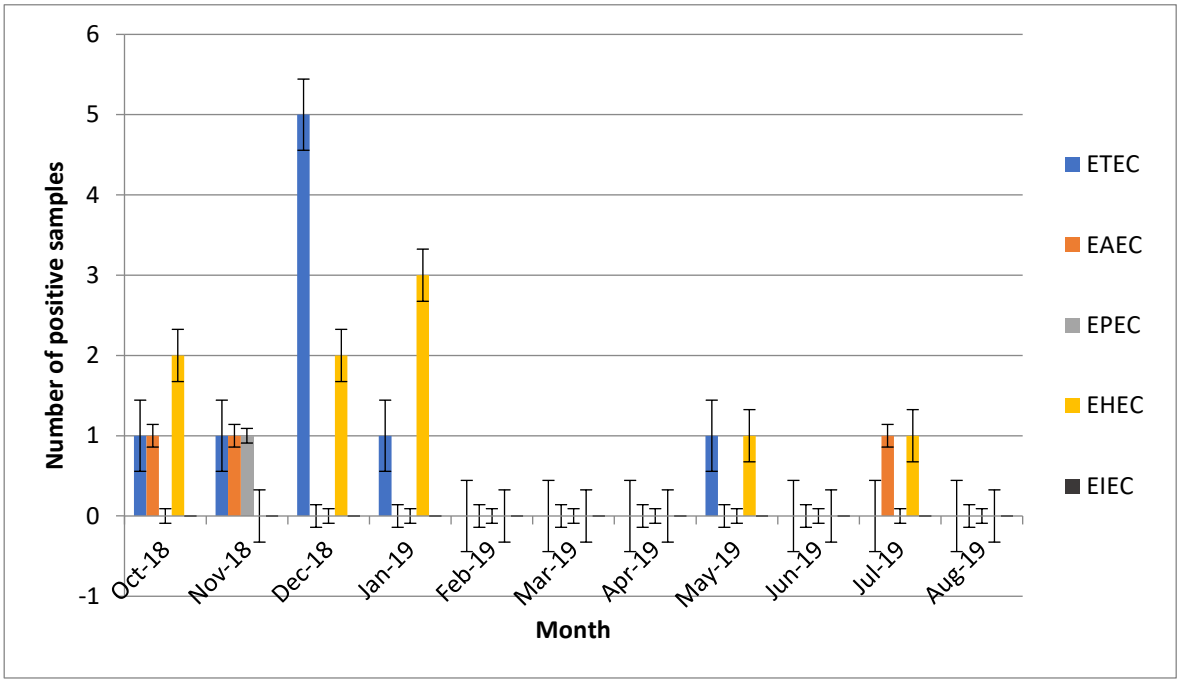

Fig 3.2.11.1 PCR results of raw water samples of Area 11

<u>Monthly variation</u>

Positive *E.coli* samples were detected in the raw water samples of Area 11 throughout the sampling duration. In October 2018, 4 positive *E.coli* samples were detected. 2 of these were EHEC, 1 was identified as EAEC and 1 was identified as ETEC. 3 positive samples were detected in the following month. These were identified as ETEC, EPEC and EAEC. The greatest numbers of *E.coli* positive samples were detected in December of 2018. 7*E.coli* positive samples were detected this month. Majority of the samples detected in this month was identified as ETEC. In other words, 5 ETEC samples were detected and identified in December of 2018. 2 EHEC samples were also detected in December 2018. 4 positive samples were detected in January 2019, 3 of which were identified as EHEC and 1 of which was identified as ETEC. *E.coli* positive samples were not detected in February, March and April of 2019. A single ETEC and a single EHEC was detected in May 2019. Positive samples were not detected in June of 2019 and again in August of 2019. In July 2019, 1 EAEC and 1 EHEC was detected.

Seasonal variation

Positive samples were detected throughout the sampling period. The greatest number of positive samples were detected in the winter season. 14*E.coli* positive samples were detected during this season, half of which were identified as ETEC. 5 EHEC, 1EPEC and 1 EAEC were also identified during this season. Only 2 positive samples were detected during the pre-monsoon season, which were identified as ETEC and EHEC. 3 EHEC, 2 EAEC and 1 ETEC sample were detected in the monsoon season. A total of 6*E.coli* positive sample were detected in the monsoon season.

