Comparative Study on Antibiotic Resistance Profiles and Plasmid Presence in Water Samples Focusing Water Bodies of Dhaka City (Hatirjheel & Gulshan Lake)

By

Marzia Mahzabin Mahi, ID: 20326002

Faria Jahan Noumi, ID: 20336011

Tasfiah Tasnim, ID: 20336009

Tazin Islam, ID: 20336002

A thesis report submitted to the 'Department of Mathematics & Natural Science' in partial fulfillment of the requirements for the degrees of 'Bachelors of Science in Biotechnology'.

Department of Mathematics & Natural Science

BRAC University

30th April, 2025

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Declaration

It is hereby declared that

1. The thesis report submitted is our own original work while completing a degree at BRAC University.
2. The report 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 report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's full Name & Signature:

Tasfiah Tasnim Barisha (Student ID :20336009)

Tazin Islam (Student ID: 20336002)

Marzia Mahzabin Mahi (Student ID: 20326002)

Faria Jahan Noumi (Student ID: 20336011)

Approval

The thesis titled “Comparative Study on Antibiotic Resistance Profiles and Plasmid Presence in Water Samples Focusing Water Bodies of Dhaka City (Hatirjheel & Gulshan Lake)” submitted by

1. Tazin Islam (20336002) of Summer 2020

2. Faria Jahan Noumi (20336011) of Summer 2020

3. Tasfiah Tasnim Barisha (20336009) of Summer 2020

4. Marzia Mahzabin Mahi (20326002) of Summer 2020

has been accepted as satisfactory in partial fulfillment of the requirement for the degree of

B.Sc. in Biotechnology & Microbiology on 30th April 2025.

Examining Committee:

Supervisor: (Member)

_____

Iftekhar Bin Naser, PhD

Associate Professor,

Department of Mathematics and Natural Sciences (MNS)

Brac University

Program Director: (Member)

_____

Munima Haque, PhD

Associate Professor,

Department of Mathematics and Natural Sciences (MNS)

Brac University

Departmental Head: (Chair)

_____

Md. Firoze H. Haque, PhD

Associate Professor and Chairperson

Department of Mathematics and Natural Sciences (MNS)

Brac University

Ethics Statement

This study was carried out in line with the ethical norms and guidelines to conduct environmental and microbiological research. The study involved sampling and testing of water from Hatirjheel and Gulshan Lake of Dhaka City. This study did not have any human or animal subjects and therefore did not need approval by an institutional review board. All sample collection procedures were conducted in such a way as to reduce environmental disruption. All safety measures were taken during sampling, transport, and laboratory work to protect researchers and the environmental integrity. There was no genetic modification or release of any biological agent into the environment employed in the study. Data from this research were utilized solely for scientific and educational intents. Results and conclusions were outlined clearly, and data were not falsified or fabricated. This research was conducted under the umbrella of scientific integrity, environmental stewardship, and data protection principles.

Acknowledgment

Above everything, we would like to wholeheartedly express our gratitude to Allah, who granted us the potential and power to bear our thesis responsibilities to the fullest and present this report in your honor.

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Abstract

Global concern has been expressed for antibiotic-resistant bacteria (ARB) and their resistant genes. Hotspots of antimicrobial resistance (AMR) have also been reported from aquatic ecosystems of urban areas, especially in fast-growing megacities, i.e., Dhaka, Bangladesh. The purpose of the present study was to compare the prevalence of plasmids and antibiotic resistance patterns of isolates of *Salmonella spp., Escherichia coli*, and *Vibrio cholerae* recovered from two urban lakes, Hatirjheel Lake and Gulshan Lake. Systematically, water samples were taken for six months, and selective culture and polymerase chain reaction (PCR) were used to confirm the existence of bacterial isolates. Antibiotic resistance to 14 antibiotics of principal classes was assessed by Kirby-Bauer disk diffusion. Plasmid DNA was extracted to determine if mobile genetic elements of resistance existed. The findings were such that the two lakes had an incredibly high prevalence of multidrug-resistant (MDR) bacteria, and Hatirjheel isolates had much higher levels of resistance. Nearly 70% of the *V. cholerae* isolates, 65% of *Salmonella spp.*, and 85% of *E. coli* were resistant to two or more antibiotic classes. Worse yet, 23% of Hatirjheel *E. coli* isolates and 18% of Gulshan Lake isolates had resistance to last-resort carbapenem antibiotics like imipenem. Plasmid analysis had a strong correlation with resistance patterns. Hatirjheeel strains of *E. coli* had 80% carriage of plasmids, whereas Gulshan isolates had 60%. Hatirjheeel had high carriage of plasmids in *Vibrio cholerae* as well as *Salmonella spp.,* supporting environmental factors resulting in horizontal gene exchange as well as transmission of AMR. The findings have implications for wastewater system reforms and the need to harmonize environmental monitoring to limit the spread of AMR in the environment. Urban lakes like Hatirjheel and Gulshan are not only scenic and recreational areas but also areas of high transmission of AMR, which may have direct and indirect effects on the health of the local population. It steers the One Health initiative as a whole towards facilitating intersectoral actions to control antibiotic resistance and provides essential baseline data on the environmental AMR status in Dhaka.

Keywords: Antibiotic Resistance (AR), urban water bodies, Hatirjheel Lake, Gulshan Lake, plasmid, *Escherichia coli*, *Salmonella spp.*, *Vibrio cholerae*, Antimicrobial Resistance (AMR), Multidrug-Resistant (MDR), environmental microbiology, public health, .

Chapter 1

Introduction

1.1: Aquatic Systems and the Spread of AMR (Antimicrobial resistance)

The global health crisis of antibiotic resistance (AR) is escalating at an alarming rate, threatening to undermine existing medical treatments and leaving millions of patients vulnerable to untreatable infections. While the clinical dimensions of antimicrobial resistance (AMR) have been extensively studied, increasing attention is now being given to the environmental pathways of AMR transmission, particularly through aquatic systems (Berendonk et al., 2015). Aquatic environments act as reservoirs and conduits for antibiotic-resistant bacteria (ARB) and associated antibiotic resistance genes (ARGs), especially in developing countries where wastewater treatment facilities are either limited or ineffective (Rizzo et al., 2013).

In the context of Bangladesh, Dhaka city presents a critical case study where rapid urbanization, dense population, and inadequate waste management converge to exacerbate environmental AR risks. Notably, Gulshan Lake and Hatirjheel Lake—two major water bodies within Dhaka—serve not only domestic and industrial functions but also recreational purposes, making them significant exposure points for human interaction. Studies have shown that these lakes harbor high concentrations of fecal coliforms and multidrug-resistant strains of *Escherichia coli* and *Vibrio cholerae* (Ahmed et al., 2019; Islam et al., 2017). The contamination of Gulshan and Hatirjheel Lakes with ARB and ARGs illustrates how urban water systems can become hotspots for resistance dissemination, reinforcing the urgent need for systematic microbial and resistance profiling in such aquatic environments.

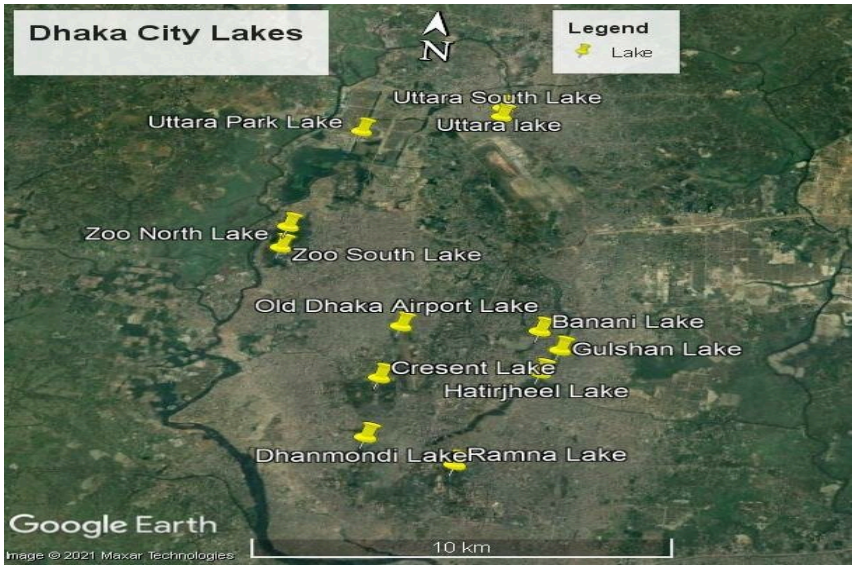


Fig 1: Location of Gulshan lake and Hatirjheel lake in Dhaka city.

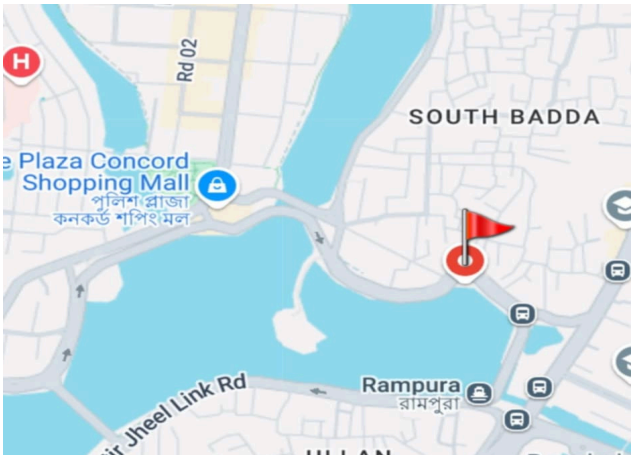
1.2: Significance of Urban Water Bodies in AR (Antibiotic Resistance) Dissemination

Urban lakes, particularly those embedded in the city center, bear a disproportionate brunt of anthropogenic activities. Hatirjheel and Gulshan Lake are two of Dhaka's largest and most crowded lakes, located in the business and residential center of the city. Both lakes have stormwater runoff, municipal waste, and unauthorized dumping of waste from adjacent industries and clinics in some cases (Parvin et al., 2022).

Recurrent release of antibiotics imposes selective pressure on microbial communities in water and hence encourages the growth and survival of antibiotic-resistant microorganisms (Kümmerer, 2009). Horizontal gene transfer (HGT) promotes the development of resistance in environmental bacterial populations through plasmids, which are small circular DNA molecules that can transfer genetic information between various bacterial species (Carattoli, 2009). Plasmid-mediated spread of antibiotic resistance genes (ARGs) in environmental isolates poses gigantic challenges to therapeutic approaches in the clinic and represents an expanding environmental reservoir of such resistance (Baker-Austin et al., 2021).

1.3: Study Area Selection

The densely populated commercial, residential, and industrial areas of Dhaka city impose human pressure on two big public lakes, Hatirjheel Lake and Gulshan Lake. These reservoirs, ideal for the development and growth of bacteria as well as the development and dissemination of genes that render bacteria resistant to antibiotics, are regularly polluted by surface runoffs, raw domestic sewage, and industrial waste waters (Miah et al., 2017). Several studies have confirmed widespread microbial pollution of the lakes, with high fecal coliform and antibiotic-resistant bacteria like *Vibrio cholerae* and *Escherichia coli.* Joint use of the lakes for residential and recreational activities ensures higher likelihood of human contact and a public health concern (Some et al., 2021b). A number of studies have confirmed widespread microbial contamination of the lakes with high fecal coliform and antibiotic-resistant bacteria like *Vibrio cholerae* and *Escherichia coli*. Joint use of the lakes for domestic and recreational activities ensures increased human contact probability and a public health problem (Salamandane et al., 2021).



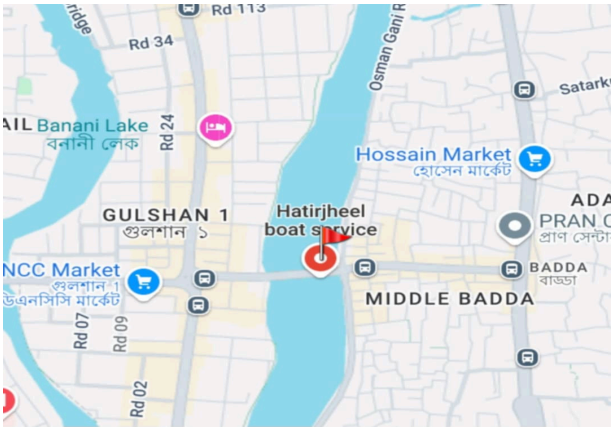


Fig 2: Water sample collected from Hatirjheel Lake.

Fig 3: Water sample collected from Gulshan Lake

1.4 Focus Organisms: *E. coli*, *Salmonella spp.*, and *Vibrio cholerae*

This study focuses on three clinically and environmentally significant bacterial genera:

- ***Escherichia coli (E. coli):*** A common fecal indicator and opportunistic pathogen. The majority of *E. coli* strains exist as harmless types but STEC produces Shiga toxin which leads to dangerous gastrointestinal illness. Research shows *E. coli* bacteria exist frequently in contaminated surface water in Bangladesh where they demonstrate strong resistance abilities against β-lactam antibiotics and fluoroquinolones (Ahmed et al., 2019).According to World Health Organization [WHO] (2022) reports reveal that E. coli strains complicate 26% of diarrheal cases among children below age five throughout Bangladesh each year. The small amounts of bacteria required to cause infections lead to increased outbreak risks particularly through water contamination (Ahmed et al., 2019). Environmental studies discovered substantial antibiotic-resistant *E. coli* strains in national surface water sites that resist both β-lactam and fluoroquinolone drugs which confirms health risks to both the environment and humans (Ahmed et al., 2019).

- ***Salmonella spp*:** The bacteria *Salmonella spp*. results in enteric fever and gastroenteritis and its transmission happens through food and water sources. The number of environmental strains showing resistance to multiple antibiotic treatments continues to increase especially throughout low- and middle-income countries (Islam et al., 2023). *Salmonella* belongs to the Enterobacteriaceae family as rod-shaped gram-negative bacteria and researchers identify it through the Kauffman-White system that distinguishes over 2,500 serotypes according to H and O surface antigens. Two primary species belong to the *Salmonella* genus consisting of *Salmonella* enterica and *Salmonella* bongori.

Salmonella exists primarily in human and animal intestinal systems then proceeds to cause acute diarrheal illness on a worldwide scale and results in severe enteric fever and additional infective conditions (Popa & Popa, 2021). Current research exhibits rising salmonellosis infection rates after it was detected in 35% of patients having acute gastroenteritis (Islam et al., 2023). The presence of *Salmonella* in contaminated food and water supply elements creates major health risks because it generates serious gastrointestinal complications and invasive illnesses in infected individuals.

- ***Vibrio cholerae:*** *Vibrio cholerae* functions as the agent that causes cholera and it exists best in aquatic environments. The incidence of cholera persists as an endemic disease throughout Bangladesh while multidrug-resistant *Vibrio* species detected in rivers and lakes have increased notably during recent years (Faruque et al., 2007; WHO, 2023).*Vibrio* bacterial infections mostly occur on human because people interact with water that contains contamination or consume seafood raw or undercooked. The waterborne pathogen V. cholerae causes cholera while passing through fresh waters in addition to causing severe diarrhea through direct person-to-person contact. The two *Vibrio* species *Vibrio*parahaemolyticus and *Vibrio Vulnificus* trigger vibriosis in humans although their infections vary from simple gastrointestinal illness to dangerous blood infections (Baker-Austin et al., 2018). World Health Organization statistics show that Bangladesh exists among the nations experiencing the most severe impact from cholera cases which affect a yearly total of 1.3 million individuals worldwide (WHO, 2022).

1.5: Commonly Used Antibiotics for Treatment in Bangladesh

The chemical substances known as antibiotics emerge naturally from bacteria and fungi microorganisms which have the ability to destroy or halt microbial growth. These substances perform essential functions in stopping and treating bacterial infections. Doctor-prescribed antibiotics operate as either bacteria-specific agents or broad-spectrum medications effective against many bacterial pathogens which aid treatment of complex multi bacterial infections (Antibiotic, 2023).

- ➢ **Penicillins:** Bacteria generation of cell walls is blocked by antibiotics which contain a β-lactam ring element responsible for their antibacterial effects. These antibiotics treat respiratory tract and urinary tract infections and also work against meningitis and endocarditis as their prescribed medical purposes.
- ➢ **Cephalosporins**: The antimicrobial mechanism of penicillins matches exactly with the action of cephalosporins because they both signal cell wall destruction. These drugs provide medical treatment against infections affecting both the urinary and respiratory tracts together with blood, skin and bones and pelvic inflammatory disease.

- **Aminoglycosides:** Bacterial protein production becomes blocked through these medications. Such antibiotics successfully treat infections affecting the urinary and respiratory tracts as well as blood infections and pelvic inflammatory disease.
- **Fluoroquinolones:** The DNA replication process of bacteria becomes disrupted by agents within this substance group. These antibiotics are prescribed to treat the condition of diarrhea and infections of pneumonia and gonorrhea alongside respiratory and urinary tract infections.
- **Tetracyclines:** Bacterial protein synthesis becomes inhibited due to these antibiotic medications. Pharmacological treatment of rickettsial diseases, pneumonia, intestinal amebiasis and minor skin injuries includes these antibiotics.
- **Macrolides**: The protein synthesis inhibition aspect of macrolides enables them to cure respiratory tract and skin infections alongside sexually transmitted diseases as well as Legionnaires disease, whooping cough and diphtheria and ear infections.
- **Chloramphenicols:** The productivity of bacterial proteins gets blocked by these substances which medical professionals use to treat skin infections together with eye infections and cystic fibrosis and minor wounds (Antibiotic, 2023).

1.6: Plasmid-Mediated Resistance and Environmental Spread

The rapid bacterial evolution process occurs through plasmid action which allows resistance gene transfer between unrelated bacterial species (Bennett, 2008). The strains thrive in Dhaka's urban lake environments together with bacterial communities because they face elevated selection from antibiotic residues and metal contamination. For instance, Mountain-harbored conjugative plasmids contain resistance genes against penicillins and tetracyclines and aminoglycosides simultaneously which facilitates formation of multidrug-resistant bacterial strains (Carattoli, 2009).

The combined location of Hatirjheel and Gulshan Lake next to medical facilities and drug stores puts these waters at significant danger of retaining bacteria that harbor plasmids. The lakes function as probable Hotspots for Horizontal Gene Transfer activities yet modern research lacks data about plasmid-mediated bacterial resistance occurrence among water microbes.

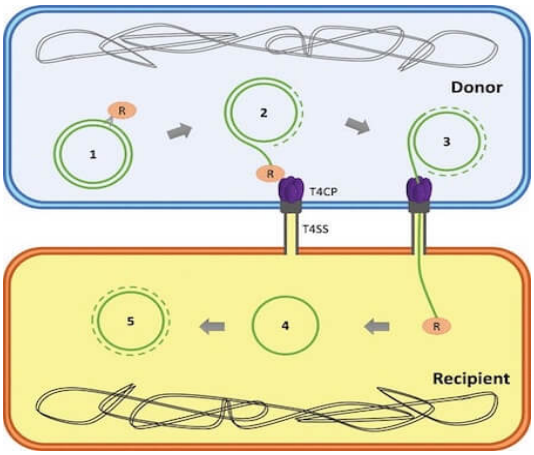


Fig 4: Spread of antibiotic resistant gene through one plasmid to another (*Plasmids and the Spread of Antibiotic Resistance Genes | ASM.org*, n.d.).

This figure indicates the process of gene transfer between two bacterial cells by plasmid through conjugation. The donor bacterium possesses a chromosome and a second plasmid containing an antibiotic resistance gene. During the process of conjugation, the plasmid is replicated and a copy is made for transferring. These specialized entities, T4CP and T4SS, create a direct connection between the donor and recipient bacterial cells. The plasmid is introduced into the host cell, where it forms a circle and is an independent entity. This renders the recipient antibiotic-resistant, such that it will be able to survive in the presence of antibiotics.

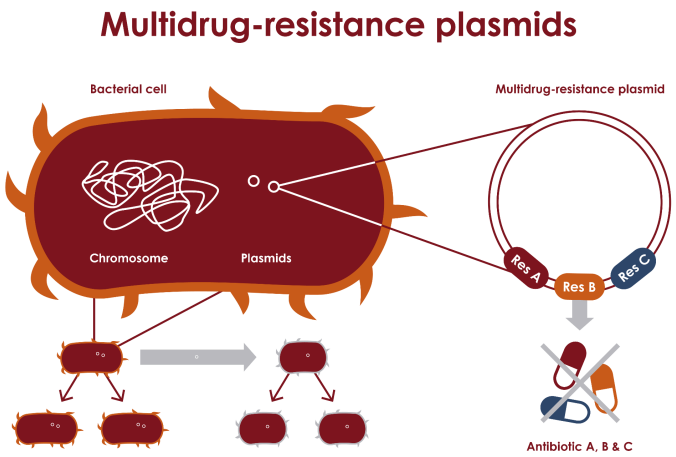


Fig 5: Action of multidrug-resistance plasmids (*Plasmids and Co-selection – Antibiotic Resistance – ReAct*, 2025).

The chart shows how plasmids causing multidrug-resistance are responsible for bacterial populations becoming resistant to antibiotics. It shows two types of genetic material in a cell: the central chromosome and the small circular plasmids. A specific plasmid, Res A, Res B, and Res C, contains a number of resistance genes, thus the bacterium is able to resist a variety of antibiotics simultaneously. These plasmids are passed on during bacterial replication through binary fission such that daughter cells inherit resistance traits. They also pass on from cell to cell so that antibiotic resistance is spread horizontally quickly. The diagram shows the important role played by plasmids in rendering bacterial infections more resistant to treatment.

1.7: Literature Review

Antibiotic resistance is an urgent global public health concern, and aquatic environments represent significant reservoirs of resistant bacterial strains and resistance genes. Domestic wastewater, hospital waste, industrial pollutants, and agricultural run-off increasingly contaminate urban water systems, especially in developing nations such as Bangladesh, thereby disseminating antibiotic-resistant microbes (Pruden et al., 2006). This has caused antibiotic resistance to become a prominent global health issue, as aquatic environments always serve as significant reservoirs for resistant bacteria and respective resistance genes (Rizzo et al., 2013). Dhaka, as one of the most densely populated cities in the world, faces huge exposure to water pollution owing to uncontrolled urbanization and absence of proper wastewater treatment systems. The aquatic bodies of Dhaka come in contact with high levels of untreated household wastewater along with medical waste that contains antibiotic-resistant bacteria (Parvin et al., 2022). The research has revealed high rates of antibiotic resistant *E. coli* and *Salmonella* in

aquatic bodies, which is a significant risk to environmental quality and human health (Ahmed et al., 2019).

