Comparative Analysis of Plasmid Presence and Antibiotic Resistance Profiles in Water Samples with a Focus on Dhaka City's Water Bodies (Dhanmondi & Gulshan Lake)

By

Addrota Khan Progga

ID: 21236061

Subhat Sumaila Ahmed

ID: 21336005

Tazkia Rahman

ID: 21236051

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

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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:

_____

Addrota Khan Progga (Student ID :21236061)

_____

Subhat Sumaila Ahmed (Student ID:21336005)

_____

Tazkia Rahman (Student ID:21236051)

Approval

The thesis titled "Comparative Analysis of Plasmid Presence and Antibiotic Resistance Profiles in Water Samples with a Focus on Dhaka City's Water Bodies (Dhanmondi & Gulshan Lake)" submitted by

1. Addrota Khan Progga (21236061) of Fall 2021

2. Subhat Sumaila Ahmed (21336005) of Summer 2021

3. Tazkia Rahman (21236051) of Fall 2021

has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. in Biotechnology & Microbiology on 18th December 2025.

Examining Committee:

Supervisor and Departmental Head: (Chair)

_____

Iftekhar Bin Naser, PhD

Associate Professor and Chairperson,

Department of Biotechnology

School of Life Science

Brac University

Program Director: (Member)

_____

Munima Haque, PhD

Associate Professor,

Department of Mathematics and Natural Sciences (MNS)

Brac University

BRAC University

Ethics Statement

This study was conducted in accordance with established ethical standards and guidelines for environmental and microbiological research. The work involved the collection and analysis of water samples from Dhanmondi and Gulshan Lake in Dhaka City. As the study did not include human or animal participants, approval from an institutional review board was not required. All sampling activities were performed carefully to minimize any disturbance to the environment. Appropriate safety precautions were followed during sample collection, transportation, and laboratory procedures to ensure the protection of both researchers and the surrounding ecosystem. No genetic modification or environmental release of biological agents was carried out in this study. The data obtained were used exclusively for scientific and educational purposes. The findings and conclusions were reported transparently, with no manipulation or fabrication of results. Overall, this research was performed under the principles of scientific integrity, environmental responsibility, and data protection.

Acknowledgment

Above all, we wish to express our deepest gratitude to Allah, whose mercy, guidance, and strength enabled us to carry out our thesis work and present this report with dignity.

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Abstract

Antimicrobial resistance (AMR) is a growing global public health concern, with environmental reservoirs contributing significantly to the spread of antibiotic-resistant bacteria. Urban surface water bodies in densely populated cities are recognized as important hotspots due to continuous exposure to domestic, medical, and industrial waste. In Dhaka, Bangladesh, rapid urbanization and inadequate wastewater management have intensified this problem. This study comparatively analyzed the antibiotic resistance profiles and plasmid presence of *Escherichia coli*, *Salmonella* spp., and *Vibrio cholerae* isolated from water samples collected from Dhanmondi Lake and Gulshan Lake.

Samples were processed using selective culture methods, followed by molecular confirmation through polymerase chain reaction (PCR). Antibiotic susceptibility testing was conducted using the Kirby–Bauer disk diffusion method, and plasmid DNA isolation was performed to assess the potential role of plasmids in resistance dissemination.

Results indicated a high prevalence of antibiotic-resistant isolates in both lakes, with several strains exhibiting multidrug resistance. Differences in resistance patterns between the two sites suggest variation in environmental contamination pressures. A substantial proportion of isolates carried plasmids, highlighting the potential contribution of horizontal gene transfer to environmental AMR spread.

These findings underscore the role of urban water bodies as reservoirs of antibiotic-resistant bacteria and resistance-associated plasmids, emphasizing the need for continuous monitoring and improved waste management strategies in rapidly urbanizing regions.

Keywords: Antimicrobial resistance (AMR), antibiotic-resistant bacteria, urban water bodies, plasmid, *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, Dhanmondi Lake, Gulshan Lake.

Chapter 1

Introduction

1.1: Role of Aquatic Environments in the Spread of Antimicrobial Resistance

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges, reducing the effectiveness of antibiotics and increasing the burden of difficult-to-treat bacterial infections worldwide. The extensive and often inappropriate use of antimicrobial agents in human healthcare, agriculture, and animal husbandry has accelerated the emergence of antibiotic-resistant bacteria. Although AMR has traditionally been examined in clinical settings, growing evidence indicates that environmental pathways—particularly aquatic environments—play a crucial role in the persistence and dissemination of antimicrobial resistance (Martí et al., 2014). Aquatic ecosystems such as rivers, lakes, and urban surface waters function as important environmental reservoirs of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). These water bodies commonly receive untreated or partially treated domestic sewage, hospital effluents, industrial discharges, and agricultural runoff. Such effluents frequently contain antibiotic residues and resistant microorganisms, creating selective pressure that favors the survival and proliferation of resistant bacterial populations in aquatic systems (Okoye et al., 2022). Consequently, aquatic environments contribute significantly to the maintenance and amplification of antimicrobial resistance beyond clinical boundaries. Urban surface waters are particularly vulnerable to antimicrobial contamination due to high population density, intense anthropogenic activity, and inadequate wastewater management. Studies have shown that urban lakes act as reservoirs and transmission routes for resistant bacteria, increasing the risk of resistance dissemination through direct or indirect human exposure (Meradji et al., 2025). Environmental investigations have further demonstrated that contaminated water systems can harbor clinically relevant resistance determinants, including multidrug-resistant bacteria, posing serious public health concerns (Walsh et al., 2011). In Bangladesh, rapid urbanization and limited wastewater treatment infrastructure have intensified the contribution of aquatic

environments to AMR dissemination. Dhaka city, in particular, contains several urban lakes that are subjected to continuous anthropogenic pressure. Among these, Dhanmondi Lake and Gulshan Lake represent important surface water bodies that are widely used for recreational, domestic, and surrounding commercial activities. Previous studies conducted in Dhaka have reported the presence of antibiotic-resistant bacteria in urban surface waters, highlighting the environmental burden of AMR in densely populated settings (Islam et al., 2025; Sharif et al., 2025). The frequent human interaction with these lakes increases the potential risk of exposure to antibiotic-resistant microorganisms. In addition to serving as reservoirs, aquatic environments facilitate horizontal gene transfer among bacterial communities. Mobile genetic elements such as plasmids play a central role in this process by enabling the exchange of resistance genes between different bacterial species (Carattoli, 2013). The coexistence of diverse microbial populations within aquatic systems enhances the efficiency of gene transfer, accelerating the emergence and spread of multidrug-resistant bacteria (Partridge et al., 2018).

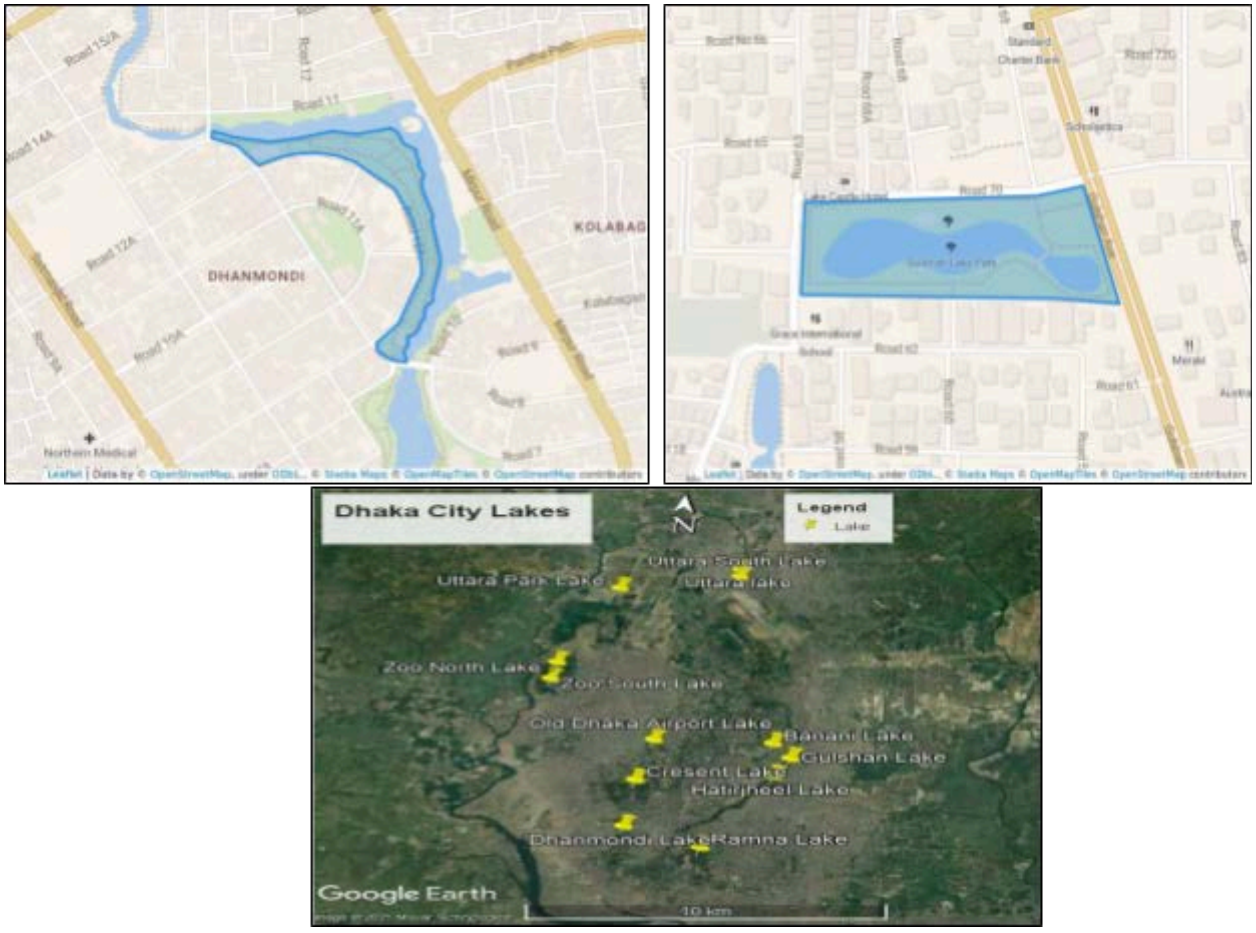


Figure 01: Location of Gulshan lake and Dhanmondi lake in Dhaka city.

