Bacteriological Study of Balu and Turag River

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

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Declaration

It is hereby declared that

1. The thesis submitted titled "**Bacteriological Study of Balu and Turag River"** is our own original work while completing our 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.
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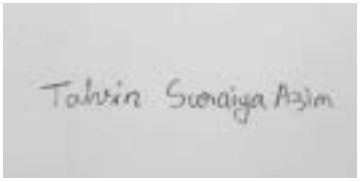

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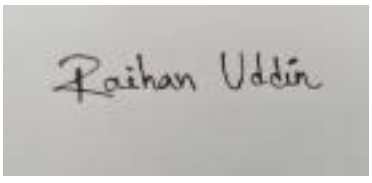

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Abstract

Bangladesh, being a riverine country, depends highly on the river water for various purposes including agriculture, domestic use and industrial usage. Thus, water pollution is also a massive conundrum for this country. Furthermore, antibiotic resistance is a globally rising topic nowadays. In this study we have tried to address these two major problems and conducted this research based on Dhaka city to demonstrate which kind of bacteria is responsible for the water pollution in the two most prominent rivers of Dhaka and to what extent these bacteria are resistant to antibiotics. The target sites for this study were the starting and ending points of the Balu and Turag river. Selective agar media were used to isolate different species of bacteria and then a set of biochemical tests were performed on these isolates for identification purposes. Using the Kirby-Bauer Disk Diffusion method, we checked for antibiotic susceptibility of these bacteria to different antibiotics. And finally, we wanted to confirm which bacteria were prominent in the ending points of these rivers especially, as the confirmed isolates from the end points represent what kind of bacteria are entering into Dhaka with the river flow and residing in them.

We found that among 43 isolates, all of them were susceptible towards Amikacin and Meropenem. The resistance rate for Ceftriaxone (6.98%), Tetracycline (6.98%), Amoxicillin-clavulanic acid (4.65%) and Ciprofloxacin (2.33%) were not very alarming but still to be concerned about. However, the resistance rate of these organisms isolated from rivers showed an alarming rate of resistance against Erythromycin and Cefixime at 39.53% and 90.70%. We ran PCR on the isolates found in site A and site D. These two sites mark the end point of the rivers which shows the state of the water in Dhaka. We found that 66% suspected cases of *Staphylococcus aureus* had positive results and 20% were identified as *Vibrio spp*. Finally, the PCR test yielded negative results for all three of the suspected cases of *Salmonella typhi.*

From this study we conclude that *Staphylococcus aureus* was the most prevalent in the Balu and Turag river water. And the second most prominent species was *Vibrio spp.* Thus, these river water can cause diseases like Cholerae and Bloodstream Infections (BSI) if people use it for a long time for domestic and drinking purposes. We have also found a significant number of fecal coliforms in these samples. It was also demonstrated that almost all the bacteria have become

resistant to Cefixime which is also a strong antibiotic. Thus, necessary steps should be taken immediately to treat these river water and set up a treatment plant to purify the water and also to stop industrial and chemical wastes from being dumped in these rivers.

Dedication

Dedicated to each other, for being there through the ups and downs for this period of time which felt like an eternity and for finishing this enormous commitment, which was unlike anything we had ever done before.

Acknowledgment

To begin with, we would like to convey our thankfulness and heartfelt gratitude to the Almighty for giving us all the guidance, strength, and blessings to conduct the research diligently.

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The completion of this thesis work is highly indebted to our beloved family, and friends. We would like to express our profound gratitude to our parents and siblings for all their sacrifices, understanding and support they gave us during the hard times. A special thank you to all the friends, especially Noshin Nabila Prothoma, Farzana Akter Mithil, Huma Hassan, Razit Kabir, Maliha Bushra Hoque, Sanjoy Dev, Rahnuma Masud and loved ones who believed in us, helped us and was there for us in every ups and downs we had during the time. Also, thanks to all the labmates for their support and help while working together and for maintaining a healthy and peaceful lab environment which helped us to complete the work successfully.

Finally, we would like to convey our heartfelt love and thanks to each other. We could not have undertaken this journey without the continuous support, advice, patience, and faith in each other. We thank each other for always keeping one another sane and holding one another during all the panic attacks and anxiety phases in this long thesis journey.

Table of Contents:

Chapter 1

Introduction

1.1 Background (Importance of Water)

More than over 70% of the Earth's surface is covered by water. Because life on Earth began with water, it is not surprising that all living organisms on our blue planet require water. Clean water is many things: it is a necessity, a shelter, a local and global resource, a transportation corridor, and a climate regulator. And, during the last two centuries, it has become the destination for numerous contaminants dumped into the environment, as well as a newly discovered mine rich in minerals to be mined. To continue to reap the benefits of clean water and healthy oceans and rivers, we must fundamentally alter how we use and treat clean water (Bruyninckx, H. (2018) 'The environment: Clean water is life, health, food, leisure and energy'). Bangladesh is a riverine country. There are almost 700 rivers in the country. Most of the rivers originate in India and flow through different parts of Bangladesh and ultimately fall in the Bay of Bengal. But most of the rivers are so polluted that it is impossible to drink from, thus leaving the urban and rural population in a shortage of safe water. Surface water is frequently contaminated by bacteria as a result of the accumulation of trash and pollution from both human and animal origins. This contaminated water can endanger the health of residents who are exposed to it.

1.2 Possibility of Waterborne Diseases in Bangladesh

The most common waterborne disease is diarrhea, which produces excessive stooling and can lead to dehydration and death. Bangladesh sees more than a hundred thousand child deaths every year due to diarrhea-related diseases (Hasan et al. 2019). According to the 2004 Bangladesh Demographic and Health Survey (BDHS), diarrhea was responsible for 5.1% of children under the age of five and 1.2% of neonatal fatalities. Another study found that diarrhea was responsible for 6.9% of fatalities in children under the age of five (Hasan et al. 2019). Another study found that diarrhea was responsible for 1% of newborn fatalities, 15% of post-neonatal deaths, and 6% of deaths in children under the age of five (WHO, 2016). Waterborne disease is a global burden that is estimated to cause over 2.2 million premature deaths per year and more cases of illness every day. It includes, among other things, diarrhea (including cholera and dysentery), jaundice, respiratory infections, hepatitis A and E, typhoid fever, gastrointestinal diseases, and systemic illnesses. According to the World Health Organization, diarrheal illness accounts for an estimated 4.1% of the total daily global disease burden and kills 1.8 million people each year.

Drinking contaminated water raises the risk of certain diseases such as typhoid, dysentery, diarrhea, hepatitis A and hepatitis B (Shar et al. 2007). According to one study, among the bacterial origin aquatic diseases, typhoid, bacillary dysentery, and diarrhea are highly common (Parveen et al. 2008). Another study discovered a link between the microbiological quality of water and gastrointestinal illnesses (Oguntoke et al. 2009). In Bangladesh, over 80% of all infections are due to contaminated drinking water, and around 28% of children's deaths are attributed to waterborne diseases caused primarily by pathogenic microbes (Shittu et al. 2010).

1.3 Geographical Analysis of Balu and Turag river

The study was conducted on two of Dhaka's major rivers Turag and Balu. The Turag River is the Buriganga's upper tributary. It gets its name from the Bangshi River. The Turag runs through Gazipur district before joining the Buriganga at Mirpur in Dhaka. The Turag River is connected to the Balu River by the Tongi Khal (Mirza et al. 2013). The average width of the river is 218m and the average depth is about 13.5m. It has a total area of 386 square miles. On the other hand, The Balu River winds its way predominantly through the vast marshes of Beel belai and the eastern region of Dhaka. It converges with the Shitalakshya close to Demra. It is also connected to the Turag river, another major river in Dhaka as described above through the Tongi Khal. Also, it is connected to the river Shitalakshya.

1.4 Analysis of Industries near Balu and Turag river

Sample site B, the starting point of the Turag river is situated in Ashulia, Dhaka which is very close to Savar. Hence, the surrounding areas of this sampling site are filled with Textile factories. However, though the ending point of this river, sampling site A does not have any big industries, it has quite a lot of brick fields. Moreover, the initial point of the Balu river, site C is located in a remote area of Dhaka containing no major industries. The ending point however has a huge jute mill adjacent to the sample site D.

1.5 Literature Review

Bangladesh is a highly populated country and Dhaka, being the capital city, accommodates about 23 million people according to the World Population Review of 2023. Bangladesh is a country surrounded by rivers and river water has been used for agriculture, domestic and industrial purposes for many years. Severe polluting conditions have been observed in the peripheral rivers surrounding Dhaka in the last 40 years (Uddin & Jeong, 2021). The main rivers surrounding Dhaka city are Balu, Turag, Buriganga, Dhaleshwari, Tongi Khal and Sitalakhya. According to the research of (Uddin & Jeong, 2021) "Dissolve oxygen (DO) was nearly zero in Buriganga River and several points in Turag, Balu, Sitalakhya and Karnaphuli River." The quality of water of the rivers in Dhaka city has been compromised for many years due to the disposal of industrial waste, solid pollutants and untreated sewage wastes (Hoque et al., 2021). The severity of this pollution was so extreme that in 2009, the Department of Environment (DoE) declared the Buriganga, Sitalakhya, Balu and Turag rivers as ecologically critical areas (The Daily Star 2009) and the Planning Commision mentioned that these river waters are unsuitable for human use (General Economics Division 2015, p.423).

That being said, knowing the microflora residing in these rivers is very important for the assessment of health hazards and probable prevention or treatment plan. The contamination of these rivers can lead to not only water-borne diseases in humans, but also polluted agricultural yield in this country. The disposal of industrial waste into the rivers lead to heavy metal contamination in the river water. These heavy metals eventually get accumulated into the crops when the agricultural field is irrigated with the river water. According to (Chowdhury et al., 2020), a high Cr concentration has been found in rice plants that have been irrigated with Turag river water. Moreover, very high coliform and low DO has been found in the water of Balu river which indicates a high level of organic and human waste presence (Roy et al., 2014).

Along with the heavy metal contamination in the rivers of Dhaka, an abundance of fecal coliforms and *Enterobacter* are also present in these water bodies. According to (Sikdar et al., 2019), abundance of *E. coli, Enterobacter sp., Klebsiella sp., Salmonella sp., Shigella sp* etc. have been found in the Buriganga and Turag river water. If this contaminated water is consumed by humans, it can cause severe diseases including cholera, typhoid, polio etc. The contaminated

water is linked to 40 out of 50 widespread diseases in Bangladesh, including typhoid, parasitic worm infection, and diarrhea (Acharjee et al., 2011). According to research (Shumy et al., 2015), the rapid urbanization and industrialization is causing more pollution of the rivers and thus, making typhoid endemic in Bangladesh.

