Comparative study of the microbiological qualities between tap water of Old Dhaka and New Dhaka with emphasis on the presence of fecal coliform bacteria

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DECLARATION

This is to declare that the research work embodying the results reported in this thesis entitled **"Comparative study of microbiological qualities between tap water of old Dhaka and new Dhaka with emphasis on the presence of fecal coliform bacteria"** submitted by Muniara Juthi, has been carried out under the joint supervision and able guidance of Dr. M. Mahboob Hossain, Coordinator, Microbiology Program, BRAC University in partial fulfillment of Undergradutation in Microbiology, at BRAC University, Dhaka. It is further declared that the research work presented here is original, has not been submitted anywhere else for any degree or diploma.

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Dedication

To My Beloved Parents
&
My Thesis Supervisor Dr. Mahboob Hossain

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At the beginning, I am grateful to the God for the good health and well being that were necessary to complete this book.

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Abbreviations

NA: Nutrient Agar

Mac: Macconkey Agar

XLD: Xylose Lysine Deoxycholate Agar

EMB: Eosine Methylene Blue Agar

MSA: Mannitol Salt Agar

MHA: Mueller Hinton Agar

MIU: Motility Indole Urea

TSI: Triple Sugar Iron Agar

WHO: World Health Organization

mm: Millimeter

µl: Microliter

MR: Methyl red

VP: Voges- Proscauer

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

ABSTRACT

Water is one of the most important elements for survival. For leading a healthy life, water should be contamination free from any kind of water-borne organism. Water-borne organisms are a leading cause of disease and death worldwide. So the objectives of this study were to determine the microbiological quality of tap water collected from old Dhaka and new Dhaka, Bangladesh. Altogether 40 tap water samples from 10 different areas of old Dhaka and 10 different areas of new Dhaka were analyzed during summer and fall season. Through the investigation it was observed that, 9 tap water samples of 20 areas contained fecal coliform in summer and 13 were in fall. Tap water samples of 7 areas showed fecal coliform both in summer and in fall. Altogether out of 40 samples, 22 samples contained fecal coliform. Besides fecal coli-form bacteria, Coli-form such as *Escherichia coli, Shigella* spp. and others bacteria were also present. Out of 40 samples of 20 areas, 23 samples of 16 areas, 7 samples of 5 areas and 3 samples of 3 areas showed the growth of *E.coli, Shigella* spp. and *Salmonella* spp. respectively. In most of the samples total viable count (TVC) was above the WHO drinking water guideline value. Furthermore, there was no correlation between fecal coliform and the presence of *Shigella* and *Salmonella* spp. The study also revealed that the microbial count in summer season was more than fall season. In contrast, the fecal coliform count was more in the fall season than in the summer season. In summary by observing the results it can be concluded that the tap water quality of old Dhaka city is not satisfactory.

Chapter 1

Introduction

1.1 Introduction

Water is the key to life, a crucial resource for humanity and the rest of the living world. Safe drinking water is essential to humans and other life-forms even though it provides no calories or organic nutrients. We depend on good quality and good quantity of water for household work (drinking, washing, cooking etc), agriculture, industrial and environmental activities. So the importance of tap water is huge but most of the time it is contaminated by different types of potential microbes. Tap water is susceptible to biological or chemical contamination for this it needs to be purified and contamination free from all types of pathogens. Although, tap water is treated with chemical compounds like chlorine but sometimes harmful microbes present in tap water can create water-borne diseases especially in sensitive and immune-compromised persons. That is why it is necessary to know the water quality. Water must be free from any pathogenic microorganisms as well as chemical contamination because it can be hazardous to public health.

Any kinds of illness caused by consuming drinking water contaminated by human or animal feces that contains pathogenic microorganisms are called water-borne diseases. (http://www.lenntech.com). The presence of non-pathogenic organisms in water sources is not the real concern, but intestinal contaminates of fecal origin are significant. These pathogens are responsible for intestinal infections like dysentery, bacillary, typhoid fever, paratyphoid fever, cholera, salmonellosis, shigellosis etc. In as long as decades, the picture for water-related human health issues need get to be progressively comprehensive, for the development for new water-related contamination illnesses and the re-emergence for ones already known. According to the WHO, 1.7 million deaths per year causes from unsafe water supplies. Most of these are from diarrheal diseases. In developing countries 90% of these deaths occur in children due to minimum level of sanitary and potable water facilities. WHO also reported, around 3.4 million deaths annually caused by different types of dangerous waterborne enteric bacterial pathogens for examples, *E.coli, Shigella* spp, *Campylobacter* spp*, Salmonella* spp*,* and *Vibrio* spp. Not only bacterial but also numerous parasitological infections, including helminth diseases like schistosomiasis and especially guinea worm infects about 200 million people worldwide each year caused by contaminated water (Cappuccino,2005,7th edition).

Behind waterborne infections, fecal contaminations either from human or animals are the one of the main reasons and they are known as indicator organisms. Specifically, it can said that, indicator organisms are bacterial species that are present in high numbers in human and animal

feces and also present in sewage effluent. There are some requirements for being indicator organisms for example,

- They should not originate from other sources.
- They should not be able to multiply in water supplies or aquatic environments.
- And they should be easily isolated from water samples.

It is unfortunate that, no organisms totally meets all these requirements but only *E. coli* most closely matches those criteria. Except *E. coli*, other members of coliform group like *Klebsiella* spp. and *Enterobacter* spp. are fecal indicators. However, they are not exclusively faecal origin. Others organisms such as *Enterococci* and *Clostridium perfringens* also used as indicator organisms. Moreover, it is not mandatory that the absence of indicators indicates the absence of pathogens (Water- Microbiological Analysis of Water, 2011). So it is necessary to test the quality of water and isolate the organisms before use.

The current study was done to observe the microbiological quality of tap water collected from different location of Dhaka city those are mainly used for household purposes. For this study, first of all the samples were collected aseptically from different location of old Dhaka and new Dhaka. After that, samples were cultured in different agar media for isolation of fecal coliform or other pathogenic organisms. Next, organisms those are isolated go through biochemical identification. Antibiotics are being used frequently and for this organisms also becoming antibiotic resistant. To see the antibiotic sensitivity pattern of bacteria, antibiotic sensitivity test was also done.

1.2 Water microorganisms

Water can support the growth of many types of organisms. Water microbiology concerned with the microorganisms that live in water and causes different types of water-borne diseases. Sometimes these water-borne disease causing microorganisms become life threatening. For example, contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. From previous report it was observed that, from the contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people. Water can be contaminated by viruses, bacteria, protozoa and algae (http://science.jrank.org/pages/7311/Water-Microbiology.html). Contamination by

bacteria is the main concern of water contamination. Bacteria which can be found generally in water are given below.

- ***E.coli*:** *Escherichia coli (E. coli)* bacteria normally live in the large intestine of human and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons. *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 *E.coli,* 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.:** *Pseudomonas* spp. are gram-negative rod bacteria commonly found in soil, ground water, plants and animals. Pseudomonal infection causes a necrotising inflammation. Infectious species include *P. aeruginosa*, *P. oryzihabitans*, and *P. plecoglossicida*. *Pseudomonas. aeruginosa* flourishes in hospital environments and is a particular problem in this environment, since it is the second-most common infection in hospitalized patients.
- ***Shigella spp.*:** *Shigella* is a species of enteric bacteria that causes disease in humans. The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis which is most typically associated with diarrhea and other gastrointestinal symptoms. In general, *Shigella* is one of the most communicable and severe forms of the bacterial-induced diarrheas. *Shigella* can easily cause infection, because the bacteria thrive in the human intestine and are commonly spread both by person-to-person contact and through the contamination of food.
- ***Salmonella* spp.:** *Salmonella* spp. is a bacterium that causes one of the most common enteric infections. Strains of *Salmonella* cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning known as salmonellosis. *Salmonella* infection is a common bacterial disease that affects the intestinal tract. *Salmonella* bacteria typically

live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food. In some cases, the diarrhea associated with *salmonella* infection can be so dehydrating as to require prompt medical attention. Life-threatening complications also may develop (Mayo Clinic Family Health Book, 4th Edition).

- ***Klebsiella spp.:*** Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiell aoxytoca,* and *Klebsiella granulomatis*. Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. In particular, the medically most important *Klebsiella* species is *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections (Podschun, 1998).
- ***Serratia spp.*:** *Serratia* species are opportunistic gram-negative bacteria classified in the tribe Klebsielleae and the large family Enterobacteriaceae. *Serratia* are widespread in the environment, but are not a common component of the human fecal flora. *Serratia. marcescens* is involved in hospital acquired infections (HAIs), particularly catheter-associated bacteremia, urinary tract infections and wound infections.
- ***Corynebacterium jeikeium:*** *Corynebacterium jeikeium*, an opportunistic pathogen, commonly colonizes in the skin, especially in immune compromised hosts. Because of its broad-spectrum resistance to antimicrobial agents, the susceptibility of *C. jeikeium* strains is studied to a wide range of antibiotics. Patients who were diagnosed with *Corynebacterium jeikeium* show signs of normal bacterial infection such as fever.
- ***Janthinobacterium lividum*:** *Janthinobacterium lividum* is an aerobic, gram-negative, soil-dwelling bacterium that has a distinctive dark-violet colour. This colour is due to a compound called violacein. Violacein has anti-bacterial, anti-viral, and anti-fungal properties. *Janthinobacterium lividum* occasionally cause food spoilage.
- ***Proteus vulgaris:*** *Proteus* species are mainly soil inhabitants, particularly common in decomposing organic matter. *Proteus* and the related genus *Providencia* can quite frequently cause urinary tract infections.
- ***Acinetobacter hemolyticus*:** *Acinetobacter* spp. is a pleomorphic aerobic gram-negative bacillus. *Acinetobacter* species have low virulence but are capable of causing infection in organ transplants and febrile neutropenia. When *Acinetobacter* infections occur, they

usually involve organ systems that have a high fluid content (eg, respiratory tract, CSF, peritoneal fluid, urinary tract). These infections may occur as outbreaks rather than isolated cases of nosocomial pneumonia.

- ***Buttiauxella izardi:*** It is a gram negative bacterium isolated from water, soil, intestine of snails and some human samples, also in raw milk, cheese, and the intestinal tract of trout. The pathogenicity of *Buttiauxella izardi*is unknown. It may be pathogenic for snails. *B. agrestis* and *B. noackiae* were associated with human infections.
- ***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 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. *Staphylococcus* is killed by cooking and pasteurization (www. Food safety.gov)
- ***Enterococcus faecalis:*** *Enterococcus faecalis* is a nonmotile, gram-positive, spherical bacterium and most often found in the large intestine of humans. *E. faecalis* is listed as the first to the third leading cause of nosocomial infections. Most of these infections occur after surgery of the abdomen or a puncturing trauma, but can also be linked to the increased use of IV's and catheters, which are considered compromising devises. It is also responsible for urinary tract infections, bacterimia, endocarditis, meningitis, and can be found in wound infections along with many other bacteria (http://web.mst.edu).
- ***Citrobacter sedlakii:*** *Citrobacter* species are commonly found in water, soil, food, and the intestinal tracts of animals and humans. *Citrobacter* are rare opportunistic nosocomial pathogens. *Citrobacter* normally cause urinary tract infections, blood stream infections, intra-abdominal sepsis, brain abscesses, pneumonia and other neonatal infection, such as meningitis, neonatal sepsis, joint infection or general bacteremia.
- ***Morganella morganii*****:** *Morganella morganii* is a gram-negative rod commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora. Despite its wide distribution, it is an uncommon cause of community-acquired

infection and is most often encountered in postoperative and other nosocomial settings. *M. morganii* often involve the urinary tract, skin and soft tissue and hepatobiliary tract. It also can cause intra-abdominal infections, skin and soft tissue infections.