Overall, aquatic environments constitute a critical component of the environmental dimension of antimicrobial resistance. Urban surface waters, including Dhanmondi Lake and Gulshan Lake in Dhaka city, not only reflect the extent of environmental contamination but also act as dynamic systems that promote the persistence and dissemination of antibiotic resistance. Understanding the role of such aquatic environments is essential for effective environmental surveillance and the development of integrated One Health strategies aimed at mitigating antimicrobial resistance at the human–environment interface (United Nations Environment Programme [UNEP], 2023; World Health Organization [WHO], 2025). in urban aquatic environments to better understand the environmental contribution to antimicrobial resistance and to inform effective control strategies.

1.2 Significance of Urban Water Bodies in Antibiotic Resistance Dissemination

Urban freshwater systems play a crucial role in the dissemination of antimicrobial resistance (AMR) in the environment. Rivers, lakes, and other surface water bodies act as reservoirs for antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), receiving continuous inputs from untreated domestic sewage, hospital effluents, industrial discharge, and agricultural runoff. These waste streams often contain antibiotic residues that exert selective pressure on microbial communities, promoting the survival and persistence of resistant bacteria (Kümmerer, 2009; Martí et al., 2014). In densely populated cities, urban lakes are particularly vulnerable to contamination due to intense anthropogenic activity and inadequate wastewater management. Studies have shown that urban water bodies function as hotspots for resistance development and transmission, facilitating the spread of ARB through direct human contact, recreational use, or indirect exposure via food and water sources (Meradji et al., 2025; Okoye et al., 2022). The persistence of antibiotics at sub-inhibitory concentrations in aquatic systems further accelerates resistance selection and maintenance. In Bangladesh, the risk associated with urban water bodies is amplified by rapid urbanization and limited sewerage infrastructure. Dhaka city exemplifies this situation, where most domestic wastewater is discharged untreated into surrounding water bodies. Previous investigations have identified widespread contamination of surface waters with multidrug-resistant bacteria, highlighting the environmental contribution to AMR dissemination

in urban settings (Islam et al., 2025; Sharif et al., 2025). These findings emphasize the importance of studying urban lakes as key environmental reservoirs of antimicrobial resistance.

1.3 Study Area Selection

The present study selected Dhanmondi Lake and Gulshan Lake as representative urban water bodies within Dhaka city. Both lakes are situated in densely populated residential and commercial areas and are subjected to continuous anthropogenic pressure. Untreated domestic sewage, stormwater runoff, and surrounding urban activities contribute significantly to the degradation of water quality in these lakes, creating favorable conditions for microbial growth and resistance development. Comparative studies have reported differences in pollution levels between these two lakes, with Gulshan Lake generally exhibiting higher organic pollution and poorer water quality than Dhanmondi Lake. Such contrasts make them suitable sites for a comparative investigation of antibiotic resistance and plasmid presence in environmental bacterial populations. The frequent use of these lakes for recreational, domestic, and surrounding commercial activities further increases the relevance of these sites from a public health perspective.

Water samples were collected over a six-month period from January 6 to June 20, 2025, covering seasonal variation from winter to early monsoon. Sampling was conducted on ten confirmed dates, always during the early morning hours (between approximately 7:00 and 7:30 a.m.) to maintain temporal consistency and minimize disturbance. Environmental conditions such as temperature, weather, and observable water characteristics were recorded during each sampling event. This longitudinal and systematic sampling approach allowed assessment of temporal trends in antibiotic resistance and plasmid occurrence in both lakes.

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

This study focuses on three clinically and environmentally significant bacterial genera: *Escherichia coli, Salmonella spp.*, and *Vibrio cholerae*. These organisms were selected due to

their strong association with fecal contamination, waterborne disease transmission, and documented capacity to harbor antibiotic resistance genes.

Escherichia coli (E. coli) is a widely used indicator organism for fecal pollution in water bodies. Although many strains are harmless, pathogenic variants can cause severe gastrointestinal and extra-intestinal infections. Environmental studies in Bangladesh have frequently reported multidrug-resistant *E. coli* in surface waters, with resistance commonly observed against β-lactams and fluoroquinolones (Sharif et al., 2025). The high prevalence of resistant *E. coli* in urban lakes highlights both environmental contamination and potential public health risks.

Salmonella spp. are important enteric pathogens responsible for gastroenteritis and enteric fever. Transmission typically occurs through contaminated food and water. In Bangladesh, increasing resistance among *Salmonella* isolates has been documented, particularly against fluoroquinolones and third-generation cephalosporins (Islam et al., 2023). The detection of resistant *Salmonella* in environmental waters indicates the potential for dissemination beyond clinical settings.

Vibrio spp., the causative agent of cholera, is naturally adapted to aquatic environments and remains endemic in Bangladesh. Recent studies have shown the emergence of multidrug-resistant *V. spp.* strains in rivers and lakes, raising concerns regarding effective outbreak management (Baker-Austin et al., 2018; WHO, 2023). Environmental reservoirs of V. cholerae therefore play a critical role in sustaining cholera transmission cycles.

1.5 Commonly Used Antibiotics for Treatment in Bangladesh

Antibiotics are essential for the treatment and prevention of bacterial infections; however, their widespread and often inappropriate use contributes significantly to antimicrobial resistance. In Bangladesh, broad-spectrum antibiotics are commonly prescribed for both community-acquired and hospital-acquired infections. The frequent use of such antibiotics increases selective pressure on bacterial populations, accelerating resistance development.

Commonly used antibiotic classes in Bangladesh include β-lactams (penicillins and cephalosporins), macrolides, fluoroquinolones, tetracyclines, aminoglycosides, and chloramphenicol. Third-generation cephalosporins such as cefixime and ceftriaxone are widely used for respiratory infections and enteric fever. Fluoroquinolones like ciprofloxacin are frequently prescribed for gastrointestinal and urinary tract infections, while doxycycline remains a standard treatment for cholera in adults. Azithromycin, a macrolide, is commonly used for diarrheal and respiratory infections.

The extensive consumption of these antibiotics in human medicine, along with their use in agriculture and aquaculture, contributes to the accumulation of antibiotic residues in surface waters. This environmental exposure plays a key role in shaping resistance profiles observed in urban water bodies.

1.6 Plasmid-Mediated Resistance and Environmental Spread

Plasmids play a central role in bacterial evolution by enabling the transfer of antibiotic resistance genes between bacteria, including unrelated species. These extra-chromosomal DNA elements replicate independently and facilitate horizontal gene transfer, allowing bacteria to rapidly acquire resistance traits that enhance survival under selective pressure (Bennett, 2008). As a result, plasmid-mediated resistance significantly accelerates the emergence and spread of multidrug-resistant bacterial populations in both clinical and environmental settings (Carattoli, 2009). Urban aquatic environments provide favorable conditions for plasmid-mediated resistance dissemination due to continuous exposure to antibiotic residues, heavy metals, and diverse microbial communities. Sub-inhibitory concentrations of antibiotics in polluted water bodies impose selective pressure that promotes the persistence of plasmid-harboring bacteria. Many plasmids carry multiple resistance genes simultaneously, conferring resistance to several antibiotic classes such as penicillins, tetracyclines, and aminoglycosides. This co-localization of resistance determinants allows a single plasmid transfer event to generate multidrug-resistant strains (Carattoli, 2009; Partridge et al., 2018).

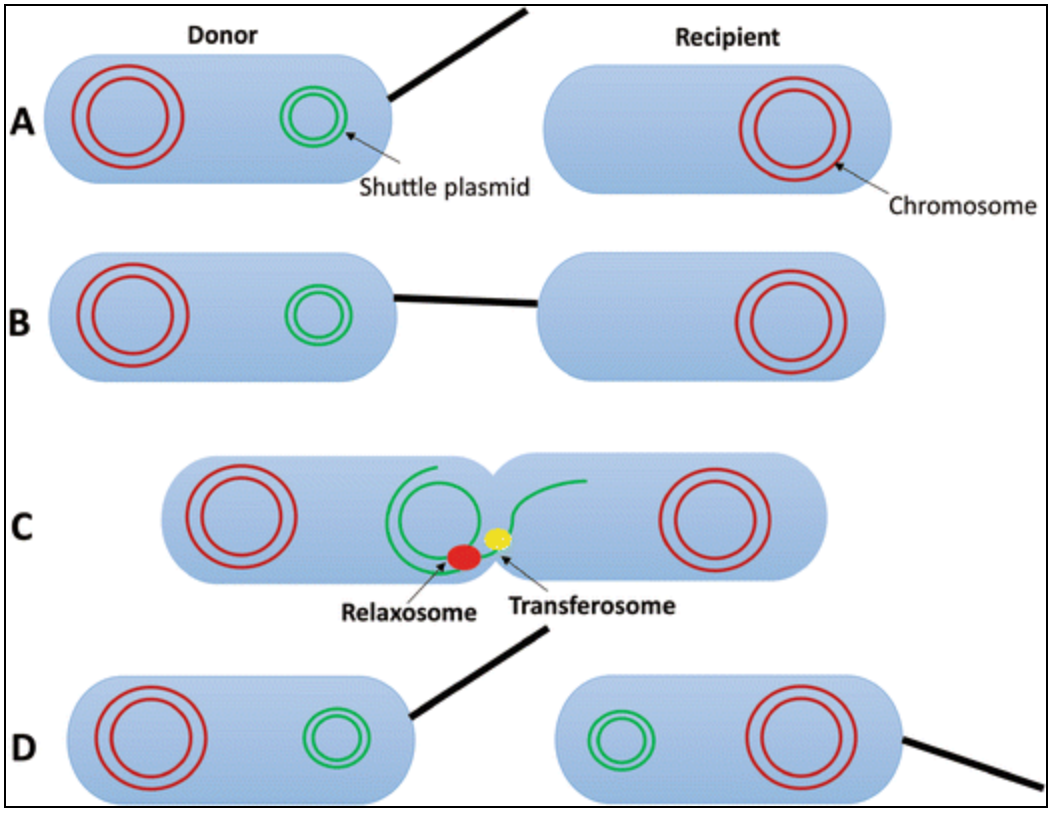


Figure 02: Plasmid-mediated horizontal gene transfer through bacterial conjugation. The donor bacterium transfers a resistance-carrying plasmid to the recipient cell, resulting in the acquisition of antibiotic resistance (adapted from Bennett, 2008).

