# Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Mohakhali, Niketon and Uttara


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# Declaration

I hereby declare that the thesis project titled “Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Mohakhali, Niketon and Uttara” has been written and submitted by me, Nowrin Hossain and has been carried out under the supervision of Mahbubul Hasan Siddiqee, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

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

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# Abstract

Dhaka, the capital city of Bangladesh, is the most densely populated city of the world. So, it is always a challenge to meet the ever increasing demand for pure drinking water of this huge population. This study aims to detect the presence of fecal coliform (FC) and *Salmonella* spp. in the supplied and domestically treated waters in Mohakhali, Niketon and Uttara zones. A total of 108 samples were collected from October 2018 to April 2019. The supplied water samples collected from these three zones produced variable FC counts (cfu/100mL); zero, 1-5, 6-30, 31-100, and >100 were detected in 50%, 6%, 13%, 11%, and 20% samples respectively. While 67% of the boiled water samples yielded no FC, others showed variable degrees of FC counts (cfu/100mL); 1-5 in 7%, 6-30 in 13% and >100 count was detected in 13% samples. In case of the filtered waters 57% samples had zero FC counts. However, FC counts (cfu/100mL) of 1-5, 6-30, 31-100, and >100 were detected in 9%, 4%, 26% and 4% samples respectively. PCR-based detection of *Salmonella* spp. suggests occasional contamination in water samples from both Mohakhali and Uttara (five and four samples respectively). Results from this study reveals possibility of transmission of fecal pathogens via drinking water and generates evidence that contaminated drinking water is an overlooked health hazard in Mohakhali, Niketon, Uttara.

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# Introduction

## 1.1 Importance of water

Water is the key part for survival of living association. Without water, it is difficult to endure. Human body utilize water in the entirety of its cells, organs, and tissues to help manage its temperature and keep up other real capacities. Water is associated with each substantial capacity from digestion and circulation through to the control of body temperature and the discharge of waste items. The water in our bodies is constantly being utilized or lost from the body. Some is utilized or consumed by the capacities it performs and some is lost through perspiration, urine and feces. Since the water in our bodies is ceaselessly being utilized or lost, it should be constantly supplanted, and the best liquid to supplant it with is water. Thus, the water that we are drinking must be pure. Usually we drink water by boiling or filtering it.. Domestic water use incudes consumption of water, food preparation, bathing, washing clothes and dishes, brushing teeth, watering the yard and garden etc. Now the question comes, the water that we use for our daily work and drinking whether it is pure to use or not.

## 1.2 Possibility of fecal contamination

High level of contamination in drinking water has been accounted for several studies though issue stays unchanged. Disease-causing organisms (pathogens) transmitted by means of drinking water are dominatingly of fecal origin. Despite the fact that, the direct isolation of intestinal pathogens is unfeasible; rather public health inspectors determine the number of indicator bacteria. Frequent examination of fecal indicator organisms remain the easiest method for evaluating the sanitation conditions of water. The ideal fecal indicator should satisfy all of the specific criteria, for example, consistently presence in the feces, inability to multiply outside the intestinal tract, is at least as resistant as the pathogens to environmental conditions and to disinfection, have a strong association with the presence of pathogenic microorganisms, and permit simple laboratory methodology (Savichtcheva & Okabe, 2006). Indicator organisms of fecal pollution include the coliform group as a whole and especially *Escherichia coli.*

Data on microbiological water quality may usefully be divided into a number of categories; the dimensions of pollution associated with each category should be selected in the light of local conditions. The following table represents increasing orders of magnitude of fecal contamination

**Table 1.2 Illustration of category and color-code scheme for thermo tolerant (fecal) coliforms or *E. coli* in water supplies (World Health Organization, 1997).**

| Count per 100ml | Category and color code | Remarks |
|---|---|---|
| 0 | A (blue) | In conformity with WHO guidelines |
| 1–10 | B (green) | Low risk |
| 10–100 | C (yellow) | Intermediate risk |
| 100–1000 | D (orange) | High risk |
| >1000 | E (red) | Very high risk |

It is suggested that the bacteriological category scheme should be based on thermo tolerant (fecal) coliform bacteria or *E. coli*. Grouping of point sources into classes of the sort appeared in Table 1.2 is generally straightforward. Incidentally, however, where a number of samples are taken each year, the levels of fecal contamination may vary widely between progressive samples. The explanations behind this are often obvious and may be related to seasonal influences such as rainfall. However, where piped small-community water supplies are being analyzed and samples are taken at different focuses in the framework, water quality may contrast in various pieces of the framework at any one time. Once more, the explanations behind this may become obvious during the sanitary inspection or if these distinctions are the result of cross-contamination or contamination brought about by leaks in pipework after resampling.

EPA (Environmental Protection Agency) distinguishes contaminants to regulate in drinking water to ensure public health. The Agency sets administrative limits for the measures of specific contaminants in water provided by public water systems. The total coliform count including fecal coliform and *E. coli* should be zero.

## 1.3 Possibilities of infection via drinking water

Diarrheal disease is a noteworthy reason of morbidity and mortality in developing countries, including Bangladesh. Among 50 diseases common in Bangladesh, 40 of them including diarrhea, dysentery, typhoid, parasitic worm infection so on are related to the contaminated food and water. Different strains of *E. coli* in drinking water are responsible for a variety of diseases including diarrhea, dysentery, hemolytic uremia syndrome (kidney failure), bladder infections, septicemia, pneumonia, meningitis (Acharjee et al., 2011). Shiga toxins (Stx) which is known as verotoxins (Vtx), comprises two major subtypes, shiga toxin 1 (Stx1) and shiga toxin 2 (Stx2) are produced by several enteric pathogens, *Shigella dysenteriae* (serotype 1 only) and enterohaemorrhagic *Escherichia coli* (EHEC). They are facultatively anaerobic which are able to ferment sugars with the production of organic acid and gas. These three genera o a kind of fermentation called "mixed-acid fermentation," however contrast in various physiological characteristics. Over 45,000 under-five children die every year in Bangladesh from diarrhea caused by contaminated water, says a report of World Health Organization. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. It also reflects the microbiological quality of water.

Spreads of waterborne diseases are regularly observed to be associated with the consumption of contaminated water possibly because of their ignorance, poverty and unavailability of clean 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-organisms | Major diseases | Major reservoirs and primary sources |
|---|---|---|
| *Salmonella typhi* | Typhoid fever | Human feces |
| *Salmonella paratyphi* | Paratyphoid fever | Human feces |
| Other *Salmonella* | Salmonellosis | Human and animal feces |
| *Shigella* spp. | Bacillary dysentery | Human feces |
| *Vibrio cholera* | Cholera | Human feces and freshwater zooplankton |

| | | |
|---|---|---|
| Enteropathogenic *E. coli* | Gastroenteritis | Human feces |
| *Yersinia enterocolitica* | Gastroenteritis | Human and animal feces |
| *Campylobacter jejuni* | Gastroenteritis | Human and animal feces |
| *Legionella pneumophila* and related 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 |

Nonetheless, the World Health Organization (WHO) evaluates that about 1.1 billion individuals globally drink unsafe water (Kindhauser, 2003) and most by far of diarrheal disease in the world (88%) is attributable to unsafe water. Moreover, social and environmental changes keep on bringing about new or re-emerging waterborne pathogen issues. For instance, climate change was assessed to be responsible in 2000 for approximately 2.4% of world-wide diarrhea, 6% of malaria in some middle-income countries and 7% of dengue fever in some industrialized nations. Altogether, the inferable mortality was 154 000 (0.3%) deaths and the attributable burden was 5.5 million (0.4%) (Ashbolt, 2004). Climate change instigated flooding and droughts can impact household water and sanitation infrastructure and related health risks. For example, flooding can scatter fecal contaminants, increasing risks of outbreaks of waterborne diseases such as cholera. Moreover, water deficiencies due to drought can increase risks of diarrheal disease.

