Effectiveness of Water Filters used in the households of Dhaka City

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
BS in Microbiology and Biotechnology

Department of Mathematics and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing a 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 that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all of the main sources of help.

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Abstract

This research aims to determine the microbiological quality of tap and filtered water and figure out the efficiency of filters used in the households of Dhaka city. To start, 51 individual samples of tap and filtered water from different areas were collected. The total Viable Count was done by spreading the samples on Nutrient Agar media. According to the study, the minimum TVC count for the tap water sample was 8.6×10^2 CFU/ml and the maximum was 3.4×10^5 CFU/ml, whereas the minimum TVC count for the filtered water sample was 5.7×10^2 CFU/ml and the maximum was 2.26×10^5 CFU/ml. The criterion for TVC is 1×10^3 CFU/ml, as stated by WHO and BDS (2009). WHO and BDS (2018) set 1×10^3 CFU/ml as the benchmark for TVC. Only 8 tap water samples and 15 filtered water samples were found to be within the range of WHO and BD standards based on the TVC values of all 51 samples. The Coliform count was performed after membrane filtration on MFC agar. In total, 51 tap water samples and 51 respective filtered water samples were spread over XLD and MacConkey agar plates. Among the samples, only 2 samples of tap water were not contaminated by *E. coli*, and only 4 samples of filtered water were not contaminated by *E. coli.* Next, different biochemical tests were performed to identify the organisms. After that, the research moved on to the Antibiotic Susceptibility Test with 12 different antibiotics. Afterward, a blood agar test and coagulase test were done to find out the pathogenicity of the organisms. These very tests were done to identify the pathogen more closely and precisely and to understand its species. All the suspected organisms showed Alpha hemolysis in the blood agar test, and 74% of organisms showed coagulase-positive results, which is alarming. Finally, to figure out the pathogenic organisms, PCR was performed. After the study of different types of filters, and testing the boiled water, it could be said that the boiled water showed less pathogenicity than some filters with high pathogenic rates.

Keywords: Water, Tap, Filter, Fecal coliforms, CFU/ml, Antibiotic Susceptibility, Pathogenicity

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List of Acronyms

NA: Nutrient Agar

Mac: Macconkey Agar

XLD: Xylose Lysine Deoxycholate Agar

EMB: Eosin Methylene Blue Agar

MHA: Mueller Hinton Agar

MIU: Motility Indole Urea

TSI: Triple Sugar Iron Agar

MR: Methyl Red

VP: Voges-Proskauer

FCC: Fecal Coliform Count

WHO: World Health Organization

mm: Millimeter

ml: Milliliter

µl: Microliter

e.g: For example

et al: And others

CFU: Colony Forming Unit

spp: Species

%: Percentage

ºC: Degree Celsius

TVC: Total Viable Count

TNTC: Too Numerous to Count

TFTC: Too Few to Count

ARB: Antimicrobial Resistant Bacteria

WASA: Water Supply & Sewerage Authority

Chapter 1

Introduction

1.1 Introduction

Dhaka is a city that is ancient among all other developing countries. The density of the population of this city is one of the most notable and alarming issues. This huge population has to utilize one of the most useful natural resources, which we call "Life", which is water. There is no doubt that the next connected shortcomings will provide quality and drinkable water access to this huge population. Needless to say, water is one of the most precious natural resources to keep us hydrated and refreshed. However, the condition of tap water and filter water is not up to par; rather, the presence of pathogenic microbes is huge in these water handles. According to a report from the World Health Organization in 2022, microbe contamination must be the primary concern for potential health concerns, which should not be avoided in any other way. More specifically, tap water is used in almost all household chores, such as cleaning utensils, washing meats, fish, fruits, vegetables, and so on. For this very reason, tap water must be pathogen free. It is a matter of sorrow that most of the tap water is unhygienic and contains pathogenic organisms, and it is nearly unusable. The World Health Organization also notes that fecal contamination is a prime cause of skin diseases and waterborne diseases. Added to that, fecal coliforms are said to be indicators.

The criteria for indicator microorganisms are

I. Indicator organisms should not originate from different sources.

II. They must not be able to multiply in water supplies or aquatic environments.

III. And they should be easily isolated from water samples.

Yet we can not say confidently that indicator organisms can fulfill the above checklists if one or two criteria match. This particular study was performed to understand the water quality of filters in Dhaka city, which are mainly used for household purposes.

Also, the comparison between tap and filter water from the same areas was measured. For this, first of all, the samples were collected aseptically from 51 households of 17 different thanas of Dhaka city. Next, the samples were cultured in different agar media for the isolation of fecal coliforms and other pathogenic organisms. Eventually, organisms that are isolated from the cultures go through biochemical identification. Different types of antibiotics are being consumed frequently by us, and for this reason, organisms are also becoming antibiotic resistant. To see the antibiotic sensitivity pattern of these organisms, an antibiotic sensitivity test was also done.

Finally, Polymerase chain reaction (PCR) was done to identify the strains of the organisms.

1.2 Water Microbes

Water microbes are high in terms of causing diseases like diarrhea, cholera, and so many other water-borne diseases. The most popular yet pathogenic microbe would be *Escherichia coli,* a rod-shaped pathogenic bacteria found in daily usable filters and tap water.

- ***Escherichia coli*:**

Escherichia coli (E. coli) bacteria usually live in the large intestines of humans and animals. Although *E. coli* are harmless and are an important part of a healthy human intestinal tract, some *E. coli* are pathogenic, meaning they can cause illness. Added to that, the types of *Escherichia coli* that can cause diarrhea can be transmitted through contaminated water or food, or contact with animals or people. *E. coli* consists of a diverse group of bacteria. Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic, they are Shiga toxin-producing *E. coli* (STEC), enterotoxigenic E. *coli* (ETEC), enteropathogenic E. *coli* (EPEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), and diffusely adherent *E. coli* (DAEC).

- ***Pseudomonas spp***:

The species *Pseudomonas* can be found in the environment. A Gram-negative bacteria that causes health risks to humans. In medical conditions, this bacterial species can be spread from hospitals, shabby places beside houses, and other worse environmental conditions. Some urinary tract infections are supported by *Pseudomonas*. In a medical journal, Iglewski (reference needed) noted that there are altogether 25 species of it.

- ***Shigella spp***

Shigella belongs to the family Enterobacteriaceae. A study conducted at the Cleveland Clinic (2022) found that symptoms of Shigella include bloody diarrhea and sometimes swallowing water while swimming.

- ***Salmonella spp***

Salmonella is a rod-shaped gram-negative bacteria mostly responsible for the typhoid disease caused by *Salmonella typhi*. During the monsoon, the symptoms of water-borne disease caused by *Salmonella spp.* are being seen. Ashurst et al. (2022) claimed that *Salmonella enterica serotype typhi* is generally contracted by ingestion of food or water that is contaminated with the excrements of humans that carry the organism and must survive the gastric pH barrier in the stomach before adherence in the small intestine.

- ***Klebsiella spp***

Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiella oxytoca,* and *Klebsiella granulomatis.* The author, *Podschun* (1998), stated that bacteria belonging to the genus *Klebsiella* frequently cause nosocomial infections in humans. In particular, the medically most important *Klebsiella* species is *Klebsiella pneumoniae,* which accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections

- ***Faecal Streptococci***

Fecal streptococci are marked as water microbes. They spread mostly through water if there is a fecal infection. To elaborate more, the human intestine, liver, and stomach are the most favorable areas for these pathogenic strains to grow, according to a study conducted by the United Kingdom-based water treatment solution company Lenntech.

- ***Staphylococcus aureus***

Staphylococcus aureus is a type of bacteria commonly found on the skin and hair, as well as in the noses and throats of people and animals. These bacteria are present in up to 25 percent of healthy people and are even more common among those with skin infections such as abscesses, respiratory infections such as sinusitis, eye, nose, or throat infections, and food poisoning. *Staphylococcus* can cause food poisoning when a food handler contaminates food and then the food is not properly refrigerated. Other sources of food contamination include the equipment and surfaces on which food is prepared. These bacteria multiply quickly at room temperature to produce a toxin that causes illness. A government organization for food safety argued over the fact that *Staphylococcus* is killed by cooking and pasteurization.

1.3 Antibiotic resistance

Antibiotic resistance is the examination of pathogens to determine if they are susceptible, intermediate, or resistant to antibiotics given upon prescription. According to Ventola

(2015), vancomycin was first used in 1972 to treat methicillin-resistant *Staphylococcus aureus* and coagulase-positive *Streptococcus*. The author also mentions that humans ingest antibiotics when they are in livestock. Frequent use of antibiotics is the main reason behind antibiotic resistance around the world. Antibiotics are the most popular drugs prescribed for the treatment of infectious diseases. Moreover, they might be lifesaving drugs. However, antibiotics are not optimally prescribed up to 50% of the time. Physicians sometimes prescribe antibiotics when they are not actually needed. A report published by the Centers for Disease Control and Prevention claimed that the spreading of resistant strains of bacteria from person to person or from non-human sources in the environment is the other major reason for developing antibiotic resistance.

1.4 Literature review:

Water pollution is one of the most dangerous factors for health issues. Water pollution happens mostly due to the free mixing of microbes in the water body. Another factor for waterborne disease is claimed to be climate change. In a literature review on the topic of "Waterborne disease and climate change," the author Lavy et al. (2018) an early recognition of climate change affecting diarrhea which estimates the effects of anthropogenic climate change. The author cited in Philipsborn et al (2016) projected that 800,000 cases of enterotoxigenic *Escherichia coli* in association with 2.2 million additional cases by the end of the century under future climate scenarios in Bangladesh, using a comparative risk assessment approach that is conducted in the context of Bangladesh.

1.5 Objectives of the Study:

The whole paper very precisely focuses on some particular key points. The main objectives of this research are:

1. Determine the microbiological quality of both tap water and filtered water in different areas of Dhaka city
2. Achieving the minimum contamination level of water where the water will no longer be unsafe to drink
3. To understand the susceptibility and resistance patterns of the pathogens that are found in water

Chapter 2

Materials and Methods

2.1 Materials

All investigations and experiments related to this project were performed in the Microbiology Laboratory of the Department of Mathematics and Natural Sciences, BRAC University, Mohakhali, Dhaka.

2.1.1 General Procedure and Equipment

By autoclaving at 121ºC for 15 minutes all media were sterilized in an autoclave. All glassware such as pipettes, Petri dishes, test tubes, etc. were sterilized at 160ºC for 1 hour in a hot-air oven before use. All agar media, agar slant, and biochemical reagents were prepared freshly and kept in a refrigerator at 3-4ºC except T_1N_1 culture media. It was stored at room temperature. All inoculations and subculturing were done under aseptic conditions in a laminar airflow cabinet. The inoculated cultures were incubated in the incubator at 37ºC and 44ºC. Accurate weights were measured by using an analytical balance. Optical density was taken by spectrophotometer.

A membrane filter unit was used for the isolation of fecal coliform from the samples.

2.1.2 Culture media

For total viable count (TVC), enteric pathogen, *Staphylococcus,* fecal coliform, and *Pseudomonas* different types of media were used. Media like Nutrient agar (NA), MacConkey agar, Xylose lysine deoxycholate (XLD), MFC agar, and Eosin-Methylene Blue (EMB) were used for bacterial isolation purposes. Details of these cultural media are as below:

- **Nutrient Agar (NA):** Nutrient Agar is used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for bacterial growth.
- **MacConkey Agar:** MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae. It also can distinguish between lactose fermenting from non-fermenting bacteria. After 24-48 hours at 37ºC of incubation period *E.coli* and *Klebsiella* will produce pink colonies. Bacteria that can not ferment lactose like *Pseudomonas aeruginosa, Salmonella* species, and *Proteus*

species will appear colorless on the medium and the agar surrounding the bacteria remains relatively transparent.