In Dhaka city, urban lakes such as Dhanmondi Lake and Gulshan Lake are surrounded by densely populated residential areas, commercial zones, pharmacies, and healthcare facilities. These surrounding activities increase the likelihood of antibiotic residues and resistant bacteria entering lake water through untreated wastewater discharge and surface runoff. The convergence of bacteria from human, animal, and environmental sources within these lakes creates ideal conditions for horizontal gene transfer. Consequently, these urban lakes function as potential hotspots for plasmid-mediated resistance exchange among environmental bacteria (Martí et al., 2014; Sharif et al., 2025). The process of plasmid-mediated gene transfer occurs primarily through bacterial conjugation, in which a donor bacterium transfers a resistance-carrying plasmid to a recipient cell via direct cell-to-cell contact. During conjugation, the plasmid is replicated, and a copy is transferred to the recipient bacterium, where it circularizes and functions independently, thereby conferring antibiotic resistance (Bennett, 2008). This mechanism is

illustrated in Figure 2, which demonstrates the transfer of resistance genes through conjugative plasmids. In addition to horizontal transfer, plasmids are also inherited vertically during bacterial cell division, ensuring the persistence of resistance traits within bacterial populations. Multidrug-resistance plasmids often carry multiple resistance genes, allowing bacteria to withstand exposure to different antibiotics simultaneously. These plasmids can spread rapidly within microbial communities, significantly reducing treatment effectiveness and increasing the burden of resistant infections. The role of multidrug-resistance plasmids in promoting both vertical and horizontal transmission of resistance is illustrated in Figure 3 (Partridge et al., 2018).

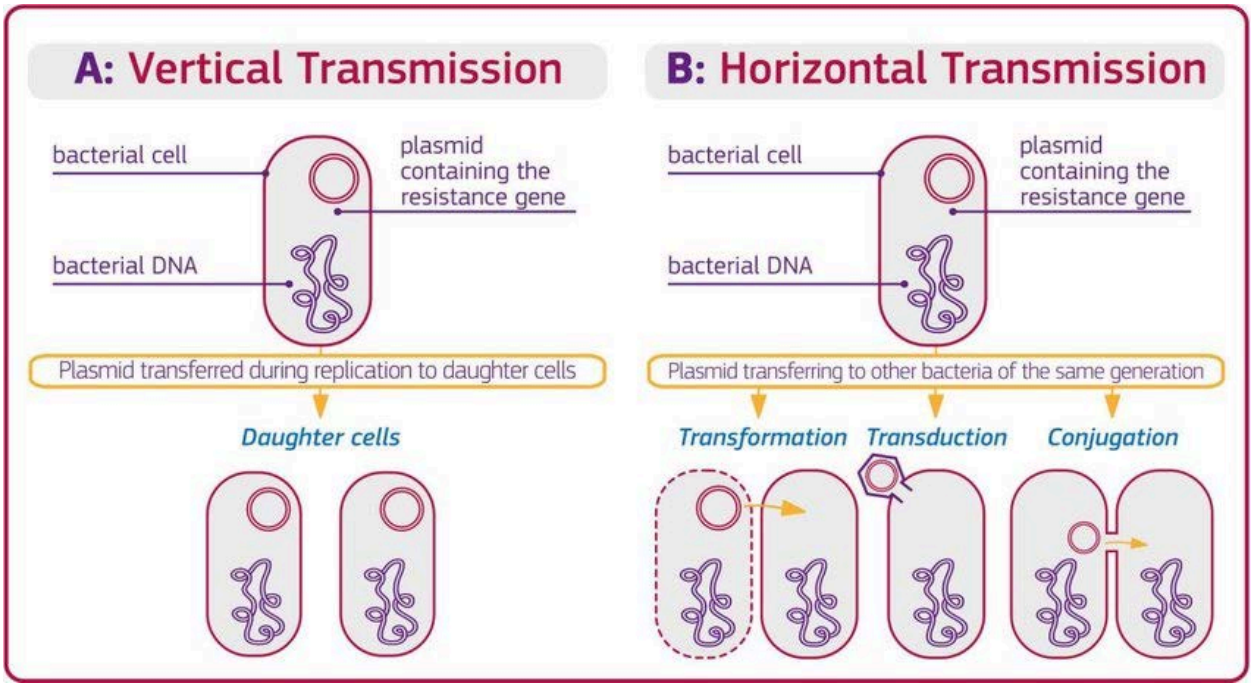


Figure 03: Vertical and horizontal transmission of plasmid-mediated antibiotic resistance in bacterial cells. Resistance-carrying plasmids are inherited by daughter cells during cell division (vertical transmission) and transferred between bacteria through transformation, transduction, and conjugation (horizontal transmission).

Although plasmid-mediated resistance has been extensively studied in clinical isolates, comparatively limited research has focused on its occurrence in environmental water bodies. This lack of environmental data is particularly evident in developing countries like Bangladesh. Investigating plasmid presence among bacterial isolates from Dhanmondi and Gulshan Lakes is therefore essential for understanding how environmental reservoirs contribute to the broader

antimicrobial resistance cycle. Such insights are critical for designing effective environmental monitoring and intervention strategies within a One Health framework.

1.7 Literature Review

Antimicrobial resistance (AMR) is widely recognized as a critical global health concern due to its capacity to compromise the effectiveness of existing antimicrobial therapies. While clinical misuse and overuse of antibiotics remain key drivers of resistance, there is growing recognition that environmental compartments play an equally important role in the emergence, maintenance, and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Among environmental reservoirs, aquatic systems are of particular concern because they integrate microbial inputs from human, animal, and industrial sources and provide conditions that facilitate bacterial survival and genetic exchange (Bennett, 2008; Carattoli, 2009).

Urban surface waters are continuously exposed to complex mixtures of contaminants, including domestic sewage, hospital effluents, industrial discharges, and stormwater runoff. These inputs introduce both antibiotics and resistant microorganisms into aquatic environments, generating selective pressure that favors resistant phenotypes. Even low, sub-therapeutic concentrations of antibiotics have been shown to promote resistance development and enhance horizontal gene transfer among bacterial populations (Kümmerer, 2009; Martí et al., 2014). As a result, surface waters in urban settings may function not only as passive recipients of resistant bacteria but also as active sites of resistance amplification.

In low- and middle-income countries such as Bangladesh, environmental AMR risks are further intensified by limited wastewater treatment infrastructure and rapid urban expansion. Dhaka city, characterized by high population density and extensive anthropogenic activity, experiences chronic pollution of its lakes and drainage systems. Previous studies investigating Dhaka's surface waters have reported high bacterial loads and widespread resistance among coliform and enteric bacteria, including the presence of multidrug-resistant and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (Haque et al., 2014; Mondal et al., 2024). These findings indicate that urban water bodies in Dhaka may serve as environmental reservoirs of clinically relevant resistance traits.

A central mechanism underlying the environmental dissemination of AMR is plasmid-mediated horizontal gene transfer. Plasmids are mobile genetic elements capable of carrying multiple resistance determinants and transferring them between bacteria, including across species boundaries. This ability enables rapid spread of resistance traits within heterogeneous bacterial communities and contributes to the emergence of multidrug-resistant strains (Bennett, 2008; Carattoli, 2009). In aquatic environments subjected to antibiotic and pollutant exposure, plasmid-bearing bacteria may gain a selective advantage, allowing resistance genes to persist even in the absence of direct antibiotic pressure (Martí et al., 2014).

Within Dhaka city, **Gulshan Lake and Dhanmondi Lake** represent prominent urban water bodies embedded within residential, commercial, and healthcare-dense zones. Both lakes are subject to varying degrees of anthropogenic influence and receive runoff and wastewater inputs from surrounding areas. Despite their environmental and public relevance, comparative assessments of antibiotic resistance patterns and plasmid occurrence between these two lakes remain limited. Addressing this gap is important for understanding how urban freshwater systems contribute to environmental AMR dynamics and for identifying potential differences in resistance burden associated with site-specific pollution pressures.

Building upon existing evidence of environmental AMR in Dhaka, the present study integrates culture-based isolation, antimicrobial susceptibility testing, and plasmid screening to generate a comparative profile of bacterial resistance in Gulshan Lake and Dhanmondi Lake. This approach provides a framework for linking phenotypic resistance patterns with potential genetic mechanisms of dissemination, thereby informing the objectives outlined in the following section.

1.8 Objectives of the Study

The primary objective of this study is to investigate and compare the antibiotic resistance profiles and plasmid occurrence among bacterial isolates of *Salmonella spp., Escherichia coli*, and *Vibrio spp* recovered from two major urban water bodies of Dhaka city, namely Gulshan Lake and Dhanmondi Lake. By integrating phenotypic antimicrobial susceptibility testing with plasmid

screening, the study aims to provide insight into the role of urban freshwater environments in the persistence and dissemination of antimicrobial resistance. Specifically, this study seeks to isolate bacteria from water samples collected from Gulshan Lake and Dhanmondi Lake over a defined sampling period and to characterize their resistance patterns using standardized antimicrobial susceptibility testing procedures. The study further aims to assess the prevalence of multidrug resistance among these isolates and to determine the distribution of plasmid-bearing bacteria within and between the two lakes. Through comparative analysis, the research also aims to identify potential differences in resistance burden that may be associated with site-specific environmental conditions and anthropogenic pressures. In addition, the study intends to explore the relationship between plasmid presence and observed resistance phenotypes, thereby contributing to an improved understanding of plasmid-mediated resistance dissemination in urban aquatic systems. Collectively, these objectives are designed to generate baseline data that can support future environmental surveillance efforts and inform strategies to mitigate the spread of antimicrobial resistance in urban environments.

1.9 Justification of the Study

Antimicrobial resistance represents a growing global threat with serious implications for public health, environmental sustainability, and disease management. While clinical surveillance of resistant pathogens is well established, environmental surveillance remains comparatively limited, particularly in developing countries. Urban surface waters, which receive continuous inputs of wastewater and runoff, provide a critical interface where resistant bacteria and resistance genes can persist and circulate beyond clinical settings. Investigating these environments is therefore essential to achieving a comprehensive understanding of AMR from a One Health perspective.