- ***Legionella Pneumophila*:** *Legionella Pneumophila* bacteria naturally occur in surface water all over the world. The bacteria cannot be completely removed during the purification process and are therefore frequently found in shower plumbing. They can reproduce to high levels in the slimy layer (biofilm) which forms on the inside of water pipes that are filled with stagnant or slow moving water. The *Legionella* bacteria are released from the bio film into the water stream and reach users when taps or showers are opened. To cause an infection, the contaminated water must be vaporized and inhaled, after which the bacteria can settle in the lungs and multiply. This can easily happen if someone takes a shower or inhales contaminated water over a kitchen sink. The fever variant is the mildest form. Legionnaires' disease on the other hand can deteriorate into a chronic condition of the lungs with a fatality rate of approximately 15% (Kreps, 2012).
- ***Cryptosporidium*:** *Cryptosporisium* is known as a protozoan, a single-celled organism and is most famous for giving people bouts of crippling diarrhea. The protozoa works like a parasite, latching onto the intestines and laying eggs in a person's fecal matter and that is how it spreads: when drinking water becomes contaminated with infected fecal matter, crypto moves on to new hosts.
- ***Anabaena sp.:*** *Anabaena*, a cyanobacteria that lives in freshwater reservoirs around the world, notably Australia, Europe, Asia, New Zealand, and North America. Cyanobacteria like this are believed to be some of the first multicellular organisms on earth, and as such have evolved to do some very curious things. In the case of Anabaena spp., those things are the production of neurotoxins. An outbreak in the 1950's got into the drinking water supply and was responsible for a series of mass die-offs at cattle farms across the U.S. In Australia, freshwater Anabaena bacteria have been found producing saxitoxins, a type of neurotoxin that causes respiratory arrest, followed by death.
- **Rotifers:** Rotifers are a relatively common microorganism that can be found pretty much everywhere in the world. And they're also one of the most common drinking water contaminants.

- **Copepods:** Copepods are larger, and possibly even more common. They are actually a type of crustacean or sort of like miniature shrimp. In 2009, residents began finding thousands of them in small samples of water. Copepods are beneficial because they often feed on toxins.

1.3 Antibiotic Resistance

For the treatment and prevention of bacterial infections antibiotics are used in health care. In present day antibiotics resistant bacteria are emerging in an increasing level. Antibiotic resistance is the ability of any microbes to resist effects of drugs. It means that, the growth of germs is not killed and growth is not stopped. Resistant bacteria can survive exposure to the antibiotic (Dewan, et al.,2010). If someone get infected with resistant organism then it will be difficult to treat. Concerns have been raised about the antibiotic resistance of bacteria. No one can completely avoid the risk of antibiotic resistant infections but some people are at greater risk than others.

Using antibiotics frequently is the main reasons behind antibiotic resistance around the world. Antibiotics are the most popular drugs which are prescribed in the treatment of infectious diseases furthermore might be lifesaving drugs. However, antibiotics are not optimally prescribed up to 50% of the time. Physician sometime prescribe antibiotic when it is not actually need. Also the spreading of resistant strains of bacteria from person to person or from the non-human sources in the environment is the other major reasons of developing antibiotic resistances (WWW.Centers for disease control and prevention; About Antimicrobial Resistance).

1.4 Literature review

K.R.Mahbub, A.Nahar, M.M.Ahmed and A.Chakraborty: Quality Analysis of Dhaka WASA Drinking Water: Detection and Biochemical Characterization of the Isolates.

Result from this article found total viable count in 62% of house tap water, 60% pipeline water and 45.45% WASA pump water were exceeded the BDS standard and WHO guideline for drinking. Among all the tested samples, 57.78% water samples were positive for coliform and

51.11% samples were positive for *E.coli* bacteria. Also the antibiotics sensitivity pattern of the isolates was determined. Most of them were found resistant to Ampicillin, Amoxicillin, kanamycin, Penicillin and Sulphomethoxazole antibiotics.

K.M. Rahman. I.H. Khan. J.A. Huq: A Bacteriological Study of Drinking Water of Different Hospitals and Hotels of Dhaka, Narayanganj, Munshiganj & Manikganj Towns. From this article it was observed that, in the hotels and hospitals of Dhaka city and its three former sub-divisional towns (Narayangan, Munshiganj and Manikganj) fecal contamination rate was higher in summer and rainy seasons than in winter. In general, the contamination rate was higher in hotels than hospitals. However, water supply of 4-5-star standard hotels and one private hospital were free from fecal contamination during winter and summer seasons but fecal contamination was present in rainy season. On an average 90'%, of the water supply examined were unsatisfactory because of presence of coliform.

Another study which was conducted by Farhana Akter, M. Mahboob Hossain, Afifur Rahman, et al., named "Antimicrobials Resistant Pattern of *Escherichia coli* Collected from Various Pathological Specimens**".**
Purpose of the study of this article was to know the effectiveness of commonly used antimicrobials against *Escherichia coli.* Here, 163 isolates of *Escherichia* colifrom pathological specimens were used for the study. From urine sample most of the isolates (77%) were collected. From the study it was observed that, Imipenem was most effective (154 out of 163 isolates) and cloxacillin was least effective. Also 4 isolates showed resistance to 13 of the 14 antimicrobials.

Also an article named "Microbiological study of drinking water qualitative and quantitative approach" by Tasnia Ahmed, Mrityunjoy Acharjee, MD. Shohanur Rahman et al (Received 7 August, 2013; Accepted 30 August, 2013).This article informed that developing countries are more affected by water borne disease outbreaks by focally originated pathogenic bacteria. Twenty-five treated water samples from different of Dhaka were tested and among them 5 samples found to be non-potable. They were contaminated with *E.coli, Klebsiella* spp.*, Alcaligenes faecalis, Pseudomonas* spp. and *Aeromonas* spp. Moreover, huge amount of other pathogenic bacteria was also detected as well.

One another article “Bacteriological Safety Assessment of Municipal Tap Water and Quality of bottle Water in Dhaka City: Health Hazard Analysis” by Saiful Islam, Housen Ara Begum and Nilufar Yeasmin Nili reported that, 50% of mineral water, 87.5% of filtered water and 100% of tap water samples were exceeded the drinking water guideline of WHO. This revealed that municipal tap water of Dhaka city was contaminated with a number of enteric bacteria like *E.coli.* So, only sensitivity test for *E.coli* was done.

1.5 Objectives of the study

Water borne illness is a common phenomenon in developing countries. In Bangladesh 90% cases of diseases (typhoid, cholera, dysentery and diarrhea) have long been reported due to the water borne micro-organisms diseases. (Islam, et al.,2013). In everyday life the important of tap water is huge. The aims of the present study are……

- Demonstrate the microbial quality of tap water of old Dhaka and new Dhaka.
- Identifying the bacteria present in the tap water.
- To see the antibiotics sensitivity of selected bacteria isolated from tap water.

Chapter 2

Materials and Method

2.1 Materials

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

2.1.1 General Procedure and Equipment

By autoclaving at 121ºC for 15 minutes all media were sterilized in an autoclave (Vertical type system sterilizer, Model: HL-340, Company: GermmyIndestrialcorp, Made in Taiwan). All glassware such as pipette, Petri dish, test-tubes etc., were sterilized at 160ºC for 1 hours in a hot-air oven (Mo: No-02G JERO TECH, Korea) prior to use. All agar media, agar slant, biochemical reagents were prepared freshly and kept at refrigerator (Samsung) at 3-4ºC except T_1N_1 culture media. It was stored at room temperature. All inoculations and subculturing were done under aseptic condition in a laminar air flow cabinet (Model: SLF-V, Vertical, SAARC Group, Bangladesh). The inoculated cultures were incubated in incubator (Model-05L-500D, DigisystemLaboratory Instrementsinc,Taiwan) at 37ºC and 44ºC. Accurate weights were measured by using an analytical balance (Model-WTB 200 RADWAG). Optical density was taken by spectrophotometer. For isolation of fecal coliform from sample membrane filter unit (Mo: AS-20 Dynair) was used.

2.1.2 Culture media

For total viable count (TVC), enteric pathogen, *Staphylococcus,* fecal coliform and *Pseudomonas* different types of media were used. Medias like Nutrient agar (NA), MacConkey agar, Xylose lysine deoxycolate (XLD), MFC agar, Cetrimide agar, Mannitol salt agar (MSA) and Eosin-Methylene Blue (EMB) were used for the bacterial isolation purpose. Details of these culture media are as below.

I. **Nutrient Agar (NA):** Nutrient Agar is used for the cultivation of microbes supporting 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 the bacterial growth.

II. **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 produced pink colonies. Bacteria which can't 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.

III. **Xylose Lysine Deoxycholate (XLD):** Xylose lysine deoxycholateisused 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 coloured for *E.coli.*

IV. **MFC Agar:** The mFC agar method is a one-step membrane-filtration method for enumeration of fecal coliform. Agar plates are incubated at 44.5°C for 22-24 hours. Fecal coliform will give blue colonies after incubation.

V. **Cetrimide Agar:** This media is known as cationic detergent which is selective media for *Pseudomonas aeruginosa*. If growth is occurring with greenness colonies in this media than it will be positive result for *Pseudomonas aeruginosa.*

VI. **Mannitol salt agar (MSA):** This media is selective and differential function for *Staphylococcus aureus*. *Staphylococci* will ferment mannitol and exhibit a yellow zone surrounding their growth. Non mannitol fermenters will give colorless zone.

VII. **EMB (eosin methylene blue):** This media can differentiate among lactose fermenters and lactose non fermenters bacteria. In case of lactose fermenters such as *E.coli*, the colonies will be blue/black in color with a metallic green sheen and for lactose non fermenters colorless and transparent colonies will be obtained. Other coliform such as *Enterobacter aerogenes*can also ferment lactose and grow on EMB media. They will give thick mucoid pink coloured colonies.

VIII. **T_1N_1 Agar:** Effective maintenance of stock cultures is essential for quality control, method validation and research purposes. Repeated subculturing may eventually lead to contamination, loss of viability and genotypic/phenotypic changes. (www.rapidmicrobiology.com).T_1N_1 Agar media is use to stock bacteria which are found from the samples.

IX. **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 use to see 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)

- **Casein agar:** Casein agar is a growth medium used for the detection of hydrolytic microorganisms. If clear zone observes around the colony, then it is positive result and if not then it is negative result.

