Comparative Study on Antibiotic Resistance Profiles and Plasmid Presence in Water Samples Focusing Inside and Outside Dhaka District

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

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October 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing the degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

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Acknowledgement

First of all, we want to express our sincere gratitude to the Almighty for giving us the chance to conduct this research and for giving us the strength and patience to complete this project.

We would like to thank Prof. Dr. A. F. M. Yusuf Haider, Chairperson of the Department of Mathematics and Natural Sciences, Brac University, Dhaka, for giving us the opportunity to do our thesis at the Brac University thesis lab.

We would like to express our sincere gratitude and respect to Dr. Munima Haque, ma'am, Associate Professor, Program Director, Biotechnology Program, Department of Mathematics and Natural Sciences, School of Data and Sciences, Brac University, for her valuable advice and support.

We earnestly thank and give our heartfelt gratitude and respect to our supervisor, Dr. Iftekhar Bin Naser, sir, Associate Professor, Biotechnology Program, Department of Mathematics and Natural Sciences, School of Data and Sciences, Brac University, for his constant guidance, support, encouragement, and valuable advice throughout our thesis days. Without his constant monitoring and pieces of advice in this research project we would have not been able to complete our thesis smoothly. We thank him for being a helpful faculty with a kind soul and for his expert guidance throughout our thesis days at Brac University.

Most importantly, we would like to appreciate and thank our respective lab officers, lab attendants and research assistant, Fariya Akter for their constant help and encouragement during our thesis days. And our heartfelt appreciation goes to our family members and all the people who have helped us with our project for their constant support and encouragement.

Abstract

One of the most pressing issues of recent times is antimicrobial resistance or AMR. Nowadays, treating infections with susceptible antibiotics has become a major concern as antibiotic resistance is rapidly rising. And due to a lack of susceptible antibiotics for a specific infection, millions of deaths are occurring worldwide. This antibiotic resistance is increasing mainly as a result of the excessive use of antibiotics and improper disposal in the environment.

For assessing the present state of antibiotic resistance in Bangladesh, water samples were collected from different areas of Bangladesh and a comparative analysis was done between inside and outside Dhaka district. We have collected four water samples from inside Dhaka district and four water samples from outside Dhaka district. A total of eight water samples were collected, and from those, 160 colonies were isolated. For the isolation of different bacterial species, we have used selective media, including MacConkey, SS, TCBS, and EMB. The general identification of the isolates was done using Gram staining and HiMedia morphology identification techniques. We have found different species of bacteria, including *Escherichia coli, Vibrio, Shigella, Salmonella, Klebsiella, Pseudomonas,* and *Enterobacter.*

The primary goal of this research project was to conduct a comparative analysis of the antibiotic resistance profiles between inside and outside Dhaka district water sources. Here, antibiotic susceptibility testing (AST) was performed using 14 different antibiotic groups. Many multidrug-resistant bacteria were found in both inside and outside Dhaka district samples. However, the antibiotic resistance rate was found to be higher in inside Dhaka district water samples. Moreover, we have obtained plasmids from the bacterial isolates and visualized those plasmids via the gel electrophoresis method. As we know, bacteria can pick up resistant genes containing plasmids and

become resistant to antibiotics. And after analyzing our results, similarly, we have found a correlation between the presence of plasmids in bacteria and their antibiotic resistance rate.

Keywords: Water pollution, Antimicrobial Resistance (AMR), Antibiotic Susceptibility Testing (AST), Gram-positive, Gram-negative, plasmid

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Chapter 1:

Introduction

1.1. Water bodies of Bangladesh

Bangladesh is a country with many rivers. Groundwater and surface water resources are abundant in Bangladesh. The surface water includes ponds, lakes, and haors (Water resources, n.d.). The Bangladesh Water Development Board (BWDB) states, approximately 405 rivers flow across Bangladesh, but many have dried up or gone extinct. The numbers can vary since the names of the same rivers can change over time. Out of the 54 rivers that originate from India, 17 are in danger of going extinct (Bangladesh water development board, n.d.). The Himalayas are the principal sources of rivers that flow through China, Bhutan, and India and pass through the Bay of Bengal. The largest rivers of Bangladesh are the Padma, Jamuna, Meghna, and Brahmaputra, with the latter enlarging to about eight kilometers (Water resources, n.d.). In Dhaka city, there are six rivers that encircle it. On the east there are Sitalakhya and Balu rivers, on the west Turag and Buriganga rivers, on the north Tongi Khal, and to the south is the Dhaleshwari river (Irfanullah, 2020).

For our research project, we have chosen water sources situated at eight different places of Bangladesh. Four of which are from inside Dhaka and four of them are from outside Dhaka district.

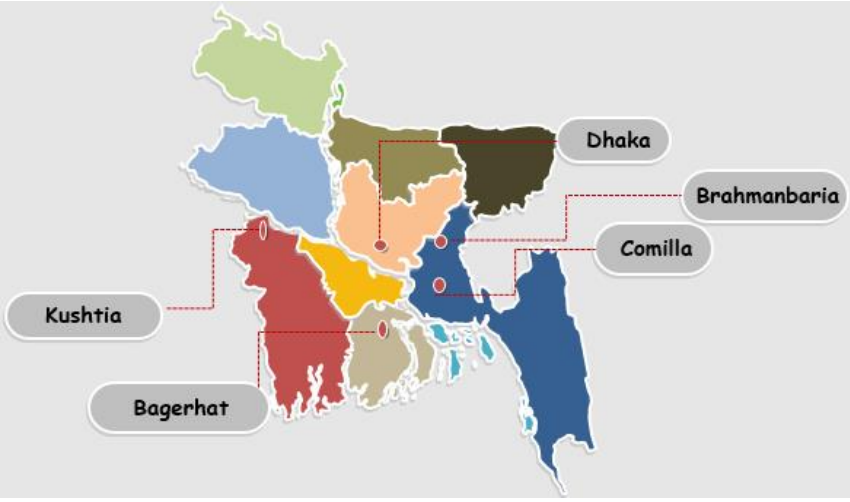


Figure 1.1: Water samples collected from Kushtia, Bagerhat, Comilla, Brahmanbaria and Dhaka.

1.2. Surface water pollution: the current situation of Bangladesh

Water pollution frequently results in death, especially among children. Surface water is thought to be the best option for wastewater discharge due to its accessibility. In Bangladesh, a large portion of the population acquires their primary water supply from untreated surface water. This is the case because there is an endless supply of drinkable water and the water supply systems are insufficient in many places. Thus, surface water now consists of many pathogenic bacteria, pharmaceutical products, and heavy metals, ultimately, causing harm to the aquatic ecosystem and humans. Several studies showed a significant number of fecal coliforms in the surface waters of Bangladesh. In the bank of Dhaleshwari River, 860 CFU per 100 mL fecal coliforms was reported. In Padma River, 640 CFU per 100 mL and in Karnaphuli River it was 680 CFU per 100 mL were reported. Also, in the pond water of Sherpur, high number of fecal coliforms was found. In the southwest coastal areas of Bangladesh, fecal coliform was found in the harvested rainwater, which is 465 CFU per 100 mL (Parvin et al., 2022).

1.3. Pathogenic bacteria in the surface water of Bangladesh

Fecal coliform and other pathogenic bacteria have regularly been discovered in Bangladeshi ponds and river water. Dhaka's residential areas are also vulnerable to fecal contamination. The presence of E. coli in various environmental samples has been identified by researchers to indicate the presence of pathogenic bacteria and the potential risk of enteric disease among residents of Dhaka (Parvin et al., 2022). Two tropical water sources of Dhaka, a pond and the Lake of Dhanmondi, were evaluated in a study. *Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Escherichia coli,* and *Shigella* spp were identified in those water samples. Even the *E. coli* that cause diarrhea, such as EPEC and ETEC, were found in the samples. Also, a small

number of EHEC and EAEC were detected. Adaptation of these diarrheagenic pathogens can cause recurrent outbreaks of diarrheal diseases in Bangladesh (Azmuda et al., 2019).

1.3.1. *E. coli (Escherichia coli)*:

E. coli, the rod-shaped bacteria belongs to the Enterobacteriaceae family. The majority of *E. coli* infections are minor and do not pose a serious risk to health. Some strains of *E. coli* can result in serious fatal complications, like hemolytic uremic syndrome, and can cause renal failure and death. The most common *E. coli* strains cause disease by secreting Shiga toxin. This toxin harms the lining of the small intestine and causes diarrhea. Shiga toxin-producing *E. coli* or STEC mainly causes food contamination. This strain is also called verocytotoxin-producing *E. coli* (VTEC) and enterohemorrhagic *E. coli* (EHEC) (E. coli: What is It, How Does it Cause Infection, Symptoms & Causes, n.d.).

1.3.2. *Salmonella spp*:

Salmonella is a genus of the Enterobacteriaceae family, is rod-shaped gram-negative bacteria. The first classification of *Salmonella spp*. was proposed by Kauffman-White according to the O and H antigens principle. The *Salmonella* genus is sub-divided into more than 2500 species as a result of this classification system. There are currently two species of *Salmonella*, *Salmonella enterica* and *Salmonella bongori*. *Salmonella* is primarily found in the intestines of people and other animals. The main reason for acute diarrheal disease is still *Salmonella*. And worldwide reports of enteric fever and serious infections were reported because of *Salmonella* (Popa & Popa, 2021).

1.3.3. *Shigella spp*:

Shigella spp. is a type of gram-negative bacteria that can result in life-threatening diarrhea if consumed. Shigellosis is a bacterial infection that is primarily caused by the pathogenic strains of Shigella bacteria. *Shigella* species enter the body through contaminated food or water and are spread via the fecal-oral pathway. The bacteria enter the large intestine after passing the small intestine and cause infection. *Shigella* species are among the common pathogenic bacteria that cause diarrhea. 5-15% of total diarrheal cases including 1.1 million fatal cases are caused by *Shigella* infections worldwide. This species of bacteria is extremely infectious because only 10 to 100 of *Shigella* are needed to spread an infection (Schroeder & Hilbi, 2008).

1.3.4. *Vibrio spp*:

Vibrio spp. is a pathogenic group of rod-shaped, gram-negative bacteria found in freshwater and marine habitats. The most prevalent pathogenic species are *Vibrio cholerae, Vibrio vulnificus,* and *Vibrio alginolyticus. Vibrio* species are responsible for many human infections attributable to the natural microbiota of aquatic environments. Cholera and non-cholera infections are the two main categories of diseases in humans brought on by the pathogenic *Vibrio* species. Infections are caused by contact with water or intake of raw or undercooked seafood, contaminated with *Vibrio spp*. The main agent of cholera is *Vibrio cholerae*. Cholera is a severe diarrheal illness that can soon become fatal if left untreated and is mainly spread through contaminated water and direct human contact. Also, *Vibrio cholerae* is found in freshwater samples. Non-cholera *Vibrio* species include *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which cause vibriosis, and can induce primary septicemia or mild gastroenteritis (Baker-Austin et al., 2018).

1.3.5. *Pseudomonas spp*:

Pseudomonas is a member of the *Pseudomonadaceae* family and gram-negative bacteria. *Pseudomonas* pathogenic strains can cause disease. The most common type is *Pseudomonas aeruginosa*, and it frequently causes human diseases. The most typical environments for *Pseudomonas aeruginosa* include water, plants, and soil. However, it can also be found in wet or humid places like sinks. A potentially fatal infection brought on by *Pseudomonas aeruginosa*, can spread to other parts of the body and lead to serious illnesses like sepsis and organ failure (What is a Pseudomonas aeruginosa infection?, n.d.).

1.3.6. *klebsiella spp*:

klebsiella belongs to a group of rod-shaped bacteria of the *Enterobacteriaceae* family. *Klebsiella* are classified as gram-negative, and facultative anaerobic bacteria. *Klebsiella spp.* is found in soil, water, and plants, and some strains are thought to be the human gastrointestinal tract's normal flora. *Klebsiella pneumonia* is a human respiratory pathogen, mostly known for causing pneumonia. *Klebsiella pneumonia* has been found in hospitals, where it may behave like other *klebsiella* strains, as an opportunistic pathogen (Klebsiella, 2023).

1.4. Factors affecting the quality of water bodies in Bangladesh

In Bangladesh, different kinds of pollutants are found in both surface water and groundwater (Azmuda et al., 2019). The six major rivers that encircle Dhaka, are being alarmingly polluted by industrial wastes. The University at Buffalo (UB) and icddr,b conducted a study together that found Dhaka's rivers to be highly contaminated with pesticides and medicines (Chemicals, industrial waste contamination turn 6 Bangladesh rivers untreatable, n.d.).

- Municipal sewage and industrial waste that has not been treated and dumped directly into the water bodies are the main sources of surface water pollution in Bangladesh. The Buriganga River in Dhaka is considered to be highly polluted due to domestic sewage and waste. Other contaminated water bodies outside Dhaka city include Savar, Ashulia, Tongi, and Gazipur. Additionally, some contaminated rivers are found close to Khulna and Chittagong, the other industrial areas (Water resources, n.d.).
- The environment and public health are both threatened by pharmaceutical pollution. Pharmaceutical pollution is also endangering Bangladesh's rivers. The antibiotics released from the pharmacies, and clinics in the water bodies, are polluting the water, which is creating a great concern (Parvin et al., 2022).

1.5. The effects of surface water pollution in Bangladesh

Microorganism-caused water pollution can affect people directly by drinking the water or bathing in that water source. Additionally, can affect indirectly by eating the vegetables or fish that grew in that polluted water.

- In Bangladesh, cholera, diarrhea, and typhoid are the main diseases that are spread by polluted water. Particularly, people between the ages of 0 and 4 are most susceptible to typhoid. According to a 2017 WHO report, in Bangladesh, contaminated water causes more than 45,000 children to die from diarrhea each year (Parvin et al., 2022).
- Antimicrobial resistance (AMR) has become a serious problem worldwide, due to this environmental exposure of antimicrobial APIs produced from antibiotics. Because of this, bacteria are becoming more resistant to antibiotics. Our ability to use antibiotics is seriously threatened by these resistant pathogenic bacteria. In a study of Barishal,

Bangladesh, researchers found significant concentrations of metronidazole, which was more than 300 times above the acceptable range (Bangladesh rivers in grave danger due to pharmaceutical pollution, 2022).

1.6. Importance of AMR & AST

1.6.1. Antimicrobial Resistance (AMR)

Antimicrobials are medicines, used for the treatment of different types of infections in plants and humans, it includes antibiotics, antivirals, and antifungals. Antimicrobial Resistance (AMR) is a situation in which bacteria, and fungi evolve over time and fail to respond to antibiotics, ultimately, makes infections more difficult to cure and raises the risk of disease transmission, life-threatening sicknesses, and deaths. Antibiotic resistance makes it harder to treat infections and makes antibiotics useless. Thus, our ability to cure common diseases is under great threat due to the spread of pathogenic bacteria that have developed new resistance mechanisms against antibiotics. The increasing global development of multi-resistant bacteria, commonly referred to as "superbugs," which cause diseases that cannot be treated with many current antibiotics, is very concerning (Antimicrobial resistance, n.d.).

AMR develops throughout time, typically by genetic alterations. Nowadays, in humans, animals, environment, and in plants, everywhere antimicrobial-resistant microorganisms are found. They can be transmitted from person to person, or via animal products in food. The main reasons for antimicrobial resistance are the overuse and misuse of antibiotics, lack of clean water, inadequate infection and disease control, the cost of pharmaceuticals, and a lack of awareness about antibiotics. All the countries and their healthcare systems are impacted by the

shortage of sensitive antibiotics. This lack of access to new high-quality antimicrobials continues to be a big problem (Antimicrobial resistance, n.d.).

1.6.2. Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing (AST) usually performed by medical technologists. It is a laboratory procedure to identify which antimicrobial or antibiotic is effective for each patient. This testing aids in the evaluation of treatment processes, by the hospitals, and clinics for the control of infectious diseases. The principle for using antibiotics is to make sure the patient receives a specific antibiotic that the target pathogenic bacteria is sensitive to, and at a dosage high enough to be effective but low enough to not have side effects, and for long enough to make sure that the infection is completely eradicated (Antibiotic, 2023).

The word "susceptibility" is employed when one or more antimicrobial medications prevent microbes like bacteria and fungi from growing. Antibiotic susceptibility testing is done to see if certain antibiotics could be effective against a certain strain of bacteria or if the bacteria have become resistant to a particular antibiotic. And the results after this test, aid in choosing the antibiotic or antibiotics that are most likely to successfully treat the infection. Resistance to antibiotics and antifungals in bacteria and fungi can develop at any time. Thus, AST is performed on every strain of bacteria or fungus that might be important for the patient's therapy and whose susceptibility may not be known. To see the effectiveness of antimicrobials, each pathogenic strain is tested separately. This test can be directly assessed by combining the pathogen and the antibiotics in a nutrient media. Or by testing a gene, that is known to produce resistance to a particular antibiotic to identify the resistance (Antibiotic susceptibility testing, 2020).

1.7. Common antibiotics used for treatments in Bangladesh

An antibiotic is a chemical compound that is produced by a living microorganism but harmful to other microorganisms. The bacteria and fungi can produce antibiotics that are helpful in preventing or treating disease. The range of action varies among antibiotics, some are specific, and some work against different types of bacteria. These broad-spectrum antibiotics are helpful in treating mixed infections (Antibiotic, 2023).

- **Penicillins:** Works by inhibiting the cell wall synthesis. The penicillins have a β-lactam ring, responsible for the antibacterial activity. This group of antibiotics are used for the respiratory and urinary tract infections, meningitis and endocarditis.
- **Cephalosporin:** Also works by inhibiting the cell wall synthesis. This group of antibiotics are also used for the infections of the respiratory and urinary tract. And also, for the infections of skin, blood, bones and pelvic inflammatory diseases.
- **Aminoglycosides:** Also works by inhibiting protein synthesis. This group of antibiotics are also used for respiratory and urinary tract infections, including, blood infections and pelvic inflammatory diseases.
- **Fluoroquinolone:** Works by interfering with DNA synthesis. This group of antibiotics are used for diarrhea, gonorrhea, and pneumonia. And also, for treating the respiratory and urinary tract infections.
- **Tetracyclines:** Works by inhibiting protein synthesis. This group of antibiotics are used for the rickettsia, pneumonia, intestinal amebiasis, and minor wounds.
- **Macrolides:** Works by inhibiting protein synthesis. This group of antibiotics are used for the infections of the respiratory tract, and skin. Also used for STDs, Legionnaire disease, pertussis, diphtheria, and otitis media.

- **Chloramphenicols:** Also works by inhibiting the protein synthesis. This group of antibiotics are used for the Chloromycetin infections of the eyes, and skin. Also used for treating cystic fibrosis and minor wounds (Antibiotic, 2023).

1.8. The role of plasmids in producing multi-resistant bacteria

A plasmid is a double-stranded, circular, small DNA that differs from a chromosomal DNA. Bacteria and certain eukaryotic cells naturally contain plasmids. All of the plasmids in a bacterial cell are duplicated during bacterial division. Another feature of plasmids is the ability to transfer the entire plasmid to different types of bacteria. These implies that by acquiring a single plasmid, a bacterium can develop resistance to several antibiotics at once, and eventually develops multidrug-resistance. Pathogenic bacteria with multidrug-resistant plasmids have become more common and are now a serious global health concern as there are not many treatments left for this type of infection (Plasmids and co-selection, 2016).

The R plasmids demonstrate that not only do bacteria have naturally occurring resistance genes, but they can also develop resistance to protect themselves from threats to their survival. This resistance is transmitted not only vertically, but also horizontally, that is, between different species. The ability of bacteria to pick up and transmit foreign genes by plasmids and transposons is regarded to be the main contributor to the spread of antibiotic resistance. Also, the wastewater treatment plants or WTPs, are significant repositories of microorganisms that are likely to infect humans and animals by entering the environment again by the plant outlet. The wastewater from WTPs contains different types of bacteria, which encourages bacterial bonding and the transfer of antibiotic-resistance genes by the plasmids (Li et al., 2019).

1.9. Objectives

1. The objective of this research was to find the comparison between different bacterial species from inside and outside Dhaka district water samples.
2. This study aimed to discover *Escherichia coli, Enterobacteriaceae, Vibrio cholerae, Salmonella spp*., and *Shigella spp.* bacterial species from inside and outside Dhaka district water samples.
3. This study aimed to find the antibiotic resistance profiles of isolated bacterial species. As we know that, inside Dhaka district there are more pharmacies and clinics and people here are using more antibiotics, than outside Dhaka districts. So, in inside Dhaka district water sources, more multi-drug resistant bacteria can be found.
4. It is aimed to obtain plasmids from the bacterial isolates and compare the plasmid containing bacteria with antibiotic resistance profiles of inside and outside Dhaka district water samples.