Dhaka city presents a particularly relevant context for environmental AMR research due to its high population density, rapid urbanization, and inadequate wastewater management infrastructure. Lakes within the city are frequently exposed to domestic sewage, hospital effluents, and urban runoff, creating conditions conducive to the survival and spread of resistant bacteria. Gulshan Lake and Dhanmondi Lake are widely used and publicly accessible water

bodies, making them potential points of human–environment interaction and exposure. Comparative assessment of these lakes is justified by their differing levels of anthropogenic influence and management, which may impact microbial composition and resistance patterns.

The inclusion of plasmid screening in this study is especially important, as plasmids play a central role in horizontal gene transfer and the rapid dissemination of resistance determinants across bacterial populations. Understanding whether resistant bacteria in urban lakes carry plasmids provides valuable insight into the potential for resistance genes to spread within environmental microbial communities and beyond. By combining culture-based isolation, standardized antimicrobial susceptibility testing, and plasmid detection, this study addresses a critical gap in environmental AMR research in Bangladesh.

Ultimately, the findings of this research are expected to contribute to baseline knowledge on antibiotic resistance in urban aquatic systems of Dhaka. Such information is necessary for risk assessment, environmental management, and the development of targeted interventions aimed at reducing the environmental spread of antimicrobial resistance. The study also provides a foundation for future research incorporating molecular characterization and long-term monitoring of resistance dynamics in urban water bodies.

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 was conducted over a six-month period, from January to June 2025. Water samples were taken at regular intervals during the study period to capture seasonal change and ensure representative bacterial isolation from both study sites.

2.3: Overview of the experimental procedure

The overall experimental workflow of the study was conducted in a stepwise manner as outlined below:

▪ Collection of water samples from Gulshan Lake and Dhanmondi Lake at designated sampling points during the early morning hours (7:00–7:30 AM).

▪ Proper labeling, handling, and transportation of collected water samples to the laboratory under sterile conditions.

▪ Pre-culture preparation of samples followed by inoculation onto selective and differential culture media (EMB, SS, and TCBS agar).

▪ Incubation of inoculated plates at 37 °C for 18–24 hours to allow bacterial growth.

▪ Selection and purification of distinct bacterial colonies based on morphological characteristics.

▪ Assignment of unique sample identification codes for each isolate based on lake, month, medium, and isolate number.

▪ Isolation of a total of 390 bacterial colonies, among which 367 isolates exhibited sufficient growth for antimicrobial susceptibility testing.

▪ Performance of antibiotic susceptibility testing (AST) using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar following CLSI 2020 guidelines.

▪ Measurement of zones of inhibition and interpretation of results as Susceptible (S), Intermediate (I), or Resistant (R).

▪ Extraction of bacterial DNA using the boiling lysis method by exposure to 100 °C in a water bath.

▪ Amplification of target DNA segments using the Polymerase Chain Reaction (PCR) technique.

▪ Isolation of plasmid DNA from selected bacterial isolates using standard plasmid extraction procedures.

▪ Analysis of genomic DNA and plasmid DNA using agarose gel electrophoresis.

▪ Documentation, compilation, and analysis of experimental data for comparative assessment between Gulshan Lake and Dhanmondi Lake.

2.4: Laboratory Equipment and Instruments

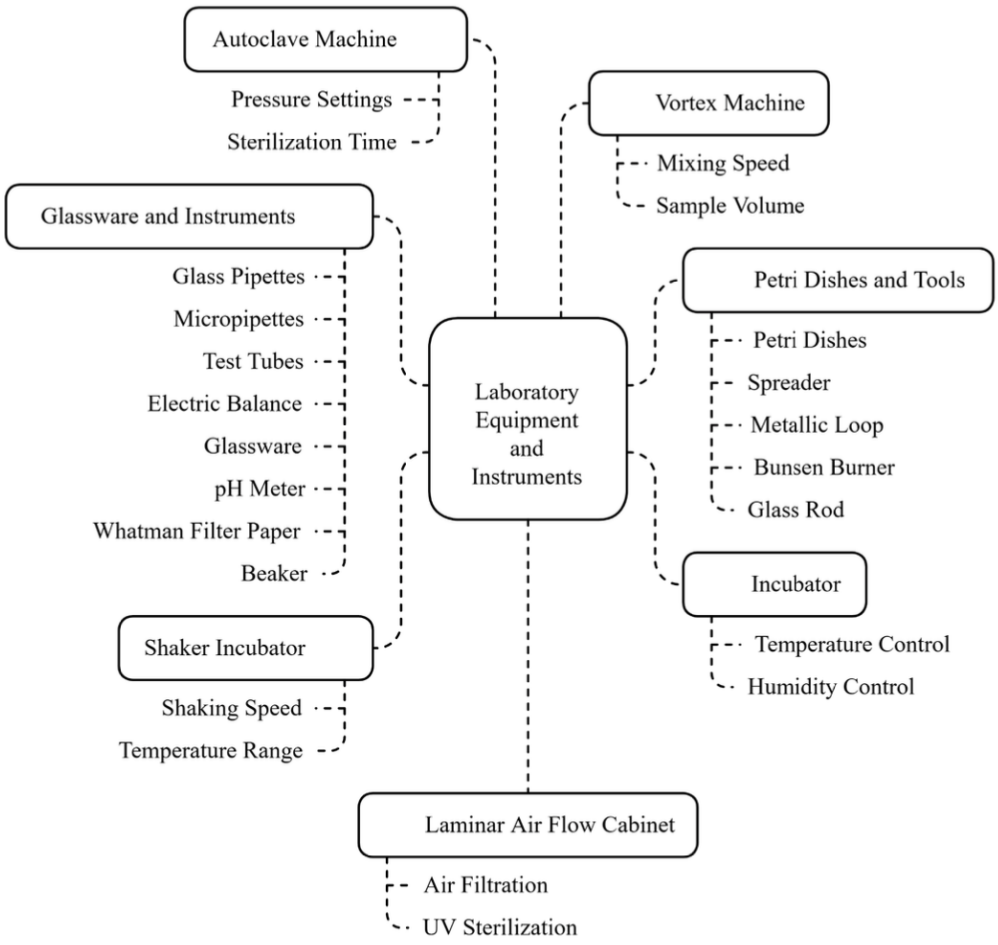


Figure 04: Laboratory equipment and instruments used for this experiment.

2.5 Collection, Handling and Transportation of Water Samples

Water samples were collected from Dhaka's Gulshan Lake and Dhanmondi Lake, both located in Dhaka city of Bangladesh. The selected areas have substantial anthropogenic activity and contact with pollutants, which may impact diversity of microbes and antibiotic resistance trends in water bodies. The surface water was collected at each site with sterile, pre-labeled sampling containers. Upon arrival at BRAC University's Biotechnology, Microbiology, and Molecular Biology Laboratory, samples were examined right away to ensure correct microbiological examination and prevent degradation or contamination.

2.6: Pre-culture preparation of bacteria

To facilitate bacterial separation from water samples, at first filtration step was performed using 0.4 mm Whatman filter paper, which successfully removed particulate matter and debris. The filtered 4 ml sample was pipetted into a sterile autoclaved Falcon tube with 10 ml of LB broth to increase the bacterial population. After that, the enrichment culture was cultivated for 4 hours at 37°C in a shaking incubator to promote bacterial growth. Following that, a serial dilution method was used to lower bacterial concentration and determine viable counts. To dilute the enriched culture, 1 ml was added to 9 ml of LB broth and serially diluted in sterile autoclaved test tubes with a 10^{-1} dilution ratio. Additionally, 80 µl of raw (unprocessed) material was plated on selective culture medium to multiply the target bacterial species.

2.7 Culture Media Employed for Bacterial Growth

Selective and differential culture media were used to isolate specific groups of bacteria from the water samples. The use of multiple media allowed effective recovery of enteric and aquatic bacteria with distinct biochemical characteristics.

2.7.1 Eosin Methylene Blue (EMB) Agar

EMB agar was employed for the isolation and differentiation of coliform bacteria, particularly *Escherichia coli*. The medium contains eosin and methylene blue dyes, which inhibit Gram-positive bacteria and differentiate lactose fermenters. After incubation, colonies displaying

a metallic green sheen or dark-centered appearance were considered presumptive *E. coli* and selected for further analysis.

Figure 05: Fresh plate of EMB agar.

2.7.2 *Salmonella Shigella* (SS) Agar

SS agar was used for selective isolation of *Salmonella* and *Shigella* species. The medium suppresses Gram-positive organisms and differentiates bacteria based on lactose fermentation and hydrogen sulfide production. Colorless colonies with or without black centers were identified as presumptive *Salmonella* isolates and subjected to sub-culturing.

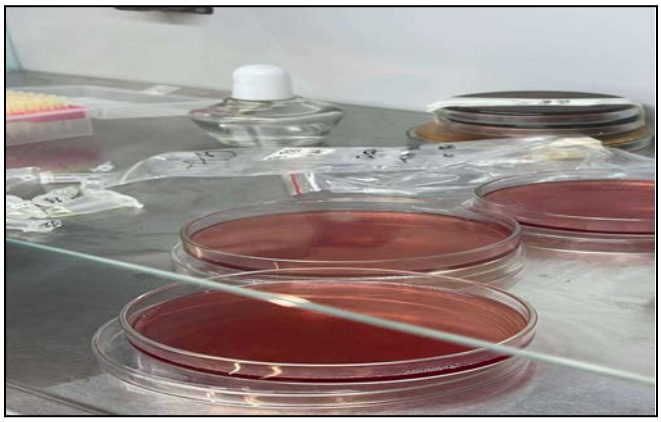


Figure 06: Fresh plate of SS agar.

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

TCBS agar was used for isolation of *Vibrio* species from water samples. The high pH and bile salts inhibit non-*Vibrio* organisms. Yellow colonies indicated sucrose fermentation, whereas green colonies represented non-sucrose fermenting *Vibrio* species. Morphologically distinct colonies were selected for purification.