## 1.4 Water borne disease in Dhaka

In Dhaka people are troubled with water borne diseases through drinking water just intensifies the circumstance. In spite of governmental plans and actions, non-governmental activities or personal awareness, the issue of safe water access as well as pervasiveness of water borne diseases are still very common.(Islam et al., 2001). A number of variables may be included for contamination of drinking water. Ground water can become contaminated from natural sources or various sorts of human exercises. Residential, civil, commercial, industrial, and agricultural activities would all be able to influence ground water quality. Contamination of tube well water

seems identified with various variables, including proximity of latrines or drains to the tube wells, tube well depth or method of completion, and factors such as the practice of tube well priming may also be involved. Supplied water might be contaminated through the dissemination pipes due to leakage. It can be prescribe keeping away from releases of wastewater without treatment, mainly from septic tanks, which are extensively used in the area (Parvez, Liza, & Marzan, 2016).

Diarrheal disease is a noteworthy reason of morbidity and mortality in developing countries, including Bangladesh. Among 50 diseases common in Bangladesh, 40 of them including diarrhea, dysentery, typhoid, parasitic worm infection so on are related to the contaminated water (Md Shahidul, Mehadee, & Sunjukta, 2014). As children are the most at-risk group, it is important to perceive what sorts of pathogens result in their diarrhea. Studies in Dhaka, Bangladesh have demonstrated that in the stools some 75% of diarrheal children and 44% of control children have an enteric pathogen. (Ashbolt, 2004) The main organisms associated with diarrhea being rotavirus, *Cryptosporidium parvum* and the following bacterial pathogens: *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* [ETEC], enteropathogenic *E. coli* [EPEC], *Shigella* spp. and *Vibrio cholerae* O1 or O139 and to a lesser degree *Aeromonas* spp., *Bacteroides fragilis* and *Clostridium difficile* (Albert et al., 1999). In any of the children in the Dhaka studies, some other potential bacterial pathogens, *Plesiomonas shigelloides*, *Salmonella* spp. diffusely adherent *E. coli* and enteroaggregative *E. coil*, along with the parasitic protozoa *Entamoeba histolytica* and *Giardia lamblia* were not significantly associated with diarrhoea and enteroinvasive *E. coli*, enterohemorrhagic *E. coli* [EHEC] and *Cyclospora cayetanensis* were not detected. (Ashbolt, 2004)

## 1.5 Selected zones of this study (Mohakhali, Niketon and Uttara)

Dhaka is the largest and most populated city in Bangladesh. Not only in Bangladesh but also it is one of the most populated cities in the world. The total area of Dhaka city is 300 square kilometers where 18.237 million of people are living. It is one of the most densely populated areas in the world, with a density of 23,234 people per square kilometer. Dhaka city is divided into different zone.

Mohakhali, Niketon and Uttara are the three metropolitan and residential areas in Dhaka city. Uttara and Gulshan are the most reputed area of Dhaka city. Total area of Uttara area is 7 km 2 where 179,907 people are living. Population density of this area is 25,701/ km 2 which is more than the population density of the Dhaka city. Another reputed area of Dhaka city is Gulshan area. Mohakhali and Niketon are the part of this Gulshan area. Total area of Gulshan area is 8.70 km 2 where 253,050 people are living. Population density of this area is 29,086/ km 2 .That means the population density of Gulshan area is more than both the Uttara are and the whole Dhaka city.

## 1.7 Aim of the study

This paper provides a brief review of microbiological quality of tap water supplied by DWASA and domestically treated water in Dhaka city on account of its significance in public health. This study will create awareness amongst the population living in the selected zones.

The specific objective of this study is to determine the total load and levels of contamination, thermo tolerant *E. coli* were quantitated in the water samples tested and to detect *Salmonella* spp., the prominent organism for typhoid fever. As the conventional techniques dependent on bacterial culture and serological tests take few days thus molecular techniques based on genotype of the bacteria are chosen over traditional method in this study to detect *Salmonella* spp.

# Chapter: 2

# Materials and Method

## 2.1. Sampling sites:

A total of 108 water samples from Niketon, Uttara and Mohakhali zones were analyzed.