Chapter 2:

Materials and Methods

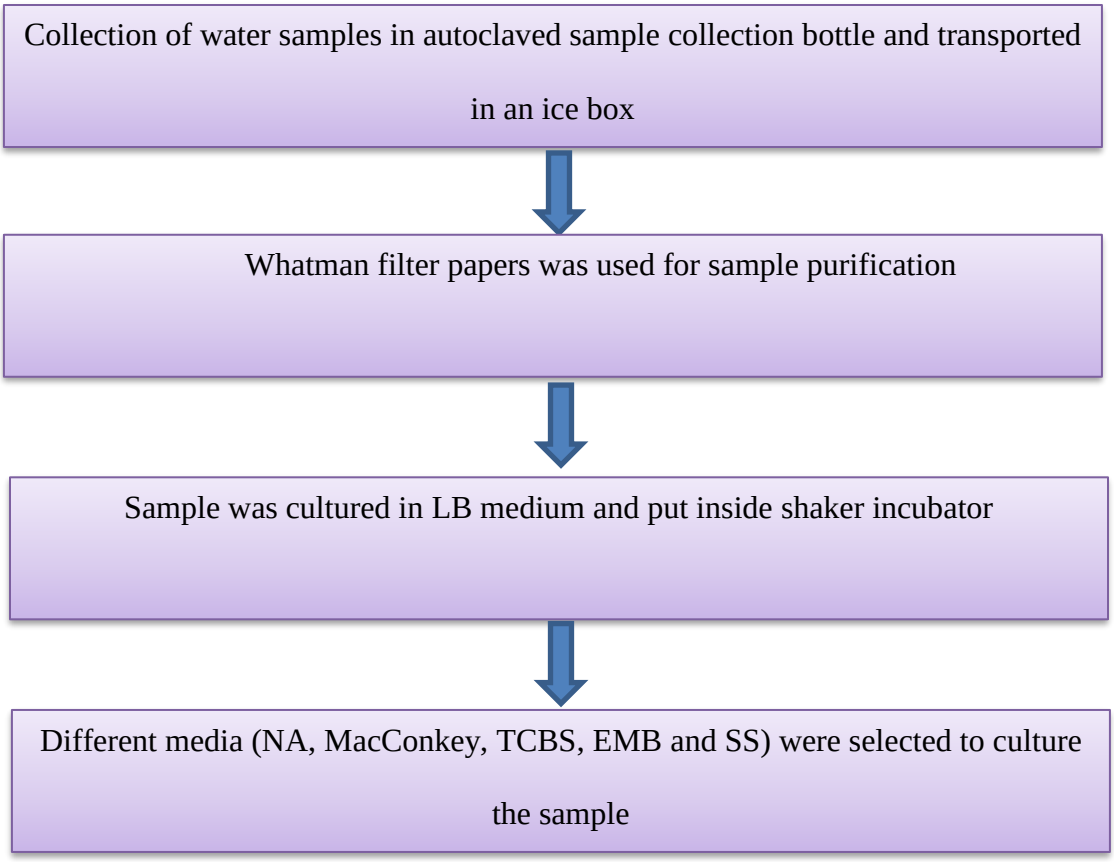
2.1. Study Place

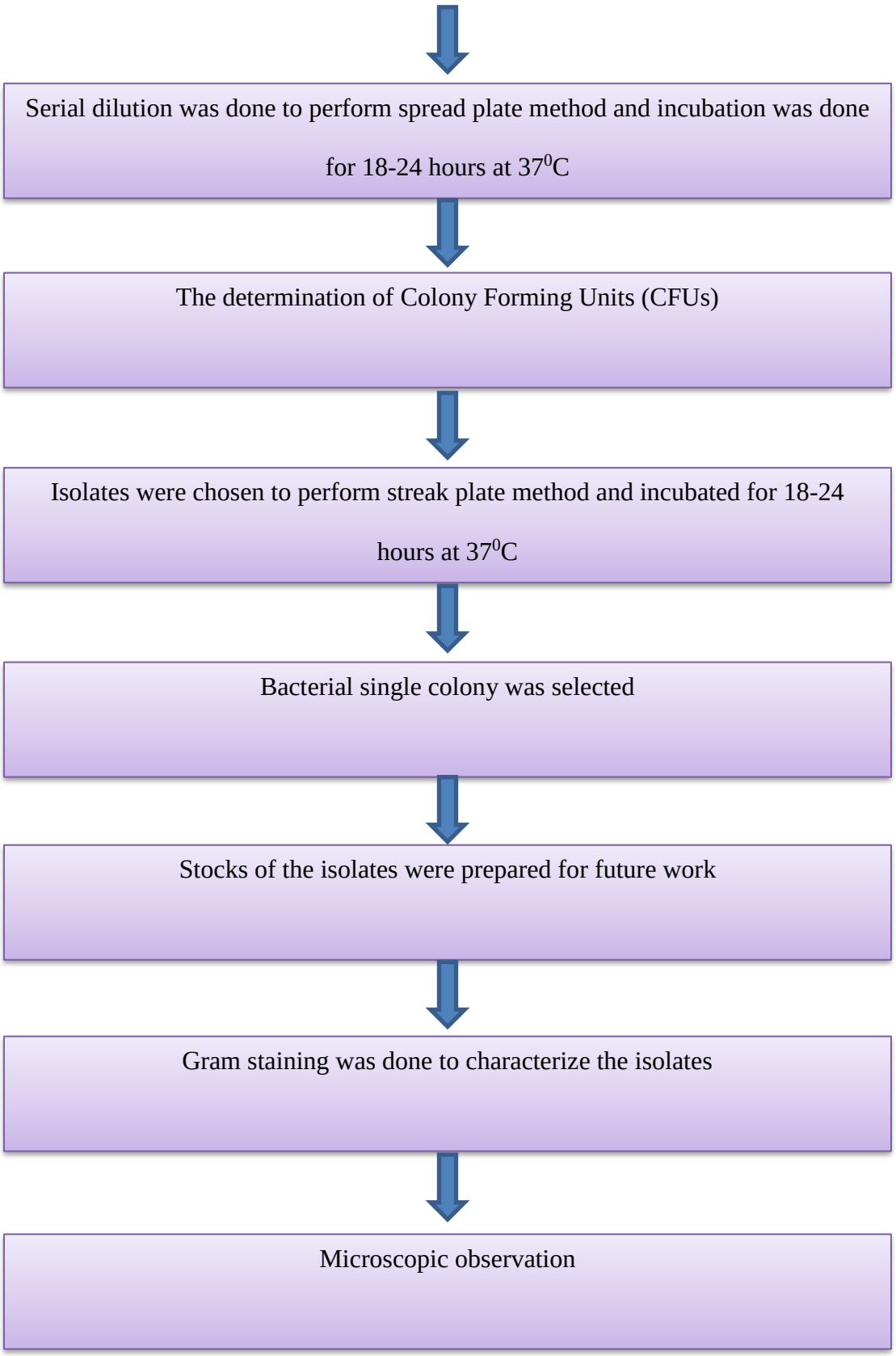
The laboratory work conducted for the research project took place in the Biotechnology, Microbiology, and Molecular Biology Laboratory, which is part of the Mathematics and Natural Sciences Department of Brac University.

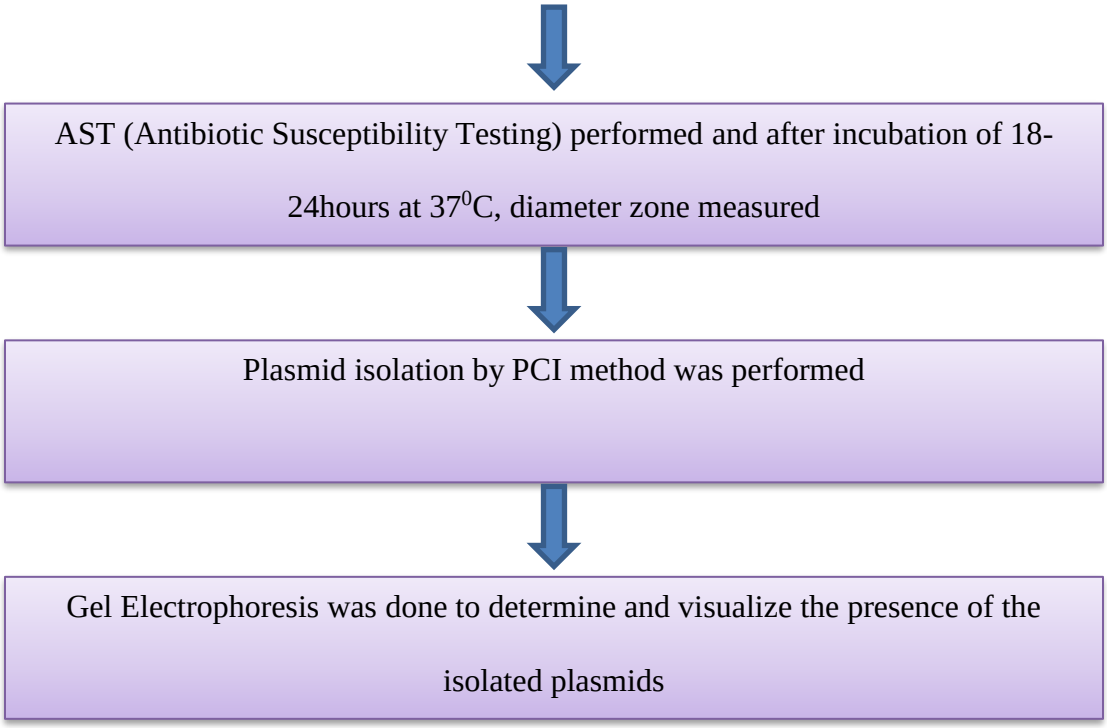
2.2. Study Duration

The study project spanned from October 2022 through August 2023.

2.3. Experimental Workflow




Serial dilution was done to perform spread plate method and incubation was done for 18-24 hours at 37^{0}C
The determination of Colony Forming Units (CFUs)
Isolates were chosen to perform streak plate method and incubated for 18-24 hours at 37^{0}C
Bacterial single colony was selected
Stocks of the isolates were prepared for future work
Gram staining was done to characterize the isolates
Microscopic observation



2.4. Types of Equipment

- Incubator
- Shaker incubator
- Autoclave machine
- Vortex machine
- Microscope
- Laminar air flow cabinet
- Petri dishes, spreader, metallic loop, Bunsen burner
- Glass pipettes, micro pipettes, test tubes and electric balance
- Glassware, pH meter, Whatman filter paper

2.5. Water Sample Collection and Processing

The water samples were collected from 08 different places in Bangladesh. The places include Hatir Jheel, Banani Lake, Dhanmondi Lake, and Balu River (Rupgonj) from Dhaka district; Modhumoti River (Kushtia), Titash River (Brahmanbaria), Kumar River (Bagerhat), and Shalda River (Comilla) which are situated in different parts of Bangladesh.

Samples were collected in an autoclaved sample collecting bottle and labeled appropriately. Then, the bottle was placed inside an ice box for transportation so that we could get accurate results. After transportation, the sample was processed immediately in the microbiology lab.

2.6. Collection of Bacteria

To collect bacteria from the water sample, the sample was first filtered by using 0.4mm Whatman filter paper. After filtration, in an autoclaved falcon tube, 3ml sample was taken and mixed with 5ml of LB (Luria Broth) medium for enrichment. The mixture was placed inside a shaking incubator at 37^0c for 4 hours. This was done to ensure optimum growth.

Afterwards, serial dilution was done. For that, 1ml sample was taken and added to sterilized 0.9% NaCl (sodium chloride) solution. Serial dilution was done till 10^{-5} in five autoclaved test tubes. Moreover, 150µl of the raw sample was taken and inoculated to culture media.

2.7. Culture Media Used for Bacterial Isolation

The choice of media is reliant upon the specific cell types to be grown, as well as the intended goal of the culture and the available resources within the laboratory setting. Various cell types exhibit distinct growth requirements, requiring the experimental determination of the most appropriate medium for each cell type (Arora, 2023). As each cell have unique nutrition requirement and different growth factors, it is important to choose culture media according to the necessity of the cells.

There were four selective media used in our research work namely MacConkey agar, TCBS agar, SS agar, and EMB agar. Nutrient agar was also used so that each type of bacteria can grow and we can determine the overall bacterial growth as well. After the serial dilution, the sample was inoculated in each of these five agar media using the spread plate method and incubated in 37^0c for 18-24 hours. After the incubation period, colony forming unit (CFU) was counted for each dilution factor and bacterial colony was isolated to perform the streak plate method, again on these five culture medias.

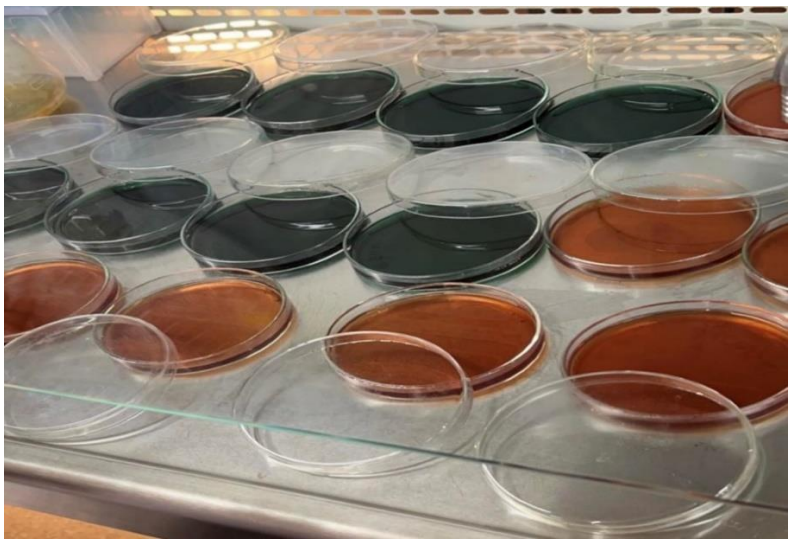


Figure 2.1: Different types media poured inside the laminar flow cabinet

2.7.1. Nutrient Agar (NA):

Nutrient Agar is a frequently used medium that serves as a substrate for the cultivation of many microorganisms, facilitating the growth of a diverse array of non-fastidious organisms. Nutrient agar is widely utilized due to its ability to support the development of diverse bacterial and fungal species, owing to its rich nutrient composition that fulfills the essential requirements for bacterial proliferation (Aryal, 2015). This medium is comprised of peptone, beef extract, sodium chloride, and agar, and it provides the necessary nitrogen molecules, carbon, vitamins, and trace elements that are crucial for bacterial development. *Salmonella*, *E. coli*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* are some of the common microbes that grow on NA medium (Pathak, 2023).

To prepare nutrient agar media, 28 grams of nutrient agar powder is dissolved in 1 liter of distilled water in a conical flask and boiled until the mixture become clear. Then, the media is autoclaved and poured onto petri dishes.

Figure 2.2: Nutrient agar and distilled water was boiled in a conical flask

2.7.2. MacConkey Agar:

MacConkey agar is a type of culture medium that possesses four essential components, including lactose, bile salts, crystal violet, and neutral red. These constituents contribute to the selective and differential properties of the agar. Bile salts and crystal violet function as selective agents, effectively inhibiting the proliferation of Gram-positive microorganisms while facilitating the development of non-fastidious gram-negative bacteria. Lactose functions as a provider of dietary carbs. The colonies produced by lactose-fermenting bacteria have a pink-red coloration as a result of their ability to ferment lactose into acids, hence reducing the pH of the medium containing the neutral red indicator. Due to the inability of non-fermenters to metabolize lactose, their colonies exhibit a colorless or transparent appearance. This media is mainly used to isolate Gram-negative enteric bacteria (Tankeshwar, 2013).

E. coli, Klebsiella spp., Enterobacter spp., Proteus spp., Shigella spp., Salmonella spp., Pseudomonas spp., Staphylococcus spp., are some of the common microbes that grow on MacConkey agar (HiMedia Laboratories, n.d.).

To prepare MacConkey agar medium, 52 grams of MacConkey agar powder is dissolved in 1 liter of distilled water followed by boiling. The conical flask is then sealed with aluminum foil paper and autoclaved. After autoclaving the media is then transferred to petri dishes and used after solidification.

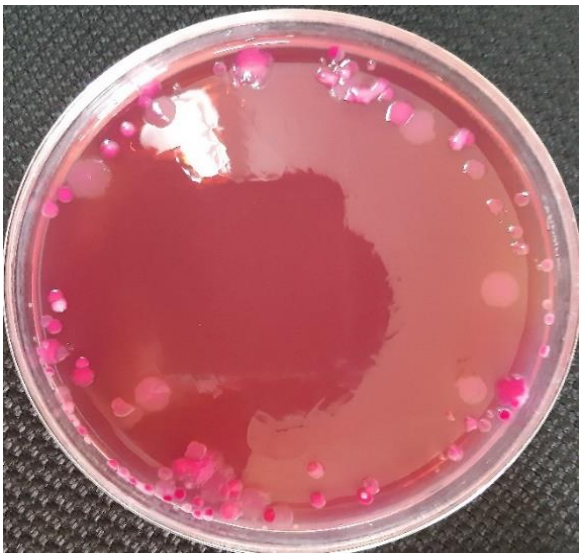


Figure 2.3: Colonies observed on MacConkey agar after spread plate method

➢ 2.7.3. Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar:

TCBS Agar is employed to selectively isolate *Vibrio cholerae* and other entero-pathogenic *vibrios*. The growth of *Enterobacteria* is significantly inhibited by thiosulfate, sodium citrate, and the alkalinity of the media. The growth of *enterococci* and the development of Gram-positive bacteria is inhibited by the presence of ox bile and sodium cholate. When *Vibrio* ferments sucrose, the medium becomes more acidic. This causes bromothymol blue to turn yellow. Bromothymol Blue and thymol blue are commonly employed as pH indicators. The presence of sodium chloride is essential for promoting optimal growth and metabolic activity in halophilic *Vibrio spp*. (Aryal, 2015).

Vibrio cholerae, Vibrio fluvialis, Vibrio parahaemolyticus, and *Vibrio vulnificus* are the organisms that grow on TCBS agar media (HiMedia Laboratories, n.d.).

To prepare TCBS agar medium, 89.08 grams of TCBS agar powder is dissolved in 1 liter of distilled water followed by boiling in a conical flask. This mixture should not be autoclaved. So, after boiling, the media is then transferred to Petri dishes and used after solidification.

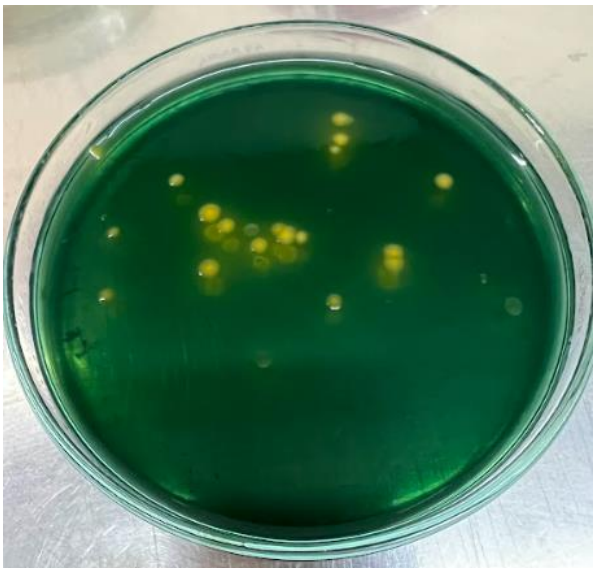


Figure 2.4: Colonies observed on TCBS agar after spread plate method

➢ 2.7.4. Salmonella Shigella (SS) Agar:

The SS Agar medium has been specifically designed to selectively and differentially cultivate and isolate *Salmonella spp*. as well as certain strains of *Shigella spp*. The inclusion of bile Salts, sodium citrate, and brilliant green in the medium serves to prevent the proliferation of Gram-positive and coliform microorganisms, while also restraining the swarming capability of *Proteus spp.* Meanwhile, it facilitates the growth of *Salmonella spp.* Beef Extract, enzymatic digestion of casein, and enzymatic digestion of animal tissue are considered suitable sources of nitrogen, carbon, and vital vitamins, which are crucial for the growth of organisms. Lactose is the source of the carbohydrate in Salmonella

Shigella agar. The identification of hydrogen sulfide is achieved by utilizing thiosulfate and ferric citrate, which leads to the creation of colonies that display black cores. The dye known as neutral red exhibits a shift in hue to red when it is subjected to an acidic pH environment. This alteration in color serves as an indicator of the presence of fermentation (Aryal, 2016).

E. coli, Enterobacter aerogenes, Proteus mirabilis, Salmonella choleraesuis, Salmonella Typhi, Salmonella Typhimurium, Salmonella Enteritidis, and *Shigella flexneri* are the microbes that grow on SS agar medium (HiMedia Laboratories, n.d.).

To prepare SS agar medium, 63 grams of SS agar powder is dissolved in 1 liter of distilled water followed by boiling in a conical flask. This mixture should not be autoclaved. So, after boiling, the media is then transferred to Petri dishes and used after solidification.

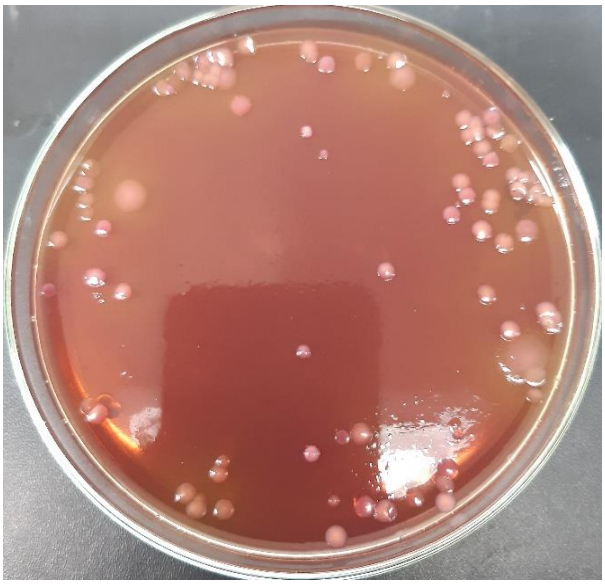


Figure 2.5: Colonies observed on SS agar after spread plate method

➢ 2.7.5. Eosin Methylene Blue Agar:

The Eosin Methylene Blue Agar (EMB agar) is a commonly utilized medium in microbiology laboratories because of its capacity to serve as both a differential as well as a selective medium. EMB agar, being a selective medium, comprises inhibitors, particularly eosin and methylene blue, which effectively inhibit the proliferation of Gram-positive bacteria. As a differential medium, it is capable of distinguishing between several types of gram-negative bacteria by assessing their capacity to undergo lactose fermentation (*EMB Agar : Principle, Composition, Uses and Interpretation*, n.d.).

E. coli is classified as a powerful lactose fermenter, as evidenced by the formation of colonies exhibiting a noticeable green metallic sheen when cultured on EMB agar. Certain lactose-fermenting bacteria, namely those with a slower fermentation process, can generate colonies that exhibit a brown-pink coloration. Non-lactose fermenting bacteria, such as *Shigella* and *Salmonella*, exhibit the absence of acid production and give rise to colonies that are clear, colorless, or amber in appearance, enabling their differentiation from coliforms (*EMB Agar : Principle, Composition, Uses and Interpretation*, n.d.).

Enterobacter aerogenes, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Salmonella Typhimurium* are the organisms that typically grows on EMB agar (HiMedia Laboratories, n.d.).

To prepare EMB agar medium, 36 grams of EMB agar powder is dissolved in 1 liter of distilled water followed by boiling in a conical flask. So, after boiling, the media is then transferred to Petri dishes and used after solidification.

2.8. Bacterial Inoculation Methods

For our research work, after the serial dilution, we have subsequently followed spread plate method to inoculate the organisms to the culture medium and later, streak plate method to isolate bacteria.

➢ 2.8.1. Spread Plate Method:

The spread plate method is a microbiology laboratory technique used to find and count the number of living microbes in a liquid sample. This is achieved by evenly distributing a specific volume of the sample onto a suitable solidified culture medium. After the incubation period, a successful spread plate will exhibit the development of uniformly distributed distinct colonies along the entirety of the culture media's surface. This method is employed to isolate and quantify the overall population of viable microorganisms, specifically by determining the colony-forming units per milliliter (CFU/mL) inside the provided sample (Dahal, 2022).

For our research work, we have taken our water sample and mixed it with LB medium for optimum growth. Afterward, before plating, the samples were serially diluted. As our objective was to count the CFU/mL, the sample was diluted to make the microbial load in the sample between 20 – 300 CFU/mL (The acceptable range for colony counts is typically reported as 20-200, but some sources may suggest a range of 30-300. On average, however, the range of 25-250 is commonly used) (Dahal, 2022).

Then, 150µl of the sample was transferred using a pipette onto the central portion of the solidified agar medium, and subsequently distributed uniformly across the whole surface of the media using a spreader. The plates were incubated under optimal conditions, after which the colony counts were determined.