Figure 07: Fresh plate of TCBS agar

2.8 Buffer Formulation

Buffers required for molecular and electrophoretic procedures were prepared using analytical-grade reagents and distilled water. All solutions were prepared following standard laboratory protocols to ensure accuracy and reproducibility. Prepared buffers were stored under appropriate conditions to maintain stability and effectiveness during experimental use.

Table 01: Formulation of buffer

1x TE Buffer	50x TAE Buffer	Running Buffer
● The solution was supplemented with 10	● A measured volume of 150 milliliters of	● A measured volume

mM Tris-HCl and 10 mM EDTA. ● 10 mM of distilled water was then added. ● The pH was adjusted to 8.3. If the pH was higher than 8.3, hydrochloric acid (HCl) was added to lower it, while sodium hydroxide (NaOH) pellets were added to raise the pH. ● To reach the desired volume of 20 ml, more distilled water was added. ● pH was measured to ensure accuracy.	distilled water was poured into a clean beaker. ● Tris base (24.2 g), glacial acetic acid (5.71 mL), and EDTA (3.72 g) were added to the distilled water. The ig solution was mixed thoroughly using a magnetic stirrer for 3 to 5 minutes until all components were properly dissolved. ● The pH of the solution was adjusted to 8.0. When the pH exceeded 8.0, glacial acetic acid was added dropwise to lower the pH. Conversely, when the pH fell below 8.0, sodium hydroxide (NaOH) pellets were added carefully to raise the pH to the desired level. ● After pH adjustment, the volume of the solution was increased to 250 milliliters by adding additional distilled water. ● Finally, the pH was re-measured to confirm accuracy, and the prepared 50× TAE buffer was stored under appropriate laboratory conditions for subsequent use in agarose gel electrophoresis.	of 490 milliliters of distilled water was poured into a clean beaker. ● 10 milliliters of 50× TAE buffer was added to the distilled water to achieve a final working concentration of 1× TAE. The solution was mixed thoroughly using a magnetic stirrer for 2 to 3 minutes to ensure uniform dilution. ● The pH of the solution was measured using a pH meter to confirm that it remained close to pH 8.0. Minor adjustments were made only if necessary to maintain optimal buffering conditions. ● The final volume of the solution was adjusted to 500 milliliters using additional distilled water. ● The prepared 1× TAE running buffer was then used for agarose gel electrophoresis, ensuring proper ionic strength and conductivity during DNA migration.

2.9 Microbial Inoculation Procedures

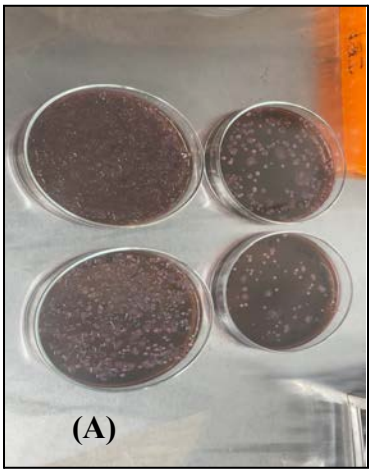
Microbial inoculation was carried out using aseptic techniques under laminar airflow conditions. Both spread plate and streak plate methods were employed to achieve optimal isolation and purification of bacterial colonies.

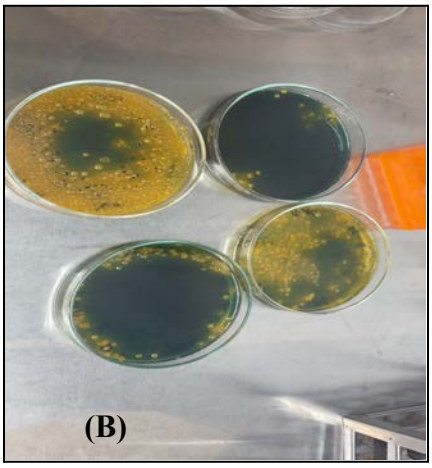
2.9.1: Spread Plate Method

The spread plate method was employed to ensure uniform distribution of bacterial cells on the surface of solid agar media for effective colony development. Initially, water samples were inoculated into Luria–Bertani (LB) broth and incubated in a shaker incubator under controlled conditions to promote bacterial growth and homogeneity of the culture. After shaker incubation, both raw (undiluted) LB broth cultures and 10^{-1} serially diluted samples were prepared for plating.

From each prepared sample, 80 microliters of the bacterial suspension were aseptically transferred onto the surface of pre-solidified selective agar plates. A sterile glass spreader was used to evenly distribute the inoculum across the agar surface. Prior to use, the spreader was sterilized by dipping in 70% ethanol followed by flaming to ensure aseptic conditions. The inoculum was spread gently in a circular motion to avoid damaging the agar surface and to achieve uniform bacterial dispersion.

After spreading, the plates were allowed to stand for a short period to permit absorption of the inoculum into the agar. The inoculated plates were then incubated at 37°C for 18–24 hours, following which discrete bacterial colonies were observed and selected for further purification and analysis.





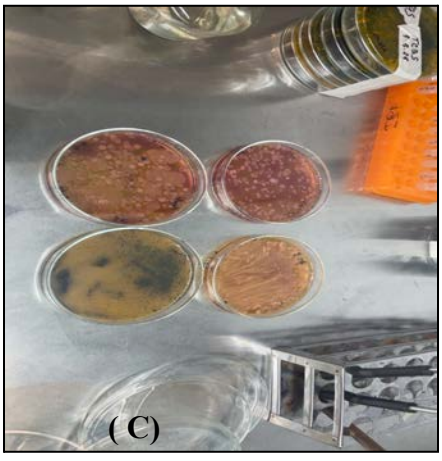


Figure 08- A) *E.coli* spread in EMB media, B) *Vibrio spp.* spread in TCBS media, C) *Salmonella spp.* spread in SS media by spread plate method .

2.9.2: Streak Plate Method

The streak plate technique is a simple microbiological method for isolating individual bacterial cultures and colonies from a population. The process involves gradually reducing bacterial density on the agar plate surface, allowing only a few cells to form isolated colonies in consecutive streaks. It can be used for both single-species culture separation and species isolation (Tankeshwar & Tankeshwar, 2024). The experiment involved streaking a spread plate technique colony. To create an initial smear, a tiny colony was streaked down the side of the agar plate using a sterile inoculating loop. The loop was used to streak along the agar plate and diminish bacterium concentrations in a methodical manner. After 18-24 hours of incubation at 37°C, we chose individual colonies and produced pure cultures. Pure cultures provided the foundation for later morphological and characterisation tests.

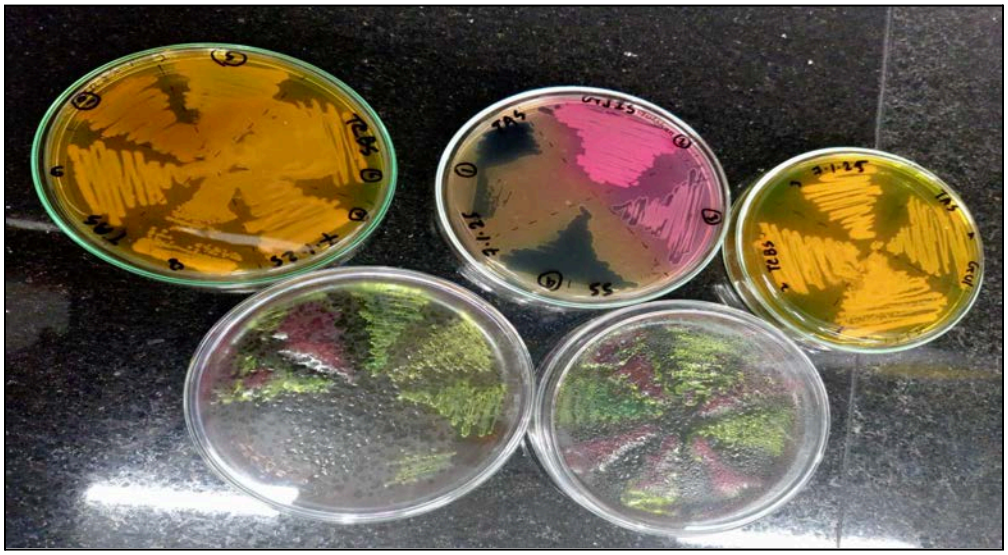


Figure 09: *Vibrio cholerae, Salmonella, E.coli* streaked from spread plate in another fresh plate

2.10: Morphological Identification of Cultured Organisms

Himedia analysis was used to identify cultivated microorganisms. The study compared colony morphology, including shape, size, color, edge, elevation, and texture, across several selected and differential culture conditions. Himedia's HiCromeTM Universal Agar Plate was used to compare colony growth patterns and looks. This method was used to group bacterial isolates and

assess their diversity before further confirmation through biochemical or molecular analysis (Libretexts, 2024).

Bacteria name	**Growth medium**	**Colony appearance**	**Metabolic activity**	**Identification of result**
Escherichia coli	Eosin Methylene Blue (EMB)	Green metallic sheen	Lactose fermentation	Positive for *E.coli*
Salmonella spp	*Salmonella-Shigella* (SS)	Black	Sucrose fermentation	Positive for *Salmonella*
Vibrio cholerae	Thiosulfate-Citrate-Bile-Salts-Sucrose (TCBS)	Yellow	H_2S	Positive for *Vibrio cholerae*

Table 02: Details of bacteria studied for the research

2.11: Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) evaluates the effectiveness of different antibiotics in preventing microbial growth under controlled laboratory circumstances (Khan et al., 2019). This study used the Kirby-Bauer disk diffusion method, a well-known method for assessing antimicrobial susceptibility. Using sterile cotton swabs, a consistent bacterial suspension was equally distributed over Mueller-Hinton Agar plates.

Antibiotic disks were applied to the inoculated agar and incubated at 37°C for 18-24 hours. After incubation, the zones around each disk were examined to evaluate bacterial isolates' susceptibility to antibiotics (resistant, intermediate, or susceptible).

2.11.1: Preparation of Bacterial Suspension

Prior to conducting the antibiotic susceptibility test, a bacterial suspension was prepared. Saline was utilized in this process. Each isolated sample received 10 ml of saline in an autoclaved glass vial. Using a sterile metallic loop, one loopful of bacteria from the 18-hour-old culture was collected and mixed. The saline and colony mixture was vortexed and pure bacterial colonies were emulsified in sterile normal saline solution to prepare a suspension matching the 0.5

McFarland turbidity standard. This standardization ensured uniform bacterial density for reliable susceptibility testing.

