A Survey on Fecal Coliform Count, Total Coliform Count and Ammonia Concentration Using Filter Drinking Water Samples Collected from Restaurants of Shantinagar And Tongi, Dhaka

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

Department of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/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 instruction.
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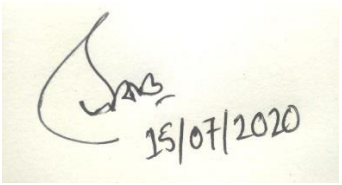

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Approval

The thesis project titled "A Survey on Fecal Coliform Count, Total Coliform Count and Ammonia Concentration Using Filter Drinking Water Samples Collected from Restaurants of Shantinagar And Tongi, Dhaka" submitted by Mahdi Mubin Shaikat (ID-16146046) of Spring, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 27.02.2020.

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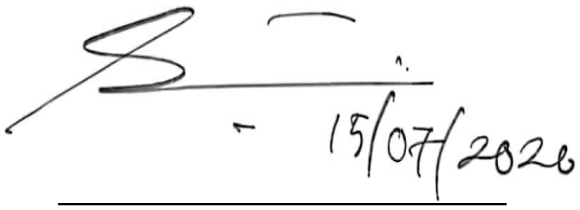

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Ethics Statement

This study does not involve any kind of animal trial and human trial.

Abstract

Sixteen filtered drinking water samples were collected following the protocol of collection of water samples from the different restaurants of Shantinagar and Tongi for the analysis of fecal coliform count, total coliform count and ammonia concentration by Membrane Filtration Method (MFM) and UV-VIS Spectrophotometry. All of these tests were performed by the central Lab of the Department of Public Health Engineering, Mohakhali, Dhaka. All of the samples are found to be meeting both the WHO and Bangladesh standards for fecal coliform, total coliform and ammonia concentration. Antibiotic resistance tests were also conducted with Azithromycin, Cefuroxime, Cefuroxime and Amoxicillin which are macrolides, cephalosporin and penicillin being antibiotics respectively.

Keywords: Water, fecal coliform, total coliform, ammonia concentration, antibiotic resistance

Dedication

Dedicated to my parents and supervisor.

Acknowledgement

Foremost, all the praises to Allah, Who is the source of our knowledge and He Who has led me in my studies, my academic career, and this very project.

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List of Acronyms

FCT	Fecal Coliform Bacteria
TCT	Total Coliform Bacteria
MFM	Membrane Filter Method
DGWQ	Guidelines for the Quality of Drinking Water
MICS	Multiple Indicator Cluster Survey
BBS	Bangladesh Bureau of Statistics
EPA	Environmental Protection Agency
ARB	Antibiotic-Resistant Bacteria
DSCC	Dhaka South City Corporation
DNCC	Dhaka North City Corporation
WASA	Water Supply and Sewerage Authority
HIV	Human Immunodeficiency Virus
DPHE	Central Laboratory of Public Health Engineering Department

Chapter 01

Introduction

Like every other animal, human life also relies on water for its existence. Water preserves various types of biological functions of the body and ensures the survival of the livestock. In addition, water is also used in various ways, such as the experiment in sciences, industries, factories and food production. However, water-borne diseases are still one of the world's major health issues. Around 4 billion incidents of diarrhea accounted for 5.7% of the global burden of disease in 2000 (WHO, 2002). Building safe and secured sources for instance boreholes, standpipes or wells for better quality water supply is one of the important strategies for handing this problem (J. Wright, Gundry, & Conroy, 2004). Since Bangladesh is a riverine country so the availability of water sources is very common. The significance of rivers, the main source of fresh water, is well demonstrated by the historical fact that significant civilizations have been settled through a river's bank. The indispensability of water, however, is compromised by its emissions, which renders it is unusable (Mishra et al., 2018). Water-related diseases in Bangladesh remain a major cause of mortality and morbidity due to the presence of massive pollution of water. In Bangladesh the most common types of water borne diseases which are bacterial originated are typhoid fever, bacillary dysentery and diarrhea (Islam, Begum, & Nili, 1970). Bacteria that are available in the environment are very dangerous to humans and all other animals, and most of the time these bacteria are resistant to antibiotics, including antibiotics of the narrow and board spectrum. The current health care system of the country is in question because of this sort of antibiotic-resistant bacteria, and the number is growing tremendously. The mortality rate is rising so high and the increasing number of bacteria entering the population is one of the major reasons behind. The water portability of bacteria is selected from the Enterobacteriaceae family as a predictor. (Fewtrell, Bartram, Ashbolt,

Grabow, & Snozzi, 2001); (Behera & Mishra, n.d.-b). Bacteria of this family are able to replicate in mammal stomach because they assist to predict the existence of pathogens, and detect it in the feces. Such bacteria are resistant to the genes of the medication and can convert into other pathogens in a short period of time. Therefore, they are causing risk to human health.

1.1 Emerging of Water-Borne Diseases and Pathogens

Waterborne disease is a global threat that causes more than 2.6 million deaths annually and incidents of disease are increasing day after day, including diarrhea, gastrointestinal diseases and systemic diseases (Work, 2018). Most importantly, of these deaths, about 1.4 million are children. (Ramírez-Castillo et al., 2015). Worldwide, there is a projected economic loss worth approximately US$ 12 billion per year. (Alhamlan, Al-Qahtani, & Al-Ahdal, 2015). Waterborne diseases are caused by a number of infectious agents, including bacteria, viruses, helminths and protozoa, ingestion, airborne or contact with contaminated water. (Leclerc, Schwartzbrod, & Dei-Cas, 2002). Around 780 million people have no exposure to safe source of water as well as a projected 2.5 billion people worldwide lack exposure to better sanitation (World Health Organization (WHO) and The United Nations Children's Fund (UNICEF), 2017). It is reported that 3.2 percent of deaths worldwide were caused by substandard sanitation and hygiene caused by contaminated water. A Bangladesh-based study shows that 40 percent of the complementary foods for children are tainted with Escherichia Coli. Fecal coliform levels and total coliform levels are largely affected by drinking water quality. The side effects for each drug are not simple to independently classify and are related by the reference factor fecal oral-pathway (Shields, Bain, Cronk, Wright, & Bartram, 2015)

Table 1: The classification of water-related diseases

Category	Example
1. Water borne (A) Classical (B) Non-classical	 Typhoid Infectious hepatitis
2. Water-washed (A) Superficial (B) Intestinal	 Trachoma Scabies Shigella dysentery
3. Water-based (A) Water-multiplied percutaneous (B) Ingested	 Schistosomiasis Guinea worm
4. Water-related insect vectors A) Water-biting B) Water-breeding	 Gambian Sleeping Sickness Onchocerciasis