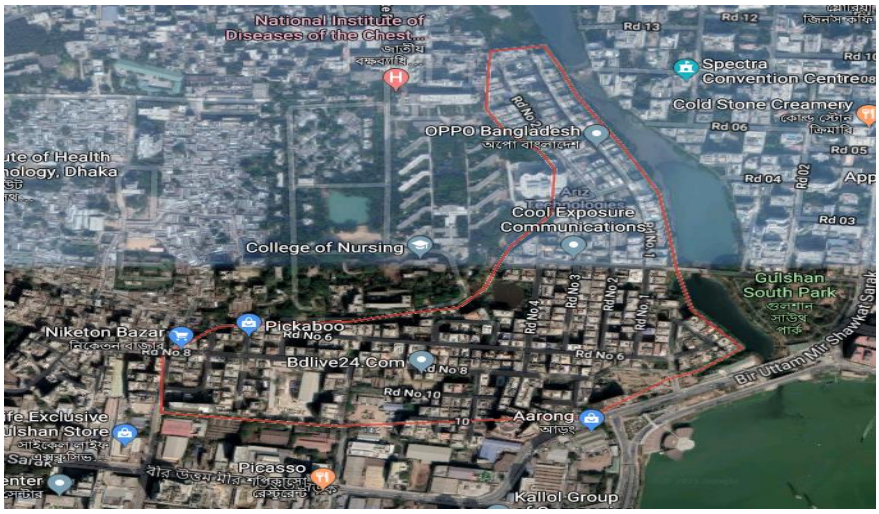


**Figure 2.1.1: Sampling sites of Niketon zone**

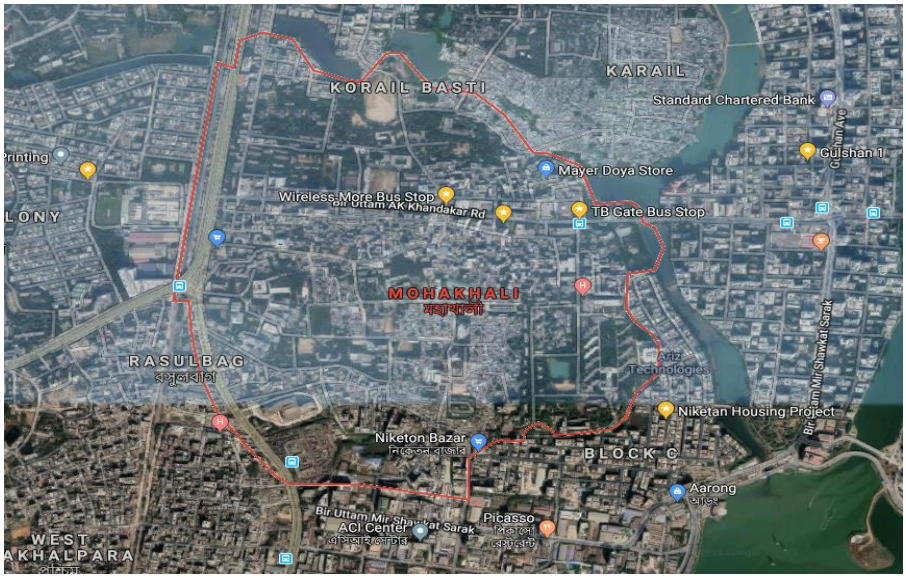


**Figure 2.1.2: Sampling sites of Mohakhali zone**

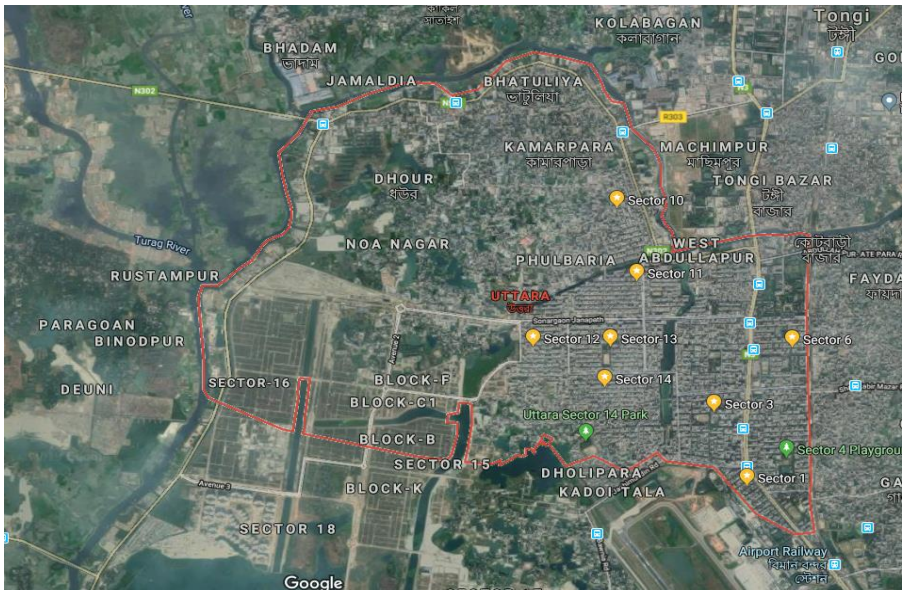


**Figure 2.1.3: Sampling sites of Uttara zone**

## 2.2. Sample collection:

Water samples were monthly collected from October 2018 to April 2019. Samples were collected from different sites of three targeted zones (Mohakhali, Niketon and Uttara). For sample collection, the collection bottles were previously autoclaved.

## 2.3. Sample processing

### 2.3.1. Filtration

By using membrane filter papers, 100ml of collected samples were filtered and placed on m-FC agar plates and the plates were incubated at 44.5°C for 24 hours. After 24 hours, the plates were observed. Colonies created by fecal coliforms can seem as variable shades of blue-colored colonies on the membrane filter. The colonies were confirmed by sub culturing them on EMB agar. Only the colonies that had appeared as metallic green sheen are *E. coli.* Same collected samples about 100ml of were filtered again. Membrane filter papers were placed in conical flasks containing 50ml of TSB supplemented with 2.5% NaCl and the flasks were incubated at 37◦C for 12 hours. 300µl of autoclaved glycerol were placed in autoclaved micro-centrifuge

tubes. After incubation was completed, TSB were transferred to micro-centrifuge tubes and vortexed. Micro-centrifuge tubes were stored at -20˚C. 1.5ml of incubated TSB were transferred to an autoclaved micro-centrifuge tubes and centrifuged at 14,000 rpm for 10 minutes. After centrifugation the supernatants were discarded. The pellets were stored at -20°C after the mouth of the micro-centrifuge tubes had been wrapped with paraflim for DNA extraction.

### 2.3.2. DNA extraction protocol

Some microorganisms require the execution of specific steps for genomic analysis, such as in cellular DNA extraction. It is known that simply boiling a suspension of *E.coli* or *Salmonella* spp, for instance, is an effective method for inducing cellular lysis, for carrying out Polymerase Chain Reactions (PCR) (Ranjbar, Naghoni, Afshar, Nikkhahi, & Mohammadi, 2016).

After 12 hours of incubation 1.7ml of TSB were transferred to a 2ml micro-centrifuge tubes and stocked with 0.3ml of glycerol. TSB containing bacteria were transferred to micro centrifuge tubes about 1.5ml and that centrifuged for 10 minutes at 14,000 rpm (Kobayashi et al., 2009). The pellet were collected and added 400µl of distilled water that were stored at room temperature an inverted the mixture for washing. The mixtures were centrifuged for 5 minutes at 13,000 rpm and then removed supernatant. Then the pellets were re-suspended with 400µl of distilled water. The cells were lysed at 100˚C for 7 minutes. After heat shocks were performed micro-centrifuge tubes were transferred in ice for 10 minute to performed cold shock. After 10 minutes, centrifuges were performed for 5 minutes at 13,000 rpm. The supernatant contained with DNA were transferred into new micro-centrifuges tubes and wrapped with paraflim and stored at -20˚C.

## 2.4. Raw DNA gel run

The supernatant was subjected to gel run to check for the presence of DNA. Preparation of primers for PCR (stock solution and working solution):

**Table 2.4 Sequences of primers used for amplification by PCR.**

| **Primer name** | **Sequence (5'-3')** | **Product (bp)** | **Target** | **Reference** |
|---|---|---|---|---|
| *Salmonella*-F | GTATTGTTGATTAATGACATCCG | 403 | *invA* | (Ranjbar, Naghoni, |

| | | | | |
|---|---|---|---|---|
| *Salmonella*-R | ATATTACGCTACGGAAACACGTT | | | Afshar, Nikkhahi, & Mohammadi, 2016) |

bp = base pair; PCR = polymerase chain reaction

Stock solution of *Salmonella* forward primer was prepared by adding 317µl of PCR water with 0.22mg of oligo (primer powder) for 100µM [concentration]. Stock solution of *Salmonella* reverse primer was prepared by adding 302µl of PCR water with 0.21mg of oligo (primer powder) **f**or 100µM [concentration]. Working solution of *Salmonella* forward and reverse primer was prepared by adding 900µl of PCR water with 100µl of stock solution for 100µM [concentration]

## 2.5. Preparation of control for PCR

Reference bacterial strains *Salmonella typhi* were streaked onto selective Medias XLD agar and incubated for 24 hours at 37ºC. After incubation, single colonies were picked and inoculated in LB broth. This was incubated for 24 hours at 37ºC. After incubation LB containing micro-centrifuge tubes were centrifuged for 10 minutes at 14,000 rpm. The pellets were collected. These pellets were used for DNA extraction. After DNA extraction, gel electrophoresis was carried out to observe if DNA were extracted from these samples properly. After conformation PCR was performed.

### 2.6. PCR

PCR assay were 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. Pipetting was done in careful manner so that no bubbles present and perform spinning. After the initial preparation was taken, PCR were performed under the following conditions: 35 cycles with initial denaturation at 94˚C for 5 minutes heat denaturation at 95°C for 30 seconds, primer annealing at 60°C for 30 seconds, and DNA extension at 72°C for 60 seconds and final extension at 72˚C for 8 minutes in micro-centrifuge tubes gradient master cycler. Sterile water was used instead of template DNA to provide a negative control to monitor the contamination of external DNA in the PCR reagents in PCR reaction.

## 2.7. Gel electrophoresis

Conventional agarose gel electrophoresis was performed to confirm that the PCR reaction amplified the correct target gene. The amplified DNA were separated by 1% agarose gel electrophoresis at 70 voltages, stained with ethidium bromide, and visualized by UV trans-illuminator. 1500 base-pair of DNA ladder was used.

# Chapter: 3

# Results

## 3.1. Fecal coliform counts

Fecal coliforms are the organisms used to indicate the presence of fecal contamination and to monitor the removal of pathogens from wastewater treatment plants. The detection of *E. coli* gives distinct proof of fecal pollution; in practice, the identification of thermo tolerant (fecal) coliform bacteria is an acceptable alternative. Normally increased levels of fecal coliforms provide a warning of negligence in water treatment, a break in the integrity of the distribution system, conceivable pollution with pathogens. At the point when levels are high there might be a raised danger of waterborne gastroenteritis. The acceptable dimension of *E. coli* is determined by risk analysis dependent on statistics to protect human health. Drinking water ought to have no *E. coli* after treatment.