Variation in strain

EIEC is the only strain that was not detected amongst the raw water samples of Area 11. 9 ETEC samples were detected throughout the entire sampling duration, with 5 ETEC samples being detected in December 2018 itself. 9 EHEC samples were also detected. The greatest numbers of EHEC samples were detected in January 2019. 3 EAEC and 1 EPEC sample were also detected throughout the entire

sampling period.

Standard deviation

Standard deviation for ETEC is 1.47093, indicating that the number of ETEC detected throughout the months varies the most from the average number of ETEC detected. Standard deviation for EPEC is 0.301511. This is the smallest standard deviation calculated for the different strain detected in raw water samples of Area 11. This indicates that the number of EPEC samples detected varies the least from the average number of positive EPEC detected. Standard deviation for EHEC is 1.07872, while the standard deviation for EAEC is 0.467099.

Table 3.2.11.2 Percentage of raw water samples detected for marked virulence gene

Strain	**Target gene**	**Percentage (%)**
EHEC	*stx*2	37.50
	*stx*1	16.67
ETEC	*st*h	37.50
	*st*p	0
	lt	0
EPEC	*eae*A	0
	*bfp*A	4.17
EAEC	*agg*R	12.50
EIEC	*ipa*H	0

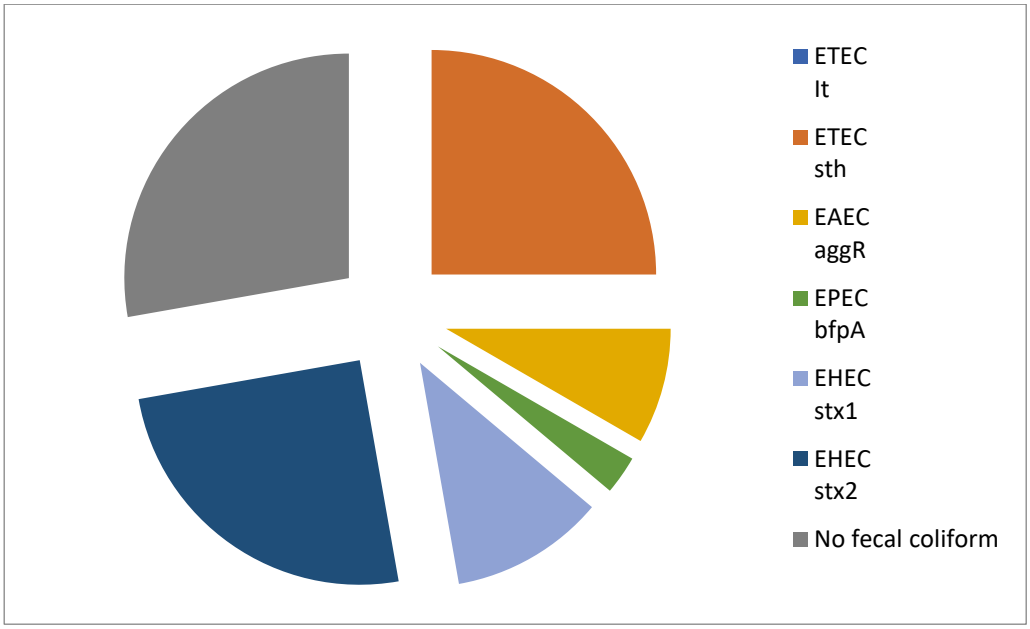

Fig 3.2.11.2 Pie chart representing the results of Table 3.2.11.2

Variation in the genes detected

41.67% of the samples do not have any marker virulence gene. *stx*2 gene and *st*h gene, each, makes 37.50% of the samples. 16.61% of the samples have *stx*1 gene, 12.50% have *agg*R gene and 4.17% of the samples have *bfp*A gene. None of the samples have*lt* gene, *st*p gene and *ipa*H gene. Many of the *E.coli* positive samples have more than 1 marker virulence gene.