Waterborne pathogens have arisen frequently for various reasons incorporating polluted water, expanded vulnerable population, improvements in drinking water treatment technologies, trade and travel globalization, and the advancement of molecular identification and origin methods. The need of commercial and industrial resources in developing countries devotes to the occurrence of waterborne disease. There are currently an estimated 1407 species of human-infected organisms, including, viruses (208 species), bacteria (538 species), parasitic protozoa (57 species), and several types of fungi and helminths (Leclerc et al., 2002)

1.2 Access to Safe Water

By 2000, one-sixth of humankind (1.1 billion people) were reported to have no exposure to any form of enhanced water supply within 1 kilometer of their home (WHO and UNICEF, 2000). Access to water utilities is a key element of developing countries in the UNDP Human Poverty Index (UNDP, 1999). It is human rights of all human to access to safe water throughout their life. In 2013, 97% of the country had exposure to enhanced water sources, which is a major step forward for Bangladesh in terms of widespread access to safe drinking. (Ya-Ying, 1996). Above 41 percent of people consume water containing fecal contaminated water, which

increases to 61.7 percent at the point of ingestion, according to the 2013 MICS report. The study also suggests that the nature of bacterial contamination is aggravating the water since it is delivered to households. The amount of pollution is increasing significantly due to inadequate hygiene practices in residences. UNICEF also found proof that there are still important differences in the standard of water in various areas of Bangladesh. For example, the division of Rangpur has around 71.8 percent safe water, while the division of Sylhet can only receive the same water at a level of 31.6 percent. During periods of emergencies such as earthquakes, landslides as well as cyclones, drinking water supplies are very much polluted. (BBS & UNICEF Bangladesh, 2014)

1.3 Safe Drinking Water Standards

Those with the highest threat of waterborne disease are babies and young children, individuals who are frail or live in unhealthy environments, also elderly people (WHO, 2003)(Sayato, 1989). To order to guarantee health and safety by recommendations that are used as a framework for legislation and standard development around the world, the World Health Organization (WHO) has developed international standards for water quality and human water usage. These guidelines are referred to as the "Guidelines for the quality of drinking water"(DGWQ). They encourage community protection as well as public health as they advocate developing local regulatory and regulatory requirements (for health purposes) through the adoption of some inhibitory risk management measures. According to the recommendations, any 100 ml water sample must not be identifiable as E.coli. The measurement process is much better when using E.coli or any thermal coliform, while the new enzyme process is known to be easier and cheaper. Hygiene inspection is one of the most commonly used and efficient ways in which risk management is detected and improves. The results of the hygienic inspection will vary by time (mostly seasonal) and region.

Table 2: Different types of risk classification of an organism in 100 ml of water

Level of risk	Coliform present in 100ml of water
Very lower risk	1-10
Lower risk	10-100
Medium risk	100
High risk	>100

While this table is not considered ideal to determine the exact coliform number as it varies depending on time and region. Standardized methods provide health risk evaluation and safety rating indicators.

1.4 Coliform Contamination

Coliforms are those bacteria which are found in the digestive tract of humans and other animals as well in their feces. It is doubtful that coliform bacteria can cause illness. Presence of coliform in drinking water, therefore, recommends that the water system can consist of disease producing organisms (Tominaga, 2019) (Cabral, 2010). Such organisms can adulterate the water supply from animal manure, as well as from improper sanitation and infrastructure maintenance. It is a very difficult, costly and time-wasting procedure to classify all the pathogen by analysis so that it is quite easy to search for the coliform bacteria. If coliform bacteria are detected after analysis, the water system provider must attempt to find the cause of contamination to maintain a healthy drinking water system. Coliform bacteria are categorized into three classes, including coliform, fecal coliform and E.coli.

Total coliform bacteria are usually and normally innocuous in the atmosphere (ground or plant life). If total coliform bacteria are solely found in potable water by a sample, the source is expected to be eco-friendly and fecal pollution is doubtful. Nevertheless, if contaminants of the

ecosystem could enter the system, a pathogenic organism could also pass into the system. Finding and mitigating the cause of the contaminants is critical. Another type of coliform bacteria is fecal coliform bacteria. These bacteria are present in humans and animals ' intestines and feces. E. coli bacteria are the last type of coliform bacteria. Most of the E. coli bacteria are innocuous and are present in humans and warm-blooded mammals in the gut. Many strains, however, may cause disease.

Water systems examine how the contaminants entered into the water where coliform bacteria are detected. They obtain extra samples of water and periodically check the whole process. The collection of additional samples is necessary because it helps to identify if there is a real problem or not. The initial results are confirmed if the lab observes any kind of error in the additional samples.

1.5 Total Coliform Test and Variation in Count

Total coliform consists of a large number of gram-positive bacteria which are rod-shaped containing various characteristics. This group consists of thermo-tolerant coliforms and fecal bacteria besides few bacteria derived from natural sources. Therefore, the existence of total coliform bacteria in a sample does not specify the presence of fecal coliform bacteria in that sample. It is very rare to find a little or zero thermo-tolerant bacteria in a huge number of total coliform bacteria. This kind of finding is not a requirement of the presence of fecal coliform bacteria. Maybe this is due to the presence of soil or other organic substances in the water which results in the growth of different types of bacteria. The growth of total coliform bacteria is seen on a lactose-containing medium at a temperature of 35 or 37 ° C in the laboratory (Tancini et al., 2012).

It is possible to introduce bacterial contamination into the water from several causes, which include natural source, person-made or by its own activities. Microorganisms which are

extracted from the feces of hot-blooded mammals which includes human that can contain disease-causing bacteria and viruses (Alarcon Falconi et al., 2017) (Sarkar et al., 2013). Nevertheless, not all total coliform bacteria are injurious to human, sometimes they help in the identification of other pathogenic bacteria. Although there is a substantial variation in the total number of coliforms as sewer areas have increased in the last few years and the rise in bacterial drainage has occurred in the use of sophisticated methods of waste treatment (Watson, 2010). One of the main reasons of pollution is the handling of untreated and non-infected human waste. Rising interaction with surface water, though, has resulted in more human access to this bacterially polluted water, including drinking water (Eshcol, Mahapatra, & Keshapagu, 2009). In all 100 ml samples, no coliform will occur in WHO GDWQ standards and studies have shown that although a high degree of care is taken of the health of the water used for direct use, the coliform does not yet exist.(J. Wright et al., 2004)(Bengal, Bhunia, Ramakrishnan, Hutin, & Gupte, 2009).

1.6 Fecal Coliform Test and Variation in Count

These bacteria are known as thermo-tolerant bacteria and they grow at between 40 and 44.5 C and ferment lactose as a result of acid and gas are produced. Practically, bacteria with these characteristics may not be from the fecal origin is, therefore, the more proper and correct names of those bacteria are thermo-tolerant bacteria nowadays. Nonetheless, the presence of thermo-tolerant bacteria confirms the presence of fecal contamination. In general, the intestinal organism of Escherichia coli, whose presence is definitive evidence of fecal contamination, is more than 95% of the thermo-tolerant coliforms segregated from water. Therefore, additional testing to verify the exact existence of E. Coli is often totally unnecessary. Fecal coliform bacteria are grown in the laboratory at a temperature of 44 to 44.5 ° C on media containing lactose (Tancini et al., 2012).