### 3.1.1. Fecal coliform counts in Mohakhali zone

**Table 3.1.1 Fecal coliform counts in raw, boil and filtered water**

| Months | Raw Water | Boil Water | Filtered Water |
|---|---|---|---|
| October | 164 | 0.3 | 128 |
| November | 153.25 | 45.3 | 70 |
| December | 185.5 | 0 | 34 |
| January | 183.25 | NA | 56 |
| March | 281 | NA | NA |
| April | NA | NA | NA |
| Average | 193.4 | 15.2 | 72 |
| SD | 50.78281 | 26.0678 | 40.16632 |
| SEM | 22.71076 | 15.05025 | 20.08316 |

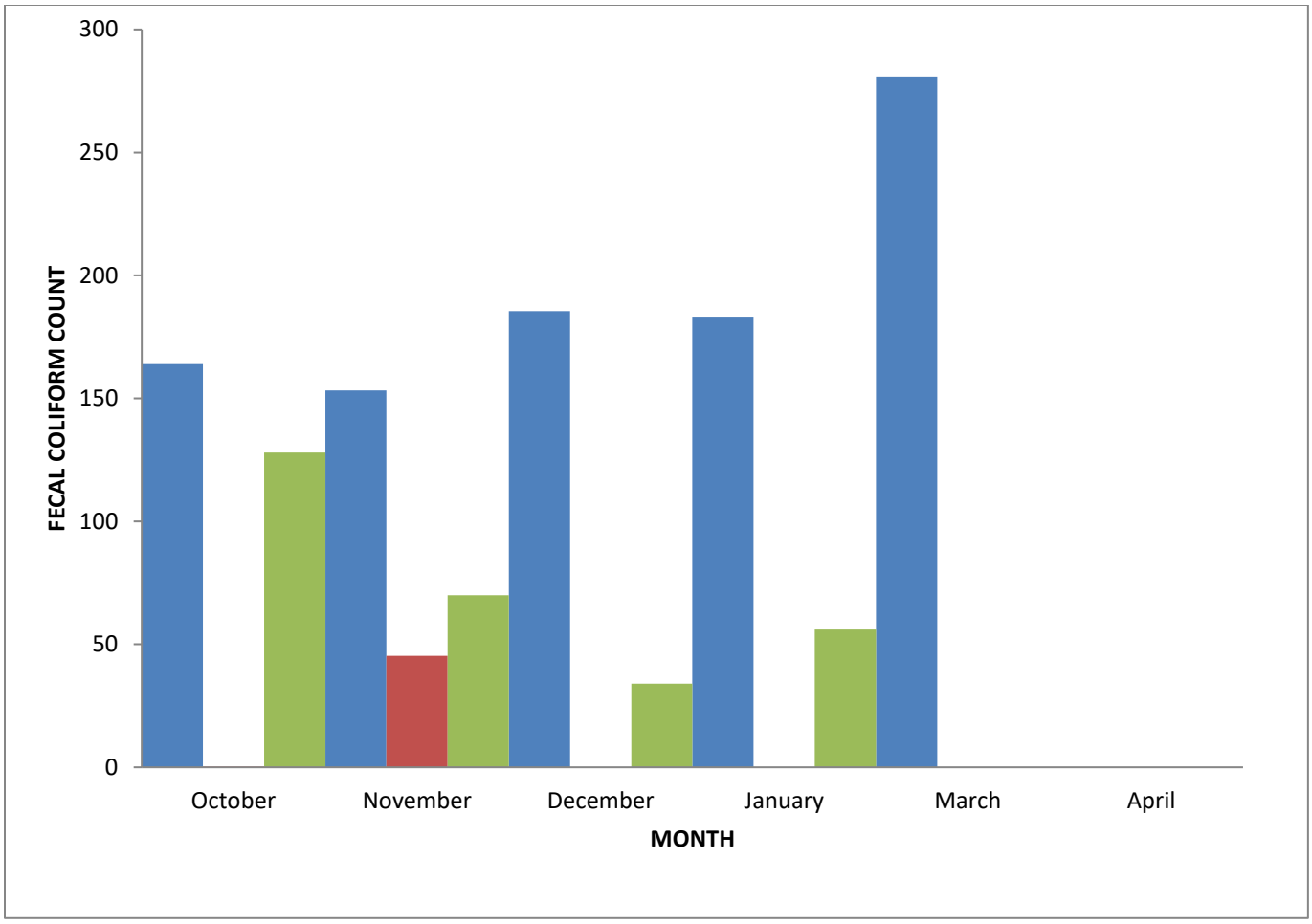


**Figure 3.1.1: Fecal coliform counts in raw, boil and filtered water**

## Monthly variation:

A total of 30 samples were collected from Mohakhali zone. In October, the average count for fecal coliform in raw water was 164, in boil water 0.3 and in filtered water 128. In November the average count for fecal coliform in raw water was 153.25, in boil water 45.3 and in filtered water 70; in December the average count for fecal coliform in raw water was 185.5, in boil water 0 and in filtered water 34; in January the average count for fecal coliform in raw water was 183.25 and in filtered water 56. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count for fecal coliform in raw water was 281. Again from the graph it had been observed that the fecal coliform count in

raw water was 164 during October and it decreased in November. However, the figure experienced a steady growth during the next two months December and January and finally it rose to 281 in March. It can be said that the highest count for fecal coliform appeared in March, followed by December, January, October then finally November. For boil water samples, the fecal coliform count was 0.3 during October which increased to 45.3 in November and fluctuated sharply to zero in December. Thus it can be said that the highest count for fecal coliform appeared in November, followed by October then December for boil water samples. Additionally, the fecal coliform count in filtered water was 128 during October which increased to 70 in November and 34 in December and then slightly increased to 56 January. Thus, it can be said that the average count of fecal coliform was higher in March for raw and filtered water samples, November for boil water samples.

### Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were 50.78281, for boil water samples 26.0678 and for filtered water samples 40.16632. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more spread out or dispersed than filtered and boil water samples data.

### Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during summer then spring.

### 3.1.2. Fecal coliform counts in Niketon zone

**Table 3.1.2 fecal coliform counts in Niketon zone**

| Months | Raw Water | Boil Water | Filtered Water |
|---|---|---|---|
| October | 10.25 | NA | 0.75 |
| November | 0 | NA | 0 |
| December | 0 | NA | NA |

| | | | |
|---|---|---|---|
| January | 0 | NA | NA |
| March | NA | NA | NA |
| April | NA | NA | NA |
| Average | 2.5625 | | 0.375 |
| SD | 5.125 | | 0.53033 |
| SEM | 2.092272489 | | 0.375 |