Different studies have also documented the high presence of *Escherichia coli*, *Vibrio cholerae*, and *Salmonella spp*. in surface waters of Bangladesh that are not just indicators of fecal contamination but also etiologic agents of acute gastrointestinal illness (Chowdhury et al., 2011). Zero tolerance of fecal coliforms in drinking water is recommended by WHO, but contamination above allowable standards has been documented in most urban water bodies in studies (Ahmed et al., 2018). Plasmids also play a role in propagating antibiotic resistance through horizontal gene transfer between bacteria. Conjugative plasmids also carry several resistance genes (ARGs), which are involved in promoting the emergence of multidrug-resistant (MDR) strains (Carattoli, 2009). The occurrence of such plasmids in aquatic environments has been linked to antibiotic use in hospital and agricultural environments. Excessive consumption of antibiotics, even off prescription, and poorly organized waste disposal systems in Dhaka have most likely accelerated the emergence and spread of resistant organisms (IEDCR, 2023). AST is also a significant method in environmental microbiology to determine resistance patterns and effectiveness of regular antibiotics against environmental isolates. AST methods like the Kirby-Bauer disk diffusion are very common in susceptibility testing of bacteria (CLSI, 2020). AST findings inform public health policy while the tool assists researchers in monitoring regional trends of resistance. Recent studies have highlighted the gravity of AMR in Bangladeshi water bodies. Country-wide studies by Ahmed et al. (2019) revealed extensive resistance among surface water bacterial isolates with 84% carbapenem resistance. Pharmaceutical contamination of Dhaka rivers was also noted in another study by Parvin et al. (2022), and plasmid-mediated resistance was detected in E. coli and *Vibrio* species of urban water samples. These results align with international interests within the environmental aspect of AMR, especially in fast urbanizing areas where environmental governance is weak (WHO, 2023).

While numerous researchers have examined the prevalence of antibiotic-resistant bacteria in rural or farm settings in Bangladesh, few have focused on Dhaka's urban water bodies specifically. The specific epidemic patterns of resistance dissemination within the city due to high population density and concentrated health centers remain to be well-studied. This study will fill that gap by conducting an extensive comparative study of antibiotic resistance patterns and plasmid presence in bacterial isolates from different water sources in Dhaka city. The research community has extensively studied antibiotic-resistant bacteria in rural regions of Bangladesh but not in the water bodies of Dhaka's urban area. The research community lacks complete information about how the resistance is spread throughout the city where the overpopulation and the concentration of healthcare centers exacerbate it. The research activity aims at the study of antibiotic resistance profiles as well as plasmid detections from different bacterial isolates collected in different Dhaka city water sources.

1.8: Objectives of the Study

The goal of the current study is to conduct comparative profiling of the antibiotic resistance profile of *Salmonella* spp., *Escherichia coli*, and *Vibrio spp*. from water samples collected from Gulshan Lake and Hatirjheel Lake in Dhaka city. Furthermore, the study also aims to investigate the occurrence and distribution of plasmids among the bacterial isolates and to analyze their role in mediating antibiotic resistance. By comparison of resistance patterns and plasmid carriage, the current work attempts to clarify environmental dissemination of multidrug-resistant bacteria in aquatic urban ecosystems and indicate potential public health risks caused by contaminated water bodies with such bacteria in regions of rapid urbanization.

1.9: Justification

Notwithstanding the existing research on antibiotic resistance in Bangladesh's water bodies, this specific area of study investigated Dhaka's urban lakes exclusively. These lakes demonstrate both the microbial pollution levels from the city and directly affect the health of urban residents because many citizens reside nearby and obtain lake water to perform their daily tasks. This research investigates Hatirjheel and Gulshan Lake specifically to deliver localized results that also demonstrate general principles about AMR patterns and plasmid behaviors in urban water ecosystems.

Chapter 2

Materials & Method

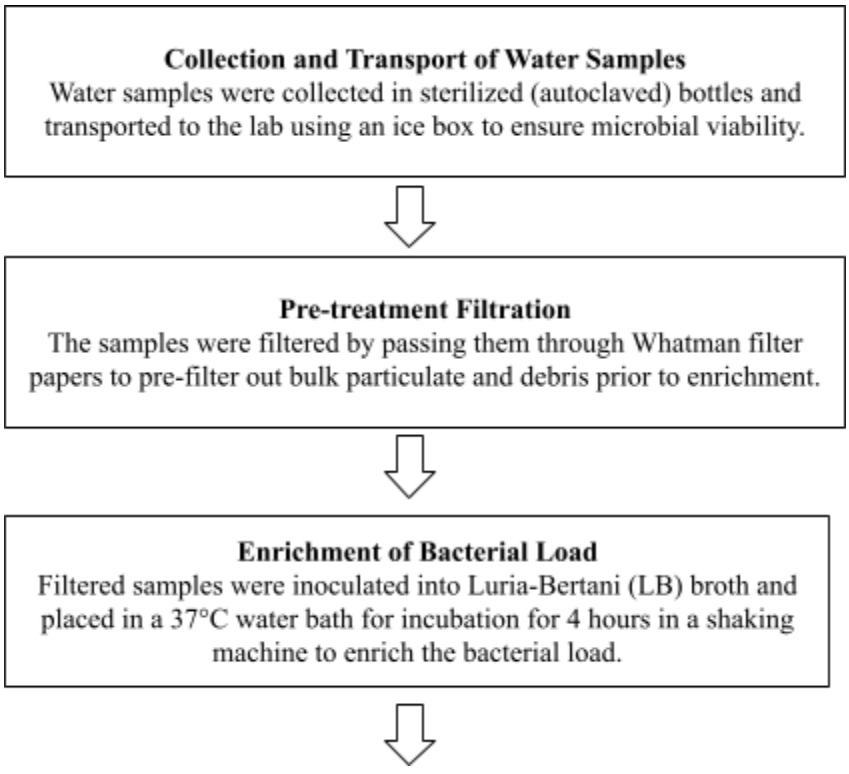
2.1: Site of research

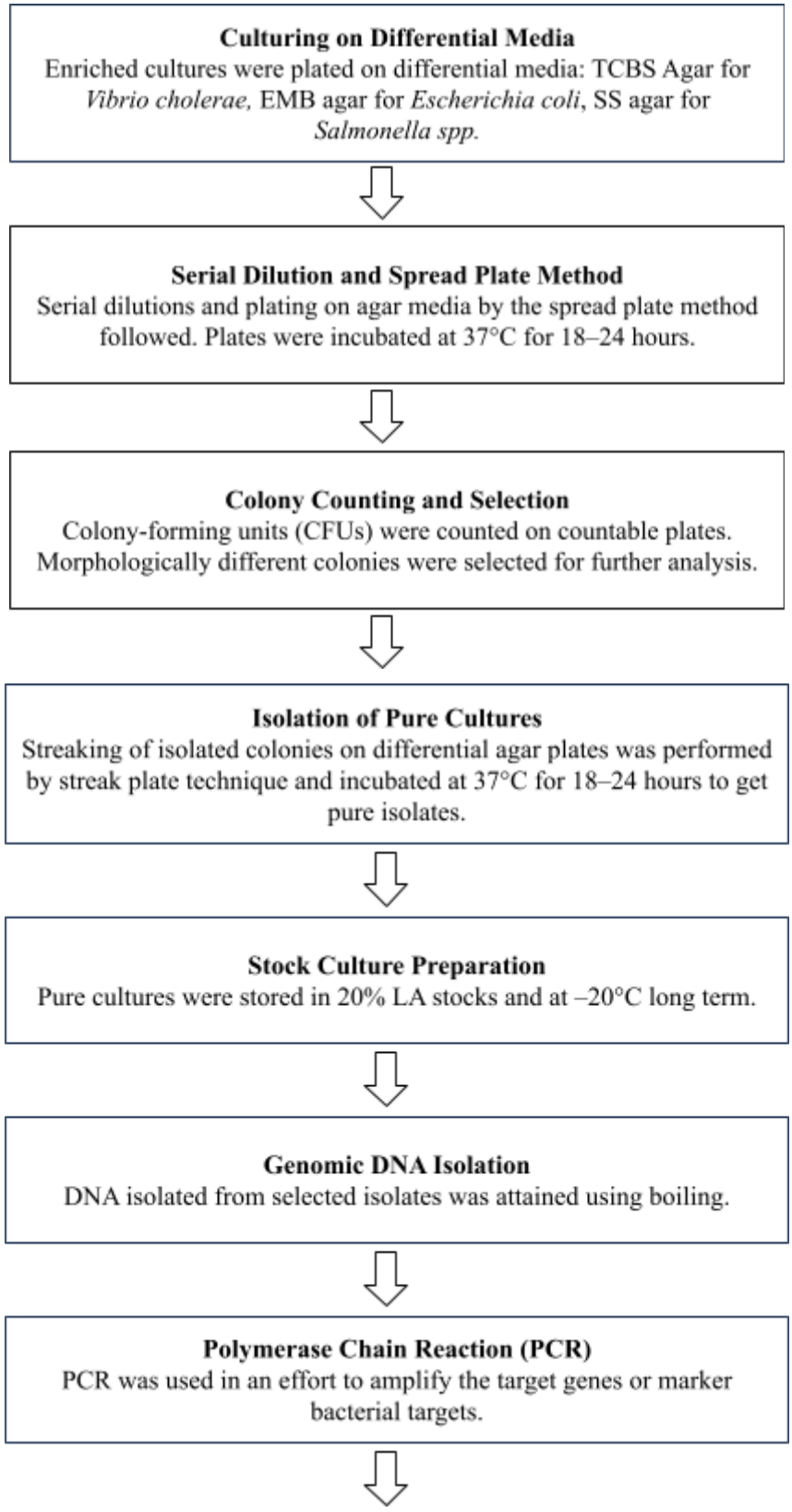
The laboratory tests in this study were conducted in the Department of Mathematics and Natural Sciences' Biotechnology, Microbiology, and Molecular Biology Laboratory at BRAC University.

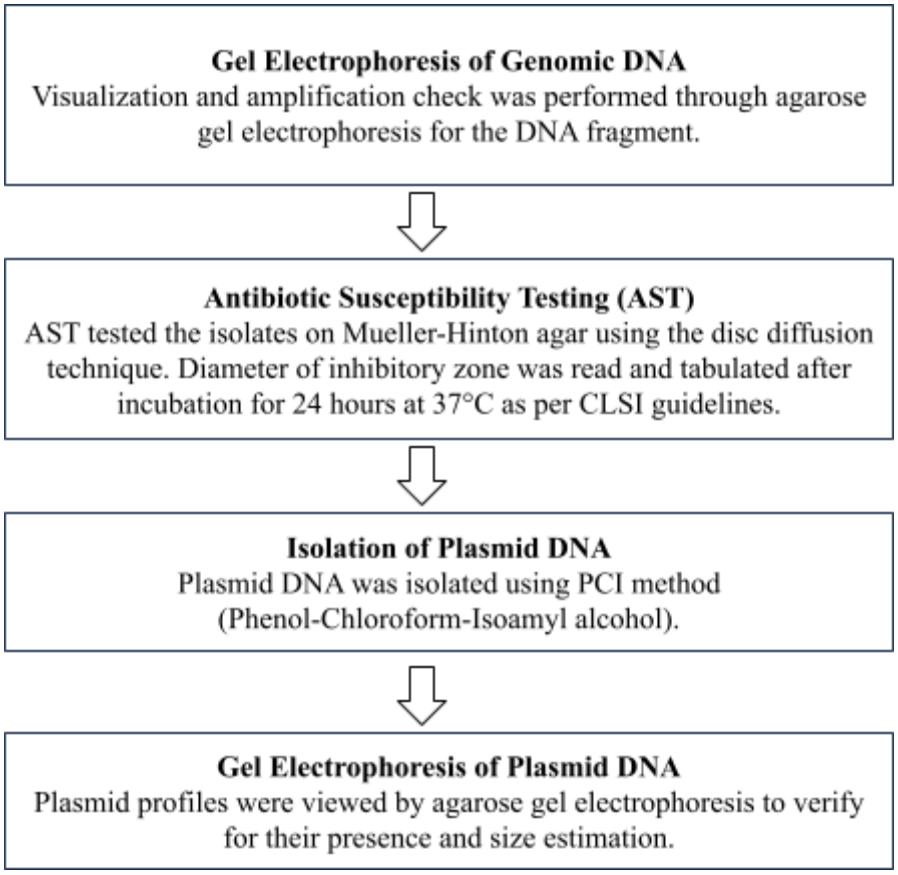
2.2: Research period

The research study was carried out between April 2024 and March 2025.

2.3: Overview of the experimental procedure




Culturing on Differential Media
Enriched cultures were plated on differential media: TCBS Agar for Vibrio cholerae, EMB agar for Escherichia coli, SS agar for Salmonella spp.
Serial Dilution and Spread Plate Method
Serial dilutions and plating on agar media by the spread plate method followed. Plates were incubated at 37°C for 18–24 hours.
Colony Counting and Selection
Colony-forming units (CFUs) were counted on countable plates. Morphologically different colonies were selected for further analysis.
Isolation of Pure Cultures
Streaking of isolated colonies on differential agar plates was performed by streak plate technique and incubated at 37°C for 18–24 hours to get pure isolates.
Stock Culture Preparation
Pure cultures were stored in 20% LA stocks and at –20°C long term.
Genomic DNA Isolation
DNA isolated from selected isolates was attained using boiling.
Polymerase Chain Reaction (PCR)
PCR was used in an effort to amplify the target genes or marker bacterial targets.


Gel Electrophoresis of Genomic DNA
Visualization and amplification check was performed through agarose gel electrophoresis for the DNA fragment.
Antibiotic Susceptibility Testing (AST)
AST tested the isolates on Mueller-Hinton agar using the disc diffusion technique. Diameter of inhibitory zone was read and tabulated after incubation for 24 hours at 37°C as per CLSI guidelines.
Isolation of Plasmid DNA
Plasmid DNA was isolated using PCI method (Phenol-Chloroform-Isoamyl alcohol).
Gel Electrophoresis of Plasmid DNA
Plasmid profiles were viewed by agarose gel electrophoresis to verify for their presence and size estimation.

2.4: Laboratory Equipment and Instruments

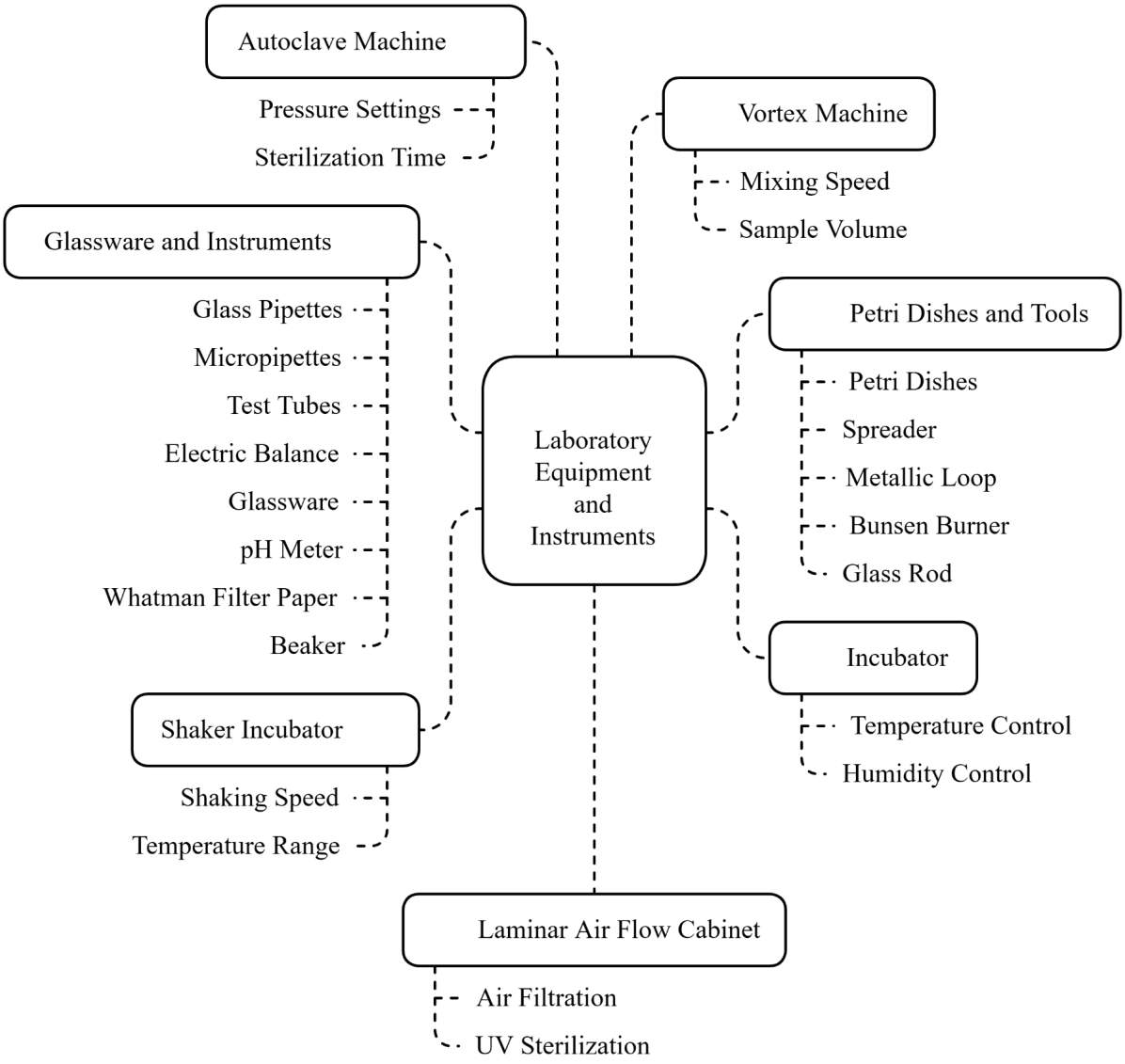


Fig 6: Laboratory equipment and instruments used for this experiment (Created using Napkin).

2.5: Collection, Handling, and Transportation of Water Samples

Water samples for analysis were taken from two water bodies of Dhaka city: Gudaraghat Lake and Hatirjheel Lake, both of which are in the Dhaka district of Bangladesh. Both of the selected sites have high anthropogenic activity, as well as the likelihood of exposure of their water bodies to anthropogenic contaminants, which are expected to influence microbial diversity and antibiotic resistance patterns. Surface water was collected at each station using sterile pre-labeled sampling bottles. To maintain microbial integrity and reduce changes in bacterial populations, the samples collected were promptly kept in an ice box at around 4°C during transportation to the laboratory. Temperature-controlled transport suppressed microbial growth before processing. On arrival at the Biotechnology, Microbiology, and Molecular Biology Laboratory of BRAC University, the samples were examined immediately to obtain accurate microbiological analysis and prevent degradation or contamination.

2.6: Pre-culture preparation of bacteria

For ease of bacterial isolation from the water samples, an initial filtration process was carried out using 0.4 mm Whatman filter paper, successfully removing particulate matter and debris. Further, the filtered sample of 4 ml was pipetted into a sterile autoclaved Falcon tube with 10 ml of LB broth, thus enhancing the bacterial population. After that, the enrichment culture was grown for 4 hours at 37°C using a shaking incubator for optimal bacterial growth.

Thereafter, serial dilution procedure was followed to reduce bacterial concentration for viable count assessment. 1 ml of the enriched culture was passed into 9 ml of sterile 0.9% NaCl solution and serially diluted through five successive sterile autoclaved test tubes, with a dilution factor of 10-6. In addition, an 80 µl of raw (unprocessed) sample was plated directly onto selective culture medium for the multiplication of the target bacterial species.

2.7: Culture Media Employed for Bacterial Growth

Selection of culture media varies with cell types under investigation, the reason for the culture method, and availability of laboratory facilities. Due to differences in the nutritional and growth factor requirements between cell types, experimental determination often is necessary to determine the ideal medium for growth (*How to Select the Right Cell Culture Media - Eppendorf US*, 2024). Three selective media were employed in the current study: Eosin Methylene Blue (EMB) agar, *Salmonella-Shigella* (SS) agar, and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar to favor the growth of different groups of bacteria and facilitate a complete determination of bacterial growth. Samples, after serial dilution, were spread plated onto selective media and incubated at 37°C for 18–24 hours. After incubation, bacterial colonies were isolated by the streak plate method on the same selective medium for identification. Nutrient agar, a generalized

medium for various microorganisms, was also utilized prior to antibiotic susceptibility testing (AST). Mueller-Hinton Agar (MHA) was employed for AST.

2.7.1: Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue agar is a common differential and selective culture media in microbiology. Selectivity is obtained by using eosin and methylene blue dyes, which have the capability to suppress the growth of Gram-positive bacteria and therefore enable the growth of Gram-negative bacteria (Sharebiology, 2023). As a differential medium, EMB agar permits differential Gram-negative bacteria according to lactose fermentation activity. Lactose-fermenting organisms such as *Escherichia coli* produce greenish metallic colonies due to acid production, while poor fermenters produce brown or pink-colored colonies. Non-lactose fermenters such as *Salmonella* and *Shigella* are not acid-producing and therefore yield colorless or clear-colored colonies, which can be used to distinguish them from coliform bacteria. Some common bacterial species *E. coli*, Enterobacter aerogenes, and *Klebsiella pneumoniae* may be cultivated using EMB agar (Editorial Team, 2022). 37.5 grams powder of EMB agar is made soluble in one liter of distilled water for preparation. The powder is dissolved by boiling to dissolve it completely and transferred to sterile petri plates to be sterilized.

Fig 7: EMB fresh agar plate.

2.7.2: *Salmonella Shigella* (SS) Agar

Salmonella-Shigella (SS) agar is a selective and differential culture medium used for the isolation of *Salmonella* and certain *Shigella* species. The medium has bile salts, sodium citrate, and brilliant green, which inhibit the growth of Gram-positive bacteria and coliforms and also inhibit swarming of ‘Proteus’ species to a great extent. The inhibitory agents permit selective

growth of *Salmonella* species (Aryal, 2022b). The medium has nutritional components such as beef extract, casein hydrolysate, and enzymatic digests of animal tissue as nitrogen, carbon, and vitamin sources for the growth of bacteria. Lactose is the carbohydrate source in the medium. Hydrogen sulfide production can be detected by the development of colonies with black centers due to the presence of sodium thiosulfate and ferric citrate in the medium. Neutral red is a pH indicator which turns red under acid conditions to signify lactose fermentation. SS agar is selective for the growth of many organisms like *Escherichia coli*, *Salmonella spp.*, *Salmonella enteritidis*, and *Shigella flexneri* (Aryal, 2022). For preparation, 63 grams of SS agar powder is mixed with one liter of distilled water. It is boiled and transferred into sterile Petri dishes where it is left to solidify. Notably, autoclaving for sterilization is not required.

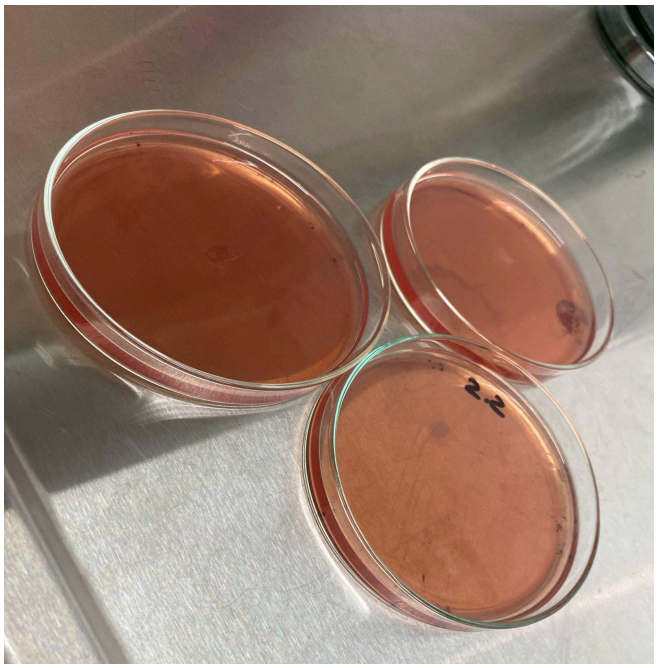


Fig 8: SS fresh agar plate.