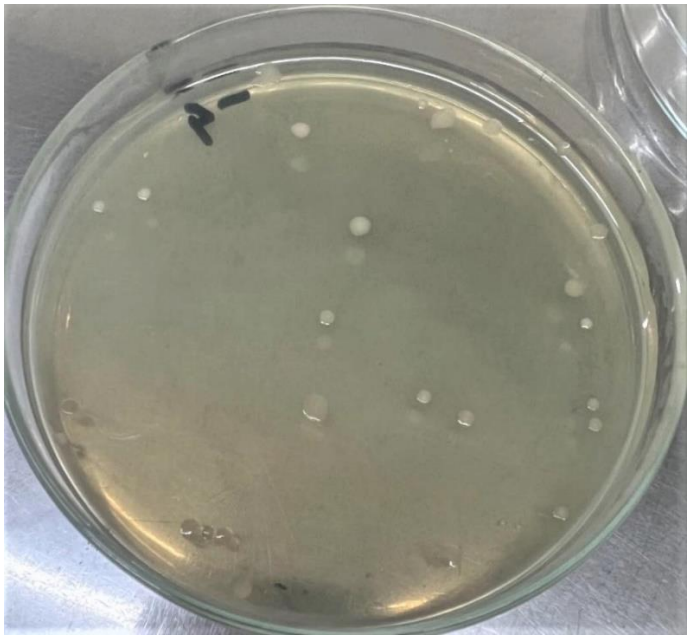


Figure 2.6: CFU/ml was counted following spread plate method at 10^{-4} dilution

➢ 2.8.2. Streak plate method:

The streak plate method is a widely employed microbiological laboratory technique utilized to isolate pure cultures and obtain well-isolated colonies of bacteria from an array of populations. This method is mostly employed to obtain pure cultures of bacteria. This technique involves the progressive dilution of bacterial concentration throughout the agar medium's surface during the streaking process. As a result, only a limited number of bacterial cells are finally introduced, leading to the formation of distinct and isolated colonies in the final streaks. So, this method is used to separate the bacteria from a group of different or the same species. Following inoculation, the terminal streaks exhibit identical colonies if the specimen contains a single species, however, the presence of many species may result in the observation of distinct colony types (Dahal, 2023).

For our research project, after we got colonies from spread plating, colonies were chosen to perform the streak plating method. Using a sterile inoculating loop, the sample was carefully positioned onto the surface of a sterile solid medium located at one side of the petri dish, resulting in the creation of a smear. The loop was utilized to consecutively streak the smear throughout the agar media, implementing various patterns. During the progression of streaking, the concentration of the inoculum steadily decreased, resulting in the dispersion of bacterial cells into distinct individual entities. The inoculated culture plates were incubated at 37^0c for 18-24 hours and the isolated bacterium would give rise to a well-isolated colony. As a result, the bacteria that had been isolated would generate a colony that was well-separated from other colonies. This enabled the acquisition of a pure culture, facilitating the subsequent characterization of the organism's colony morphology.

2.9. Possible Identifications

During our research project, we had performed Gram staining as a part of biochemical testing and also done Himedia analysis to closely identify the organisms we have isolated and worked with. Depending on the appearance of the organisms in different culture media and observing their colony pattern in Gram staining, we have tried to closely identify the organisms.

➢ 2.9.1. Gram Staining

Gram stain is a commonly utilized microbiological staining technique that plays a crucial role in the identification and classification of bacteria. At first, the primary stain crystal violet was used, and it stained all the cells in purple color. Next, the secondary stain, Gram's iodine was used and the crystal violet and iodine form a complex known as CV-I

complex. Then, acetone was used as a decolorizer. The Gram-positive cells have a thick peptidoglycan layer, so the decolorizer cannot break the CV-I complex, hence the cells are not decolorized. However, the Gram-negative cells, have thin peptidoglycan layers so they are decolorized easily. And afterward, the de-stainer, safranine was used, the Gram-positive cells retain their purple color, but the Gram-negative cells are stained in pink or red color.

Examples of Gram-negative organisms include:

- Cocci: *Neisseria gonorrheae, Neisseria meningitidis,*
- Bacilli: *Escherichia coli, Pseudomonas* species, *Proteus* species, and *Klebsiella* species (Tripathi et al., 2023).

Figure 2.7: Gram-negative sample showing pink to red color

Examples of Gram-positive organisms include:

- Cocci: Staphylococcus species, and Streptococcus species
- Bacilli: Clostridium species, and Listeria species (Tripathi et al., 2023).

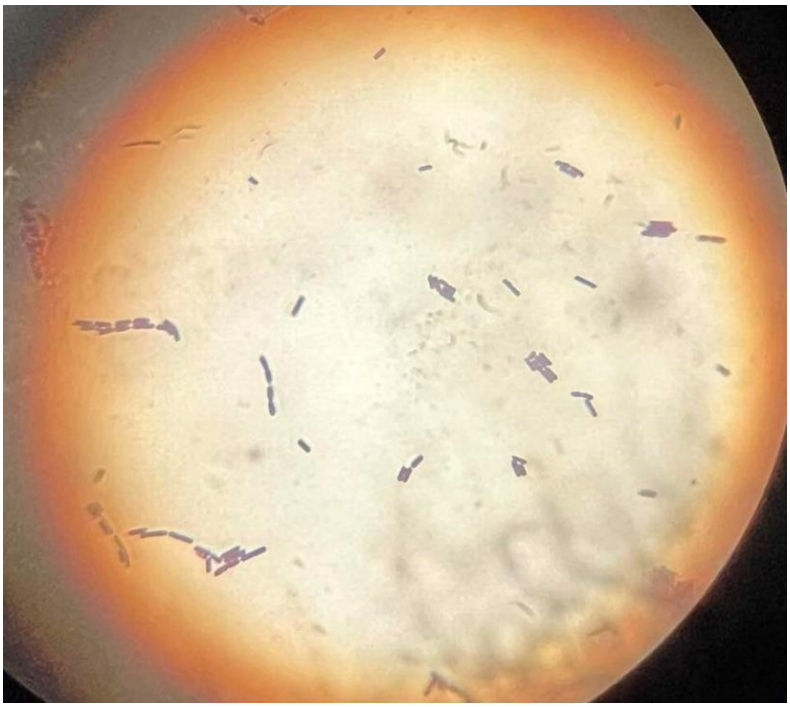


Figure 2.8: Gram-positive sample showing purple color

2.9.2. HiMedia Analysis

Upon careful examination of the microscopic findings, then we had done streaking on culture media plates. Seeing the colony growth pattern and their color, we have tried to identify the organisms following the "HiMedia Publication". The organisms present in MacConkey agar media, TCBS, SS, and EMB agar media have been identified by analysis of this paper. According to the publication, the organism can be identified based on the color of its colony.

The following chart demonstrates the method of identification for each culture media: (HiMedia Laboratories, n.d.).

Culture Media Name	**Color of the Colony**	**Organism Name**
MacConkey Agar	pink to red, matte with bile precipitate	*Escherichia coli*
MacConkey Agar	pink to red	*Klebsiella aerogenes*
MacConkey Agar	colorless	*Salmonella Typhimurium*
MacConkey Agar	colorless	*Salmonella Enteritidis*
MacConkey Agar	colorless	*Salmonella Paratyphi A*
MacConkey Agar	colorless	*Salmonella Paratyphi B*
MacConkey Agar	colorless	*Salmonella Typhi*
MacConkey Agar	colorless	*Proteus vulgaris*
TCBS Agar	yellow	*Vibrio cholerae*
TCBS Agar	yellow	*Vibrio fluvialis*
TCBS Agar	bluish green	*Vibrio parahaemolyticus*
TCBS Agar	greenish yellow	*Vibrio vulnificus*

Table 2.1: HiMedia Analysis (HiMedia Laboratories, n.d.)

Culture Media Name	Color of the Colony	Organism Name
SS Agar	colorless with black center	*Salmonella Choleraesuis*
SS Agar	colorless with black center	*Salmonella Typhi*
SS Agar	colorless with black center	*Salmonella Typhimurium*
SS Agar	colorless with black center	*Salmonella Enteritidis*
SS Agar	colorless	*Shigella flexneri*
SS Agar	Colorless, may have black center	*Proteus mirabilis*
SS Agar	pink with bile precipitate	*Escherichia coli*
EMB Agar	purple with black center and green metallic sheen	*Escherichia coli*
EMB Agar	pink, without sheen	*Enterobacter aerogenes*
EMB Agar	pink, mucoid	*Klebsiella pneumoniae*
EMB Agar	colorless	*Proteus mirabilis*
EMB Agar	colorless	*Salmonella Typhimurium*

Table 2.1: (Continued) HiMedia Analysis (HiMedia Laboratories, n.d.)

2.10. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) involves subjecting the microorganism to various antibiotics inside a controlled laboratory setting to assess their efficacy in inhibiting bacterial growth (*Antimicrobial Susceptibility Testing (AST): Définition, Protocol, Interpretation*, n.d.).

For our research project, we followed the Kirby-Bauer disk-diffusion method which is widely used to do antibiotic susceptibility testing. In this method, a bacterial suspension was prepared and with the help of a cotton swab, the suspension was lawned onto the MHA agar medium. Then, the antibiotic disks were placed on the culture medium and the culture plates put into an incubator for 18-24 hours. After the incubation period, the diameter zone was measured to determine whether the particular sample isolate was resistant, intermediate, or susceptible to the particular antibiotics.

➢ 2.10.1. Bacterial Suspension Preparation

Before doing the antibiotic susceptibility testing, a bacterial suspension was prepared. For this, Luria Broth was utilized. In an autoclaved test-tube, 2 ml of LB was taken for each isolated sample. With this, using a sterile metallic loop, the bacterial colony from the 18-hour-old culture was taken and mixed. The LB and colony mixture was vortexed and kept in a shaker incubator for 2 hours for optimum growth of bacteria.

➢ 2.10.2. Muller-Hinton Agar

To perform AST, MHA medium is the most preferred culture medium as it is not a differential, as well as a selective medium. Moreover, it allows better diffusion of antibiotics than most other medium. Additionally, it comprises starch. Starch has been

observed to possess the capability to absorb toxins that are generated by bacteria, hence preventing their interference with the efficacy of antibiotics (Aryal, 2015).

For our work, we took 38 grams of MHA powder and mixed with 1 liter of distilled water. The mixture was boiled and later autoclaved. Afterward, it was transferred to sterile Petri plates.

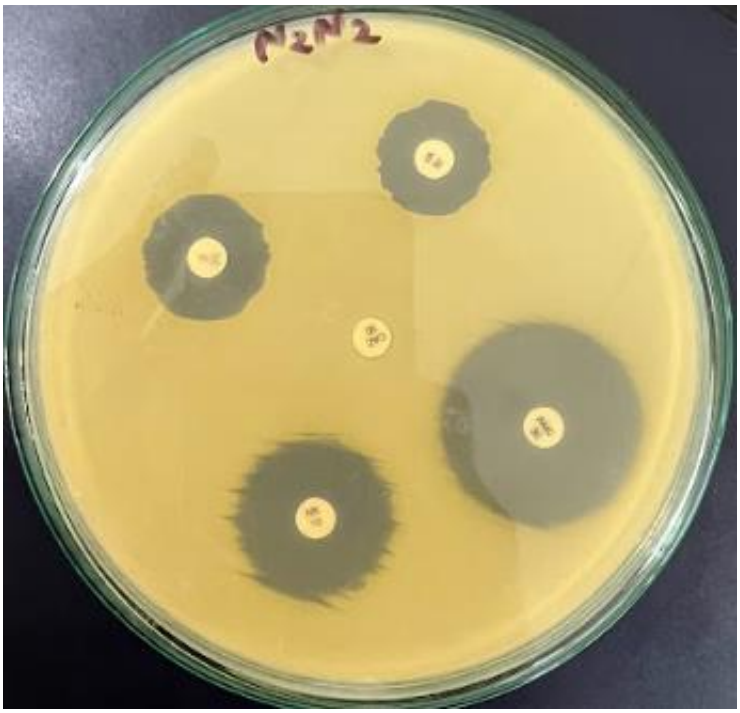


Figure 2.9: Antibiotic Susceptibility Testing (AST)

➢ 2.10.3. List of Antibiotics

The selection of antibiotics for the susceptibility test was based on their recommended usage. The table below presents the list of antibiotics utilized in this research study, along with their corresponding zone of inhibition-

Antibiotic group	**Antibiotic Name**	**Disc Concentration**	**Diameter of zone of inhibition**		
			Resistant < or = (mm)	Intermediate (mm)	Sensitive = or > (mm)
Penicillins	Ampicillin (AMP)	25 mcg	13	14-17	20
Monobactams	Aztreonam (AT)	30 mcg	17	18-20	21
Phenicols	Chloramphenicol (C)	30 mcg	12	13-17	18
Cephalosporin	Cefixime (CFM)	30 mcg	15	16-18	19
Sulfonamides-Trimethoprim	Co-Trimoxazole (COT)	25 mcg	10	11-15	16
Tetracyclines	Doxycycline (DO)	30 mcg	10	11-13	14
Macrolides	Erythromycin (E)	15 mcg	13	14-22	23
Carbapenem	Imipenem (IPM)	10 mcg	13	14-15	16
Aminoglycosides	Kanamycin (K)	30 mcg	13	14-17	18
Fluoroquinolone	Levofloxacin (LE)	5 mcg	16	17-20	21
Oxazolidinones	Linezolid (LZ)	30 mcg	20	21-22	23
Glycopeptides	Vancomycin (VA)	5 mcg	14	15-16	17
Pinicillin combination	Amoxicillin / Clavulanic acid (AMC)	30 mcg	13	14-17	20
Drugs against mycobacteria	Streptomycin (S)	10 mcg	11	12-14	15

Table 2.2: Antibiotic List with their diameter zone of inhibition

2.11. Plasmid Isolation via PCI Method

Plasmid isolation is a process that utilizes the difference in topology between plasmids and bacterial chromosomal DNA. Plasmids are small, supercoiled DNA molecules, while chromosomal DNA is larger and less supercoiled. This allows for selective precipitation of chromosomal DNA and cellular proteins from plasmids and RNA molecules. For plasmid isolation, bacterial cultures should be grown to the late logarithmic/early stationary phase so, cells should be grown overnight in proper condition to obtain the most viable culture. We inoculated the sample in LB medium and kept it overnight in shaking incubator. After that, the sample was used to isolate the plasmid.

Consecutively, 3 lysis solutions were used to isolate the plasmid. At first, with the addition of lysis solution I, the cells undergo lysis in an alkaline environment, resulting in the denaturation of nucleic acids and proteins. As the lysis solution II, comprised of sodium hydroxide and sodium dodecyl sulfate (SDS), they were utilized to induce cell lysis and promote the denaturation of genomic and plasmid DNA, as well as any proteins present in the solution. The cell membranes are effectively disrupted and the double-stranded DNAs (dsDNA) are converted into single-stranded DNAs (ssDNA) through the use of a strongly alkaline solution containing NaOH and SDS. Then, lysis solution III was added. Upon neutralization of the solution with the introduction of Potassium Acetate, chromosomal DNA and proteins precipitate due to their inability to properly renature, possibly because of their huge size. Plasmids exhibit proper renaturation and remain in solution, efficiently segregating them from chromosomal DNA and proteins. Lastly, ethanol precipitation is used to get the plasmid DNA out of the cell. Once the plasmid DNA had been precipitated, it was rinsed with 70% ethanol which was as cold as ice, and was allowed to air dry for about 10 minutes so that the alcohol could evaporate. Then, the plasmid DNA pellet was

resuspended in a buffer solution containing Tris-EDTA and stored in -20^0c freezer (Man, T. P., n.d.).

In addition, we have utilized DH5α cells as positive control. As we know that, the *E. coli* strain DH5α is commonly employed as a host strain for gene cloning due to its favorable characteristics, including robust growth and possess a high degree of transformation efficiency, enabling them to effectively maintain plasmid quantities and integrity (*E. Coli Cloning Strains for Molecular Biology: What They Are and Why They Matter*, n.d.). We used the DH5α because we knew we would surely get plasmid from this cell and be able to use it as a positive control to compare our results.

2.12. Plasmid Visualization by Gel Electrophoresis

After we have done plasmid isolation, gel electrophoresis was performed to verify the presence of plasmids and visualize them. Some steps were followed to perform this technique.

➢ 2.12.1. Agarose Gel preparation

To prepare 100 ml of 1% agarose solution, 1g agarose powder was measured into a flask and 98 ml distilled water along with 2 ml 50X TAE buffer was added to it. The solution was put inside a microwave on a hot plate until agarose was dissolved and the solution became clear. Subsequently, 4 µl EtBr was added to the gel when it became a little bit cool. Later, the comb was placed into the gel casting tray. The liquid gel was poured into the gel casting tray, then allowed to be cool in room temperature. It was made sure that, there was no bubble in the gel. The comb was placed about 1 inch from one end of the tray and positioned

vertically such that the teeth were about 1- 2 mm above the surface of the tray. When the gel became solidified, the comb was carefully removed.

2.12.2. Gel electrophoresis

The tray was placed in electrophoresis chamber, and covered with electrophoresis buffer or running buffer till the max fill line. The isolated plasmid samples were prepared for electrophoresis, by the addition of 1µl of gel loading dye for every 5µl of plasmid DNA solution. Total 6µl sample was loaded into each well. Also, for each water sample, when we ran the gel electrophoresis, we have added the isolated plasmid from DH5α cell at the very last lane, as the positive control. Afterward, the gel was run at 90V for 60minutes. Lastly, the plasmid bands were visualized under short wave UV light.

3. Results

➢ Labelling the isolated colonies:

We have collected water samples from 08 (eight) different water sources of Bangladesh. 04(four) samples were collected from inside Dhaka district and 04(four) samples were collected from outside Dhaka district water sources.

From the samples, isolated strains of bacteria were named as:

- First letter of the area, from where the water sample was collected. For example: Hatir Jheel: H
- Secondly, the serial number of isolated colonies from the sample. For example: Hatir Jheel's 1st colony: H1
- Then, according to the first letter of the media name. Here, Nutrient Agar (NA)=N, Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar=T, Eosin Methylene Blue Agar (EMB agar) =E, MacConkey agar=M, Salmonella Shigella (SS) Agar=S.
- Lastly, the number of isolated colonies from a specific media.

So, H1N1means, Hatir Jheel's 1st isolated colony, from NA media, colony number 1.

➢ The percentages of Gram-positive and Gram-negative bacteria:

As we have used selective medias for Gram-negative bacteria, thus, we did not find too many Gram-positive bacteria from our 180 isolated colonies. Only in the Nutrient agar (NA) media, we have isolated only 4 colonies of Gram-positive bacteria. So, there is only 2.5% of Gram-positive bacteria, and 97.5% of Gram-negative bacteria found in our total isolated colonies from the water samples.

3.1. Inside Dhaka district water sample results:

3.1.1. Results of Hatir Jheel water sample:

H= Hatir jheel water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Hatir Jheel water sample, we got 35 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-3}.

Calculation:

Here,

Number of colonies= 35

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-7}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies*reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(35*3.75x10^{7}) / 0.15= 8.75x10^{9}$

So, total colony forming units for HatirJheel water sample was $8.75x10^{9}$ CFU/mL

➢ Identification by colony morphology

Sample Name	**Characteristics**	**Gram staining**			
		Result		Shape	Species
H1E1	Pink, without sheen	Pink	(-)	Rod	Enterobacter spp.
H2N2	Yellow in TCBS agar	Pink	(-)	Rod	Vibrio spp.
H3E3	Pink, mucoid	Pink	(-)	Rod	Klebsiella spp.
H4N4	Pink to red in MacConkey Agar	Pink	(-)	Rod	*Klebsiella spp.*
H5E5	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
H6S1	Colorless	Pink	(-)	Rod	*Shigella spp.*
H7S2	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
H8S3	Colorless	Pink	(-)	Rod	*Shigella spp.*
H9S4	Colorless	Pink	(-)	Rod	*Shigella spp.*
H10S5	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
H11M1	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
H12M2	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
H13M3	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
H14M4	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
H15M5	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
H16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
H17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
H18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
H19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
H20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.1: Probable identification of colonies isolated from Hatir Jheel water sample

- AST Results:

Antibiotic group	H1E1	Result	H2N2	Result	H3E3	Result	H4N4	Result	H5E5	Result	H6S1	Result	H7S2	Result	H8S3	Result	H9S4	Result	H10S5	Result
	Enterobacter spp.		*Vibrio spp.*		*Klebsiella spp.*		*Klebsiella spp.*		*E. coli*		*Shigella spp.*		*E. coli*		*Shigella spp.*		*Shigella spp.*		*Proteus spp.*	
Penicillins	0	R	0	R	0	R	0	R	17	S	0	R	0	R	0	R	0	R	0	R
Monobactams	26	S	0	R	24	S	30	S	25	S	24	S	27	S	21	S	24	S	29	S
Phenicols	25	S	27	S	19	S	26	S	27	S	20	S	24	S	28	S	25	S	22	S
Cephalosporin	35	S	0	R	0	R	30	S	22	S	18	I	23	S	25	S	22	S	21	S
Sulfonamides-Trimethoprim	19	S	25	S	0	R	27	S	24	S	0	R	28	S	31	S	26	S	0	R
Tetracyclines	16	S	21	S	16	S	17	S	0	R	12	I	16	S	16	S	16	S	16	S
Macrolides	16	I	0	R	0	R	13	R	19	I	0	R	10	R	16	I	0	R	0	R
Carbapenem	24	S	12	R	16	R	22	I	25	S	12	R	29	S	0	R	0	R	19	R
Aminoglycosides	18	S	22	S	17	S	22	S	17	S	18	S	18	S	17	S	17	S	19	S
Fluoroquinolone	20	I	24	S	16	R	30	S	31	S	17	I	25	S	19	I	23	S	13	R
Oxazolidinones	0	R	13	R	0	R	0	R	11	R	R	R	0	R	0	R	0	R	0	R
Glycopeptides	13	R	11	R	0	R	11	R	0	R	0	R	0	R	0	R	0	R	0	R
Pinicillin combination	30	S	33	S	24	S	35	S	26	S	0	R	21	S	0	R	9	R	0	R
Drugs against mycobacteria	18	S	20	S	17	S	22	S	20	S	18	S	18	S	19	S	18	S	19	S

Table 3.2: Antimicrobial susceptibility testing (AST) results of Hatir Jheel water sample

Antibiotic group	H11M1	Result	H12M2	Result	H13M3	Result	H14M4	Result	H15M5	Result	H16T1	Result	H17T2	Result	H18T3	Result	H19T4	Result	H20T5	Result
	Klebsiella spp.		*Klebsiella spp.*		*Klebsiella spp.*		*Klebsiella spp.*		*E. coli*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Monobactams	0	R	0	R	0	R	24	S	26	S	0	R	9	R	10	R	24	S	0	R
Phenicols	15	I	15	I	15	I	23	S	26	S	27	S	30	S	29	S	20	S	26	S
Cephalosporin	0	R	0	R	0	R	25	S	23	S	0	R	0	R	0	R	18	I	0	R
Sulfonamides-Trimethoprim	0	R	0	R	0	R	28	S	30	S	27	S	31	S	30	S	0	R	28	S
Tetracyclines	14	S	15	S	15	S	15	S	18	S	19	S	21	S	20	S	16	S	20	S
Macrolides	0	R	0	R	0	R	0	R	16	I	0	R	0	R	0	R	0	R	0	R
Carbapenem	0	R	0	R	0	R	25	S	29	S	13	R	17	R	16	R	15	R	11	R
Aminoglycosides	0	R	0	R	0	R	17	S	17	S	20	S	19	S	21	S	18	S	19	S
Fluoroquinolone	0	R	0	R	0	R	25	S	34	S	22	S	22	S	22	S	16	R	22	S
Oxazolidinones	0	R	0	R	0	R	0	R	0	R	0	R	11	R	12	R	0	R	0	R
Glycopeptides	0	R	0	R	0	R	0	R	13	R	11	R	11	R	16	I	0	R	0	R
Pinicillin combination	0	R	0	R	0	R	21	S	17	I	11	R	16	I	13	R	0	R	0	R
Drugs against mycobacteria	17	S	16	S	10	R	18	S	18	S	19	S	18	S	25	S	19	S	21	S