- **Xylose Lysine Deoxycholate (XLD):** Xylose lysine deoxycholate is used for the isolation of *Salmonella* and *Shigella*. After 24-48 hours at 37ºC incubation, the growth of *Salmonella* will give black-centered colonies, red-colored colonies for *Shigella,* and yellowish-white colored for *E.coli.*
- **MFC Agar:** The MFC agar method is a one-step membrane-filtration method for the enumeration of fecal coliform. Agar plates are incubated at 44.5°C for 22-24 hours. Fecal coliform gives blue colonies after incubation.
- **EMB (Eosin Methylene Blue):** This media can differentiate between lactose fermenters and lactose non-fermenters bacteria. In the case of lactose fermenters such as *E.coli*, the colonies will be blue/black with a metallic green sheen, and for lactose non-fermenters colorless and transparent colonies will be obtained. Other coliform such as *Enterobacteraerogenes* can also ferment lactose and grow on EMB media. They will give thick mucoid pink-colored colonies.
- **T_1N_1 Agar:** For quality control, method validation, and research, stock cultures must be properly maintained. Subculturing the same colonies multiple times may eventually result in contamination, a loss of viability, and changes to the genotype or phenotype. (www.rapidmicrobiology.com). T_1N_1 agar media is used to stock bacterial colonies that are found in the samples.
- **Mueller-Hinton Agar:** Mueller-Hinton Media is used for the antibiotic susceptibility test of bacteria.

2.1.3 Biochemical test media

- **Simmon's citrate agar:** Simmons citrate agar tests were used to observe the ability of organisms to utilize citrate as a carbon source. If the medium turns blue, the organism is citrate-positive. If there is no color change, the organism is citrate-negative. (http://www.austincc.edu)
- **Blood agar:** It is used to see the lysis of blood cells by organisms. Lysis are three typesα-hemolysis, β-hemolysis, and γ-hemolysis. By observing clear zone hemolysis is determined.
- **TSI (Triple Sugar Iron) agar:** Triple sugar iron agar is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and

pH indicator phenol red. It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates.

- **MIU (Motility, Indole, Urease) agar:** This is best described as a multi-purpose medium for the differentiation of *Enterobacteriaceae* that combines three individual tests into a single medium. It is used to see whether the organisms are motile or not and if they can produce indole and can utilize urease or not.
- **MR (Methyl Red) broth:** Methyl Red test determines the microbe performs mixed acids fermentation when glucose is supplied. After the addition of methyl red reagent to the culture media, if it turns pink then it is a positive result and if it remains yellow then it is a negative result.
- **VP (Voges-Proskauer) broth:** This test is used to detect acetoin in a bacterial broth culture. The test is performed by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth which has been inoculated with bacteria. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result.

2.2 Methods

2.2.1 Samples Collection

Fifty-one different tap water and filter water samples were collected from fifty-one different households in seventeen different places in Dhaka city. Samples were collected aseptically (rinsed with the water sample) in autoclaved plastic bottles during different seasons. Bacterial growth may occur in taps so it is good practice to disinfect the tap with alcohol. Water should be allowed to run through the tap for several seconds to flush out any contamination within the tap. Filter water should be collected in the same manner. All samples should be brought to the laboratory for culture as soon as possible.

2.2.2 Sample Processing and Inoculation

After the collection of the sample, it was processed and inoculated in the culture media as soon as possible. First of all, the tap water sample was transferred to a sterile conical flask, and from that it was serially diluted in sterile physiological saline up to 10 times. Then to all the media used in this study, a 100 µl sample was added to the media. Next, a sterile glass spreader was used to spread the sample thoroughly The spreader was burnt before spreading the sample. Ethanol containing the breaker where the spreader was kept was away from the

burner. Each medium was inoculated by this method except MFC media. For fecal coliform count MFC agar was used and the membrane filtration method was followed.

2.2.3 Sample spreading

Equipment: Vortex machine, micro-pipette, spreader, test tube, physiological saline solution, spirit lamp, 70% alcohol

Media: Nutrient agar, MacConkey agar, XLD agar

Sample: Water sample collected from tap and filter

Procedure:

I. At first, the hand was cleaned with 70% ethanol and all media plates were labeled by a glass marker.

II. One milliliter raw sample of tap water was taken in a test tube containing 9 ml physiological saline (0.9%NaCl solution). Then it was mixed well by vortexing and labeled as dilution 10^{-1}.

III. Now by a 1 ml micropipette, a 100 µl sample was taken from 10^{-1} diluted tap water and spread on a nutrient agar plate by a spreader. Before spreading, the spreader was burnt into the burner to avoid contamination.

IV. For filter water samples, the same technique was followed.

V. Samples were spread by the same process on MacConkey agar, and XLD agar.

VI. Next, the plates were incubated for 24-48 hours at 37°C.

VII. After the incubation period, the results were observed and recorded.

2.2.4 Membrane filtration

For fecal coliform count membrane filtration technique was done.

Equipment:

Sterile millipore membrane apparatus (base, funnel, and clump)

One-liter suction flask

Sterile millipore membrane filter

Small beaker of 95% alcohol

Forceps

Spreader

Sample: Water samples collected from tap and filter.

Media: M-FC agar plates

Procedure:

1. M-FC agar plates were labeled for FCC (Fecal coliform count).
2. The sterile paper-wrapped membrane filter unit was assembled as follows:
   I. The centered glass filter base was unwrapped and inserted into the neck of a 1-liter side arm suction flask.
   II. A sterile membrane filter disc grid side up, on the center glass platform, was placed with sterile forceps.
   III. The funnel suction of the apparatus on top of the filter disc was unwrapped and placed carefully. By using a filter clamp the funnel to the filter base was secured.
   IV. A rubber hose was attached from the sidearm of the vacuum flask to a vacuum source.
3. One hundred milliliters of water sample was added through the funnel and the vacuum was started.
4. After the filtration of the entire sample, the inner surface of the funnel was washed with 10 ml of sterile water.
5. Then, the vacuum was disconnected, the filter assembly was unclamped, and the membrane filter was removed carefully with sterile forceps. Then it was placed on the surface of the medium labeled as FCC (The grid side was kept up).
6. Plates were incubated for 24-48 hours at 44.5ºC.
7. After the incubation period results were observed and recorded.

2.2.5 Stock culture method

T_1N_1 Agar media was used to stock bacteria that were found in the samples. T_1N_1 media was preserved at room temperature after the growth of bacteria. First, the bacterial colony will be taken by a needle from 24 hours of subculture plate. Subculture should be done in the nutrient agar plate. Then bacterial colonies were inoculated by stabbing and incubated for 24 hours. After 24 hours if growth was observed then 200 µl paraffin oil was added to the T_1N_1 agar. Now this stock culture can keep for several months at room temperature.

2.2.6 Biochemical test methods

Different types of unknown bacteria were found after spreading the sample on different types of agar plates. For the identification of those unknown bacteria, different types of biochemical tests were performed. They are as below:

- **Gram-staining:** At first, clean glass slides were obtained. Using a sterile technique smears of the organisms were prepared. The smear was allowed to air dry and then heat fixed. The smear was flooded with crystal violet for 1 minute. Then the smear was gently washed with tap water. Then the smear was gently flooded with Gram's iodine mordant for 1 minute. After that, the smear was gently washed with tap water again. In the next step, the smear was decolorized with 95% ethyl alcohol. The reagent was added 10 drops for 10 seconds until crystal violet failed to wash from the smear. Later the smear was gently washed with tap water. After that smear was counterstained with safranin for 45 seconds. Again the smear was gently washed with tap water. Finally, the slide was air-dried and examined under a microscope with oil immersion. Under the microscope if the bacterial cells appear pink then it is gram-negative and if they appear purple then gram-positive. Shapes of the cells can be different types like rods, cocci, spiral, bacilli, etc.

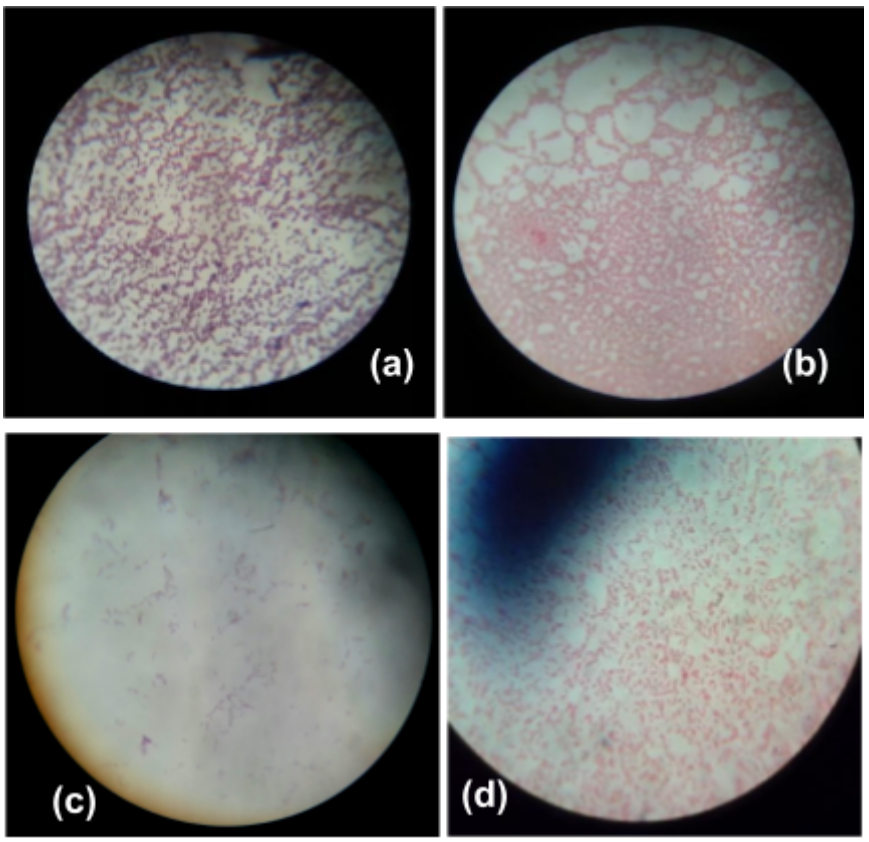


Figure 2.1: (a) Gram-positive cocci, (b) Gram-negative cocci, (c) Gram-positive rods, (d) Gram-negative rods

- **Oxidase test:** During aerobic respiration, Oxidase enzymes play an important role in the operation of the electron transport system. The oxidase test is used to identify bacteria that produce cytochrome oxidase, an enzyme of the bacterial electron transport chain. At first, enough quantity of bacteria from the nutrient agar plate was picked and placed on a piece of filter paper. One drop of the oxidase reagent (Tetramethyl-p-phenylenediaminedi-hydrochloride) was added at the place of the culture on the filter paper and mixed with a sterile toothpick. A positive reaction turned the bacteria from violet to purple within 20 seconds. No such coloration indicates a negative result.