Fecal contamination of potable water is determined by the fecal indicator bacteria. According to WHO, E. coli is the most preferable and appropriate bacteria to identify fecal contamination (Kostyla, Bain, Cronk, & Bartram, 2015). However, the seasonal change is a major problem with fluctuating fecal coliform quantities. This can be split according to moisture, temperature as well as in various seasons such as summer, winter and fall. Microorganisms are found more susceptible in the rainy season because of enhanced environmental exposure.(Onda, Lobuglio, & Bartram, 2012). Their versatility is at its highest point in the humid season (Wolf et al., 2018). Most studies of potable water are performed for travel versatility in dry or winter season. Unless seasonal variations are taken into consideration, the pollution level results will be misleading and in more inexact results. In earlier research, a widely common pattern in assessing water quality in the following seasonal trends.(Ouyang, Nkedi-Kizza, Wu, Shinde, & Huang, 2006). Depending on seasonal changes, the recommendations can be improved regarding the sample collection, which will ultimately help to improve the sample collection region and produce more correct and precise results. While in developing countries, there is no analysis of the overall seasonal trend on fecal contamination and cumulative pollution by enhanced water sources (Alarcon Falconi et al., 2017) (Kostyla et al., 2015). Research in developed countries includes extensive sampling in various seasons that involves both existing and degraded supplies of potable water (R. C. Wright, 1986).

1.7 Significance of Fecal Coliform and Total Coliform Count Test

Despite of being drinking water, the prevalence of bacteria as well as other microorganisms that kill species often identified as pathogen is a big concern. Microorganisms which are pathogenic result in intestinal diseases, diarrhea, dysentery, typhoid, cholera etc. Waste products of humans and other mammals are recognized as the primary source of this type of contamination.(Khan, Beattie, & Knapp, 2016)(Wellington et al., 2013)(Gaze et al., 2011). Such causes may involve septic tank ingestion or disposal, wastewater treatment systems, and

natural soil and bacteria from plants. Those bacteria can penetrate or have capped on wells or any other open water surfaces that are not moisture-tight enclosures. Each water supply source must be checked at least four times a year, as indicated by studies. The EPA has set drinking water requirements, which are divided into two categories known as Primary and Secondary Standards. Primary requirements are focused on environmental criteria and are intended to keep safe human beings against three categories of toxic pollutants, including bacteria, radioactive elements as well as toxic substances. Bacterial contamination falls under the category of pathogen. As monitoring is relatively inefficient and expensive for all specific pathogens, EPA has developed total coliform bacterial counts as a criterion for evaluating water safety. This check works by tracking the coliform bacteria to calculate the rise or fall in the number of pathogenic bacteria. For non-pathogenic, non-coliform bacteria besides the general heterotrophic number or regular count plate of < 500 Colonies per ml, no particular sanitary requirements or health recommendations are suggested. Checking and recording of a water sample as non-compliant with the EPA bacteriological norms level of nil are deemed to be unsafe for use. Certain bacteria may sometimes interact in a sample when measuring the categories of coliforms. All the above samples are categorized as "too many to count" or "confluent growth."(Coleman et al., 2013)(Tominaga, 2019)(Lyimo, Buza, Subbiah, Smith, & Call, 2016).

1.8 Ammonia in Water

Ammonia is a nitrogenous compound that is greatly soluble in water. It is a colorless and strong gaseous compound and a biologically active compound in water. It is found as a biologically degradable waste product of nitrogenous organic matter such as protein. Most likely, it is found in different types of water such as ground and surface water from industrial wastage and fertilizers. The presence of ammonia increases the formation of chloramines which leads to

decreases formation of chlorination which may be carcinogenic (WQA, 2013). For groundwaters, natural levels normally are less than 0.2 mg per liter of ammonia and up to 12 gm/L are found in surface water(World Health Organization (WHO), 2003). The standard level of ammonia in drinking water is 0.5 in 100 ml of water (Alam et al., 2007).

1.9 Effects of Ammonia on Human

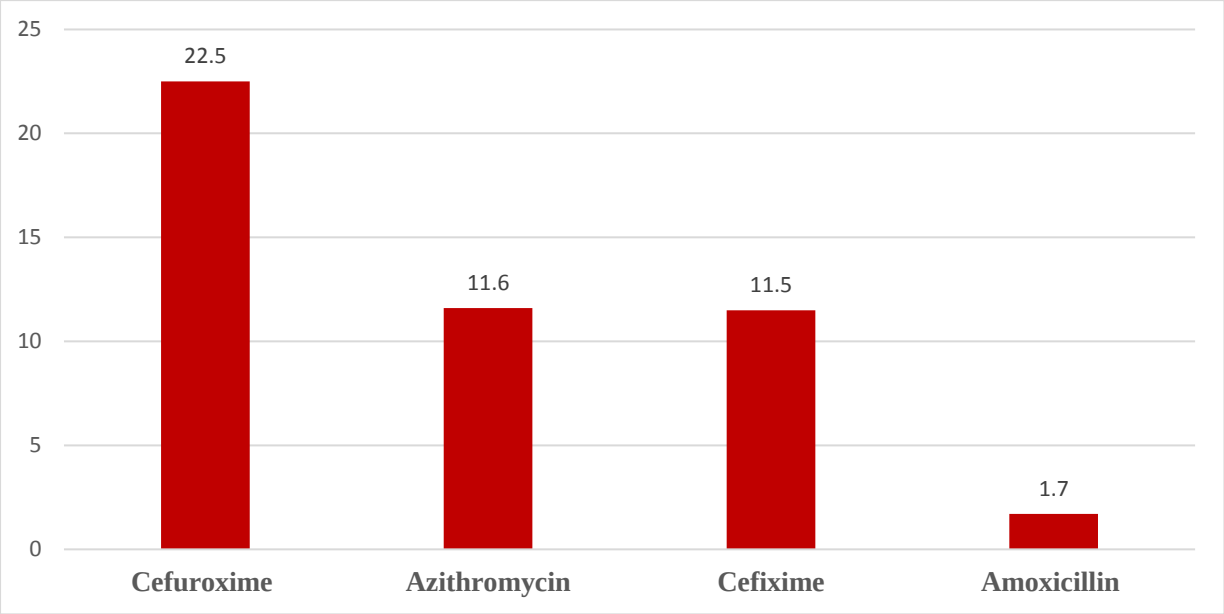
Only when the intake is higher than the ability to detoxify has a toxic effect on healthy people. When ammonia is supplied in ammonium salts, it also must take into consideration the effects of the anion. The acidic actions of chloride ions tend to be stronger than that of the ammonium ion with ammonium chloride. Ammonium chloride influences the metabolic rate at a dosage of more than 100 mg/kg body mass daily by changing the acid-base balance, disrupting the absorption to glucose, and decreasing tissue sensitivity towards insulin (World Health Organization (WHO), 2003).