Table 3.2.11.3 PCR results of treated water samples of Area 11

Month	ETEC	EAEC	EPEC	EHEC	EIEC
Oct-18	0	0	0	0	0
Nov-18	0	0	0	0	0
Dec-18	0	0	0	1	0
Jan-19	1	0	0	1	0
Feb-19	0	0	0	0	0
Mar-19	0	0	0	0	0
Apr-19	0	0	0	0	0
May-19	0	0	0	0	0
Jun-19	0	0	0	0	0
Jul-19	0	0	0	0	0
Aug-19	0	0	0	0	0

SD	0.301511	0	0	0.40452	0

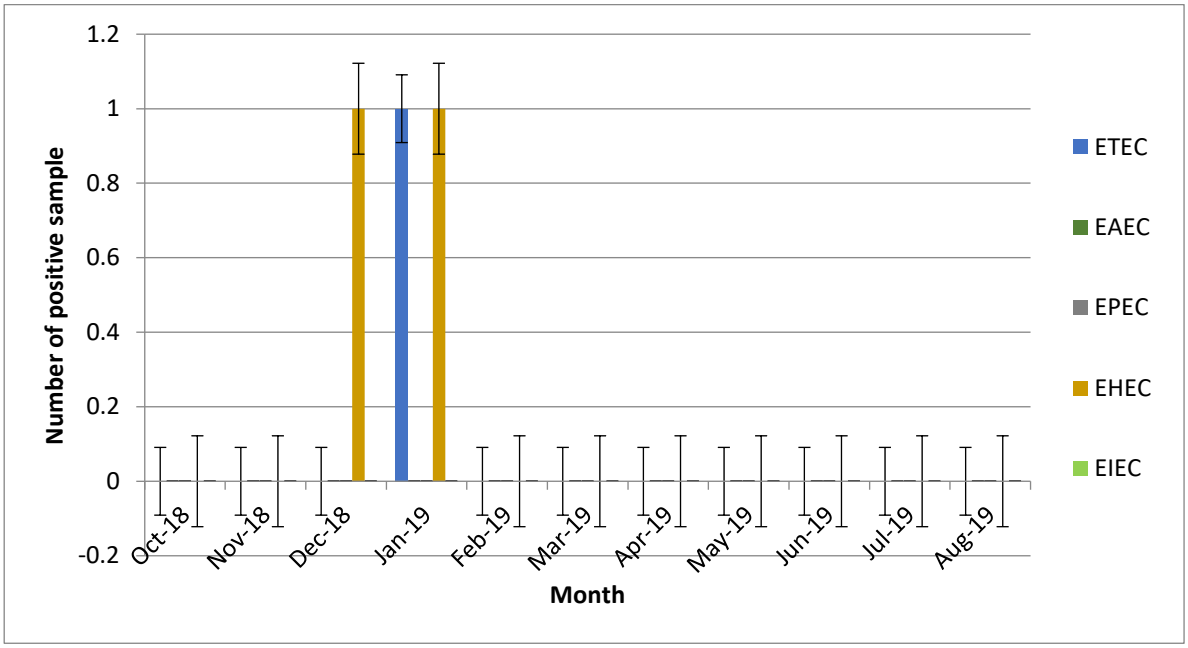

Fig 3.2.11.3 PCR results of treated water samples of Area 11

Monthly variation

When treated water samples of Area 11 is taken into consideration, *E.coli* positive samples were detected in December 2018 and January 2019 only. *E.coli* positive samples were not detected before December 2018 or after January 2019. 1 EHEC sample was detected in December of 2018. 2*E.coli* positive samples were detected in January of 2019. One of the samples were identified as ETEC while the other was identified as EHEC.

Seasonal variation

E.coli positive samples were detected in the winter season only. 3 positive samples were detected in the winter season, 2 of which were identified as EHEC and 1 was identified as ETEC. Positive samples were not detected in the pre-monsoon or monsoon season.

Variation in strain

EHEC and ETEC were the only strains detected in the treated water samples of Area 11. No other strains were detected. 2 EHEC positive sample were detected in the winter season, in December 2018

and in January 2019. 1 ETEC positive sample was also detected in winter, in January 2019.

Standard deviation

Standard deviation of EHEC is 0.4042 while standard deviation of ETEC is 0.301511. This indicates that number of EHEC samples detected throughout the sampling period varies more from its average than the number of ETEC samples does.