- **Gelatin agar:** It was used to see if the microbe can used the protein gelatinas a source of carbon and energy for growth or not. Use of gelatinis accomplished by the enzyme gelatinase. Liquefaction of the gelatin agar indicates positive result and no liquefaction indicate negative result.

- **Starch agar:** Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyze starch. After addition of gram's iodine if clear zone are observed surrounding the bacterial colony then it is positive result and if not then it is negative result for starch hydrolysis.

- **Blood agar:** It is used to see the lysis of blood cells by organisms. Lysis are three types- α-hemolysis, β-hemolysis and γ-hemolysis. By observing clear zone hemolysis is determined.

- **Lactose broth:** It is used to see whether the organisms can ferment lactose or not.

- **Nitrate reduction broth:** Nitrate broth is used to determine the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2). If the medium turns red after the addition of the nitrate reagents, it is considered a positive result for nitrate reduction. If no color change occurs, then zinc powder will be added. After that if colour changed then it is a negative result.

- **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 the 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 differentiation of enterobacteriacae that combines three individual tests into a single medium. It is used to see whether the organisms are motile or not. Is it able to 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 addition of methyl red reagent to the culture media if it turns pink then it is positive result and if remains yellow then it is a negative result.

- **VP (Voges-Proskaure) 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 colour indicates a negative result.

2.2 Methods

2.2.1 Samples Collection

Twenty different tap water samples were collected from twenty different places of old Dhaka and new Dhaka. Samples were collected aseptically (rinsed with the water sample) in plastic bottles during summer and winter season. Bacterial growth may occur in taps and 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. All samples were brought to the laboratory for culture as soon as possible.

2.2.2 Sample processing and inoculation

After the collection of sample, it was processed and inoculated in the culture media as soon as possible. First of all, the sample was transferred to sterile conical flask and from that it was serially diluted in sterile physiological saline up-to 10^2 times. Then all the media used in this study, 100 µl sample was added and transferred to the media. Next by a sterile glass spreader sample was spread until it becomes dry. Always spreader was burnt before spread the sample. Ethanol containing breaker where 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 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, EMB agar, MSA agar, Cetrimide agar

Sample: Water sample collected from tap water

Procedure:

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

2. 1 ml raw sample was taken into 9 ml of saline test tube. Then it was mixed well by vortexing and labeled as dilution 10^{-1}. Then from dilution 10^{-1}, 1 ml sample was taken and mixed with 9 ml physiological saline in another test tube and labeled as dilution 10^{-2}.
3. Now by a one ml micro-pipette, 100 µl sample was taken from raw sample and spread on nutrient agar plate by a spreader. Before spreading, spreader was burnt into burner to avoid contamination.
4. For dilution 10^{-1} and 10^{-2}, same technique was followed.
5. Sample was spread by this same process on MacConkey agar, XLD agar, MSA agar, EMB agar and cetrimide agar.
6. Next the plates were incubated for 24-48 hours at 37 °C.
7. 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 water.

Media: M-FC agar plates

Procedure:

1. For FCC (Fecal coliform count) M-FC agar plates were labeled.
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 filter clamp the funnel to the filter base was secured.
IV. A rubber hose was attached from the side arm on the vacuum flask to a vacuum source.

3. 100 ml of water sample was added through the funnel and the vacuum was started.
4. After the filtration of 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 with sterile forceps the membrane filter was removed carefully. Then it was placed on the surface of the medium labeled as FCC (The grid side was kept up).
6. A new membrane filter was aseptically placed on the platform, the filtration apparatus was reassembled and repeated step 2 through 4 was repeated. Here 50 ml water sample was taken instead of 100 ml. The filter disc was placed to the plate.
7. The plates were incubated in an inverted position as follows: Plates were incubated for 24-48 hours at 44.5ºC.
8. After the incubation period results were observed and recorded.

2.2.5 Stock culture method

Effective maintenance of stock cultures is essential for quality control, method validation and research purposes. Repeated subculturing may eventually lead to contamination, loss of viability and genotypic/phenotypic changes (www.rapidmicrobiology.com). T_1N_1 Agar media was used to stock bacteria which were found from the samples. T_1N_1 media was preserved at room temperature after growth of bacteria. First, bacterial colony will be taken by a needle from 24 hours of subculture plate. Subculture should be done in nutrient agar plate. Then bacterial colonies was 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 tests methods

Different types of unknown bacteria were found after spreading the sample on different types of agar plates. For 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 sterile technique smear 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 next step, smear was decolorized with 95% ethyl alcohol. The reagent was added 10 drops for 10 second until crystal violet failed to wash from 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 then examined under microscope with oil-immersion. Under the microscope if the bacterial cells appear pink then it is gram negative and if appears purple then gram positive. Shapes of the cells can be different types like: rods, cocci, spiral, bacilli etc.

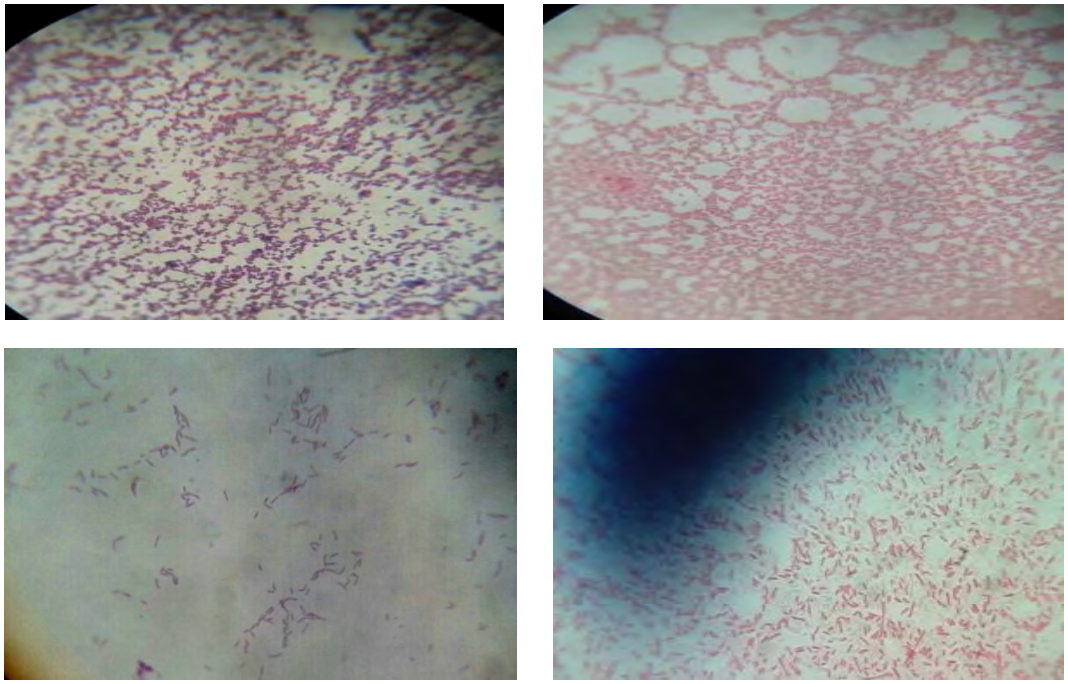


Figure 2.1: (a) Gram positive cocci, (b) Gram negative cocci(c) Gram positive rods (d) Gram negative rods

- **Oxidase test:** During aerobic respiration Oxidase enzymes play an important role in the operation of 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-phenylenediamine di-hydrochloride) was added at the place of the culture on the filter paper and mixed by a sterile tooth pick. Positive reaction turned the bacteria from violet to purple within 20 seconds. No such colouration indicates a negative result.

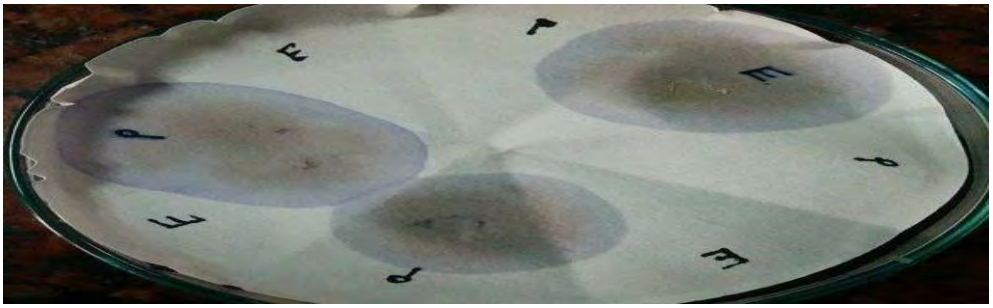


Figure 2.2: Oxidase test

- **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 amount of a bacterial colony (18 to 24 hours old) was places 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 bubbling reaction. A negative result showed no bubble.

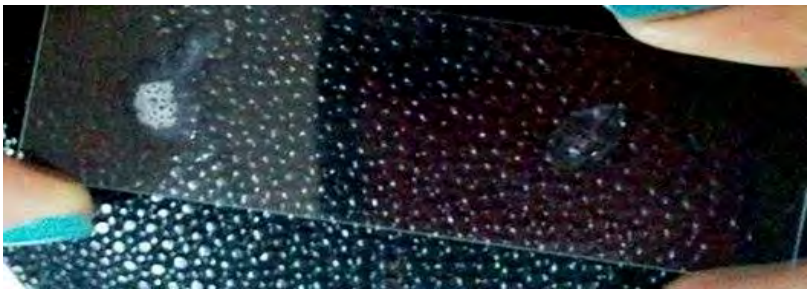


Figure 2.3*:* Left one is a positive and right one is a negative results of catalase test

- **TSI (Triple Sugar Iron) test:** TSI media was used for determining whether bacteria can ferment glucose, lactose or they can produce Hydrogen sulfide or other gases. This will help to differentiate between various *enterobacteriacae* including intestinal pathogens *Salmoneela* and *Shigella*. TSI media have two parts one is butt with poorly oxygenated area at the bottom and the other is slant with well oxygenated area on 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 colour from red to yellow.

ii. If gas is produce, then there will be crack in media.