1.10 Antibiotic Resistance

Antibiotic resistance happens once the bacteria modify their reaction to the antibiotic and therefore do not function as intended. Resistant bacteria are much more difficult to treat rather than non-resistant ones. To resolve the issue the world desperately needs to change the approach antibiotics are distributed and used. If the behavior is not modified, the use of new medicine will not be healthy. Changes in behavior include steps to decrease infection spread through vaccination, washing hands, safer health practice, and good hygiene throughout the food. Usually, in countries that do not have a recommended treatment plan, the healthcare workers and veterinarians sometimes over-prescribe antibiotics and the 10 people ending up overuse it. Antibiotics are the most widely prescribed medicine in hospitals today, worldwide (Rubin, 2007). Absence of strict regulation on unnecessary and improper use of antibiotics

resulting in increased tolerance to the medication (Kim, Hwang, Kim, Lee, & Pai, 2018). Therefore the appropriate use of antibiotics is a major challenge and becomes a significant health imperative against the creation of resistance for healthcare professionals (Ahmad, Khan, Moorthy, Jamshed, & Patel, 2015). Many variables that support the development of antibiotic resistance which are overprescribed including for viral infections, overuse and insufficient antibiotic length, over-the-counter supply of antibiotics, ineffective medical care and patients who purchase only as many tablets as they can manage and doctors often tend to prescribe antibiotics according to patient request. (Ata et al., 2019). In 2015, the White House published The National Action Plan to Fight Antibiotic-Resistant Bacteria (ARB), that set a target of eliminating unnecessary outpatient antibiotic use through at least a majority by 2020 (USA, 2015). In developing nations such as Bangladesh, 55.57 per cent of physicians prescribe antibiotics of presumed infection whilst in actual cases only 33.46 per cent of prescribe antibiotics. 40.22 percent of physicians prescribe cold and fever antibiotics prior to any diagnostic test. In fact, 37.31 percent of physicians prescribe antibiotics to appease patients while 62.44 percent opposed any undue influence (Ata et al., 2019). Resistance to antibiotic has now become a global issue where Bangladesh is one of the significant contributors, leading to its substandard healthcare and high levels of abuse or overuse of antibiotics. It affects the successful treatment and prevention of the rising range of infectious diseases caused by bacteria, viruses, parasites and even fungi. One of the reasons behind antimicrobial resistance is the extremely expensive and time-consuming work on antibiotics, so that very few scopes of antibiotic testing on recent high-class antibiotics stay out of control (Khan et al., 2016)

Figure 1: Percentage of antibiotic usage in Chattogram between January 2018 to June 2018.



1.11 Objective of the Study

High percentage of people all over towns and cities are quite probable to consume street meals. The prospect of having at least safe and clean drinking water is maybe still a big challenge. Microbially contaminated water remains an alarming threat in drinking water and has its wild impact on quality of human life. Although nowadays people are very conscious of the fact regarding waterborne diseases and are quite careful about their preferences in terms of safe drinking water because many events of municipal water supply have been reported. The causes of this sort of pollution include poor maintenance of transport and pipelines, as well as leakage in that pipelines. Several companies are coming forward to supply bottles of water and appear to have purified water but it is expensive for ordinary people. The option for this approach was to supply water in large sealed containers at a minimal cost. Such bins are simple to use as they must be connected to the dispensing unit and there is no need for cabling or heavy maintenance. Consumers can get the water out of that dispenser directly in small glasses. Now since drinking water from such a sort of dispenser is quite widespread and has gained prominence in recent

times, it became an essential part of checking whether that water is clean enough to consume directly and ensuring that the water is free of microbial contamination. Previously, three students from the Department of pharmacy, Brac University conducted a survey on the same project consisting of physical properties and FECAL COLIFORM and TOTAL COLIFORMcount of water in Mohakhali and Brac University premises. So, it was necessary to test water from other areas of Dhaka city so that we can compare the results and come up with a piece of new information and ideas to reduce the causes of water-borne diseases. The objective of this study was to count the fecal and, coliform count, ammonia which was found high on their data previously. Antibiotic resistance test was also performed on the samples which were collected from Shantinagar and Tongi.

Chapter 2

Methodology

2.1 Site Location and Area

Shantinagar and Tongi are two different types of the site of Dhaka city. Shantinagar is located under the area of Dhaka South City Corporation (DSCC) which is a more likely residential area as well as considered as a commercial area which includes banks, shopping malls, restaurants, hospitals, private office etc. On the other hand, Tongi is located under the area of Dhaka North City Corporation (DNCC) which is one of the largest industrial areas consisting of the feature of BSCIC and produced about 1500 corer industrial products yearly. The foremost reason behind choosing these two areas so that this project can cover both DSCC and DNCC of Dhaka City as well as both residential and industrial areas.

2.2 Sampling site and sources

A total of 16 samples were collected from both places. Out of 16 samples, 12 and 4 samples were collected from Shantinagar and Tongi respectively. All samples were collected from different types of restaurants of both places and these restaurants were selected by the degree of usage of people. All the samples were collected on Sunday dated 17-11-2019. Three kinds of water sources include WASA, drinking water from private companies which are usually provided with a blue jar and some restaurants use their own sources of water such as deep tubes that were used to collect those samples. These water samples were collected to perform antibiotic-resistant test, fecal coliform count and total coliform count.

Figure 2: Map showing the locations of restaurants, hotels and tea-stall of Shantinagar using Arc GIS

Figure 3: Map showing the locations of restaurants, hotels and tea-stall of Shantinagar using Google Map

Figure 4: Map showing the locations of restaurants, hotels and tea-stall of Tongi using Arc GIS