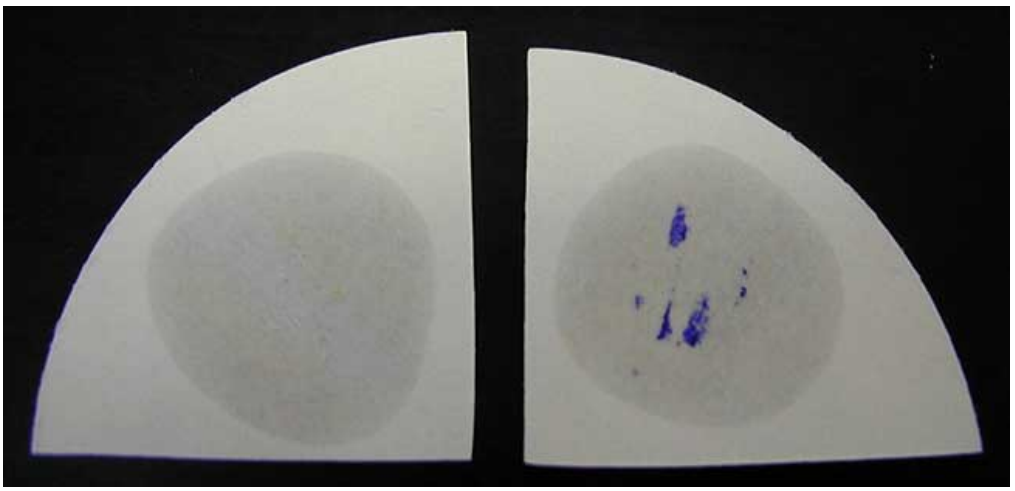


Figure 2.2: Oxidase test; the Left one is for negative result and the Right is for positive

- **Catalase test:** The catalase test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. At first, the inoculating loop was flamed and cooled. Then a small bacterial colony (18 to 24 hours old) was placed on a clean glass slide. The inoculating loop was flamed and cooled again. One or two drops of 3% hydrogen peroxide (H_2O_2) were added. A positive result gave a rapid evolution of oxygen within 5-10 seconds and was evidenced by a bubbling reaction. A negative result showed no bubble.

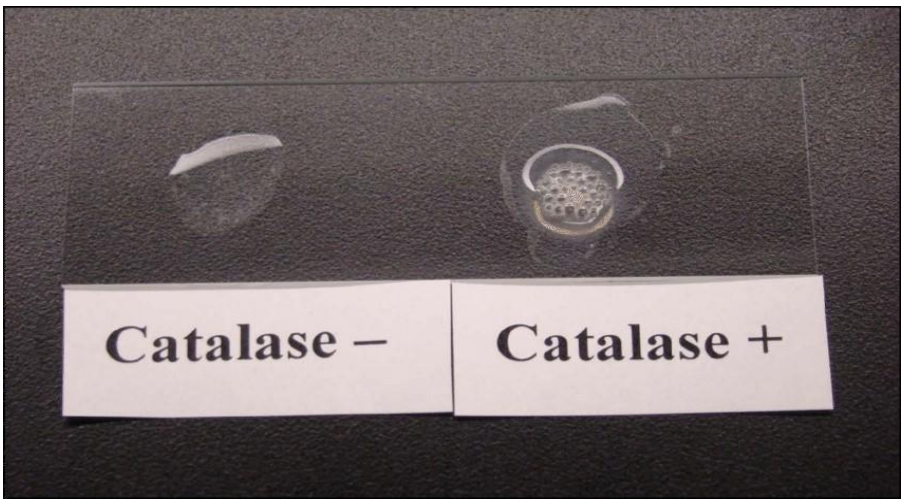


Figure 2.3: Catalase test

- **TSI (Triple Sugar Iron) test:** TSI media was used for determining whether bacteria can ferment glucose, and lactose or they can produce Hydrogen Sulfide or other gas.

This will help to differentiate between various *Enterobacteriaceae* including intestinal pathogens *Salmonella* and *Shigella*. TSI media have two parts one is a butt with a poorly oxygenated area at the bottom and the other is a slant with a well-oxygenated space at the top.

Interpretation of Triple Sugar Iron Agar Test:

i. If lactose or sucrose is utilized, then a huge amount of acid production is indicated by changing the color from red to yellow.

ii. If gas is produced, then there will be a crack in the media.

iii. If H_2S is produced, then there will be black precipitation.

iv. If lactose is not fermented but a small amount of glucose is fermented, then the butt will be Yellow and the slant will be Red.

v. If neither glucose nor lactose nor sucrose is fermented then both butt and slant will remain red.

vi. If Ammonia is produced, then the slant can become a deeper red-purple.

Equipment: Bunsen burner, inoculating loop

Procedure:

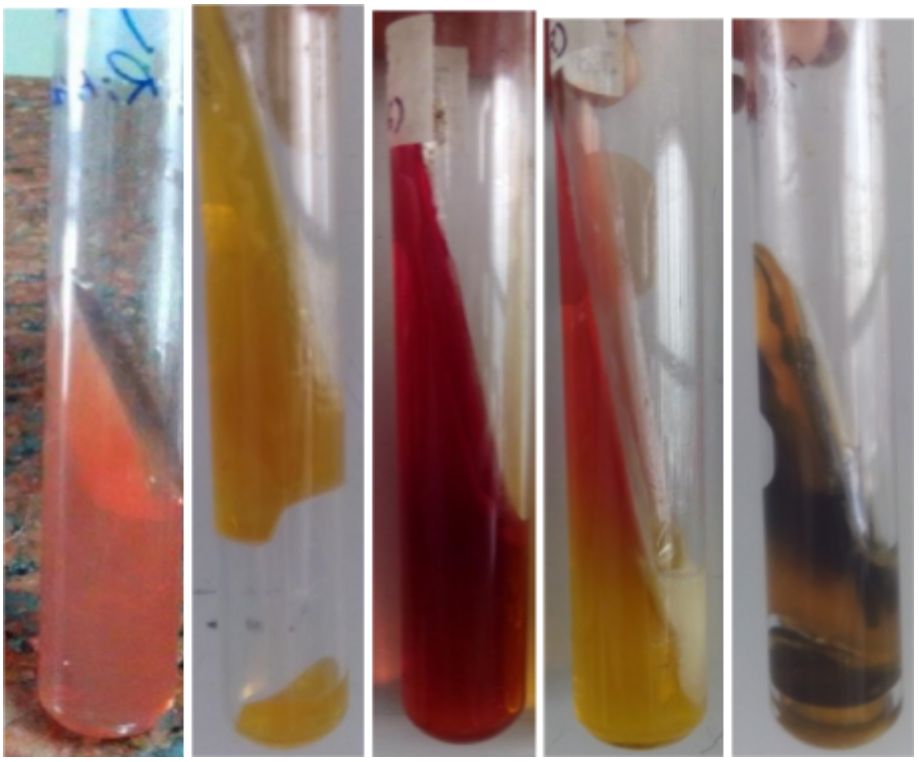
1. The TSI tubes were labeled using a marker.
2. Using a sterile technique, each experimental organism was inoculated into its appropriately labeled tube using stab and streak inoculation (inoculated by stabbing into the agar butt with an inoculating wire and streaking the slant in a wavy pattern).
3. The tubes were incubated for 24 to 48 hours at 37°C.
4. After the incubation period results were observed and recorded.

The expected results of the TSI agar test are:

- Red slant/red butt/no black color: No fermentation and no H_2S formation
- Red slant/black butt: No lactose fermentation but H_2S production
- Red slant/yellow butt: No lactose fermentation but glucose fermentation, noH_2S production
- Yellow slant/yellow butt with black precipitation: Lactose/sucrose/glucose fermentation

and H_2S production

- Yellow slant/yellow butt/media cracking: Lactose/sucrose/glucose fermentation and gas production.

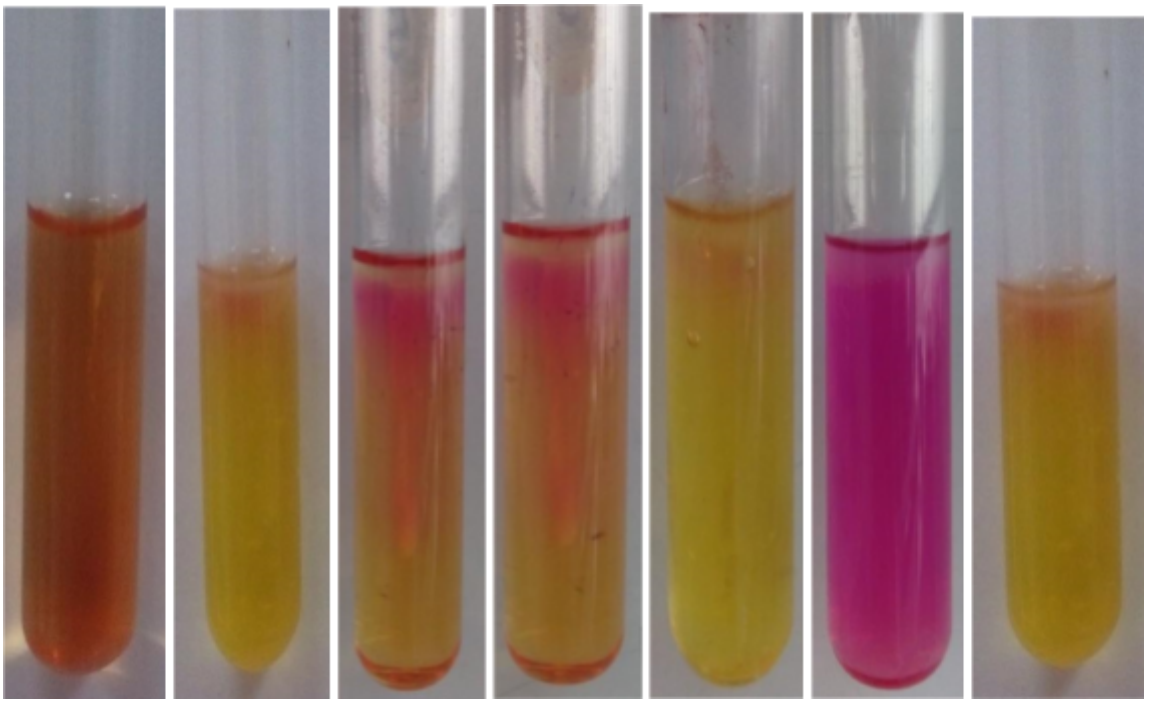


(a) **(b)** **(c)** **(d)** **(e)**

Figure 2.4: (a) Un-inoculated, (b) Lactose/ sucrose/ glucose fermentation and gas production but no H_2S, (c) No fermentation, no gas, and no H_2S production, (d) No lactose fermentation but glucose fermentation, no H_2S production, (e) Lactose/ sucrose/ glucose fermentation and H_2S production

- **MIU (Motility, Indole, Urease) test:** It is used to see whether the organisms are motile or not. If the organisms can produce indole and can they utilize urease or not. For this test MIU agar media was first prepared. Then by a sterile needle bacterial colonies were taken and inoculated by a single stab. After that, it was incubated at 37ºC for 24 hours. After the incubation period by adding reagent results were observed and recorded. For motility, if hazy growth was observed around the stab line or throughout the media then it is positive for motility test. If the media was transparent and no hazy growth was observed, then it is a negative result for the motility test. For the indole test result, 5 drops of Kovac's reagent were added and waited for the result. The formation of a pink to cherry red color ring indicates a

positive result. On the other hand, if the media remain yellow then it is a negative result. For the urease test, if the media becomes deep pink color from orange yellow color after 24 hours of incubation then it is a positive result. If no color change occurs, then it is a negative result.