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

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

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

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

The expected results of TSI agar test are:

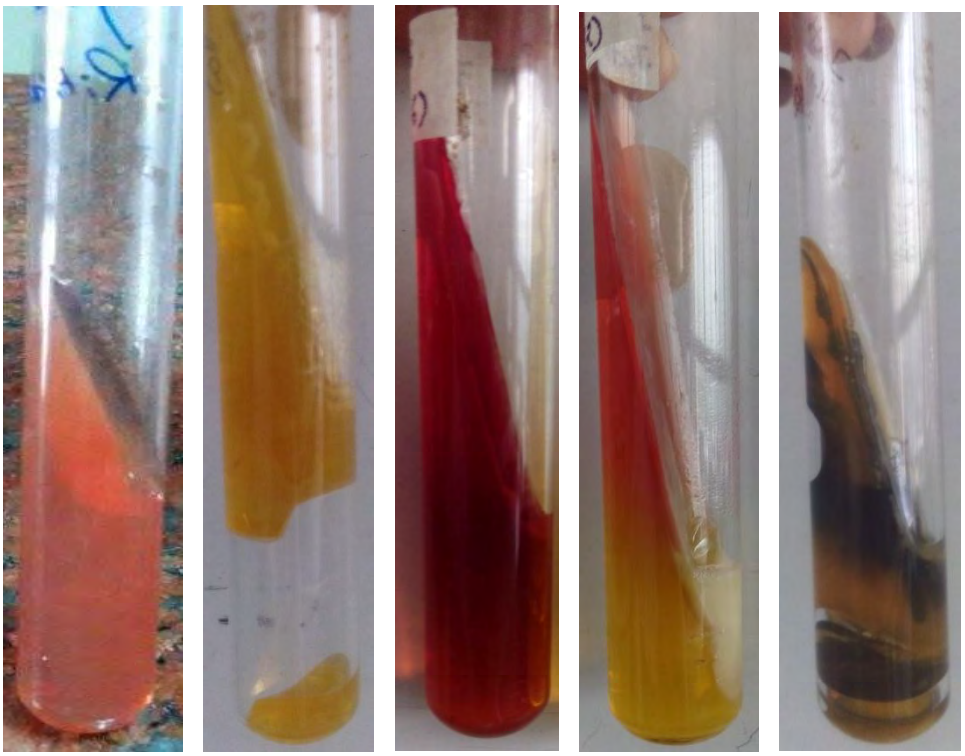
- Control: Red slant/ red butt/ no black color= No fermentation and no H_2S formation
- Red slant/ black butt= No lactose fermentation but H_2S production
- Red slant/ yellow butt= No lactose fermentation but glucose fermentation, noH_2S production
- Yellow slant/ yellow butt with black precipitation=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

Equipment: Bunsen burner, inoculating loop

Procedure:

1. Using a marker, the TSI tubes were labeled.

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

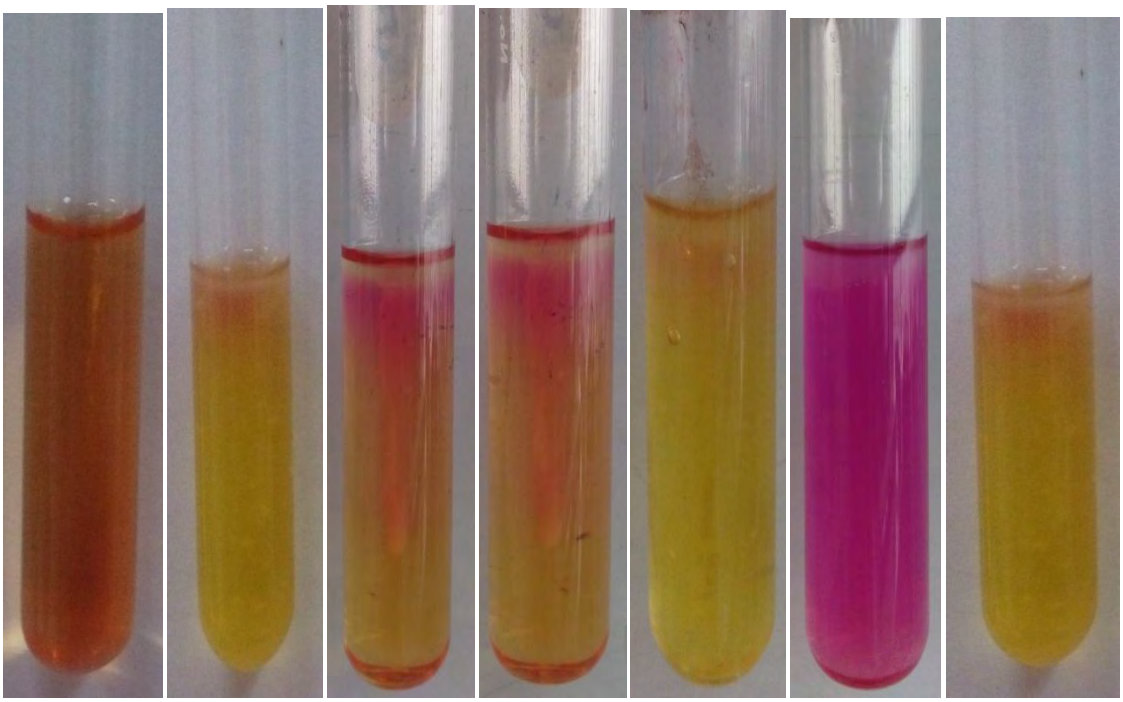


(a) (b) (c) (d) (e)

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, noH_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. Are organisms being able to 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 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 clearly transparent and no hazy growth was observed, then it is negative result for motility test. For indole test result, 5 drops of Kovac's reagent was added and waited for result. Formation of pink to cherry red color ring indicates positive result. On the other hand, if the media remain yellow then it is negative result. For 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 negative result.



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

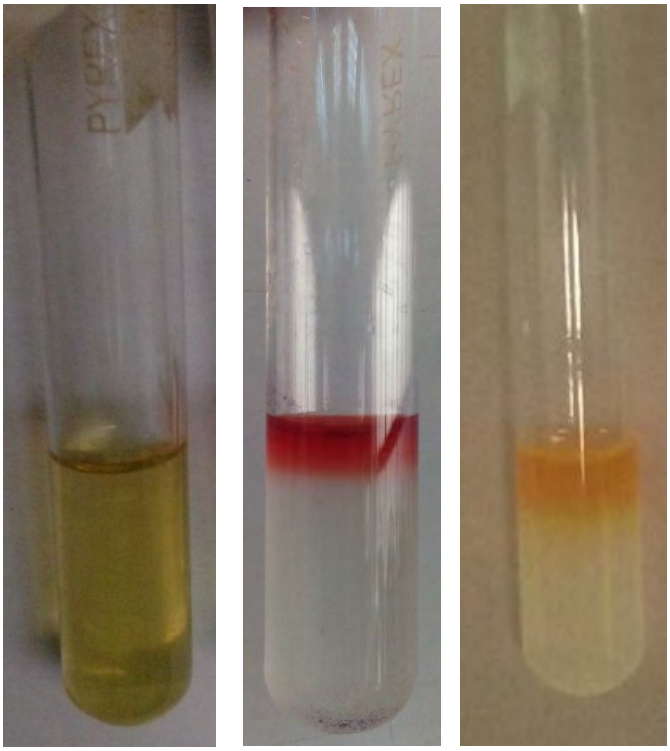
Figure 2.5: MIU test result, (a) Control (b) Motility positive (c) Motility negative (d) Indole positive (e) Indole negative (f) Urease positive (f) Urease negative

- **Simmon's citrate test:** This test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as the sole source of carbon and energy. Citrate is used by microorganisms as 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 performing this test bacterial culture was obtained from a 24 hours' subculture plate. Sterile citrate agar media was taken and using a marker it was labeled.

Then using an inoculating needle and aseptic technique, 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 incubation period, the simmon citrate slants were taken out of the incubator and observed for colour changes. Prussian blue colour of the agar indicates positive results and the green color indicates a negative result.

Figure 2.6: First one is a negative result and second one is a positive result

- **Methyl red (MR) test:** Methyl red test detect 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 large concentration of acidic end products. For performing this test fresh 24 hours' 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 was 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 ring indicated positive results for MR test and the yellow colour ring indicated negative results.

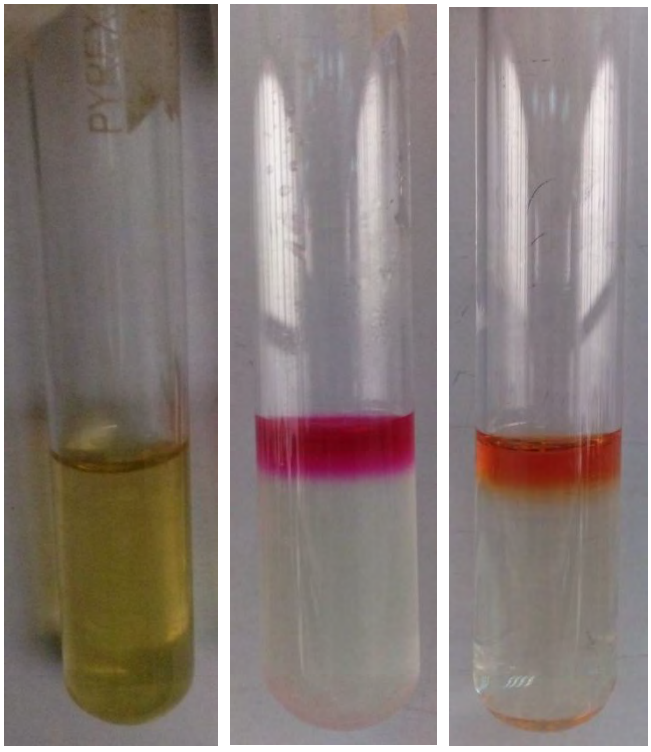


(a) **(b)** **(c)**

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 mehyl carbinol, from organic acids that result from glucose metabolism. When 40% KOH and 5% alpha-naphthol is 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 colour in the medium within 30 minutes after the addition of reagent which is a positive VP test. On the other hand, yellow or absence of rose/pink colour represents a negative result. For performing this test fresh 24 hours' 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 was 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 was added to the tube. The tube was shaken gently for several minutes and was waited for 15-20 minutes for colour change. Rose/pink colour formation is a positive result and yellow or no rose/pink colour 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

- **Casein hydrolysis test:** This test is useful to determine if an organism can produce the exoenzyme casenase. This test is conducted on milk agar which is a complex media containing casein, peptone and beef extract. If an organism can produce casein, then there will be a zone of clearing around the bacterial growth. No clear zone is a negative result. For this test, 24 hours' bacterial sub-culture from nutrient agar was used. Colony from plate was taken by a sterile loop and then streaked on the casein agar media. Next it was incubated at 37ºC for 24 hours. After incubation period by observing the plates result was taken and recorded.

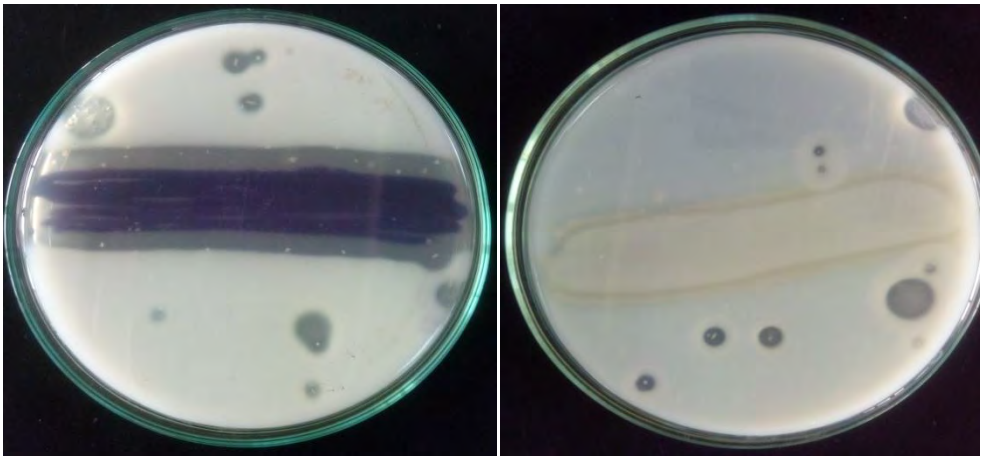


Figure 2.9 Casein hydrolysis test: First one is a positive result for casein hydrolysis test and second one is negative result.