One of the most serious worldwide public health and development problems is antimicrobial resistance (AMR). Bacterial AMR was directly responsible for 1.27 million global deaths in 2019 and has contributed to 4.95 million deaths (WHO). Antimicrobial drugs are the foundation of contemporary medicine. Drug-resistant microorganisms are threatening our ability to treat common infections and execute life-saving treatments such as cancer chemotherapy and cesarean section, hip replacements, organ transplantation, and other surgeries. Moreover, AMR doesn't only pose a health threat but also is responsible for economic threat. AMR has severe economic effects in addition to death and disability. According to the World Bank, AMR might result in $1 trillion in additional healthcare expenses by 2050, as well as $1 trillion to $3.4 trillion in annual GDP losses by 2030 (The World Bank). According to a research (Timothy et al., 2023), Prolonged antibiotic abuse and misuse has resulted in the emergence of'super-bugs' that are well-known to many, such as methicillin-resistant *Staphylococcus aureus*.

Historically, the focus of antibiotic resistance research has been on bacterial infections and the consequences of resistance for human health. Of course, from a clinical perspective, antibiotic resistance is critical because it can jeopardize advanced therapeutic procedures like immunosuppressive transplantation or anticancer therapy, which require potent anti-infective preventive therapies, as well as the treatment of infectious diseases (Jose et al., 2012). Given that the environmental microbiota is the source of antibiotic resistance, it would be required to study resistance in natural, non-clinical settings in order to completely comprehend the cycle of resistance acquisition by human infections. Furthermore, the use of functional genomic and metagenomic techniques has shown that a vast array of components found in natural ecosystems—including soils and the human gut—can impart resistance to any antibiotic when they are transmitted to a new host (D'Costa et al., 2006).

Antibiotic resistant bacteria in river water is a massive threat to the world. Especially in river irrigated countries like Bangladesh. Most of the people of Bangladesh are dependent on river water for various reasons. AMR in Bangladesh rivers might pose immense health risks to the people. Bacteria with inherent antibiotic resistance can be found in nature. Surface water bodies of Bangladesh have become contaminated in recent years as a result of uncontrolled development, industrialization, and faulty sewage systems. According to a study, 100% of *Salmonella spp*. isolates were resistant to amoxicillin and 90% were resistant towards cefepime (Chakma et al., 2018). Not only the rivers, other surface waters have also been contaminated by AMRs such as β-lactamase-producing *Escherichia coli* (ESBL-Ec) and carbapenem-resistant *E. col*i (CR-Ec). In rural homes, ESBL-Ec was found in 5% of examined drinking water samples, although it was found in a considerable proportion of wastewater (90%), pond water (76%), and river water (85%) samples. Moreover in urban houses, 38% of drinking water samples and 98% of wastewater samples from food markets were ESBL-Ec positive, whereas 30% of wastewater samples were CR-Ec positive (Asaduzzaman et al., 2022).

Measures should be taken as soon as possible to stop antimicrobial resistance. According to Nicholai Schaff, head of Stockholm International Water Institute, To fight antimicrobial resistance, antibiotics manufacturing must prioritize the prevention of pharmaceutical waste polluting water. The World Health Organization (WHO) launched the Global Action Plan on Antimicrobial Resistance (GAP) in 2015 to combat antimicrobial resistance through a comprehensive One Health approach to AMR in humans, animals, and the environment. The One Health conceptual framework analyzes human-animal-environment interactions in which resistance can traverse social, ecological, and habitat borders. The WHO's GAP has five key goals: (1) increasing awareness and understanding, (2) boosting surveillance and research, (3) reducing the frequency of illness, (4) optimizing the use of antimicrobial drugs, and (5) assuring long-term investment in resistance management.

1.6 Gaps In Previous Studies

Bangladesh being a riverine country, there have been numerous studies of the river waters of Bangladesh. Most of these researches focused on assessing the quality of the water by measuring

BOD and DO. There have also been studies regarding heavy metal contamination in the river water of Dhaka as there are many industries around the rivers in Dhaka. These researches indicated the presence of heavy metals like Cr and Pb in the water and the accumulation of these metals in the agricultural products around these river areas.

However, the complete bacteriological study of the rivers in Dhaka is what was found lacking in these previous studies. Although the microbial study of the major rivers like Padma, Jamuna and Meghna are prevailing, there was little to no data of the microbial studies of the rivers in Dhaka and especially Balu river. This lacking in the previous researches laid the foundation of the design of this particular study which focuses exclusively on the Turag and Balu river. Thus, this research was planned to get the idea of the microflora residing in the Turag and Balu rivers and to test their resistance to specific antibiotics.

Chapter 2

Materials and Methods

2.1 Study Area/Sampling Sites

The target area for this experiment was Dhaka. Two rivers of Dhaka city, Balu and Turag river are selected for the bacteriological study. For this study, water samples from the starting and ending point of both the rivers were collected. Thus, a total 4 sets of samples labeled as Site A, Site B, Site C and Site D are used to conduct this study. Where sample site B is the initial point of the Turag river and A is the ending point. Also, C and D are the initial and ending points of the Balu river, respectively.

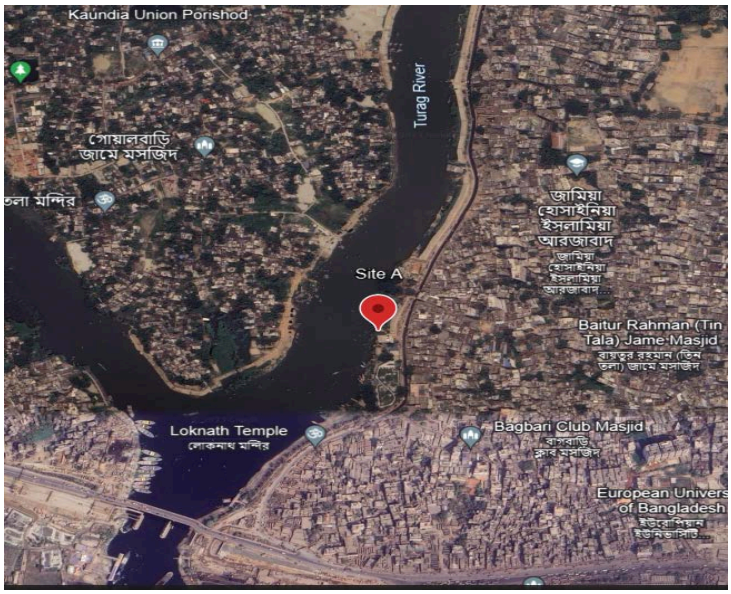
Site A: Boro Bazar Ghat, Mirpur. (23.7883 N, 90.3396 E)

Site B: Ashulia, Dhaka (23.8925 N, 90.3601 E)

Site C: Ichapura Bazar (23.8293 N, 90.4912 E)

Site D: Demra Staff Quarter (23.7229 N, 90.4979 E)

The reason for selecting these rivers is to get an idea of what types of bacteria reside in the water bodies in Dhaka city. As all the rivers are connected to each other, thus studying the microorganisms residing in the starting point and ending point of each of the rivers gives a brief idea about where the bacteria might have come from. Additionally, studying the microorganisms in the end point of a river gives an idea about which river the microorganisms are flowing into and thus contributing to the contamination. **Site A:**



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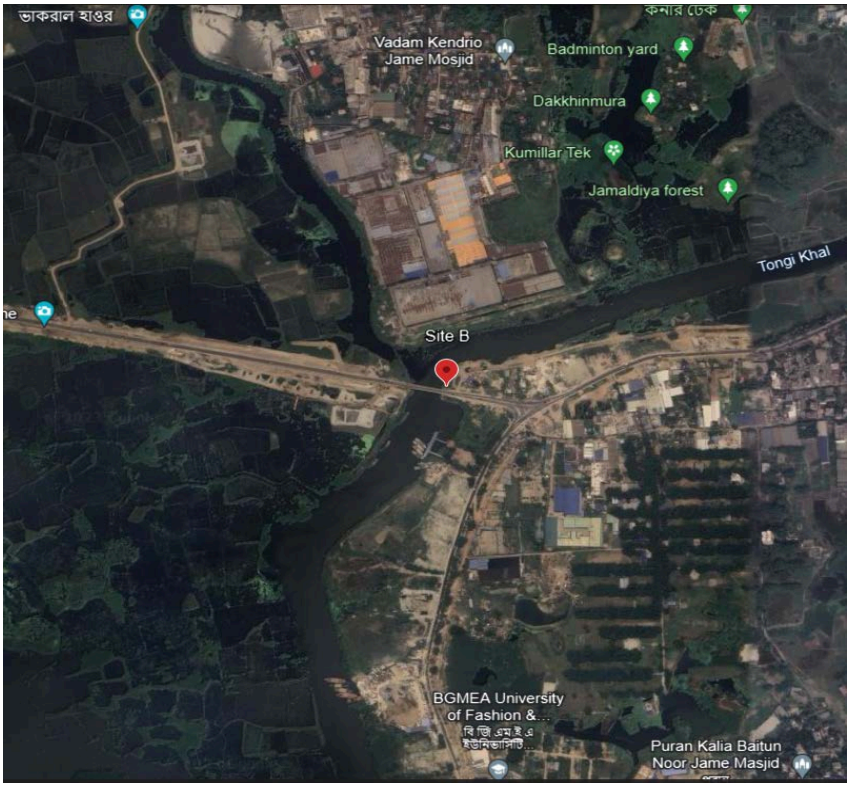
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Dakkhinmura
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Site B
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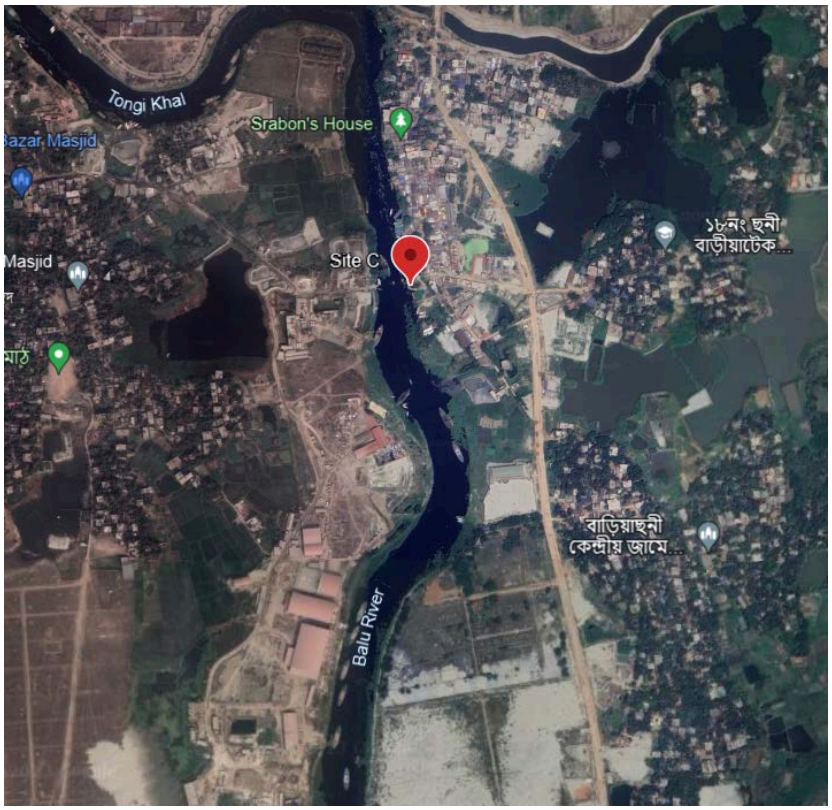
Puran Kalia Baitun
Noor Jame Masjid