Table 3.2.11.4 Percentage of treated water samples detected for marked virulence gene

Strain	Target gene	Percentage (%)
EHEC	*stx*2	40
	*stx*1	40
ETEC	*st*h	0
	*st*p	0
	lt	20
EPEC	*eae*A	0
	*bfp*A	0
EAEC	*agg*R	0
EIEC	*ipa*H	0

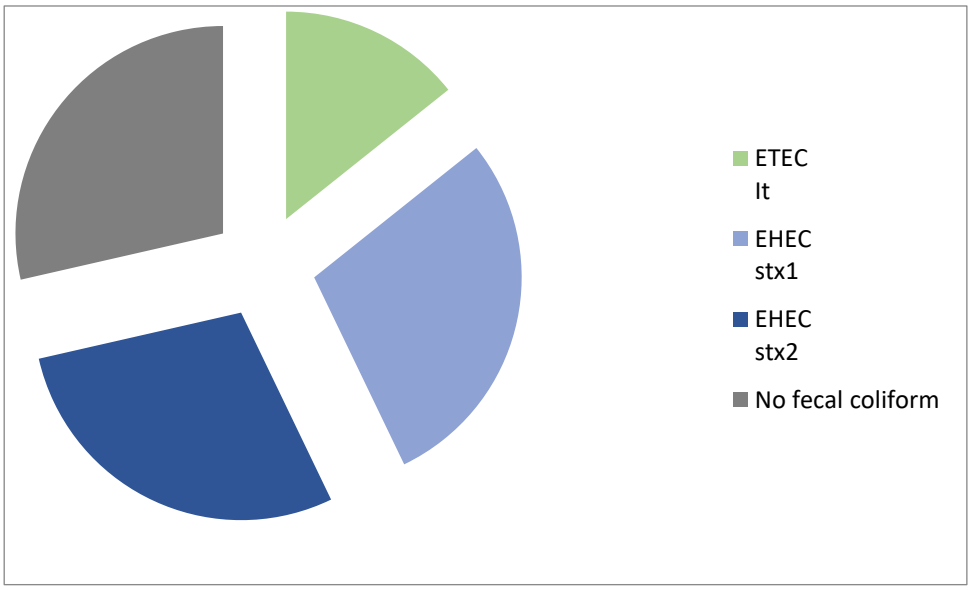

Fig 3.2.11.4 Pie chart representing the results of Table 3.2.11.4

Variation in the genes detected

40% of the treated water samples does not have any marker virulence gene. *stx*1 gene and *stx*2 gene,

each, makes up 40% of the treated water samples of Area 11. 20% of the samples have *lt* gene. Some of the positive samples have more than 1 marker virulence gene. None of the samples have*st*h gene, *st*p gene, *agg*R gene, *eae*A gene, *bfp*A gene or *ipa*H gene.

Chapter 4
Discussion

Escherichia coli is a gram-negative, rod-shaped bacteria that are capable of facultative anaerobic respiration. E.coli is a part of the natural flora found in the human colon. It is also found in the lower intestine of warm-blooded animals. According to James P. Nataro and James B. Kaper, 1998, Diarrheagenic E.coli colonises the gastrointestinal tracts of infants within a few hours after they are born. The bacteria and host start a mutually beneficial relationship after this. E.coli produces vitamin K2 and prevents pathogenic bacteria from colonising the intestines (Bentley R, et al, 1982) (Hudault S,et al, 2001) (Reid G, et al, 2001). E.coli and other groups of bacteria that are capable of facultative anaerobic respiration make up about 0.1% of the gut's natural flora (Eckburg PB, et al, 2005).

E.coli is transmitted mainly through the fecal oral route. The bacteria is expelled along with the fecal material of the host. The bacteria will multiply quickly in the fecal material for the next three days, if oxygen is present. After this, the rate of replication will start to decrease (Russell JB,*et al,* 2001).According to Guidelines for drinking-water quality by World Health Organization, 1997, any water that is intended for consumption should not have detectable fecal coliform (such as *E.coli*), when 100ml of that particular sample is tested. Coliform count of the sample would determine the quality of

the water sample.Fecal coliforms are oxidase negative. Fecal coliforms are capable of fermenting lactose to give acid and gas, within 48 hours, at a temperature of 44.5°C.