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

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar is both a differential and selective medium originally designed for the isolation of *Vibrio cholerae* and other pathogenic *Vibrio* species. It is very alkaline in pH, supplemented with thiosulfate and sodium citrate, which is inhibitory to *Enterobacteriaceae* growth (Aryal, 2022c). It also contains oxy-bile and sodium cholate to suppress Gram-positive bacteria, including *Enterococcus* species. The chief fermentable carbohydrate is sucrose. The acid is generated by the sucrose-fermenting *Vibrio* species, which changes the color of the pH indicators bromothymol blue and thymol blue from blue to yellow. Sodium chloride is included to provide the metabolic activity and growth for the halophilic *Vibrio* strains. TCBS agar is employed on a large scale for the cultivation of *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* (“Thiosulphate Citrate Bile-salt

Sucrose (TCBS) Agar," 2003). For preparation, 89.08 grams of dehydrated medium is dissolved in one liter of distilled water, boiled, and poured into sterile Petri dishes to solidify. Autoclaving of the medium is not required.

Fig 9: TCBS fresh agar plate.

2.8: Buffer formulation

Buffer solutions are essential components which are responsible for stabilizing the pH levels, which is a prerequisite for the smooth operation of most of the biological processes (Libretexts, 2023). In this study, extreme care has been taken to prepare a broad range of buffer systems for assessing the optimum conditions needed for effective DNA extraction and other essential processes in molecular biology.

Table 1: Formulation of buffer

1X TE Buffer	20X TBE Buffer	Running Buffer
➔ A 10 mM concentration of Tris-HCl was added to the solution. ➔ Also, 10 mM EDTA was added. ➔ Distilled water, 10 ml, was then added. ➔ The pH was brought to 8.3. When the pH was more than 8.3, hydrochloric acid (HCl) was added to bring it down; if less than 8.3, sodium hydroxide (NaOH) pellets were added to increase the pH. ➔ More distilled water was added to bring the volume to 20 ml. ➔ pH was re-measured to confirm accuracy.	➔ A 150 milliliters amount of distilled water was placed in a beaker. ➔ Tris base (54.6 g), boric acid (28.6 g), and EDTA (4.658 g) were added to the solution and mixed well in a magnetic stirrer for 3 to 5 minutes. ➔ The pH was brought to an end pH of 8.3. Hydrochloric acid (HCl) was added when the pH was greater than 8.3; however, when the pH fell below 8.3, sodium hydroxide pellets were added to increase the pH. ➔ The volume was increased to 250 milliliters with the use of extra distilled water. ➔ The pH was re-measured to verify its accuracy.	➔ A volume of 50 ml of 20X buffer was added to the solution. ➔ Next, 700 ml of distilled water was added. ➔ The pH was lowered to a reading of 8.3. In case the pH reading was more than that of 8.3, hydrochloric acid (HCl) was added to lower it; in case less than that of 8.3, sodium hydroxide (NaOH) pellets were employed to increase it. ➔ The last volume was prepared to 250 ml with distilled water. ➔ The pH was re-checked to make sure it was at the desired level.

2.9: Microbial Inoculation Procedures

The research started with the use of serial dilution, and then the spread plate method was used to inoculate microorganisms into the culture medium. After that, the streak plate method was used to obtain bacterial isolation.

2.9.1: Spread Plate Method

Spread plate technique is a most common microbiological procedure for isolation and counting of viable microorganisms present in liquid samples. The process involves the uniform spreading of a defined volume of the sample on the surface of a solid culture medium (Dahal, 2025). For the present study, water samples were enriched in LB medium before applying the spread plate technique to promote bacterial growth. Following enrichment, serial dilution was performed to obtain an optimum microbial concentration. 80 µL aliquot from each of the diluted samples was then plated onto agar plates and spread with a sterile spreader. The colony counts were then performed following incubation in order to determine the microbial load.

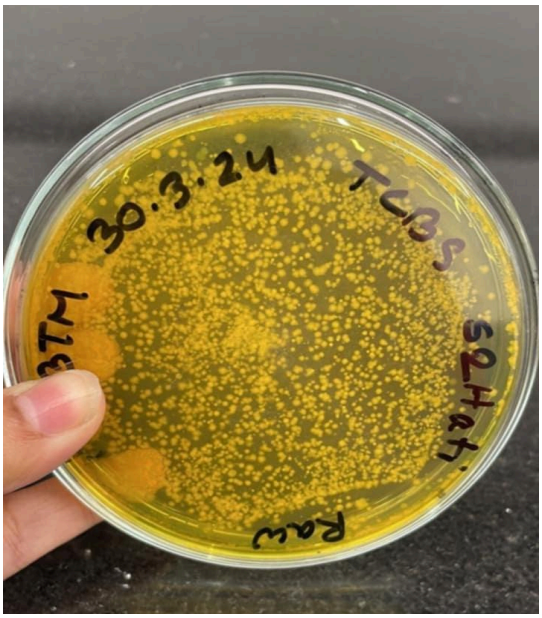


Fig 10: *Vibrio cholerae* spreaded in TCBS media through spread plate method.

2.9.2: Streak Plate Method

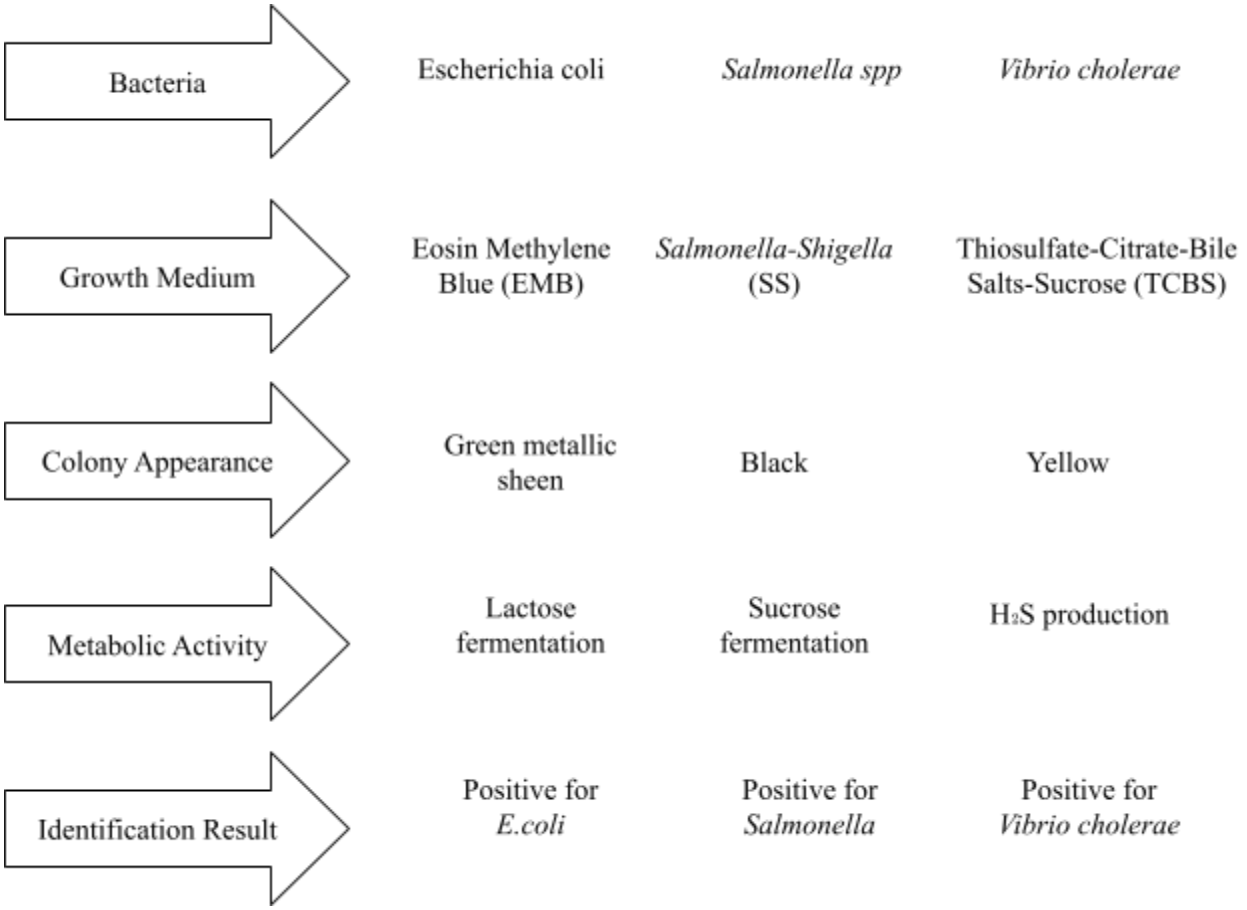
Streak plate technique is a simple microbiological method of isolating single bacterial cultures and well-isolated colonies from a population. It is achieved by decreasing bacterial density in progressive steps on the agar plate surface in a manner that a limited number of cells are permitted to grow as discrete, isolated colonies in subsequent streaks. It may be employed for species isolation or for the isolation of single-species cultures (Tankeshwar & Tankeshwar, 2024). A colony of the spread plate method was streaked in the experiment. A small amount of colony was streaked down the side of the agar plate with a sterile inoculating loop to achieve an initial smear. The loop was utilized to streak down the agar plate in a systematic manner while trying to dilute the concentration of bacteria in a systematic way. Following 18–24 hours of

incubation at 37°C, individual colonies were selected, and pure cultures were obtained. Pure cultures served as the basis for subsequent morphological and characterization experiments.

Fig 11: *Vibrio cholerae* streaking in TCBS agar by streak plate method.

2.10: Morphological Identification of Cultured Organisms

During the research, Himedia analysis was employed to aid presumptive identification of the microorganisms cultured. This included comparing the colony morphological characteristics such as shape, size, color, edge, elevation, and texture on different selective and differential culture media. Growth patterns and colony appearances were well examined and compared with standard reference information from Himedia (*HiCromeTM Universal Agar Plate*, n.d.). This method allowed the preliminary grouping of the bacterial isolates and provided a preliminary impression of the microbial diversity of the samples prior to additional biochemical or molecular confirmation (Libretexts, 2024).



2.11: Antibiotic Susceptibility Testing

Antibiotic susceptibility testing, or AST, is employed to ascertain the action of various antibiotics in inhibiting microbial growth under reproducible laboratory test conditions (Khan et al., 2019). In this research, the Kirby-Bauer disk diffusion method was employed, a well-established method utilized to assess antimicrobial susceptibility. A reproducible suspension of bacteria was spread evenly onto Mueller-Hinton Agar plates with sterile cotton swabs. Antibiotic disks impregnated with antibiotics were subsequently placed on the surface of the inoculated agar, and the cultures were incubated for 18–24 hours at 37°C. The zones around each disk were measured after the incubation period to determine the susceptibility patterns of the bacterial isolates as resistant, intermediate, or susceptible to the respective antibiotics.

2.11.1: Preparation of Bacterial Suspension

A suspension of bacteria in sterile saline was prepared before sensitivity testing to antibiotics.

- ➔ Sterile saline was added to each autoclaved test tube containing a bacterial isolate as a medium for suspension.
- ➔ A loopful of colonies of an 18-hour-old culture was removed aseptically using a sterile metallic inoculating loop and added to the saline.
- ➔ In a bid to ensure that the bacterial cells were uniformly suspended, the resulting suspension was appropriately vortexed.
- ➔ To stabilize and maximize dispersion of cells and to precondition repeatable and consistent test conditions, the suspension was allowed to stand at room temperature for fifteen minutes.

2.11.2: Mueller Hinton Agar (MHA)

Mueller-Hinton Agar (MHA) has become the gold standard medium for antibiotic susceptibility testing (AST) because it is a non-differential and selective medium that produces accurate and reproducible results. The MHA medium also gives the highest antibiotic diffusion with clear and consistent zones of inhibition (Aryal, 2023a). In addition, the starch in the medium helps to absorb bacterial toxins that may inhibit antimicrobial activity, thus improving the overall reliability of the results. To prepare the medium for use in this research, 38 grams of MHA powder were dissolved in 1 liter of distilled water. It was boiled to facilitate complete dissolution and then sterilized by autoclaving. Following sterilization, the molten medium was aseptically poured into sterile Petri dishes and left to solidify under controlled conditions before use in the test procedure.

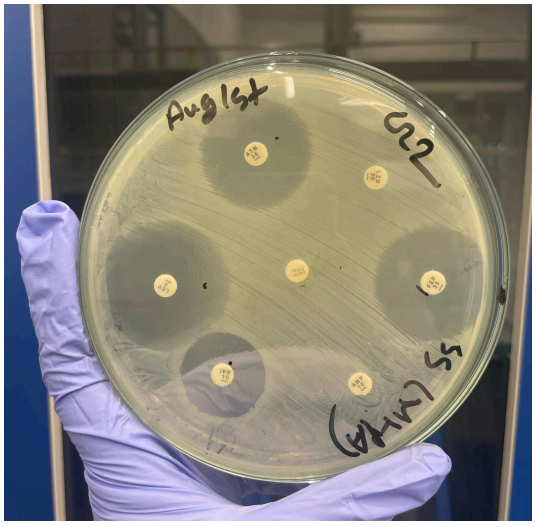


Fig 12: Antibiotic Susceptibility testing in MHA agar.

2.11.3: Lists of Antibiotics

Based on their suggested use, antibiotics were chosen for the susceptibility test. The list of antibiotics used in this investigation is shown in the table below, along with the zone of inhibition for each one.

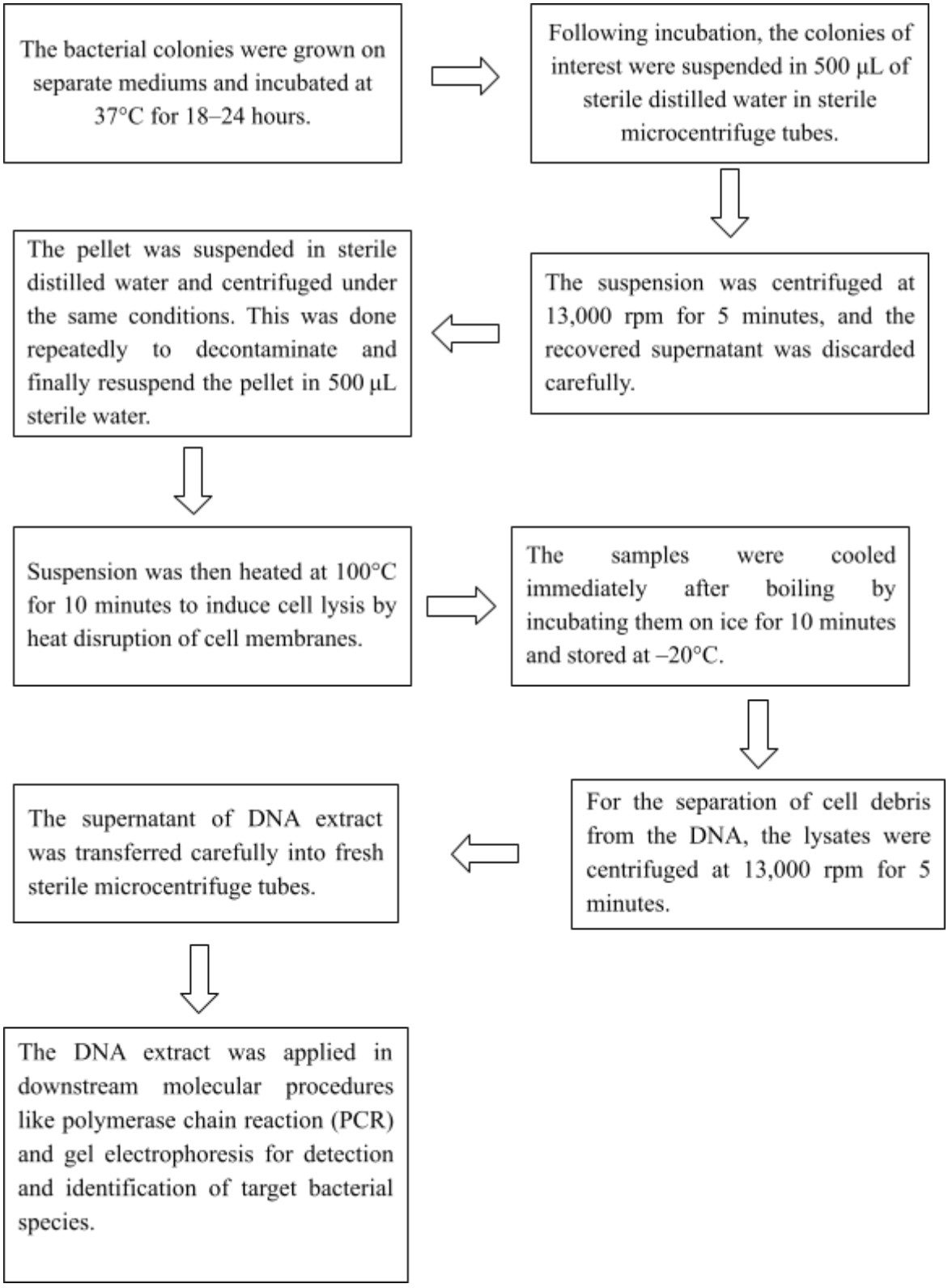
Table 2: List of antibiotics and their susceptibility and resistance level.

Antibiotic Group	**Antibiotic Name**	**Code**	**Disc Concentration (mcg)**	**Diameter of zone inhibition**		
				Susceptible (S) (mm)	**Intermediate (I) (mm)**	**Resistant (R) (mm)**
Penicillins	Ampicillin	AMP	2	≥17	14-16	≤13
Aminoglycosides	Gentamicin	CN	10	≥15	13-14	≤12
Aminoglycoside	Kanamycin	K	30	≥18	14-17	≤13
Oxazolidinones	Linezolid	LZD	30	≥23	21-22	≤20
Fluoroquinolones	Levofloxacin	LEV	5	≥17	14-16	≤13
Tetracyclines	Doxycycline	DO	30	≥16	13-15	≤12
Carbapenems	Imipenem	IPM	10	≥23	20-22	≤19

Monobactams	Aztreonam	ATM	30	≥22	16-21	≤15
Macrolides	Azithromycin	AZM	25	≥15	10-14	≤9
Lincosamides	Clindamycin	DA	2	≥21	15-20	≤14
Glycopeptides	Vancomycin	VA	30	≥17 (for gram positive like S.aureus)	N/A	N/A
Amphenicols	Chloramphenicol	C	30	≥18	13-17	≤12
Cephalosporins (4th gen)	Cefepime	FEP	30	≥26	21-25	≤20

2.12: Isolation of DNA through boiling method

Thermal lysis or boiling is a rapid and easy method of bacterial DNA extraction. It is a heat denaturation process that forces the bacterial cells to expel their genomic DNA into the liquid phase.


The bacterial colonies were grown on separate mediums and incubated at 37°C for 18–24 hours.
Following incubation, the colonies of interest were suspended in 500 µL of sterile distilled water in sterile microcentrifuge tubes.
The suspension was centrifuged at 13,000 rpm for 5 minutes, and the recovered supernatant was discarded carefully.
The pellet was suspended in sterile distilled water and centrifuged under the same conditions. This was done repeatedly to decontaminate and finally resuspend the pellet in 500 µL sterile water.
Suspension was then heated at 100°C for 10 minutes to induce cell lysis by heat disruption of cell membranes.
The samples were cooled immediately after boiling by incubating them on ice for 10 minutes and stored at –20°C.
For the separation of cell debris from the DNA, the lysates were centrifuged at 13,000 rpm for 5 minutes.
The supernatant of DNA extract was transferred carefully into fresh sterile microcentrifuge tubes.
The DNA extract was applied in downstream molecular procedures like polymerase chain reaction (PCR) and gel electrophoresis for detection and identification of target bacterial species.

2.13: PCR method

Individual fragments of DNA are very hard to study because vast quantities of DNA are needed for molecular and genetic research. Traditional polymerase chain reaction (PCR) or molecular photocopying refers to a technique of copying (amplifying) tiny amounts of RNA and DNA from a sample (Alberts et al., 2002). The PCR method has various laboratory and clinical uses. It is used in criminalistic laboratories to analyze DNA samples collected from crime scenes. Clinical laboratories also utilize it in diagnosing infected patients with the virus. Pharmaceutical laboratory facilities use PCR also to diagnose and copy DNA and RNA samples in the production of medicine and vaccines.

2.13.1: PCR procedure steps

- ➔ Initial Denaturation: It is required for heat-activating DNA polymerases, which are used by hot start PCR most commonly. The reaction mixture is heated for one to nine minutes between 94°C and 96°C. The enzyme is activated and the DNA is prepared for amplification.
- ➔ Denaturation: In this reaction, the reaction is incubated at an interval of 94°C–98°C for around 20 to 30 seconds. As the initial step of a cycle, this elevated temperature creates single-stranded DNA templates by breaking the hydrogen bonds between the complementary bases of double-stranded DNA.
- ➔ Annealing: To try to allow primers to adhere to their corresponding complementary sequences on the single-stranded DNA, the temperature is lowered to 50°C to 65°C for 20 to 40 seconds. Annealing temperature is generally set 3°C to 5°C less than the melting temperature (Tm) of the primers. This is a process in which ideally best temperature should be employed to provide specificity of primer binding; at low temperature, non-specific binding occurs, while at high temperature, binding can be completely prevented.
- ➔ Extension (Elongation): Extension is performed at the optimal temperature for the DNA polymerase being utilized. Taq polymerase is an example. It operates most efficiently at a temperature of around 72°C.By adding dNTPs in the 5' to 3' direction, the enzyme polymerizes a complementary new strand of DNA from the template strand at this temperature. The phosphodiester bonds form between the incoming dNTP's 5'-phosphate group and the 3'-hydroxyl of the chain of growing DNA through catalysis by the enzyme. The time for this step varies according to the size of the fragment and also according to the type of polymerase, with the standard polymerases being able to add about 1,000 bases per minute. In practice, this translates to doubling the target DNA sequence, which aids in reaching the exponential PCR amplification.

➔ Final Extension: At the end of all the cycles of amplification, final extension is carried out at 70°C–74°C for 5 to 15 minutes. This allows the full extension of those partially made DNA strands which remain from previous cycles.

➔ Final Hold: Following final extension, the reaction mixture is kept at 4°C to 15°C in order to facilitate short-term storage and preservation of the amplified DNA products. This avoids degradations until subsequent sample handling.

2.13.2: Components of PCR

Table 3: PCR components and their function.

Characteristics	Template DNA	Primers	DNA polymerase	Mg2+	Buffer	dNTPs
Function	Target sequence present	Hybridizes with template DNA	Builds new DNA strands	Act as cofactor	Maintains pH and ionic conditions	Building block for new strands
Length	N/A	18–25 nucleotides long	N/A	N/A	N/A	N/A
Requirement	High temperature to unzip	Hybridization with the DNA	Building new strands of DNA	Effective functioning	Best pH and ionic conditions	DNA's new strands

2.13.3: Equipments of PCR

➔ Thermal Cycler (PCR Machine): The denaturation, annealing, and extension phases of the PCR reaction need controlled and specific thermal conditions, which are offered by the thermal cycler, or PCR machine. Temperature control with high precision is key to successful DNA amplification. The term "cycler" being used implies repetitive cycling of temperature conditions, which typically equals the number of amplification cycles that is often based on the size of the target gene.