2.11.2 Mueller Hinton Agar (MHA)

Mueller Hinton Agar (MHA) was used as the standard medium for performing antimicrobial susceptibility testing due to its reproducible composition, uniform diffusion properties, and compatibility with the Kirby–Bauer disc diffusion method. The medium supports satisfactory growth of non-fastidious organisms and allows reliable diffusion of antimicrobial agents from discs into the agar matrix. Prepared MHA plates were checked for surface dryness and uniform thickness prior to inoculation to ensure consistent zone formation and accurate interpretation of inhibition diameters.

2.11.3 List of Antibiotics

A panel of antibiotics representing multiple antimicrobial classes was selected for susceptibility testing in order to evaluate resistance patterns among bacterial isolates recovered from Gulshan Lake and Dhanmondi Lake. The selection was based on the clinical relevance of these antibiotics, their widespread use in Bangladesh, and their importance in the treatment of enteric and waterborne bacterial infections. Antibiotic discs were obtained commercially and stored under recommended conditions to preserve potency.

List of antibiotics and their susceptibility and resistance level:

Antibiotic group	**Antibiotic name**	**Code**	**Disc concentration (mcg)**	**Diameter of Zone of inhibition**		
				Susceptible (S) (mm)	Intermediate (I) (mm)	Resistant (R) (mm)
Penicillins	Ampicillin	AMP	10	≥17	14–16	≤ 13
Aminoglycosides	Gentamici	CN	10	≥15	13–14	≤12

	n					
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	≥ 21	16–20	≤ 15
Macrolides	Erythromycin	E	15	≥ 18	14–17	≤ 13
Macrolides	Azithromycin	AZM	30	≥18	14-17	≤ 13
Glycopeptides	Vancomycin	VA	30	-	-	Intrinsic resistance
Amphenicols	Chloramphenicol	C	30	≥18	13-17	≤ 12

Cephalosporins (4th gen)	Cefepime	FEP	30	≥18	15-17	≤14
Cephalosporins (4th gen)	Cefepime	CPM	30	≥18	15-17	≤14

Table 3: List of antibiotics and their zones of inhibition

Note: Interpretive criteria for Azithromycin were based on published literature and internal laboratory practice. Vancomycin resistance in Gram-negative bacteria was considered intrinsic.

2.12 Isolation of DNA through Boiling Method

Genomic DNA was extracted from bacterial isolates using the boiling lysis method, a rapid and cost-effective technique commonly employed for bacterial DNA preparation. This method relies on heat-induced cell disruption to release intracellular DNA suitable for downstream molecular analysis. The workflow of this method is given below:

Bacterial stock culture was streaked on agar plates and incubated to obtain fresh bacterial growth

⬇

A loopful of fresh bacterial colonies was collected and transferred into a microcentrifuge tube (MCT) containing 500 µL of TE Buffer

⬇

The suspension was vortexed to ensure uniform mixing

⬇

The sample was centrifuged at 12,000 rpm for 5 minutes

⬇

The supernatant was carefully discarded

⬇

500 µL of TE Buffer was added to the bacterial pellet

⬇

The suspension was re-pipetted/ vortexed thoroughly

⬇

The tube was placed in a water bath at 100 °C for 10 minutes to lyse the bacterial cells

⬇

Immediately after heating, the sample was subjected to a cold shock at −20 °C for 5 minutes

⬇

The sample was then centrifuged at 12,000 rpm for 10 minutes

⬇

Approximately 400 µL of the supernatant containing crude genomic DNA was carefully transferred into a fresh microcentrifuge tube

⬇

The extracted DNA was labelled and stored appropriately at −20 °C and used as template DNA for PCR analysis

Figure 10 : Workflow of genomic DNA extraction from bacterial isolates using the boiling lysis method.

2.13 PCR Method

Individual fragments of DNA are difficult to study directly due to the limited quantity of template DNA available from environmental samples. Polymerase Chain Reaction (PCR) is a widely used molecular technique that enables selective amplification of specific DNA sequences, thereby generating sufficient quantities of target DNA for downstream analysis. PCR has extensive applications in molecular biology, clinical diagnostics, environmental microbiology, and genetic research, particularly for the detection and confirmation of bacterial species from complex samples (Saiki et al., 1988; Alberts et al., 2002).

2.13.1 PCR Procedure Steps

The PCR process involves a series of precisely controlled thermal steps that allow exponential amplification of the target DNA sequence. Each cycle consists of denaturation, annealing, and extension phases, which are repeated multiple times to increase the quantity of amplified DNA.

Initial Denaturation

Initial denaturation is required to activate the DNA polymerase and completely separate the double-stranded DNA template. In this study, the reaction mixture was heated to 95°C for 5 minutes, ensuring disruption of hydrogen bonds between complementary DNA strands and preparation of the template for amplification (Alberts et al., 2002).

Denaturation

Denaturation was performed at 95°C for 30 seconds in each cycle. During this step, the double-stranded DNA was converted into single strands, allowing primers to bind during the subsequent annealing step.

Annealing

Annealing allows primers to hybridize to their complementary sequences on the single-stranded DNA template. The temperature was lowered to 50–65°C for 20–40 seconds, depending on the melting temperature (Tm) of the primers. Annealing temperatures are typically set 3–5°C below the primer Tm to ensure specificity of binding while minimizing non-specific amplification (Saiki et al., 1988).

Extension (Elongation)

Extension was carried out at 72°C, the optimal temperature for Taq DNA polymerase activity. During this step, deoxynucleotide triphosphates (dNTPs) were incorporated in the 5′ to 3′ direction, resulting in synthesis of complementary DNA strands. The duration of this step varied depending on the expected amplicon size, with Taq polymerase extending approximately 1,000 base pairs per minute under optimal conditions (Alberts et al., 2002).

Final Extension

Following completion of the amplification cycles, a final extension step was conducted at 72°C for 5 minutes. This step ensured complete extension of partially synthesized DNA strands remaining from previous cycles, resulting in fully amplified PCR products.

Final Hold

After final extension, the reaction was held at 4°C to preserve the amplified DNA products until further processing. This step prevents degradation of PCR products prior to electrophoretic analysis.

2.13.2 Components of PCR

The PCR reaction mixture consisted of template DNA, forward and reverse primers, Taq DNA polymerase, Mg^{2+} ions, reaction buffer, and dNTPs. Each component plays a critical role in amplification: primers provide specificity, Mg^{2+} acts as a cofactor for enzyme activity, the buffer maintains optimal pH and ionic strength, and dNTPs serve as building blocks for new DNA strand synthesis.

2.13.3 Equipment of PCR

Thermo-cycler machine: This apparatus provides the optimal temperature for the three stages of the PCR procedure. The denaturation, annealing, and extension processes are made easier by PCR's precise and regulated temperature control. The word "cycler" for the equipment originated from the fact that PCR is repeated based on the size of the gene.

PCR tubes: Because they help to improve results by preventing evaporation during PCR, these tubes are especially useful to work with.

PCR reaction preparation:

Table 4: PCR components

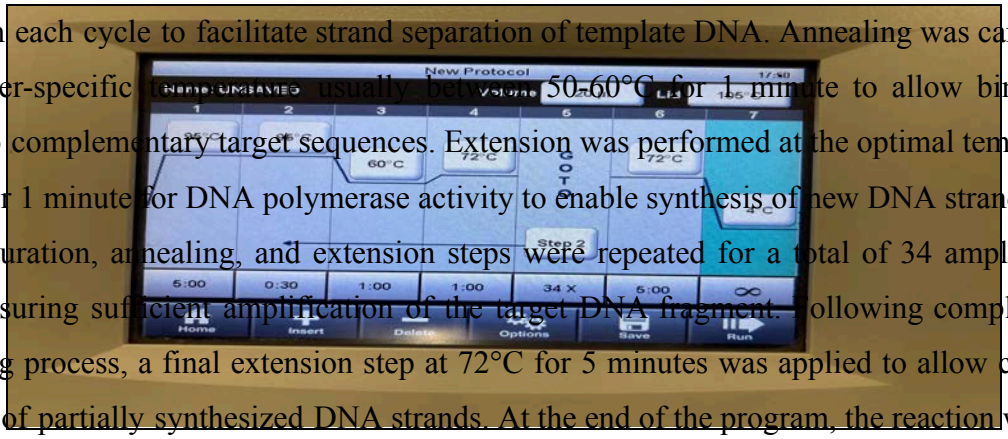
Component	Volume per sample
Nuclease-free water (NF)	1µl
Master mix	10µl
Isolated DNA	5µl
Forward primer (Fp)	2µl

Reverse Primer (Rp)	2µl
	Total volume: 20µl

2.13.4 Procedure of PCR Machine Run

PCR amplification was performed using a programmable thermal cycler following standardized operating procedures. Prepared PCR reaction mixtures with an appropriate final reaction volume (20-25 µL) were transferred into PCR tubes and placed into the thermocycler block. The heated lid temperature was set to prevent condensation and evaporation of the reaction mixture during thermal cycling.

The PCR program was initiated with an initial denaturation step at 95°C for 5 minutes, to ensure complete separation of double-stranded DNA and activation of the DNA polymerase. This step was followed by repeated amplification cycles consisting of denaturation, annealing, and extension phases.During denaturation, the reaction temperature was raised to 95°C for 30 seconds in each cycle to facilitate strand separation of template DNA. Annealing was carried out at a primer-specific [illegible] usually between 50-60°C for 1 minute to allow binding of primers to complementary target sequences. Extension was performed at the optimal temperature at 72°C for 1 minute for DNA polymerase activity to enable synthesis of new DNA strands.



The denaturation, annealing, and extension steps were repeated for a total of 34 amplification cycles, ensuring sufficient amplification of the target DNA fragment. Following completion of the cycling process, a final extension step at 72°C for 5 minutes was applied to allow complete extension of partially synthesized DNA strands. At the end of the program, the reaction was held at 4°C for an indefinite period until the PCR products were removed from the thermal cycler for subsequent analysis by agarose gel electrophoresis.

Figure 11 : Thermal cycling profile of the PCR program used for molecular detection of *Vibrio* species.