- **Starch hydrolysis test:** The purpose of this test is to see if the microbe can use starch, a complex carbohydrate made from glucose, as a source of carbon and energy for growth or not. To perform this test, 24 hours of an inoculum from a pure culture was streaked on a sterile plate of starch agar. Inoculated plate was incubated at 37ºC for 24 hours. After incubation period, Iodine reagent was then added to flood the growth. Presence of clear halos surrounding colonies is positive for their ability to digest the starch and absence of clear halos is negative result.

Figure 2.10 Starch hydrolysis test: Colony on left side positive result for starch hydrolysis test and colony on right side negative result for starch hydrolysis test

- **Blood Agar:** 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 digest 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. Inoculated plate was incubated at 37ºC for 24 hours. After incubation period, plate was observed and result was taken. After 24 hours' incubation, the medium was inspected for telltale signs of alpha- or beta-hemolysis. If the medium is discoloured or darkened after growth, the organism has demonstrated alpha-hemolysis. If the medium has been cleared under growth, the organism is beta-hemolytic. No discernible change in the color of the medium constitutes gamma-hemolysis.

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

Figure 2.11 Test for hemolysis: (a) α-hemolysis (b) β-hemolysis (c) γ-hemolysis

- **Gelatin hydrolysis test:** The gelatin hydrolysis test detects the ability of bacteria to produce gelatinases. Gelatin hydrolysis is detected using a nutrient gelatin medium. For performing this test, heavy inoculums of 24-hours-old bacterial colony was stab-inoculated into tube containing nutrient gelatin. The inoculated tube and an un-inoculated control tube were incubated at 37°C, for up to 1 week, and checked everyday for gelatin liquefaction. Gelatin normally liquefies at 28°C and above, so to confirm that liquefaction was due to gelatinase activity, the tubes were immersed in an ice bath for 15 to 30 minutes. Afterwards, tubes were tilted to observe if gelatin has been hydrolyzed. Hydrolyzed gelatin will result in a liquid medium even after exposure to cold temperature (ice bath), while the un-inoculated control medium will remain solid. For weak positive results, incubate the inoculated nutrient gelatin tube longer until complete liquefaction was observed. The hydrolysis of gelatin indicates the secretion of gelatinase by the test organism into the medium. No liquefaction indicates negative result.

(a) **(b)**

Figure 2.12 Gelatin hydrolysis test: (a) Left one is negative result and (b) right one is positive result for gelatin hydrolysis

- **Nitrate reduction test:** This test determines whether the microbe produces the enzymes nitrate reductase and nitrite reductase. Nitrate broth contains nutrients and potassium nitrate as a source of nitrate. For this test, 24 hours of bacterial colony was transferred by a sterile loop aseptically to a sterile tube of nitrate broth containing an inverted Durham tube. The inoculated tube was incubated at 37º C for 24 hours and the result was determined. After incubation Five drops of nitrate reagent A was added, followed by five drops of nitrate reagent B. If broth turns into red colour, then it indicates positive result.

If no red colour observed, then small amount of zinc powder would be added. After that, if it turns red then negative result for nitrate reduction test.

(a) (b) (c)

Figure 2.13 Nitrate reduction test: (a) Un-inoculated (b) Positive result without zinc powder (c) Negative result with zinc powder

2.2.7 Disc diffusion method for antibiotic susceptibility test

Mueller Hinton Media is used for the antibiotic susceptibility test of bacteria. For antibiotic susceptibility test first off all MacFarlane solution was 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% physiological saline solution and mixed by vortexing.
2. Next the turbidity of the saline solution was compared with MacFarlane solution. Turbidity was observed by OD machine at 360nm. If the turbidity of the saline solution and MacFarlane solution becomes 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 bacterial lawn was made on Muller Hinton agar media.
4. Through a sterile forceps, specifics antibiotics were placed on the inoculated agar media and disks were slightly pressed on the agar to place it well.
5. Then inoculated plates were incubated at 37ºC for 24 hours.
6. After the incubation period plates were observed and result were recorded.
7. Results were taken by observing and measuring diameter of the clear zone around the antibiotics discs. According to the diameter of clear zone, it was determined whether the organisms were susceptible, intermediate or resistant to the antibiotics. No clear zone also indicates resistance to the antibiotic.

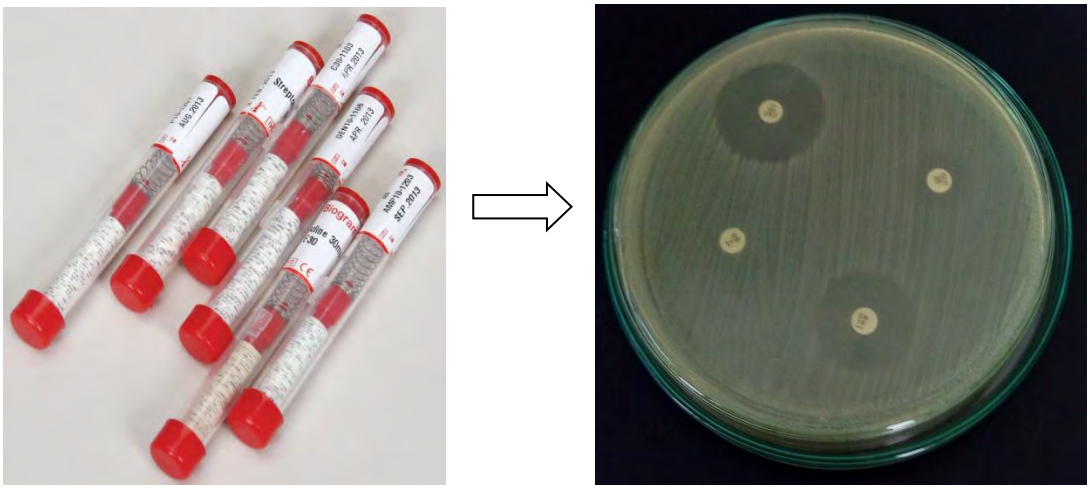


Figure 2.14 Antibiotic susceptibility test: Clear zone indicates susceptibility to antibiotic and no clear zone indicates resistance to the antibiotic

In this study, different types of antibiotics were used to see the antibacterial pattern of *Escherichia coli* and *Buttiaxuella izardii* 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
Aztreonam (30µg)	≤ 27	28-36	≥ 37
Ceftriaxone (30µg)	≤ 24	25-26	≥ 27
Ceftrazidime (30µg)	≤ 14	15-17	≥ 18
Ciprofloxacin (5µg)	≤ 15	16-20	≥ 21
Chloramphenicol (30µg)	≤ 12	13-17	≥ 18
Cloxacillin (5µg)	≤ 15	16-17	≥ 18
Co-trimoxazole (25µg)	≤ 10	11-15	≥ 16
Doxicycillin (30µg)	≤ 12	13-15	≥ 16
Gentamicin (10µg)	≤ 12	13-14	≥ 15
Impienem (10µg)	≤ 13	14-15	≥ 16
Nalidixic Acid (30µg)	≤ 16	17-19	≥ 20
Netilmicin (30µg)	≤ 13	14-18	≥ 19
Rifampicin (5µg)	≤ 12	13-14	≥ 15
Tetracycline (30µg)	≤ 14	15-18	≥ 19

Chapter 3

Results

3.1 Isolation of the organisms

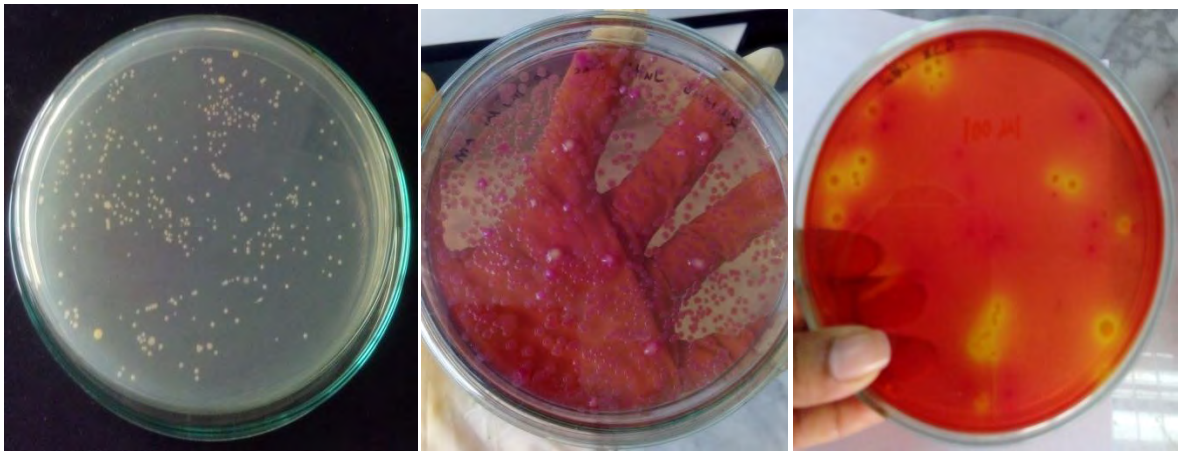
Tap water was collected from different areas of old Dhaka and new Dhaka. To observe the microbial loads of different organisms, different types of selective, non-selective and differential media were used. For isolation purpose, water samples were incubated on Nutrient agar plates, MacConkey agar plates, XLD agar plates, MFC agar plates, MSA agar plates, EMB agar plates and cetrimide agar plates by spread plate and streak plate technique. After 24-48 hours of incubation results were taken and recorded.