➔ PCR tubes: Because PCR is exposed to such radical fluctuations in temperature, PCR tubes are designed to take the extremes. Their shape reduces evaporation of the sample during heat cycling, which makes the reaction both more efficient and consistent in outcome overall.

➔ PCR reaction preparation:

Table 4: Reaction mixture of PCR and their amounts.

Component	Master mix	Nuclease free water	Forward primer	Reverse primer	Isolated DNA	Total
Volume per sample	10µl	2µl	2µl	2µl	5µl	21µl

2.13.4: Procedure of PCR machine run

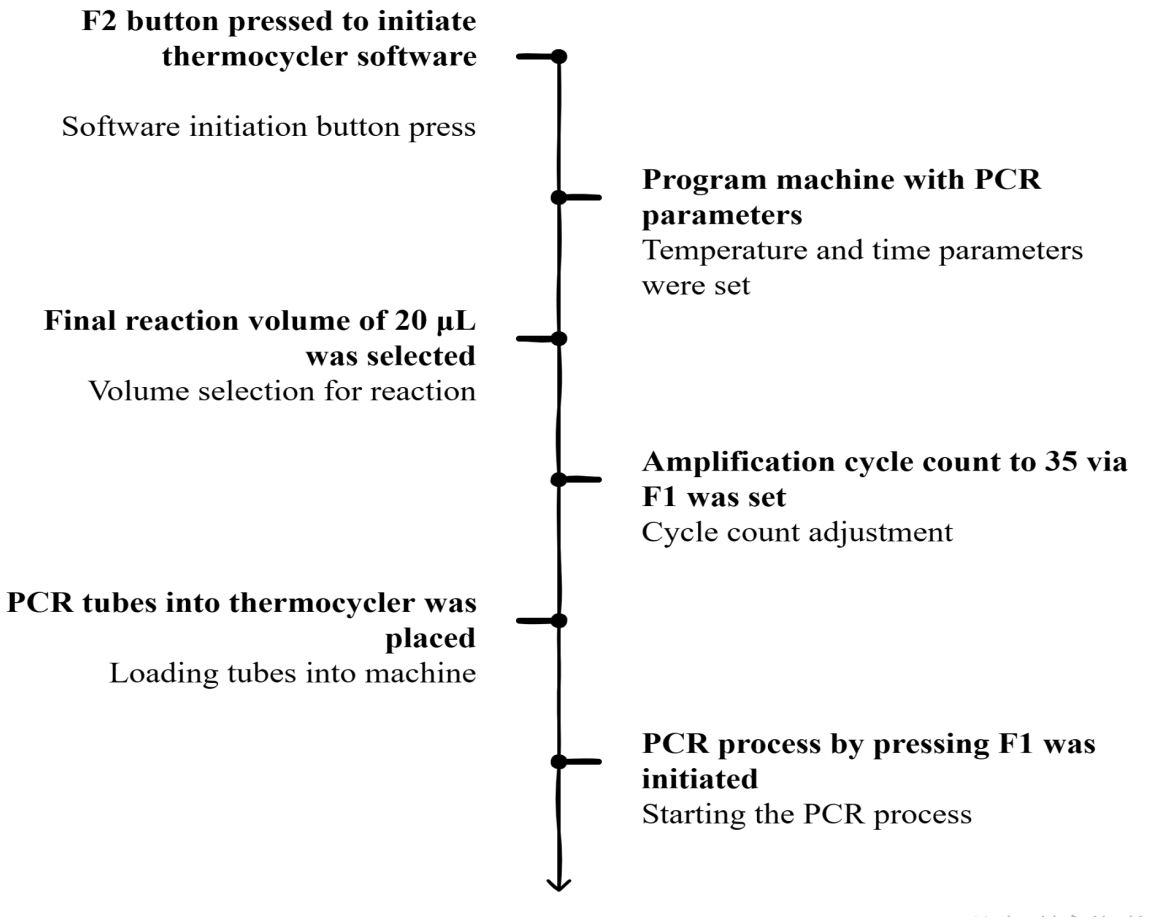
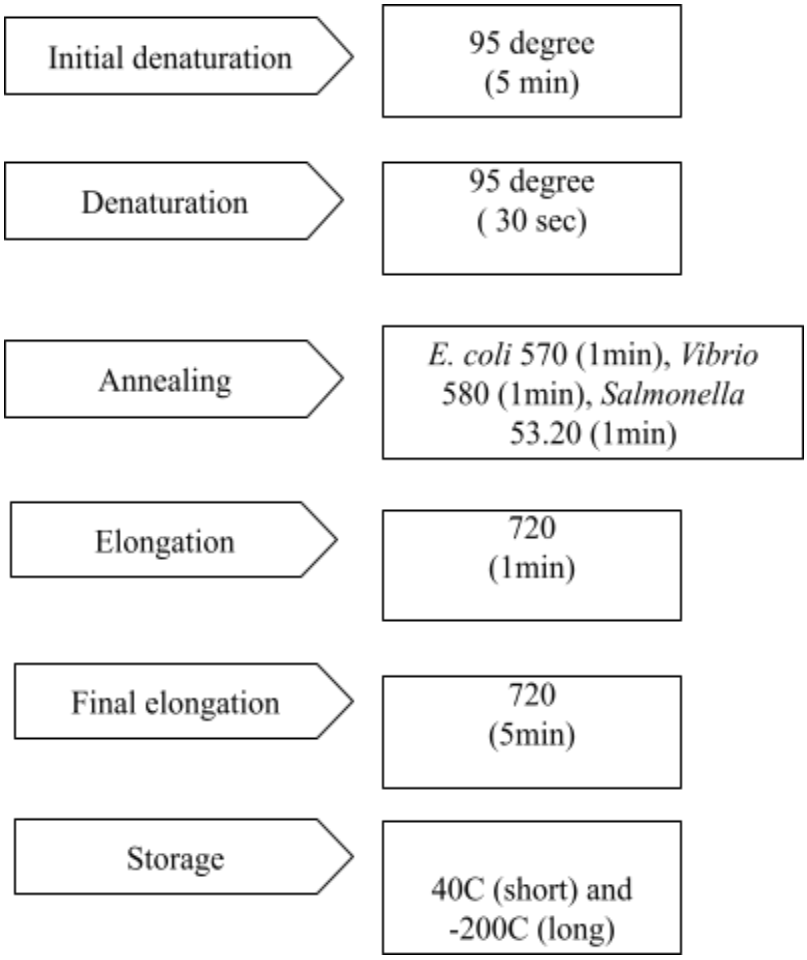


Fig 13: Steps of PCR machine run (Created using Napkin).

2.13.5 Thermal cycle



2.13.6: Condition of Primer

Table 5: Primer sequence of three different bacteria.

Primer	Primer sequence
E.coli forward	5'-GACCTCGGTTTAGTTCACAGA-3'
E.coli reverse	5'-CACACGCTGACGCTGACCA-3'
Salmonella forward	5'-GTGAATTATCGCCACGTTCGGGCAA-3'
Salmonella reverse	5'-TCATCGCACCGTCAAAGGAACC-3'
Vibrio forward	5'-ATAATGGCTCACCAAGAAGG-3'
Vibrio reverse	5'-TTAGAACTTATAACCACC-3'

2.14: Plasmid isolation

Plasmid isolation is a simple molecular biology method that utilizes the differences in the topology and structure of the plasmid and chromosomal DNA of bacteria (Libretexts, 2025). The bacterial chromosomal DNA is larger in size and often less supercoiled or relaxed. Plasmids, however, are small circular DNA molecules that are highly supercoiled. Upon separation, these differences are utilized. Some of the chemical and physical conditions used in plasmid isolation denature the chromosomal DNA and cause it to precipitate along with cellular proteins (Sahoo, 2024). Both plasmids and RNA are found in the soluble fraction because they are tightly coiled and supercoiled. By precipitating out the majority of the genomic DNA and cellular debris quite effectively, this selective precipitation can leave behind a purer isolated plasmid sample for downstream applications such as cloning, sequencing, or transformation.

2.14.1: Solutions needed for plasmid isolation

Table 6: Solutions for plasmid isolation.

Solution I	Solution II	Solution III
The main buffer used in this protocol, referred to as Solution I, consists of 50 mM Tris-HCl, 10 mM EDTA, and 50 mM glucose, with the pH adjusted to 8.	For lysis of cells and induction of denaturation of genomic and plasmid DNA and proteins, a solution of 0.2 M NaOH and 1% SDS is employed. Synergistic effect of the extremely alkaline condition induced by NaOH and detergent activity of SDS facilitates effective degradation of double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA).	Neutralization with a solution containing 3 M potassium acetate and glacial acetic acid to pH 5.2 is used to neutralize alkaline lysate. This leads to precipitating out of the chromosomal DNA and proteins and are incapable of renaturing properly due to large molecular sizes.

2.14.2: Plasmid Isolation Procedure

- ➔ The bacteria streak plate was incubated overnight, and a loopful of colonies of bacteria was transferred to an MCT containing 200 µl of Solution I. To the solution, 400 µl of freshly prepared Solution II was added and incubated at room temperature for 5 minutes.
- ➔ Then, ice-cold Solution III was added, and the contents were mixed by inverting the mixture gently and then incubated on ice for 10 minutes. The tubes were centrifuged for 15 minutes at 12,000 rpm. Following centrifugation, 700 µl of the supernatant was carefully transferred to a new tube, and the pellet was discarded.
- ➔ An equal amount (700 µl) of phenol:chloroform:isoamyl alcohol (PCI) was then added to each tube, and there was a continuous inversion for 3 to 5 minutes. The solution was centrifuged at 12,000 rpm for 2 minutes. A volume of 250–500 µl of the resulting supernatant was transferred into new tubes and 625 µl of 100% ethanol added. The tubes were mixed gently.
- ➔ The samples were placed at –20°C for maximum precipitation of the DNA and then centrifuged at 12,000 rpm for 2 minutes. The ethanol was discarded, and the tubes were left to air-dry for 5 minutes.
- ➔ Finally, 10–15 µl of TE buffer was added to all the tubes to resuspend the DNA, and the samples were stored at –20 °C for further use.

2.15: Agarose Gel Electrophoretic Analysis of DNA and Plasmid Constructs

Agarose gel electrophoresis was used to identify the resultant fragments and confirm the integrity of the genomic and plasmid DNA after plasmid extraction (Lee et al., 2012). A suitable concentration agarose gel was prepared, each DNA sample was mixed with loading dye and carefully dispensed into wells, the gel was subjected to an electric field to allow for the size-based separation of nucleic acid fragments, the gel was stained with an intercalating dye (such as SYBR Safe or ethidium bromide), and the illuminated bands were then examined under UV or blue-light transillumination to assess yield and integrity.

2.15.1: Formulation of gel in agarose gel electrophoresis

Fig 14: Gel formation for agarose gel electrophoresis (Created using Napkin).

2.15.2: Gel electrophoresis

After PCR amplification, agarose gel electrophoresis was carried out to determine the presence of DNA bands. The following was the procedure carried out:

Table 7: Steps of agarose gel electrophoresis for genomic DNA and plasmid DNA

Gel electrophoresis process for genomic DNA	Gel electrophoresis process for genomic plasmid
➔ An agarose gel was poured according to the established protocol, with the comb being inserted before gel polymerization and removed upon complete solidification. ➔ The gel tray was inserted into the electrophoresis chamber and immersed in a TBE running buffer to completely cover the wells. ➔ Ten microliters of each PCR product were loaded slowly into the separate wells. ➔ Electrophoresis was carried out at 80 V for 45 minutes. ➔ DNA bands were visible under short-wave ultraviolet light.	➔ The gel tray was set into the electrophoresis chamber and loaded with a running buffer to the highest fill line. ➔ Each plasmid prep was mixed by adding 6 µl of DNA solution and 2 µl of loading dye, and 8 µl of resultant mixture was loaded into separate wells. ➔ The water samples were also prepared and charged. The electrophoresis was run for 50 minutes at 120 V. ➔ The plasmid bands were then viewed under short-wave UV light.

Chapter: 3

Result

3.1: Labeling the isolated colonies:

The water samples were collected from two sources: one from Hatirjheel lake, and the other one from Gulshan lake; both situated at the Dhaka city. We collected samples twice a month from each spot for 6 months. Therefore, samples were collected 10 times and 20 samples in total throughout the course of six months. According to the samples, the isolated bacterial colony stocks were named as:

- For Hatirjheel, Hati was written in short.
- For Gulshan lake, Gul was written.

➢ The samples from the first spot of the source were named as 'Hati_S1'

➢ The samples from the second spot of the source were named as 'Gul_S1'

For each month there were two samples, so the Second samples were S2. The months were labeled separately. From April to October, samples of 6 months were taken. Due to political unrest in the country, samples of July were not able to be collected.

3.2: Conformation checked of the PCR positive result of bacterial genomic DNA via Agarose Gel-electrophoresis

All the gel show good resolution, allowing for differentiation between DNA fragments of different molecular weights. However, some faint or smeared bands indicate potential issues, such as degraded DNA, improper loading, or gel running conditions that need optimization.

3.2.1: Gel Electrophoresis result of *E.coli*

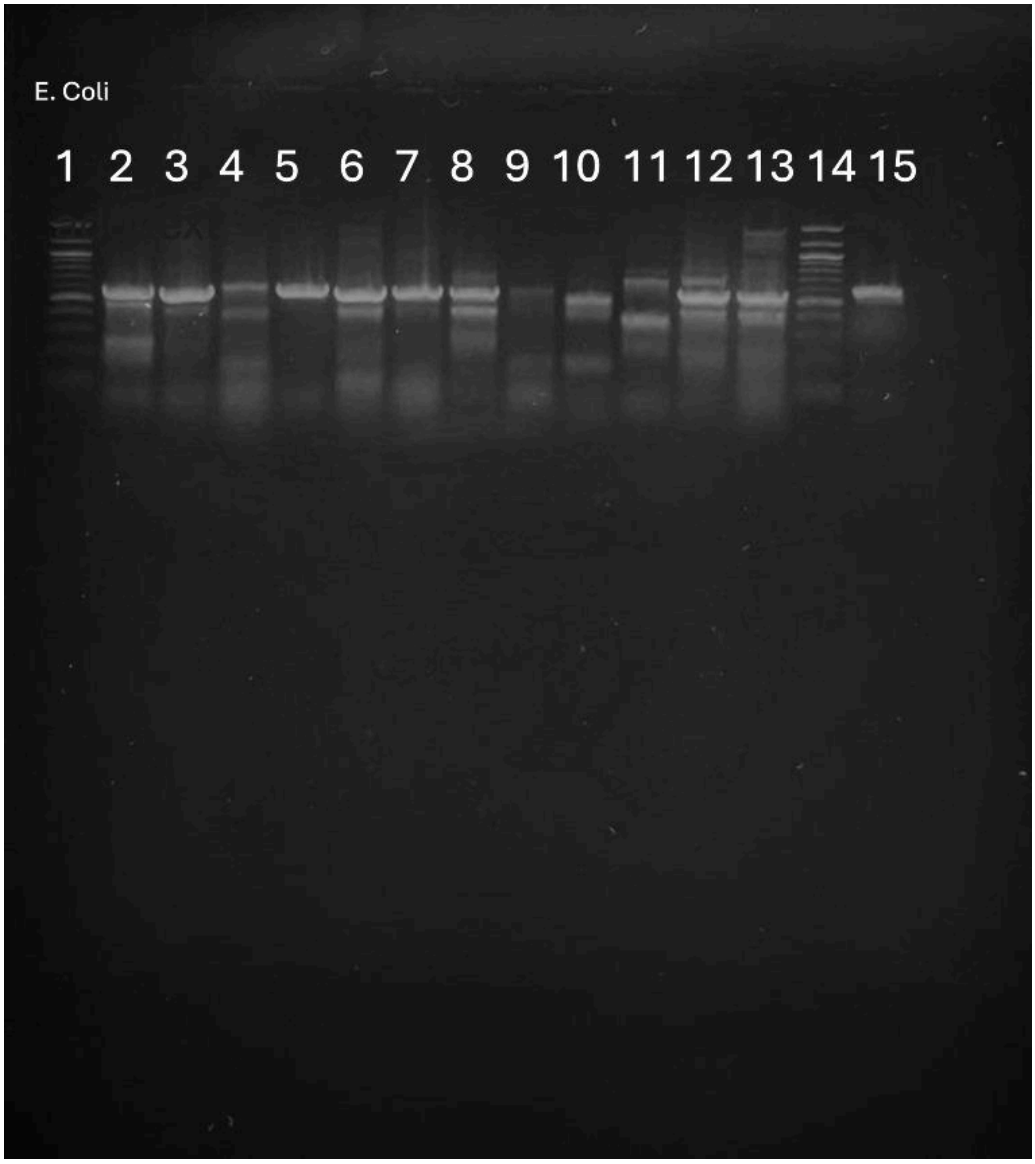


Fig 15: PCR result (via Agarose gel electrophoresis method) of genomic DNA of the *E. coli* isolated colonies.

The *E. coli* genomic DNA amplification appears on the gel electrophoresis photo, a 100 bp DNA ladder. The wells 1 and 14 contain the ladders which are indications of size markers. DNA ladder bands are clearly visible and used as a crisp marker to help identify the fragment size. The target DNA band of *E. coli* would be approximately 500-600 base pairs. In the gel, strong and intense bands at 500–600 bp in wells 2, 3, 5, 6, 7, 8, 10, 12, 13 and 15 indicate positive detection of *E. coli* genomic DNA in those samples. Wells 4, 9 and 11 contain weaker or negative amplification results as they lack any band or contain very weak bands. Generally, the outcome confirms the

observation that 10 out of the 13 test samples had strong positive tests for *E. coli,* confirming that the target DNA fragment was indeed of the predicted size.

3.2.2: Gel Electrophoresis result of *Salmonella spp.*

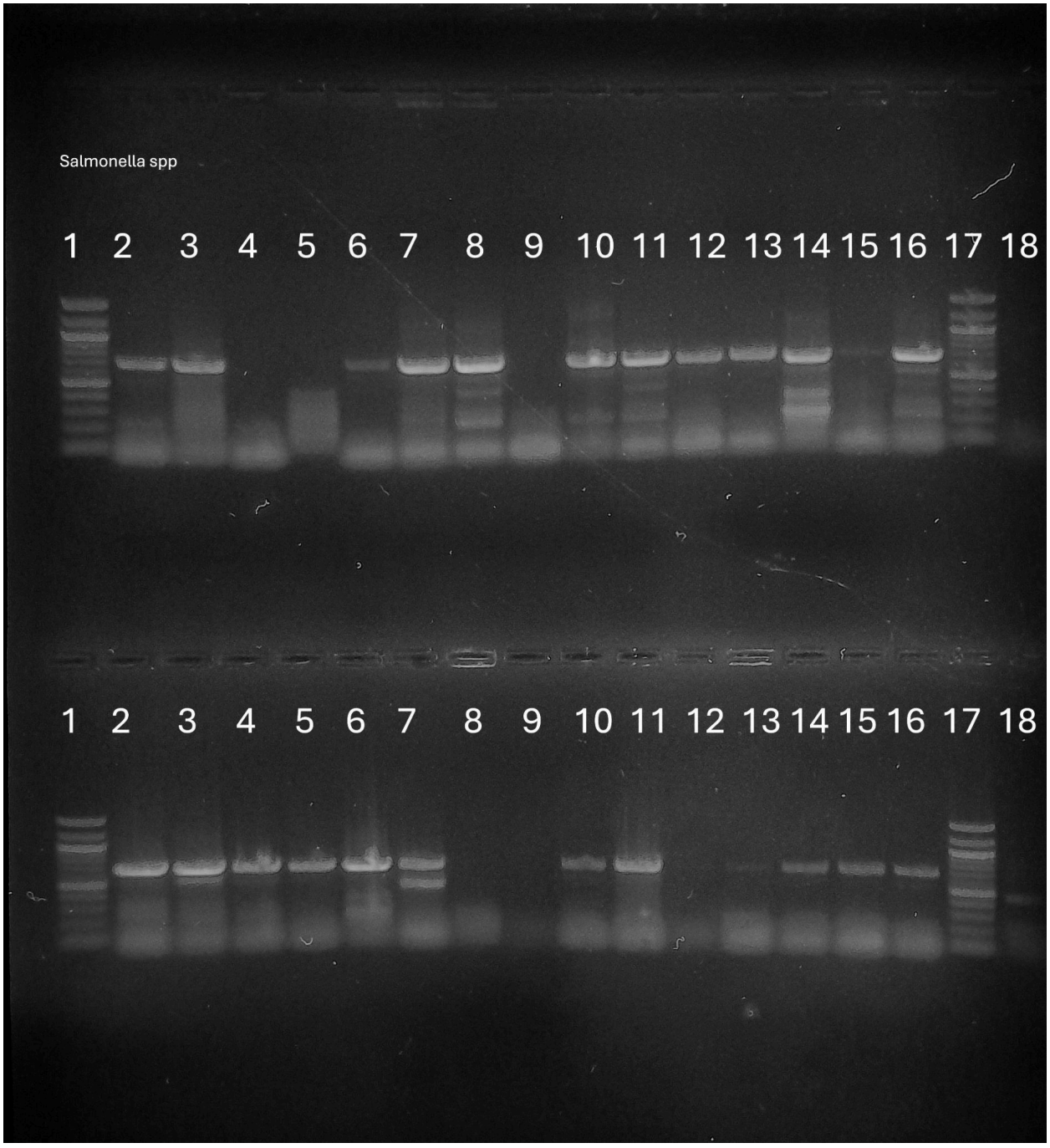


Fig 16: PCR result (via Agarose gel electrophoresis method) of genomic DNA of the *Salmonella spp.* isolated colonies.