(a) **(b)** **(c)** **(d)** **(e)** **(f)** **(g)**

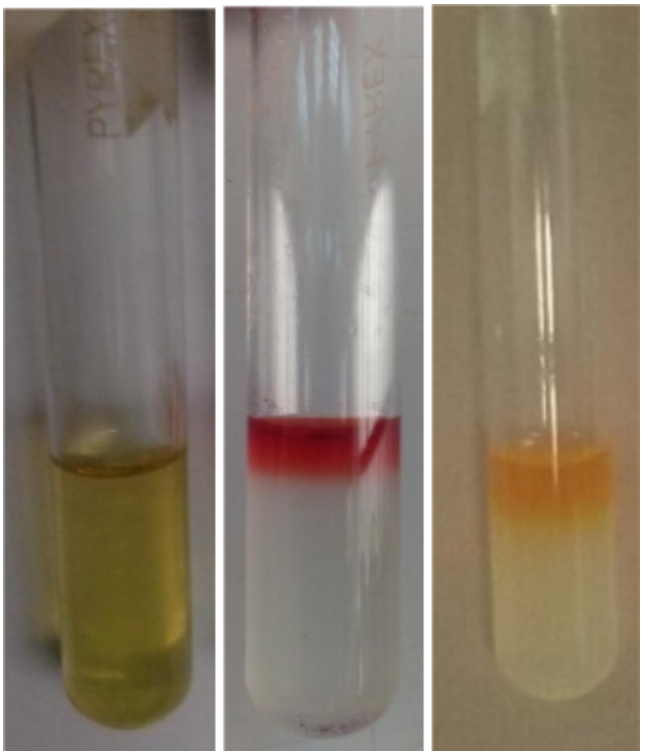
Figure 2.5: MIU test result, (a) Control (b) Motility positive (c) Motility negative (d) Indole positive (e) Indole negative (f) Urease positive (f) Urease negative

- **Simmon's citrate test:** This test was done to differentiate among enteric organisms based on their ability to ferment citrate as the sole source of carbon and energy. Citrate is used by microorganisms as a carbon source for their energy when there is no easily fermentable glucose or lactose. This can be possible if that organism contains citrate permease. For this test bacterial culture was obtained from a 24-hour subculture plate. Sterile citrate agar media was taken and using a marker it was labeled. Then using an inoculating needle and aseptic technique, a bacterial colony was taken. The needle was inserted into the butt of the citrate and as the needle was pulled out of the butt, the slant was streaked in a zigzag manner. Next, it was incubated for 24 to 48 hours at 37°C. After the incubation period, the simmon citrate slants were taken out of the incubator and observed for color changes. The Prussian

blue color of the agar indicates positive results and the green color indicates a negative result.

Figure 2.6: Citrate test results; Green one is a negative result, and Blue ones are the positive results

- **Methyl Red (MR) test:** Methyl red test detects the ability of an organism to produce and maintain stable acid end products from glucose fermentation. In this test methyl red is the pH indicator which detects the presence of a large concentration of acidic end products. For performing this test fresh 24-hour bacterial subculture was used. One sterile tube of MR broth was taken. Then, using a marker it was labeled. With an inoculating loop and ascetic technique, a loop full of bacterial culture was transferred into the MR broth. It was assured that the loop was shaken and touched to the side of the tube to remove excess broth. Next, this tube was placed in the incubator for 24 to 48 hours at 37°C. After incubation, inoculated MR broth was taken out of the incubator. Five drops of methyl red indicator were added to the tube and rolled between the hand palms to mix. The red color rings indicated positive results for the MR test and the yellow color ring indicated negative results.

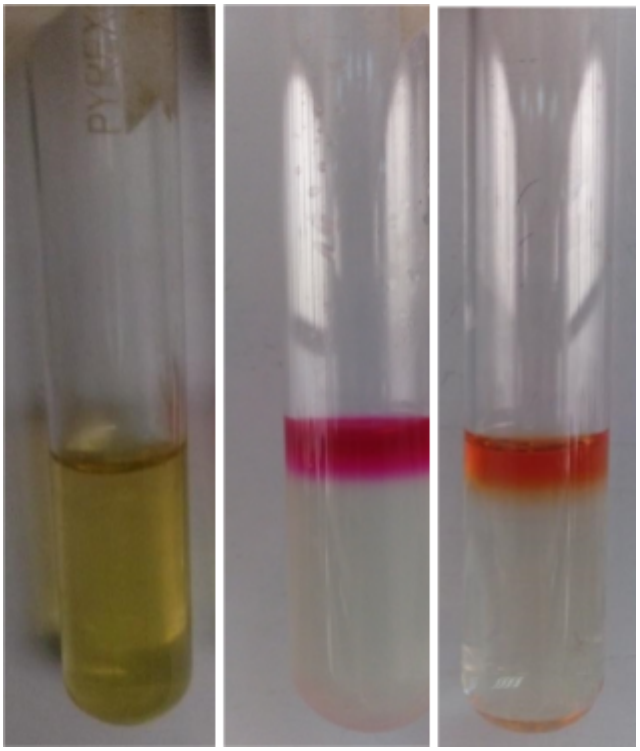


(a) **(b)** **(c)**

Figure 2.7: (a) Un-inoculated MR broth (b) Positive result for MR test (c) Negative result for MR test

- **Voges-Proskauer (VP) test:** VP test detects some organisms that produce neutral or non-acidic end products like acetyl methyl carbinol, from organic acids that result from glucose metabolism. When 40% KOH and 5% alpha-naphthol are added to the solution which is known as Barritt's reagent, it reacts with the acetoin produced by the bacteria in the solution. This reaction will produce diacetyl and a deep rose/pink color in the medium within 30 minutes after the addition of the reagent which is a positive VP test. On the other hand, yellow or the absence of rose/pink color represents a negative result. For performing this test fresh 24-hour bacterial subculture was used. One sterile tube of VP broth was taken. Then, using a marker it was labeled. With an inoculating loop and ascetic technique, a loop full of bacterial culture was transferred into the VP broth. It was assured that the loop was shaken and touched to the side of the tube to remove excess broth. Next, this tube was placed in the incubator for 24 to 48 hours at 37°C. After incubation, inoculated VP broth was taken out of the incubator, and 10-14 drops of Barritt's reagent were added to the tube. The tube was shaken gently for several minutes and waited for 15-20

minutes for color change. Rose/pink color formation is a positive result and yellow or no rose/pink color formation is a negative result.



(a) **(b)** **(c)**

Figure 2.8: (a) Un-inoculated VP broth (b) Positive result for VP test (c) Negative result for VP test

2.2.7 Pathogenicity test

Blood Agar test: This test provides information on what hemolytic enzymes a bacterium possesses. By providing a culture medium enriched with red blood cells, it is possible to determine whether a bacterium can destroy the cells and whether it can break down the hemoglobin inside. To perform this test, 24 hours of an inoculum from a pure culture was streaked on a sterile plate of blood agar. The blood agar was made in the laboratory using human blood. The inoculated plate was incubated at 37ºC for 24 hours. After the incubation period, plates were observed and the result was taken. After 24 hours of incubation, the medium was inspected for telltale signs of alpha- or beta-hemolysis. If the medium is discolored or darkened after growth, the organism has demonstrated alpha-hemolysis. If the medium has been cleared of undergrowth, the organism is beta-hemolytic. No discernible change in the color of the medium constitutes gamma-hemolysis (Libretexts, 2020).

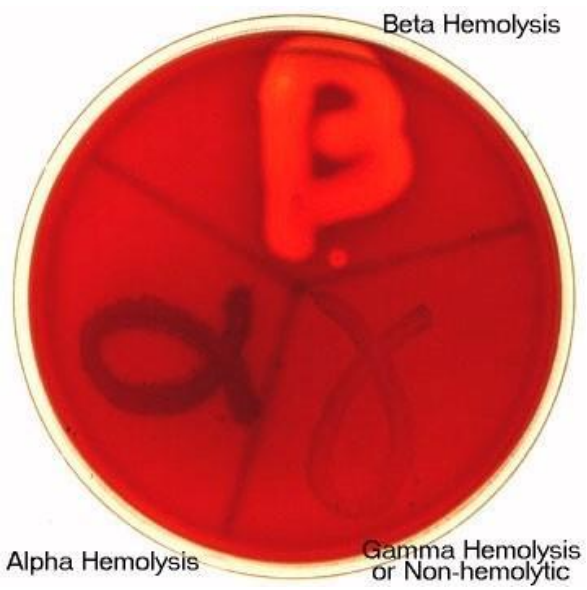


Figure 2.9: Test for hemolysis: α-hemolysis, β-hemolysis, and γ-hemolysis

Coagulase test: The coagulase test is a biochemical test that is performed to differentiate between coagulase-positive *Staphylococcus aureus* and coagulase-negative *Staphylococcus CONS*. Coagulase is an enzyme that is generally produced by *S. aureus*. *Staphylococcus aureus* produces this coagulase that converts the soluble fibrinogen in plasma to insoluble fibrin. *Staphylococcus* produces two forms of coagulase bound and bound-free. Since there are 2 kinds of coagulase enzymes - bound and free, there are 2 different tests that can be used to identify these enzymes: tube test and slide test. Both of the enzymes activate fibrinogen in plasma, in different ways. A slide test was done in the lab for results. Human plasma was used for this test.

Procedure:

I. A 1-inch diameter circle was made on a clean glass slide using a marker.

II. Two drops of plasma were laced into the circle, using a wooden pick or a clean loop.

III. A heavy inoculum was added and emulsified in the plasma (should be milky-looking).

IV. Fibrin threads form between the cells, causing them to agglutinate, or clump.

V. There will be a visible clumping of cells within 10-15 seconds.

VI. This test is for the bound coagulase enzyme.

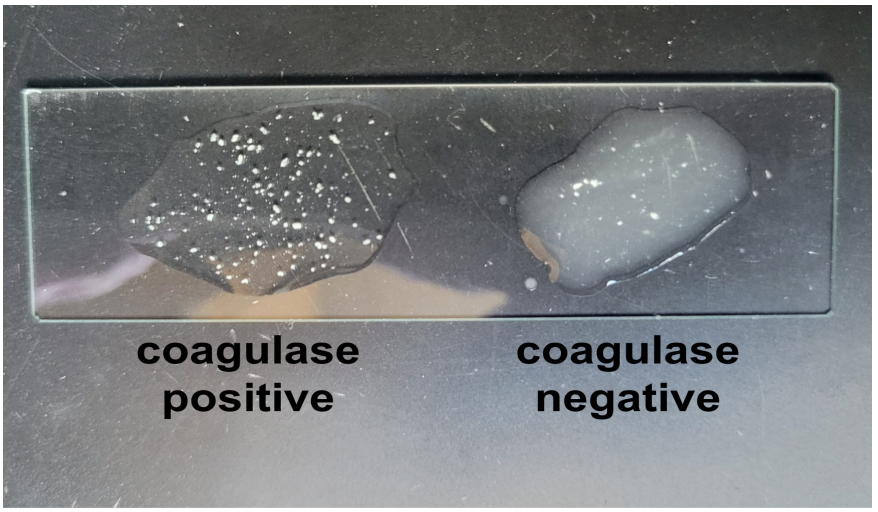


Figure 2.10: Coagulase test result

2.2.8 Disk diffusion method for antibiotic susceptibility test

Mueller Hinton Media is used for the antibiotic susceptibility test of bacteria. For the antibiotic susceptibility test first off all MacFarlane solutions were made. (www.microbiologyinfo.com)

Equipment: Antibiotic disks, forceps, burner, OD (Optical density) machine, vortex machine, loop, cuvette, saline solution, sterile cotton stick.

Media: Mueller Hinton agar

Sample: Bacteria collected from tap water

Procedure:

1. One to two specific bacterial colonies were taken by a sterile loop from 24 hours of fresh subculture plate. Then it was inoculated into 0.9% NaCl solution (physiological saline) and mixed by vortexing.
2. Next, the turbidity of the saline solution was compared with the MacFarlane solution. Turbidity was observed by the OD machine at 360 nm. If the turbidity of the saline solution and MacFarlane solution becomes the same then this saline solution containing bacteria can be used for the test.

3. After taking turbidity, a cotton swab was dipped into the turbid saline solution, and the bacterial lawn was made on Muller Hinton agar media.
4. Through sterile forceps, specific antibiotics were placed on the inoculated agar media, and disks were slightly pressed on the agar to place them well.
5. Then inoculated plates were incubated at 37ºC for 24 hours.
6. After the incubation period plates were observed and results were recorded.
7. Results were taken by observing and measuring the diameter of the clear zone around the antibiotics disks. According to the diameter of the clear zone, it was determined whether the organisms were susceptible, intermediate, or resistant to antibiotics. No clear zone also indicates resistance to the antibiotic.