Although it's mostly harmless, certain highly adapted strains of *E.coli* are capable of causing infection in individuals even with the strongest immune systems. After the bacterium infects the host, it may be confined to the mucosal surfaces or can spread throughout the body of the host. Pathogenic strains of *E.coli* usually cause:

I. Urinary tract infections

II. Sepsis or meningitis

III. Enteric or diarrheal diseases (Nataro, J. P, et al, 1998)

In this thesis, the water quality of certain areas was evaluated by the presence of E.coli, as well as the presence of pathogenic strains of *E.coli*- i.e. ETEC, EPEC, EIEC, EHEC, EAEC- was observed. Tap water and water meant for consumption were collected mainly from households in different area of Dhaka city. A few water samples were also collected from restaurants, roadside tea stalls and slums. The water samples were filtered and the filter paper were placed on mFC plates. This was used to get the fecal coliform count. The presence of fecal coliform was confirmed by subjecting the samples to multiplex PCR.

If Guidelines for drinking-water quality by World Health Organization, 1997 is followed and only the average fecal count is taken into consideration, raw water samples of all of the 11 different areas are not considered potable. The raw water samples are from all of the areas would be classified as unsatisfactory. Only the treated water samples from Area 7 can be considered potable and be classified as highly satisfactory. Treated water samples from Area 1, 2, 3, 6, 8 and 10 are classified as doubtful while treated water samples from Area 4, 5, 9 and 11 are classified as unsatisfactory.

If average fecal count is taken into consideration, raw water and treated water from Area 11 has the greatest average fecal coliform count. Average fecal count for raw water of Area 11 is 265.37 while the average fecal count for treated water of this area is 48.

In all 11 areas, the percentage of positive samples in raw water samples is always greater than the percentage of positive samples in treated water samples. *E.coli*was detected in 91.67% of the raw water samples of Area 11. 48.64 % of the raw water samples of Area 5 also tested positive for *E.coli*. Area 11 and Area 5 have the greatest percentages of samples that tested positive for *E.coli*. This is also reflected in the treated water samples from these areas. 60% of the treated water samples from Area 11 have tested positive for *E.coli*, while 14.94% of the treated water samples from Area 5 have tested positive for *E.coli*.

IfraTunNur, RakhiBaishnab and Niger Sultana Tethee investigated the water quality in the slums of Dhaka, Bangladesh. 90% of their sample was contaminated with fecal material. *Escherichia coli*was detected in 16 out of the 20 samples, or in 80% of their samples. *E.coli*was detected in 91.67% of the samples of Area 11. Sample size of Area 11 was much smaller than the sample size of IfraTunNur, RakhiBaishnab and Niger Sultana Tethee. This might be why the results vary. Numerous factors can explain why water in slums is not of the highest quality. A large number of slums in Dhaka city start to form when people begin to settle illegally on large unoccupied stretches of land. Due this reason, the poverty-stricken people that are residing in the slums receive subpar services. Sewage pipelines and water pipelines run parallel in the city. It is possible that the sewage pipelines and the water pipelines in the slum area are not properly maintained. As a result, contents of the sewage pipelines might leak into the water pipelines, contaminating it with fecal material. Slums are also extremely overcrowded. Only a few taps are present to meet the demands of hundreds of individuals residing in the slum. This makes accessing water quite difficult for the residents of slums. Only a limited number of toilets are present in the slum area, which makes it difficult for the residents to maintain personal hygiene. These factors contribute in deteriorating the quality of water available in slums.

MrityunjoyAcharjee, Farjana Rahman, Fahmida Jahan and Rashed Noor carried out a research where they studied the bacterial proliferation in municipal water supplied in Mirpur. Fecal coliform and E.coli were not detected in samples from the supply points. However, *E.coli* and fecal coliform were detected in the samples collected from consumer points. Fecal coliform were detected in 11 out of the 30 samples while *E.coli* were detected in 8 out of the 30 samples. Fecal coliform was detected in 36.67% of their samples while E.coli was detected in 26.67% of their samples. A complete comparison cannot be made between this research and our thesis due to a number of reasons. The research mentioned above does not differentiate between raw water samples and treated water samples obtained from consumer point. Our thesis does not differentiate between samples obtained from supply points or from consumer points. In our thesis, Area 5 represents Mirpur, Mugdapara and Tolarbagh. *E.coli*was detected in 48.64% of the raw water samples and 14.94% of the treated water samples of Area 5. The research mentioned above and our thesis both indicate that water supplied to the residents of these areas is of not of the greatest quality and is not considered potable. The difference in results between the two researches is mainly due to two reasons. The research mentioned above was conducted before 2013, while our sampling process was carried out in 2018- 2019. A considerable amount of time had passed between the two researches, which can account for the difference in the result obtained. Our thesis might include a greater area in Area 5 than the area covered by the research carried out in 2013. This might also help in explaining as to why there is a difference between the researches. There are many reasons why *E.coli* might be present in the water supply of Area 5. As mentioned before, contents of the sewage pipeline might have leaked into the water pipelines. The bacteria might have settled and formed biofilm in the housing tanks and pipelines, thus contaminating the supplied water.