Salmonella spp. Genomic DNA amplification appears on the gel electrophoresis photo, a 100 bp DNA ladder. The wells 1 and 17, both on the well sets above and below are the ladders used as size markers. DNA ladder bands are clearly visible and used as a crisp marker to help identify the fragment size. The target DNA bands would be approximately 200-300 base pairs. In the gel, strong and intense bands shown in wells 2, 3, 6, 7, 8, 10, 11, 12, 13, 14, 16 in the top and 2, 3, 4, 5, 6, 7, 10, 11, 14, 15 and 16 indicate positive detection of *Salmonella* genomic DNA in those samples. Wells 4, 5, 9, 15 and 18 above and 8, 9, 12, 13 and 18 below contain weaker or negative amplification results as they lack any band or contain very weak bands. Generally, the outcome confirms the observation that 22 out of the 30 test samples had strong positive tests for *Salmonella.*

3.2.3: Gel Electrophoresis result of *Vibrio cholerae*

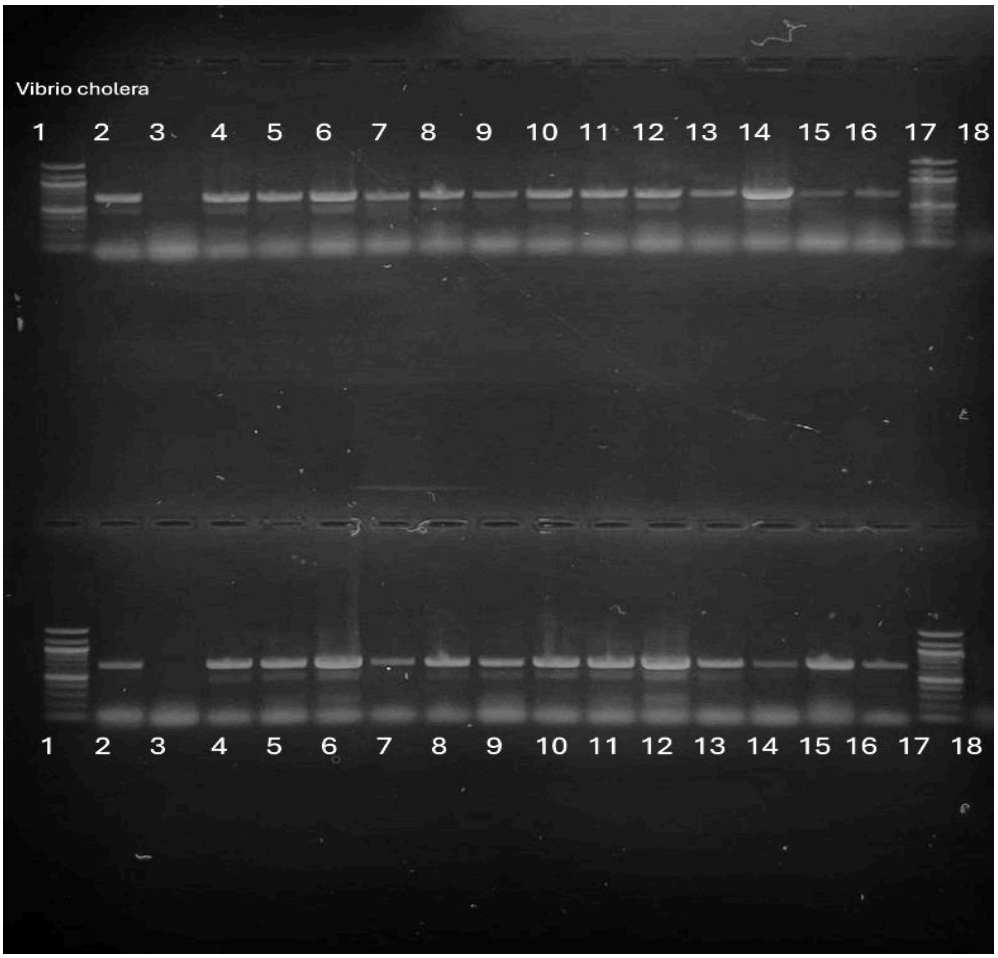


Fig 17: PCR result (via Agarose gel electrophoresis method) of genomic DNA of the *Vibrio cholerae* isolated colonies.

The *Vibrio cholerae* Genomic DNA amplification appears on the gel electrophoresis photo, a 100 bp DNA ladder as size marker. The ladders are 1 and 17 on both of the well sets. DNA ladder bands are clearly visible and used as a crisp marker to help identify the fragment size. The target DNA bands would be approximately 200-300 base pairs. In the gel, strong and intense bands shown in wells 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 on the well above and 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 indicate positive detection of *Vibrio* genomic DNA in those samples. Wells 3 on top and 3 at the bottom contain negative amplification results as they lack any band or contain very weak bands. Generally, the outcome confirms the observation that 28 out of the 30 test samples had strong positive tests for *Vibrio.*

3.3: Antibiotic Susceptibility Results and Analysis:

E. coli

Table 8: Antibiotic Susceptibility Result of *E.coli* April

Antibiotics	G4	H2	G3	G6	G5	G2	H10	H1
AMP10	0	0	10	15	15	0	20	0
CN10	17	20	20	20	20	20	25	20
K30	15	20	20	24	20	25	28	20
LZD30	0	0	0	0	5	0	0	0
LEV5	10	25	28	30	30	35	4	30
DO30	0	0	30	15	15	15	15	15
IMP10	35	30	14	0	0	35	30	30
ATM30	33	20	30	35	20	35	40	30
E15	0	0	0	0	0	0	10	0
AZM15	0	20	20	10	15	25	20	20
DA2	0	0	0	0	0	0	0	0
VA30	0	0	0	0	0	0	8	0
C30	0	30	28	30	30	27	35	30
FEP30	35	20	30	35	35	34	40	33

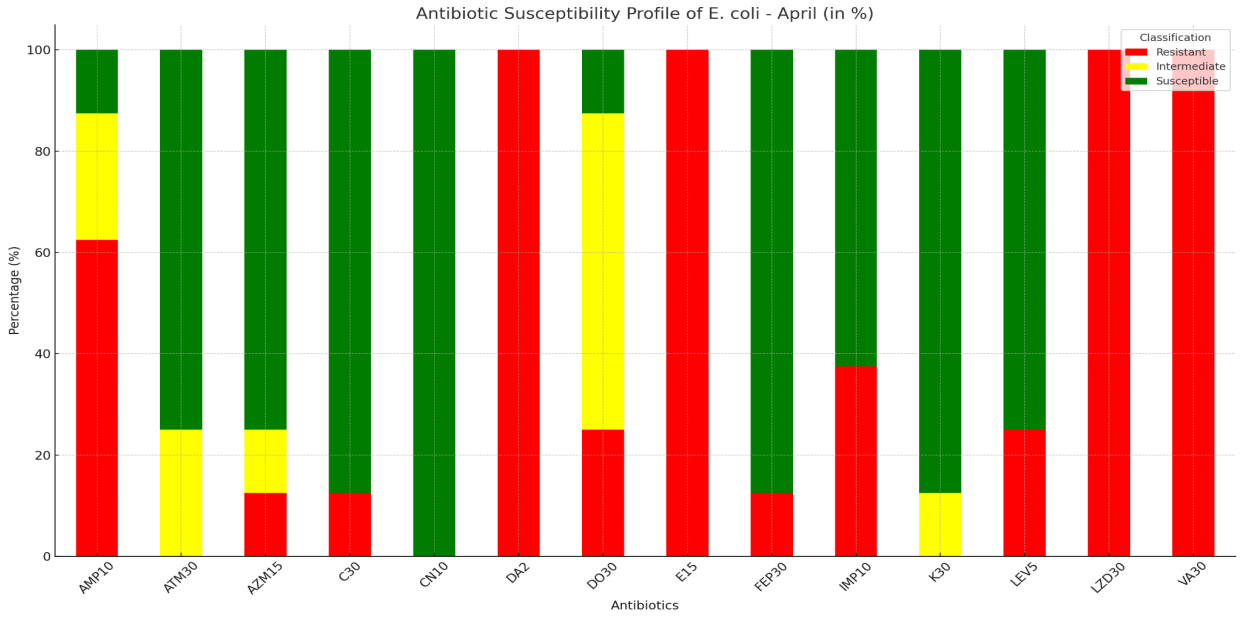


Fig 18: Antibiotic Susceptibility Profile of *E.coli* - April (%).

Table 9: Antibiotic Susceptibility Result of *E.coli* May

Antibiotics	H2	G2	G5	G8	G6	H8	H1	G4	H6	G1
AMP10	0	0	0	15	0	12	0	16	0	10
CN10	20	20	20	17	20	17	17	20	22	20
K30	20	20	20	20	22	20	20	22	20	23
LZD30	0	0	0	0	0	0	10	15	0	0
LEV5	25	30	30	35	25	25	20	35	35	25
DO30	10	15	15	15	9	0	17	18	15	13
IMP10	30	3.5	30	30	35	30	30	35	30	30
ATM30	20	20	30	30	0	30	20	30	25	35
E15	0	16	0	0	0	0	20	0	10	0
AZM15	0	20	23	20	20	0	22	15	20	20
DA2	30	0	0	0	0	0	0	0	0	0
VA30	0	10	0	0	0	0	0	0	0	0
C30	30	30	25	25	25	25	15	28	25	25
FEP30	35	40	34	34	13	30	30	35	40	35

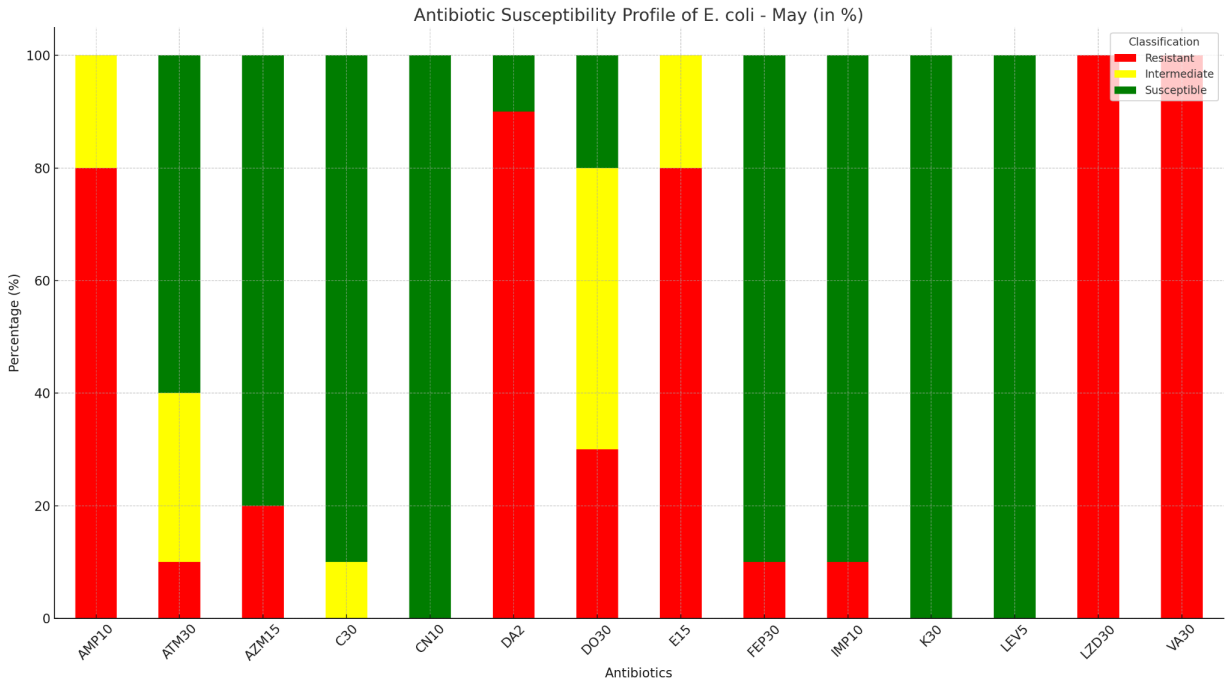


Fig 19: Antibiotic Susceptibility Profile of *E.coli* - May (%).

Table 10: Antibiotic Susceptibility Result of *E.coli* June

Antibiotics	G2	G1	G6	G5	G4	H3	H6	H4	G3
AMP10	0	0	0	20	0	0	30	0	0
CN10	20	19	20	20	20	0	20	20	20
K30	25	9	7	20	24	15	20	20	20
LZD30	0	0	0	0	0	0	0	0	0
LEV5	35	25	23	30	30	18	35	25	33
DO30	15	14	15	15	15	8	13	13	10
IMP10	20	22	20	23	30	30	25	26	30
ATM30	25	20	25	35	30	5	30	30	30
E15	0	0	0	0	0	0	0	0	0
AZM15	21	0	0	23	20	18	20	25	15
DA2	0	0	0	0	0	0	0	0	0
VA30	0	0	0	0	0	0	0	0	0
C30	29	26	0	20	26	25	25	25	20
FEP30	35	17	18	35	30	17	35	35	35

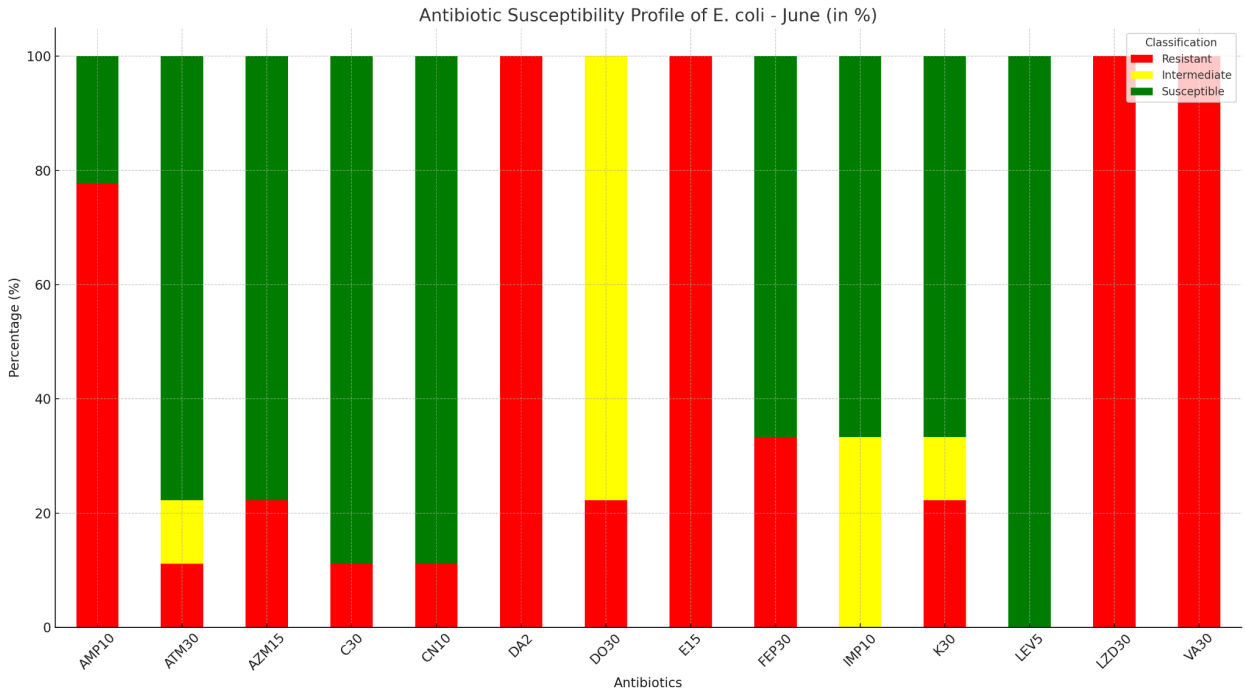


Fig 20: Antibiotic Susceptibility Profile of *E.coli* - June (%).

Table 11: Antibiotic Susceptibility Result of *E.coli* August

Antibiotics	G1	H10	H4	H7	H2
AMP10	0	0	0	20	0
CN10	20	15	20	20	18
K30	20	25	20	15	17
LZD30	0	0	0	0	0
LEV5	25	30	25	30	30
DO30	15	10	15	15	14
IMP10	26	25	30	31	30
ATM30	30	35	25	30	30
E15	0	0	0	0	0
AZM15	25	0	0	0	12
DA2	0	0	0	0	0
VA30	0	0	0	0	0
C30	28	30	25	25	20
FEP30	35	35	30	16	30

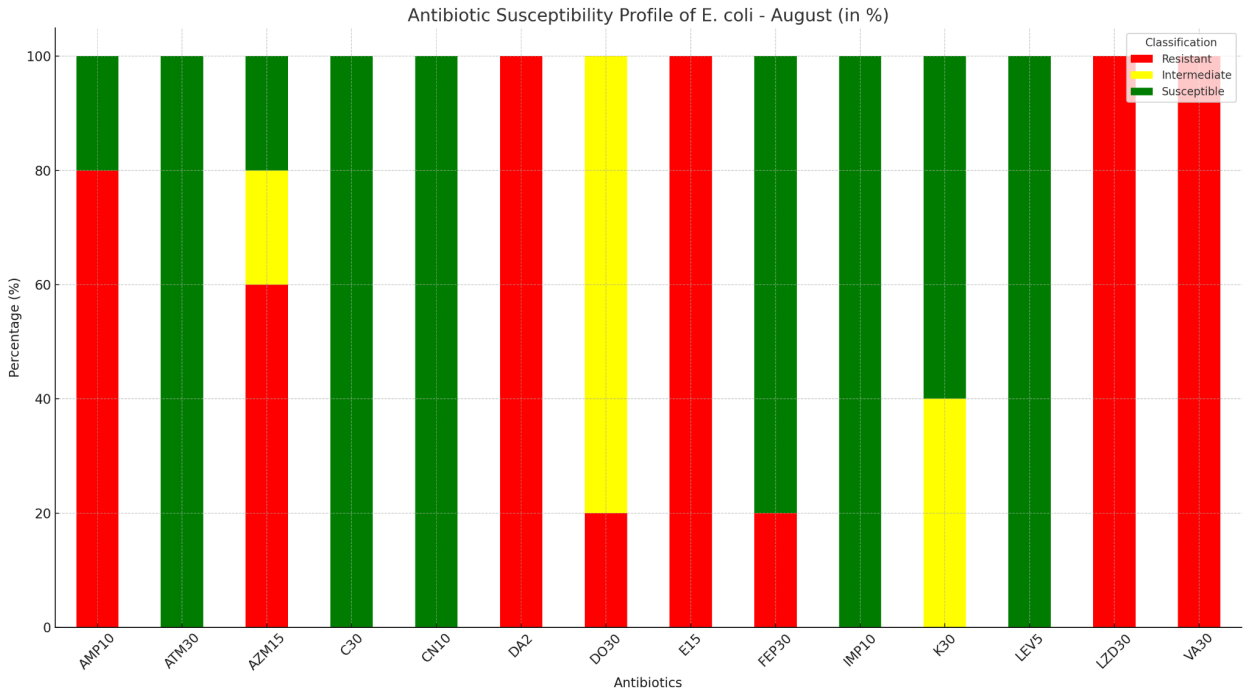


Fig 21: Antibiotic Susceptibility Profile of *E.coli* - August (%).

Table 12: Antibiotic Susceptibility Result of *E.coli* September

Antibiotics	H5	H8	G5
AMP10	0	0	15
CN10	20	20	20
K30	15	15	18
LZD30	0	0	0
LEV5	28	25	25
DO30	15	15	15
IMP10	30	30	25
ATM30	30	28	25
E15	0	0	0
AZM15	0	15	12
DA2	0	0	0
VA30	0	0	0
C30	20	25	25

Antibiotics	H5	H8	G5
AMP10	0	0	15
FEP30	30	31	30

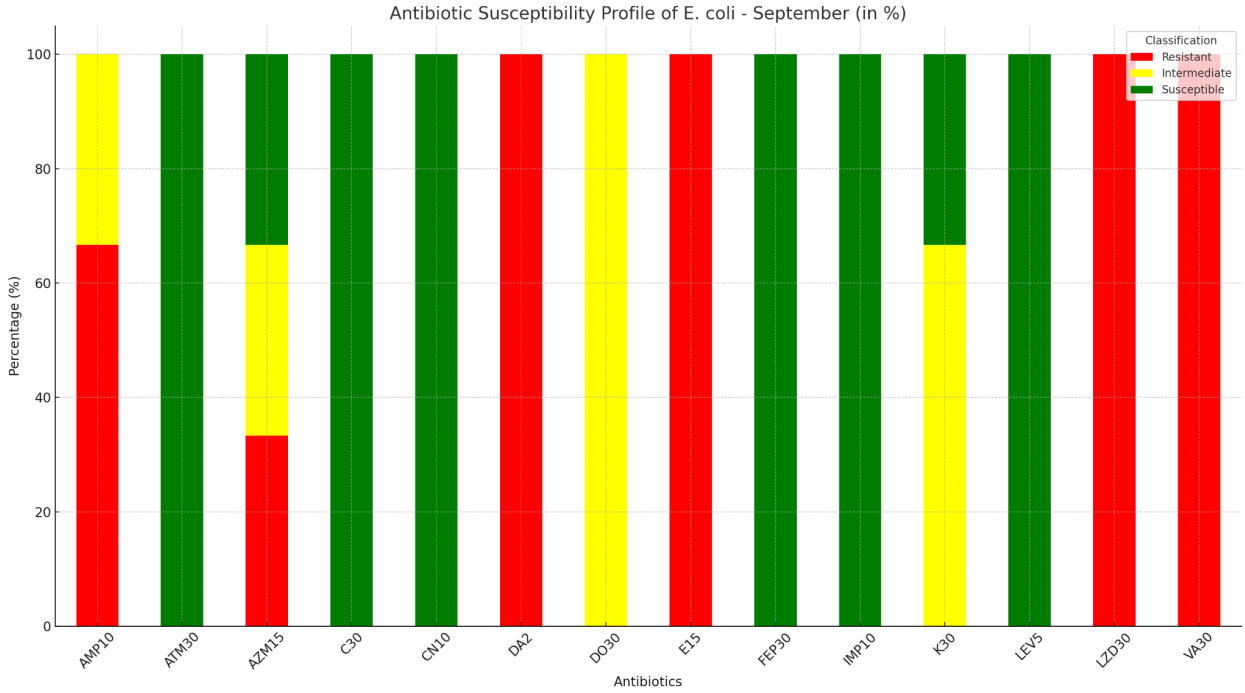


Fig 22: Antibiotic Susceptibility Profile of *E.coli* - September (%).

Table 13: Antibiotic Susceptibility Result of *E.coli* October

Antibiotics	H1	G2	H9	G1
AMP10	0	10	0	0
CN10	20	20	15	20
K30	15	17	15	18
LZD30	0	0	0	0
LEV5	25	27	25	28
DO30	15	15	15	15
IMP10	30	30	25	30
ATM30	30	28	25	30
E15	0	0	0	0
AZM15	15	10	12	15

DA2	0	0	0	0
VA30	0	0	0	0
C30	25	25	20	25
FEP30	30	30	30	30

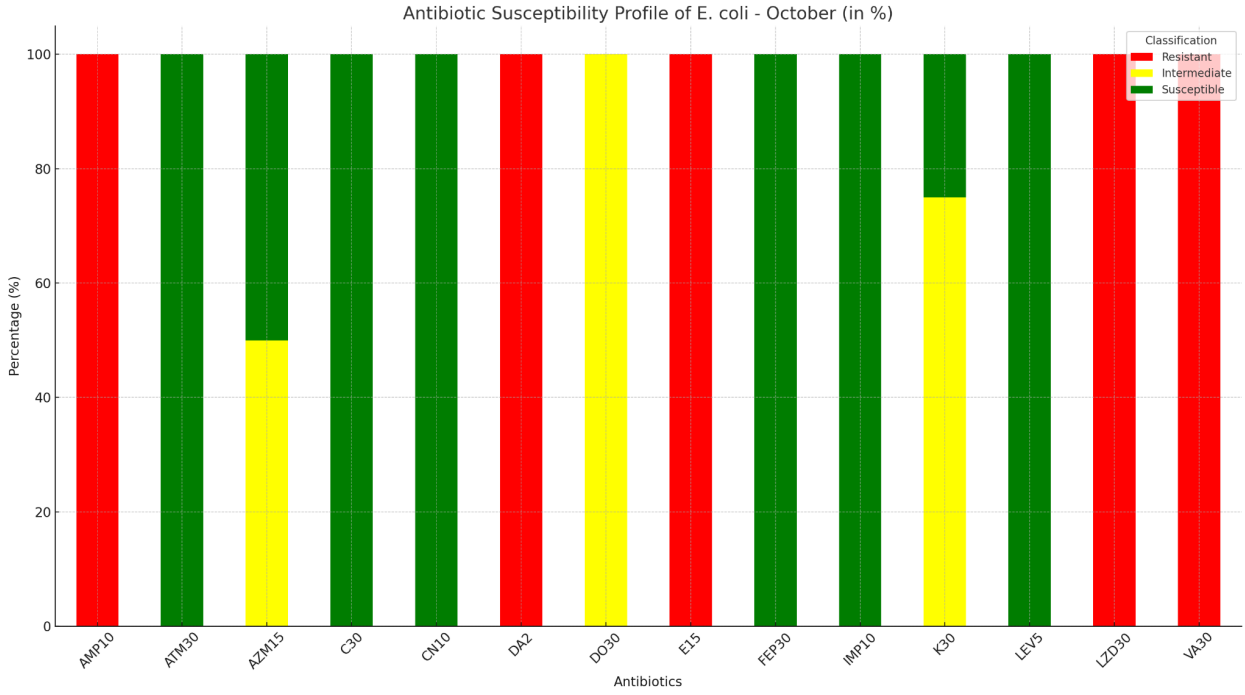


Fig 23: Antibiotic Susceptibility Profile of *E.coli* - October (%).