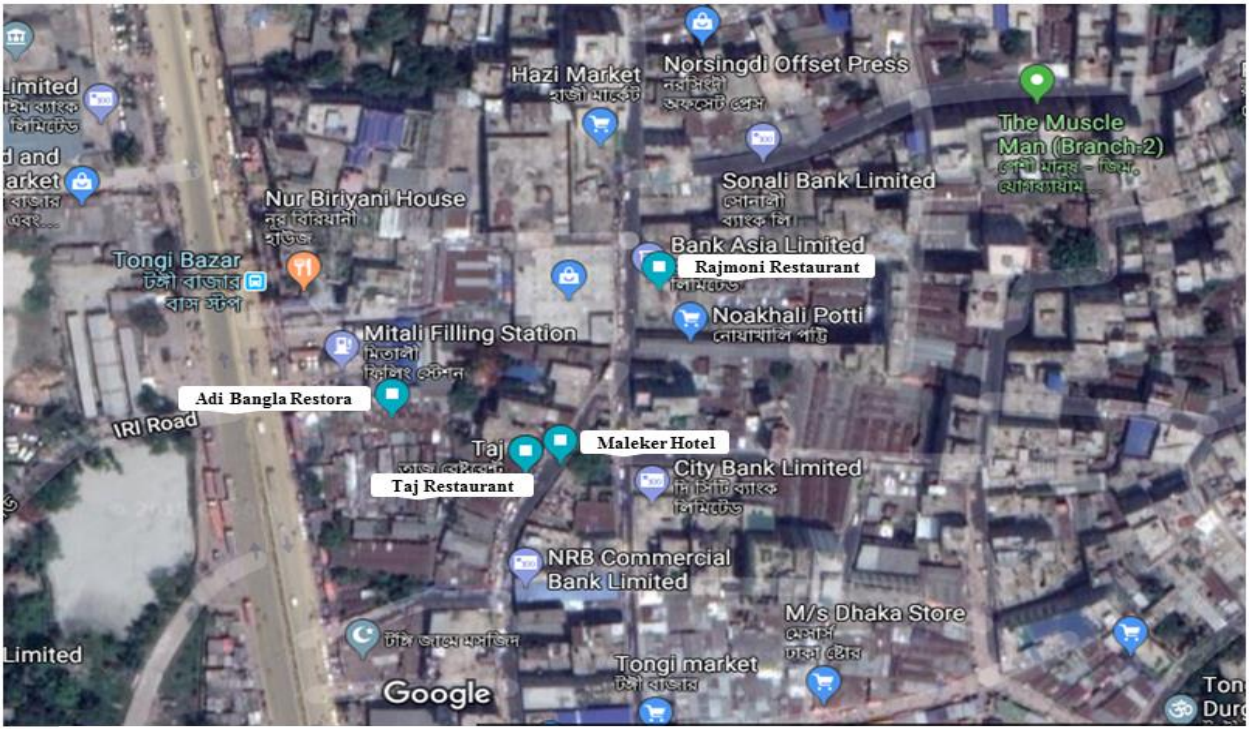


Figure 5: Map showing the locations of restaurants, hotels and tea-stall of Tongi using Google Map

Figure 1.0;1.1 and 1.2;1.3 showing the locations of restaurants, hotels, and tea-stall around the Shantinagar and Tongi respectively. These are the most crowded and busiest restaurants, hotels and tea-stalls in those areas. Every day thousands of people visit these places to have a meal. The samples were collected from all the marked places in maps. For the identification purpose of the samples site, these figures are used.

2.3 Collection of Samples

According to WHO 1984 standard, all the filtered drinking water samples were collected and tested. The internal portion of the containers was sterilized by ethanol to make them ready for collecting the samples. Samples were collected on those sterile containers and about 250ml of drinking water samples were collected from each source. All of the samples were instantaneously moved to the Central Laboratory of Public Health Engineering Department (DPHE) of Mohakhali in a segregated box. These filtered drinking water from different restaurants were consumed by an ample number of people in this city every day.

2.4 Antibiotic Resistant Test

Antibiotics are on the most available and common medicines in Bangladesh. A vast number of antibiotics are used in our country. The percentage of usage of antibiotics is increased due to the lack of regulation in the prescribing of antibiotics. One can easily buy an antibiotic without any prescription just by saying the brand name of antibiotics. I have selected 4 antibiotics for my project to perform an antibiotic resistance test and these are Cefuroxime, Azithromycin, Cefixime and Amoxicillin. These four antibiotics are widely used in Dhaka city for the last few years (Ata et al., 2019).

Cefuroxime is a cephalosporin antibiotic grouping under the second generation of cephalosporin. It exerts activity by interfering with the bacterial wall synthesis and by

inhibiting cross-linkage of the peptidoglycan. Cefuroxime is certainly used to treat some bacterial infections which include bronchitis (an infection of the lung), gonorrhea (sexually transmitted infection), lime disease and infection of the skin, sinuses, ears, throat, tonsils as well as urinary tract infection. Although, it has some serious side effects such as rash, itching, bloody stools etc. However, it has been one of the most effective antibiotics throughout the years.

Azithromycin is the class of macrolide antibiotics. The mechanism of action this antibiotic is by impeding the protein synthesis. Azithromycin is used to treat the infection of mouth, ears, sinuses, skin and reproductive system as well as bacterial infection such as bronchitis, pneumonia and sexually transmitted diseases. Azithromycin is also used in people affected by HIV (Human Immunodeficiency Virus). Despite having some serious side effects, Azithromycin proved as mostly used antibiotics in this era.

Cefixime is a third-generation cephalosporin antibiotic. It acts the same as cefuroxime but having more effectiveness than cefuroxime. It is a very stable antibiotic due to the presence of beta-lactamase enzymes. Cefixime is used to treat infection in ear, tonsillitis, pharyngitis, laryngitis, bronchitis, pneumonia and urinary tract infection.

Amoxicillin is a penicillin antibiotic that is used to treat mild to moderate infections that includes pneumonia, infection of ear, throat, nose, urinary tract. It works by inhibiting the growth of bacteria. It is a narrow-spectrum antibiotic.

2.5 Methodology for Antibiotic Resistance Test

Step 1: All the samples were collected from the sources.

Step 2: 25 ml of nutrient agar was prepared in the conical flask for the formation of bacterial culture.

(Continued)

Step 3: The prepared agar was autoclaved and then poured into a petri dish while agar was bearable hot so that bubble cannot be formed.

Step 4: 50µl of the sample was taken using a micropipette (range 1-100µl) and spread into the agar with the help of a spreader.

Step 5: Petri-dish was then kept upside down and the dish was taken into the incubator at 37degree Celsius (overnight) that allow growth and formation of a bacterial colony.

Step 6: To do the dilution of samples as well as to take absorbance three solutions of nutrient broth were prepared in the conical flask containing 10ml water and broth powder for each sample.

Step 7: Visible quantity of bacterial colony was taken in one of the three conical flasks with the aid of an inoculation loop and two rest are held as blank and dilution purpose.

Step 8: The conical flask with the culture of bacteria was kept overnight in the shaking incubator. (Note: The shaking motion speed should be at a constant rate. Nor can interruption or break cause bacterial culture growth problems)

Step 9: The following day conical flask with bacterial culture was removed from the incubator after that placed inside the laminar flow cabinet. 2-3ml of the solution was taken in cuvette from that flask.