Site C:


Tongi Khal
Srabon's House
Site C
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বাড়ীয়াটেক...
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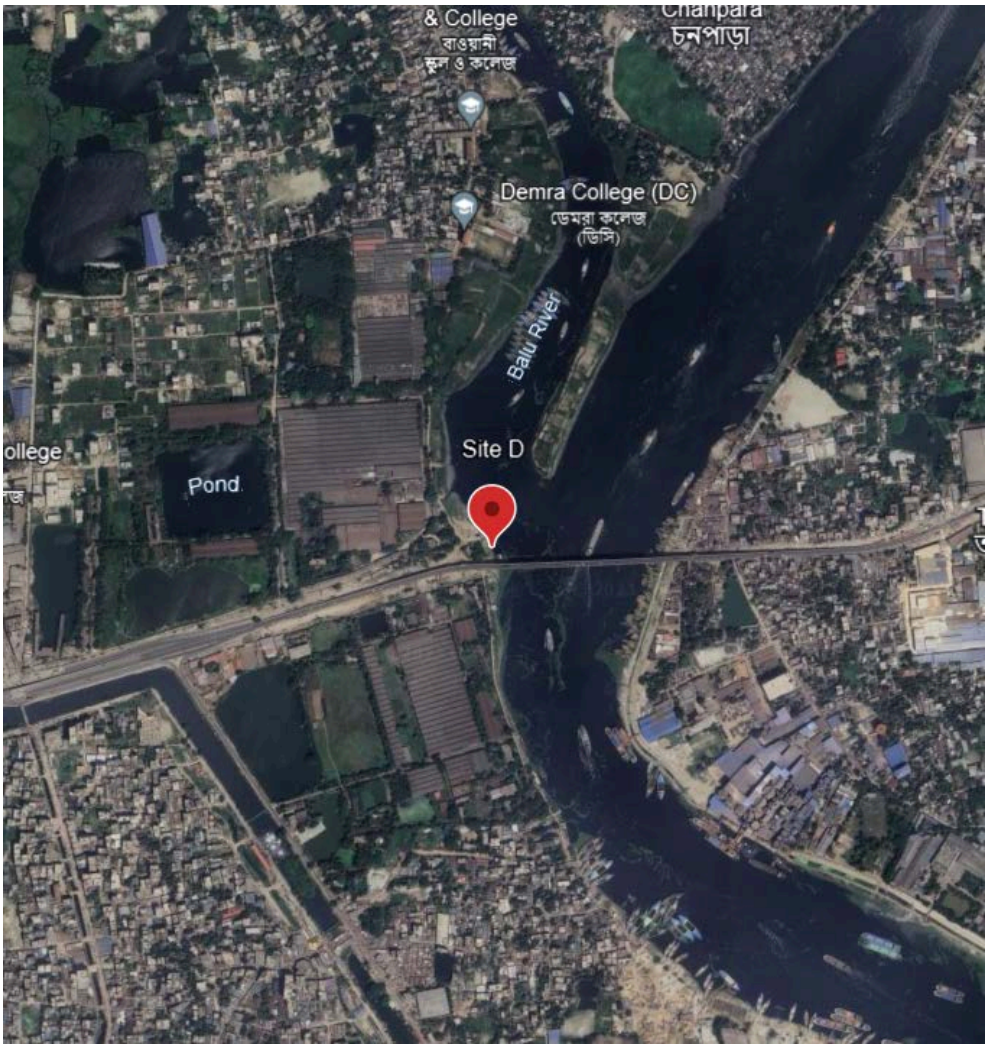
কেন্দ্রীয় জামে...
Balu River

Site D:


& College
বাওয়ানী
স্কুল ও কলেজ
Chanpara
চনপাড়া
Demra College (DC)
ডেমরা কলেজ
(ডিসি)
Balu River
Site D
Pond

2.2 Sample Collection

For each river, two sampling sites were determined. So a total of 4 sets of samples were collected.

Equipment: The equipment used for sample collection were: One 400ml sample collecting bottle and two 15ml falcon tubes. All the equipment used for sample collection were autoclaved beforehand.

Collection: While collecting the water sample from the desired site, the water bottles and falcone tubes were first submerged into the water and then uncapped to allow only mid-stream water to enter into the bottles and tubes. Caps were closed under the water before the sample collecting bottles were brought out of water in order to avoid any contamination. After that, the bottles and tubes were sealed properly and labeled with the site name and number of samples.

2.3 Sample Transportation

The samples were carried and transported to the laboratory maintaining cold chain. As soon as the samples were collected, they were placed inside an ice box and transported to the laboratory maintaining 4-8 degree celsius temperature. All the samples were processed within 6 hours after collection.

2.4 Sample Processing

Among the collected samples, the 400ml water bottle and one falcon tube is used for sample processing. The other falcon tube sample was stored at 4 degree celsius temperature as a backup. After collecting the samples, they were processed in two ways: Membrane filtration and Inoculation.

2.4.1 Membrane Filtration

The water samples collected in the 400ml bottle were used for the membrane filtration. The objective for using this technique is to detect the presence and enumeration of fecal coliform bacteria.

In this procedure, 100ml water from the collected samples was passed through a porous membrane with the help of the membrane filtration system. The membrane can trap microorganisms that are larger than 0.45 μm.

Figure 2.4.1: Membrane Filtration System

After filtering the water through the membrane, the membrane is placed on a selective media. In this study, the membrane was placed on M-FC agar media. The M-FC agar media is selective for the fecal coliforms. After placing the membrane on the M-FC agar plate, the plate is incubated at 44.5 degree celsius for 24 hours. Finally the plates were observed for presence of fecal coliform colonies and subsequent enumeration if countable.

2.4.2 Inoculation

The second processing step, inoculation can be divided into three parts: Serial dilution, Inoculating selective media and transferring to Nutrient Agar.

Serial Dilution:

The 15 ml falcon tube water sample was used for serial dilution. The undiluted sample was labeled as raw. The water sample was diluted to a factor of 10^7 in sterile saline water.

Inoculation in Selective Media:

After the samples were diluted, they were plated on different selective media using the spread plating method. The chart below shows the list of selective media used, presumptive isolation and the factor of dilution used while spread plating.

Table 2.4: This table shows the list of selective media used for this experiment and the Presumptive Identification of the isolates

Agar Media	Presumptive Identification	Dilutions Used
Cetrimide Agar (HiMedia, Mumbai, India)	*Pseudomonas aeruginosa* display fluorescence underneath UV light	10^{-2}, 10^{-3}
TCBS Agar (Himedia, Mumbai, India)	*Vibrio cholerae* is yellow with a dense center and glowing edges (Routh et al., 2018).	Raw sample, 10^{-1}
MSA agar (HiMedia, Mumbai, India)	*Staphylococcus aureus* gives golden yellow color colonies	Raw sample, 10^{-1}

MacConkey Agar (HiMedia, Mumbai, India)	*Klebsiella pneumoniae* is pink mucoid colony *Escherichia coli* is red or pink non-mucoid	10^{-7}
XLD agar (Oxoid, UK)	*Salmonella typhi* gives black centered red colony *Shigella* spp has red colony	10^{-2}, 10^{-3}

Along with these selective media, 10^{-6} dilution was used to inoculate NA media. All the inoculated plates were incubated at 37 degree celsius temperature for 24 hours.

Colony Selection:

After the incubation period, the plates were checked for distinct colonies. Colonies with distinguishable morphologies were selected from each plate. A total of 43 colonies or isolates were selected for further studying. Each of the colonies were given a codename which contains initials of the growth media and a number.

Transferring to Nutrient Agar media:

All the selected colonies were then transferred to NA media by using streak plating method. These transferred colonies on NA are then used for further tests and research. The colonies were subcultured each 3-4 days on NA in order to use fresh cultures always for testing.

2.5 Stock Preparation

After all the isolates to be used for this research were selected and transferred to NA media, it was important to prepare a stock of these colonies to be used as a backup or revival purpose. The stock was prepared in sterile T1N1 media which is also called soft agar.

Components of T1N1:

I. Tryptone
II. NaCl

III. Agar Powder
IV. Distilled Water

The sterile T1N1 media that has been prepared in vials was inoculated with each isolate by using a stabbing method and then incubated for 24 hours. After 24 hours, if growth is observed, then sterile paraffin oil was added into the vials. Finally, the vials were completely sealed using parafilm and each vial was labeled properly with the codenames previously generated for each isolate.

2.6 Microbial Identification

The isolates transferred on NA media were then subjected to different biochemical tests and PCR. The purpose of performing these biochemical tests was to study different properties of the microorganisms and identify them. And the purpose of performing PCR test was to identify the isolates at the molecular level and confirming the biochemical test results.

2.6.1 Biochemical Test

The biochemical tests that were performed for this study are:

I. Oxidase Test
II. Catalase Test
III. IMViC Test
    A. Indole Test
    B. Methyl Red Test (MR)
    C. Voges-Proskauer Test (VP)
    D. Citrate Utilization Test
IV. Motility-Indole-Urease test (MIU)
V. Triple Sugar Iron test (TSI)
VI. Nitrate Reduction Test

I. Oxidase Test

Purpose: The oxidase test is performed to detect the presence of cytochrome oxidase in a bacteria. If a bacterium uses the cytochrome oxidase enzyme as a part of its respiration system, then it will render a positive result in oxidase test. The cytochrome oxidase catalyzes the

oxidation of cytochrome c. What happens in this reaction is, the oxidase test reagent (tetra-methyl-p-phenylenediamine dihydrochloride) acts as an artificial electron donor for cytochrome c. When the reagent is oxidized by cytochrome c, a compound named indophenol blue is formed which renders a dark purple color. Thus, a positive result is determined by observing the dark purple color formation. A negative result gives no color.