2.13.5 Thermal cycle

Initial denaturation	95℃ (5 min)
Denaturation	95℃ (30 sec)
Annealing	*E.coli:* 58℃, *Salmonella:* 51.7℃, *Vibrio:* 60℃
Elongation	72℃ (1 min)
Final elongation	72℃ (5 min)
Storage	4℃ (short term), -20℃ (long term)

Table 5: Required step for each step of thermal cycle

2.13.6: Condition of Primer

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’-GTATTGTTGATTAATGACATCCG-3’
Vibrio reverse	5’-ATATTACGCTACGGAAACACGTT-3’

Table 6: Primer sequence of three different bacteria

2.14. Plasmid Isolation

Plasmid isolation is based on the structural and topological differences between bacterial chromosomal DNA and plasmid DNA. Plasmids are small, circular, and highly supercoiled extrachromosomal DNA molecules, whereas chromosomal DNA is larger and less compact. When bacterial cells are exposed to alkaline lysis conditions, chromosomal DNA and cellular proteins undergo denaturation and precipitation, while plasmid DNA remains soluble due to its covalently closed circular structure. This differential behavior allows selective separation and purification of plasmid DNA from cellular components (Birnboim & Doly, 1979).

The alkaline lysis method remains one of the most widely used approaches for plasmid extraction because it is simple, efficient, and suitable for routine molecular analysis. Subsequent organic extraction and alcohol precipitation steps further enhance the purity of plasmid DNA by removing residual proteins, lipids, and other contaminants, yielding DNA suitable for downstream applications such as PCR and agarose gel electrophoresis (Sambrook & Russell, 2001).

2.14.1 Solutions used for Plasmid Isolation

Solution I: This protocol's main buffer, Solution I, contains 50 mM Tris-HCl, 10 mM EDTA, and 50 mM glucose, with a pH of 8.

Solution II: A 0.2 M NaOH and 1% SDS solution is used to lyse cells and denaturate genomic and plasmid DNA, as well as proteins. The combination of NaOH's alkaline conditions and SDS's detergent activity effectively breaks down double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA).

Solution III: Alkaline lysate is neutralized using a solution that contains glacial acetic acid and 3 M potassium acetate to pH 5.2. Large molecular sizes prevent adequate renaturation, resulting in precipitation of chromosomal DNA and proteins.

2.14.2 Plasmid Isolation Procedure (Workflow)

Bacterial isolates were processed for plasmid extraction following the workflow outlined below:

Bacterial stock culture streaked on agar plates

⬇

Isolates showing poor growth were inoculated into LB broth

⬇

LB broth cultures incubated in a shaker incubator for 24–48 hours

⬇

Properly grown cultures selected for plasmid isolation

⬇

Microcentrifuge tubes (MCTs) labeled and 200 µL of Solution I added

⬇

A loopful of fresh bacterial colony transferred into MCT

⬇

Contents vortexed to ensure complete resuspension

⬇

400 µL of freshly prepared Solution II added

⬇

Mixed by gentle inversion

⬇

Incubated at room temperature for 5 minutes for alkaline lysis

⬇

300 µL of Solution III added

⬇

Mixed by gentle inversion

⬇

Incubated at 4°C for 10 minutes

⬇

Centrifuged at 12,000 rpm for 15 minutes

⬇

Supernatant collected carefully into a fresh MCT

⬇

Equal volume of Phenol-Chloroform-Isoamyl alcohol (PCI) added

⬇

Mixed by vortexing or gentle inversion

⬇

Centrifuged at 12,000 rpm for 10 minutes

⬇

Upper aqueous layer transferred into a new MCT

⬇

Equal volume of Chloroform-Isoamyl alcohol (CI) added

⬇

Mixed by gentle inversion for 3-4 minutes

⬇

Centrifuged at 12,000 rpm for 10 minutes

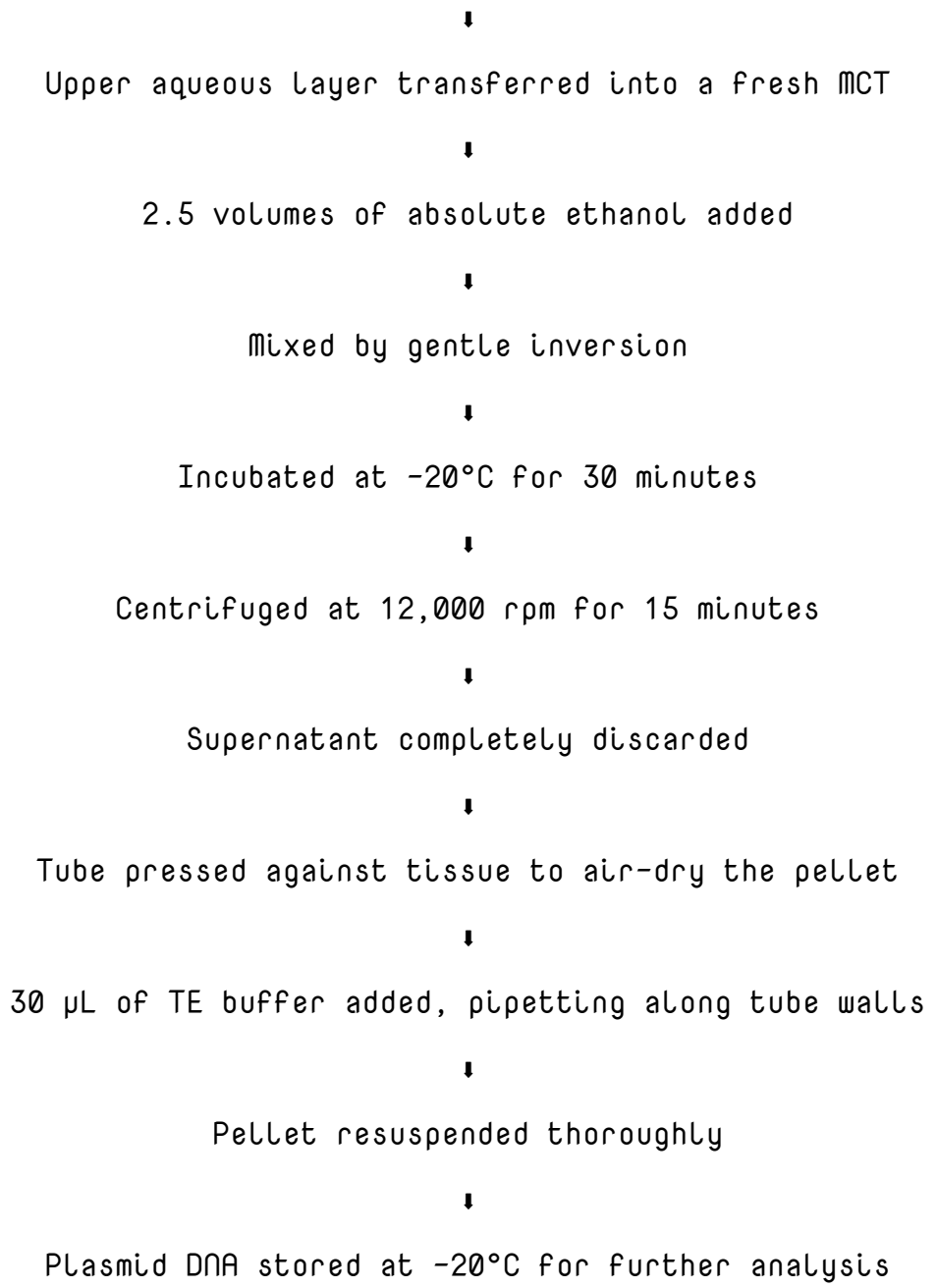


Figure 12: Procedure of plasmid isolation.

2.15 Agarose Gel Electrophoretic Analysis of DNA and Plasmid Constructs

Agarose gel electrophoresis was performed to analyze amplified PCR products as well as isolated plasmid DNA and to assess their integrity and approximate molecular size. This technique separates nucleic acid fragments based on size by applying an electric field through an

agarose matrix, allowing DNA fragments to migrate toward the anode due to their negatively charged phosphate backbone. Following separation, DNA bands were visualized using an intercalating dye under ultraviolet illumination to confirm successful amplification and plasmid isolation (Lee et al., 2012).

2.15.1 Formulation of Gel in Agarose Gel Electrophoresis

Agarose gel was prepared following standard electrophoretic procedures to allow effective separation of DNA fragments.

One gram (1 g) of agarose powder was accurately weighed and transferred into a clean conical flask.

↓

98 mL of distilled water and 2 mL of 50× TAE buffer were added to the flask and mixed gently to ensure uniform suspension.

↓

The mixture was heated until the agarose powder dissolved completely, producing a clear and homogeneous solution without visible particles.

↓

The molten agarose solution was allowed to cool slightly to a tolerable temperature.

↓

A suitable amount of ethidium bromide (EtBr) was added to the cooled agarose solution and mixed gently to ensure even distribution of the intercalating dye.

↓

The gel casting tray was prepared, and the molten agarose solution was carefully poured into the tray.

↓

A comb was placed appropriately to form wells for sample loading.

↓

The gel was allowed to solidify at room temperature for approximately 20–25 minutes.

↓

After complete solidification, the comb was gently removed, and the gel was made ready for electrophoretic analysis.

Figure 13: Workflow of gel formation.

2.15.2 Gel Electrophoresis

After obtaining PCR products and isolated plasmid DNA, agarose gel electrophoresis was carried out to confirm the presence of DNA bands. The following procedures were performed:

For PCR DNA

1. Agarose gel was prepared according to the standard protocol, and the comb was inserted before gel solidification. After complete solidification, the comb was carefully removed to form wells.
2. The gel tray was placed into the electrophoresis chamber and filled with an electrophoresis buffer until the wells were completely submerged.
3. PCR products were directly loaded into the wells without the addition of any extra reagents.
4. DNA molecular weight markers (1 kb ladder and 50 bp ladder) were loaded into separate wells to estimate the size of amplified PCR products.
5. The gel was run at 100 V for approximately 40 minutes.
6. After completion of electrophoresis, DNA bands were visualized under short-wave ultraviolet (UV) light.