Step 10: Another 2-3ml nutrient broth solution was taken as blank as previously prepared in another cuvette. First, the UV spectroscopy should be standardized using a blank solution in both cuvettes and then one cuvette is replaced with the sample

(Continued)

Step 11: These two cuvettes were then placed to measure the absorbance with UV spectroscopy. (Note: the wavelength should be 600nm and cuvette should be washed by a rinse and sterilized properly with ethanol).

Step 12: Upon having the absorbance, it was used to measure the amount of bacterial culture in microliter MIC (minimum inhibitory concentration) that required to be diluted in the previously prepared nutrient broth solution.

Step 13: Using a micropipette, the desired quantity of solution was taken from the conical flask from which absorbance was determined and transferred to the conical flask containing 10ml of nutrient broth for 2nd-time dilution.

Step 14: Then three Petri dishes were prepared and set properly with Muller Hinton agar at the correct concentration level in the container.

Step 15: In the second diluted solution, a sterile cotton swab was dipped and spread evenly on the surface of the petri dish containing Muller Hinton media.

Step 16: Antibiotic disks were placed on top of the Muller Hinton media and one piece of autoclaved punched paper for comparison was also placed as blank.

Step 17: Now, at 37°C, the Petri dishes were put inside the incubator to check antibiotic resistance.

Step 18: The Petri dishes were removed from the incubator the next morning. The lysis demonstrates a dead bacterial colony and measurement of the diameter of the dead colony.

Step 19: Bacterial inhibition circle values in mm diameter are measured after standard deviation using an average of three values.

(Continued)

Precautions:

1. The safety measures of the laboratory have been strictly followed.
2. The whole process was done side the laminar flow.
3. Before using, all the materials and instruments were autoclaved.
4. Weight of the all materials was taken carefully to keep the concentration accurate.
5. All the instruments were handled and washed properly.
6. Sterilization of the cuvettes of UV spectroscopy was done by using ethanol,
7. As a protocol of standard, time and temperature were maintained carefully.
8. The samples were used within four hours of collection.
9. After shaking it properly, all the agar media were poured out before each time it poured while still warm liquid to prevent bubble formation and solidification.
10. Antibiotics discs were stored in a refrigerator.

2.6 Fecal Coliform Test

Before starting:

1. The temperature of the incubator was maintained at 44±0.5°C.
2. Before the addition of the sample, the temperature of the incubator was stabilized.
3. Hands are required to be sanitized with soap and water.
4. Until beginning the process, the workstation must be sterilized and washed with germicides, bacterial spray, and weak bleach.
5. All the materials that will come into contact with the sample are made sterilized.
6. The vacuum was removed as soon as the funnel was empty during filtration to prevent the filter from drying.

Materials and Ingredients

1. mFECAL COLIFORM agar
2. Bacto Agar
3. Distilled water
4. Rosalic Acid
5. Petri dish
6. Membrane filter, 0.45 micron
7. Incubator
8. Pipets

Preparation of Fecal Coliform Plate

Step 1: In a conical flask, 37 gm mFECAL COLIFORM powder was taken.

Step 2: It was then added to 15.6 gm of Bacto Agar.

Step 3: Powder and agar were then added to 1000 ml of distilled water.

Step 4: 10 ml of Rosalic Acid solution was added with the mixture.

Step 5: The mixture was mixed well.

Step 6: The mixture was the boiled in a heater and was cooled at room temperature

Fecal Coliform Count

Step 1: At first 100 ml of the sample was filtered with 0.45 nm filter paper.

Step 2: The filter paper was put on the FECAL COLIFORM plate.

Step 3: The plate was placed in the incubator at 44.5℃ for 24 hours.

Step 4: Finally, the blue colonies were counted which indicated FECAL COLIFORM colonies.

Source: Central Laboratory of Public Health Engineering

2.7 Total Coliform Test

Preparation of Total Coliform Plate

Step1: At first 37 gram of MTC powder is taken into a conical flask.

Step 2: Then 1000 ml of distilled water is added so that the MTC powder is dissolved.

Step 3: The mixture is stirred gently to dissolve the solute.

Step 4: The mixture is then boiled for a couple of minutes.

Step 5: After boiling the mixture for a while it is kept aside to cool down slightly.

Step 6: The light warm mixture is then placed in an absorption pad.

Total Coliform Count

Step 1: 100ml of sample is filtered by 0.45nm filter paper.

Step 2: The filter paper is then placed on the absorption pad which has the MTC mixture.

Step 3: The absorption pad is placed on a plate and placed in the incubator at 37 degree Celsius for 24 hours.

Step 4: The next day counting of the dark blue/purple/black colony are done.

Source: Central Laboratory of Public Health Engineering

2.8 Ammonia Test

Ammonia is accountable for the microbial protein catabolism of any kinds of organic substances. Therefore, decomposition of raw fish, meat and milk etc. are the results of high concentration of ammonia. Abnormal odor indicates the presence of higher level of ammonia. Concentration of ammonia are found to be high in urine and feces. Ultra Violet Spectrometer

has been used in this survey to determine the presence of ammonia. This test was done by the Central Lab, department of Public health Engineering (Mohakhali, Dhaka)

Ingredients and materials

1. Phenol sodium nitroprusside solution
2. Sodium hypochloride
3. Volumetric flask
4. UVS

Procedure

Step 1: 25 ml of the sample was taken in a 50 ml volumetric flask.

Step 2: 10 m of Phenol sodium nitroprusside solution was added.

Step 3: Deionized water was added up to the mark of 50 ml.

Step 4: 1 hour waiting for the color development.

Step 5: The absorbance was measured at 640nm.

Source: Central Laboratory of Public Health Engineering

Chapter 3

Results

Table 3: Fecal coliform count results from the central lab of DPHE

Sample source	Water quality parameter	Bangladesh standard	Concentration present	Unit	Analysis method
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM

(Continued)

Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM
Filter	Coliform (fecal)	0	0	N/100 ml	MFM

Table 3 is showing the result of the fecal coliform count of all 16 samples which were collected from different places of Dhaka city. All of the samples are showing the positive result which means all the samples have been found to be meeting the standard set by the World Health Organization (WHO) which is 0 fecal coliform in 100 ml of water. Earlier in spring and summer of 2019, a similar FECAL COLIFORM count had been conducted with a different number of samples of Mohakhali area and they both showed a moderate to high level of fecal coliform concentration in the result.

Table 4: Total coliform count results from the central lab of DPHE

Sample source	Water quality parameter	Bangladesh standard	Concentration present	Unit	Analysis method
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM

(Continued)

Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM

Table 4 is showing the result of the total coliform count of all 16 samples which were collected from different places of Dhaka city. All of the samples are showing the positive result which means all the samples have been found to be meeting the standard set by the World Health Organization (WHO) which is 0 total coliform in 100 ml of water. Earlier in the spring and summer of 2019, a similar FECAL COLIFORM count had been conducted with a different number of samples of Mohakhali area and they both showed a moderate to high level of total coliform concentration in the result.