Equipment:

1. Whatman filter papers
2. Sterile toothpick
3. Fresh colonies (24-48 hours)
4. Kovac's Oxidase Reagent

Reagent: Kovac's Oxidase Reagent - 1% tetra-methyl-p-phenylenediamine dihydrochloride mixed in distilled water.

Procedure: To perform this test, a drop of Kovac's Oxidase reagent was added on a Whatman filter paper. Using a sterile toothpick, a fresh single colony is picked up from nutrient agar plates. Then the colony that was picked up is smeared on the Whatman filter paper where the drop of reagent was previously added.

Positive control: *Pseudomonas aeruginosa*

Negative control: *Escherichia coli*

II. Catalase Test

Purpose: The purpose of performing catalase test on the found isolates is to differentiate between the bacteria by detecting the presence of the catalase enzyme in them. As a part of the bacterial defense mechanism, some bacteria produce catalase enzyme that helps them escape oxidative damage caused by hydrogen peroxide (H_2O_2). The catalase enzyme breaks down H_2O_2 and produces water and oxygen thereby neutralizing the bactericidal effects of hydrogen peroxide.

Equipment:

1. Glass slides
2. Dropper
3. Fresh culture (24-48 hours)
4. Sterilized inoculating loop

Reagent: 3% Hydrogen Peroxide solution

Procedure: Using a sterile inoculating loop, a fresh single colony of 24-48 hours from nutrient agar is picked up and smeared on a sterile glass slide. Next, 2-3 drops of 3% H_2O_2 solution is added on the smear. The positive results are determined by observing bubbles formation from the smear. Negative results show no bubble formation.

Positive control: *Staphylococcus aureus*

Negative control: *Streptococcus pyogenes*

III. IMViC Tests

A. Indole Test

Purpose: The purpose of the Indole test is to distinguish the bacteria that can degrade the amino acid tryptophan and produce indole. This test is a part of IMViC, a set of tests designed to distinguish among the members of Enterobacteriaceae. Only the bacteria that can express tryptophanase enzyme can cause the deamination of tryptophan and give a positive result by producing indole.

Equipment:

1. Sterile test tubes
2. Tryptone broth
3. Fresh cultures (24-48 hr)
4. Sterile inoculating loop
5. Kovac's Reagent

Procedure: At first in sterile test tubes tryptone broth is taken and autoclaved. Tryptone broth is made by adding tryptone and NaCl in distilled water. Using a sterile loop the tryptone broth is inoculated with a small amount of pure culture. The inoculated broth is incubated at 37°C for 24-48 hours. After the incubation, Kovac's reagent is added to check for indole production. Kovac's reagent contains isoamyl alcohol, HCL and p-dimethylaminobenzaldehyde. After adding 5 drops of Kovac's reagent, the positive results will be determined by a cherry ring (pink or red color) at the top of the media. As isoamyl alcohol is not water soluble, the red color forms as an oily layer at the top and creates a ring. In this biochemical reaction, the indole reacts with p-dimethylaminobenzaldehyde and forms a red dye named rosindole.

Positive control: *Escherichia coli*

Negative control: *Pseudomonas*

B. Methyl Red Test (MR)

Purpose: The Methyl Red (MR) test detects the acid production by bacteria during fermentation of glucose. Some bacteria ferment glucose by the mixed acid pathway and produce stable acids like lactic acid and acetic acid, CO_2 and H_2 gas. As a result of the production of acids in a large quantity, the pH of the culture media decreases significantly. This pH change can be indicated by adding Methyl Red, a pH indicator which gives a red color when the pH is 4.5 or lower. The MR test is also a part of IMViC tests that are used to differentiate between the members of the Enterobacteriacea family.

Equipment:

1. Glucose Phosphate Broth
2. Sterile test tubes
3. Fresh culture (24-48 hr)
4. Sterile inoculating loop
5. Methyl Red

Procedure: MR broth contains peptone, dipotassium phosphate and dextrose. MR broth is inoculated with a fresh culture using a sterile loop. The culture is then incubated at 37°C for 24-48 hours. After the incubation, 5 drops of methyl red are added into the media. A red color formation in the media indicates acid production and thus renders a positive result. A negative result is determined by a yellow color in the medium.

Positive Control: *Escherichia coli*

Negative Control: *Klebsiella pneumoniae*

C. Voges-Prokauer Test (VP)

Purpose: VP test is performed together with MR test to detect which bacteria uses the mixed acid pathway and which ones use the butylene glycol pathway to ferment glucose. VP is also a part of IMViC set of tests for indicating distinguishable properties between members of Enterobacteriaceae.

The VP test is a biochemical procedure used to determine if bacteria can convert pyruvate into a neutral intermediate product called acetylmethylcarbinol or acetoin. In the presence of KOH, $\propto$ – naphthol catalyzes the oxidation of the intermediate product acetoin into diacetyl. This diacetyl reacts with the guanidine component of peptone in the presence of $\propto$ – naphthol and forms a red or pinkish color. This distinct color formation indicates a positive VP result.

Equipment:

1. Glucose Phosphate Broth
2. Sterile test tubes
3. Fresh culture (24-48 hr)
4. Sterile inoculating loop
5. Barrit's reagent A
6. Barrit's reagent B

Procedure: The VP broth contains the same components as MR broth. Barrit's reagent A contains α-naphthol reagent in 95% ethanol and Barrit's reagent B contains 40% KOH dissolved in distilled water.

The autoclaved broth is inoculated with pure culture and incubated at 37°C for 24-48 hours. Following the incubation, 12 drops of Barrit's A and 4 drops of Barrit's B are added into the media. Within 30 minutes, a color formation is observed. A pink or red color formation indicates a positive result.

Positive control: *Klebsiella pneumoniae*

Negative control: *Escherichia coli*

D. Citrate Utilization Test

Purpose: The Citrate Utilization Test is used to screen for those bacteria that can use the citrate as their sole source of carbon and energy for survival. The Simmons Citrate Agar used in this test contains Bromothymol Blue, a pH indicator. When the bacteria can use the citrate as their carbon and energy source, the pH of the media will increase due to the production of alkaline byproducts. When the pH of the media is increased above 7.6, the pH indicator will change its color from green to blue. The positive result is indicated by observing growth on the media and the blue color of the media. If the media remains green after incubation, then it is considered as citrate negative bacteria.

Equipments:

1. Sterilized Vials
2. Simmons Citrate Agar
3. Fresh Culture
4. Sterilized Inoculating Loop

Procedure:

The Simmons Citrate Agar is prepared by mixing with appropriate amount of distilled water and heated till boiled. After autoclaving, the media is poured into the vials in a sterilized environment and cooled down by keeping in a slanted position. After the media solidifies, it is inoculated with fresh culture using a sterilized loop and incubated for 24-48 hours at 37℃. Then bacterial growth and the color change of the media is observed. Blue colored media after incubation indicates a positive result and no color change indicates a negative result.

Positive Control: *Klebsiella pneumoniae*

Negative Control: *Escherichia coli*

IV. Motility-Indole-Urease test (MIU)

Purpose: The MIU test is performed to identify bacteria species on the basis of their motility, indole and urease production. This is an identification test that is especially used to differentiate between the members of Enterobacteriaceae.

The MIU agar used for this test contains Phenol Red as a pH indicator and it is a semi-solid media. Motile bacteria give a scattered growth from the stab line or render a diffused growth or turbidity in the media while the non-motile bacteria will only grow along the stab line.

The bacteria that contain urease can hydrolyse the urea present in the MIU media and release ammonium carbonate as a by-product. The alkaline ammonium carbonate increases the pH of the media and thus, the pH indicator phenol red turns from yellow to pink-red. So the pink-red color change of the media indicates a urease positive result.

Bacteria that contain tryptophanase enzyme can degrade the tryptophan present in the media and produce indole. To check for the production of indole, Kovac's reagent is added in the media after incubation. The indole reacts with the p-dimethyl amino benzaldehyde of the reagent to form a red ring on top of the media.

Equipment:

1. Sterilized Test Tubes
2. MIU Agar
3. Inoculating Needle
4. Fresh Culture
5. Kovac's Reagent

Procedure: At first MIU media is prepared. MIU agar is dissolved in distilled water and heated until boiled and autoclaved to make it sterile. After the media is cooled down to about 50℃, 5 ml

sterile 40% urea solution (per 95ml media) is added in a sterile environment. Then the media is poured into the sterile test tubes and set to cool down.

After the media is cooled down, the semi-solid media is inoculated using a sterile needle with fresh culture and incubated for 24-48 hours. Post incubation the color of the media and the growth of the organism along the stab line is observed. If there is scattered growth of bacteria extended away from the stab line, then the result is considered to be motility positive. Growth only along the straight stab line indicates motility negative result.

If the color of the media is changed from yellow to pink or red, the result is urease positive , otherwise it is urease negative result. And finally, Kovac's reagent is added in the test tubes for indole detection. If a red ring forms on top of the media, it is indole positive and no ring means indole negative result.

Positive Control: *Escherichia coli* (Motility- Positive, Indole- Positive, Urease- Negative)

Negative Control: *Klebsiella pneumoniae* (Motility- Negative, Indole- Negative, Urease- Weakly Positive)

V. Triple Sugar Iron test (TSI)

Purpose: TSI test is used to differentiate among the Enterobacteriaceae based on their ability to ferment Glucose, Sucrose and Lactose; and also their ability to produce H_2S. Gas production from carbohydrate fermentation can also be detected through this test.

The TSI agar contains glucose, sucrose and lactose. Phenol red is present in the media as a pH indicator. When the bacteria ferments any of these sugars, the acid produced from the fermentation reduces the pH of the media and the phenol red changes the media color from orange-red to yellow. If the bacteria only ferments glucose, the fermentation will take place in the butt and turn the butt color yellow while the slant will remain red as the oxidative degradation of peptone prest in the media will occur in the slant. And if the bacteria ferments both sucrose and lactose, it produces enough acid to turn both the butt and slant yellow. This will mean that the bacteria can ferment all three sugars. In case of no fermentation the color of the media will remain unchanged.