General Trend of *Escherichia coli:*

- Moderate to high susceptibility to Carbapenems (IMP) and the Cephalosporins (FEP).
- Very high resistance to Ampicillin (AMP) — about 100% resistance through all months (April – October).
- Gentamicin (CN) at least was fairly good--moderate susceptibility levels maintained over several months, but started to decline slightly after June.
- Doxycycline (DO) showed low performance with very high %R starting in April.
- Levofloxacin (LEV) had an excellent susceptibility in the April–June period but began to decrease slightly in the August–October period.

Observations:

- AZM susceptibility was quite variable — some months were very susceptible, others fewer so, and all more resistant than macrolides typically are.

- C30 (chloramphenicol) remained reasonably active during the months (mainly 60–80% sensitive).
- Vancomycin (VA) and Linezolid (LZD) have no clinical utility for E. coli (nearly 0% activity).

Salmonella spp.

Table 14: Antibiotic Susceptibility Result of *Salmonella spp.* May

Antibiotics	H4	G10	H5
AMP10	0	0	0
CN10	20	20	20
K30	22	20	21
LZD30	0	0	0
LEV5	30	34	29
DO30	10	13	15
IMP10	30	24	25
ATM30	30	32	35
E15	10	10	13

AZM15	20	20	18
DA2	0	0	0
VA30	0	0	0
C30	25	25	23
FEP30	30	30	30

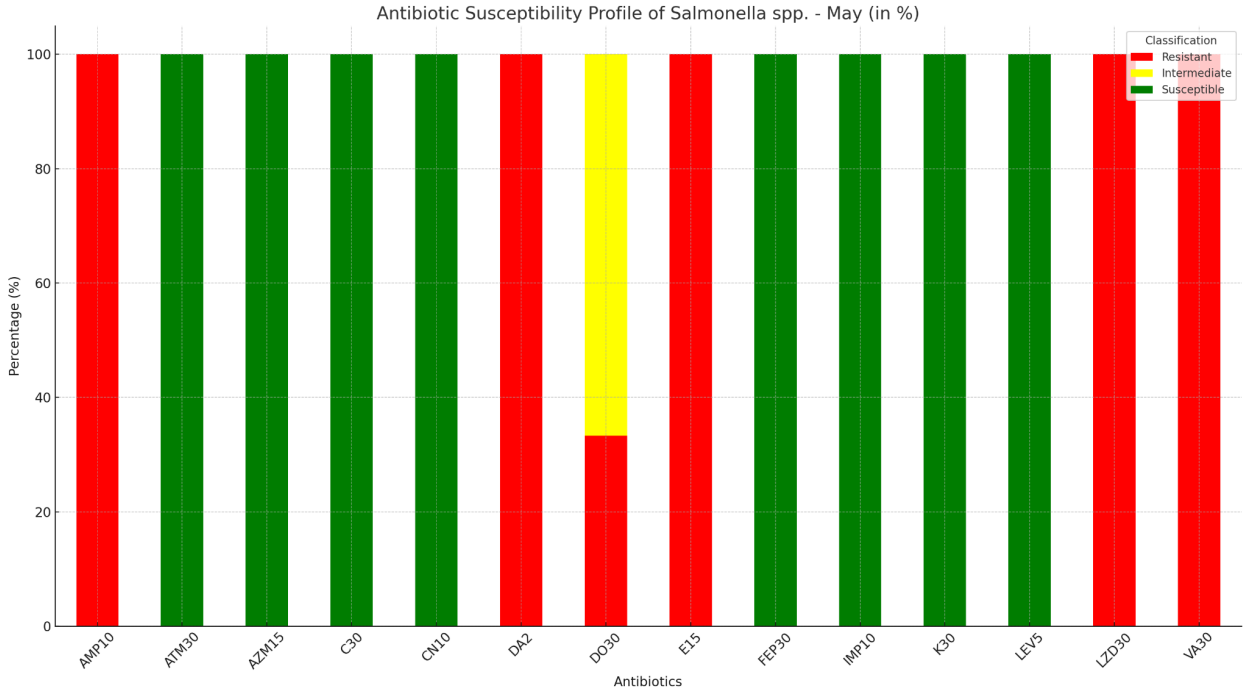


Fig 24: Antibiotic Susceptibility Profile of *Salmonella spp.* - May (%).

Table 15: Antibiotic Susceptibility Result of *Salmonella spp*. June

Antibiotics	G3	G10	H11	H2
AMP10	0	0	0	0
CN10	22	20	20	20
K30	20	20	24	20
LZD30	0	0	0	0
LEV5	30	29	33	30
DO30	15	16	15	14
IMP10	24	14	35	30
ATM30	35	14	35	30
E15	13	10	10	0
AZM15	20	28	21	20

DA2	0	0	0	0
VA30	0	0	0	0
C30	23	28	27	25
FEP30	30	30	32	32

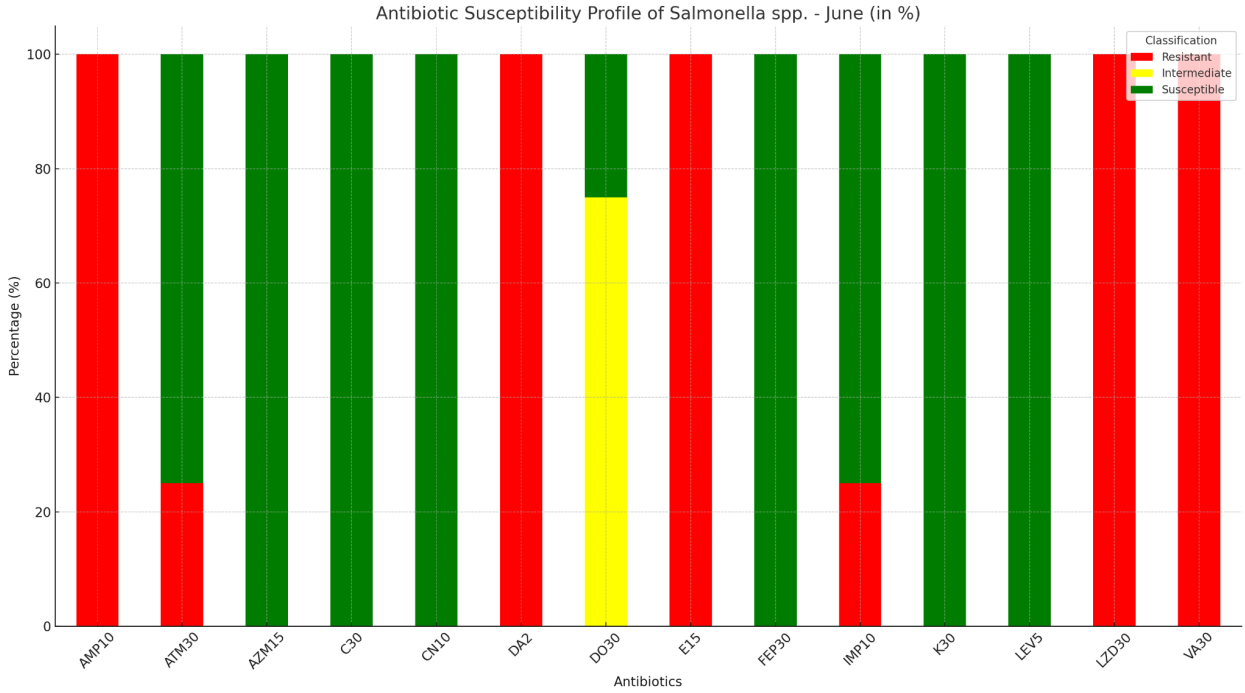


Fig 25: Antibiotic Susceptibility Profile of *Salmonella spp.* - June (%).

Table 16: Antibiotic Susceptibility Result of *Salmonella spp* August

Antibiotics	G2	H8	G4	G7
AMP10	0	0	0	0
CN10	20	20	20	20
K30	22	22	20	20
LZD30	0	0	0	0
LEV5	35	28	35	82
DO30	11	15	28	10
IMP10	24	26	30	25
ATM30	29	32	35	32
E15	9	10	10	12

AZM15	20	19	20	19
DA2	0	0	0	0
VA30	0	0	0	0
C30	20	25	25	23
FEP30	30	30	30	30

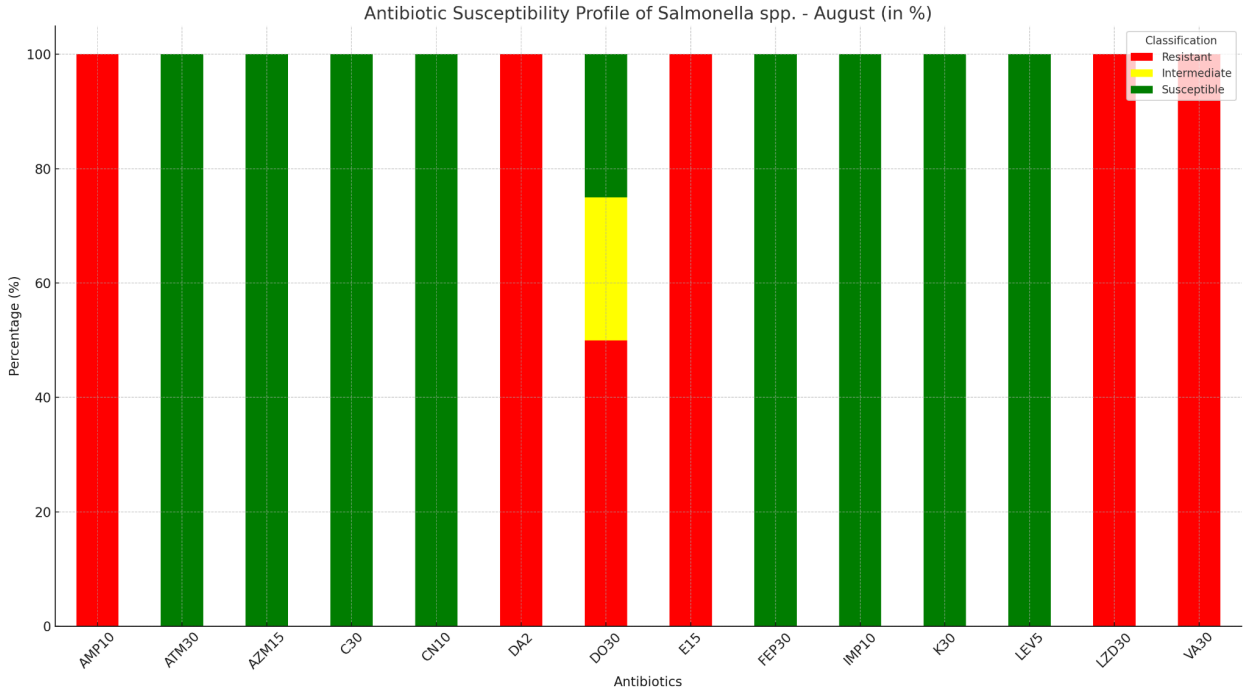


Fig 26: Antibiotic Susceptibility Profile of *Salmonella spp*. - August (%).

Table 17: Antibiotic Susceptibility Result of *Salmonella spp* September

Antibiotics	H5	G5.2	G5.1	H3	G6.1	G8.1	H10	H1	H9	**G7.1**
AMP10	15	0	0	0	0	0	0	0	0	13
CN10	20	20	21	21	25	20	20	20	20	25
K30	20	23	22	22	23	20	20	24	20	20
LZD30	0	0	0	0	0	0	0	0	0	10
LEV5	34	29	30	31	28	31	29	33	30	12
DO30	15	13	15	15	13	13	16	15	14	10
IMP10	27	27	30	33	29	30	14	35	30	29

ATM30	30	30	30	33	29	30	14	35	30	28
E15	9	10	0	10	0	0	10	10	0	0
AZM15	23	15	30	23	28	13	28	21	20	17
DA2	0	0	0	0	0	0	0	0	0	0
VA30	0	0	0	0	0	0	0	0	0	0
C30	25	23	30	26	25	25	28	27	25	30
FEP30	30	33	33	32	31	29	30	32	32	30

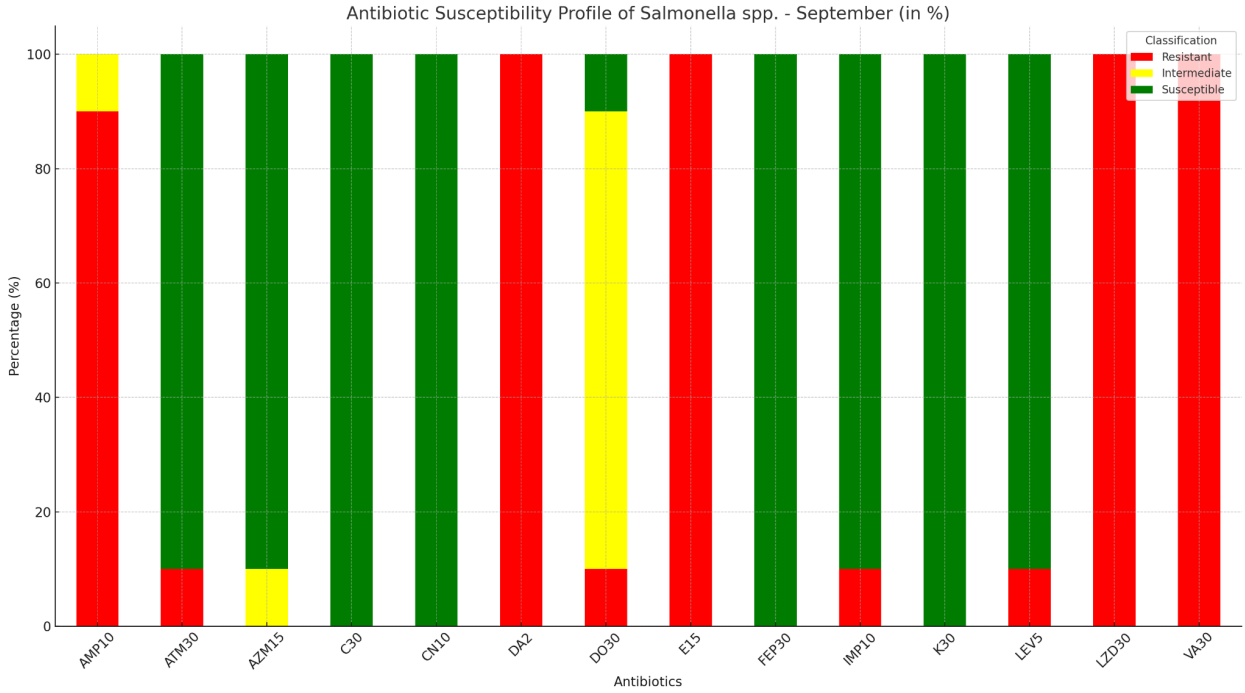


Fig 27: Antibiotic Susceptibility Profile of *Salmonella spp*. - September (%).

Table 18: Antibiotic Susceptibility Result of *Salmonella spp* October

Antibiotics	H4	G3	H2	G6	G4
AMP10	0	15	0	0	0
CN10	20	22	21	25	20
K30	24	23	22	23	20
LZD30	0	0	0	0	0
LEV5	32	34	31	28	31
DO30	15	12	15	13	13
IMP10	29	29	33	29	30

ATM30	35	18	33	29	30
E15	10	10	10	0	0
AZM15	23	18	23	28	13
DA2	0	0	0	0	0
VA30	0	0	0	0	0
C30	30	27	26	25	25
FEP30	30	32	32	31	29

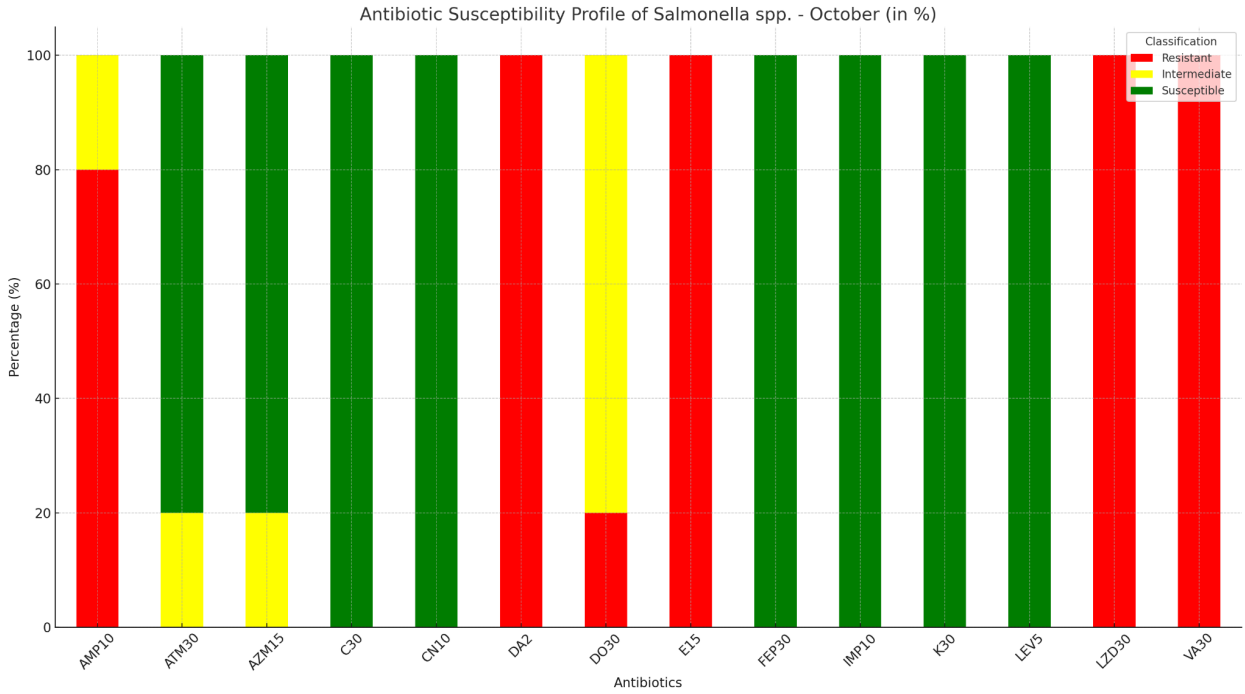


Fig 28: Antibiotic Susceptibility Profile of *Salmonella spp.* - October (%).

General Trend of *Salmonella spp.*

- High susceptibility rate was recorded for Imipenem (IPM), Cefepime (FEP), Aztreonam (ATM) in all months (at least 100% susceptible).
- To a very good resistance against Ampicillin (AMP) always (nearly 100% resistant all months).
- Mixed findings were observed for Gentamicin (CN) and Kanamycin (K), which exhibited moderate susceptibility, despite still having a proportion of resistant strains.
- From May to September, DO exhibited an alarming rise in resistance.
- Levofloxacin (LEV) showed a maintained high susceptibility though the emergence of resistant strains was occasionally observed.

Observations:

- Vancomycin (VA) and Linezolid (LZD) are generally not effective, 0% both in many strains.
- Azithromycin (AZM) performance was variable: moderate susceptibility but some variability on a monthly basis.

Vibrio Cholera

Table 19: Antibiotic Susceptibility Result of *Vibrio Cholerae* April

Antibiotics	H8	G5	G3
AMP10	0	0	20
CN10	25	25	20
K30	22	22	20
LZD30	0	0	0
LEV5	25	25	23
DO30	18	15	15
IMP10	25	20	20
ATM30	16	15	15
E15	20	20	21
AZM15	20	20	20
DA2	0	0	0
VA30	15	18	15
C30	25	25	20
FEP30	0	0	0

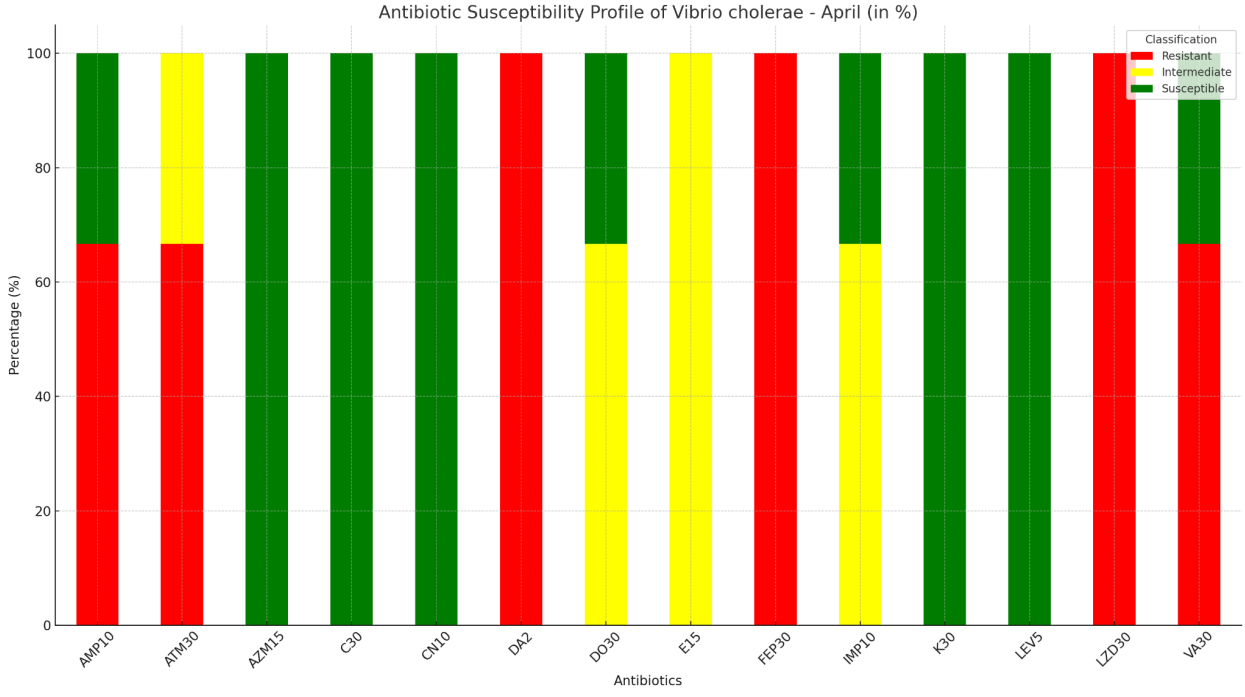


Fig 29: Antibiotic Susceptibility Profile of *Vibrio cholerae* - April (%).