Table 3.2: (Continued) Antimicrobial susceptibility testing (AST) results of Hatir Jheel water sample

- AST results in a pie chart:

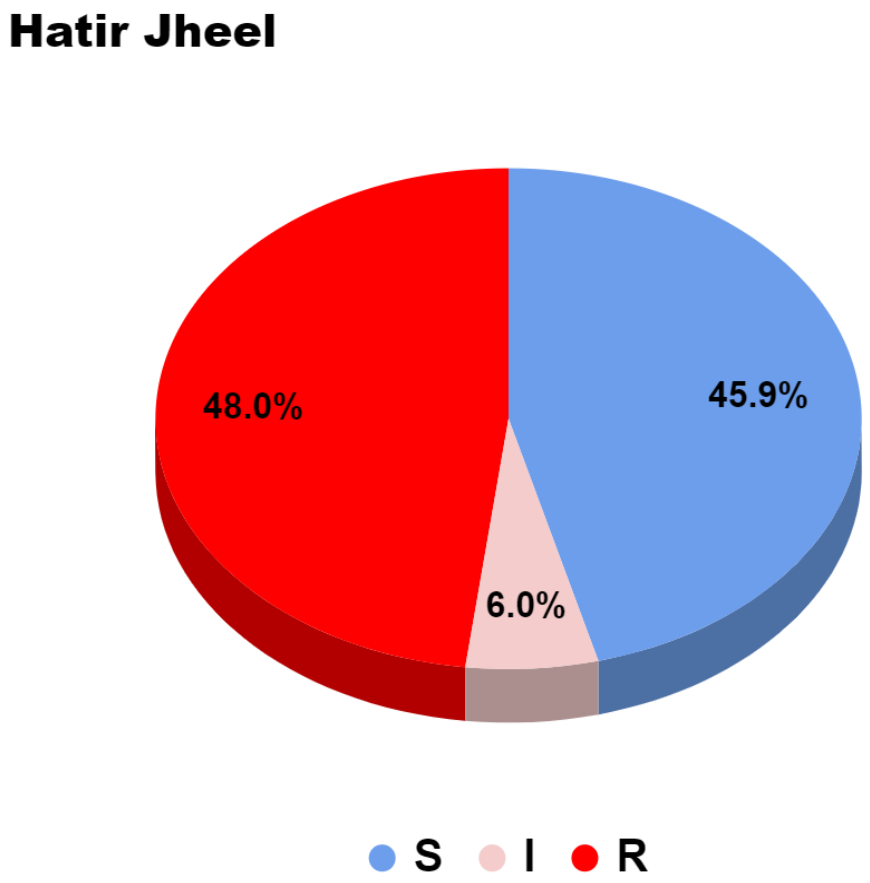


Figure 3.1: Pie chart showing AST results of Hatir Jheel

From the pie chart, it is found that from the isolated colonies of the Hatir Jheel water sample, 48% of the bacteria showed resistance, 45.9% showed sensitivity, and 6% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Hatir Jheel water sample

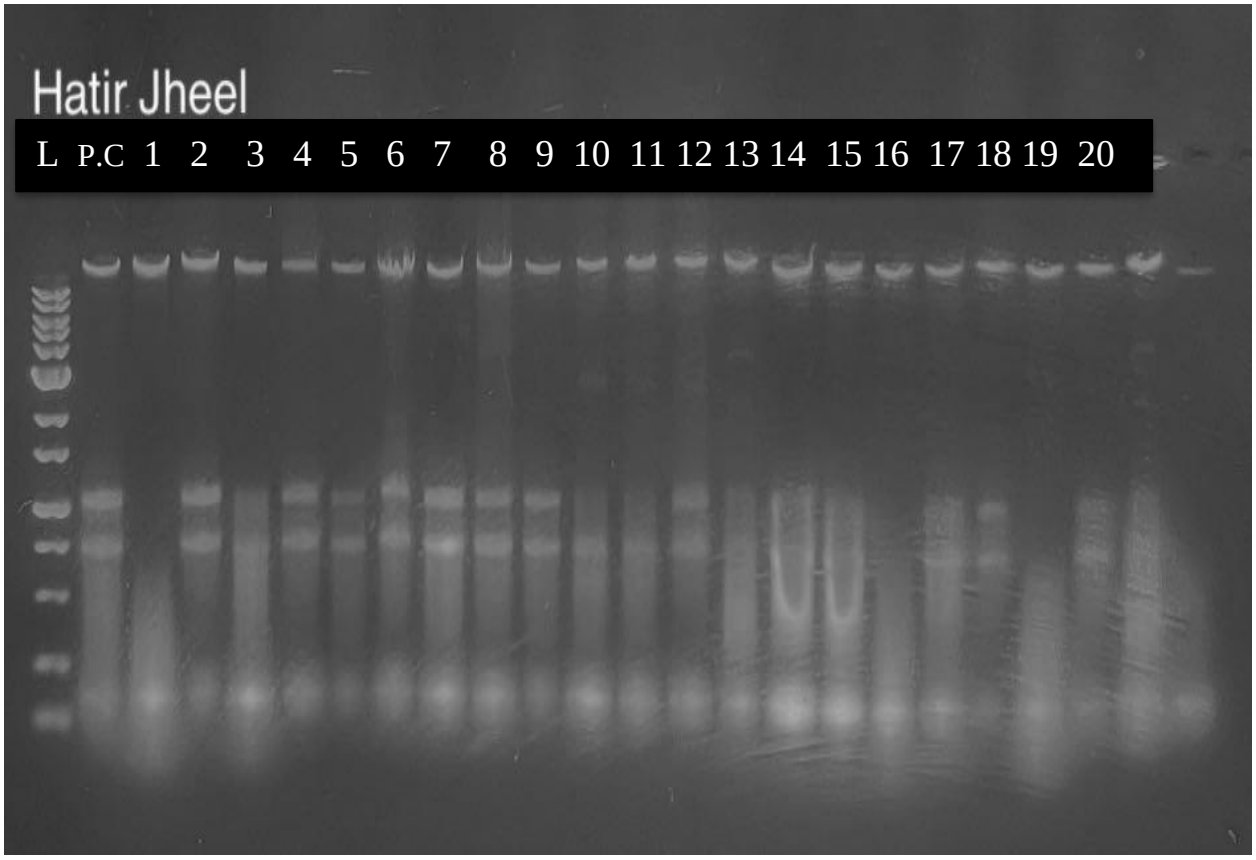


Figure 3.2: Plasmids isolated from the colonies of Hatir Jheel water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Hatir Jheel water sample. From the 20 colonies, 17 colonies showed plasmid bands. And the other 03 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 1Kb PLUS DNA ladder has been used.

3.1.2. Results of Banani Lake water sample:

B= Banani lake water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Banani Lake water sample, we got 32 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-3}.

Calculation:

Here,

Number of colonies= 32

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-7}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(32*3.75x10^{7}) / 0.15= 8x10^{9}$

So, total colony forming units for Banani Lake water sample was $8x10^{9}$ CFU/mL

➢ Identification by colony morphology

Sample Name	**Characteristics**	**Gram staining**			
		Result		Shape	Species
BIEI	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
B2E2	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
B3E3	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
B4E4	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
B5E5	Pink, mucoid	Pink	(-)	Rod	*Klebsiella spp.*
B6S1	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
B7S2	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
B8S3	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
B9S4	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
B10S5	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
B11M1	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
B12M2	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
B13M3	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
B14M4	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
B15M5	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
B16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
B17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
B18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
B19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
B20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.3: Probable identification of colonies isolated from Banani Lake water sample

➢ AST Results

Antibiotic group	BIEI	Result	B2E2	Result	B3E3	Result	B4E4	Result	B5E5	Result	B6S1	Result	B7S2	Result	B8S3	Result	B9S4	Result	B10S5	Result
	E. coli		*E. coli*		*E. coli*		*E. coli*		*Klebsiella spp.*		*Salmonella spp.*		*E. coli*		*Salmonella spp.*		*Salmonella spp.*		*Salmonella spp.*	
Glycopeptides	11	R	0	R	12	R	15	I	0	R	0	R	0	R	0	R	0	R	0	R
Oxazolidinones	0	R	10	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Aminoglycosides	16	I	18	S	0	R	16	I	17	I	15	I	17	I	17	I	17	I	17	I
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Fluoroquinolone	28	S	31	S	28	S	28	S	26	S	25	S	28	S	25	S	22	S	22	S
Cephalosporin	13	R	14	R	15	R	13	R	12	R	0	R	12	R	0	R	13	R	14	R
Macrolides	14	I	15	I	19	I	14	I	9	R	10	R	9	R	0	R	15	I	14	I
Sulfonamides-Trimethoprim	27	S	31	S	30	S	0	R	29	S	30	S	30	S	27	S	0	R	0	R
Tetracyclines	17	S	19	S	11	I	11	I	17	S	17	S	17	S	16	S	11	I	10	R
Monobactams	24	S	28	S	27	S	25	S	27	S	25	S	27	S	0	R	28	S	26	S
Phenicols	24	S	29	S	27	S	12	R	25	S	24	S	24	S	24	S	20	S	19	S
Carbapenem	24	S	29	S	25	S	25	S	25	S	22	I	25	S	25	S	26	S	27	S
Pinicillin combination	18	S	21	S	20	S	15	I	21	S	0	R	21	S	19	S	18	S	19	S
Drugs against mycobacteria	20	S	18	S	19	S	18	S	20	S	19	S	18	S	12	I	20	S	19	S

Table 3.4: Antimicrobial susceptibility testing (AST) results of Bnani Lake water sample

Antibiotic group	B11M1	Result	B12M2	Result	B13M3	Result	B14M4	Result	B15M5	Result	B16T1	Result	B17T2	Result	B18T3	Result	B19T4	Result	B20T5	Result
	Enterobacter spp.		*Klebsiella spp.*		*Enterobacter spp.*		*E. coli*		*Klebsiella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Glycopeptides	0	R	0	R	0	R	12	R	0	R	13	R	14	R	14	R	11	R	14	R
Oxazolidinones	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Aminoglycosides	16	I	15	I	16	I	16	I	16	I	20	S	20	S	19	S	21	S	23	S
Penicillins	0	R	0	R	0	R	9	R	0	R	0	R	0	R	0	R	0	R	0	R
Fluoroquinolone	35	S	27	S	28	S	32	S	23	S	29	S	30	S	29	S	24	S	29	S
Cephalosporin	16	R	12	R	16	R	15	R	10	R	12	R	0	R	0	R	0	R	14	R
Macrolides	9	R	9	R	10	R	17	I	10	R	13	R	15	I	14	I	14	I	15	I
Sulfonamides-Trimethoprim	29	S	26	S	29	S	30	S	0	R	22	S	25	S	0	R	23	S	22	S
Tetracyclines	18	S	16	S	19	S	20	S	8	R	18	S	17	S	20	S	19	S	19	S
Monobactams	29	S	25	S	30	S	30	S	22	S	29	S	25	S	27	S	26	S	24	S
Phenicols	27	S	24	S	26	S	27	S	20	S	26	S	27	S	14	I	27	S	25	S
Carbapenem	27	S	25	S	26	S	26	S	24	S	23	S	24	S	24	S	25	S	26	S
Pinicillin combination	21	S	21	S	21	S	22	S	20	S	12	R	15	I	20	S	16	I	17	I
Drugs against mycobacteria	19	S	18	S	17	S	20	S	16	S	19	S	20	S	17	S	15	S	24	S

Table 3.4: (Continued) Antimicrobial susceptibility testing (AST) results of Banani Lake water sample

- AST results in a pie chart:

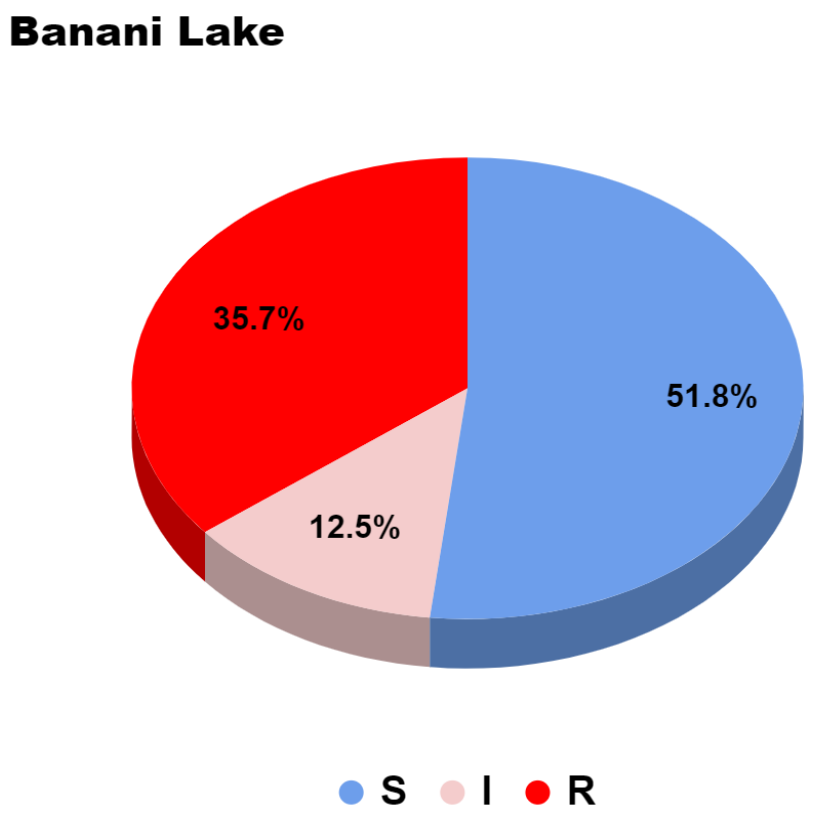


Figure 3.3: Pie-chart showing AST results of Banani Lake

From the pie chart, it is found that from the isolated colonies of the Banani Lake water sample, 35.7% of the bacteria showed resistance, 51.8% showed sensitivity, and 12.5% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Banani Lake water sample

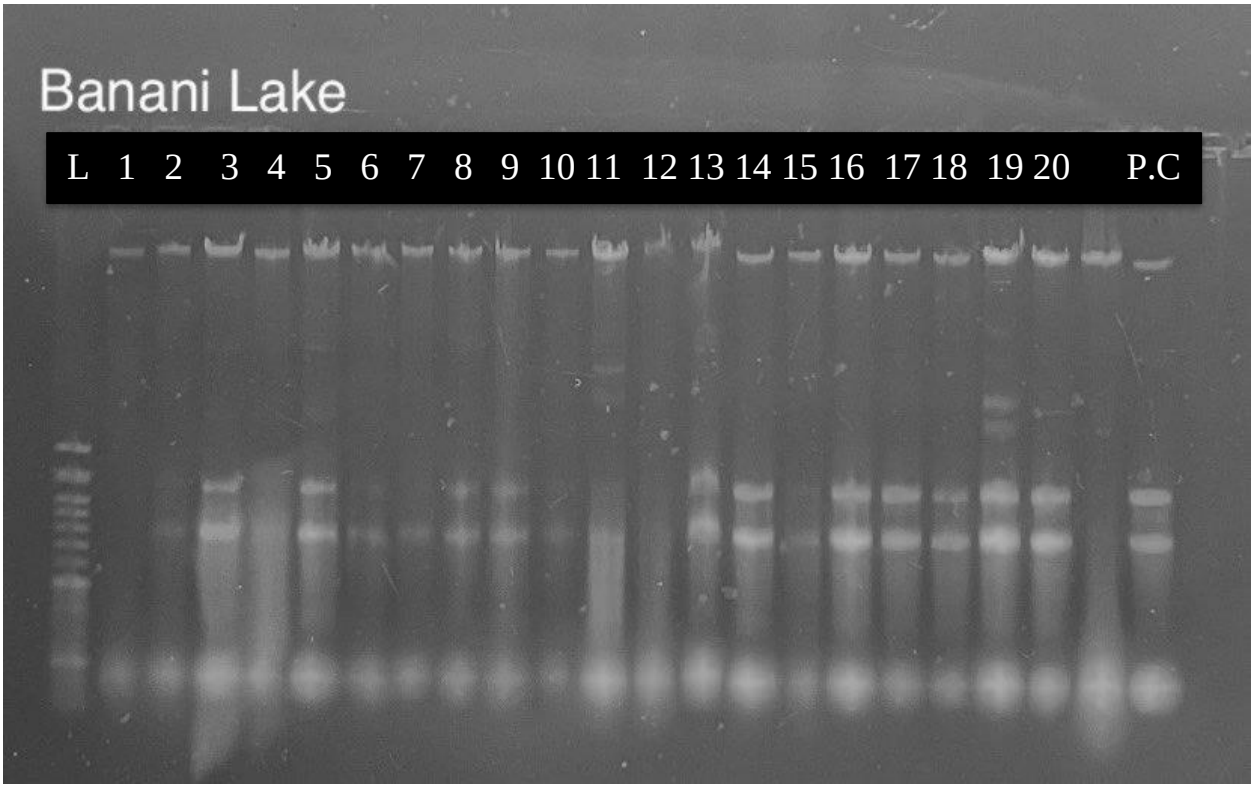


Figure 3.4: Plasmids isolated from the colonies of Banani Lake water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Banani Lake water sample. From the 20 colonies, 16 colonies showed plasmid bands. And the other 04 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 50BP DNA ladder has been used.

3.1.3. Results of Dhanmondi Lake water sample:

D= Dhanmondi lake water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Dhanmondi Lake water sample, we got 182 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-1}.

Calculation:

Here,

Number of colonies= 182

Total Dilution Factor = (Current Dilution x Previous Dilution) = 0.0375

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = (182*26.7) / 0.15= $3.3x10^4$

So, total colony forming units for Dhanmondi Lake water sample was $3.3x10^4$ CFU/mL

➢ Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
D1N1	Colorless in MacConkey Agar	Pink	(-)	Rod	*Proteus spp.*
D2E2	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
D3E3	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
D4E4	Pink, mucoid	Pink	(-)	Rod	*Klebsiella spp.*
D5E5	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
D6S1	Colorless	Pink	(-)	Rod	*Shigella spp.*
D7S2	Colorless	Pink	(-)	Rod	*Shigella spp.*
D8S3	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
D9S4	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
D10S5	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
D11M1	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
D12M2	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
D13M3	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
D14M4	Colorless	Pink	(-)	Rod	*Pseudomonas spp.*
D15M5	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
D16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
D17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
D18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
D19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
D20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.5: Probable identification of colonies isolated from Dhanmondi Lake water sample

➢ AST Results:

Antibiotic group	D1N1	Result	D2E2	Result	D3E3	Result	D4E4	Result	D5E5	Result	D6S1	Result	D7S2	Result	D8S3	Result	D9S4	Result	D10S5	Result
	Proteus spp.		*E. coli*		*E. coli*		*Klebsiella spp.*		*E. coli*		*Shigella spp.*		*Shigella spp.*		*E. coli*		*E. coli*		*E. coli*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	13	R	0	R	0	R	0	R
Glycopeptides	20	S	20	S	0	R	0	R	12	R	0	R	0	R	0	R	0	R	0	R
Oxazolidinones	12	R	9	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Aminoglycosides	21	S	22	S	16	I	18	S	21	S	16	I	18	S	16	I	16	I	17	I
Fluoroquinolone	41	S	28	S	21	S	16	I	34	S	21	S	28	S	23	S	28	S	22	S
Cephalosporin	32	S	22	S	24	S	13	R	16	R	12	R	29	S	25	S	15	R	25	S
Sulfonamides-Trimethoprim	27	S	19	S	29	S	0	R	24	S	26	S	17	S	27	S	0	R	28	S
Monobactams	28	S	10	R	25	S	19	I	29	S	22	S	28	S	27	S	26	S	26	S
Macrolides	17	I	19	I	0	R	0	R	17	I	0	R	0	R	0	R	17	I	0	R
Phenicols	28	S	20	S	22	S	22	S	28	S	24	S	14	I	22	S	25	S	22	S
Tetracyclines	24	S	29	S	18	S	17	S	21	S	9	R	18	S	17	S	12	I	17	S
Carbapenem	13	R	23	S	19	R	16	R	29	S	21	I	22	I	21	I	26	S	19	R
Pinicillin combination	11	R	27	S	9	R	10	R	27	S	0	R	18	S	0	R	20	S	0	R
Drugs against mycobacteria	21	S	26	S	18	S	21	S	20	S	16	S	20	S	20	S	0	R	18	S

Table 3.6: Antimicrobial susceptibility testing (AST) results of Dhanmondi Lake water sample

Antibiotic group	D11M1	Result	D12M2	Result	D13M3	Result	D14M4	Result	D15M5	Result	D16T1	Result	D17T2	Result	D18T3	Result	D19T4	Result	D20T5	Result
	Enterobacter spp.		*E. coli*		*E. coli*		*Pseudomonas spp.*		*Klebsiella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Glycopeptides	0	R	13	R	0	R	13	R	0	R	0	R	12	R	0	R	0	R	0	R
Oxazolidinones	0	R	0	R	0	R	12	R	0	R	0	R	0	R	0	R	0	R	0	R
Aminoglycosides	17	I	18	S	16	I	19	S	16	I	15	I	20	S	15	I	15	I	17	I
Fluoroquinolone	25	S	21	S	22	S	43	S	30	S	20	S	22	S	21	S	21	S	22	S
Cephalosporin	22	S	27	S	26	S	36	S	28	S	17	R	15	R	15	R	16	R	15	R
Sulfonamides-Trimethoprim	26	S	28	S	33	S	28	S	29	S	28	S	25	S	28	S	38	S	26	S
Monobactams	28	S	28	S	29	S	31	S	26	S	26	S	30	S	29	S	40	S	27	S
Macrolides	11	R	11	R	19	I	19	I	0	R	0	R	17	I	0	R	0	R	0	R
Phenicols	23	S	28	S	29	S	30	S	25	S	27	S	29	S	27	S	33	S	25	S
Tetracyclines	11	I	15	S	20	S	26	S	18	S	17	S	20	S	17	S	23	S	16	S
Carbapenem	20	I	21	I	27	S	18	R	22	I	25	S	27	S	27	S	31	S	23	S
Pinicillin combination	18	S	11	R	0	R	12	R	20	S	15	I	20	S	0	R	0	R	0	R
Drugs against mycobacteria	18	S	18	S	17	S	24	S	19	S	16	S	20	S	16	S	17	S	19	S

Table 3.6: (Continued) Antimicrobial susceptibility testing (AST) results of Dhanmondi Lake water sample

- AST results in a pie chart:

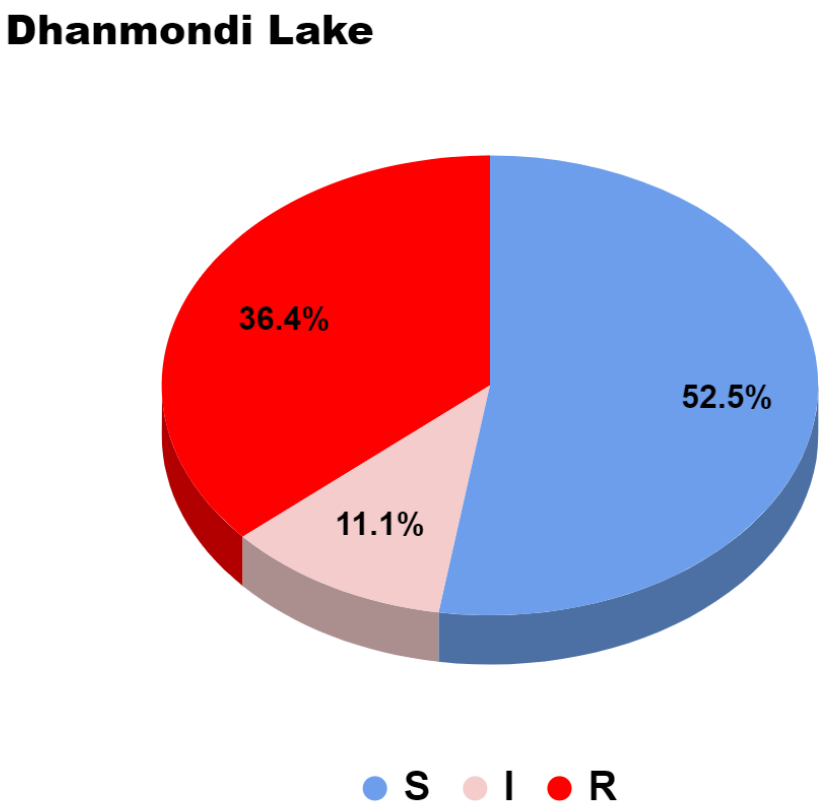


Figure 3.5: Pie-chart showing AST results of Dhanmondi Lake

From the pie chart, it is found that from the isolated colonies of the Dhanmondi Lake water sample, 36.4% of the bacteria showed resistance, 52.5% showed sensitivity, and 11.1% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Dhanmondi Lake water sample

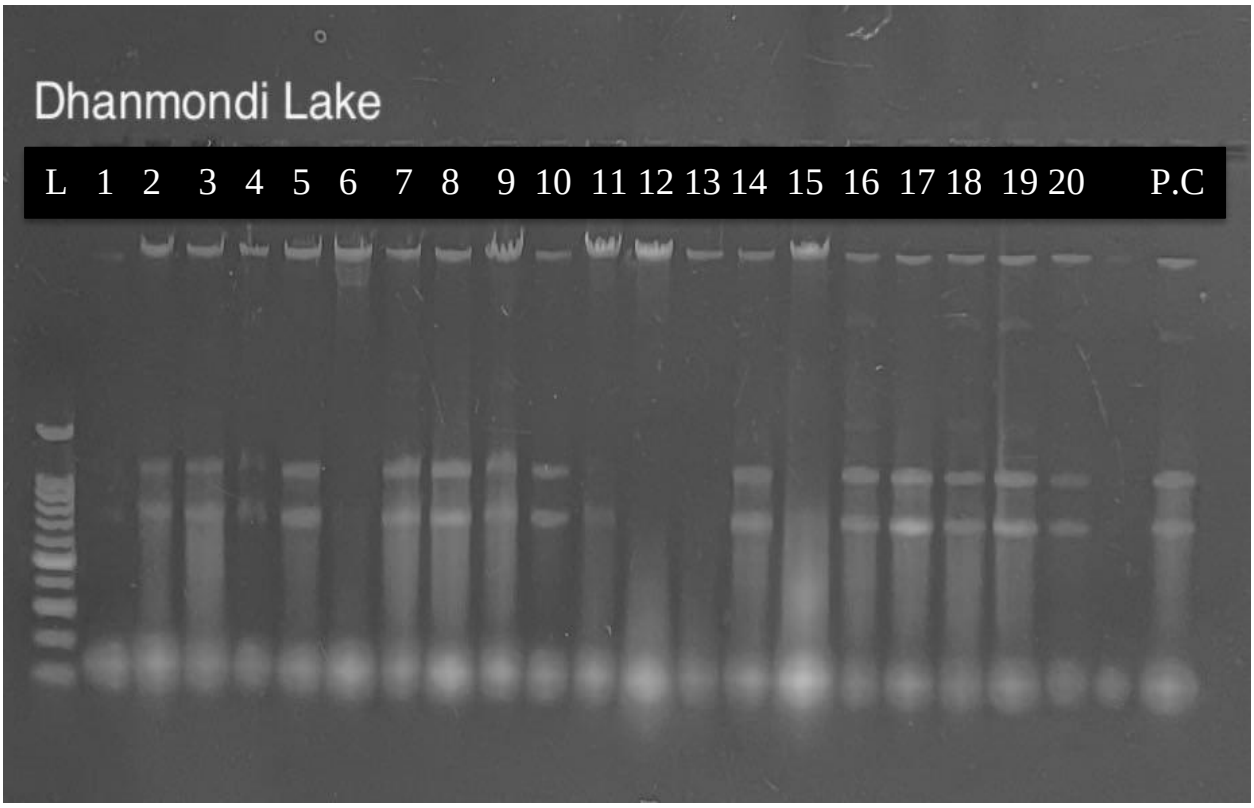


Figure 3.6: Plasmids isolated from the colonies of Dhanmondi Lake water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Dhanmondi Lake water sample. From the 20 colonies, 15 colonies showed plasmid bands. And the other 05 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 100BP DNA ladder has been used.

3.1.4. Results of Balu River water sample:

N= Nandi Para/Balu river water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Balu River water sample, we got 94 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-2}.

Calculation:

Here,

Number of colonies= 94

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-4}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(94*3.75x10^{4}) / 0.15= 2.35x10^{7}$

So, total colony forming units for Balu River water sample was $2.35x10^{7}$ CFU/mL

➢ Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
N1N1	Yellow	Purple	(+)	Cocci	*Staphylococcus spp.*
N2N2	Yellow	Purple	(+)	Cocci	*Staphylococcus spp.*
N3E3	Pink, mucoid	Pink	(-)	Rod	*Klebsiella spp.*
N4N4	Yellow	Purple	(+)	Cocci	*Staphylococcus spp.*
N5N5	Yellow	Purple	(+)	Cocci	*Staphylococcus spp.*
N6S1	Colorless	Pink	(-)	Rod	*Shigella spp.*
N7S2	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
N8S3	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
N9S4	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
N10S5	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
N11M1	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
N12M2	Colorless	Pink	(-)	Rod	*Salmonella spp.*
N13M3	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
N14M4	Colorless	Pink	(-)	Rod	*Salmonella spp.*
N15M5	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
N16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
N17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
N18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
N19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
N20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.7: Probable identification of colonies isolated from Balu River water sample

➢ AST Results:

Antibiotic group	N1N1	Result	N2N2	Result	N3E3	Result	N4N4	Result	N5N5	Result	N6S1	Result	N7S2	Result	N8S3	Result	N9S4	Result	N10S5	Result
	Staphylococcus spp.		*Staphylococcus spp.*		*Klebsiella spp.*		*Staphylococcus spp.*		*Staphylococcus spp.*		*Shigella spp.*		*Proteus spp.*		*E. coli*		*Salmonella spp.*		*E. coli*	
Cephalosporin	0	R	0	R	0	R	0	R	0	R	0	R	10	R	14	R	10	R	0	R
Glycopeptides	20	S	19	S	17	S	20	S	20	S	13	R	0	R	0	R	0	R	14	R
Penicillins	23	R	17	R	0	R	15	R	18	R	0	R	0	R	0	R	14	I	0	R
Aminoglycosides	19	S	21	S	19	S	22	S	22	S	17	I	17	I	15	I	9	R	16	I
Oxazolidinones	32	S	30	S	14	R	29	S	25	S	0	R	0	R	0	R	0	R	0	R
Macrolides	25	S	19	I	9	R	0	R	20	I	0	R	0	R	0	R	0	R	0	R
Tetracyclines	29	S	24	S	31	S	30	S	25	S	27	S	0	R	12	I	12	I	11	I
Phenicols	24	S	25	S	30	S	27	S	26	S	0	R	24	S	28	S	26	S	28	S
Sulfonamides-Trimethoprim	27	S	23	S	30	S	25	S	25	S	0	R	0	R	23	S	29	S	0	R
Fluoroquinolone	25	S	20	I	25	S	25	S	20	I	25	S	13	R	19	I	25	S	24	S
Carbapenem	35	S	36	S	42	S	36	S	33	S	31	S	22	I	21	I	22	I	24	S
Monobactams	0	R	0	R	0	R	0	R	0	R	18	I	28	S	30	S	27	S	20	I
Pinicillin combination	30	S	27	S	20	S	27	S	24	S	12	R	0	R	0	R	19	S	16	I
Drugs against mycobacteria	20	S	22	S	23	S	22	S	22	S	24	S	25	S	21	S	15	S	26	S

Table 3.8: Antimicrobial susceptibility testing (AST) results of Balu River water sample

Antibiotic group	N11M1	Result	N12M2	Result	N13M3	Result	N14M4	Result	N15M5	Result	N16T1	Result	N17T2	Result	N18T3	Result	N19T4	Result	N20T5	Result
	Klebsiella spp.		*Salmonella spp.*		*E. coli*		*Salmonella spp.*		*E. coli*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Cephalosporin	12	R	15	R	11	R	11	R	0	R	14	R	14	R	19	I	11	R	12	R
Glycopeptides	0	R	10	R	0	R	0	R	0	R	21	S	16	I	10	R	0	R	14	R
Penicillins	0	R	0	R	10	R	13	R	0	R	0	R	0	R	0	R	0	R	11	R
Aminoglycosides	19	S	17	I	16	I	15	I	14	I	19	S	18	S	16	I	15	I	17	I
Oxazolidinones	0	R	9	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Macrolides	0	R	10	R	11	R	10	R	0	R	14	I	18	I	12	R	0	R	11	R
Tetracyclines	12	I	19	S	17	S	19	S	18	S	20	S	20	S	20	S	10	R	20	S
Phenicols	20	S	27	S	27	S	26	S	27	S	28	S	25	S	29	S	24	S	30	S
Sulfonamides-Trimethoprim	25	S	30	S	30	S	29	S	30	S	22	S	28	S	20	S	26	S	24	S
Fluoroquinolone	26	S	25	S	30	S	25	S	28	S	24	S	27	S	32	S	23	S	32	S
Carbapenem	20	I	24	S	25	S	24	S	24	S	28	S	30	S	27	S	21	I	37	S
Monobactams	25	S	30	S	27	S	29	S	25	S	30	S	29	S	30	S	24	S	30	S
Pinicillin combination	24	S	22	S	17	I	19	S	16	I	21	S	23	S	19	S	0	R	26	S
Drugs against mycobacteria	25	S	19	S	17	S	17	S	19	S	14	I	19	S	18	S	16	S	19	S

Table 3.8: (Continued) Antimicrobial susceptibility testing (AST) results of Balu River water sample

- AST results in a pie chart:

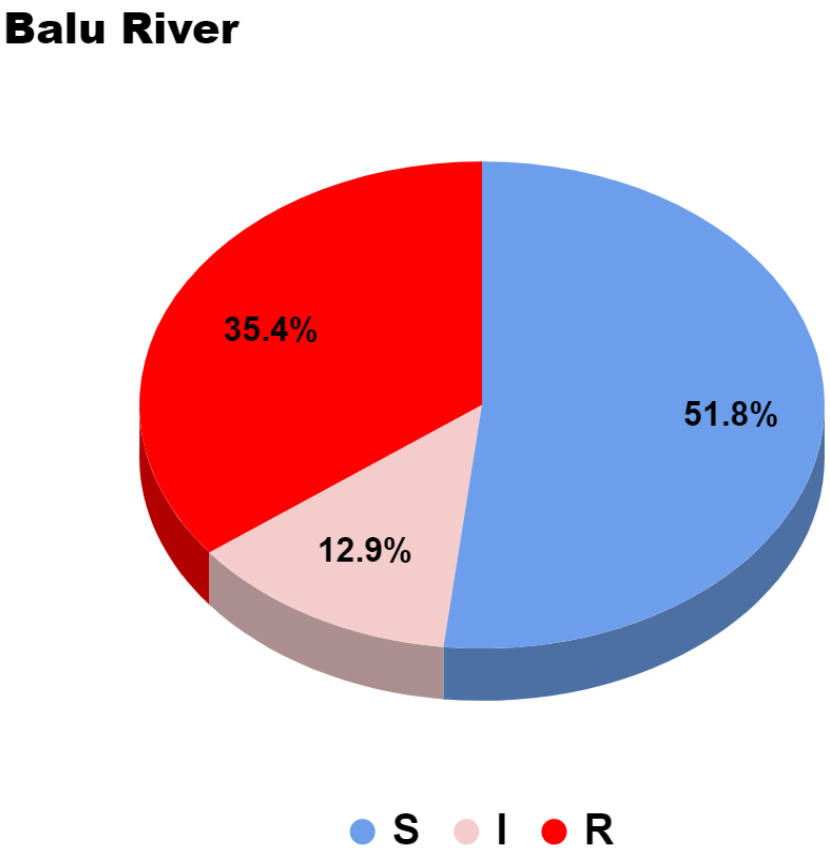


Figure 3.7: Pie-chart showing AST results of Balu River

From the pie chart, it is found that from the isolated colonies of the Balu River water sample, 35.4% of the bacteria showed resistance, 51.8% showed sensitivity, and 12.9% showed intermediate results against the antibiotics used.

➢ Plasmids isolated from the Balu River water sample

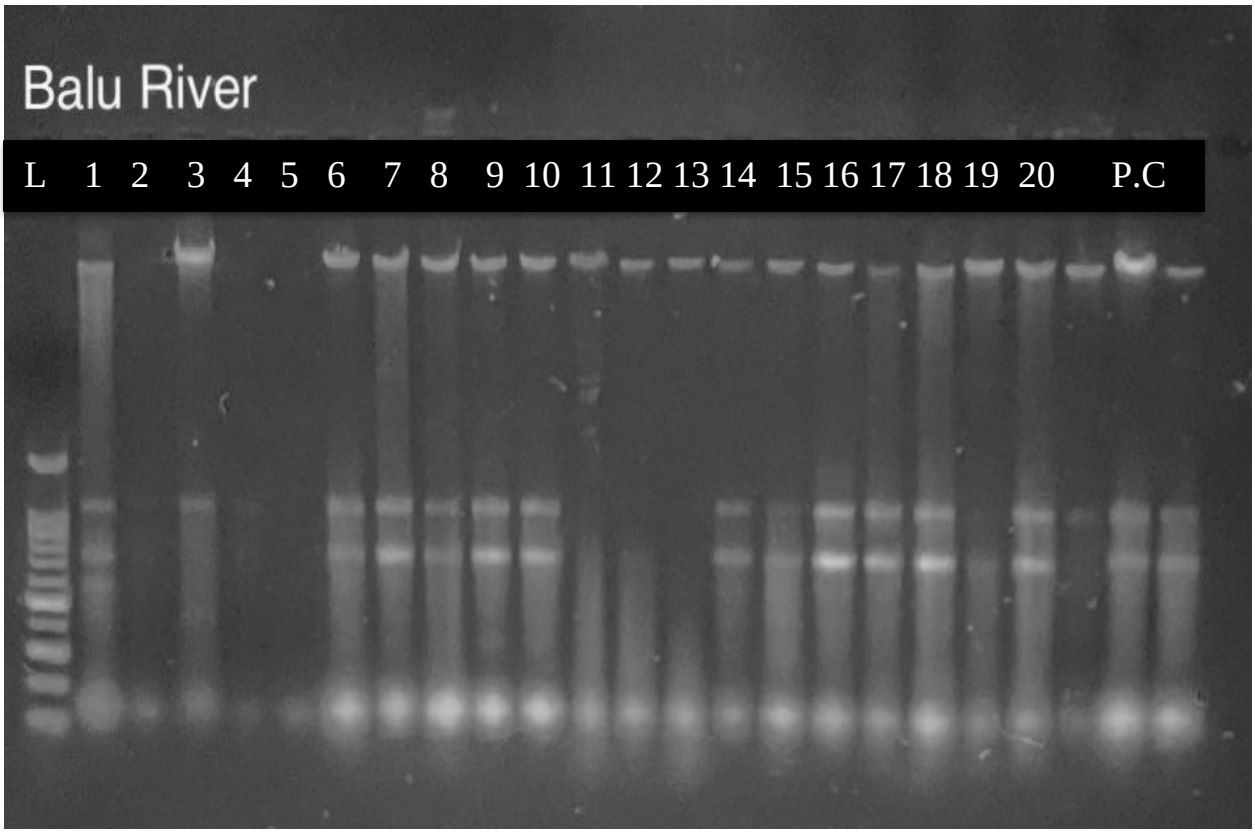


Figure 3.8: Plasmids isolated from the colonies of Balu River water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Balu River water sample. From the 20 colonies, 14 colonies showed plasmid bands. And the other 06 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 100BP DNA ladder has been used.

3.2. Outside Dhaka district water sample results:

3.2.1. Results of Shalda River water sample:

S= Shalda river water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Shalda River water sample, we got 37 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-3}.

Calculation:

Here,

Number of colonies= 37

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-7}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(37*3.75x10^{7}) / 0.15 = 9.25x10^{9}$

So, total colony forming units for Shalda River water sample was $9.25x10^{9}$ CFU/mL

- Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
S1E1	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
S2E2	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
S3E3	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
S4E4	Pink, without sheen	Pink	(-)	Rod	*Enterobacter spp.*
S5E5	Pink, mucoid	Pink	(-)	Rod	*Klebsiella spp.*
S6S1	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S7S2	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
S8S3	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
S9S4	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S10S5	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S11M1	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S12M2	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
S13M3	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S14M4	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S15M5	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
S16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
S17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
S18T3	Bluish green	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
S19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
S20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.9: Probable identification of colonies isolated from Shalda River water sample

➢ AST Results:

Antibiotic group	S1E1	Result	S2E2	Result	S3E3	Result	S4E4	Result	S5E5	Result	S6S1	Result	S7S2	Result	S8S3	Result	S9S4	Result	S10S5	Result
	E. coli		*E. coli*		*E. coli*		*Enterobacter spp.*		*Klebsiella spp.*		*E. coli*		*Salmonella spp.*		*Proteus spp.*		*E. coli*		*E. coli*	
Cephalosporin	15	R	14	R	18	I	13	R	16	I	19	S	15	R	18	I	26	S	28	S
Glycopeptides	17	S	15	I	15	I	16	R	0	R	0	R	0	R	0	R	0	R	0	R
Penicillins	18	S	18	S	23	S	15	I	11	R	0	R	0	R	13	R	0	R	0	R
Aminoglycosides	21	S	24	S	21	S	21	S	17	I	15	I	18	S	17	I	16	I	15	I
Oxazolidinones	0	R	14	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Sulfonamides - Trimethoprim	28	S	26	S	37	S	30	S	25	S	29	S	25	S	29	S	25	S	27	S
Carbapenem	34	S	40	S	36	S	34	S	20	I	19	R	19	R	19	R	24	S	26	S
Macrolides	21	I	0	R	21	I	21	I	0	R	0	R	0	R	0	R	0	R	0	R
Phenicols	21	S	24	S	13	I	21	S	24	S	24	S	24	S	22	S	25	S	28	S
Fluoroquinolone	27	S	31	S	32	S	29	S	26	S	24	S	22	S	23	S	25	S	24	S
Monobactams	17	R	0	R	32	S	24	S	25	S	28	S	23	S	27	S	31	S	30	S
Tetracyclines	21	S	32	S	29	S	23	S	15	S	12	I	17	S	17	S	13	I	17	S
Pinicillin.combination	26	S	24	S	30	S	26	S	13	R	0	R	0	R	16	I	23	S	24	S
Drugs against mycobacteria	25	S	0	R	0	R	22	S	15	S	15	S	15	S	16	S	15	S	16	S

Table 3.10: Antimicrobial susceptibility testing (AST) results of Shalda River water sample

Antibiotic group	S11 M1	Result	S12 M2	Result	S13 M3	Result	S14 M4	Result	S15 M5	Result	S16 T1	Result	S17 T2	Result	S18 T3	Result	S19 T4	Result	S20 T5	Result
	E. coli		*Klebsiella spp.*		*E. coli*		*E. coli*		*E. coli*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Cephalosporin	17	I	9	R	16	I	18	I	17	I	32	S	32	S	15	R	37	S	33	S
Glycopeptides	0	R	0	R	0	R	0	R	0	R	10	R	11	R	0	R	12	R	12	R
Penicillins	0	R	0	R	0	R	0	R	12	R	0	R	0	R	13	R	0	R	0	R
Aminoglycosides	10	R	19	S	18	S	19	S	17	I	20	S	19	S	12	R	19	S	19	S
Oxazolidinones	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Sulfonamides-Trimethoprim	29	S	18	S	27	S	26	S	20	S	28	S	31	S	26	S	28	S	28	S
Carbapenem	16	R	21	I	20	I	18	R	16	R	34	S	36	S	21	I	32	S	30	S
Macrolides	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	8	R
Phenicols	25	S	22	S	22	S	20	S	21	S	35	S	38	S	24	S	33	S	33	S
Fluoroquinolone	24	S	17	I	25	S	24	S	24	S	41	S	41	S	23	S	27	S	35	S
Monobactams	25	S	13	R	24	S	25	S	25	S	44	S	47	S	26	S	40	S	40	S
Tetracyclines	16	S	16	S	16	S	16	S	16	S	16	S	17	S	16	S	25	S	16	S
Pinicillin combination	0	R	20	S	11	R	16	I	0	R	14	I	19	S	16	I	26	S	20	S
Drugs against mycobacteria	16	S	18	S	16	S	16	S	15	S	19	S	19	S	15	S	21	S	19	S

Table 3.10: (Continued) Antimicrobial susceptibility testing (AST) results of Shalda River water sample

- AST results in a pie chart:

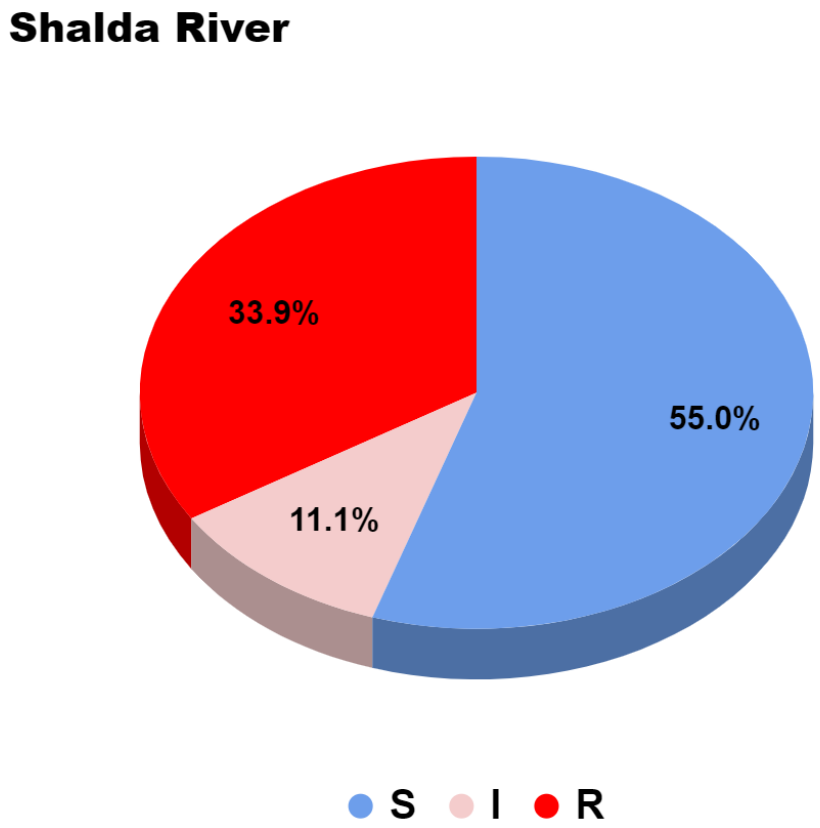


Figure 3.9: Pie-chart showing AST results of Shalda River

From the pie chart, it is found that from the isolated colonies of the Shalda River water sample, 33.9% of the bacteria showed resistance, 55% showed sensitivity, and 11.1% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Shalda River water sample