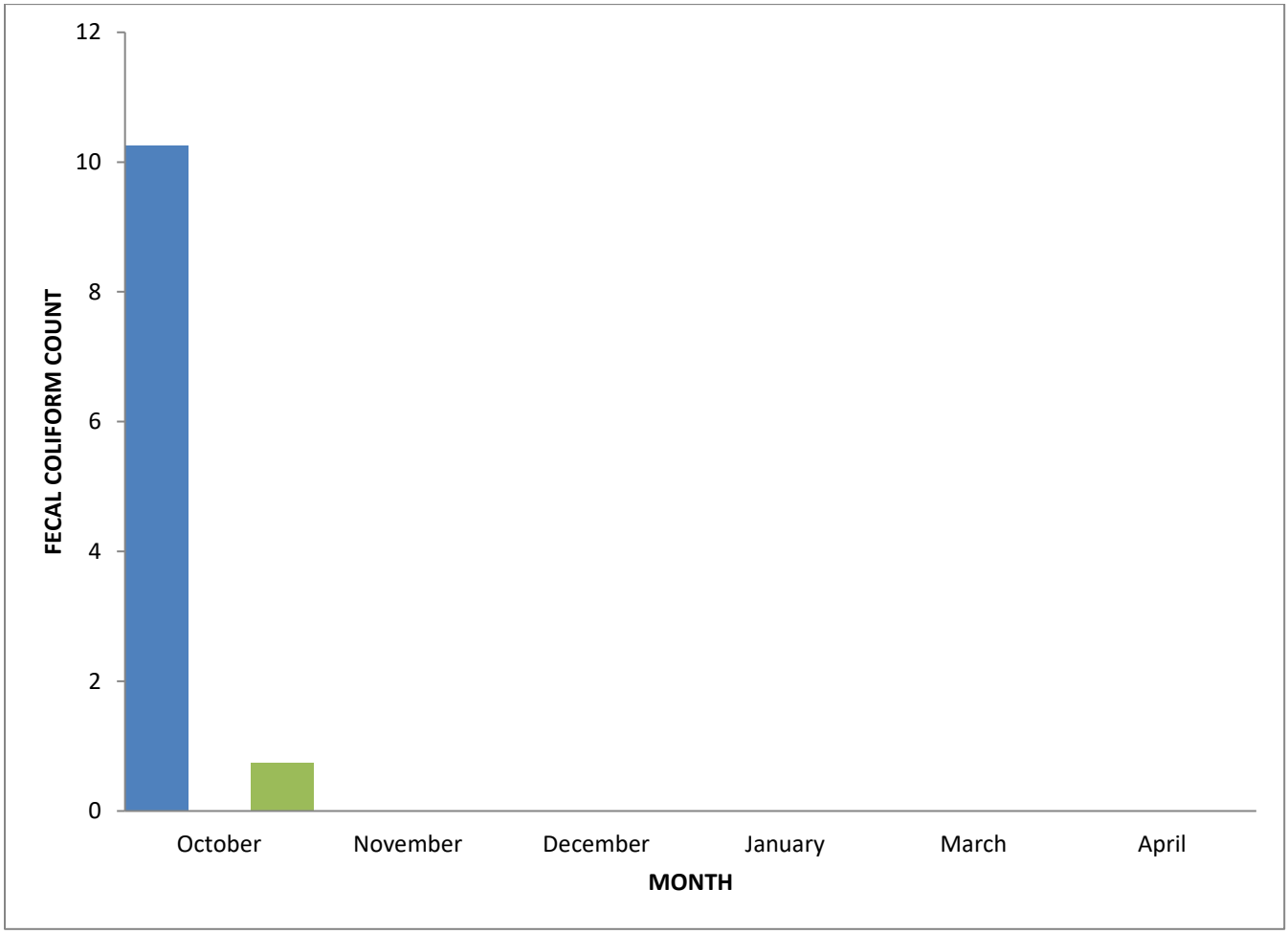


**Figure 3.1.2: Fecal coliform counts in raw, boil and filtered water**

### Monthly variation:

A total of 24 samples were collected from Niketon zone. In October, the average count for fecal coliform in raw water was 10.25 and in filtered water 0.75. In November, December and January the average count for fecal coliform in raw water and filtered water samples were zero. From the graph it had been observed that the fecal coliform count in raw water was 10.25 during October and reached a low during following months November, December and January. It can be said that the highest count for fecal coliform appeared in October only. Furthermore, the fecal coliform counts in filtered water samples were 0.75 during October and after that bottomed out in November.

### Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were 2.092272489 and for filtered water samples 0.375. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more dispersed than filtered water samples data.

### Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during spring then summer.

### 3.1.3. Fecal coliform counts in Uttara zone

**Table 3.1.3 Fecal coliform counts in raw, boil and filtered water**

| Months | Raw Water | Boil Water | Filtered Water |
|---|---|---|---|
| October | 13.625 | 5 | 1.125 |
| November | 10.875 | 0 | 25 |
| December | 8.5 | 0 | 70 |
| January | 9 | NA | 87 |
| March | 27.5 | NA | NA |

| | | | |
|---|---|---|---|
| April | 136.5 | NA | NA |
| Average | 34.33333 | 1.666667 | 45.78125 |
| SD | 50.54303 | 2.886751 | 39.62985 |
| SEM | 20.63411 | 1.666667 | 19.81492 |

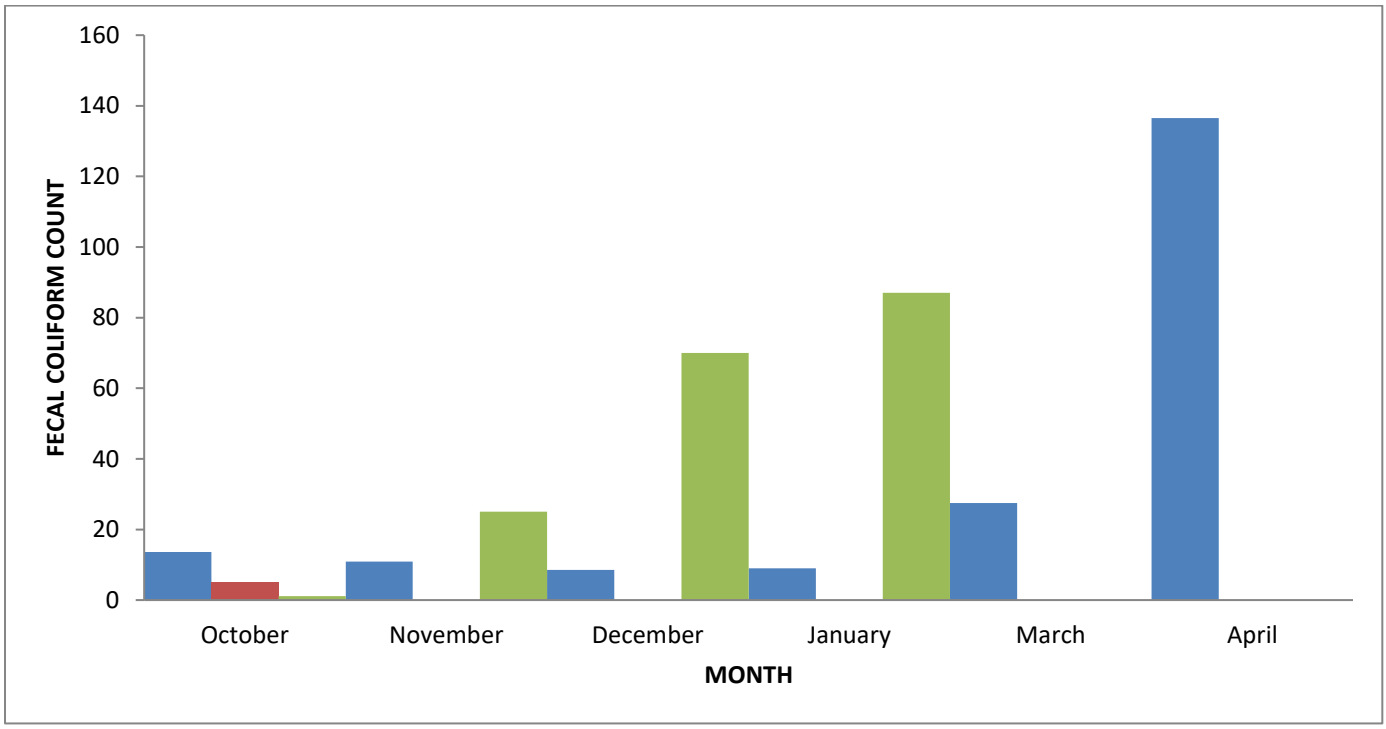


**Figure 3.1.3: Fecal coliform counts in raw, boil and filtered water**

## Monthly variation:

A total of 54 samples were collected from Uttara zone. In October, the average count for fecal coliform in raw water was 13.625, in boil water 5 and in filtered water 1.125. In November the average count for fecal coliform in raw water was 10.875, in boil water 0 and in filtered water

25; in December the average count for fecal coliform in raw water was 8.5, in boil water 0 and in filtered water 70; in January the average count for fecal coliform in raw water was 9 and in filtered water 87. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count for fecal coliform in raw water was 27.5 and in April it was 136.5. From the graph it had been observed that the fecal coliform count in raw water was 13.625 during October and a steady decline during the next two months November and December. In January it slightly rose to 9 and 27.5 in March. Finally, it increased dramatically to 136.5 in April. It can be said that the highest count for fecal coliform appeared in April, followed by March, October, November, January, and then finally December. For boil water samples, the fecal coliform count was 5 during October which bottomed out in November and December. Thus it can be said that the highest count for fecal coliform appeared in October for boil water samples. Additionally, the fecal coliform count in filtered water was 1.125 during October and a constantly increased in November, December and eventually in January. Thus, it can be said that the average count of fecal coliform was higher in April for raw water samples, October for boil water samples and January for filtered water samples.

### Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were 50.54303, for boil water samples 2.886751 and for filtered water samples 39.62985. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more scattered than filtered and boil water samples data.

### Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during summer then spring.

### 3.1.4. Comparison between water samples of Mohakhali, Niketon and Uttara

**Table 3.1.4 Comparison of fecal coliform counts in raw, boil and filtered water**

| Zones | Raw Water | Boil Water | Filtered Water |
|---|---|---|---|
| Mohakhali | 193.4 | 15.2 | 72 |
| Niketon | 1.71 | NA | 0.375 |
| Uttara | 34.33 | 1.67 | 45.78 |
| Average | 76.48 | 8.435 | 39.385 |
| SD | 102.5609 | 9.567155 | 36.2382 |
| SEM | 59.21354 | 6.765 | 20.92213 |

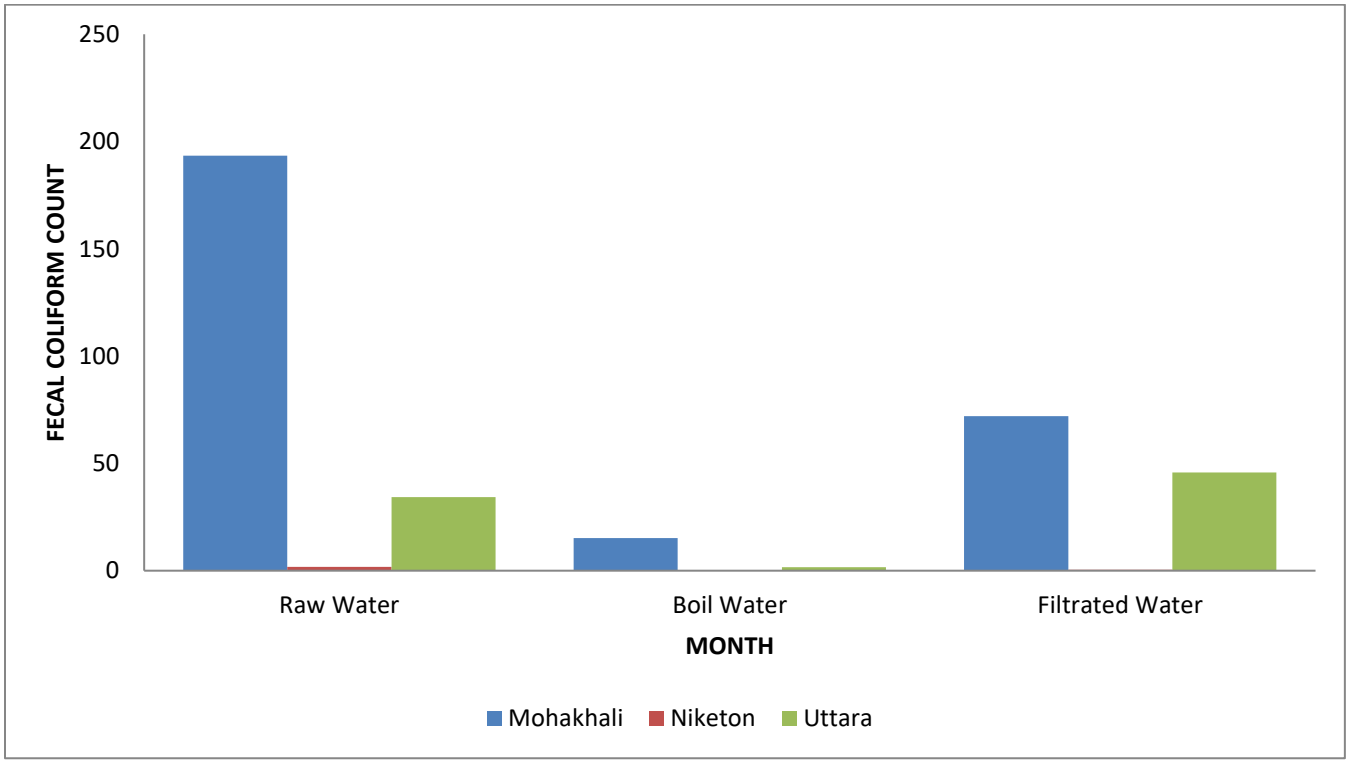


**Figure 3.1.4: Comparison of fecal coliform counts in raw, boil and filtered water**

The average fecal coliform count in raw water for Mohakhali zone was 193.4, for Niketon 1.71 and for Uttara 34.33; in boil water 15.2 for Mohakhali and 1.67 for Uttara; in filtered water 72 for Mohakhali, 0.375 for Niketon and 45.78 for Uttara. From the bar chart, it clearly seen that Mohakhali zone contained the most fecal coliform counts followed by Uttara and then Niketon for raw, boil and filtered water.

Furthermore, the standard deviation for raw water samples among three zones were 102.5609, for boil water samples 9.567155 and for filtered water samples 36.2382. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more disseminated than filtered and boil water samples data.

## 3.2. PCR result:

PCR was done using one set of primer pairs targeted for the *invA* gene that correctly identified *Salmonella* spp. by the size of bands products; positive bands consist of gene *(*403 base pair).

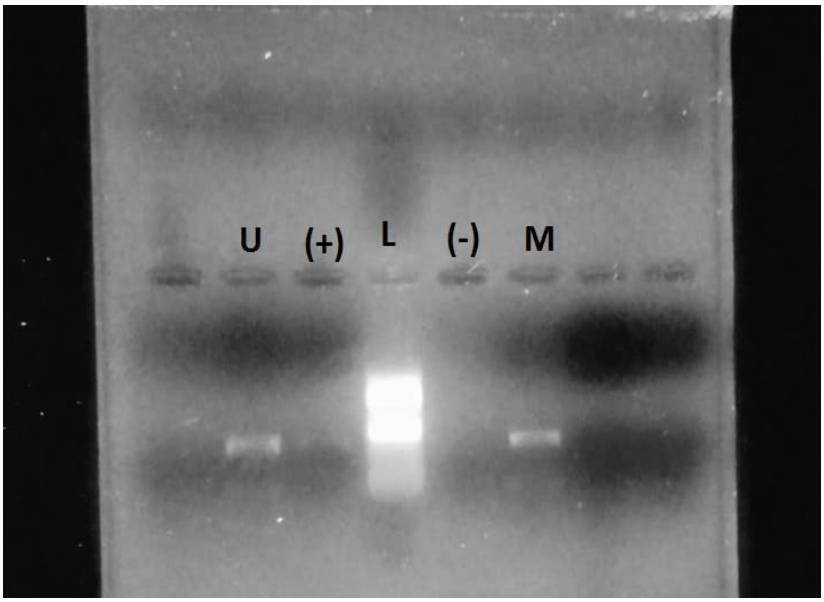


**Figure 3.2.1: The PCR results. Lanes U represented strains Salmonella spp. in Uttara zone and M represented strains Salmonella spp. in Mohakhali zone. L represented ladder which was 1500 base pair. The reference strain *Salmonella typhi* used as positive control and *Shigella dysenteriae* as negative control. The two positive bands came from the samples that had fecal coliform count in between 1 to 30.**