Table 20: Antibiotic Susceptibility Result of *Vibrio Cholerae* May

Antibiotics	G4	H3	G1
AMP10	15	20	20
CN10	25	20	20
K30	20	20	20
LZD30	0	0	0
LEV5	25	23	22
DO30	15	15	15
IMP10	20	20	20
ATM30	15	15	16
E15	20	20	20
AZM15	20	20	20
DA2	0	0	0
VA30	15	15	15
C30	25	22	25
FEP30	0	0	0

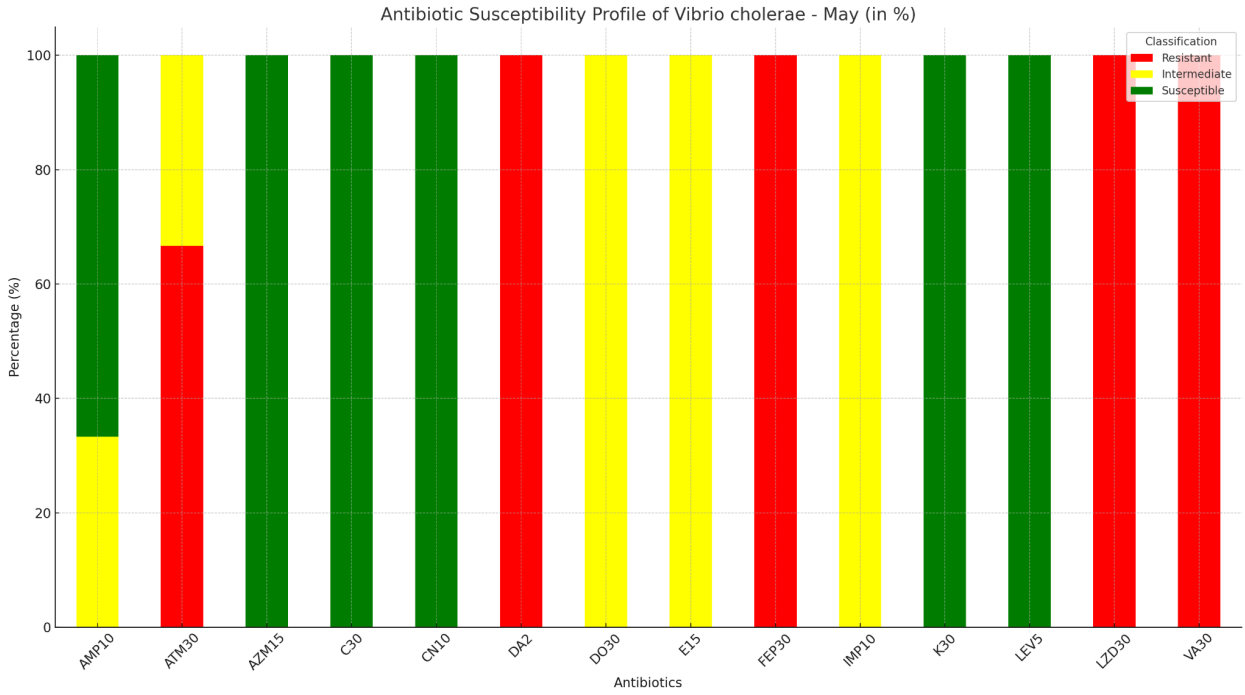


Fig 30: Antibiotic Susceptibility Profile of *Vibrio cholerae* - May (%).

Table 21: Antibiotic Susceptibility Result of *Vibrio Cholerae* August

Antibiotics	H8	G3
AMP10	25	20
CN10	20	20
K30	22	20
LZD30	34	0
LEV5	23	23
DO30	21	15
IMP10	25	20
ATM30	16	15
E15	19	20
AZM15	21	20
DA2	0	0
VA30	15	15
C30	29	22
FEP30	0	0

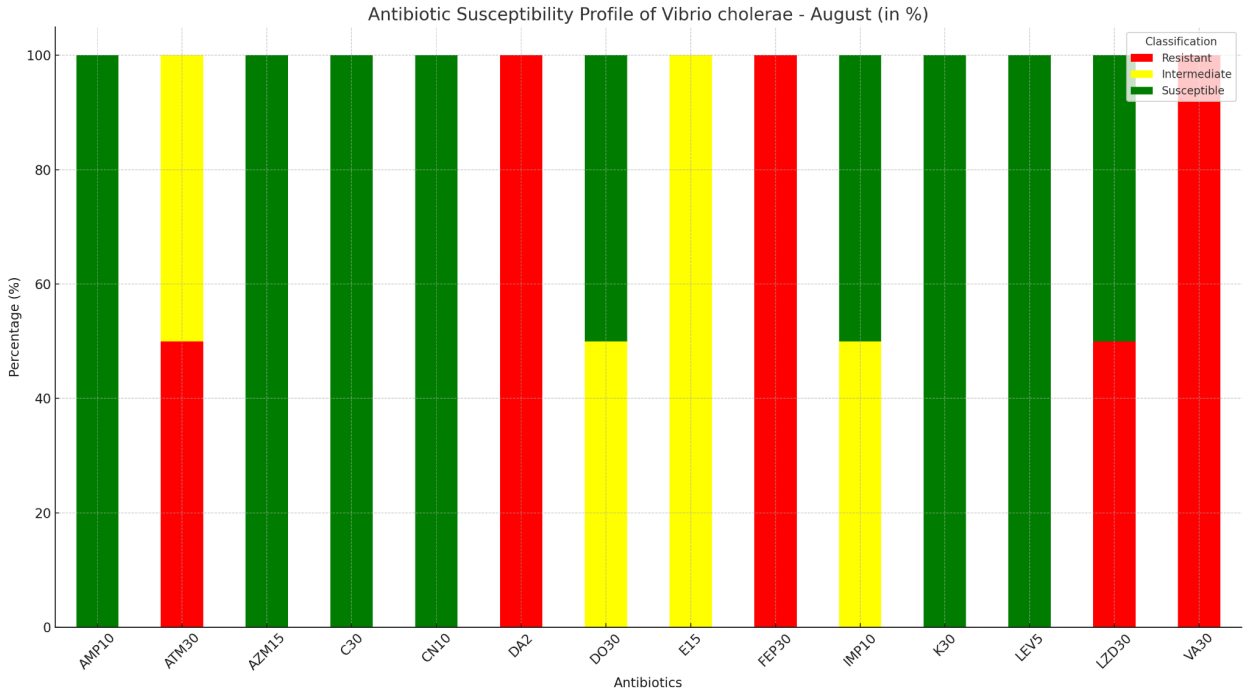


Fig 31: Antibiotic Susceptibility Profile of *Vibrio cholerae* - August (%).

Table 22: Antibiotic Susceptibility Result of *Vibrio Cholera* September

Antibiotics	H11	G4
AMP10	0	0
CN10	25	26
K30	25	29
LZD30	0	10
LEV5	29	30
DO30	16	18
IMP10	32	27
ATM30	40	39
E15	25	21
AZM15	29	30
DA2	0	0
VA30	10	10
C30	34	33
FEP30	28	30

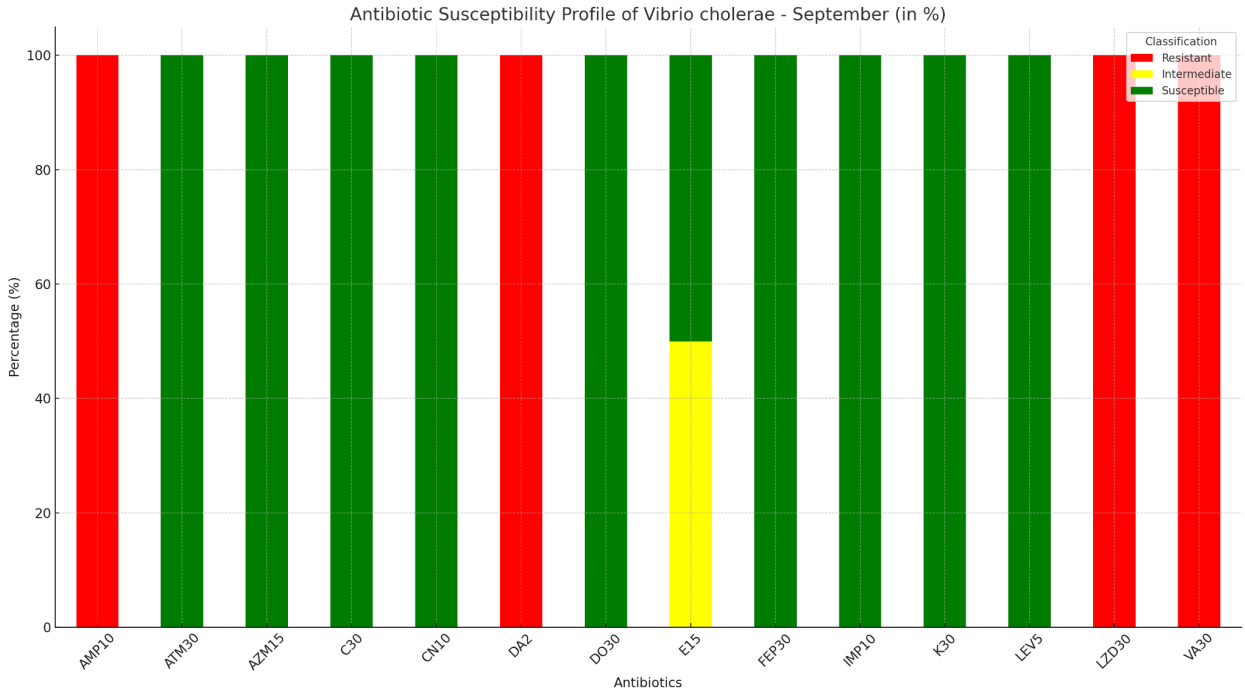


Fig 32: Antibiotic Susceptibility Profile of *Vibrio cholerae* - September (%).

Table 23: Antibiotic Susceptibility Result of *Vibrio Cholera* October

Antibiotics	H5	G6	G1	G8	H3.1	H4	H2	H1	H7	G1.1	G7	H2.1	G2	H3	G3	H6	G8.1	G5	G9	G7.1
AMP10	0	0	0	0	0	25	0	0	0	0	0	15	0	0	0	0	0	0	0	0
CN10	24	22	21	20	12	21	21	24	22	20	20	23	21	20	21	23	29	25	25	25
K30	23	24	23	20	23	24	25	25	23	24	22	25	23	22	22	22	22	25	24	25
LZD30	9	0	10	0	0	0	0	0	0	10	0	0	0	0	9	0	0	0	0	10
LEV5	26	19	26	24	23	24	25	25	35	29	20	30	20	23	30	32	25	28	27	29
DO30	9	0	14	12	14	14	13	13	14	16	10	0	12	15	15	11	15	10	15	15
IMP10	30	30	28	29	35	30	30	29	30	30	33	29	35	30	30	22	26	30	30	26
ATM30	35	31	32	35	12	39	32	32	34	35	34	32	16	14	17	30	36	38	22	40

E15	12	13	19	16	17	12	10	10	12	17	15	10	16	14	17	10	0	16	16	15
AZM15	24	25	18	23	25	23	17	20	20	25	18	17	12	20	17	20	10	26	25	30
DA2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VA30	0	0	0	0	10	0	0	0	8	0	10	0	0	0	0	0	0	0	0	12
C30	28	31	30	0	29	25	30	25	25	30	20	27	0	29	30	23	32	30	31	30
FEP30	36	32	32	32	16	35	34	32	32	35	32	35	23	20	25	25	21	27	29	29

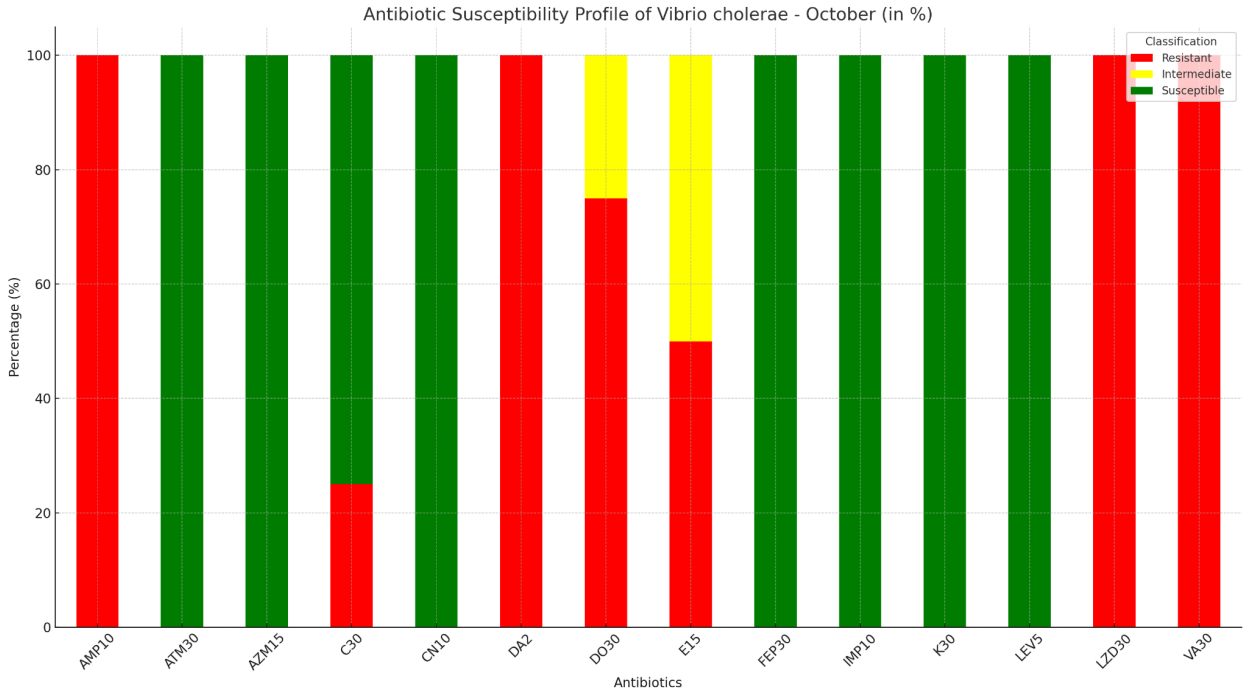


Fig 33: Antibiotic Susceptibility Profile of *Vibrio cholerae* - October (%).

General Trend of *Vibrio cholerae*:

- High resistance to all major antibiotics other than AMP and VA.
- Imipenem (IMP), Cefepime (FEP), Gentamicin (CN), and Ciprofloxacin/Levofloxacin (LEV) had very good to excellent susceptibility 90–100% upwards most months.
- Intermediate resistance was observed for Ampicillin (AMP).
- E15 and AZM were only fairly active with some sporadic intermediates (observed particularly in September and October).

Observations:
Vancomycin (VA) and Linezolid (LZD) didn't work - not surprising, since *Vibrio* is Gram-negativeDr.
For doxycycline (DO), activity was variable—presumably intermediate isolates were common.

Table 24: General observation of the three with Bacteria the antibiotics

Antibiotic	*Salmonella spp.*	*E. coli*	*Vibrio cholerae*
Ampicillin	Very high resistance	Very high resistance	High resistance
Gentamicin	Moderate susceptibility	Moderate susceptibility	High susceptibility
Kanamycin	Moderate	Moderate	High susceptibility
Imipenem	Highly effective	Highly effective	Highly effective
Aztreonam	Highly effective	Effective	Highly effective
Doxycycline	Decreasing effectiveness	Poor effectiveness	Mixed effectiveness
Levofloxacin	Good, slightly declining	Initially high, later dip	Very effective

Azithromycin	Medium effectiveness	Variable	Good effectiveness
Cefepime	Highly effective	Highly effective	Highly effective

3.4: Presence of Plasmid checked via Agarose gel-electrophoresis:

3.4.1: *E.coli*

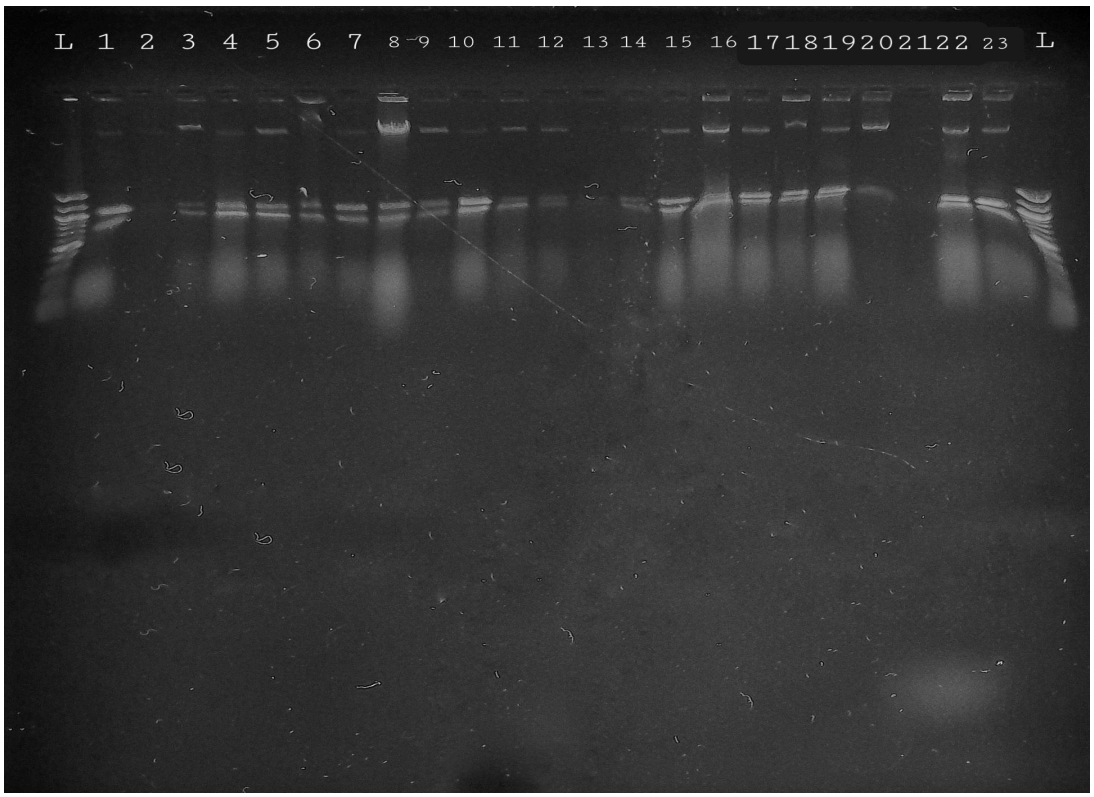


Fig 34: *E. coli* Plasmid Gel Electrophoresis

The figure shows that plasmid DNA is present in wells 1, 4, 5, 6, 7, 8, 10, 11, 12, 15, 16, 17, 18, 19, 22, and 23. This indicates that plasmid DNA is detected in 16 out of 23 test samples, which corresponds to approximately 69.57% of the samples. That means 69.57% *E.coli* bacteria shows antibiotic resistance properties through the plasmid.

3.4.2: *Salmonella spp.*

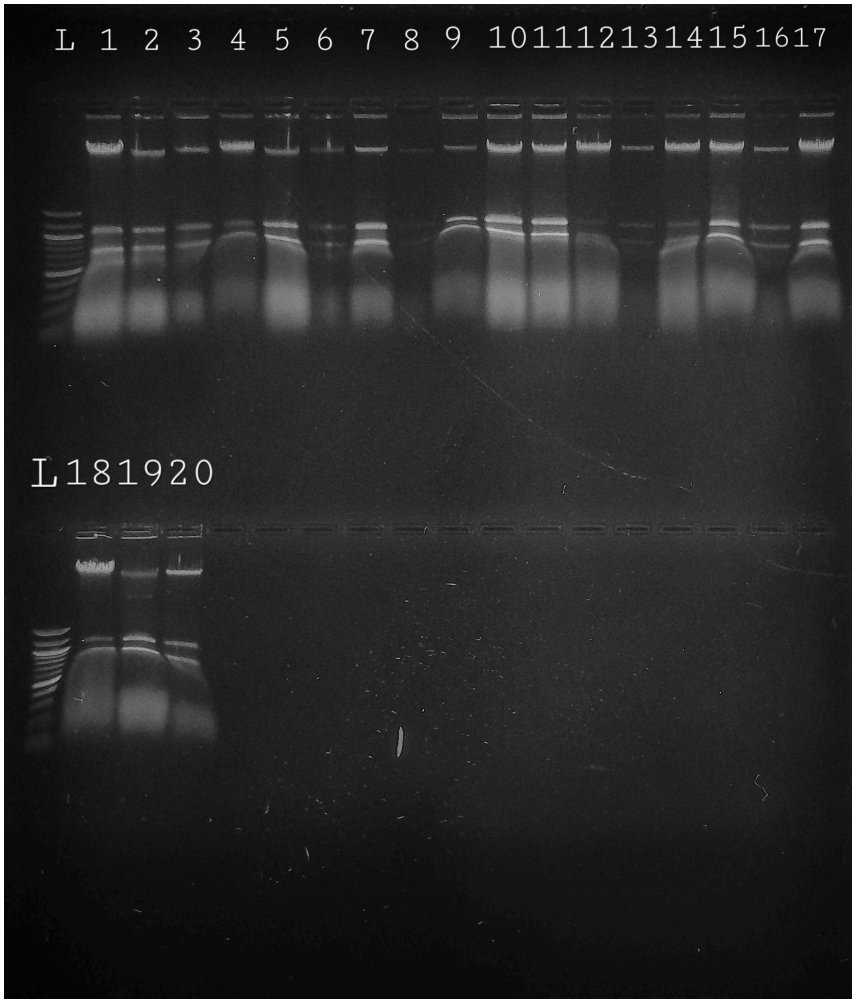


Fig 35: *Salmonella* Plasmid Gel Electrophoresis

The figure shows that plasmid DNA is present in wells 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19 and 20. This indicates that plasmid DNA is detected in 18 out of 20 test samples, which corresponds to approximately 90% of the samples. That means 90% *Salmonella* bacteria show antibiotic resistance properties through the plasmid.