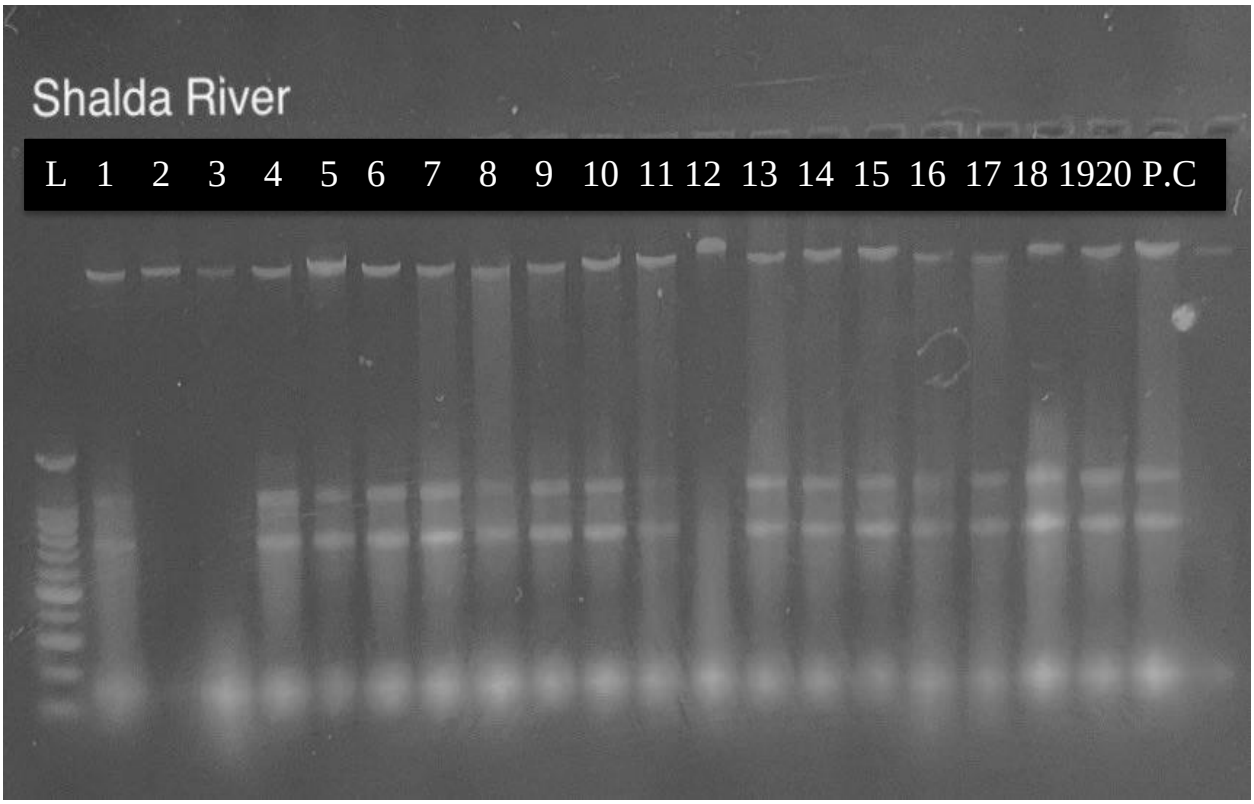


Figure 3.10: Plasmids isolated from the colonies of Shalda River water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Shalda River water sample. From the 20 colonies, 17 colonies showed plasmid bands. And the other 03 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 100BP DNA ladder has been used.

3.2.2. Results of Titash River water sample:

T= Titash river water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Titash River water sample, we got 34 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-2}.

Calculation:

Here,

Number of colonies= 34

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-4}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(34*3.75x10^{4}) / 0.15= 8.5x10^{6}$

So, total colony forming units for Titash River water sample was $8.5x10^{6}$ CFU/mL

➢ Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
T1E1	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
T2E2	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
T3E3	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
T4N4	Pink in MacConkey Agar	Pink	(-)	Rod	*Enterobacter spp.*
T5E5	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
T6S1	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
T7S2	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
T8S3	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
T9S4	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
T10S5	Colorless	Pink	(-)	Rod	*Shigella spp.*
T11M1	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
T12M2	Slightly pink	Pink	(-)	Rod	*Shigella spp.*
T13M3	Slightly pink	Pink	(-)	Rod	*Shigella spp.*
T14M4	Colorless	Pink	(-)	Rod	*Salmonella spp.*
T15M5	Slightly pink	Pink	(-)	Rod	*Shigella spp.*
T16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
T17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
T18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
T19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
T20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.11: Probable identification of colonies isolated from Titash River water sample

➢ AST Results:

Antibiotic group	T1N1	Result	T2E2	Result	T3E3	Result	T4N4	Result	T5E5	Result	T6S1	Result	T7S2	Result	T8S3	Result	T9S4	Result	T10S5	Result
	E. coli		*E. coli*		*E. coli*		*Enterobacter spp.*		*E. coli*		*Proteus spp.*		*Proteus spp.*		*E. coli*		*E. coli*		*Shigella spp.*	
Penicillins	0	R	19	S	10	R	16	I	20	S	0	R	0	R	0	R	0	R	0	R
Cephalosporin	20	I	23	S	15	R	25	S	30	S	0	R	27	S	36	S	25	S	29	S
Glycopeptides	20	S	22	S	17	S	28	S	24	S	12	R	11	R	13	R	14	R	11	R
Aminoglycosides	23	S	27	S	23	S	25	S	28	S	18	S	18	S	24	S	16	I	0	R
Oxazolidinones	21	S	12	R	14	R	0	R	29	S	0	R	0	R	0	R	0	R	0	R
Phenicols	28	S	29	S	26	S	27	S	23	S	34	S	36	S	33	S	32	S	27	S
Macrolides	0	R	0	R	18	R	19	I	22	I	0	R	12	R	15	R	0	R	0	R
Sulfonamides-Trimethoprim	29	S	30	S	25	S	26	S	30	S	29	S	24	S	29	S	33	S	0	R
Tetracyclines	29	S	27	S	26	S	24	S	28	S	24	S	22	S	25	S	15	S	10	R
Monobactams	18	I	21	S	18	I	21	S	15	R	15	R	33	S	39	S	35	S	28	S
Carbapenem	28	S	31	S	32	S	31	S	36	S	32	S	30	S	28	S	30	S	29	S
Fluoroquinolone	25	S	14	R	25	S	25	S	23	S	24	S	25	S	24	S	27	S	0	R
Pinicillin combination	28	S	29	S	21	S	30	S	33	S	15	I	20	S	14	I	16	I	20	S
Drugs against mycobacteria	26	S	28	S	23	S	26	S	27	S	19	S	20	S	21	S	18	S	16	S

Table 3.12: Antimicrobial susceptibility testing (AST) results of Titash River water sample

Antibiotic group	T11M1	Result	T12M2	Result	T13M3	Result	T14M4	Result	T15M5	Result	T16T1	Result	T17T2	Result	T18T3	Result	T19T4	Result	T20T5	Result
	Enterobacter spp.		*Shigella spp.*		*Shigella spp.*		*Salmonella spp.*		*Shigella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Cephalosporin	25	S	27	S	27	S	26	S	25	S	26	S	27	S	20	I	28	S	21	S
Glycopeptides	0	R	0	R	13	R	0	R	0	R	11	R	12	R	12	R	16	I	14	R
Aminoglycosides	16	I	18	S	19	S	16	I	15	I	17	I	19	S	18	S	18	S	17	I
Oxazolidinones	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	9	R
Phenicols	25	S	27	S	29	S	24	S	24	S	28	S	30	S	34	S	32	S	25	S
Macrolides	0	R	0	R	12	R	0	R	0	R	10	R	11	R	11	R	10	R	10	R
Sulfonamides-Trimethoprim	26	S	29	S	25	S	27	S	26	S	28	S	25	S	26	S	23	S	0	R
Tetracyclines	16	S	16	S	19	S	16	S	15	S	21	S	21	S	23	S	22	S	19	S
Monobactams	25	S	27	S	28	S	26	S	26	S	34	S	39	S	36	S	38	S	32	S
Carbapenem	16	R	18	R	20	I	14	R	18	R	23	S	29	S	34	S	30	S	25	S
Fluoroquinolone	24	S	27	S	23	S	25	S	25	S	21	S	23	S	34	S	30	S	21	S
Pinicillin combination	0	R	0	R	15	I	0	R	0	R	12	R	13	R	14	I	14	I	14	I
Drugs against mycobacteria	17	S	19	S	19	S	18	S	17	S	18	S	20	S	13	I	19	S	18	S

Table 3.12: (Continued) Antimicrobial susceptibility testing (AST) results of Titash River water sample

- AST results in a pie chart:

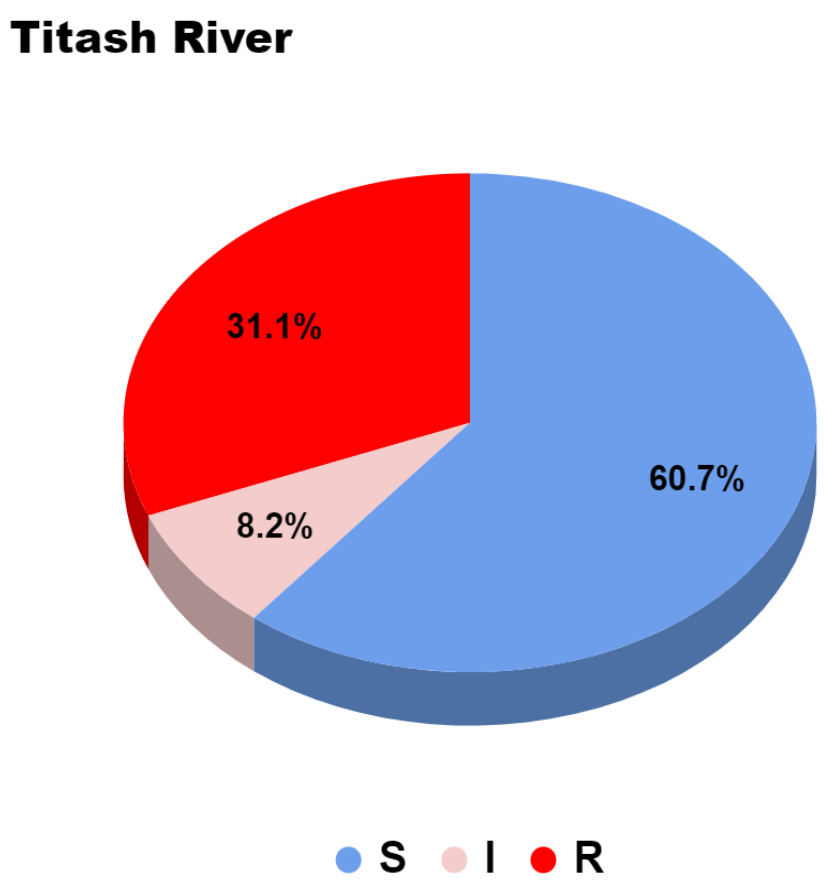


Figure 3.11: Pie-chart showing AST results of Titash River

From the pie chart, it is found that from the isolated colonies of the Titash River water sample, 31.1% of the bacteria showed resistance, 60.7% showed sensitivity, and 8.2% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Titash River water sample

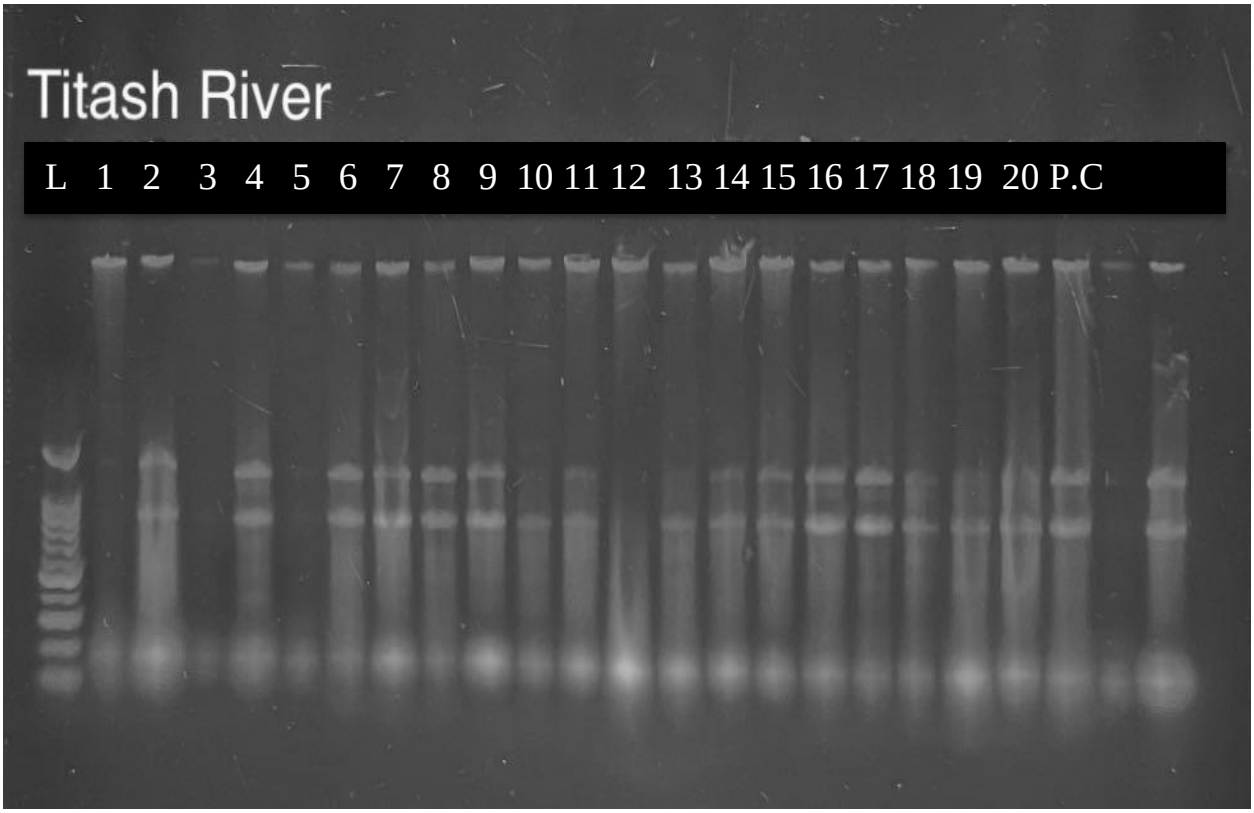


Figure 3.12: Plasmids isolated from the colonies of Titash River water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Titash River water sample. From the 20 colonies, 16 colonies showed plasmid bands. And the other 04 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 100BP DNA ladder has been used.

3.2.3. Results of Modhumoti River water sample

M= Modhumoti river water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Modhumoti River water sample, we got 98 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-3}.

Calculation:

Here,

Number of colonies= 98

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-7}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(98*3.75x10^{7}) / 0.15= 2.45x10^{10}$

So, total colony forming units for Modhumoti River water sample was $2.45x10^{10}$ CFU/mL

➢ Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
M1N1	Pink to red in MacConkey	Pink	(-)	Rod	*Escherichia coli*
M2N2	Pink to red in MacConkey	Pink	(-)	Rod	*Escherichia coli*
M3N3	Yellow in TCBS	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
M4E4	Pink, mucoid	Pink	(-)	Rod	*Klebsiella spp.*
M5N5	Pink to red in MacConkey	Pink	(-)	Rod	*Escherichia coli*
M6S1	Pink with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
M7S2	Colorless	Pink	(-)	Rod	*Shigella spp.*
M8S3	Colorless	Pink	(-)	Rod	*Shigella spp.*
M9S4	Colorless	Pink	(-)	Rod	*Shigella spp.*
M10S5	Colorless	Pink	(-)	Rod	*Shigella spp.*
M11M1	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
M12M2	Pink to red	Pink	(-)	Rod	*Enterobacter spp.*
M13M3	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
M14M4	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
M15M5	Pink to red	Pink	(-)	Rod	*Klebsiella spp.*
M16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
M17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
M18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
M19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
M20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.13: Probable identification of colonies isolated from Modhumoti River water sample

➢ AST Results:

Antibiotic group	M1 N1	Result	M2 N2	Result	M3 N3	Result	M4E 4	Result	M5 N5	Result	M6 S1	Result	M7 S2	Result	M8 S3	Result	M9 S4	Result	M10 S5	Result
	E. coli		*E. coli*		*Vibrio spp.*		*Klebsiella spp.*		*E. coli*		*E. coli*		*Shigella spp.*		*Shigella spp.*		*Shigella spp.*		*Shigella spp.*	
Penicillins	23	S	21	S	0	R	0	R	45	S	0	R	0	R	0	R	0	R	0	R
Cephalosporin	25	S	27	S	29	S	27	S	41	S	27	S	21	S	27	S	29	S	19	S
Fluoroquinolone	21	S	27	S	29	S	27	S	19	I	23	S	13	R	33	S	23	S	25	S
Oxazolidinones	9	R	15	R	13	R	11	R	0	R	0	R	0	R	9	R	0	R	0	R
Glycopeptides	19	S	9	R	9	R	9	R	13	R	13	R	13	R	13	R	0	R	0	R
Tetracyclines	20	S	22	S	23	S	21	S	38	S	21	S	0	R	9	R	15	S	14	S
Carbapenem	25	S	30	S	0	R	0	R	52	S	30	S	27	S	30	S	19	I	20	I
Sulfonamides-Trimethoprim	26	S	29	S	27	S	28	S	44	S	25	S	20	S	23	S	25	S	23	S
Phenicols	30	S	32	S	32	S	32	S	44	S	30	S	21	S	25	S	24	S	22	S
Macrolides	20	I	20	I	19	I	19	I	34	S	14	I	15	I	16	I	0	R	0	R
Monobactams	27	S	28	S	29	S	28	S	50	S	18	I	26	S	29	S	25	S	25	S
Aminoglycosides	16	I	17	I	14	I	14	I	26	S	17	I	15	I	15	I	14	I	14	I
Pinicillin combination	29	S	31	S	25	S	21	S	47	S	21	S	27	S	21	S	25	S	23	S
Drugs against mycobacteria	15	S	17	S	17	S	13	I	27	S	15	S	0	R	13	I	13	I	15	S

Table 3.14: Antimicrobial susceptibility testing (AST) results of Modhumoti River water sample

Antibiotic group	M11M1	Result	M12M2	Result	M13M3	Result	M14M4	Result	M15M5	Result	M16T1	Result	M17T2	Result	M18T3	Result	M19T4	Result	M20T5	Result
	Enterobacter spp.		*Enterobacter spp.*		*Klebsiella spp.*		*Klebsiella spp.*		*Klebsiella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Penicillins	0	R	0	R	0	R	16	I	11	R	0	R	0	R	0	R	0	R	0	R
Cephalosporin	21	S	23	S	25	S	17	I	19	S	19	S	19	S	29	S	27	S	25	S
Fluoroquinolone	21	S	23	S	25	S	23	S	15	R	33	S	21	S	19	I	23	S	21	S
Oxazolidinones	0	R	0	R	9	R	9	R	9	R	9	R	9	R	15	R	11	R	0	R
Glycopeptides	0	R	0	R	0	R	0	R	0	R	11	R	15	I	11	R	11	R	11	R
Tetracyclines	17	S	18	S	19	S	20	S	18	S	17	S	11	I	18	S	23	S	23	S
Carbapenem	24	S	25	S	25	S	22	I	20	I	23	S	24	S	23	S	30	S	30	S
Sulfonamides-Trimethoprim	26	S	27	S	27	S	34	S	29	S	24	S	0	R	20	S	28	S	25	S
Phenicols	27	S	27	S	26	S	29	S	27	S	30	S	30	S	27	S	34	S	31	S
Macrolides	0	R	0	R	10	R	12	R	11	R	15	I	0	R	14	I	14	I	14	I
Monobactams	27	S	29	S	28	R	34	S	29	S	27	S	28	S	27	S	35	S	39	S
Aminoglycosides	14	I	16	I	9	R	18	S	17	I	15	I	16	I	17	I	20	S	23	S
Pinicillin combination	23	S	25	S	27	S	25	S	27	S	23	S	23	S	27	S	23	S	23	S
Drugs against mycobacteria	15	S	15	S	15	S	13	I	15	S	15	S	15	S	27	S	23	S	17	S

Table 3.14: (Continued) Antimicrobial susceptibility testing (AST) results of Titash River water sample

- AST results in a pie chart:

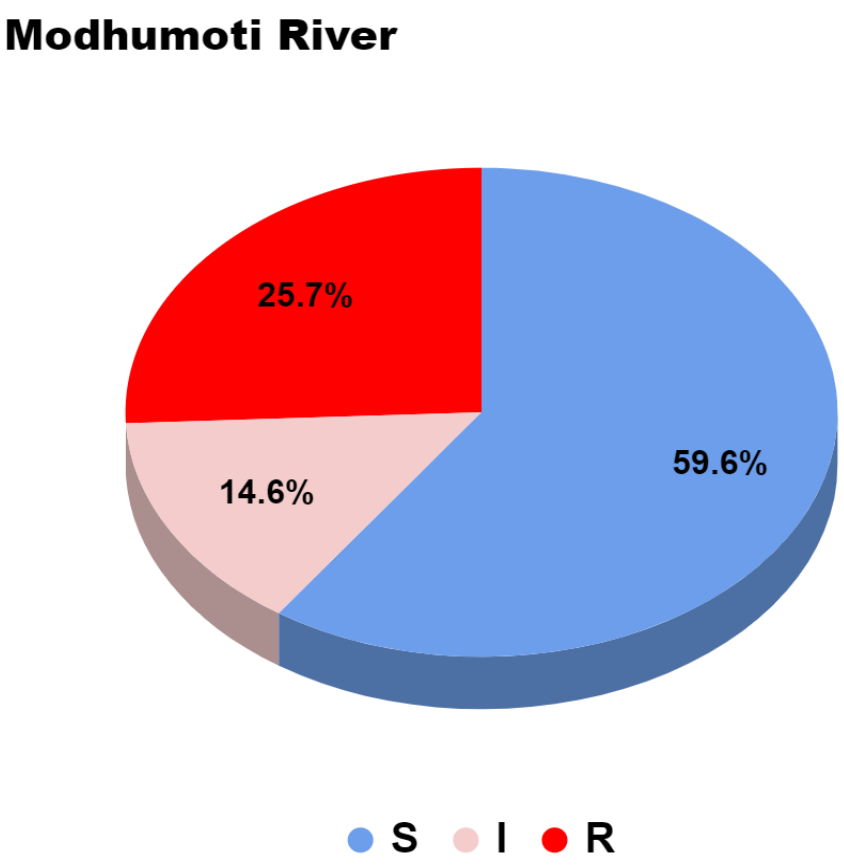


Figure 3.13: Pie-chart showing AST results of Modhumoti River

From the pie chart, it is found that from the isolated colonies of the Modhumoti River water sample, 25.7% of the bacteria showed resistance, 59.6% showed sensitivity, and 14.6% showed intermediate results against the antibiotics used.