Ammonia

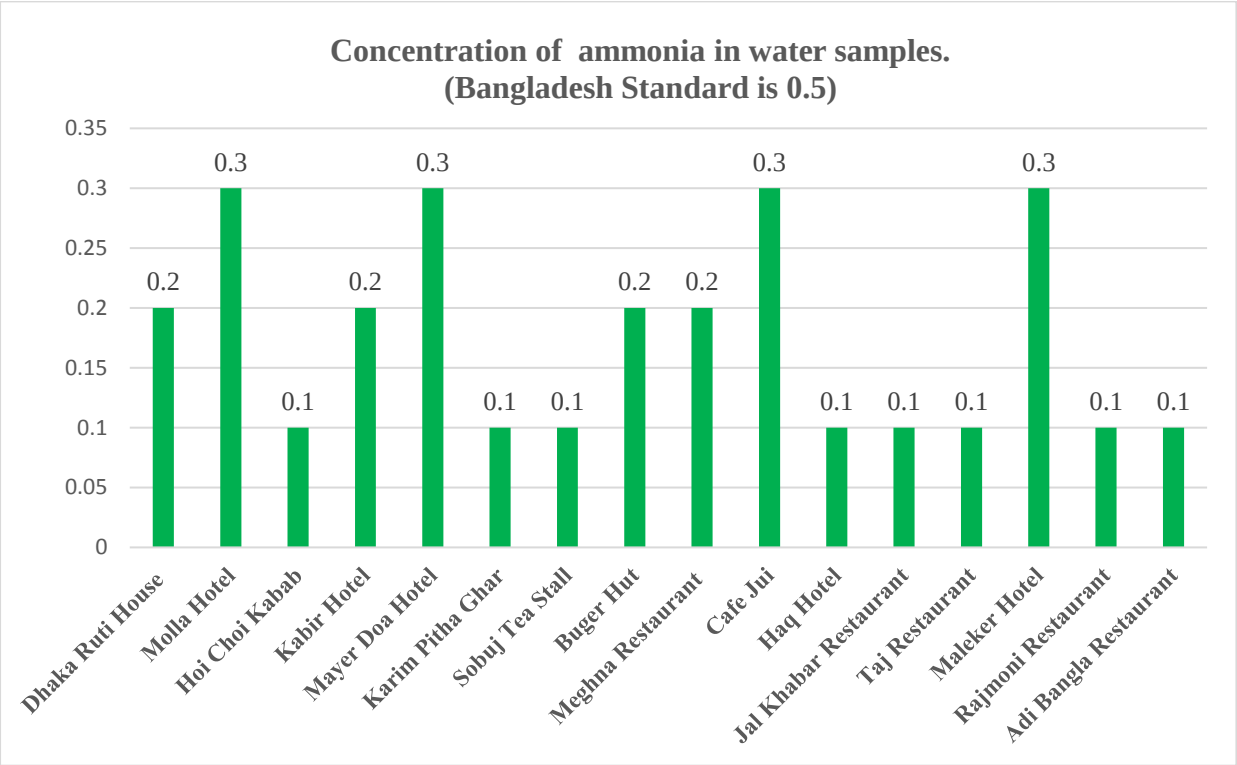


Figure 06: Bar chart showing the concentration of ammonia in water samples

All samples containing Ammonia are under the Bangladesh Standard of Ammonia.

Antibiotic Resistance Test

After performing the antibiotics resistant test of 16 samples, the study shows that a great number of samples are resistant and most of them are moderately sensitive. In case of 16 samples, Azithromycin is 25% sensitive, 56.25% moderately sensitive and 18.75% resistant. Cefixime is 12% sensitive, 38% moderately sensitive and 50% resistant. Cefuroxime is 31.25% moderately sensitive and 68.75% resistant. Lastly. Amoxicillin is 6.25% moderately sensitive and 93.75% resistant. Out of 16 samples, 7 (44%) samples are resistant to both Cefuroxime and Amoxicillin.

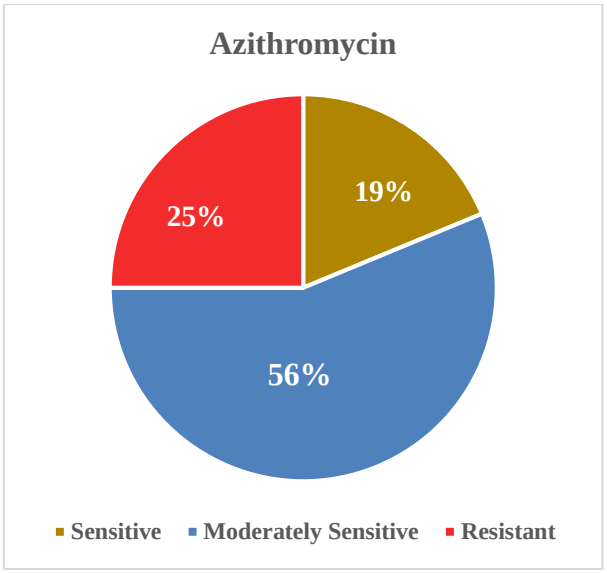


Figure 07: Pie chart representing the antibiotic resistant test result of Azithromycin

Figure 07 is showing that Azithromycin is 19% sensitive, 56% moderately sensitive and 25 % resistant.

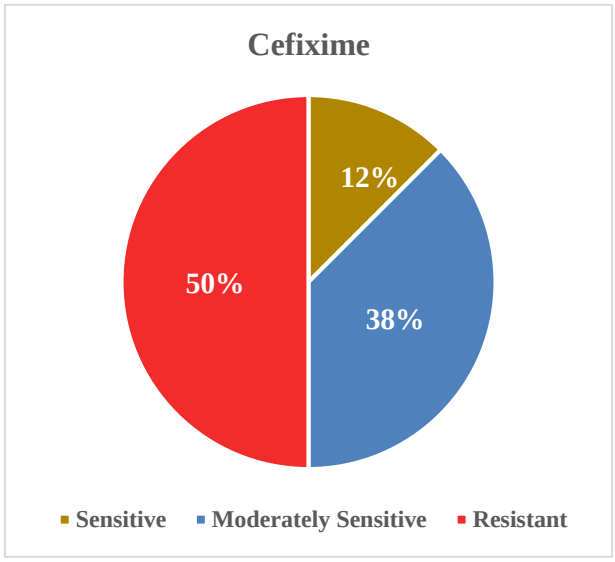


Figure 08: Pie chart representing the antibiotic resistant test result of Cefixime

Figure 08 is showing that Cefixime is 12% sensitive, 38% moderately sensitive and 50% resistant.