Raw water of Area 4 has a very low average fecal coliform count. Average fecal coliform count of Area 4 is 16.38. The lowest average fecal coliform count is observed for treated water of Area 7, which is only 0.09.

Area 7 has the smallest percentage of samples that tested positive for *E.coli*. Only 11.11% of the raw

water samples of Area 7 have *E.coli*. *E.coli*was not detected in the treated water samples of Area 7. Similar to the treated water samples of Area 7, none of the treated water sample of Area 1 have*E.coli*. Majority of the *E.coli* positive raw water sample were identified as ETEC. A very small portion of the *E.coli* positive raw water samples were identified as EIEC. Ahmed ElsadekFakhr, Maha Kamal Gohar, and Amal Hassan Atta investigated the quality of drinking water in Zagazig city in Egypt. 33.33% of their samples had *eae*A gene. This indicates that most of their samples were identified as EPEC. ETEC was the second most common strain detected in their sample. 26.27% of their samples contained LT gene, half of which also contained the ST gene. A research was conducted studying the genetic diversity of *E.coli* isolated from water supply from different households in Dhaka city. The results found in this research is similar to our results. Majority of their samples were identified as ETEC. 6 their samples contained the *st* gene while 3 of the samples contained the *lt* gene. 2 of the samples contained both of this gene.

The same phenomenon is observed when treated water is taken into consideration. A much smaller portion of the treated water samples tested positive for *E.coli* than raw water samples. Majority of the treated water samples that were confirmed to have *E.coli* were identified as ETEC. None of the treated water samples were identified as EIEC or EPEC.

Majority of the positive raw water samples were detected in the winter season. This is observed for all of the 11 areas from which sample was collected. The smallest numbers of positive samples were detected in the pre-monsoon season. When we consider treated water samples, a similar phenomenon is observed. The greatest numbers of treated water samples were detected in the winter season while the smallest numbers of samples were detected in the pre-monsoon season. An investigation was carried out bySikdar, M. S. S., Abony, M., Zerin, T., Banik, A., and Datta, S where the bacterial load of rivers and lake waters of Dhaka city was investigated through the seasons in the year of 2016 till 2017. A greater bacterial load was found in the spring and winter season while the bacterial load decreases during the monsoon season.The weather remains almost constant throughout the whole year in Dhaka

city. Even though the temperature decreases in the winter months, the drop is not significant. Rainfall decreases in the winter months. It is possible that *E.coli* was more concentrated in the water supply during this time of the year, thus easily detected in the winter months. The study mentioned above also states that the bacterial load could have increased in late autumn because of the lack of rainfall causing the bacterial load to be more concentrated in the waterbodies. In the research conducted in Zagazig City in Egypt, the greatest numbers of positive samples are detected in the spring season. It is possible that the weather changes more drastically through the seasons in Egypt than what is observed in Dhaka city. This might contribute to the difference in results.

Diarrheal diseases are one of the leading causes of death in children under the age of five. This is especially true for low and middle income countries, like Bangladesh (Mashoto KO, Malebo HM, Msisiri E, Peter E, 2014). Mortality and morbidity of diarrheal diseases is linked to access to safe and clean water and proper sanitation, (Montgomery M, Elimelech M, 2007) socioeconomic status, high population density, and proximity of household to contaminated water bodies. Relative humidity and temperature affect the rate of replication of agents that cause diarrheal diseases (Black RE, Lanata CF, 1995). Studies have shown that there is a steep rise in the number of diarrheal cases after flood occurs (Schwartz BS, Harris JB, Khan AI, LaRocque RC, Sack DA, Malek MA, et al. 2006).