- Plasmids isolated from the Modhumoti River water sample

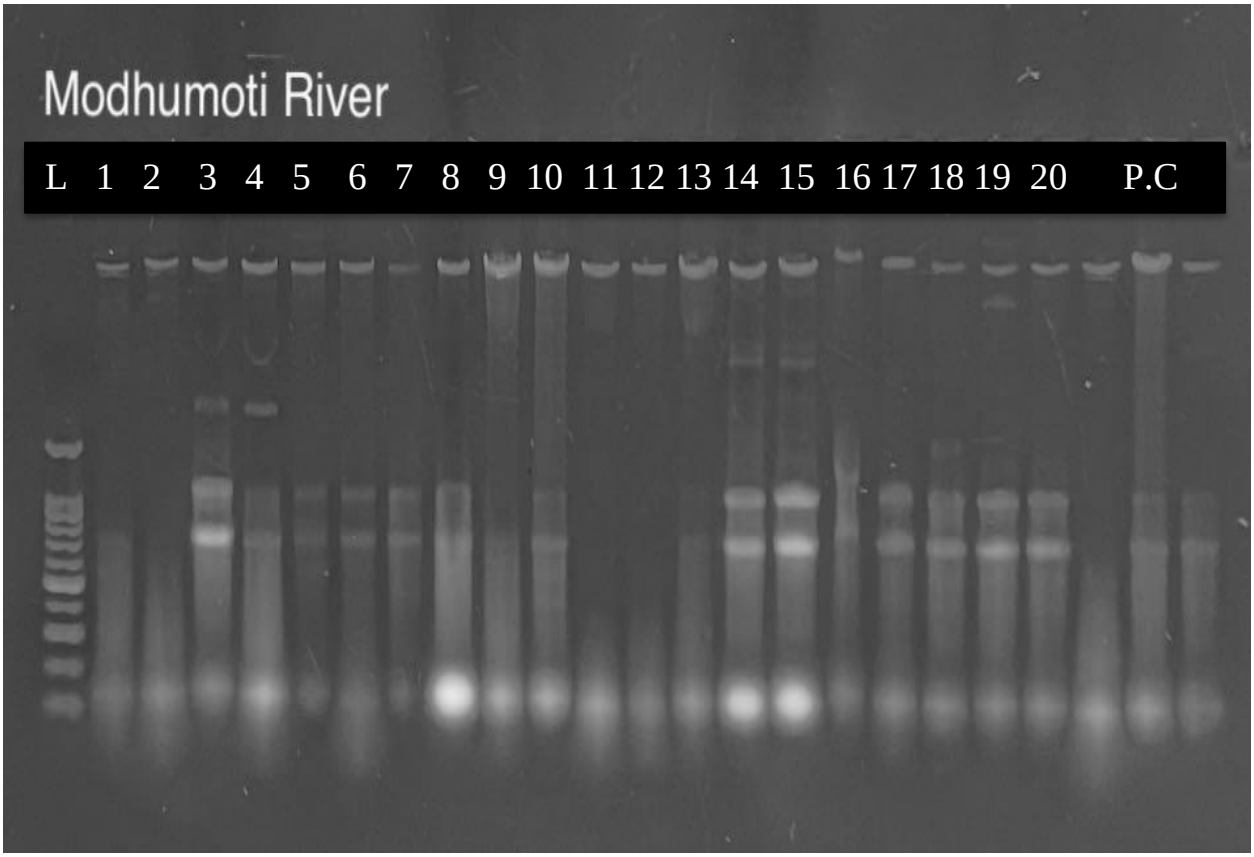


Figure 3.14: Plasmids isolated from the colonies of Modhumoti River water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Modhumoti River water sample. From the 20 colonies, 14 colonies showed plasmid bands. And the other 06 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 100BP DNA ladder has been used.

3.2.4. Results of Kumar River water sample:

K= Kumar river water sample, N= Nutrient Agar, E= EMB agar, M= MacConkey Agar, S= SS agar, T= TCBS agar

- CFU/mL

From Kumar River water sample, we got 106 colonies from Nutrient Agar (NA) plate, when the serial dilution was 10^{-3}.

Calculation:

Here,

Number of colonies= 106

Total Dilution Factor = (Current Dilution x Previous Dilution) = $3.75x10^{-7}$

Volume of culture plate= 0.15mL

Formula:

CFU/mL= (Number of colonies* reciprocal of Dilution Factor) / volume of culture plate

CFU/mL = $(106*3.75x10^{7}) / 0.15= 2.65x10^{10}$

So, total colony forming units for Kumar River water sample was $2.65x10^{10}$ CFU/mL

➢ Identification by colony morphology

Sample Name	Characteristics	Gram staining			
		Result		Shape	Species
K1E1	Green metallic sheen	Pink	(-)	Rod	*Escherichia coli*
K2N2	Pink to red in MacConkey agar	Pink	(-)	Rod	*Klebsiella spp.*
K3E3	Pink to red in MacConkey agar	Pink	(-)	Rod	*Klebsiella spp.*
K4N4	Bluish green in TCBS agar	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K5N5	Bluish green in TCBS agar	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K6S1	Colorless, may have black center	Pink	(-)	Rod	*Proteus spp.*
K7S2	Colorless	Pink	(-)	Rod	*Shigella spp.*
K8S3	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
K9S4	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
K10S5	Colorless with black center	Pink	(-)	Rod	*Salmonella spp.*
K11M1	Pink to red, matte with bile precipitate	Pink	(-)	Rod	*Escherichia coli*
K12M2	Colorless	Pink	(-)	Rod	*Pseudomonas spp.*
K13M3	Colorless	Pink	(-)	Rod	*Pseudomonas spp.*
K14M4	Colorless	Pink	(-)	Rod	*Pseudomonas spp.*
K15M5	Colorless	Pink	(-)	Rod	*Salmonella spp.*
K16T1	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K17T2	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K18T3	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K19T4	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*
K20T5	Yellow	Pink	(-)	Comma-shaped rod	*Vibrio spp.*

Table 3.15: Probable identification of colonies isolated from Kumar River water sample

- AST Results:

Antibiotic group	K1E1	Result	K2N2	Result	K3E3	Result	K4N4	Result	K5N5	Result	K6S1	Result	K7S2	Result	K8S3	Result	K9S4	Result	K10S5	Result
	E. coli		*Klebsiella spp.*		*Klebsiella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Proteus spp.*		*Shigella spp.*		*Salmonella spp.*		*Salmonella spp.*		*Salmonella spp.*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Fluoroquinolone	11	R	17	I	19	S	27	S	21	S	19	S	29	S	25	S	27	S	35	S
Oxazolidinones	0	R	11	R	9	R	13	R	0	R	9	R	0	R	9	R	0	R	0	R
Glycopeptides	17	S	9	R	15	I	15	I	11	R	9	R	15	I	15	I	15	I	19	S
Cephalosporin	15	R	19	S	9	R	11	R	13	R	13	R	15	R	15	R	19	S	21	S
Aminoglycosides	20	S	14	I	13	R	18	S	20	S	18	S	18	S	19	S	16	I	17	I
Phenicols	19	S	30	S	26	S	15	I	24	S	32	S	21	S	19	S	19	S	20	S
Sulfonamides-Trimethoprim	26	S	25	S	21	S	21	S	25	S	26	S	24	S	24	S	26	S	28	S
Carbapenem	30	S	16	R	18	R	24	S	29	S	30	S	33	S	28	S	25	S	25	S
Macrolides	0	R	18	I	15	R	20	I	20	I	11	R	16	I	14	I	0	R	0	R
Tetracyclines	15	S	20	S	20	S	24	S	20	S	25	S	23	S	22	S	0	R	0	R
Monobactams	16	R	27	S	29	S	11	R	16	R	40	S	20	I	20	I	18	I	29	S
Pinicillin combination	25	S	21	S	11	R	21	S	21	S	21	S	31	S	27	S	33	S	35	S
Drugs against mycobacteria	21	S	17	S	15	S	19	S	27	S	19	S	27	S	23	S	25	S	31	S

Table 3.16: Antimicrobial susceptibility testing (AST) results of Kumar River water sample

Antibiotic group	K11M1	Result	K12M2	Result	K13M3	Result	K14M4	Result	K15M5	Result	K16T1	Result	K17T2	Result	K18T3	Result	K19T4	Result	K20T5	Result
	E. coli		*Pseudomonas spp.*		*Pseudomonas spp.*		*Pseudomonas spp.*		*Salmonella spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*		*Vibrio spp.*	
Penicillins	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Fluoroquinolone	19	S	17	I	24	S	15	I	17	I	35	S	21	S	31	S	17	I	21	S
Oxazolidinones	11	R	0	R	0	R	0	R	0	R	11	R	9	R	11	R	0	R	11	R
Glycopeptides	11	R	13	R	13	R	13	R	13	R	9	R	13	R	15	I	11	R	15	I
Cephalosporin	31	S	27	S	0	R	0	R	0	R	29	S	27	S	25	S	23	S	27	S
Aminoglycosides	15	I	14	I	17	I	15	I	15	I	17	I	18	S	18	S	9	R	9	R
Phenicols	32	S	25	S	31	S	26	S	26	S	30	S	27	S	29	S	30	S	30	S
Sulfonamides-Trimethoprim	30	S	26	S	25	S	25	S	23	S	24	S	24	S	24	S	0	R	29	S
Carbapenem	27	S	23	I	26	I	23	I	20	I	31	S	29	S	23	S	23	S	16	R
Macrolides	24	S	19	I	18	I	19	I	19	I	13	R	12	R	20	I	18	I	15	I
Tetracyclines	24	S	20	S	22	S	21	S	19	S	20	S	20	S	17	S	19	S	22	S
Monobactams	40	S	28	S	33	S	28	S	25	S	30	S	31	S	23	S	30	S	30	S
Pinicillin combination	25	S	17	I	17	I	19	S	17	I	31	S	25	S	23	S	21	S	11	R
Drugs against mycobacteria	13		13	I	15	S	15	S	13	I	19	S	27	S	31	S	13	I	13	I

Table 3.16: (Continued) Antimicrobial susceptibility testing (AST) results of Kumar River water sample

- AST results in a pie chart:

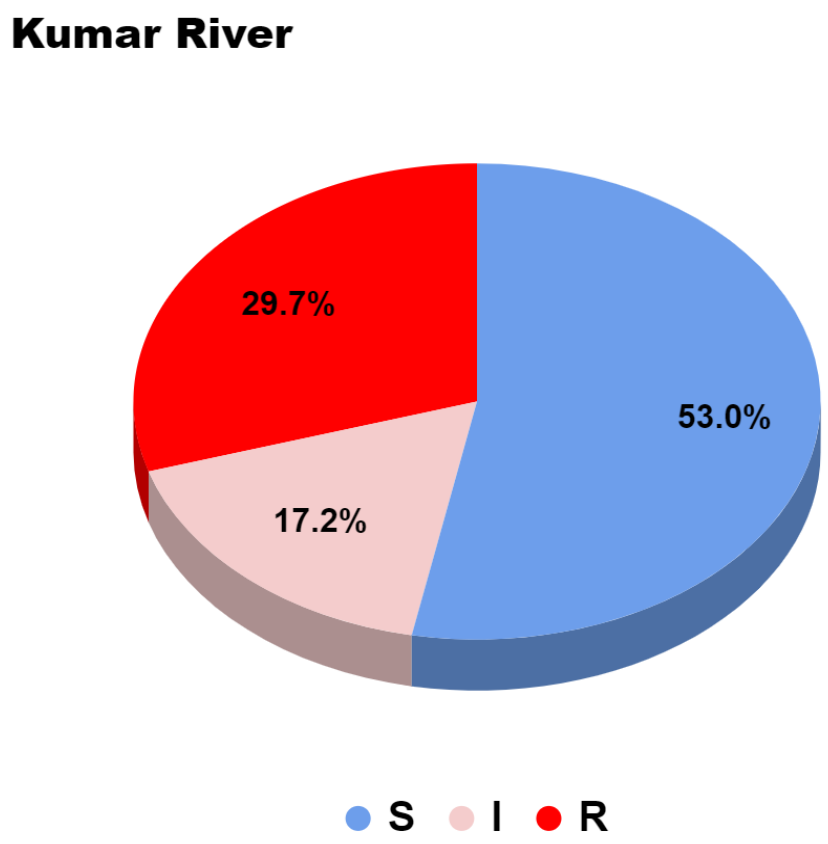


Figure 3.15: Pie-chart showing AST results of Kumar River

From the pie chart, it is found that from the isolated colonies of the Kumar River water sample, 29.7% of the bacteria showed resistance, 53% showed sensitivity, and 17.2% showed intermediate results against the antibiotics used.

➢ Plasmids isolated from the Kumar River water sample

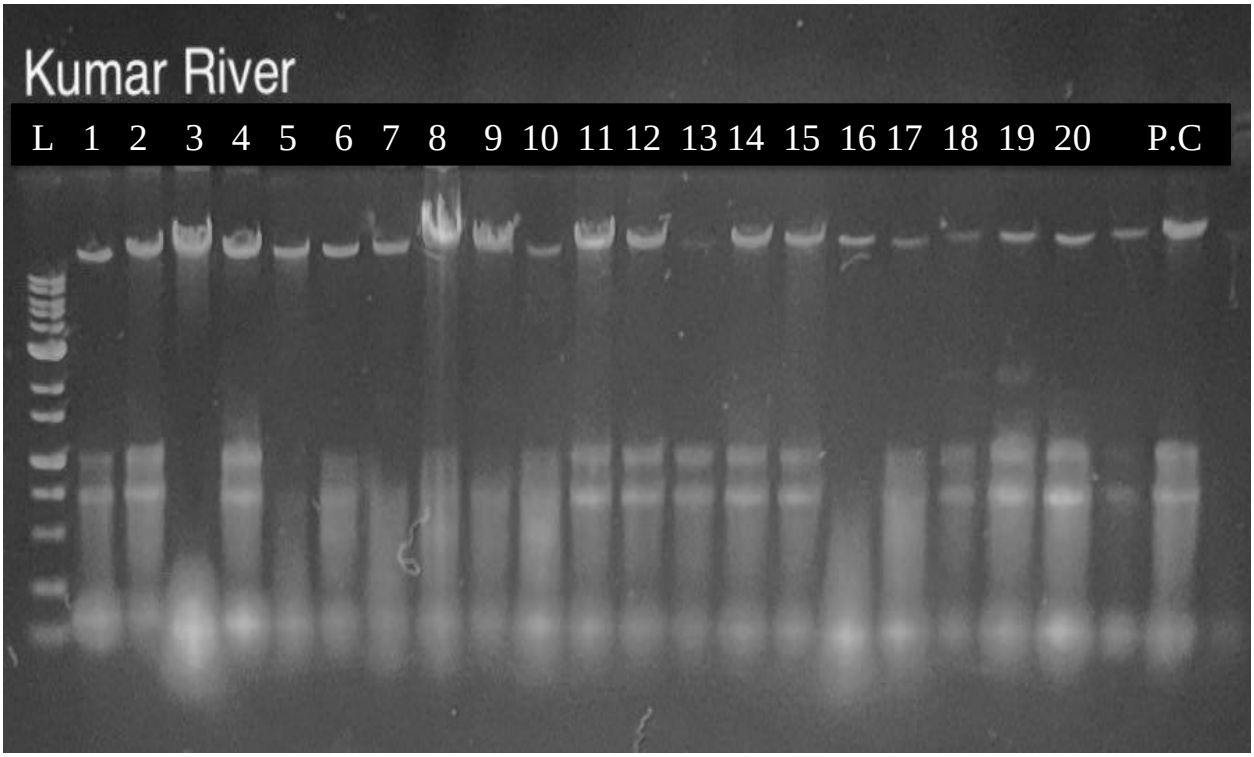


Figure 3.16: Plasmids isolated from the colonies of Kumar River water sample, and visualized via gel-electrophoresis method

Here, plasmids were isolated from the 20 colonies of bacteria taken from the Kumar River water sample. From the 20 colonies, 12 colonies showed plasmid bands. And the other 08 colonies did not show any bands for plasmids; either those colonies do not contain any plasmids, or the plasmids might have been degraded while doing the plasmid isolation and gel electrophoresis processes. To confirm our plasmid isolation process, we have used DH5α as a positive control. And on the first lane, a 1Kb PLUS DNA ladder has been used.

3.3. Comparative analysis between the results:

3.3.1. Comparison of antibiotic resistance profiles of the bacteria isolated from inside and outside Dhaka district water samples:

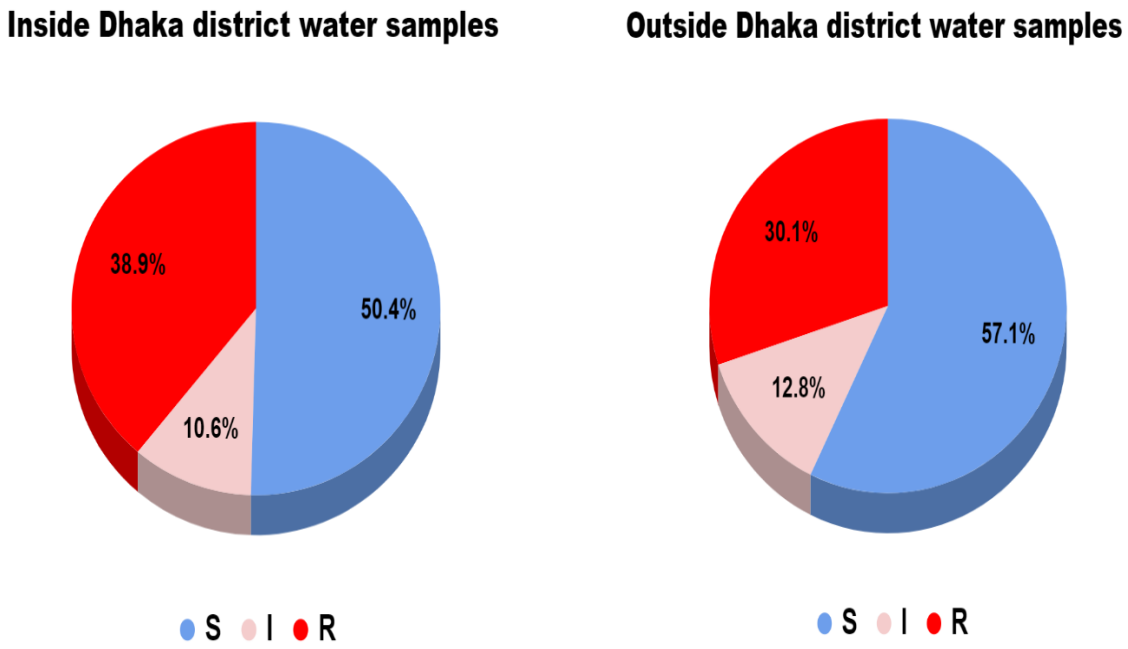


Figure 3.17: Comparison of AST results between inside and outside Dhaka district water samples

In the pie chart of inside Dhaka district water samples, it is found that, 38.9% of the isolated bacterial species showed resistance, 50.4% showed sensitivity, and 10.6% showed intermediate results against the antibiotics we used. Whereas, in the pie chart of outside Dhaka district water samples, it is found that, 30.1% of the isolated bacterial species showed resistance, 57.1% showed sensitivity, and 12.8% showed intermediate results against the antibiotics we used.

- Bacteria isolated from inside and outside Dhaka district show different percentages for antibiotic resistance:

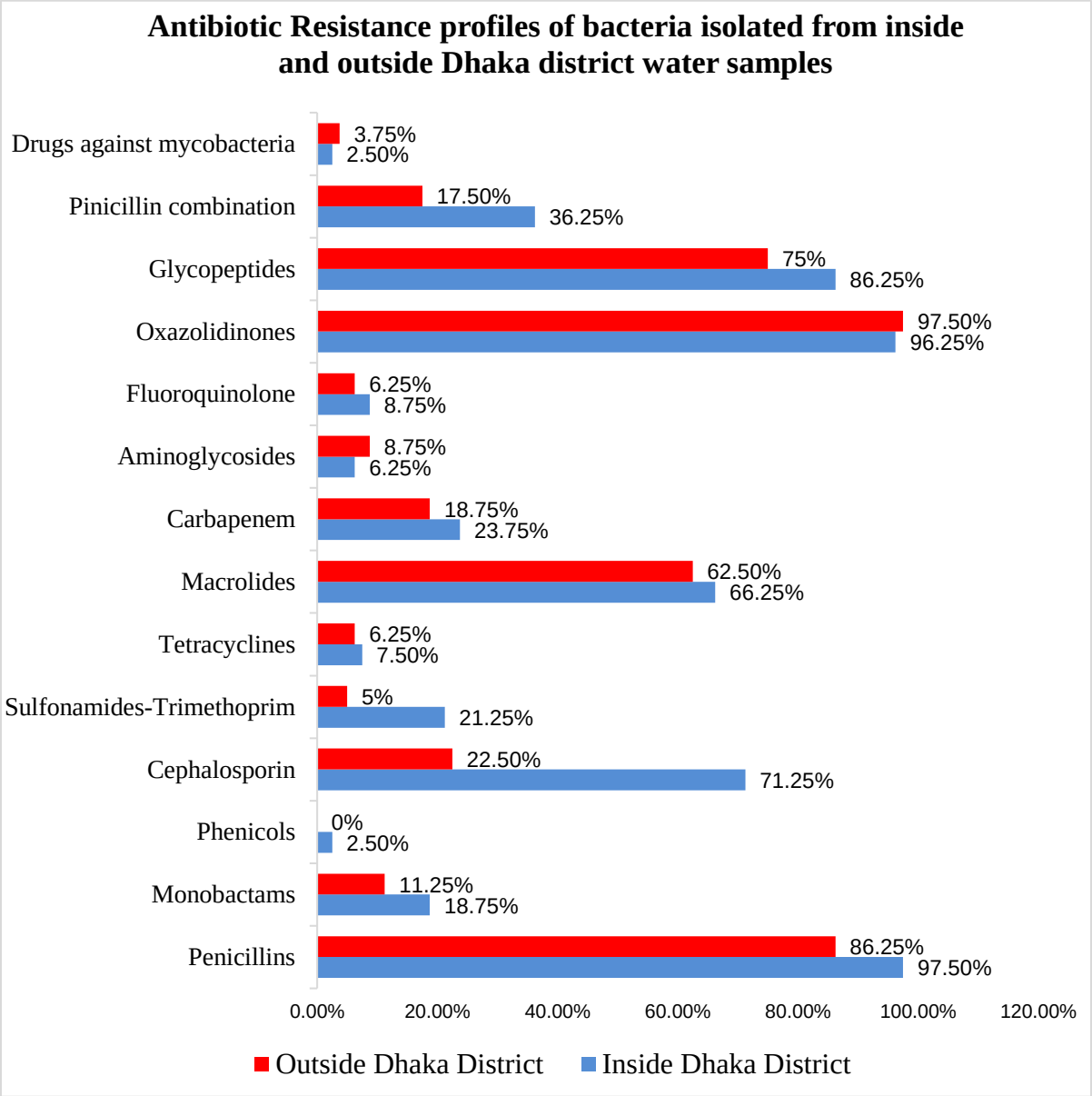


Figure 3.18: Bar chart showing the antibiotic resistance profiles of isolated bacteria from inside and outside Dhaka district water samples

Here, the bar chart shows the comparison of the resistant patterns against the 14 types of antibiotic groups, for the isolated bacteria between inside and outside Dhaka district water samples.