3.2 Morphology and interpretation of the unknown organisms

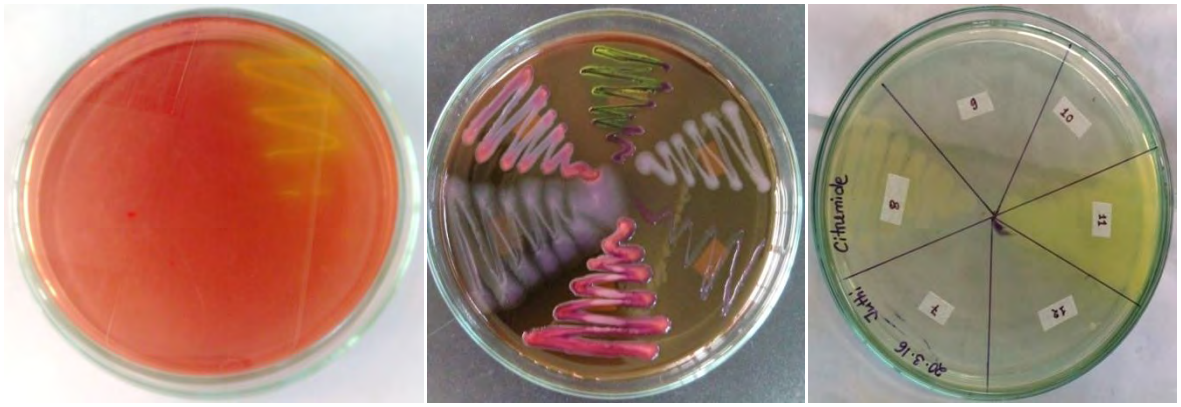
Different organisms give different types (colour, shape) of colony in different agar media. Nutrient agar was used for total viable count. In the same way, MacConkey agar was used for coliform count, XLD was used for *Shigella*, *Salmonella* and *E.coli* detection, MFC was for fecal coliform count, MSA for *Staphylococcoi* spp., EMB for *E.coli* and cetrimide for *Pseudomonas* detection.

In nutrient agar, after plating white, off-white, orange, yellow, purple and light greenness colony were observed. In macConkey agar plate deep pink (*E.coli*), light pink (*Klebsiella* spp.) and whitish pink colony were observed. Yellow (*E.coli*), red (*Shigella* spp), red with black centered (*Salmonella* spp.) and transparent colonies were observed on XLD media. On MFC media light to deep blue (Fecal coli-form) colonies were found. Yellow (*Straphylococcus aureus*) and pink/red (*Straphylococcie*) colonies also found in MSA agar. 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, Shigella spp*) colonies were observed on EMB agar. On cetrimide agar green (*Pseudomonas aeruginosa*) and transparent colony was observed.

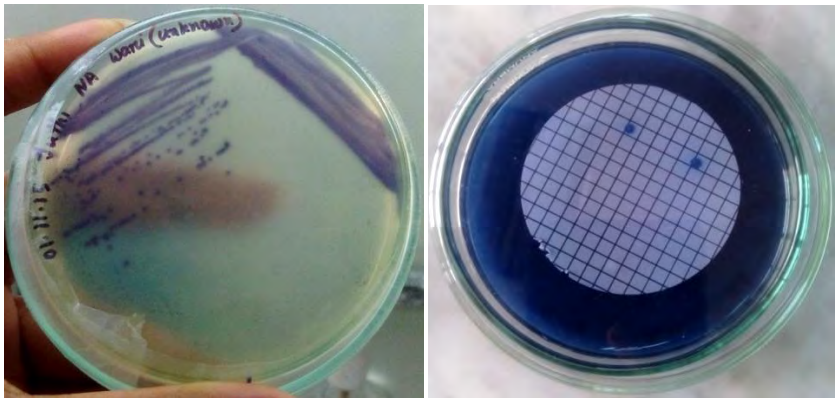
Results were recorded according to their colony morphology in different agar media (Figure 3.1).



(a) Nutient agar plate (b) MacConkey agar plate (c) XLD agar plate



(d) MSA agar plate (e) EMB agar plate (f) Cetrimide agar plate



(g) Nutrient agar plate (h) MFC agar plate

Figure 3.1: Growth of organisms on different types of selective, non-selective and differential agar media

3.3 Microbial load of tap water of old Dhaka

As the part of old Dhaka, tap water samples were collected from Shakharibazar, Narinda, Wari, Maniknagar, Gandaria, Laxmibazar, Mitford, Bijoynagar, Motijheel and Lalbagh. After the samples processing, samples inoculation and incubation results were observed and recorded in a chart. From the results, it was found that microbial load of organisms varied from one area to another area. According to Table 2, the total viable cells count of 10 areas of old Dhaka were more than 10^3 CFU/ml. Contamination by *Escherichia coli* was found in 6 areas in summer season and 3 areas in winter season. Only one area (Bijoynagar) was contaminated by *Shigella spp.* and no contamination by *Salmonella spp.* was found in any areas. It was observed that, most of the areas of tap water were contaminated by coliform bacteria. Among 10 areas, in summer 3 areas were contaminated by fecal coliform bacteria and in fall 5 areas were contaminated with the same bacteria.

3.4 Microbial load of tap water of new Dhaka

As the part of new Dhaka, tap water samples were collected from Mirpur, Khilgaon, Mohakhali, Mouchak, Cantonment, Dhanmondi, Banani, Gulshan, Monipuripara and Mohammadpur. After the samples processing, samples inoculation and incubation results were observed and recorded in a chart. From the results, it was found that microbial load varied from one area to another area. According to Table 1, the total viable cells count in some places were found more in new Dhaka than old Dhaka and vice versa for *Escherichia coli*. Three areas in summer and two areas in fall season were contaminated by *Shigella spp*. Also three areas in summer and no areas in fall season were contaminated by *Salmonella spp.* Among 10 areas of tap water of new Dhaka, 6 areas in summer and 8 areas in fall season were contaminated by fecal coliform bacteria.

According to the results, microbial load of old Dhaka and new Dhaka was recorded in a table which is given below in (Table 2).

Table 2: Total viable count (TVC) and enumeration of *Escherichia coli, Shigella spp., Salmonella spp.,* Coliform and Fecal coliform bacteria of old

Sample Numbers	Location of Samples		TVC (CFU/ml)		*Escherichia coli*(CFU/ml)		*Shigella spp.* (CFU/ml)		*Salmonella spp.* (CFU/ml)		Coli-form (CFU/ml)		Fecal coli-form (100 ml)		Potablity	
			S	F	S	F	S	F	S	F	S	F	S	F	S	F
1	Old Dhaka	Shakharibazar	1.7×10^4	1.2×10^4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Potable	Potable
2		Narinda	1.08×10^5	1.47×10^5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Potable	Potable
3		Wari	3.8×10^4	1.6×10^4	1×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	13	Nil	Non-potable	Non-potable
4		Maniknagar	2.06×10^4	2.34×10^2	8×10^3	2×10^3	Nil	Nil	Nil	Nil	5×10^2	Nil	8	28	Non-potable	Non-potable
5		Gandaria	2.6×10^4	2.5×10^4	5×10^3	Nil	Nil	Nil	Nil	Nil	9×10^3	Nil	Nil	83	Potable	Non-potable
6		Laxmibazar	1.46×10^4	1.4×10^4	9×10^3	1×10^3	Nil	Nil	Nil	Nil	4×10^3	Nil	Nil	2	Potable	Non-potable
7		Mitford	3.6×10^4	6×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Potable	Potable
8		Bijoynagar	2.03×10^5	1.37×10^4	2.40×10^4	1×10^3	2×10^3	1×10^3	Nil	Nil	Nil	Nil	Nil	4	Potable	Non-potable
9		Motijheel	1.42×10^5	1.03×10^5	2×10^3	1×10^3	Nil	Nil	Nil	Nil	Nil	2×10^3	1	9	Non-potable	Non-potable
10		Lalbagh	1.4×10^4	6×10^2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Potable	Potable
1	New Dhaka	Mirpur	5.06×10^4	1.65×10^5	4.42×10^3	1×10^3	5×10^3	1×10^3	3×10^3	Nil	7×10^4	1.5×10^4	11	19	Non-potable	Non-potable
2		Khilgaon	1.79×10^5	5.1×10^4	1.2×10^4	1×10^3	8×10^3	Nil	7.8×10^4	Nil	2×10^4	Nil	Nil	8	Potable	Non-potable
3		Mohakhali	3.2×10^4	7×10^3	2×10^3	1×10^3	Nil	Nil	Nil	Nil	3×10^3	Nil	5	3	Non-potable	Non-potable
4		Mouchak	1.7×10^5	2.1×10^5	3×10^3	Nil	1.1×10^3	Nil	4×10^3	Nil	1.4×10^3	5×10^3	92	15	Non-potable	Non-potable
5		Cantonment	1.35×10^5	3.6×10^4	2×10^3	Nil	Nil	Nil	Nil	Nil	2.2×10^4	Nil	12	Nil	Non-potable	Potable
6		Dhanmondi	2.6×10^4	5.6×10^4	3×10^2	Nil	Nil	1×10^3	Nil	Nil	1.6×10^4	2×10^3	52	6	Non-potable	Non-potable
7		Banani	1.12×10^4	3×10^4	Nil	4×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1	Potable	Non-potable
8		Gulshan	2.1×10^4	1.54×10^5	Nil	2×10^3	Nil	Nil	Nil	Nil	Nil	3×10^3	Nil	27	Potable	Non-potable
9		Monipuripara	3.9×10^4	6.4×10^4	1×10^3	Nil	Nil	Nil	Nil	Nil	Nil	1.3×10^4	31	9	Non-potable	Non-potable
10		Mohammadpur	5.7×10^4	7.2×10^4	Nil	2×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Potable	Potable

*S= Summer, *F= Fall, *CFU= Colony Forming Unit, *TVC= Total Viable Cells, *TNTC=Too Numerous to Count, *TFTC= Too Few to Count

Dhaka and new Dhaka's tap water. (Summer/Fall

3.5 Combined microbial load of old and new Dhaka

Out of 40 samples of 20 areas of Dhaka city collected in summer and fall seasons, 13 were found to contain *E.coli* in summer and 10 were in fall. Unexpectedly 9 tap water samples of 20 areas contained fecal coliform in summer and 13 were in fall. Tap water samples of 7 areas showed fecal coliform both in summer and in fall. Altogether, out of 40 samples 22 (55%) samples contained fecal coliform which is a matter of concern. Out of 40 samples of 20 areas, 23 (57.5%) samples of 16 areas, 7 (17.5%) samples of 5 areas and 3 (7.5%) samples of 3 areas showed the growth of *E.coli, Shigella* spp. and *Salmonella* spp. respectively.