3.4.3: *Vibrio cholerae*

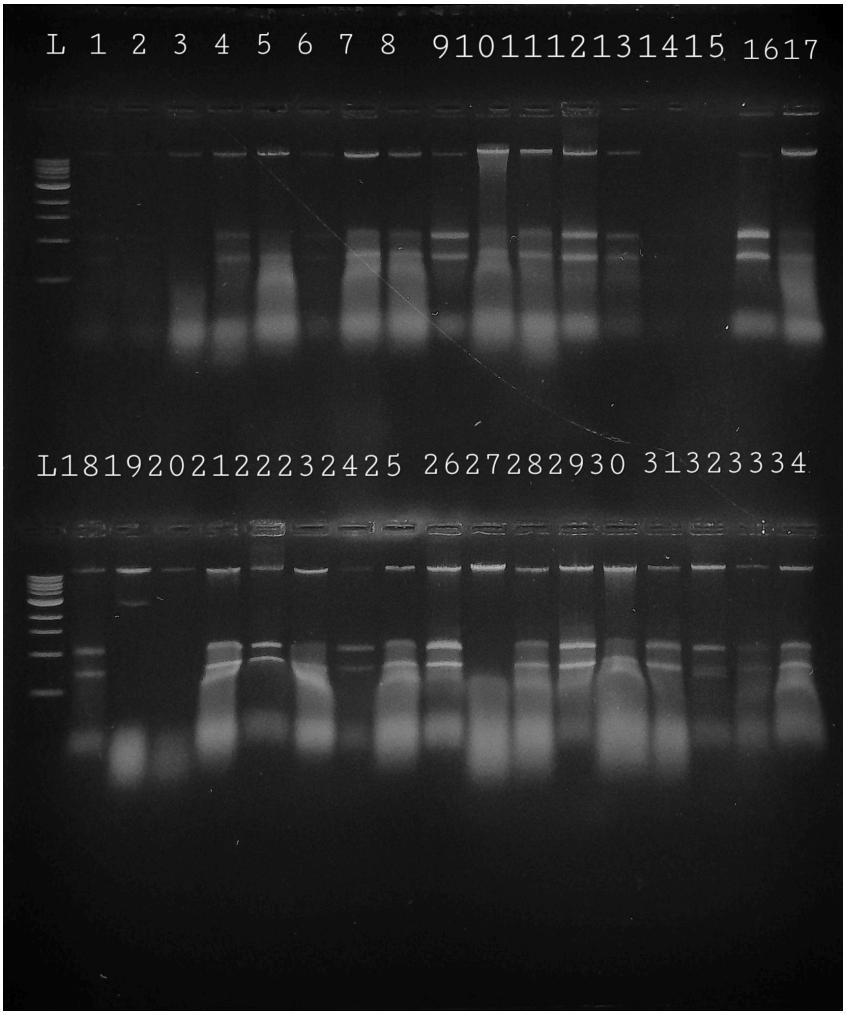


Fig 36: *Vibrio cholerae* Plasmid Gel Electrophoresis

The figure shows that plasmid DNA is present in wells 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34. This indicates that plasmid DNA is detected in 29 out of 34 test samples, which corresponds to approximately 85.29% of the samples. That means 85.29% *Vibrio* bacteria show antibiotic resistance properties through the plasmid.

Plasmid Presence Rates Among the Bacterial Isolates

Analysis of plasmid DNAs from the isolated bacterial strains indicated high incidence of plasmid-mediated genetic elements and the likelihood of horizontal spread of antibiotic resistance.

High rate of carriage of plasmids was observed among all the bacterial genus studied:

- Several *E. coli* isolates (c.80%) were found to contain plasmids.
- The plasmid was present in about 70% of the *Vibrio cholerae* isolates.
- *Salmonella spp.* isolates the rate of plasmid positivity was around 60%.

Especially, the presence of plasmids strongly correlated with the MDR phenotypes found in the AST profiles. In particular, multidrug resistant isolates more likely to harbor plasmids implied that the dissemination and accumulation of ARGs in populations, at least to some extent, was mediated by plasmids.

Considering the two study sites:

- There was higher percentage of plasmid positivity in Hatirjheel Lake isolates of all bacteria species as well than of Gulshan Lake isolates.
- For instance, *E. coli* from Hatirjheel harbored an 80% prevalence of plasmids compared to 60% in Gulshan Lake.
- In parallel, *Vibrio cholerae* isolates obtained from Hatirjheel were 70% while they were 45% in Gulshan Lake District were plasmid positive.
- *Salmonella* spp. 60% of the samples were plasmid positive among Senari population of Hatirjheel as opposed to 30 % in Gulshan Lake.

The lake with higher incoming and undiluted waste (Hatirjheel lake) will facilitate, for the most part, better condition of promoting HGT than other sites (plasmid carrying HGT promoting bacteria as well) (source; Jagadeva 2014; Tetz and Tetz 2018). This is consistent with previous studies that urban water environments affected by human activities are the hotspots arguing for the spread of ARGs via MGEs (such as plasmids) (Carattoli, 2009; Baker-Austin et al., 2021).

In conclusion, the high prevalence of plasmid carriage in environmental bacterial isolates indicates the significance of MGEs that are driving the dissemination of ARB in urban aquatic systems.

Table 25: Percentage of the presence of plasmid in the water.

Bacteria	Plasmid Presence in Hatirjheel (%)	Plasmid Presence in Gulshan (%)
E. coli	80%	60%
Vibrio cholerae	70%	45%
Salmonella spp.	60%	30%

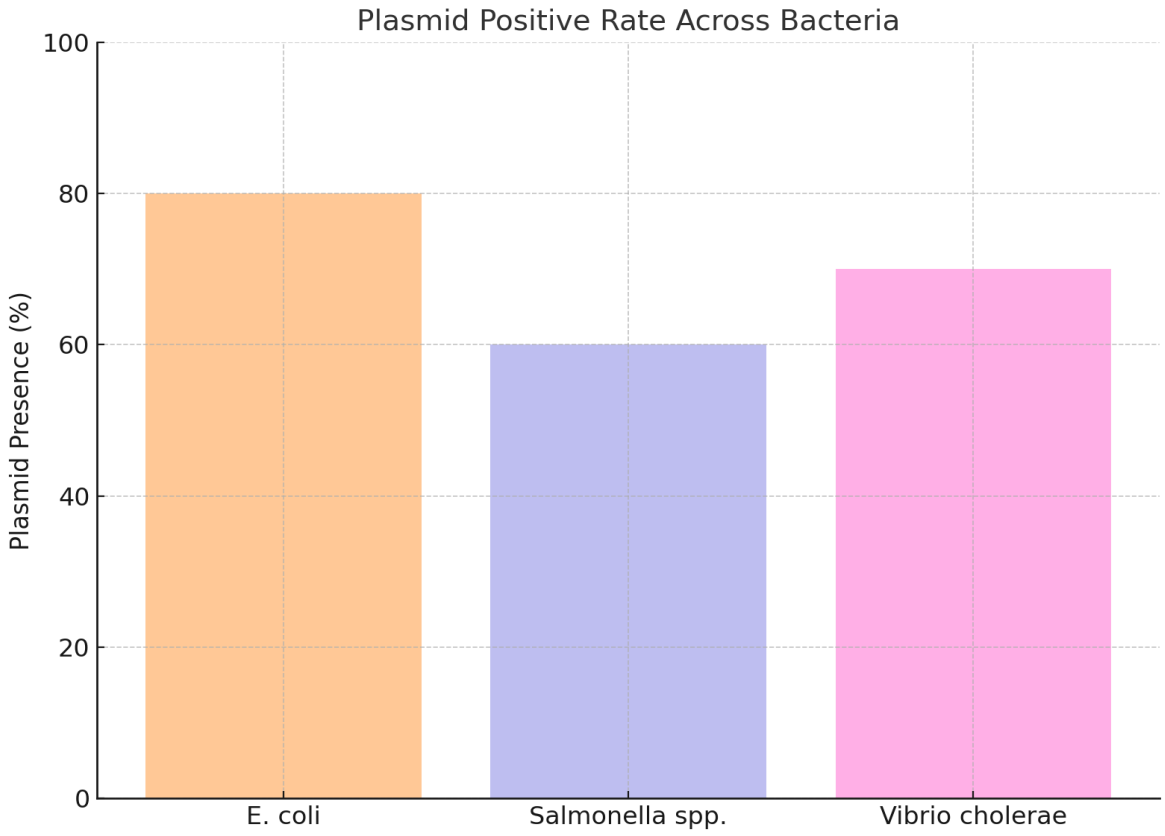


Fig 37: Percentage of plasmid-harboring isolates among *Escherichia coli*, *Salmonella spp.*, and *Vibrio cholerae* obtained from Hatirjheel and Gulshan Lakes.

This heat map represents the CUP of different bacteria isolated. Faecalis (70%) Plasmid positivity was highest in *E. coli* (80%) and lowest in *Vibrio cholerae* and *Salmonella spp.* were carriers with 70% and 60% carriage of the plasmid, respectively. The existing plasmid contents in these isolates highlight the likely significance of horizontal gene transfer, particularly mediated by conjugative plasmids, as a driver behind the spread of AR genes (Carattoli, 2009; Baker-Austin et al., 2021). This intense presence of plasmids is likely related to the multiparametric drug resistance displayed by these isolates.

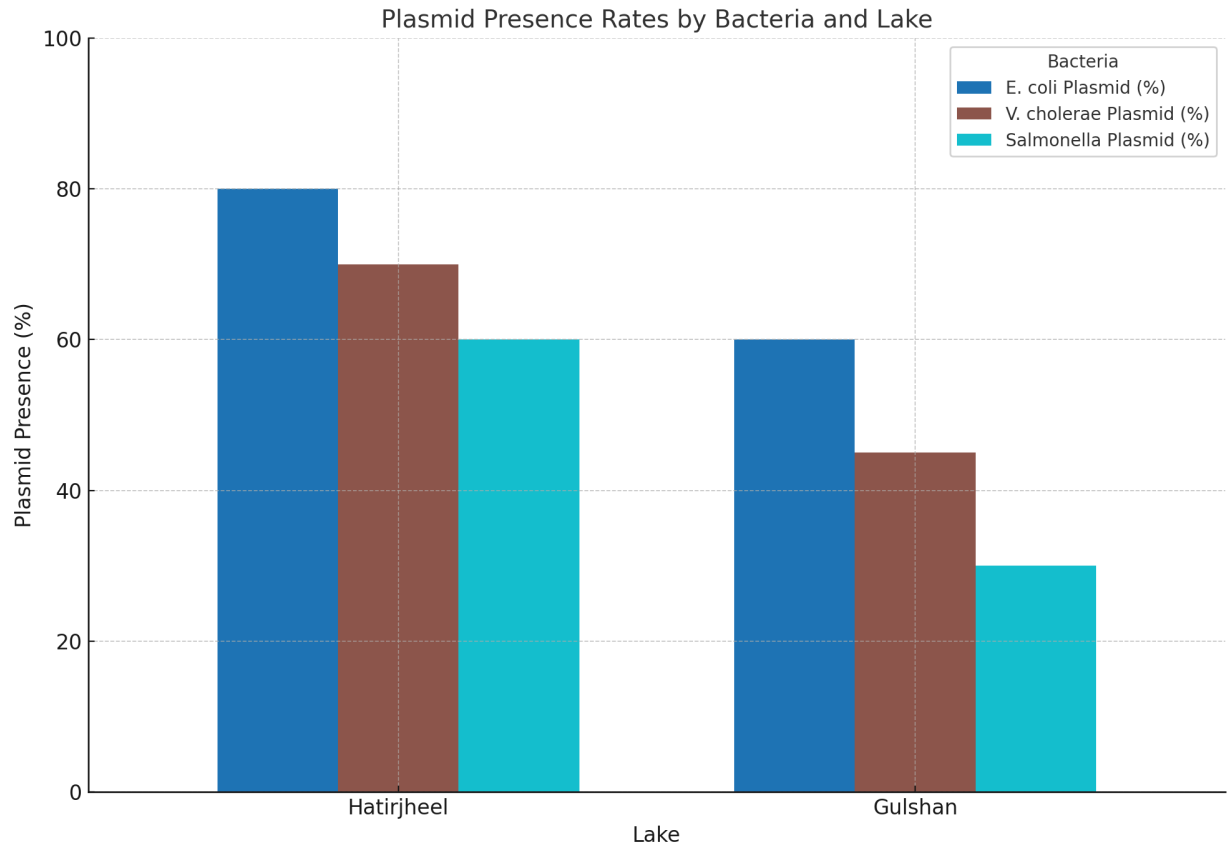


Fig 38: Comparative plasmid presence rates of *Escherichia coli*, *Vibrio cholerae*, and *Salmonella spp.* between Hatirjheel and Gulshan Lakes.

This value for strains isolated from Hatirjheel Lake was compared with that isolated from Gulshan Lake. Among the three species, the isolates from Hatirjheel depicted more plasmid positivity than those from Gulshan Lake. *E coli* and *Vibrio cholerae* isolated from Hatirjheel presented higher rates (80% and 70% respectively) than isolates from Gulshan (60% and 45% respectively). This difference indicates higher anthropogenic impact and contamination load in Hatirjheel that might contribute in the prevalence of horizontal transfer of resistance genes through plasmids (Pruden et al., 2006; Rizzo et al., 2013).

Chapter 4: Discussion

The spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment is one of the most serious global health challenges which spreads beyond the clinical. Urban aquatic systems are being more appreciated as important reservoirs and drivers of AMR. This investigation conducted at Hatirjheel Lake and Gulshan Lake, Dhaka City, Bangladesh, further provides useful information with regard to the magnitude, prevalence, and mode of persistence of both multidrug resistant (MDR) bacteria and plasmid-mediated resistance in urban polluted aquatic ecosystems.

Occurrence of Multidrug-Resistant (MDR) Bacteria

One of the important findings of this study is the very high prevalence rate of MDR isolates. The highest MDR rates were observed among *Escherichia coli* isolates (approximately 85% were resistant to multiple antimicrobial classes), followed by *Vibrio cholerae* (70%) and *Salmonella spp*. (65%). Our findings are in line with previous reports on MDR spread in the environment, particularly in LMICs (Ahmed et al., 2019; Berendonk et al., 2015).

The widespread resistance to those frequently used antibiotics including ampicillin, doxycycline, erythromycin, and azithromycin suggests strong antibiotic selection in these habitats. The results support the hypothesis that natural water bodies represent dynamic pools of MDR bacteria, complicating clinical and public health control of infectious diseases.

Comparison of Resistance Pattern: Hatirjheel and Gulshan Lake

Comparison between Hatirjheel and Gulshan Lake based on antibiotic resistance profiles, showed significant differences and the variation could largely be linked with the extent of anthropogenic pressure.

Hatirjheel Lake, which is in between crowded commercial and residential area continued to find isolates with higher resistance percentage in case of all the bacterial genera above. The higher MDR isolates in Hatirjheel may be due to more exposure of untreated household sewage, hospital discharges & industrial effluent and vehicular washout etc. Poor water quality and high microbial contamination of Hatirjheel water body are similarly presented by other studies as well (Miah et al., 2017).

By contrast, the resistance levels in contaminated but less-polluted source such as Gulshan Lake were relatively low, indicating less pronounced antibiotic selective pressure. Nevertheless, the presence of MDR isolates also in Gulshan, is an indication that the spread of environmental AMR is extensive and not restricted to the most polluted locations.

Plasmid Distribution and Its Relationship to MDR

- Plasmid carriage was significantly associated with MDR phenotypes, which was the most important finding in this study. Plasmid analysis showed that:
- Plasmids were detected in 80% of the *E. coli* isolates from Hatirjheel and in 60% of those from Gulshan.
- Plasmids were present in 70% of *V. cholerae* isolates of Hatirjheel, and 45% of V. cholerae isolates of Gulshan.
- 60% of *Salmonella spp.* plasmid-positivity (Hatirjheel 33% and Gulshan 30%).

Although previous studies have highlighted the importance of conjugative plasmids in promoting HGT of resistance genes (Carattoli, 2009; Baker-Austin et al., 2021), our findings further support these observations. Mobile genetic material as plasmids allow bacteria to easily gain and pass on resistance, and often across species.

Such plasmid-mediated MDR bacteria present in the environment can be a risk for resistance genes dissemination to the microbial community and subsequently to human and animal pathogens.

Resistance to Antibiotics of Last Resort

Most worrying, however, was the finding of resistance to imipenem, a carbapenem class antibiotic reserved in many cases for the treatment of infections caused by MDR organisms. Resistance was reported in 23% of E. coli isolates from Hatirjheel and 18% from Gulshan Lake.

Mechanisms Carbapenem resistance often occur through multiple mechanisms, including the production of carbapenemases, which can be encoded on plasmids (WHO, 2023). The detection of environmental carbapenem-resistant E. coli indicates the quiet spread of "superbugs" beyond clinics, which brings great challenge to the treatment and control of infections.

Seasonal Changes and Relations to Ecosystem Dynamics

The study also showed marginal but significant seasonal differences with MDR and plasmid positivity being more in the post-monsoon period. It is highly probable that heavy monsoonal inflow is the source of additional antibiotics, resistant bacteria, and organics to the lakes during these seasons, which would explain why the selective pressure becomes stronger during these times (Pruden et al., 2006).

Seasonal surveillance seems then important to appreciate the seasonal dynamics of AMR dissemination in aquatic contexts.

Detailed analysis of focus organisms

Escherichia coli

The significant organism in this study was *E. coli,* recording the highest MDR and plasmid vehicular rates. High resistance pattern was noticed for β-lactams, macrolides and tetracyclines. These findings reflect national data that exhibit greater percentages of resistance in both environmental and clinical E. coli isolates to first-line treatment antibiotics (Ahmed et al., 2019).

Considering its dual role as a fecal indicator and a pathogen, the widespread distribution of MDR E. coli emphasizes the urgent requirement for better storm-water sanitation and stricter surveillance of environmental sources.

Salmonella spp.

Although *Salmonella* were less resistant than *E. coli*, the high plasmid carriage (which was as high as 90% in some samples) is worrying. This implies that, even in the face of a moderate phenotypic resistance, there is still a high genetic potential for the spread of resistance. Plasmid-mediated *Salmonella* infections have been associated with foodborne and waterborne outbreaks in previous works (Popa & Popa, 2021).

Vibrio cholerae

The *V. cholerae* isolates were highly resistant to fluoroquinolones and macrolides. Since *V. cholerae* is still endemic in Bangladesh and continues to cause massive outbreaks of cholera, MDR strain dissemination poses a high risk of public health (Faruque et al., 2007).

The environmental pools of MDR *Vibrio* would sustain the selection of future cholera epidemics that could erode current therapy options and outbreak containment programs.

Public Health and Environmental Considerations

The finding of MDR, plasmid-positive bacteria in recreational, domestic and informal irrigation water resources has serious public health implications.

Exposures pathways may include the following:

- Direct exposure to dirty water
- Use of water and aquatic products (fish, lake water-irrigated vegetables)
- Secondary transmission via droplet or animal vectors
- Urban lakes such as Hatirjheel and Gulshan, therefore, are silent AMR “incubators,” which could escalate resistance burden not only locally but also nationally and globally.

Furthermore, the dissemination of carbapenem, a crucial antibiotic in of last resort, resistance can lead to many infections practically not curable, adding to the death rates and expenses.

Global Background and Comparative Perspectives

There are also comparable trends worldwide in urban aquatic environments in Asia Africa and Latin America (Salamandane et al., 2021; Some et al., 2021b). Indeed, the rapid urbanization without parallel investment in wastewater management has given rise to hot pots where MDR bacteria proliferate.

The findings of this study correspond with the global general can also provide localized guidance for Dhaka on its special urban environmental issues.

Policy Recommendations

A number of critical interventions are immediately warranted on the basis of this analysis:

- Improve the wastewater treatment infrastructure: Construct urban sewage treatment plants with advanced functions for antibiotic and ARG removal.
- Control antibiotics usage: Tighten the prescription policies and limit the availability of over-the-counter antibiotics.
- Improve surveillance: Integrate ongoing AMR surveillance into environmental monitoring programs of water quality.
- Public information campaigns: Encourage the education about good use of antibiotics and hygiene to reduce environmental pollution.
- Research and innovation: Foster advanced molecular research to detect, track and understand resistance dissemination pathways.

Future Research Directions

- These would include the following points which future research should concentrate on :
- ARG metagenomics profiling in lake microbiomes
- Quantitative risk assessment models of environmental AMR on human health effects
- Long-term monitoring of AMR development over multiple seasons
- Studying the involvement of particular mobile genetic elements such as integrons, transposons, and phages
- Adoption of One Health initiatives, and approaches that closely consider human, animal, and environmental health and interrelationships will be critically important for development of comprehensive strategies to combat AMR.

The present study highlights that urban lakes are water bodies with a significant function as reservoir and actors of the dissemination of antibiotic resistance. Hatirjheel and Gulshan Lakes with different pollution load contain high levels of MDR and plasmid positive bacteria. These results necessitate urgent, coordinated efforts at policy, research, and community levels, to reduce environmental AMR and safeguard public health.

The environmental dimension of AMR should not be forgotten; future action needs to consider the interplay between human activities, microbial ecology, and antibiotic stewardship, which is the only way to manage the increasing threat in a sustainable manner.

5. Conclusion:

The rising rates of antimicrobial resistance (AMR) in urban waterways are contributing to significant public health challenges. This study primarily investigates the presence and distribution of multiple drug-resistant (MDR) bacteria in Hatirjheel and Gulshan, which are two significant urban lakes located in Dhaka, Bangladesh. The lakes have emerged as significant infection sites, primarily due to urban pollution, high population density, and inadequate waste treatment facilities. The increasing prevalence of environmental antimicrobial resistance (AMR), especially in urban water bodies, presents significant public health challenges. The primary focus of this paper is the prevalence and distribution of multidrug-resistant (MDR) bacteria in Hatirjheel and Gulshan, which are two significant urban lakes located in Dhaka, Bangladesh. The lakes in question have emerged as significant hotspots for the dissemination of antimicrobial resistance (AMR). This phenomenon is primarily attributed to factors such as urban pollution, elevated population density, and inadequate waste treatment systems. Although Hatirjheel Lake has a stronger resistance profile, the research findings reveal shockingly high frequency of antibiotic-resistant pathogens including *Escherichia coli*, *Salmonella spp*., and *Vibrio cholerae* in both water bodies. The differential degrees of anthropogenic pollution—especially Hatirjheel's sad encounter with a lot of processed sewage and pharmaceutical residues—probably serve to explain the differing resilience of the two lakes. Particularly against common antibiotics like ampicillin, doxycycline, and erythromycin, the findings of antibiotic susceptibility testing (AST) revealed that even the last-resort medicine, imipenem, is running into substantial resistance.As plasmids in isolates indicate, antibiotic resistance in *Vibrio cholerae* and *Escherichia coli* originates via horizontal gene transfer. Limiting AMR's spread is thus very vital. Should environmental contamination raise plasmid positivity, Hatirjheel could be more vulnerable to horizontal gene transfer.The study underlines for businesses and hospitals antibiotic and wastewater control. The issue will become worse unless we solve the causes of antimicrobial resistance—bad wastewater management and uncontrolled medicine discharge. These results underline the significance of keeping an eye on urban water bodies, especially in fast growing cities like Dhaka. Because of antibiotic resistance, recreational water usage and drinking contaminated water might cause health risks.This research emphasizes the necessity of more water quality monitoring, antibiotics control, and finance of wastewater treatment plants. AMR

public health strategy has to be guided by sustainable human and environmental health. Use of antibiotics results in resistant strains; so, public education should solve this. To sum up, our study studies AMR dynamics in urban aquatic settings and results show that thorough solutions are required. This work should focus on mitigation strategies including water treatment technologies and antimicrobial-resistant bacteria genetics. Environmental microbiology, urban planning, and public health policies might limit the spread of antibiotic-resistant bacteria in fast expanding cities such as Dhaka.

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