3.3.2. Analysis of plasmids obtained from bacteria isolated from inside and outside Dhaka district water samples:

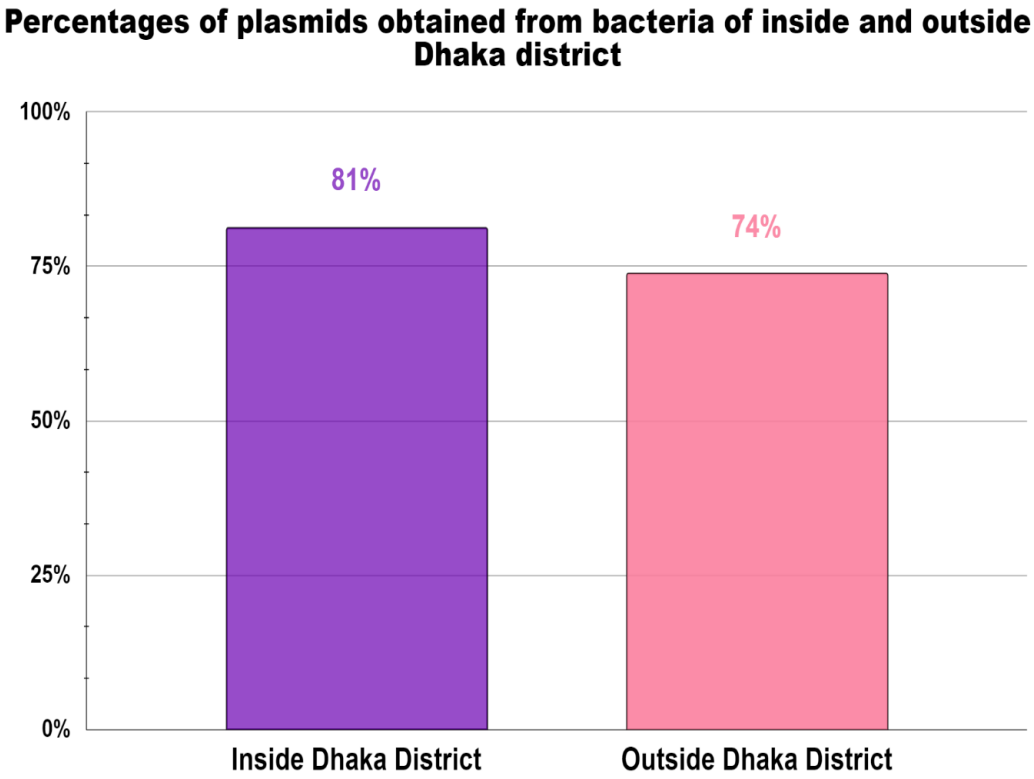


Figure 3.19: Bar chart showing percentages of obtained plasmids isolated from bacteria of inside and outside Dhaka district

From the above bar chart, we can see that, among the bacterial species obtained from inside the Dhaka district, 81% of the isolates contained plasmids and showed plasmid bands after the gel-electrophoresis method. On the other hand, among the bacterial species obtained from outside the Dhaka district, 73.75% of the isolates contained plasmids and showed plasmid bands after the gel-electrophoresis method.

➢ Plasmid percentage varied in different species from both inside and outside Dhaka district:

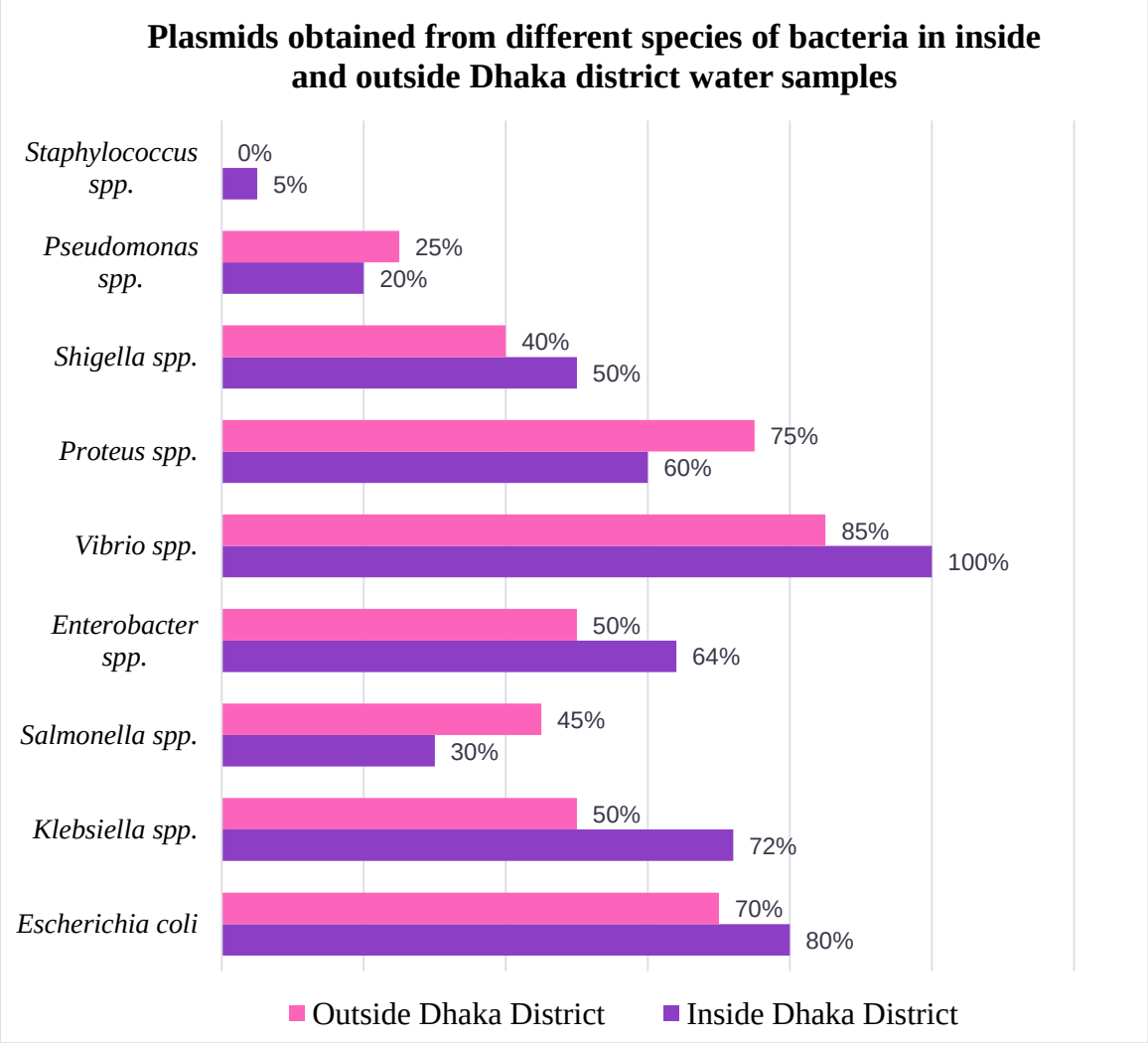


Figure 3.20: Comparison of plasmids from different bacterial species between inside and outside Dhaka district

In this bar chart, a comparative analysis is shown between the obtained plasmids from the different species of bacteria isolated form inside and outside Dhaka district water samples. Here, the comparison is shown on the basis of isolated bacterial species.

3.3.3. Difference in AST profiles between bacteria that showed plasmid presence and bacteria that did not:

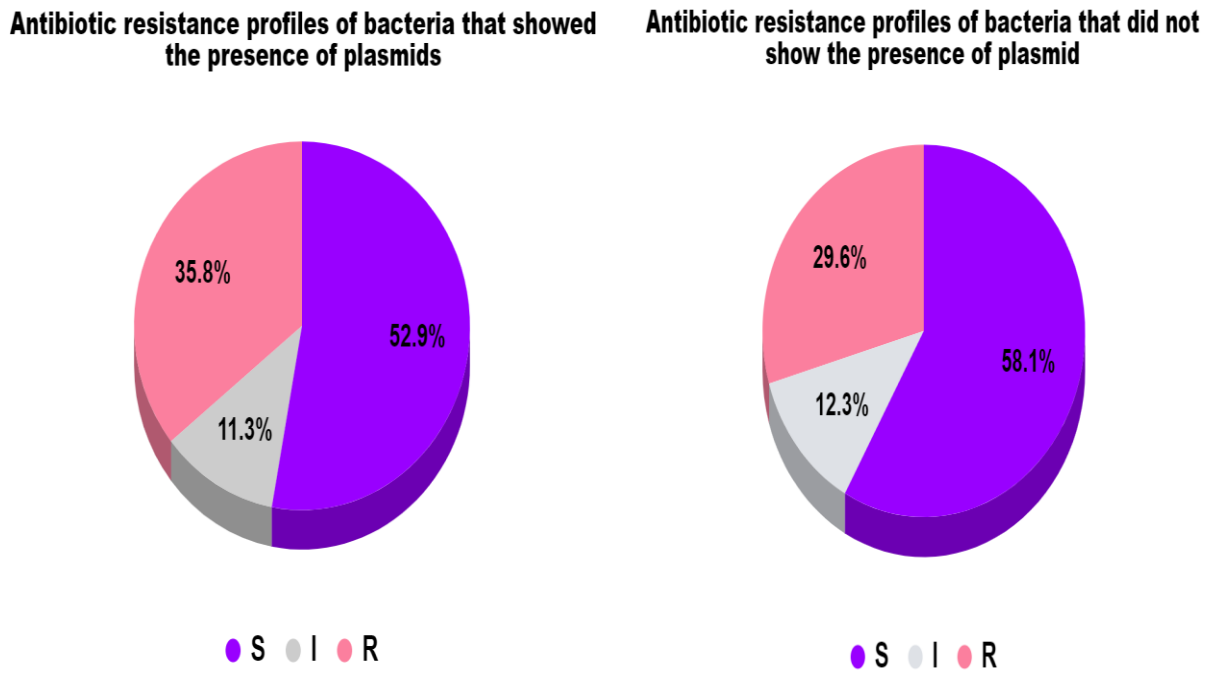


Figure 3.21: Pie charts showing the difference in AST results of isolates that contained plasmids and the isolates that did not contained plasmid

From the above pie chart, showing the antibiotic resistance profiles of isolates that produced plasmids, we can see that 52.9% of the isolates are sensitive, 11.3% are intermediate and 35.9% of them are resistant. Meanwhile, from the bacteria that did not show the presence of plasmid, 58.1% are sensitive, 12.3% are intermediate and 29.6% are resistant to antibiotics.

Chapter 4:

Discussion

Discussion

Water is essential for our lives. The world is surrounded by 80% water but only 1% of that water is consumable. In Bangladesh, the pollution of rivers is more severe and critical near urban stretches due to the huge amounts of pollution discharged by urban activities. Rivers around Dhaka district are facing severe pollution nowadays. A study of the Buriganga river found that the Biochemical Oxygen demand, or BOD, was 20–30 mg/liter, and the total coliform bacteria were found to be 104–105 MPN/100 ml. This study also indicated that the leading cause of river water pollution is the increasing trend of industrialization. The unplanned industrialization in the Dhaka district is causing inadequate sewerage of pollutants, and insufficient pollution control measures are causing water pollution in the rivers (Karn & Harada, 2001).

This river pollution is not only causing cholera and other diarrheal diseases among people but also causing Antimicrobial Resistance (AMR) among different strains of pathogenic microorganisms. The increase in Antimicrobial Resistance (AMR) in microorganisms found in the water sources is very alarming for the people of the surrounding areas, as they consume and use water from these sources. Hence, the need to know the current antimicrobial resistance (AMR) pattern of Bangladeshi water sources is enormous. A study was done in 2019 where 46 research articles were reviewed to analyze the AMR profile and the increasing trend of antibiotic resistance in Bangladesh. They found that the resistance data were available only for six districts (Ahmed et al., 2019). Thus, there is an urgent need to collect the resistance data of all the other districts of Bangladesh as soon as possible.

In our research project, we have aimed to showcase a comparison in antibiotic resistance profiles between inside and outside Dhaka district of the isolated bacterial species from eight different water sources. Also, we tried to show another comparative analysis by isolating the bacterial plasmids to indicate the connection between antibiotic resistance profiles and plasmids containing resistant genes. As per our investigation, the isolated bacteria found within the Dhaka district showed more antibiotic resistance than the bacteria isolated from outside the Dhaka district. The results were expected as the negative factors affecting water sources are distinctively present more in inside Dhaka district. After the characterization of isolated microorganisms, antibiotic susceptibility testing, and plasmid isolation, the result of our research project is determined.

To start with, water samples were taken from different locations in Bangladesh. We have taken four water samples from inside Dhaka district, which includes 'Hatirjheel', 'Banani Lake', Dhanmondi Lake, and the Balu river. And four water samples from outside Dhaka district, including the 'Modhumoti river', 'Kumar river', 'Titash river', and 'Shalda river'. In this study, we observed the morphology and characteristics of our isolated bacterial colonies by using the Gram staining method and were able to characterize the isolated colonies. We have collected 160 colonies of bacteria of various species. Among those, only 04 isolates of bacteria were found to be Gram-positive and the rest, 156 isolates, were Gram-negative. As we have used selective media for isolating Gram-negative bacterial species, we did not find too many Gram-positive bacteria in our water samples, whereas the 04 Gram-positive bacteria were isolated from Nutrient Agar (NA) media. Here, the interesting fact is that the 04 isolates that were found to be Gram-positive, were all found in the ‘Balu river’ water sample.

For the comparative study, we tried to investigate the various types of bacterial species present in our collected water samples. We got different species of coliforms and *Enterobacter*. The isolated

bacterial species include *Vibrio, Shigella, Salmonella, Staphylococcus, Pseudomonas, Proteus, Klebsiella,* and *Escherichia coli*. After analyzing our results, we found that bacterial species like *Staphylococcus, Vibrio, Salmonella, Enterobacter,* and *Klebsiella* were in higher ratios in inside Dhaka district water samples. Whereas, organisms like *Pseudomonas*, *Shigella,* and *Escherichia coli* were found in increased ratios in outside Dhaka district water samples. Also, strains of *Staphylococcus spp.* were only found from the inside Dhaka water samples, and no strain of *Staphylococcus spp.* was found from the outside Dhaka water samples. Here, strains of *Escherichia coli* were found more from the outside Dhaka water samples than inside Dhaka. But even if there are more strains of *Escherichia coli* found from outside Dhaka district, we cannot tell about the pathogenicity of the strains, as we did not run the PCR and do not know which strains of bacteria have been isolated.

In our research project our main aim was to investigate the antibiotic resistance patterns of the different types of bacterial species present in our 08 (eight) water samples taken from inside and outside Dhaka district. And after analyzing the data of our antimicrobial susceptibility testing (AST), we have found significant difference between the antibiotic resistance patterns of the isolated bacterial species from inside and outside Dhaka district. As per our research hypothesis, we have found more antibiotic resistance in inside Dhaka district water samples, and less resistance and more sensitivity in outside Dhaka district water samples.

In **Figure 3.17**, a comparative analysis is showed between inside and outside Dhaka district water samples using pie charts. After comparing the AST results, we have found that the antibiotic resistance is more in the isolates taken from the inside Dhaka district water samples, which is 8.8% or almost 9% higher than the isolates taken from the outside Dhaka district water samples. Also, we have found that the antibiotic sensitivity is more in the isolates taken from the outside Dhaka

district water samples, which 6.7% or almost 7% higher than the isolates taken from the inside Dhaka district water samples.

For the antimicrobial susceptibility testing (AST), we have utilized 14 common antibiotic groups and used them against the 160 isolates of bacteria to see whether the isolates are susceptible, intermediate or resistant against a particular antibiotic. From the **Figure 3.18**, it can be seen that the resistant percentages against most of the antibiotic groups were found to be higher for the inside Dhaka district isolates, rather than the outside Dhaka district isolates. The inside Dhaka district isolates showed higher resistance against Cephalosporin, Sulfonamides-Trimethoprim, Penicillins, Monobactams, Tetracyclines, Macrolides, Carbapenem, Floroquinolone, Glycopeptides, and Pinicillin combination antibiotic groups, than the isolates from outside Dhaka district water samples. Another interesting thing is found, that the antibiotic group Phenicols showed a total of 2.5% resistance against inside Dhaka district isolates, whereas, it did not show any resistance against outside Dhaka district isolates. As the resistance is higher in inside Dhaka district isolates, so, it can be said that, the antibiotics that work well on bacteria from outside Dhaka district, do not work well on bacteria from inside Dhaka district. Moreover, almost all of the isolated bacteria from inside and outside Dhaka showed resistance against the antibiotic group Oxazolidinones, as this particular antibiotic group works only on Gram-positive organisms. However, during our work, we isolated mostly Gram-negative bacteria, so the isolates showed more resistance toward this particular antibiotic group. All in all, sensitivity against these same antibiotics were found to be higher in the isolates of outside Dhaka district water samples rather than the isolates of inside Dhaka district water samples. These findings obtained from the AST

results, corresponded to our research hypothesis, which was to get more antibiotic resistance from inside Dhaka district isolates and more antibiotic sensitivity from outside Dhaka district isolates.

Additionally, for this research project, we have obtained plasmids from the isolated bacterial species. We know that bacteria can pick up resistant genes containing plasmids and become resistant to antibiotics. And by this process, a bacterium can immediately become resistant to multiple antibiotics by just simply taking up a single plasmid containing multi-resistant genes (Plasmids and co-selection, 2016). After analyzing our plasmid isolation results, we did find correlation between the presence of plasmids in bacteria and the antibiotic resistance rate. From **Figure 3.19**, we have found that the obtained plasmid ratio is significantly higher in the bacterial isolates of inside Dhaka district than in outside Dhaka district isolates. The plasmid isolation rate is 7.25% higher in the bacteria of inside Dhaka district water samples than the outside Dhaka district bacterial isolates.

Also, after comparing the percentages of obtained plasmids for the different types of isolated bacterial species, we have found that among our nine isolated bacterial species, six of them showed higher percentages of plasmids in inside Dhaka district isolates, than outside Dhaka district isolates. From **Figure 3.20**, we can see that the percentage of isolated plasmids from *Escherichia coli* is 80% in inside Dhaka district, whereas, it is 70% for outside Dhaka district water samples. For *Klebsiella spp.* the percentage of isolated plasmids in inside Dhaka district is 72%, whereas it is 50% for outside Dhaka district water samples. For *Salmonella spp.* the percentage of isolated plasmids in inside Dhaka district is 30%, whereas it is 45% for outside Dhaka district water samples. For *Enterobacter spp.* the percentage of isolated plasmids in inside Dhaka district is 64%,

whereas it is 50% for outside Dhaka district water samples. For *Vibrio spp.* the percentage of isolated plasmids in inside Dhaka district is 100%, whereas it is 85% for outside Dhaka district water samples. For *Proteus spp.* the percentage of isolated plasmids in inside Dhaka district is 60%, whereas it is 75% for outside Dhaka district water samples. For *Shigella spp.* the percentage of isolated plasmids in inside Dhaka district is 50%, whereas it is 40% for outside Dhaka district water samples. For *Pseudomonas spp.* the percentage of isolated plasmids in inside Dhaka district is 20%, whereas it is 25% for outside Dhaka district water samples. For *Staphylococcus spp.* the percentage of isolated plasmids in inside Dhaka district is 5%, whereas it is 0% for outside Dhaka district, as there was no *Staphylococcus spp.* found from the outside Dhaka district water samples.

Furthermore, we have tried to establish the relation between our obtained AST results and isolated plasmids. We investigated whether there was a correlation between the antibiotic resistance profiles of the bacteria that showed plasmid presence and the bacteria that did not. **Figure 3.21** shows that even though there is no discernible difference in the intermediate range, the isolates that did not show the presence of plasmids are less resistant to antibiotics and more susceptible to them. It became clear that the plasmid-producing isolates had a lower sensitivity rate and a higher resistance rate. From this, we can establish the fact that there is indeed somewhat of a correlation between the presence of plasmids in bacteria and their antibiotic resistance rate.

Moreover, from our study, we have found some strong evidences of correlation between the presence of plasmids in bacteria and their antibiotic resistance profile. In the ‘Hatirjheel’ water sample, which is from inside Dhaka district, the resistance to antibiotics showed higher ratio (48%) than sensitivity to antibiotics and here each bacterial isolate showed the presence of plasmids. In

the ‘Balu River’ water sample, the sensitivity to antibiotics is found to be in higher ratio than other locations of Dhaka district and the least number of plasmid (14 out of 20) bands were seen (in relation to inside Dhaka district). However, as from the total of 160 isolates, 36 of them did not show the presence of plasmid. The data range for establishing the antibiotic resistance profiles for isolates that did not contain plasmid; is very small. We need to further this study and the data range to completely establish the findings.

Chapter 5:

Conclusion

Conclusion

The main aim of this research project was to see the difference between the occurrence of different species of bacteria and the antibiotic resistance profiles of those bacteria and to find out the relation between the antibiotic resistance profiles and isolated plasmids from those bacteria inside and outside Dhaka district water sources. This comparative analysis was based on the fact that inside Dhaka district water sources are more polluted and are being polluted day by day, so their antibiotic resistance profiles should be different from outside Dhaka district water sources.

From the water samples, we have found nine different species of bacteria, including coliform bacteria, which indicates that the water sources are highly polluted. However, we did not run PCR for those isolates; thus, we could not find from where we had isolated more pathogenic strains of bacteria. From our AST results, we have found distinctive antibiotic resistance profiles between inside and outside Dhaka district water samples. We found that inside Dhaka district water samples contained more resistant bacteria, whereas outside Dhaka district water samples contained more sensitive bacteria against some common antibiotics, which supported our main hypothesis of this study.

Moreover, in both inside and outside Dhaka district water samples, multi-drug-resistant bacteria have been found. That reveals the urgent issue of antimicrobial resistance in the water sources of Bangladesh. The results of this research provide significant insights into the dynamics of different bacterial species and their antibiotic resistance patterns from different water sources in Bangladesh, establishing a basis for future research. Moreover, the correlative results of antibiotic resistance profiles and isolated plasmids showed the urgency of further study on this research project.

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