According to the results, combined microbial load of old and new Dhaka was recorded by a histogram which is given below

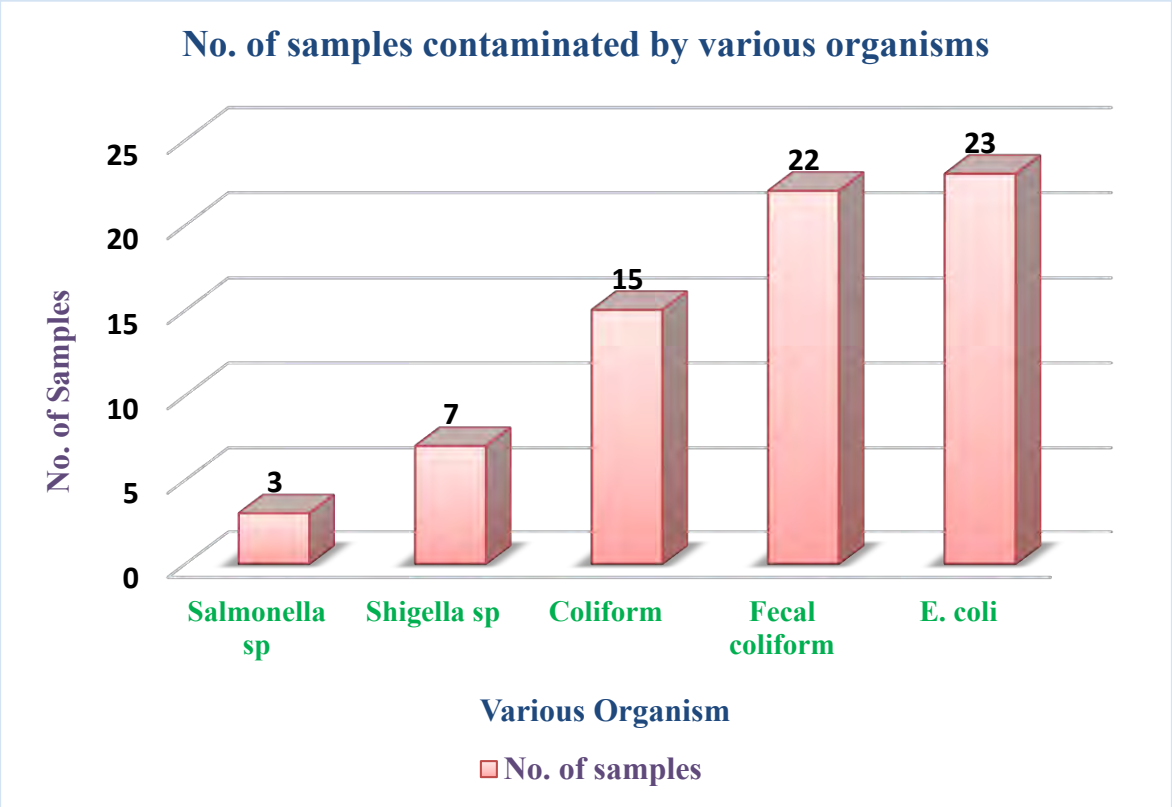


Figure 3.2: No. of samples contaminated by various organisms

3.6 Biochemical tests result of isolated bacteria

Bacteria those were isolated from old Dhaka and new Dhaka's tap water were tested by different types of biochemical tests. Biochemical tests are important for identification of the unknown organisms. After spreading and streaking microorganisms were isolated and sub-cultured for biochemical tests. Biochemical tests were done with 24 hours fresh culture. Without fresh culture organisms might gives false negative result. After subculture, required biochemical tests were done and recorded. Then with the help of references books and online software called ABIS, organisms were analyzed and identified. According to their test results, organisms were listed into a chart. After biochemical test *Shigella* spp. was found in tap water of bijoynagar, mirpur, khilgaon and mouchak. *Salmonella* spp. was found in the tap water of mirpur, khilgaon and mouchak areas. Moreover, *Pseudomonas* spp., *Enterococcus faecalis, Citrobacter sedlaki, Strephylococcus aureus, Buttiauxella izardii, Acinetobacter hemolyticus, Proteus vulgaris, Janthinobacterium lividum, Morganella morganii, Corynebacteium jeikeium, Sarratia* spp., *Klebsiella* spp. and *Raoultella ornithinolytica* were also found.

The results of those biochemical test results and name of the organisms is given below in Table 3.

Table 3: Biochemical tests results of unknown bacteria isolated from tap water

Number of isolates	Gram Staining		TSI				MIU																Suspected organisms
	Gram stain	Shape	Butt	Slant	H_2S	GAS	Motility	Indole	Urease	Lactose	Oxidase	Catalase	Citrate	MR	VP	Nitrate reduction	Gelatin hydrolysis	Blood agar or lysine	Casein hydrolysis	Starch hydrolysis	Mannitol hydrolysis	Cetrimide agar	EMB Agar	
1	-	Rod	K	K	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	Grey mucoid	*Pseudomonas* spp.
2	-	Rod	A	A	-	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	Green sheen	*Escherichia .coli*
3	+	Cocci	A	A	-	+	-	-	-	+	-	-	-	-	+	+	-	+	+	+	+	-	Purple pink	*Enterococcus faecalis*
4	-	Rod	A	A	-	+	+	+	+	+	-	+	+	+	-	+	-	-	-	-	+	-	Pinkish mucoied	*Citrobacter sedlaki*
5	+	Cocci	A	A	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-	+	-	Gray pink	*Strephylococcus aureus*
6	-	Rod	A	A	-	+	+	-	-	+	-	+	-	+	-	+	-	-	-	-	+	-	Glowing Pink	*Buttiauxella izardii*
7	-	Rod	K	K	-	+	+	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	Blue pink	*Acinetobacter hemolyticus*
8	-	Rod	A	A	+	-	+	+	+	-	-	+	+	+	-	+	+	-	+	-	-	+	Pink	*Proteus vulgaris*
9	-	Rod	K	K	-	-	+	-	-	-	+	-	-	+	-	+	+	-	+	-	-	-	Purple pink	*Janthinobacterium lividum*
10	-	Rod	K	K	-	+	-	+	+	-	+	+	-	+	-	+	-	+	-	-	-	+	Blue pink	*Morganella morganii*
11	+	Rod	K	K	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	Grey pink	*Corynebacterium jeikeium*
12	-	Cocco bacilli	A	A	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	Transparent	*Serratia spp.*
13	-	Rod	A	K	-	+	-	-	+		-	+	+	-	+	+	-	-	-	-	-	+	Brown dark centered	*Klebsiella spp.*
14	-	Cocco bacilli	K	A	-	-	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	Grey mucoid	*Shigella spp.*
15	-	Rod	K	A	+	+	+	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	Colorless	*Salmonella paratyphi*
16	-	Cocci	A	A	-	+	-	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	Deep pink	*Raoultella ornithinolytica*

*(-) = Negative * (+) = Positive *K = Alkaline reaction *A= Acidic reaction

3.7 Antibiotics susceptibility pattern results of Escherichia coli and Buttiauxella izardii

Escherichia coli and *Buttiauxella izardii* was selected for antibiotic susceptibility test. Both of them were treated by Ampicillin, Aztreonam, Ceftriaxone, Ceftrazidime, Ciprofloxacin, Chloramphenicol, Cloxacillin, Co-trimoxazole, Doxicycillin, Gentamicin, Impienem, Nalidixic Acid, Netilemicin, Rifampicin and Tetracycline. Among those 15 antibiotics *Escherichia coli* was resistant to two and *Buttiaxuella izardii* was resistant to six antibiotics.

The isolates were categorized into 3 groups: resistant, intermediate and susceptible on the basis of measuring zone diameter in mm. *Escherichia coli* was resistant to aztreonam and cloxacillin. Except this two antibiotic, *Escherichia coli* was susceptible for ampicillin, ceftriaxone, ceftrazidime, ciprofloxacin, chloramphenicol, co-trimoxazole, doxicycillin, gentamicin, impienem, nalidixic Acid, netilemicin, rifampicin and tetracycline. In case of *Buttiaxuella izardii,* it was resistant to ampicillin, ciprofloxacin, cloxacillin, co-trimoxazole, nalidixic acid, netilemicin and intermediate for aztreonam. On the other hand, it was susceptible for ceftriaxone, ceftrazidime, chloramphenicol, doxicycillin, gentamicin, impienem, rifampicin and tetracycline.

According to the zone diameter, antibiotics sensitivity results were recorded and listed into a table (Table 4). The table is given below

Table 4: Antimicrobial sensitivity pattern of *Escherichia coli* and *Buttiaxuella izardii* isolated from tap water

Antibiotics Name	Sensitivity level (mm)			*Escherichia coli*		*Buttiaxuella izardii*	
	Resistant	Intermediate	Susceptible	Zone of diameter(mm)	Comment	Zone of diameter(mm)	Comment
Ampicillin (10µg)	≤ 13	14-16	≥ 17	20	S	0	R
Aztreonam (30µg)	≤ 27	28-36	≥ 37	21	R	32	I
Ceftriaxone (30µg)	≤ 24	25-26	≥ 27	32	S	30	S
Ceftrazidime (30µg)	≤ 14	15-17	≥ 18	31	S	29	S
Ciprofloxacin (5µg)	≤ 15	16-20	≥ 21	32	S	0	R
Chloramphenicol (30µg)	≤ 12	13-17	≥ 18	26	S	32	S
Cloxacillin (5µg)	≤ 15	16-17	≥ 18	0	R	0	R
Co-trimoxazole (25µg)	≤ 10	11-15	≥ 16	33	S	0	R
Doxicycillin (30µg)	≤ 12	13-15	≥ 16	21	S	20	S
Gentamicin (10µg)	≤ 12	13-14	≥ 15	23	S	23	S
Impienem (10µg)	≤ 13	14-15	≥ 16	30	S	31	S
Nalidixic Acid (30µg)	≤ 16	17-19	≥ 20	20	S	10	R
Netilmicin (30µg)	≤ 13	14-18	≥ 19	23	S	0	R
Rifampicin (5µg)	≤ 12	13-14	≥ 15	25	S	26	S
Tetracycline (30µg)	≤ 14	15-18	≥ 19	26	S	20	S

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

Chapter 4

Discussion and Conclusion

4.1 Discussion

After performing all required tests (microbial culture and biochemical), the results of this study was revealed.

Nutrient agar plates were used for the calculation of total viable count (TVC). After 24 hours of incubation, the largest numbers of different types of microbes were grown. Bacterial colonies of different morphology and colour were observed. Maximum bacteria gave white colony except white colour also greenness, orange, yellow, purple and off-white colonies were observed. By counting those colonies, total viable cells count was done in the form of CFU/ml. According to WHO and BDS (2009) the standard for TVC is 1×10^3 cfu/ml. In this study, it was found that the minimum TVC count of the tap water sample was 6×10^2 and maximum 2.1×10^5 cfu/ml. From the results of TVC it was observed that of all 20 samples only 2 samples in fall season were within the range of WHO and BDS standard. Most of the tap water samples were contaminated. According to others research paper and study it was shown that most of the pump water of WASA which use deep tube well is free of bacterial load (Mahboob, et.,al, 2011). So the house tap water contamination may have occurred due to the contamination in pipe line. From previous study it was observed that, 100% of municipal tap water samples were exceeded the drinking water guideline value of WHO (World health organization, 1996, vol-2). In this study it was observed, of the 10 samples in old Dhaka only 2 samples in fall seasons were within the drinking water guideline value of WHO. In case of new Dhaka, all 10 samples in both summer and fall were exceeded the drinking water guideline value of WHO.

For the detection of *Escherichia coli* EMB agar was used. This media can differentiate between lactose fermenters and lactose non fermenters bacteria. In case of lactose fermenters such as *E.coli*, the colonies will be blue or black in colour with a metallic green sheen and for lactose non fermenters colourless and transparent colonies were observed. By observing green metallic sheen colony, and after performing suitable biochemical tests presence of *Escherichia coli* was conformed (Tortora,2011) From the results it was observed that in summer season 13 samples and in fall season 10 samples were contaminated by *Escherichia coli*. Minimum count was 3×10^2 cfu/ml and the maximum count was 2.40×10^4 cfu/ml.