Treatments for diarrheal diseases are usually quite simple and inexpensive. On rare occasions however, the infection maybe so severe that it can threaten the life patient, if they do not receive the proper medical attention on time. In such cases, the hospital bills can become a burden to families that are living below the poverty line. In a country like Bangladesh, such families make up a sizeable chunk of the population.The elderly, children under the age of 5 and the immuno-compromised are the most susceptible to diarrheal diseases (Sarker, A.R., Sultana, M., Mahumud, R.A. *et al.* 2018).About 2.56 million diarrheal diseases were reported from various health facilities in Bangladesh in 2015 (MOHFW. Health bulletin 2016. Dhaka: Bangladesh; 2016). The hospital survey carried out by Sarker. *et al*.

showed that about 44% of the patients received treatment while they were admitted in the hospital, while the remaining received outpatient services. The yearly expense of treatment was US$ 172.02 million from societal perspective. Healthcare facilities had spent about US$ 35.72 million (Sarker, A.R., Sultana, M., Mahumud, R.A. *et al.* 2018).

There are more than a dozen laws and regulations to ensure that the food we are consuming is of the highest quality. All of the effort of the Government to reduce the number of cases food poisoning will be for nothing if it cannot provide a safe, clean source of water to its citizens to fulfil their everyday needs.

Water that appears safe may contain minerals, organic compounds and high concentrations of pathogenic micro-organisms.

There are several categories of tests that a water source should be subjected to. These are:

- Bacteriological tests: Bacteriological tests checks for the presence of indicator bacteria in the water source. Indicator bacteria could be either fecal coliform or total coliform. Presence of indicator bacteria shows the possibility of pathogenic micro-organisms being present in the water source.
- Mineral tests: These tests are carried out to find out the mineral content of the water source. A very high mineral content can affect our health, the taste, color and odor of water and the cleaning capacity of water.
- Organic chemical tests: Organic chemical tests are carried out to detect certain types of contaminants, such as pesticides, in the water system. Organic chemical tests can detect industrial and petroleum contaminants.
- Other tests can be used to detect radiological contaminants or heavy metals.

Campaigns to inform the general population on the methods that can be used to treat water could be carried out. The campaign could also include how to handle treated water and maintain personal

hygiene. Such a campaign could help to reduce the number of cases of diarrhea in the city.

This thesis can be extended in many different directions. Fecal contamination at the supply points and the consumer points could be studied to get an indication of the source of fecal contamination in household water supply. Capability of the bacteria to form biofilm could be studied. Both of these combined together would let us know if treatments used by water treatment plants are effective or not. Furthermore, it would allow us to know if the household water storage tanks and pipelines are a source of contamination or not. *E.coli* may settle in the tanks and pipelines and form biofilms, thus contaminating the water supply. Antibiotic resistance and extended beta lactamase activity of the bacteria could be studied. Horizontal gene transfer could also be studied.

Chapter 5

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Appendix I

Media composition

Composition of the media used in the study is given below. All of the media were autoclaved at 121ºC for 15 minutes, unless mentioned otherwise.

Nutrient Agar

Ingredients	Amount(g/l)
Nutrients Agar	28

mFC Agar (Not autoclaved)

Ingredients	Amount(g/l)
mFC Agar	52.1

EMB Agar

Ingredients	Amount(g/l)
EMB Agar	35.96

Nutrient Broth

Ingredients	Amount(g/l)
Nutrient broth	13

Tryptone Soy Broth

Ingredients	Amount(g/l)
Soyabean Caesin Digest Medium	29.77
Sodium chloride	2.5

Appendix II

Reagents

TAE buffer

Ethidium bromide

Appendix III

Instruments

Equipments used to carry out the study are listed below:

Autoclave, Model no: HL-42AE : Hirayama corp, Japan

Steriliser, Model no: NDS 600D : Japan

Class II Microbiological safety cabinet : Labcaire, USA

Electric balance, Scout, SC4010 : USA

Freezer (-30ºC) : Liebherr, Germany

Refrigerator (4ºC) : Vest frost

Incubator : Japan

Micropipettes : Eppendorf, Germany

Microwave oven, Model: D90N30 ATP ; Butterfly, China

Gel run

UV

Centrifuge

Waterbath