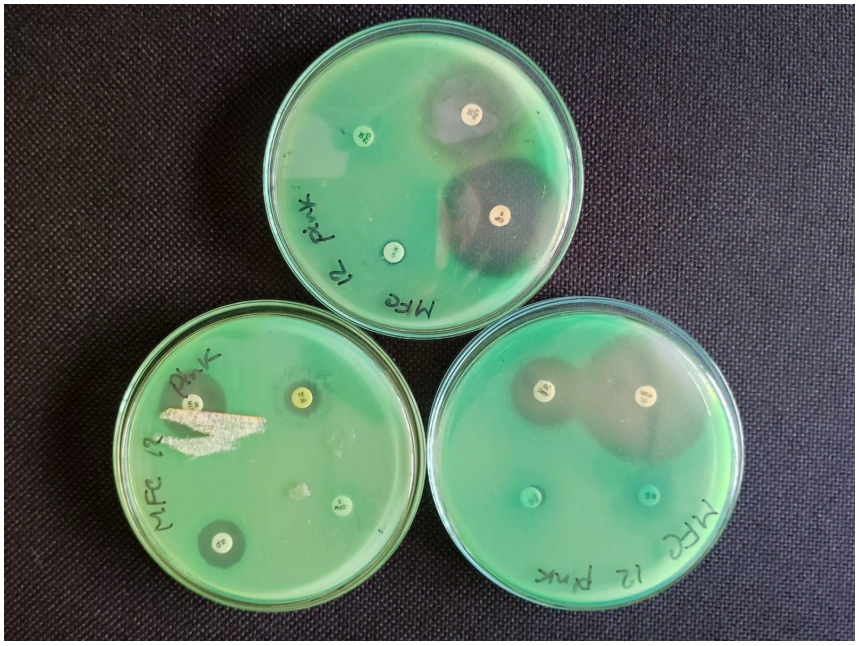


Figure 2.11: Antibiotic susceptibility test: Clear zone indicates susceptibility to antibiotics and no clear zone indicates resistance to the antibiotic

In this study, different types of antibiotics were used to see the antibacterial pattern of the colonies isolated from tap water. The list of antibiotics and their sensitivity level are given below in Table 1,

Table 1: Name of antibiotics and their sensitivity level

Antibiotics Name	Sensitivity level (mm)		
	Resistant	Intermediate	Susceptible
Ampicillin (10µg)	≤ 13	14-16	≥ 17
Amoxicillin (30µg)	≤ 27	28-36	≥ 37
Ceftriaxone (30µg)	≤ 24	25-26	≥ 27
Ceftazidime (30µg)	≤ 14	15-17	≥ 18
Ciprofloxacin (5µg)	≤ 15	16-20	≥ 21
Amoxyclav (30µg)	≤ 12	13-17	≥ 18
Cefixime (5µg)	≤ 15	16-17	≥ 18
Vancomycin (30µg)	≤ 10	11-15	≥ 16
Doxycycline (30µg)	≤ 12	13-15	≥ 16
Colistin (10µg)	≤ 12	13-14	≥ 15
Meropenem (10µg)	≤ 13	14-15	≥ 16
Azithromycin (30µg)	≤ 13	14-18	≥ 19
Kanamycin (5µg)	≤ 12	13-14	≥ 15
Tetracycline (30µg)	≤ 14	15-18	≥ 19

2.2.9 PCR and Gel Electrophoresis

A polymerase chain reaction is an in vitro method to produce a million copies of specific DNA sequences by amplifying it in less than two hours while gel-electrophoresis is the method to analyze DNA and RNA strands by separating the genetic material by size. After the pathogenicity and antibiotic susceptibility test, PCR and gel electrophoresis were performed.

Materials:

Sample: DNA extracted from suspected colonies

Reagents:

- Nuclease-free water.
- PCR master mix.
- Set of Primers: Forward and Reverse.

Equipment:

- PCR machine.
- Dry-ice.
- Micropipettes along with tips.
- PCR tubes.

Procedure:

I. Using a micropipette, 6µL of nuclease-free water was transferred inside a PCR tube.

II. 10 µL of PCR master mix was then transferred inside the tube.

III. After that, 2 µL of forwarding and 2 µL of reverse primer specific for the suspected gene were added inside the tube.

IV. 5 µL of previously isolated DNA samples were then added into the same tube.

V. After that, the PCR machine was programmed for maintaining the following conditions:

- 95 °C for 5 minutes for initialization.

- 95 °C for 1 minute for denaturation.
- 60°C for 1 minute for primer Annealing.
- 2°C for elongation for 60 seconds.
- 72°C for final elongation for 5 minutes.
- The number of cycles: 30.

VI. The tubes were then placed inside the PCR machine and the lid was closed.

VII. After completion of PCR, the tubes containing amplified DNA were stored at -20°C until further use.

VIII. For confirmatory results, Agarose gel Electrophoresis would be performed.

Figure 2.12: PCR products in tubes

Chapter 3

Results

3.1 Isolation of the organisms

Both tap and filter water samples were collected from the same household. The water samples were collected from different areas of Dhaka. To observe the microbial loads of different organisms, different types of selective, non-selective, and differential media were used. For isolation purposes, water samples were incubated on Nutrient agar plates, MacConkey agar plates, XLD agar plates, MFC agar plates, and EMB agar plates by spread plate and streak plate techniques.

After 24–48 hours of incubation, the results were taken and recorded.

3.2 Morphology and interpretation of the unknown organisms

Different organisms form different (color and shape) colonies in different agar media. Nutrient agar was used for the total viable count. In the same way, MacConkey agar was used for the coliform count, XLD was used for *Shigella*, *Salmonella, and E. coli* detection, MFC was for the fecal coliform count, and EMB was for *E. coli* detection.

On nutrient agar, after plating, white, off-white, orange, yellow, purple, and light green colonies were observed. On the MacConkey agar plate, deep pink (*E. coli*), light pink (*Klebsiella spp.),* and whitish pink colonies were observed. Yellow (*E. coli*), red with black centered (*Salmonella* spp.), and transparent colonies were observed on XLD media. Light to deep blue (Fecal coliform) colonies were found on MFC media. Metallic green sheen (*E. coli*), pink (Non-lactose fermenting), blue-pink, purple-pink, brown-pink (Enterobacter aerogenes), and pinkish mucoid (*Pseudomonas aeruginosa*, *Proteus spp., Salmonella spp., and Shigella spp.*) colonies were observed on EMB agar.

Results were recorded according to their colony morphology in different agar media,

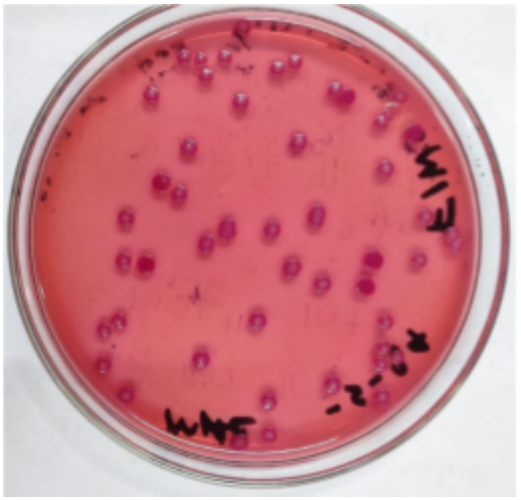


Figure 3.1: Mac Media plate

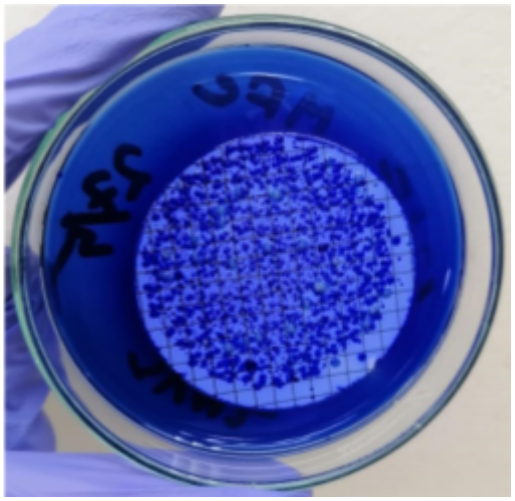


Figure 3.2: MFC Media plate

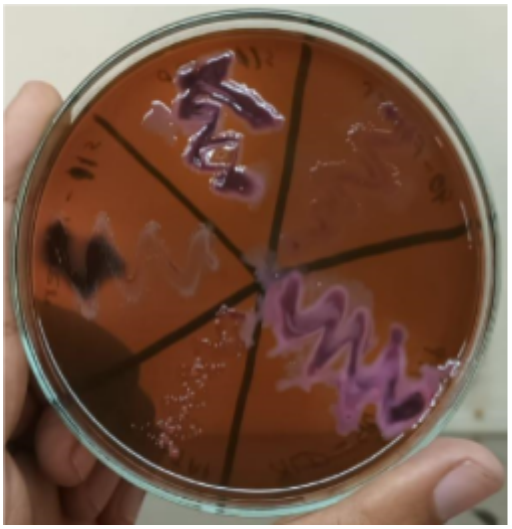


Figure 3.3: EMB Media plate

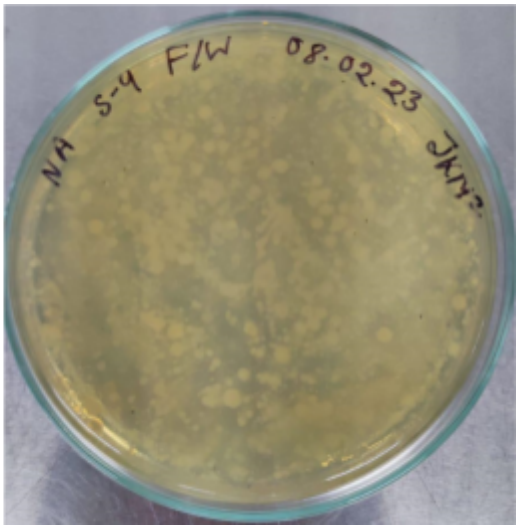


Figure 3.4: NA Media plate

Figure 3.5: XLD Media plate

3.3 Microbial load of tap water of Dhaka

As part of Dhaka, tap water samples were collected from Mohakhali, Agargaon, Hazaribagh, Dhanmondi, Azimpur, Moghbazar, Shyamoli, Rayerbazar, Tejgaon, Chawk Bazar, Zigatola, Bongshal, Mohammadpur, Kalabagan, Rampura, Banani, Gulshan. After the sample processing, sample inoculation and incubation results were observed and recorded in a chart. From the results, it was found that the microbial load of organisms varied from one area to another. According to Table 2, the total coliform count of tap water in Dhaka varied from one area to another. Contamination by fecal coliform was noticeable. Tap water samples showed significant growth both in XLD and MacConkey agar.