If gas is produced from the fermentation it will either be seen as bubbles formed in the media or will crack the media or push the media upwards leaving a gas bubble at the bottom of the tube.

The bacteria that can produce H_2S will degrade the sodium thiosulfate present in the media and release H_2S gas. The H_2S gas will then react with ferric ions and produce ferrous sulfide which is

an insoluble black precipitate in the media. Thus, the black precipitate in the media will indicate H_2S producing bacteria.

Equipment:

1. Sterilized Test Tubes
2. TSI Agar
3. Inoculating Needle
4. Fresh Culture

Procedure:

The TSI media is prepared by dissolving the agar in distilled water and autoclaving it. After the media is poured into test tubes, the tubes are cooled down in a slanted position and then stabbed with a sterile needle with fresh culture. After incubating for 24-48 hours the color change of the media is observed.

Controls:

1. *Escherichia coli* - Yellow/Yellow, Gas positive, H_2S negative
2. *Shigella* - Red/Yellow, Gas negative, H_2S negative
3. *Salmonella Paratyphi* - Red/Yellow, Gas positive, H_2S positive

VI. Nitrate Reduction Test

Purpose: This test specifically tests for the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) by an organism which aids in the identification and differentiation among various microbial species. The process of nitrate reduction by bacteria is facilitated by the enzyme nitrate reductase. If a bacteria can reduce nitrate, it means that the microorganism can use (NO_3^-) as an electron acceptor during their anaerobic respiration. Some bacteria can also reduce nitrite and produce various nitrogen products.

Equipment:

1. Nitrate broth
2. Reagent A (N,N-dimethyl-a-naphthylamine)
3. Reagent B (Sulfanilic acid)
4. Zinc Dust
5. Fresh culture
6. Wooden stick

Procedure: Nitrate broth is prepared and put into fresh test tubes and autoclaved. After the media cools down, loopful of fresh cultures are taken and the broth is inoculated. Followed by a 24-48 hour incubation, reagent A and B are added in order.

If the tested organism reduces nitrate to nitrite, the nitrite present in the media will react with the reagents and form a red azo dye called Prontosil. However, if the nitrite has also been reduced by the bacteria, then the red color will not form after adding the reagents. In that case, zinc dust is added which is a powerful reducing agent. Zinc can reduce the nitrate to nitrite therefore, a red color appearance after adding zinc dust means that there was nitrate present in the media.

Thus, a red color formation after adding reagents renders a positive result. If no color is changed after adding the reagents, then zinc dust is added. If still there is no color change in the media then it will also be included as a nitrate reducing organism thereby a positive result. However, if color appearance is caused by zinc dust then it will mean a negative result.

Controls:

Positive control: *Escherichia coli*

Negative control: *Streptococcus*

2.7 Antibiotic Susceptibility Testing

Antibiotic resistance is a worldwide problem in the present times. After only eight decades of using antibiotics, even the infections that could previously be treated easily by using antibiotics are now becoming untreatable (MacGowan & Macnaughton, 2017). The novelty of our study is to demonstrate the antibiotic resistance pattern of the microorganisms residing in the rivers of Bangladesh.

The Kirby-Bauer disk diffusion test is implied in this study to test for bacterial susceptibility towards specific antibiotics. Mueller-Hinton Agar (MHA) was used for this test. At first, bacterial suspension was prepared by dissolving loopful of the bacteria to be tested into 0.9% sterile saline solution. The bacterial suspension was evaluated against 0.5 McFarland standards to maintain the 1.5×10^8 cells/ml quantity. Next, sterile cotton swabs were dipped into the suspension and using the dipped swab, the surface of the MHA agar plates were streaked three times from different angles to make sure that the whole area is swabbed with the suspension. After that the specific antibiotic discs are placed on the agar surface and incubated at 37ºC for 24

hours. Following the incubation period, the zones of inhibition around each antibiotic disc are measured in diameters and the results were predicted as either resistant (R), intermediate (I) or susceptible (S) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Table 2.7: The following table shows the list of antibiotics used in this study to test the susceptibility and resistance of bacteria:

Name of the Antibiotic	Antibiotic Symbol	Disc content (µg)
Amikacin	AK	30
Amoxicillin-Clavulanic Acid	AMC	30
Cefixime	CFM	5
Ceftriaxone	CTR	30
Ciprofloxacin	CIP	5
Erythromycin	E	15
Meropenem	MRP	10
Tetracycline	TE	30

2.8 Molecular Identification

After microbial identification of the isolates, molecular identification by Polymerase Chain Reaction (PCR) was performed for further confirmation of the isolates. For this procedure, the DNA of the isolates were extracted using the colony extraction method and then after amplifying the DNA by PCR, Agarose Gel Electrophoresis was employed to confirm the isolates.

2.8.1 DNA Extraction

To extract DNA from the organisms in order to perform a PCR test, we used the “Colony extraction” method. In this method, initially, 150mL of TE buffer was taken in Micro Centrifuge

Tube. Afterwards, a loopful of the microorganism was mixed in the buffer. The tube was then put in the heating box at 95 degrees for 20 minutes. The next step was centrifuging it for 10 minutes at 4 degrees and 10000 rpm. Lastly, the supernatant containing the desired DNA was taken out carefully.

2.8.2 PCR confirmation

Polymerase chain reaction (PCR) is a laboratory technique for rapidly amplifying millions or billions of copies of a specific region of DNA, which may subsequently be analyzed in more detail. In a conventional polymerase chain reaction (PCR) setup, initially, the essential chemicals or master mix, as well as the DNA template is added to PCR tubes. Following that, rigorous mixing and centrifugation ensure perfect homogenization and the removal of any interfering air bubbles. The next stage is the critical amplification phase, which is carried out in accordance with the prescribed thermal cycler and primer parameters. Finally, the PCR reaction's success and specificity are assessed using agarose gel electrophoresis. The master mix contains the right amount of DNA template, Taq polymerase, all dNTPs and both forward and reverse primer.

Previously isolated organisms were primarily grouped on the basis of the media they grew into. But for further and absolute confirmation PCR test was done using organism specific primers.

Table 2.8.2: This table shows the list of forward and reverse primers used to detect specific isolates

Suspected Organism	Primer Name	Primer Sequence	PCR Conditions	No. of Cycles	Amplicon Size	Reference
Vibrio spp.	*Vibrio* genus	F 5'GTCARATTGAAAARCARTTYGGG3' R 5'ACYTTRATRCGNGTTTCRTTRCC3'	94ºC 5mins 94ºC 30sec 60ºC 30sec 72ºC 30sec 72ºC 10mins	25	689	(Spilker et al., 2004)
Staphylococcus aureus	Nuc gene	F 5'GCGATTGATGGTGGATACGGT3' R 5'AGCCAAGCCTTGACGAACTAAAGC3'	94ºC 4mins 94ºC 1mins 55ºC 1mins 72ºC 1mins 72ºC 10mins	32	275	(Brakstad et al., 1992)
Salmonella typhi	InvA gene	F 5'GTGAAATTATCGCCACGTTCGGGCAA3' R 5'TCATCGCACCGTCAAAGGAACC3'	95ºC 10mins 95ºC 30sec 60ºC 30sec 72ºC 1mins 72ºC 10mins	35	284	(Yanestria et al., 2019)

A total volume of 13µl PCR mixture was used where 6.5µl master mix (Thermofisher), 1µl forward and reverse primer in each, 2.5µl nuclease free water and 2µl template DNA was added. Here the master mix contains an adequate amount of dNTPs, $MgCl_2$ and Taq polymerase to run the reaction. Then these mixtures were run in the PCR machine on organism specific conditions as shown in table above. The PCR products were then observed in gel electrophoresis method.

Chapter 3

Results

3.1 Growth Observation From Selective Media

As mentioned above, different selective media were used in this study for a presumptive idea of what species the isolates might be. By observing the growth on the selective media we could narrow down the identification process.

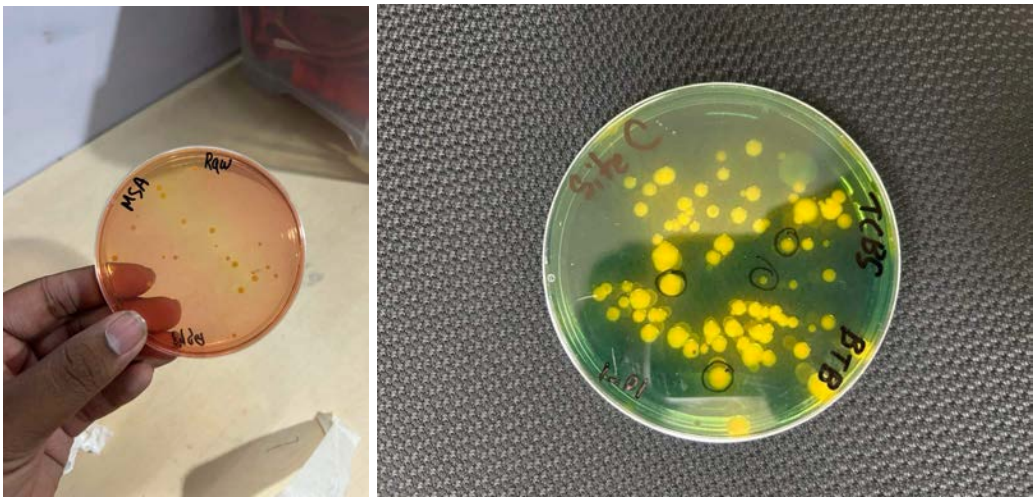


Figure 3.1: Growth Observation from Selective Media

The growth from the TCBS agar was assumed to be *Vibrio* species, similarly the growth from the XLD agar could be either *Salmonella* or *Shigella*. Similarly, MSA agar is selective for *Staphylococcus* and Cetrimide agar isolates have a high chance of being *Pseudomonas*.

Although these selective media helps to narrow down the identification process, there are also slight chances of isolates other than the presumptive ones to be grown on these selective agar. For further confirmation, a set of biochemical tests and PCR was performed.

Following the membrane filtration process, the growth in M-FC Agar was observed. All the samples were observed to contain high amounts of fecal coliforms as all of the M-FC agar growth were denoted as TNTC (too numerous to count).

Table 3.1 This table shows the number of isolates found from each site and their presumptive identification

Sample Site	Number of Isolates	Suspected Organisms
Site A	**14**	***Vibrio spp., Staphylococcus aureus, Salmonella typhi***
Site B	**5**	***Staphylococcus aureus***
Site C	**13**	***Staphylococcus aureus, Vibrio spp., Pseudomonas aeruginosa, Salmonella typhi***
Site D	**11**	***Staphylococcus aureus, Vibrio spp., Salmonella typhi***

3.2 Biochemical Test Results

A set of biochemical tests were performed for further confirmation of the isolates. Depending on the result of all the biochemical tests together, the isolates were identified accordingly.