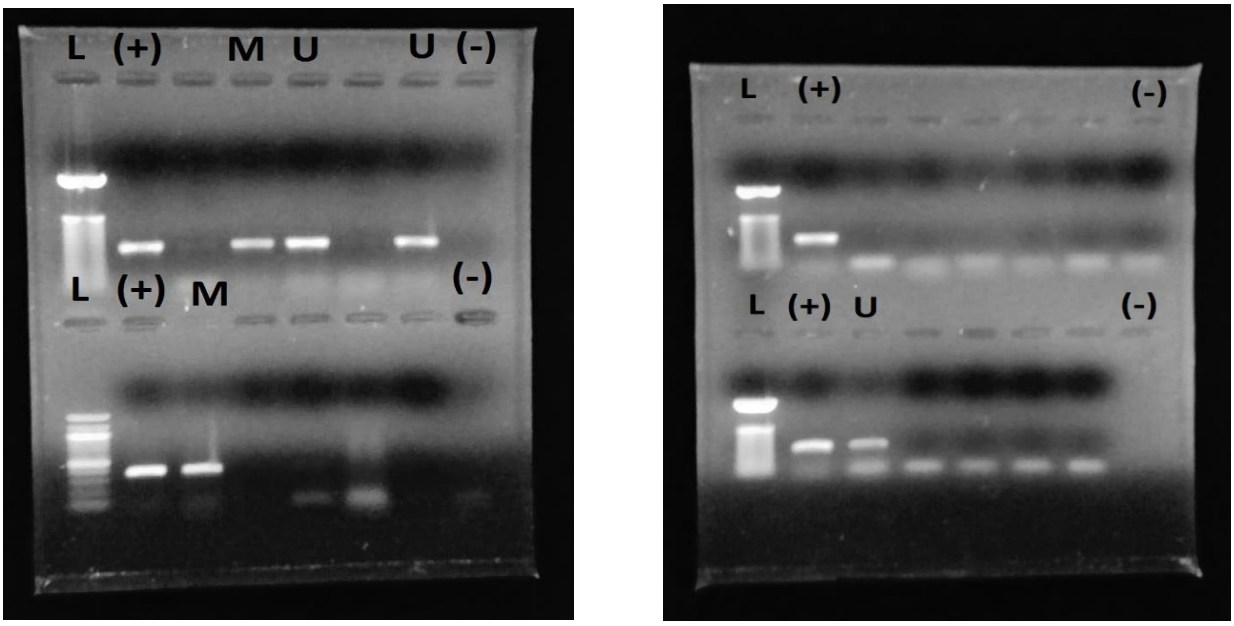


**Figure 3.2.2: The PCR results. Lanes U represented strains Salmonella spp. in Uttara zone and M represented strains Salmonella spp. in Mohakhali zone. L represented ladder which was 1500 base pair. The reference strain *Salmonella typhi* used as positive control and *Shigella dysenteriae* as negative control. The five positive bands came from the samples that had fecal coliform count in between 30 to 300.**

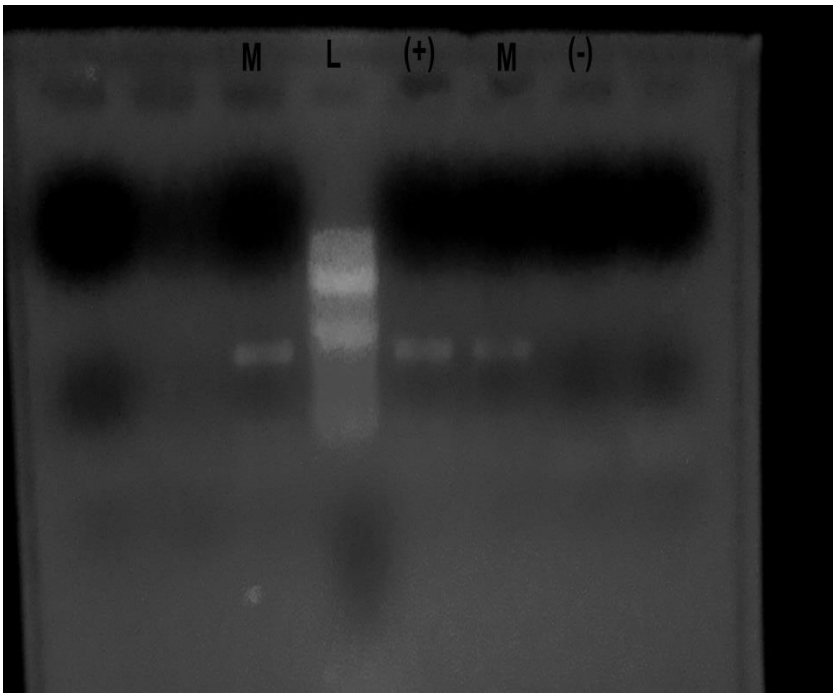


**Figure 3.2.3: The PCR results. Lanes U represented strains Salmonella spp. in Uttara zone and M represented strains Salmonella spp. in Mohakhali zone. L represented ladder which was 1500 base pair. The reference strain *Salmonella typhi* used as positive control and *Shigella dysenteriae* as negative control. The five positive bands came from the samples that had fecal coliform count in above 300.**

From 108 samples, nine samples were positive for *Salmonella* spp. Among these nine samples four samples with positive bands had been appeared from Uttara zone; one from October and March and two from November. Furthermore, five samples with positive bands had been observed from Mohakhali zone; three from October and one from November as well as March. No positive samples were found in Niketon zone as well as treated water samples. Thus, from this result it could be estimated that *Salmonella* spp. contained in raw water in October exceedingly as the four positive samples had been observed followed by November and then December.

The presence of fecal coliform counts and *Salmonella* spp. indicated the fecal contamination and eventual survival of the bacterial population in the water supply line as well as treated water. From the result it could be said that none of the water samples tested was observed to be potable based on fecal coliform counts and the presence of pathogenic bacteria *Salmonella* spp. Although *Salmonella* spp. was not specifically stable in water environments and its presence generally

indicated recent fecal contamination (WHO 2008). Fecal coliform count in water samples reflected lack of efficiency in water treatment. Where community water supplies are unchlorinated, they will definitely contain huge quantities of fecal coliform bacteria, which may be of constrained sanitary significance. Nonetheless, it might be said that the DWASA water somehow gets contaminated after entering the distribution chain although they are treated sufficiently (Mrityunjoy, 2011). As the elevated counts appeared in filtered water than standard value of drinking water it could be said that filtration might not be completely effective and the reason behind it might be problem with filter machine or lack of domestic hygiene. It shows up from the study that consumers were not aware of the dimension of pollution in the DWASA tap water and its effect on public health, otherwise they would not utilize this water for drinking, washing utensils and salads. This finding recommended the need of regular observing the quality of the DWASA water at the user end and to take necessary steps to fix the problem immediately. From the result it could be said that existence of these microorganisms can lead to the formation of biofilms and enhance the growth of contaminating microorganism and increase resistance to disinfection.

# Chapter 4

# Discussion

Typhoid fever and diarrhea are the most prominent issue that Dhaka city battles with on an everyday basis. City like Dhaka where 300 million of people are living required safe water for drinking and for other household work. Most of the people of this city drink water by boiling or by filtration process. The cause is the raw water that is supplied is not suitable or safe to drink. So it must be treated by boiling or filtration. But still people suffer from water borne diseases. Hundreds of people flock to hospitals and private practices to find a cure for diarrhea and other gastrointestinal diseases. Families living below the poverty line are at the highest risk of these diseases as they do not have access to safe food and water. Due to limited amount of money, even simple treatment for diarrhea can become a burden. It is in cases like these where a case of diarrhea can lead to the loss of a life. However, lack of treatment is not the only reason why diarrhea can lead to death. In some cases, typhoid fever maybe so severe that it can lead to death. This is especially true for young children, senior citizens or any other immune-compromised groups. The prevalence of diarrhea was 16 per 1000 individual among all ages; young children represented for 44 per 1000 persons (Chowdhury et al., 2015). Thus, it felt that drinking water in Dhaka should be checked.

People flock to Dhaka city from all over the country in search of various opportunities. As a result the population of the city is increasing constantly. There are many issues that the city deals with on an everyday basis. Being able to provide clean water to its residents is one of them. As stated earlier, hundreds of people in city suffer from food poisoning. This leads us to assume that the water being used for food preparation, cooking or consumption may not be of the highest quality.

Coliform bacteria are rod shaped gram negative bacteria which may or may not be motile. Members of the coliform group includes: *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella* and *Escherichia coli.* Fecal coliform are facultative anaerobic, rod shaped, gram negative bacteria. They do not form spores and can grow in the presence of bile salts or other similar agents. Total coliform bacteria are present in the environment. They can be found in soil, vegetation and in the feces of warm blooded animals. It can be used to detect contamination in water sources. However, water sources contaminated with vegetation, soil, or affected surface water

would give total coliform count. It is very unlikely that a water source that gives total coliform count is polluted with sewage. Fecal coliform count is used to detect the existence of sewage in water source. It indicates water source that gives a fecal coliform count is most likely to contain other pathogenic bacteria. Since the origins of fecal coliforms are more explicit than the origins of the broader total coliform group of bacteria, fecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms.