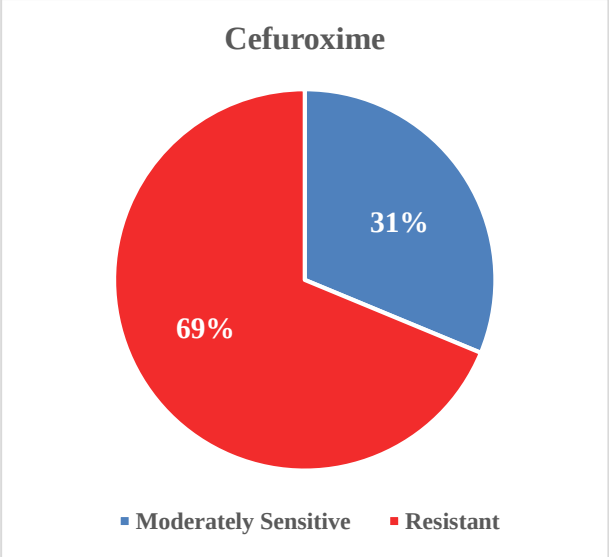


Figure 09: Pie chart representing the antibiotic resistant test result of Cefuroxime

Figure 09 is showing that Cefuroxime is 31% moderately sensitive and 69% resistant.

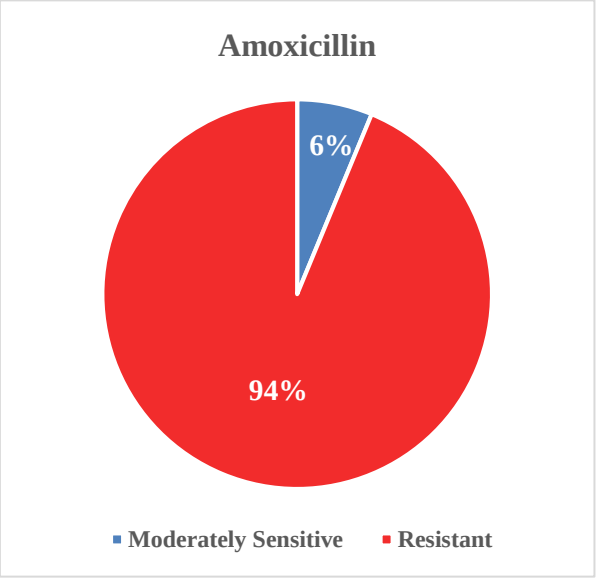


Figure 10: Pie chart representing the antibiotic resistant test result of Amoxicillin

Figure 10 is showing that Amoxicillin is 6% moderately sensitive and 94% resistant.

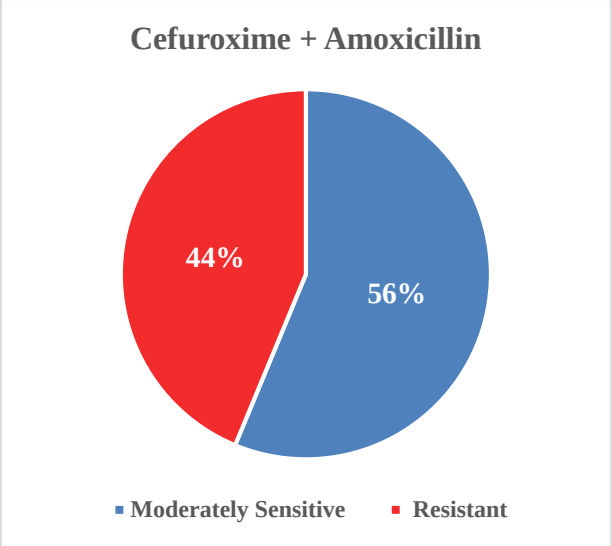


Figure 11: Pie chart representing the antibiotic resistant test result of Cefuroxime + Amoxicillin

Figure 11 is showing that out of 16 samples, 7 (44%) samples are resistant to both Cefuroxime and Amoxicillin.

Chapter 4

Discussion

The samples which were drinking water was collected from different restaurants, hotels and tea-stalls have the same water supplier. Most of the restaurants and hotels are directly connected and have a direct line of filtered water from the Water Supply and Sewerage Authority (WASA). WASA is the main department of Bangladesh administering the water supply, drainage and sanitation system. All 16 samples were tested for fecal coliform and total coliform test. For both tests, the result was zero. The previously same test was done by other students of the Department of Pharmacy, Brac University in Summer 2018 and Spring 2019. They have found a moderate to high level concentration of both fecal and total coliform count. However, in case of previous tests, the sampling sites and locations were different. The places were around Brac University premises and Mohakhali. The reason for this kind of result can be due to the sewerage system of different areas of Dhaka city as well as how the restaurants, hotels and tea-stalls personnel are providing the water to the consumer. Another reason can be the seasonal change in sample collection. This time samples were collected in the winter season whereas previous samples were collected in the spring and summer season. Rain is common during the summer season which can be a major cause of increased concentration of bacterial growth in samples. So, water samples which were collected from Shantinagar and Tongi are safer than water in Mohakhali and Brac University premises. An effectual measure for this could be restoring the pipes, daily monitoring of all water transporters and most notably and raising local awareness among the community. It is also necessary to identify and check companies that produce the dispensing water containers whether it is the jar or the main water that had been supplied that is polluted and also to test the dispenser to have a clear understanding of where the contamination is coming from. Any negligence in this regard could

be viewed as an impetus for water supplies pollution. Earlier the most contaminated water was around Brac University premises which is unfortunate for the students of Brac University. So, university authorities should have taken that in account so that they can provide safer water for their students.

In terms of ammonia, all the samples were below the Bangladesh standard which is a sign that WASA is providing safe water throughout the city.

However, in case of antibiotic resistance tests, this time the scale of resistance of antibiotics was higher. This can be due to the increment of usage of antibiotics in the community. Nowadays, antibiotic has been used in different places such as in farm, milk which is doing a great cause in antibiotic-resistant. In case of Shantinagar and Tongi, antibiotic-resistant was found higher than previously conducted study. So, people of these areas must be careful before drinking water from restaurants, hotels and tea-stalls. In order to better understand the safety of drinking water consumed by a significant number of people all over Shantinagar and Tongi area, all aspects should be considered equally.

Chapter 5

Conclusion

The significant of water is inexpressible in human life. The supplied filter drinking water to restaurants of Shantinagar and Tongi from WASA is showing a positive result in case of fecal coliform test, total coliform and ammonia concentration. All the 16 samples are within the range of both WHO and Bangladesh Standard. However, regarding antibiotic resistance test samples were showed alarming resistant result. The reason behind resistant in the pipe water supplied by WASA needs to be figure out as samples were showed a distressing result.

5.1 Future Work

Further work can be done by identifying the microorganism and their resistant pattern, changing the parameters of microbial test and changing the locations of Dhaka city in different seasons to obtain better outcome.

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