Here in figure 3.6, showed that water samples of almost all areas *E. coli, S. aureus, P. aeruginosa, Salmonella spp., Pseudomonas spp., Klebsiella spp., E. faecalis,* and *Shigella* were present

Based on the results, the microbial load of Tap water in Dhaka is recorded below,

Table 2: Microbiological status in tap water, recorded from XLD, Mac, MFC and NA media, unit CFU/ml except for Fecal coliform count in MFC in CFU/100ml

Area	XLD CFU/ml	MaC CFU/ml	Fecal coliform /100ml	Total coliform CFU/ml	Total viable count (TVC)
Mohakhali	0.42×10^{2}	0.66×10^{2}	1.58×10^{4}	4.27×10^{2}	3.2×10^{4}
Agargaon	0.63×10^{2}	0.73×10^{2}	1.59×10^{4}	3.44×10^{2}	3.9×10^{4}
Hazaribagh	1.97×10^{2}	0.09×10^{2}	0.29×10^{4}	2.35×10^{2}	2.4×10^{4}
Dhanmondi	3.40×10^{2}	3.01×10^{2}	0.98×10^{4}	1.98×10^{2}	2.6×10^{4}
Azimpur	5.37×10^{2}	4.46×10^{2}	0.01×10^{4}	3.96×10^{2}	3.4×10^{5}
Moghbazar	0	1.78×10^{2}	0	4.33×10^{2}	1.7×10^{4}
Shyamoli	0.25×10^{2}	1.89×10^{2}	0.3×10^{4}	0.40×10^{2}	2.03×10^{4}
Rayerbazar	2.14×10^{2}	1.54×10^{2}	0.96×10^{4}	4.07×10^{2}	1.7×10^{5}
Tejgaon	0.46×10^{2}	0.35×10^{2}	2.71×10^{4}	4.10×10^{2}	5.06×10^{4}
Chawk Bazar	2.67×10^{2}	1.78×10^{2}	2.35×10^{4}	4.44×10^{2}	1.42×10^{4}
Zigatola	0.44×10^{2}	2.76×10^{2}	0.04×10^{4}	0.04×10^{2}	1.35×10^{4}
Bongshal	0.1×10^{2}	1.76×10^{2}	2.29×10^{4}	4.18×10^{2}	1.79×10^{4}
Mohammadpur	3.89×10^{2}	2.42×10^{2}	2.68×10^{4}	3.98×10^{2}	5.7×10^{4}
Kalabagan	3.65×10^{2}	4.10×10^{2}	2.41×10^{4}	4.17×10^{2}	1.08×10^{4}
Rampura	3.04×10^{2}	2.62×10^{2}	2.23×10^{4}	4.36×10^{2}	3.8×10^{4}
Banani	4.58×10^{2}	4.06×10^{2}	1.69×10^{4}	2.99×10^{2}	1.12×10^{4}
Gulshan	0	0.06×10^{2}	1.46×10^{4}	1.80×10^{2}	8.6×10^{2}

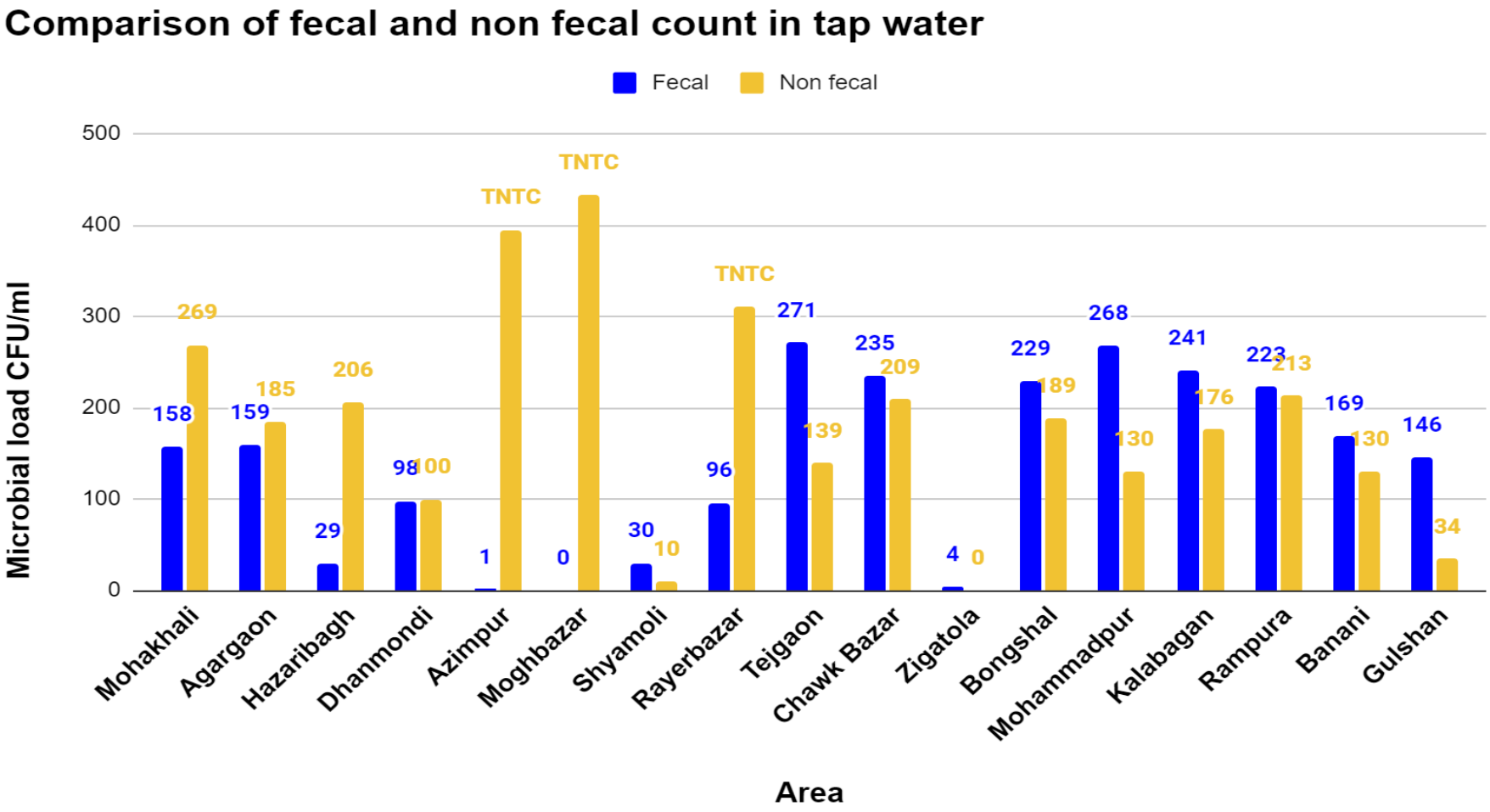


Figure 3.6: Comparative microbial load of tap water around Dhaka

3.4 The microbial load of filtered water in Dhaka

For filter water samples, different types of filtered water were collected from different households around Dhaka. For example, Drinkit, Heron Hybrid, Pureit Marvella Slim, Mechanical water filter, Pureit Ultima, Zazen Alkaline Water filter, Pureit Ultima RO+UV, CT

40, LSRO 929 CAR, Aqua Shine UV, Vision RO, Pureit Classic, Puricom RO, Heron Gold RO, Miyako, Boiled water, Wellsys UV, Pureit Ultima RO, and Pureit RO were observed here and there. After the sample processing, sample inoculation and incubation results were observed and recorded in the chart. From the analysis, it was observed that microorganisms still remained in filtered water. According to Table 3, the total coliform count of filtered water varied from one type of filter to another. Contamination by the filter itself was also observed, such as for some households tap water given lesser growth than filtered water. Coliform was noticeable also. However, as expected, in XLD and Maconkey agar lesser growth was found in some filtered water. Here in Figure 3.7, altogether microbial growth from different areas' samples indicates that there were *E. coli, S. aureus, P. aeruginosa, Salmonella spp., Pseudomonas spp., Klebsiella spp., E. faecalis,* and *shigella are* probably present in those more or less.

Based on the results, the microbial load of Filter water around Dhaka is recorded below,

Table 3: Growth of microorganisms present in filter water, recorded from XLD, Mac, MFC and NA media, unit CFU/ml except for Fecal coliform count in MFC in CFU/100 ml

Sample	Filter Model	XLD CFU/ml	MaC CFU/ml	Fecal Coliform CFU/100ml	Total coliform CFU/ml	Total viable count (TVC)
AG1F	Drinkit	0.15×10^2	0.34×10^2	2.45×10^4	3.79×10^2	3.35×10^3
AG2F	Heron Hybrid	0.92×10^2	1.40×10^2	1.40×10^4	1.40×10^2	2.22×10^3
AG3F	Pureit Marvella Slim	0.14×10^2	0.27×10^2	0.79×10^4	1.34×10^2	1.63×10^3
HB1F	Mechanical water filter	0.14×10^2	0.29×10^2	1.92×10^4	2.38×10^2	3.01×10^3
HB3F	Pureit Ultima	0.09×10^2	0.09×10^2	1.34×10^4	1.64×10^2	1.02×10^3
DN2F	Zazen Alkaline Water filter	0.8×10^2	2.27×10^2	0	4.23×10^2	4.88×10^3
AZ2F	Pureit Ultima RO+UV	1.92×10^2	1.36×10^2	0.11×10^4	1.76×10^2	1.38×10^3
AZ3F	CT 40	0.15×10^2	0.14×10^2	3.12×10^4	5.48×10^2	2.26×10^5
SM3F	LSRO 929 CAR	0	0	4.28×10^4	4.28×10^2	4.22×10^3
RB1F	Aqua shine UV	0.01×10^2	0.02×10^2	0.1×10^4	0.01×10^2	1.08×10^3
RB3F	Vision RO	0	0.32×10^2	3.50×10^4	3.53×10^2	3.51×10^3
CB2F	Pureit classic	1.28×10^2	0.88×10^2	2.17×10^4	3.87×10^2	4.45×10^3
BS1F	Puricom RO	0.54×10^2	0.65×10^2	1.55×10^4	3.04×10^2	3.61×10^3
BS2F	Heron Gold RO	0.02×10^2	0.11×10^2	3.47×10^4	3.96×10^2	4×10^3
BS3F	Miyako	0.01×10^2	0.14×10^2	2.35×10^4	3.59×10^2	3.96×10^3
MD1F	Boiled water	0.16×10^2	0.06×10^2	0	0.05×10^2	5.7×10^2
MD3F	Wellsys UV	0	0.05×10^2	0.37×10^4	1.19×10^2	1.55×10^3
BN2F	Pureit Ultima RO	0.06×10^2	0.34×10^2	2.34×10^4	3.88×10^2	4.59×10^3
BN3F	Pureit RO	0	0.04×10^2	0.1×10^4	0.16×10^2	0.71×10^2