Access to safe drinking-water is significant as a health and development issue at national, regional and local levels. In certain areas, it has been shown that investments in water supply and sanitation can yield a net economic benefit, as the reductions in adverse health effects and health-care costs outweigh the costs of undertaking the interventions. According to Guidelines for drinking-water quality by World Health Organization, 1997, any water that is expected for consumption should not have detectable fecal coliform, when 100ml of that specific sample is tested. Coliform count of the sample would decide the quality of the water sample.

**Table 4.1 Quality of drinking water based on coliform count (E. Ohanu, P. Udoh, & I. Eleazar, 2013)**

| Coliform count/100ml | Class | Quality |
|---|---|---|
| 0-1 | 1 | Highly satisfactory |
| 1-2 | 2 | Satisfactory |
| 3-10 | 3 | Doubtful |
| More than 10 | 4 | unsatisfactory |

Water has the limitless magnitude including human survival and environmental sustainability. City like Dhaka where 300 million of people are living required safe water for drinking and for other household work. Most of the people of this city drink water by boiling or by filtration process. The cause is the raw water that is supplied is not suitable or safe to drink. So it must be treated by boiling or filtration. But still there are group of people who suffer from typhoid fever. Typhoid fever is the main source of bacteremia in children aged

<5 years hospitalized in Dhaka, with an annual incidence estimated to be about 18·7/1000 persons (Rahman et al., 2010). The member of Enterobacteriaceae family *Salmonella* spp. is the causative agent of diseases such as salmonellosis, typhoid, paratyphoid, etc. The bacteria infect the intestines, from which it goes to the bloodstream. The bacteria returns back to the gut, from which it is excreted along with feces of the patient or the carrier. They spread through the fecal oral route. In other words, individual who get infected with the disease after consumption of water contaminated with sewage containing the bacteria. In Bangladesh, typhoid and paratyphoid fever are endemic. Hundreds of children in the country suffer from the disease on a regular basis. To prevent the situation from worse, it is of the utmost importance that we check the water that we consume for *Salmonella* spp. In spite of awareness of this considerable burden, knowledge of *S. typhi* transmission routes in Dhaka is minimal, and focused control efforts infrequent.

In this study, PCR was carried out to detect the presence of *Salmonella* spp. in the water samples. This PCR amplifies *invA* which is a gene in *Salmonella*. Among 24 samples from Mohakhali were subjected to detect the presence of *Salmonella* spp. in them where 5 bands were obtained which indicates that five samples of Mohakhali did contain the bacteria. Again, 30 samples from Uttara were subjected to PCR and the results showed that four samples did contain Salmonella. Samples from Niketon were also subjected to PCR, but there were no positive samples. After taking a look at the results obtained from PCR, it can assume that water from Mohakhali is most likely to contain *Salmonella* spp. while water from Niketon is the least likely to contain it. We can further assume that consumption of water from Mohakhali is most likely to cause *Salmonella* infection while water from Niketon is least likely to cause the infection. However, we do not the concentration at which strain *Salmonella* spp. is present in the water samples. Hence, we cannot pick out samples that would cause particular *Salmonella* infection.

According to the above findings of this study the water that we use for drinking that are contaminated with microorganism like coliform and Salmonella. WASA reported that they provide safe water to the people living in Dhaka city. But in reality they are not really safe. Results showed that raw water of Dhaka city in Uttara, Mohakhali and Niketon area are

contaminated with fecal coliform. 20% of the raw water shows >100 fecal coliform count where boil water showed 13.33% and filtered water showed 4.35% which means the findings of this study is not match with the WASA report. Raw water samples from Mohakhali contained the greatest fecal coliform load. This is followed by Uttara. Raw water samples from Niketon contain the least amount of fecal coliform. Assuming that the samples represent the main water supply of Mohakhali, Niketon and Uttara, thus it can be said that the water supply of Mohakhali is most contaminated while that of Niketon is the least contaminated. So, the problems must be find out that how the water is contaminated with fecal coliform and Salmonella spp. On the other hand, for boiling water samples there were no data for Niketon in this category. The number of fecal coliform decreases significantly for both Mohakhali and Uttara after being subjected to boiling. Unfortunately, water from both the areas does not meet the standard guideline, and is not drinkable. Average fecal coliform count in filtered water in Mohakhali, Niketon and Uttara is 72 colonies per 100ml of water, 0.375 colonies per 100ml of water and 45.78 colonies per 100ml of water. From the result it can be concluded none of the water quality meet the criteria for being portable water only those samples with zero count can be consider drinkable. As most of the water samples from Niketon contained zero fecal coliform count thus they might be drinkable. Again between treated water results from graphs indicated that boiling is more efficient at reducing fecal coliform count than filtration.

Pipelines run underground to supply water from Dhaka Water Supply & Sewerage Authority to various households in different areas and thus the reason contaminated water might be the problem in the pipeline of this city. The water may be contaminated by the leakage on pipeline system of this city. The water may be safe at the point it treated but contaminated when it passes through the pipeline. If it happened, then initial steps should be taken by the authority to change the pipeline system of Dhaka city. From the results it had been clearly observed that the quality of water from the 3 zones vary quite a bit. Water from Niketon is of the greatest quality. This is followed by water from Uttara. Water from Mohakhali is of the lowest quality. Mohakhali is an area that has developed years and years ago. Uttara has seen major development in the last few decades while Niketon is a residential area formed only a few years back. Pipelines that run under Mohakhali might be much older than that of Uttara or Niketon. This means that the pipelines under Mohakhali could be a lot more damaged than those present in the two other

areas. Pipelines of Mohakhali might have a lot more cracks and so, a larger quantity of sewage could leak into water supply pipelines.

A slight seasonal variation can be observed if the graphs take into consideration. Average fecal coliform count in raw water samples in Mohakhali increase slightly from November to January. However, there is a significant rise in the average fecal coliform count from January to March. This is possibly due to the fact that temperature rises from January to March. The rise in temperature causes an increased growth in fecal coliform. If we take the graph for Uttara into consideration, average fecal coliform count in raw water samples decreases slightly from November to January, but rises in March and April. Apart from the rise in temperature, there is also a small amount to rainfall during March and April. Rainfall might cause stagnant sewage material to flow and seep into the water supply pipeline. This might be the reason behind the greater fecal count in the month of March and April. There is an increase in humidity in these 2 months, which might cause the growth of fecal coliform to increase. Sampling was carried out mostly in winter and spring. To observe a more significant seasonal variation, sampling could be carried out in summer and monsoon season.

In this study the data shows fecal coliform count in treated water. There might be several reasons for this. People are unaware of the duration and the temperature at which the water samples were boiled. Hence, they cannot comment on the efficiency of the boiling treatment. Bacteria detected even after treatment might have been thermo tolerant as well. People also do not know the type of filter used to treat water. It might also be possible that these water treatments are capable of removing fecal coliform. In such a case water becomes contaminated due to poor handling after treatment. Water may become contaminated due to poor personal hygiene. Water may become contaminated from the vessel in which it is stored. Biofilm maybe present at the bottom of these vessels may contaminate the treated water.

In the future there are more opportunities to extend this work. This work could be extended by doing genus specific PCR and species specific PCR should be done by targeting a virulent gene. Finally, campaigns should be arranged for general people to train them to practice some personal hygiene to prevent waterborne diseases. Hands must be kept clean by washing thoroughly with

soap and water or using an alcohol-based hand sanitizer. Cuts and scrapes should be kept clean and covered with a bandage until healed. Any kind of contact with other people's wounds or bandages should be avoided. Since hands and transfer tools both are significant sources of total coliform bacteria and may be contribute to the formation of biofilm layers thus the formation of biofilm can be tested.

# Chapter 5

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