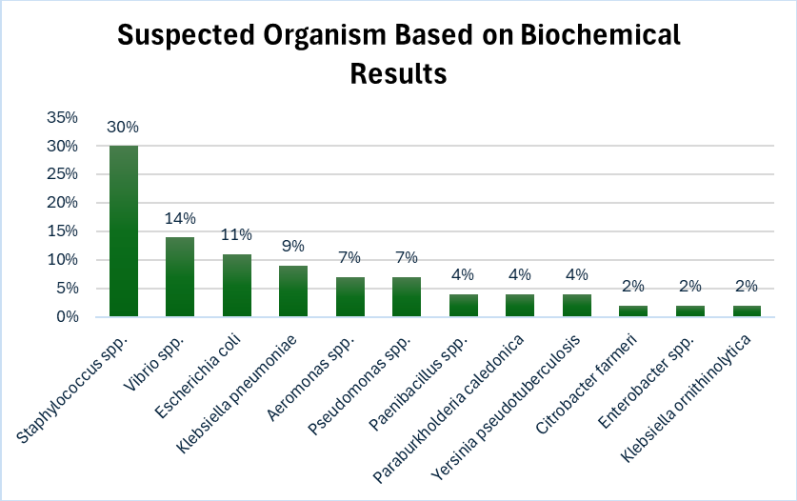


Figure 3.2: Graph of suspected organisms based on biochemical results

Based on the biochemical results of all the isolates, it can be said that *Staphylococcus spp.* was found to be predominant with the highest percentage of 30% among 43 isolates. The next species to be found abundantly in the river water was *Vibrio spp.* with a 14% and *E.coli* with a 11% prevalence. Other species were also found such as *Klebsiella pneumoniae* (9%), *Aeromonas spp.* and *Pseudomonas spp.* (7%), *Paenibacillus spp., Paraburkholderia caledonica* and *Yersinia pseudotuberculosis* (4%), and lastly *Citrobacter farmeri, Enterobacter spp.* and *Klebsiella ornithinolytica* (2%) were less prevalent among the 43 isolates.

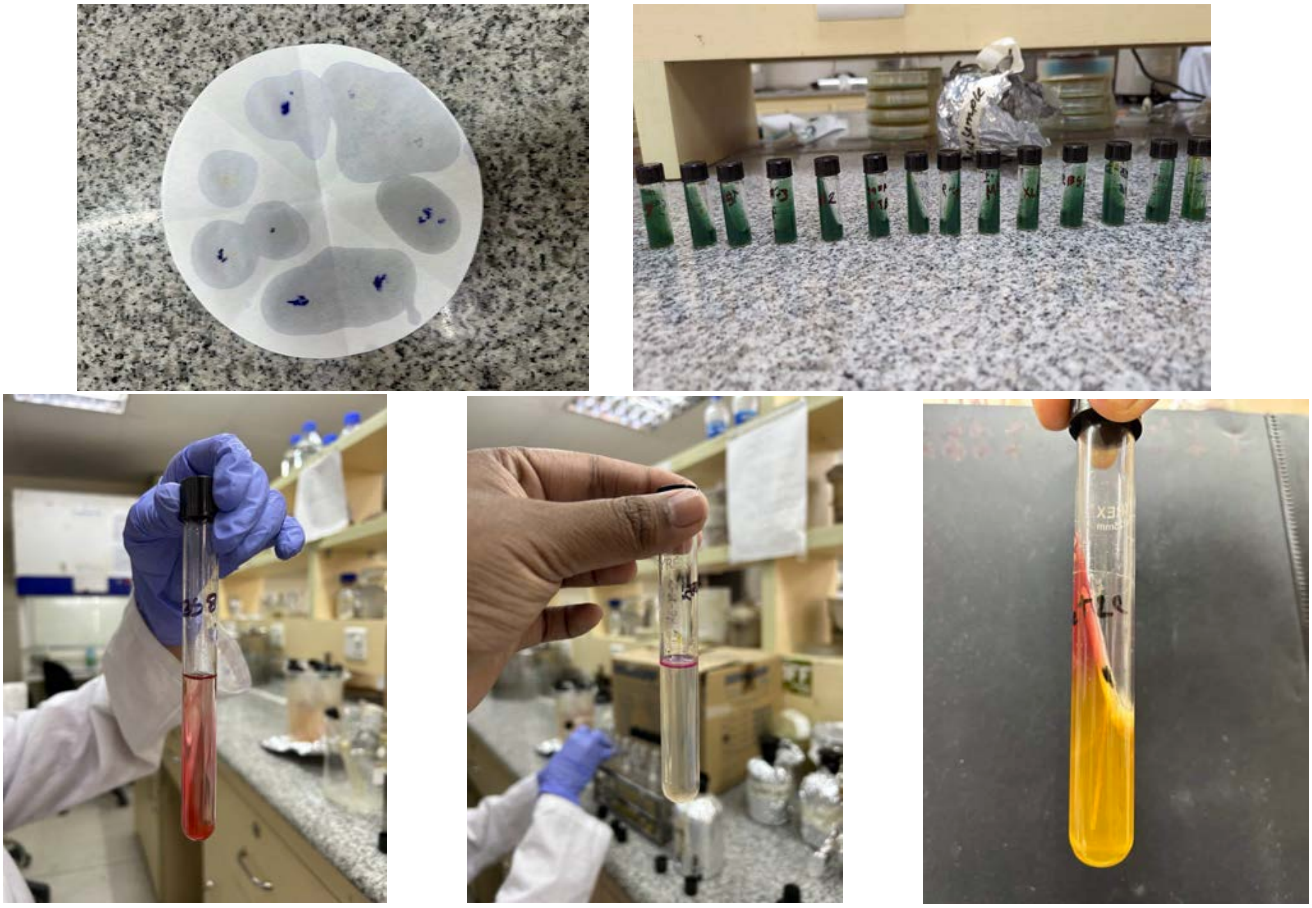


Figure 3.2: Some of the Biochemical Tests Performed for This Research

3.3 Antibiotic Susceptibility Test

The Antibiotic susceptibility test of all the isolates was conducted. Based on the graphical representation below shows almost all the isolates were resistant towards cefixime. Additionally, the isolates showed sensitivity to amoxiclav, tetracycline, erythromycin, and other antibiotics.

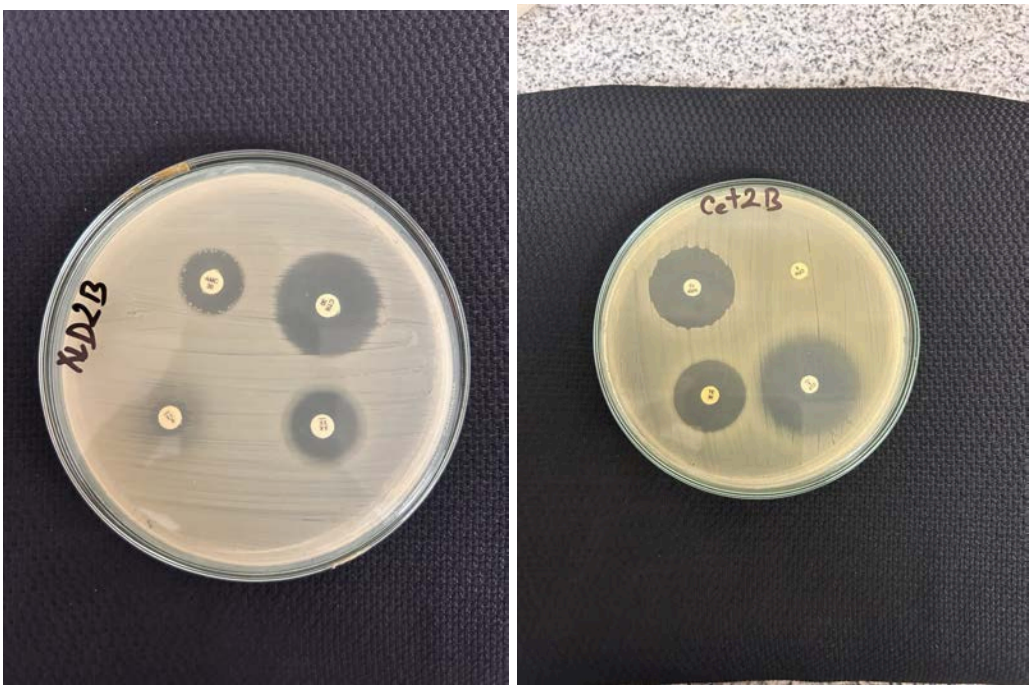


Figure 3.3.1: AST Result of Some Isolates

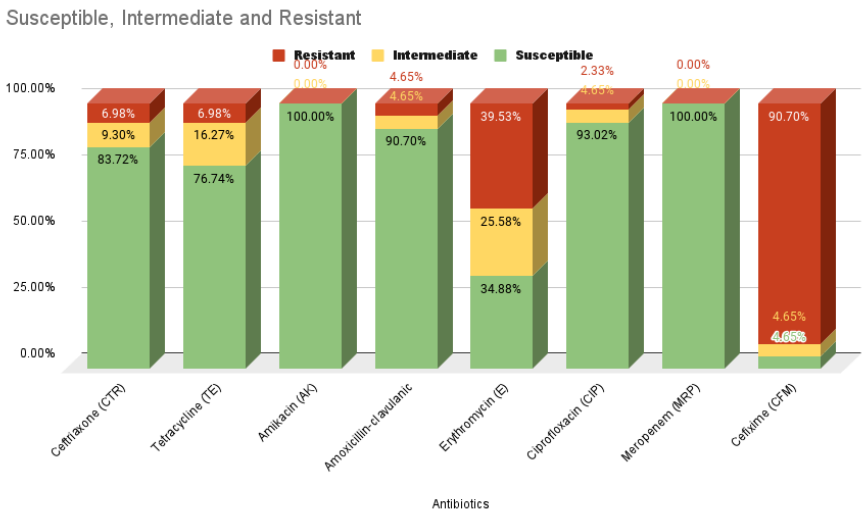


Figure 3.3.2: : Antibiotics susceptibility test ratio for all the isolates

Site A:

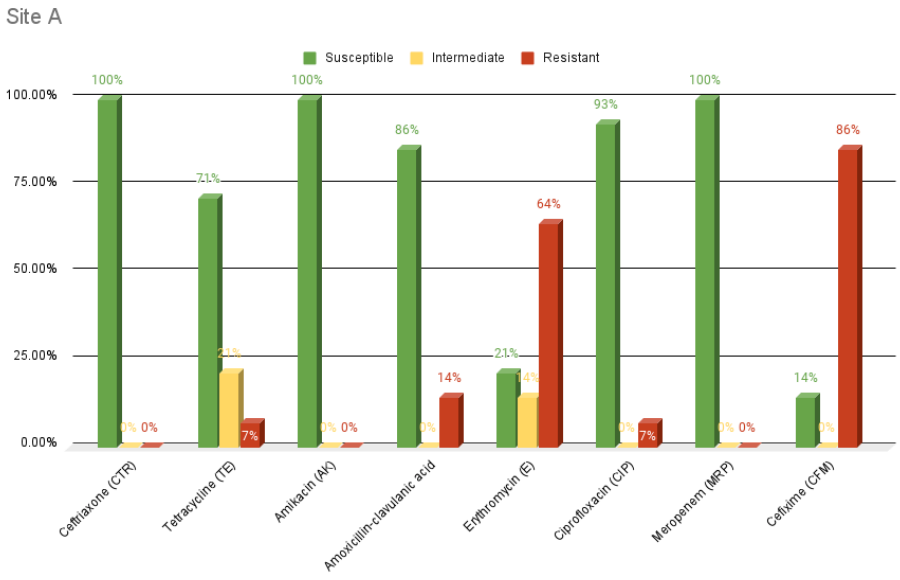


Figure 3.3.3: : Antibiotics susceptibility test ratio for site A

In this site, Site A (Turag River, Mohammadpur), we can see that all the organisms isolated from this site were susceptible to the Ceftriaxone antibiotic. In the case of the antibiotic Tetracycline, 71% of the organisms were susceptible, 21% and 8% of the organisms were intermediate and resistant respectively. Furthermore, 100% of the organisms were also susceptible towards Amikacin and Meropenem. In addition to that, 86% of the organisms isolated from this sample were susceptible towards the antibiotic Amoxicillin-clavulanic acid where 14% of them were resistant. We can see a big rise in the percentage of resistance in the next two antibiotics, Erythromycin and Cefixime with 64% and 86% of the organisms being resistant to these.

Site B:

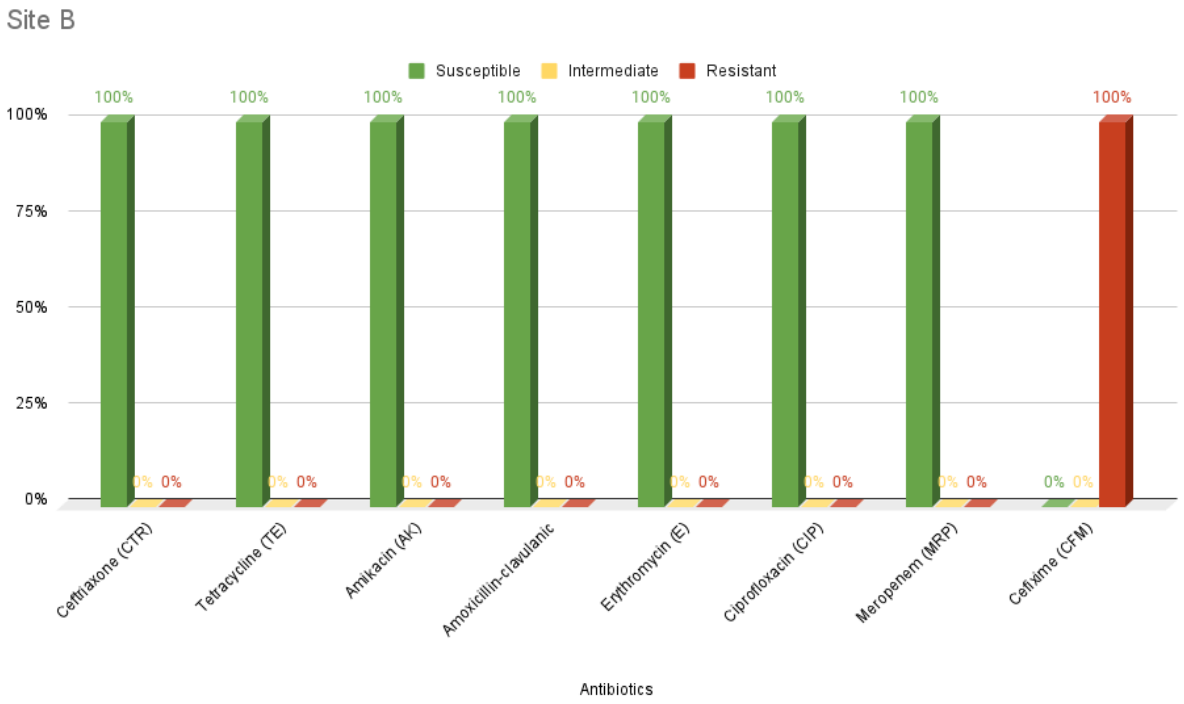


Figure 3.3.4: : Antibiotics susceptibility test ratio for site B

All the organisms isolated from Site B (Turag River, Ashulia), the entry point of the Turag River in Dhaka, were susceptible to all the antibiotics tested except for cefixime. 100% of the organisms were resistant to that.

Site C:

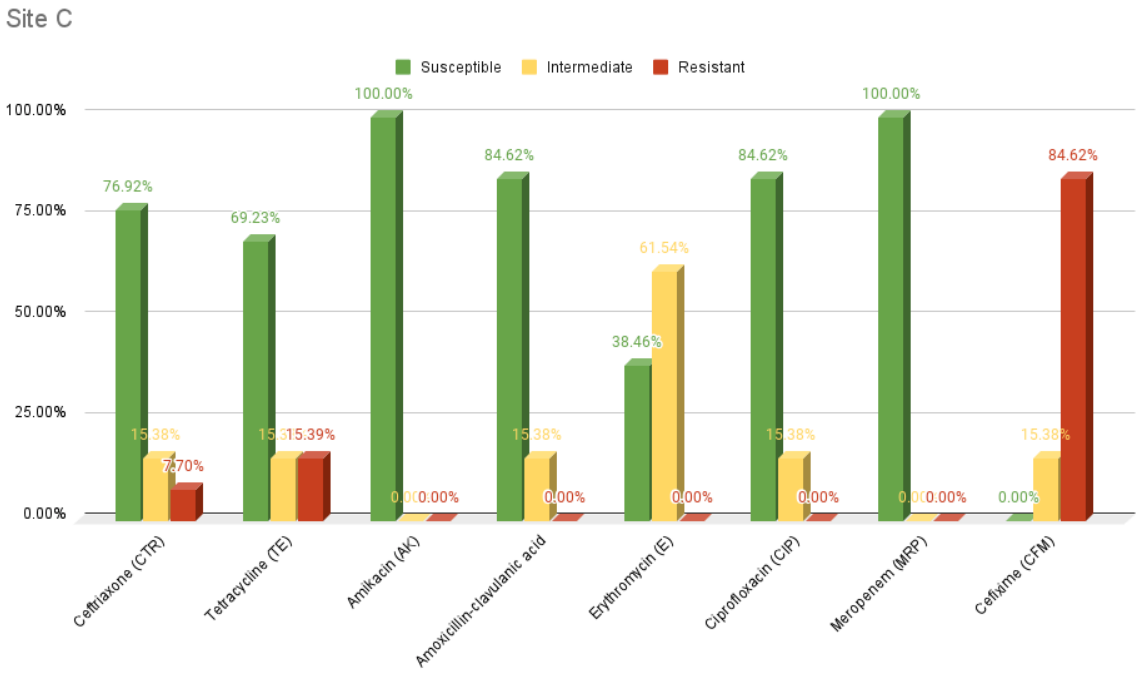


Figure 3.3.5: : Antibiotics susceptibility test ratio for site C

In sampling Site C (Balu River, Isapura Bazar), we can see that 76.92% of the organisms isolated from this site were susceptible to the Ceftriaxone antibiotic. 15.38% were intermediate and the rest (7.70%) were resistant. In the case of the antibiotic Tetracycline, 69.23% of the organisms were susceptible, 15.38% and 15.39% of the organisms were intermediate and resistant respectively. Furthermore, 100% of the organisms were also susceptible towards Amikacin and Meropenem. In addition to that, 84.62% of the organisms isolated from this sample were susceptible towards the antibiotic Amoxicillin-clavulanic acid where 15.38% were intermediate. The Erythromycin bar of the graph indicates 38.46% of the organisms being resistant to this antibiotic and 61.54% being intermediate. Furthermore, 84.62% of the isolates were susceptible towards Ciprofloxacin and the rest were intermediate. Lastly, 84.62% of the organisms were resistant to the antibiotic named Cefixime and the rest were in the intermediate region.

Site D:

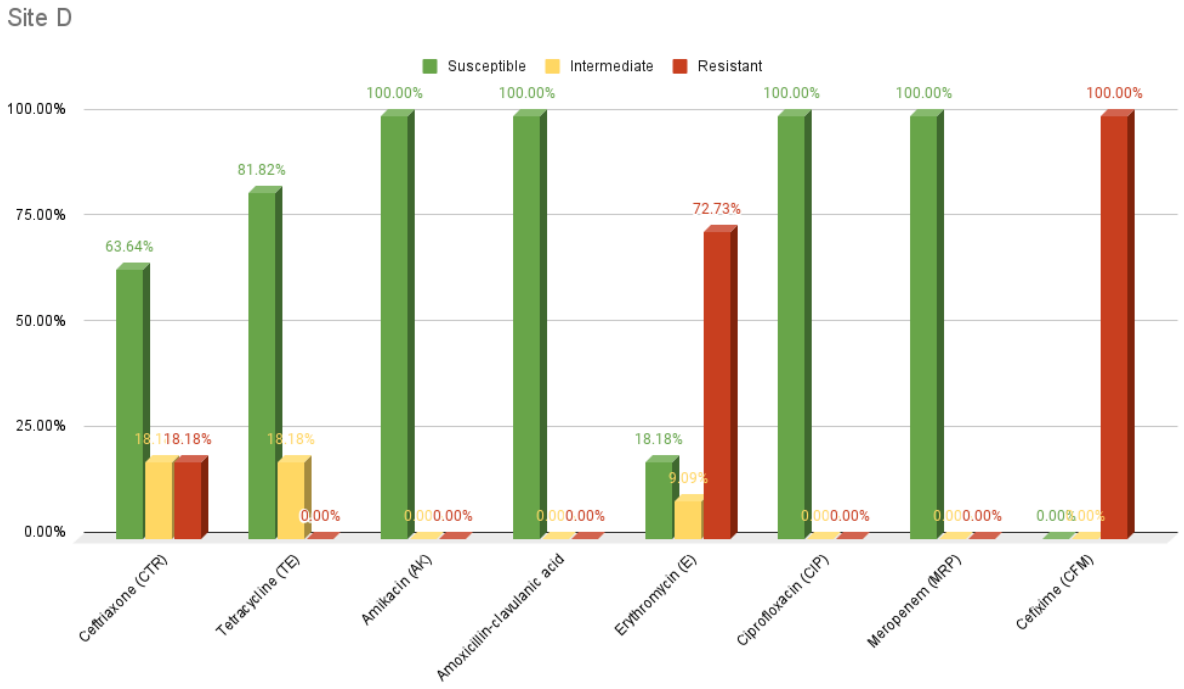


Figure 3.3.6 : Antibiotics susceptibility test ratio for site D

100% of the isolates from site D were susceptible towards Amikacin, Amoxicillin-clavulanic acid, Ciprofloxacin and Meropenem antibiotics. Ceftriaxone had 63.64% of the organisms that were susceptible to it. Both the percentage of intermediate and resistant organisms were 18.18%. In the case of Tetracycline, 81.82% were susceptible with no resistant organism. However, all the organisms that we tested from this sampling site came out to be resistant towards Cefixime antibiotics. Additionally, 72.73% of the organisms were resistant to Erythromycin antibiotics.

3.4 PCR Results:

Figure 3.4.1: Gel electrophoresis Result of *Staphylococcus aureus*

For the confirmation of the organisms based on the plates they grew in, we used PCR tests. Isolates from Site A and D (the ending points of Turag and Balu river in Dhaka) were taken and were tested for *Vibrio spp, Staphylococcus aureus and Salmonella typhi*.

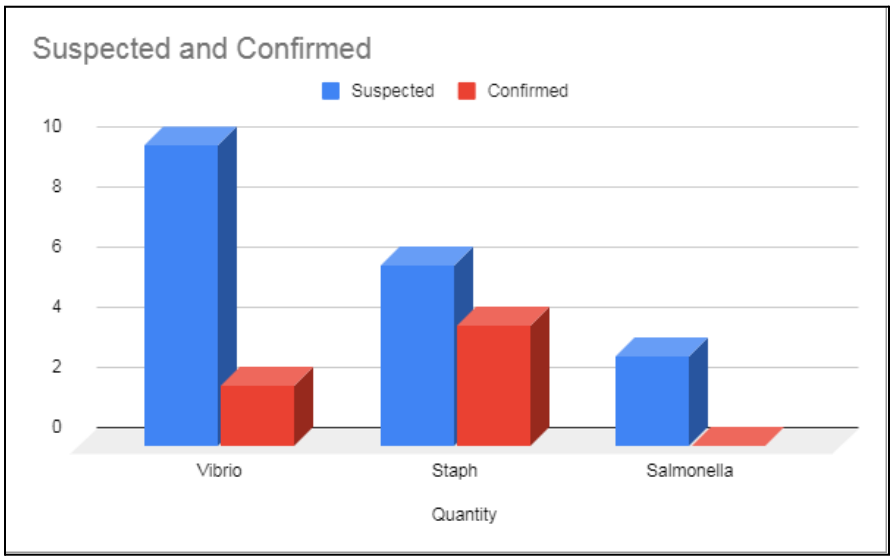


Figure 3.4.2: Ratio of Suspected and Confirmed Species from PCR

The bar chart shows that, total 10 organisms were suspected to be Vibrio from site A and D. Only 2 (20%) of them were confirmed as *Vibrio spp*. Moreover, 4 of the 6 (66%) of the suspected *Staphylococcus aureus* tested positive.

Figure 3.4.3: Gel electrophoresis result of *Vibrio Spp.*

Lastly, none of the 3 suspected *Salmonella typhi* showed positive results in the PCR test.

Chapter 4

Discussion

4.1 Result Analysis

The results show that both the rivers Balu and Turag contain high amounts of fecal coliforms and other pathogenic organisms. Because of containing a high amount of fecal coliforms, these river waters are not hygienic and not usable for drinking or cleaning purposes. The biochemical results showed the presence of many enteric bacteria as well as other pathogens. But specifically *Staphylococcus aureus* was found to be the most prevalent in these water samples. Studies show that if infections from *Staphylococcus aureus* enter the bloodstream, it can be life-threatening. According to Del RíO et al. (2009), "S. aureus BSIs are associated with a high frequency of life-threatening complications, such as metastatic infections, and S. aureus is the principal pathogen responsible for infective endocarditis (IE) in industrialized countries." The biochemical test results also reveal the presence of pathogenic species like *Vibrio spp.* and *Klebsiella pneumoniae*. *Vibrio spp.* can cause cholerae (Rodriguez et al., 2022) which has been a deadly disease for a long time now. Furthermore, pneumonia, bloodstream infection and meningitis can be caused by *Klebsiella pneumoniae* (Klebsiella Pneumoniae in Healthcare Settings | HAI | CDC, n.d.) which is also found in the samples.

For ease of comparison, we only performed PCR on the isolates from Site A and Site D. The sites are the endpoint of the Turag and Balu rivers. The purpose of performing PCR on the isolates from these two sites was to determine which bacteria were confirmedly present in the end points of these rivers. This was to demonstrate that there were still pathogenic bacteria present in the river water after entering Dhaka. Both the PCR result and the Biochemical results confirm the abundance of *Staphylococcus aureus* present in these water samples. For site A and site D, about 66% of *Staphylococcus aureus* suspected isolates are confirmed to be positive through PCR. From the other PCR results it can be stated that about 20% of the *Vibrio spp.* suscpected isolates are confirmed to be positive and no *Salmonella typhi* was present in these samples. The AST results demonstrate that all the 43 isolates from these 4 sites are 100% susceptible to Meropenem and Amikacin.

From the results of our research, we can see that 100% bacteria were susceptible for Meropenem whereas about 90% of the bacteria showed resistance for Cefixime. Both MRP and CFM are strong antibiotics. Cefixime is used to treat illnesses like Bronchitis, infection of throat, urinary tract etc (Cefixime: MedlinePlus Drug Information et al., 2014). If people get infected by the

pathogens because of using this water, the infections cannot be treated using CFM which is alarming. Also an astonishing amount of bacteria were found which were resistant towards Erythromycin. Resistance towards this specific antibiotic means it will not be able to treat pneumonia, blood infections and several STDs anymore (Farzam et al, 2023).

Antibiotic resistance is a serious threat nowadays worldwide. Because of the widespread use of the antibiotics, people not completing the course of antibiotics or after getting better they stop taking antibiotics before the suggested course has been completed - these reasons widely contribute to the antibiotic resistance problem (World Health Organization, WHO et al., 2023). This paper aimed to address this situation and spread awareness among the people of Bangladesh .

4.2 Recommendations

From the results found from this study, we can say that two of the most prominent rivers of Bangladesh, the Balu and Turag river are highly polluted with different enteric microorganisms. Prolonged usage and exposure to this polluted water can cause various life-threatening diseases in human beings and animals. As there are a few garment factories and tannery near these sites, it could be possible that the chemical and industrial waste from these are contributing to the water contamination.

Sixty percent of the population of our country is directly dependent on river water for their daily needs (Roundtables et al, 2020). Thus, the authors recommend a proper water treatment system implemented by the government before using these rivers' water for any agricultural or industrial purposes. Advanced wastewater and sewage treatment plants and proper sewage systems should be established around these rivers. Before releasing any chemical or industrial waste into rivers, these wastes must be treated properly.

As the bacterial resistance towards antibiotics is becoming a global conundrum and since a long time ago, Bangladesh has been suffering from As-induced serious health disorders, which are primarily caused by surface and groundwater (Uddin & Jeong et al, 2021), people should be made aware of this situation. The government should take proper measures to raise awareness among the people regarding this issue. Furthermore, more research facilities have to be employed for the research of wastewater and river waters.

Additionally, to prevent the spreading of the waterborne diseases from these rivers, the authorities should prohibit the usage of these river waters for any domestic cleaning or drinking purposes for the time being, at least until these river waters are purified and made usable again.

4.3 Limitations

This research solely focused on the bacteriological studies of these two rivers. However, physiochemical properties of these water samples could not be included in the research. Physiochemical properties like pH plays an important role in quality assessment of river water while DO or dissolved oxygen is necessary for good quality of the river water (M. G. Uddin et al., 2016). Additionally, due to contamination the isolates of Site B and Site C could not be assessed and confirmed through PCR. Thus, this study lacks the proper comparison between the organisms present in the starting points and ending points of these rivers. Due to these lackings only the isolates of the ending points could be confirmed.

4.4 Future Prospects

Our research focused mainly on the bacteriological studies of the Balu and Turag river. However, the physiochemical properties of these river waters such as BOD, DO, COD, temperature, pH etc. can be assessed for better understanding of the water quality and if these water bodies can be used for fish culture and agricultural purposes. PCR confirmation of the isolates of the starting points of these rivers can provide a comparison of which bacteria were present in the river before entering Dhaka and which bacteria remains after entering Dhaka from the ending points' isolates. Moreover, antibiotic resistant genes of specific bacteria can be studied as there have been increasing concerns that residues of antibiotics in surface water may be posing a threat to human health by developing antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) (Berendonk et al., 2015). Apart from the Balu and Turag river, Buriganga is also one of the main rivers in Dhaka. Thus, the bacteriological study of the Buriganga river will give a complete bacteriological mapping of the Dhaka city rivers.

Conclusion:

In this research we solely focused on the bacteriological study of Balu and Turag river. The results show that various kinds of bacteria are found in the river water that people of Dhaka use in their daily chores. A significant amount of *Staphylococcus aureus* is found in the samples of the 4 sites from different parts of Dhaka with many other types. 90% of these bacteria were resistant to Cefixime where on the other hand all of the isolates were susceptible towards Meropenem and Amikacin. Prolonged exposure to these water bodies can cause human beings or animals various kinds of deadly diseases such as bloodstream infections, pneumonia, or bone and joint infections (caused by *Staphylococcus aureus)*, Cholera (caused by *Vibrio Cholera)* etc. Further molecular analysis is needed to identify the rest of the isolates that were found in our research and to acknowledge the mechanism of these resistant genes.

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