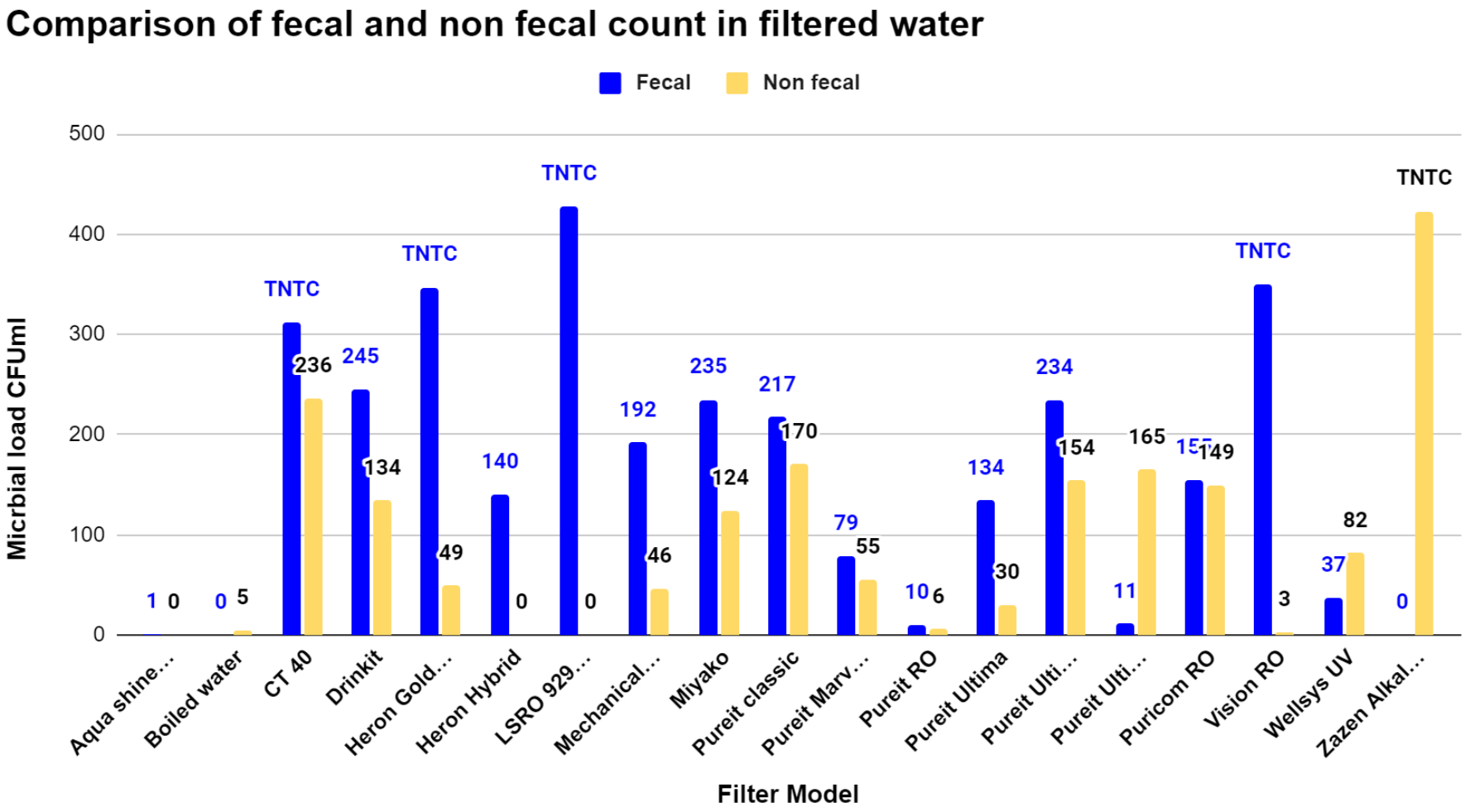


Figure 3.7: Comparative microbial load of filtered water around Dhaka

3.5 Biochemical tests results of isolated bacteria

The isolated bacteria were subjected to numerous biochemical tests. Biochemical tests are crucial for identifying unidentified organisms. Bacteria were isolated and subcultured for biochemical testing after spreading and streaking. In biochemical testing, a 24-hour fresh culture was used. Organisms could produce a misleading negative result without a new culture. The required biochemical tests were performed, and the results were recorded. After that, organisms were studied and identified with reference books. The results were listed in a chart according to the findings obtained from the tests. *E. coli, S. aureus, P. aeruginosa, Salmonella spp., Pseudomonas spp., Klebsiella spp.,* and *E. faecalis* were identified during biochemical tests.

3.6 Antibiotic susceptibility patterns of isolated microorganisms

E. faecalis, E. coli, E. coli 1, P. Aeruginosa, S. pneumoniae, S. aureus, Salmonella, and Klebsiella were selected for the antibiotic susceptibility test. Each of the organisms was treated with Amoxicillin AMX30, Ciprofloxacin CIP5, Azithromycin AZM30, Tetracycline TE30, Cefixime CFM5, Vancomycin VA30, Colistin CL10, Kanamycin K5, Meropenem MRP10, Ceftazidime CAZ30, Ceftriaxone CTR30, and Amoxiclav AMC30. The isolates were categorized into 3 groups: resistant, intermediate, and susceptible, on the basis of measuring zone diameter in mm. Among those 12 antibiotics, Escherichia coli was resistant to Amoxicillin AMX30, Cefixime CFM5, Vancomycin VAN30, and Ceftazidime CAZ30. Another strain of *E. coli* was named *E. coli* 1. *E. coli* 1 is Resistant to AMX30, TE30, CFM30, VA30, K5, CAZ30, CTR30, and AMC30. *P. aeruginosa* is resistant to AMX30, TE30, CFM5, VA30, K5, CAZ30, CTR30, and AMC30. S. pneumoniae is resistant to AMX30, CFM5, and CAZ30. S. aureus is resistant to AMX30, CFM5, VA30, K5, and CAZ30. Salmonella is resistant to AMX30, TE30, CFM30, VA30, CL10, K5, MRP10, CAZ30, CTR30, and AMC30. Klebsiella is resistant to AMX30, TE30, CAZ30, VA30, CTR30, CFM5, K5, CL10, and AMC30.The results are shown in the table below,

Table 4: AST for estimated microorganisms

Name of the antibiotics	Isolated probable organisms							
	E. faecalis	***E.coli***	***E.coli 1***	***P. aeruginosa***	***S. pneumoniae***	***S. aureus***	***Salmonella***	***K. pneumoniae***
Amoxicillin AMX30	R	R	R	R	R	R	R	R
Amoxiclav AMC30	S	S	R	R	S	S	R	R
Azithromycin AZM30	S	S	S	S	S	S	S	S
Cefixime CFM5	R	R	R	R	R	R	R	R
Ceftazidime CAZ30	R	R	R	R	R	R	R	R
Ceftriaxone CTR30	I	S	R	R	I	I	R	R
Ciprofloxacin CIP5	S	S	S	S	S	S	I	S
Colistin CL10	I	I	I	I	I	I	R	R
Kanamycin K5	I	S	R	R	S	R	R	R
Meropenem MRP10	S	S	S	S	S	S	R	S
Tetracycline TE30	S	S	R	R	S	S	R	R
Vancomycin VA30	S	R	R	R	S	R	R	R

***R= Resistant *I= Intermediate *S= Susceptible**

3.7 The pathogenicity test results of the isolated organisms

Blood Agar Test

The test was done on the isolated organisms to figure out the pathogenicity level of the organisms. To perform this test, 24 hours of an inoculum from a pure culture was streaked on a sterile plate of blood agar and the inoculated plate was incubated at 37ºC for 24 hours. After the incubation period, plates were observed and the result was taken. The medium was inspected for telltale signs of alpha- or beta-hemolysis. Almost 100% of the suspected organisms showed Alpha hemolysis. If the medium is discolored or darkened after growth, the organism has demonstrated alpha-hemolysis (Libretexts, 2020). Alpha-hemolysis is caused by damage (but not lysis) of the RBCs in the blood, representing partial damage of the red blood cells which reduces the hemoglobin to methemoglobin. So the results found in the test are significant.

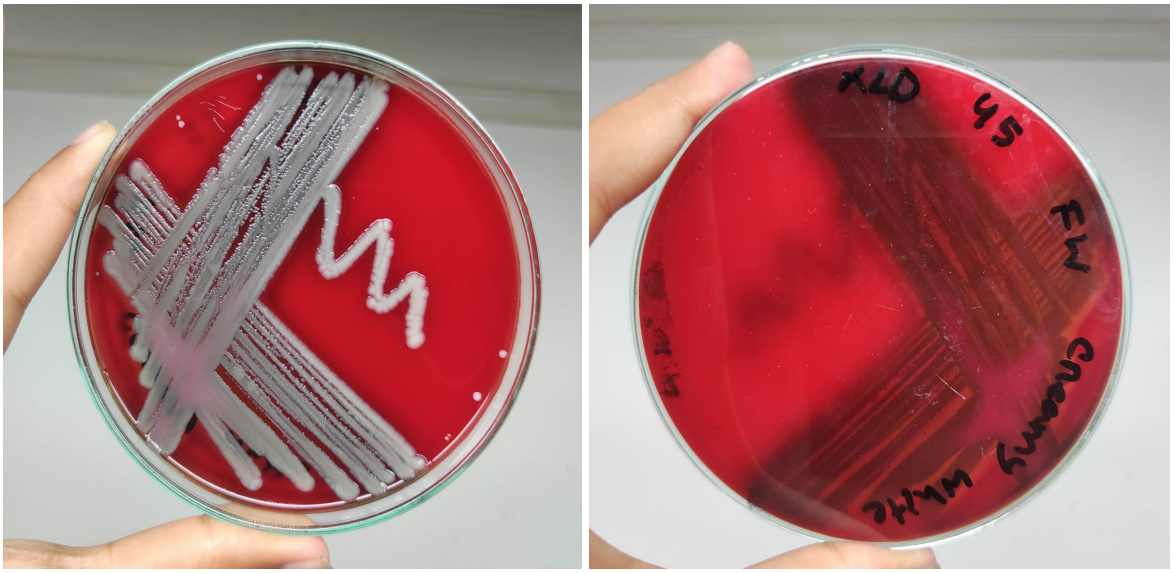


Figure 3.8: Pathogenicity test result

Coagulase Test

The coagulase test was performed to differentiate highly pathogenic organisms, like *Staphylococcus aureus*. Plasma was separated from blood by centrifugation. On a glass slide, a drop of physiological saline was placed, and with a loop, an isolated bacterial colony was emulsified. Soon after that, a drop of plasma was added and mixed gently. The colonies that showed visible clumps, were coagulase test positive, and the colonies that did not show

any clumps, were coagulase test negative. Among the suspected colonies, 74% of the organisms were coagulase test positive.

3.8 PCR

For the detection of the suspected organisms, PCR was performed. For PCR, the DNA was extracted from the bacteria by boiling method. Then the DNA was purified and PCR was done. For the detection of E. Coli, Eco primer was used. Eco primer has a base pair of 585 bp. After gel electrophoresis, DNA that binds with the primer showed a band under UV light. Almost 80% of the organisms showed bands. This means the DNA of the organisms, that showed bands were *E. Coli*.

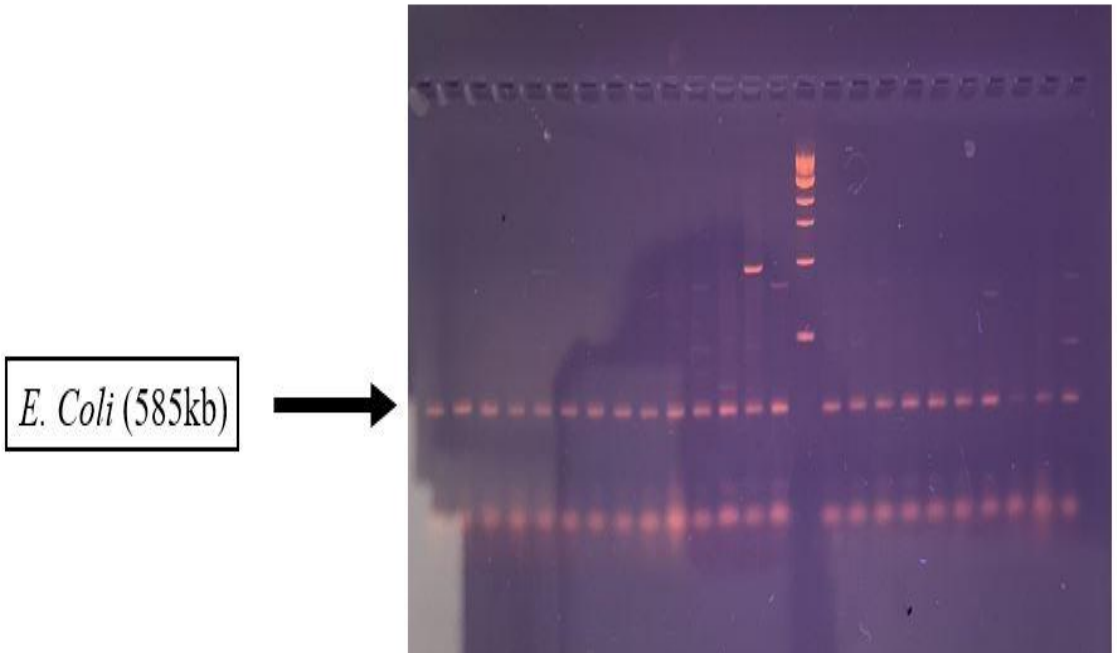


Figure 3.9: PCR bands under UV light

Chapter 4

Discussion and Conclusion

4.1 Discussion

After performing all the required tests, the results of this study were revealed.