For the detection of *Shigella* spp. and *Salmonella* spp. XLD agar was used. This is selective media for the isolation of *Shigella* spp. and *Salmonella* spp. and other non pathogenic organisms. By observing deep pink or red colour colony presence of *Shigella,* observing black centered colony presence of *Salmonella* and observing yellowish or white colony presence of *E.coli* were determined (cappuccino, 7th edition). Maximum count was 7.8 $\times 10^4$ cfu/ml and minimum count was 1×10^3 cfu/ml. It was observed that 4 samples in summer and 3 samples in fall contained *Shigella* spp.

For the detection of total coliforms MacConkey agar was used. This media selectively induces the growth of gram negative bacteria and inhibits gram positive bacteria. By observing deep pink colonies on MacConkey agar and suitable biochemical tests (table) it was concluded that the water samples were contaminated with coliform bacteria (cappuccino, 7th edition). A total count of colonies of coliform bacteria determines the potability of the water sources. From the results it was observed that in both summer and fall season 9 samples were contaminated by coliform bacteria. 5×10^2 cfu/ml was the minimum and 7×10^4cfu/ml was the maximum count of coliform bacteria in water samples.

For detection of fecal coliform mFC agar was used. Fecal coliform produced deep blue coloured colony in mFC agar. According to WHO, not a single fecal coliform bacteria should be present in water for being potable. In the present study it was observed that, in summer 9 and in fall season 13 samples were contained with fecal contamination.

Biochemical tests results showed that there were 16 different types of bacteria in water. All results were taken and recorded with the help of books and online identification software. Among them some organisms are able to create different types of waterborne and enteric diseases. From the biochemical results, *Pseudomonas* spp., *Escherichia coli, Enterococcus faecalis*, *Citrobacter sedlaki, Strephylococcus aureus, Buttiauxella izardii, Acinetobacter hemolyticus, Proteus vulgaris, Janthinobacterium lividum, Morganella morganii, Corynebacterium jeikeium, Serratia spp., Klebsiella spp, Shigella spp., Salmonella paratyphi* and *Raoultella ornithinolytica* were found. From different study it was found that, *Escherichia coli spp.* in 60%, *Klebsiella spp.* in 40%, *Enterobacter spp.* in 20%, *Pseudomonas spp.* in 70%, *Proteus spp.* in 10%, *Staphylococcus spp.* in 40% and *Salmonella spp.* were present in 0% tap water. Meanwhile, yeasts and molds were also found (Islam, et.,al, 2010).

After all biochemical tests and identification of 16 different types of organisms were found. All of the 16 organisms antibiotic sensitivity tests was done for *Escherichia coli* and *Buttiauxella izardii.* First of all, *Escherichia coli* and *Buttiauxella izardii* was cultured on nutrient agar plate the day before performing sensitivity test. It is necessary to use 24 hour culture to avoid false reading. After incubation, clear zone diameter was taken by mm scale and recorded. Clear zone indicates susceptibility of bacteria to the specific antibiotics and no clear zone indicates resistance to the antibiotic. Selected two organisms were treated by Ampicillin, Aztreonam, Ceftriaxone, Ceftrazidime, Ciprofloxacin, Chloramphenicol, Cloxacillin, Co-trimoxazole, Doxicycillin, Gentamicin, Impienem, Nalidixic Acid, Netilemicin, Rifampicin and Tetracycline. Out of the 15 antibiotics *Escherichia coli* was resistant to aztreonam and cloxacillin. Except two antibiotics, *Escherichia coli* were susceptible for ampicillin, ceftriaxone, ceftrazidime, ciprofloxacin, chloramphenicol, co-trimoxazole, doxicycillin, gentamicin, impienem, nalidixic Acid, netilemicin, rifampicin and tetracycline. In case of *Buttiaxuella izardii,* it was resistant to ampicillin, ciprofloxacin, cloxacillin, co-trimoxazole, nalidixic acid, netilemicinand intermediate for aztreonam. On the other hand, it was susceptible for ceftriaxone, ceftrazidime, chloramphenicol, doxicycillin, gentamicin, impienem, rifampicin and tetracycline. Others study reported that some strains of *E.coli* were totally resistant to rifampin and bacitracin, some were highly resistant to tetracycline and erythromycin. On the other hand, some strains were totally sensitive to gentamycin and kanamycin and highly sensitive to chloramphenicol.

The occurrence and spread of antibiotic-resistant bacteria (ARB) are pressing public health problems worldwide. It was observed in different study that, in general tap water contains less bacterial levels than source water and higher levels of ARB (antimicrobial resistant bacteria) than finished water. During water treatment and in tap water resistance of some antibiotic was observed (Xi, et al.,2013). Antibiotics resistance bacteria are the results of frequent use of antibiotics. Studies show that in the environment municipal wastewater treatment plants (WWTPs) are important point sources of antibiotics and antibiotic-resistant bacteria. Some resistant infections cause severe illness. People who are infected by these types of infections may require increased recovery time or may die from the infection. If one bacterium become resistance to any antibiotics then alternative drugs will be prescribed, but it might be less

effective, more toxic and more expensive. Antibiotic resistance occurs as part of natural process where bacteria can be slow but not stopped (WWW.Centers for disease control and prevention; About Antimicrobial Resistance). To protecting human and animal health preserving the effectiveness of antibiotics is vital. So, for preventing the development of antibiotic resistant bacteria, antibiotic should use only when it is needed.

One of the main objectives of this study was comparison between microbial quality of tap water of old Dhaka and new Dhaka. From this study, it was observed that in old Dhaka the highest microbial count was at Bijoynagar, 2.03×10^5 CFU/ml and in new Dhaka the highest microbial count was at Khilgaon, 1.79×10^5CFU/ml. It was the count in summer season. In case of fall season, in old Dhaka the highest microbial count was at Narinda, 1.47×10^5 CFU/ml and in new Dhaka the highest microbial count was at Mouchak, 2.1×10^5CFU/ml. From this data it can be suggested that the microbial count of old Dhaka and new Dhaka are not so different. *Shigella* spp. was found only in one sample at Bijoynagar of old Dhaka both in summer and fall seasons. In contrast, 3 samples (Mirpur, Khilgaon, Mouchak) in summer and 2 samples (Mirpur, Dhanmondi) in fall season were contaminated by *Shigella* spp. in new Dhaka. No *Salmonella spp.* was found in any samples of old Dhaka both in summer and fall seasons. On the other hand, *Salmonella* was detected in 3 samples (Mirpur, Khilgaon, Mouchak) of new Dhaka in summer and no *Salmonella* in fall. In case of coliform bacteria, 3 samples (Maniknagar,Gandaria, Lxmibazar) were in summer and 1 sample (Motijheel) was in fall season of old Dhaka was contaminated by coliform bacteria. In new Dhaka, 6 samples (Mirpur, Khilgaon, Mohakhali, Mouchak, Cantonment, Dhanmondi) in summer and 5 (Mirpur, Mouchak, Dhanmondi, Gulshan, Monipuripara) samples in fall were contaminated by coliform bacteria. The most important fecal coliform bacteria were found in 3 (Wari, Maniknagar and Motijheel) samples in summer and 5 (Maniknagar, Gandaria, Laxmibazar, Bijoynagar and Motijheel) samples in fall season in old Dhaka. Surprisingly, 6 samples (Mirpur, Mohakhali, Mouchak, Cantonment, Dhanmondi, Monipuripara) in summer and 8 (Mirpur, Khilgaon, Mohakhali, Mouchak, Dhanmondi, Banani, Gulshan, Monipuripara) samples in fall season in new Dhaka were contaminated by fecal coliform bacteria. We know even if water contains single fecal coliform bacteria then it cannot be potable. In summary by observing all the results it was concluded that the tap water quality of old Dhaka was better than new Dhaka. Although, the tap water of both old and new Dhaka need

more treatment. The total viable count was less in fall season but more in summer season. Moreover, the fecal contamination was observed more in fall season than summer.

4.2 Conclusion

This study was carried out to see the microbial quality of tap water of WASA and compare the microbial quality of old Dhaka and new Dhaka. From the study it can be concluded that, the microbial quality of tap water of old Dhaka was better than new Dhaka. From others earlier study it was informed that bacteriological quality of WASA source water was superior than pipeline and tap water. So tap water contamination might have taken place in distribution system, reservoirs or house tank. Before drinking tap water of WASA it should boiled or treated. This study showed that more than 50% samples were contaminated with fecal coliform bacteria which is alarming.

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Appendices

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 enzymichydrolysate 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 55 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 (Oxoid)	Peptone Lactose Dipotassium phosphate Eosin Y Methylene blue Agar	10.0 5.0 2.0 0.4 0.065 13.5
MSA (Himedia)	Beef extract Peptone Sodium chloride D-Mannitol Phenol red Agar	1.0 10.0 75.0 10.0 0.025 15.0
Cetrimide Agar (Himedia)	Cetrimide Gelatine peptone Magnesium chloride Potassium sulfate Agar	0.3 20.0 1.4 10.0 15.0
Skim Milk Agar (Himedia)	Skim milk powder Casein enzymichydrolysate Yeast extract Dextrose Agar	28.0 5.0 2.5 1.0 15.0
Starch Agar (Himedia)	Peptone Beef extract Starch (soluble) Agar	5.0 3.0 2.0 15.0
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 (Oxoid)	Casein enzymichydrolysate 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(Oxoid)	Magnesium sulphate 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
Nutrient Gelatin	Peptone Beef extract Gelatin	5.0 3.0 120.0
Trypticase nitrate broth	Trypticase Disodium phosphate Dextrose Potassium Agar	20.0 2.0 1.0 1.0 1.0
MR-VP broth	Peptone Dextrose Potassium phosphate	7.0 5.0 5.0
Lactose fermentation broth	Beef extract Peptone Lactose	3.0 5.0 5.0

Appendix 2

Different Reagents Preparation

Name of Reagents	Preparation
Catalase reagent	35% Hydrogen peroxide(H_2O_2)
Oxidase reagent	100mg of N,N,N1,N1-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10 ml of distilled water and covered with aluminum foil and stored at 4ºC.
Nitrate reagent	Solution A: 1 gm of Sulfanilic acid was dissolved in 125 ml of 5N acetic acid. Solution B: 0.625 gm of α-napthaylamine was dissolved in 120 ml 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 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 25 ml. Then it was covered with aluminum foil and stored at 4ºC.
Kovac's reagent	1.25 gm of para-dimethylaminobenzaldehyde was dissolved in 18.75 ml of amyl-alcohol. Then concentrated HCl was added to make the final volume 25 ml. Then it was covered with aluminum foil and stored at 4ºC.
MacFarlane solution (5N)	0.18M sulfuric acid and 0.048M barium chloride was added in 1000 ml 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, 2.0 gm of potassium iodide was added into 300 ml of distilled water.
Ethyl alcohol (95%)	95 ml of ethyl alcohol (100%) was added into 5 ml of distilled water.
Safranin	0.25 ml of safranin O and 95% of ethyl alcohol was added into 100 ml 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