The total viable count (TVC) was calculated using nutrient agar plates. The largest number of different kinds of microbes were grown after 24 hours of incubation. Bacterial colonies of various morphology and color were noticed. Maximum bacteria gave white colonies except for white tone likewise greenness, orange, yellow, purple, and cream colonies were noticed. CFU/ml form was used to calculate the total viable cell count by counting those colonies. Through the study, it was found that the minimum TVC count of the tap water sample was 8.6×10^2 CFU/ml and the maximum was 3.4×10^5 CFU/ml and the minimum TVC count of the filtered water sample was 5.7×10^2 CFU/ml and the maximum was 2.26×10^5 CFU/ml. According to WHO and BDS (2009), the standard for TVC is 1×10^3 CFU/ml. According to WHO and BDS (2018), the standard for TVC is 1×10^3 CFU/ml. From the results of TVC, it was found that of all 51 samples, only 8 tap water samples and 15 filtered water samples were within the range of WHO and BD standards. Most of the tap water samples exceeded the acceptable range and some filtered water samples were contaminated as well. Other studies have demonstrated that the majority of the WASA pump water that comes from deep tube wells is free of bacterial load (Hossain et al., 2011). So the contamination of household tap water may have occurred as a result of the pipeline contamination. According to a prior study, 100% of municipal tap water samples exceeded the WHO's drinking water guideline threshold (World Health Organization, 1996, vol-2). But some filtered water showed a higher bacterial load than tap water. It can occur due to the moistness of the filter membranes, by not changing the membranes of the filter when needed, or because the filter might not be of good quality, because of which, rather than filtering the water, the filter pollutes it.

Mac, XLD, and MFC media were used to culture the organisms through spreading. After 24 hours of incubation, different colored colonies were found in XLD and Mac such as Light/Dark Pink, Yellow, Purple, Off-white, Transparent, Orange, Black, and Brown. MFC media shows blue colonies that indicate fecal coliforms and white colonies that indicate non-fecal coliforms.

Colonies found in Macokey and XLD media were inoculated and streaked in EMB agar plates and were kept for incubation to detect the presence of *Escherichia coli.* Most of the

streaked colonies showed pink colonies that indicate non-lactose-fermenting, gram-negative bacteria that include *E. coli* and *Salmonella spp*. Among the samples, only 2 samples of tap water were not contaminated by *E. coli,* and only 4 samples of filter water were not contaminated by *E. coli.*

All the isolated organisms were taken for different biochemical tests to figure out their characteristics by differentiating them based on biochemical activities. The presence of different types of pathogens was suspected by analyzing the results of the biochemical tests. Among them, some organisms can create different types of waterborne and enteric diseases. All results were taken and recorded with the help of books and online articles. From the biochemical tests, *Escherichia coli, Staphylococcus aureus, Salmonella, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis,* and *Streptococcus pneumoniae* were suspected.

By completing the biochemical tests, seven different organisms were suspected and taken for the antibiotic susceptibility test. A total of twelve antibiotics were used to treat each of the organisms: Cefixime CFM5, Vancomycin VA30, Colistin CL10, Kanamycin K5, Meropenem MRP10, Ceftazidime CAZ30, Ceftriaxone CTR30, and Amoxiclav AMC30. The diameter of the zones was measured with a scale from two sides, and the average diameter was noted. Finally, the diameters were compared with the reference book to find out the susceptibility or resistance of the organisms.

After the antibiotic susceptibility test, the pathogen test was done to determine the pathogenicity of the suspected organisms. Blood agar was prepared to detect pathogenicity. The isolated colonies of the suspected organisms were streaked on the blood agar plates, and then the result was recorded after 24 hours. Almost 100% of the organisms showed alpha hemolysis, which means the pathogenicity level is high. This indicates that the results found in the test are significant.

After the bacterial pathogenicity test, PCR was performed for detecting *Escherichia coli.* Almost 80% of the organisms that were tested showed bands. This means the DNA of the organisms, that showed bands were *E. Coli*.

One of the main objectives of this study was a comparison between the microbial quality of tap water and filter water of the households of Dhaka City. Through this study, it was observed that the highest microbial count of tap water was at the Rampura area which is 4.36×10^2 CFU/ml, and the lowest microbial count of tap water was at the Zigatola area which is 0.04×10^2 CFU/ml. On the other hand, the highest microbial count of filtered water was in the Azimpur area, which was 5.48×10^2 CFU/ml and the filter that showed this much growth was CT 40, and the lowest microbial count of filter water was at the Rayerbazar area, which is 0.01×10^2 CFU/ml and the filter is Aqua UV. For better understanding, experiments were performed on boiled water as well, where almost no growth was observed. According to Harrington et al. (2003), there should not be any fecal and non-fecal coliform in boiled water. So according to the research, tap water should be boiled and then filtered by a reverse osmosis filter to get the best drinkable water. On average, Pure It Classic showed the highest coliform count (3.87×10^2 CFU/ml) which is used in most households from where samples were collected. This shows the filter fails to provide safe water, as the microbial loads and pathogen counts are alarming. On the other hand, Pureit Marvella Slim showed less microbial count on average, which is 1.34×10^2 CFU/ml, and this filter was also used in many households that were collected for testing. This shows that Pureit Marvella Slim is a better choice for safe drinking water.

The focus of the study was to check the efficiency of the filters. The microbial load and pathogens were studied. So, whether the filter could remove the nanoparticles and heavy metals from the tap water remained unchecked. *Vibrio cholerae* was not studied, because it needs a different approach to find out and isolate this pathogen. Also, algae, yeast, or molds were not studied, but these can remain in the water, which can be potentially harmful to health (Cabral, 2010).

4.2 Conclusion

This study was carried out to determine the microbial load of tap and filter water of the households of Dhaka city. From the study, it can be concluded that the tap water conditions of different areas of Dhaka City are highly contaminated. The filter water quality varied depending on the quality of the filters used in the houses. Furthermore, Studies have shown that most of the deep tube well water used for WASA pumps is free of bacterial load (Mahbub et al., 2011). So, the tap water can be contaminated in various ways. Some of them are the sewerage pipeline or the supply pipeline, which can be damaged and mixed. Because of this, fecal bacteria can contaminate the water supply. Again, reserve tanks can also contaminate tap water if they are not cleaned regularly. Moreover, in the case of filters, if the membranes of the filters remain moist, uncleaned, or unchanged, and the reserve tanks of the filters are not cleaned regularly, the water can be contaminated easily. Finally, if the filter quality is not up to the mark, it fails to turn the tap water into drinkable water. Although the tap water quality of Dhaka city is so bad, it can challenge the efficiency of any filter on the market. To conclude, this study showed that more than 50% of samples were contaminated with fecal coliform bacteria, which is alarming.

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Appendix 1

Different media composition

Name of the media	**Composition**	
	Name of the ingredients	Amount(gram per liter)
Nutrient Agar (Himedia)	Peptone Beef extract Agar	5.0 3.0 15.0
MacConkey Agar (Himedia)	Peptic digest of animal tissue Casein enzymic hydrolysate Pancreatic digest of gelatin Lactose Bile salts Crystal violet Neutral red Agar	1.5 1.5 17.0 10.0 1.50 0.001 0.03 15.0
MFC Agar (Himedia)	Biosate peptone Polypeptone peptone Yeast extract Sodium chloride Lactose Bile salts Aniline blue * Add 10 ml of rosolic acid (1% in 0.2N sodium hydroxide). Heat to boiling with agitation; do not autoclave.	10.0 5.0 3.0 5.0 12.5 1.5 0.1
XLD Agar (Himedia)	Lactose Sucrose Sodium Thiosulfate L-Lysine Sodium Chloride Xylose Yeast Extract Sodium Deoxycholate Ferric Ammonium Citrate Phenol Red Agar	7.5 7.5 6.8 5.0 5.0 3.75 3.0 2.5 0.8 0.08 15.0
EMB Agar (Himedia)	Peptone Lactose Dipotassium phosphate Eosin Y Methylene blue Agar	10.0 5.0 2.0 0.4 0.065 13.5

Blood Agar	Infusion from beef heart Tryptose Sodium chloride Agar * Add 50ml of sterile defibrinated blood into the autoclaved blood agar base media and mix well.	500.0 10.0 5.0 15.0
T_1N_1 Agar	Tryptone Sodium chloride Agar	1.0 1.0 0.6-0.75
Mueller-Hinton Agar (Himedia)	Beef, infusion Casamino acids Starch Agar	300.0 17.5 1.5 17.0
MIU Agar (Himedia)	Casein enzymic hydrolysate Dextrose Sodium chloride Phenol red Agar 40% Urea Solution	10.0 1.0 5.0 0.01 2.0 5 ml
TSI Agar (Himedia)	Peptic digest of animal tissue Casein enzymatic hydrolysate Yeast extract Beef extract Lactose Sucrose Dextrose Sodium chloride Ferrous sulfate Sodium thiosulfate Phenol red Agar	10.0 10.0 3.0 3.0 10.0 10.0 1.0 5.0 0.02 0.30 0.024 12.0
Simmon's Citrate Agar (Himedia)	Magnesium sulfate Ammonium dihydrogen phosphate Ammonium phosphate Sodium citrate tribasic Sodium chloride Bromothymol blue Agar	0.2 0.2 0.8 2.0 5.0 0.08 15.0
MR-VP broth	Peptone Dextrose Potassium phosphate	7.0 5.0 5.0

Appendix 2

Different Reagents Preparation

Name of Reagents	**Preparation**
Catalase reagent	35% Hydrogen peroxide(H_2O_2)
Oxidase reagent	100 mg of N,N,N1,N1-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10ml of distilled water and covered with aluminum foil and stored at 4ºC.
Nitrate reagent	Solution A: 1 gm of Sulfanilic acid was dissolved in 125ml of 5N acetic acid. Solution B: 0.625 gm of α-napthaylamine was dissolved in 120ml of 5N acetic acid.
Methyl red reagent	0.01 gm of methyl red was dissolved in 30 ml of 95% ethanol. The distilled water was added to make the final volume 50ml. Then it was covered with aluminum foil and stored at 4ºC.
Barritt's reagent	Solution A: 1.25 gm of α-naphthol was dissolved in 95% of ethanol with constant stirring to make a 25 ml solution. This solution was covered with aluminum foil and stored at 4ºC. Solution B: 10 gm of KOH was dissolved in distilled water. It will be warm. After cooling to room temperature, creatine was dissolved by stirring. Distilled water was added to adjust the final volume to 25ml. Then it was covered with aluminum foil and stored at 4ºC.
Kovac's reagent	1.25 gm of para-dimethylaminobenzaldehyde was dissolved in 18.75ml of amyl-alcohol. Then concentrated HCl was added to make the final volume 25ml. Then it was covered with aluminum foil and stored at 4ºC.
MacFarlane solution (5N)	0.18M sulfuric acid and 0.048M barium chloride were added in 1000ml distilled water.
Crystal violet	Solution A: 2.0 gm of crystal violet (95% dye content) added into 20. ml of 95% ethyl alcohol. Solution B: 0.8 gm of ammonium oxalate was added into 80.0 ml of distilled water. *Solution A and B was mixed
Gram's iodine	1.0 gm of iodine, and 2.0gm of potassium iodide was added into 300ml of distilled water.
Ethyl alcohol (95%)	95ml of ethyl alcohol (100%) was added into 5ml of distilled water.
Safranin	0.25ml of safranin O and 95% of ethyl alcohol were added into 100ml of distilled water.

Appendix 3

Different types of Instruments

Name of Instruments	**Model and company**
Autoclave	Vertical type system sterilizer, Model: HL-340, Company: GermmyIndestrialcorp, Made in Taiwan
Hot air oven	Mo: No-02G JERO TECH, Korea
Refrigerator	Samsung
Laminar air flow cabinet	Model: SLF-V, Vertical, SAARC Group, Bangladesh
Micropipette (100-1000µl)	Eppendorf, Germany
Micropipette (20-200µl)	Eppendorf, Germany
Incubator	(Model-05L-500D, Digisystem Laboratory Instrementsinc, Taiwan
Analytical Weight balance	Model-WTB 200 RADWAG
Membrane filter unit	Mo: AS-20 Dynair
Vortex Mixture	VWR International