For Plasmid DNA

1. The gel tray was placed into the electrophoresis chamber and covered with an electrophoresis buffer up to the maximum fill line.
2. Isolated plasmid DNA samples were prepared by mixing 8 µL of plasmid DNA with 2 µL of loading dye.
3. The prepared plasmid DNA samples were carefully loaded into the wells.
4. A 1 kb DNA ladder was loaded into a separate well to estimate plasmid DNA size.
5. Electrophoresis was carried out at 100 V for 30–40 minutes.
6. After completion of the run, plasmid DNA bands were visualized under short-wave ultraviolet (UV) light.

Chapter: 3

Result

3.1: Labeling the isolated colonies:

Water samples were collected from two urban lakes in Dhaka city: **Dhanmondi Lake** and **Gulshan Lake**. Sampling was conducted **twice per month** from **January to June 2025**, resulting in **12 sampling events per lake** and a total of **24 water samples** over the full study period. Each sampling event was assigned a short identification code so that all downstream

colony isolation, purification, and antimicrobial susceptibility testing (AST) could be traced back to the original collection time.

To label each isolate consistently, a structured code format was followed:

- **Lake code:**
  D = Dhanmondi Lake
  G = Gulshan Lake

- **Month code:**
  January = **J**, February = **F**, March = **M**, April = **A**, May = **My**, June = **Jn**

- **Sampling event number within the month:**
  1 = first collection of the month (early-month)
  2 = second collection of the month (late-month)

- **Selective/differential medium code (based on target group):**
  E = EMB agar (for presumptive *E. coli*)
  S = SS agar (for presumptive *Salmonella/Shigella group*)
  X = XLD agar (for presumptive *Salmonella/Shigella group*)
  T = TCBS agar (for presumptive *Vibrio spp*.)

- **Isolate number:**
  The final number denotes the **colony/isolate** number picked from that sample and that medium (e.g., 01, 02, 03 …).

Therefore, isolate IDs followed the general pattern:
[Lake][Month][Sampling number][Medium][Isolate number]

For example:

- **GJ1E12** indicates the **12th isolate** recovered on **EMB agar** from **Gulshan Lake**, collected during the **first sampling event of January.**

- **DJ2T05** indicates the **5th isolate** recovered on **TCBS agar** from **Dhanmondi Lake**, collected during the **second sampling event of January**.

Using this labeling system, a total of **390** bacterial isolates were obtained across both lakes and all three media types. Among these, 357 isolates were subjected to AST, which included a total of **157** ***Escherichia coli*****, 117** ***Salmonella spp***., and **83** ***Vibrio spp*****.** (presumptively *Vibrio cholerae* and related species). Standard microbiological and biochemical identification confirmed these genera. Out of the 390 isolates, the remaining 33 could not be tested due to viability loss or contamination issues.

3.2 Verification of PCR-Amplified Genomic DNA by Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to verify the presence and quality of PCR-amplified genomic DNA from the isolates. Overall, the gels produced clear band separation and acceptable resolution, enabling differentiation of DNA fragments based on molecular size. In most samples, bands were observed at the expected region, supporting successful amplification. However, a small number of lanes showed faint bands and/or slight smearing, which may indicate partial DNA degradation, suboptimal template concentration, pipetting or loading inconsistencies, or variations in electrophoresis conditions (e.g., running time, voltage, or agarose concentration). Despite these minor inconsistencies, the gel profiles were sufficient to confirm PCR positivity for the majority of tested samples, and the results were recorded accordingly.

3.2.1: Gel Electrophoresis result of *E.coli*

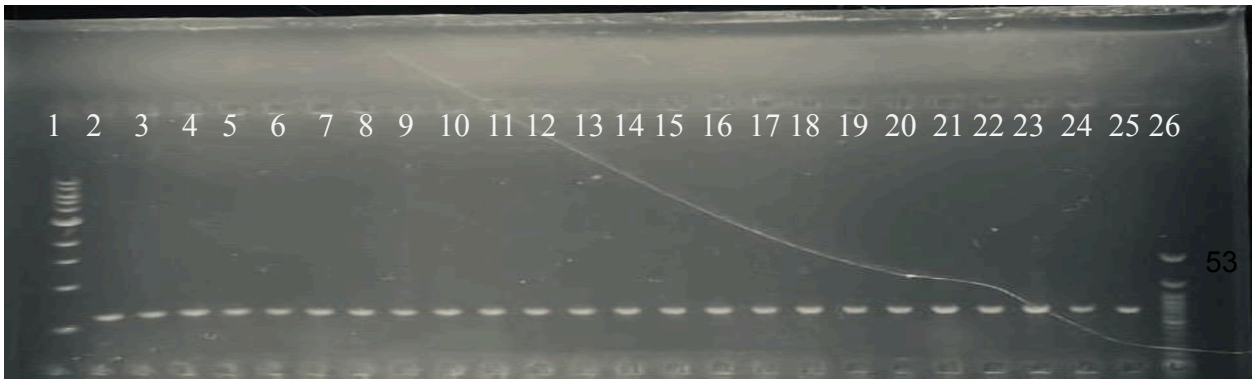


Figure 14 : Agarose gel electrophoresis image showing PCR amplification results from genomic DNA of *E. coli* isolates.

The agarose gel electrophoresis image shows PCR amplification results of genomic DNA from *Escherichia coli* isolates alongside DNA molecular weight markers. Lanes 1, 26, 27, 42 contain DNA ladders and serve as molecular size references for estimation of amplified fragment sizes. The ladder bands are clearly resolved and provide a reliable comparison standard for interpreting the migration of PCR products. Distinct and well-defined amplification bands are observed across the majority of sample lanes, indicating successful PCR amplification of *E. coli* genomic DNA. The amplified products in the sample lanes migrate at consistent positions relative to the DNA ladders, suggesting uniform amplification of the target DNA fragment. Based on comparison with the 50 bp and 1 kb DNA ladders, the amplified fragments are estimated to fall within the approximately 400–700 base pair range. Among the analyzed samples, clear amplification bands are visible in both the upper (lanes 2–24) and lower (lanes 27–40) portions of the gel, demonstrating positive PCR results for a large proportion of the tested isolates. Minor variations in band intensity were observed between lanes, which may be attributed to differences in template DNA concentration or amplification efficiency. The absence of prominent smearing or non-specific bands indicates good DNA quality and primer specificity.

Overall, the gel electrophoresis results confirm successful PCR amplification and positive molecular detection of *E. coli* genomic DNA in the majority of tested samples, with observed band migration patterns consistent with the expected amplification range.

3.2.2: Gel Electrophoresis result of *Salmonella spp*.

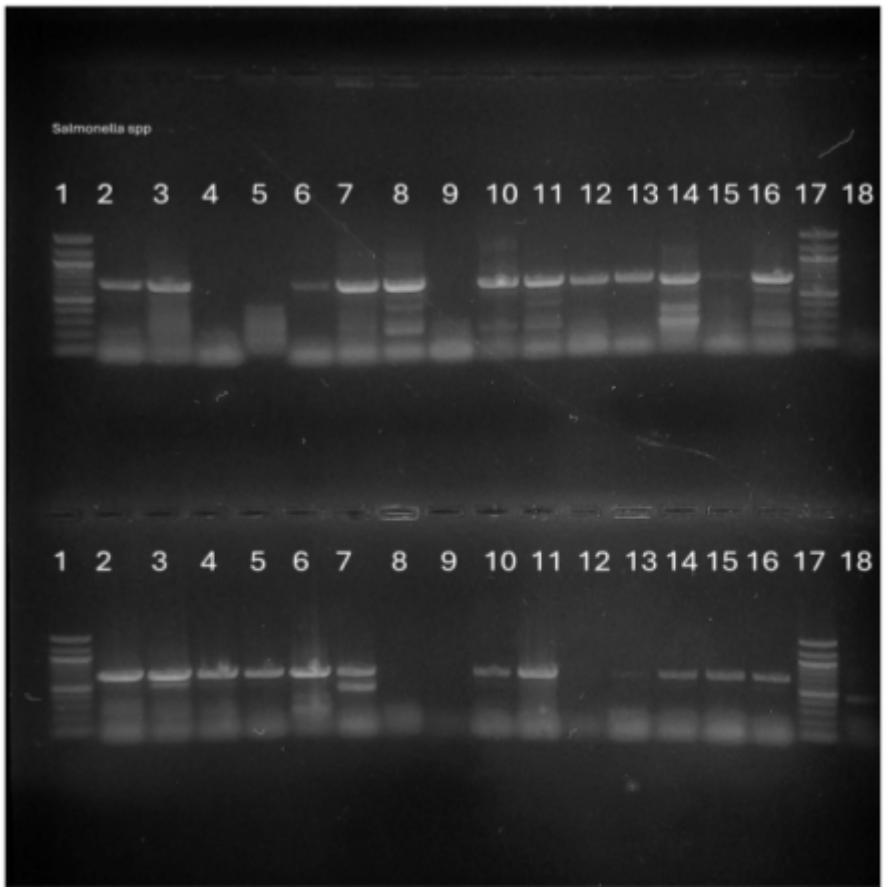


Figure 15: Agarose gel electrophoresis image showing PCR amplification results from genomic DNA of *Salmonella spp*. isolates.

The agarose gel electrophoresis image shows PCR amplification of *Salmonella spp.* genomic DNA alongside a 100 bp DNA ladder. Wells 1 and 17, positioned in both the upper and lower sets of wells, contain the ladders and serve as molecular size markers. The ladder bands are clearly resolved and provide a reliable reference for estimating the size of amplified fragments. The expected amplicon size for the target *Salmonella* DNA fragment is approximately 200–300 base pairs. In the gel, strong and distinct bands are observed in wells 2, 3, 6, 7, 8, 10, 11, 12, 13, 14, and 16 in the top set, and wells 2, 3, 4, 5, 6, 7, 10, 11, 14, 15, and 16 in the bottom set, indicating positive detection of *Salmonella* genomic DNA in those samples. In contrast, wells 4, 5, 9, 15, and 18 in the top set and wells 8, 9, 12, 13, and 18 in the bottom set show weak or negative amplification, as they lack visible bands or contain only faint bands. Overall, these

results support the observation that 22 out of the 30 test samples produced strong positive amplification for *Salmonella*.

3.2.3: Gel Electrophoresis result of *Vibrio cholerae*

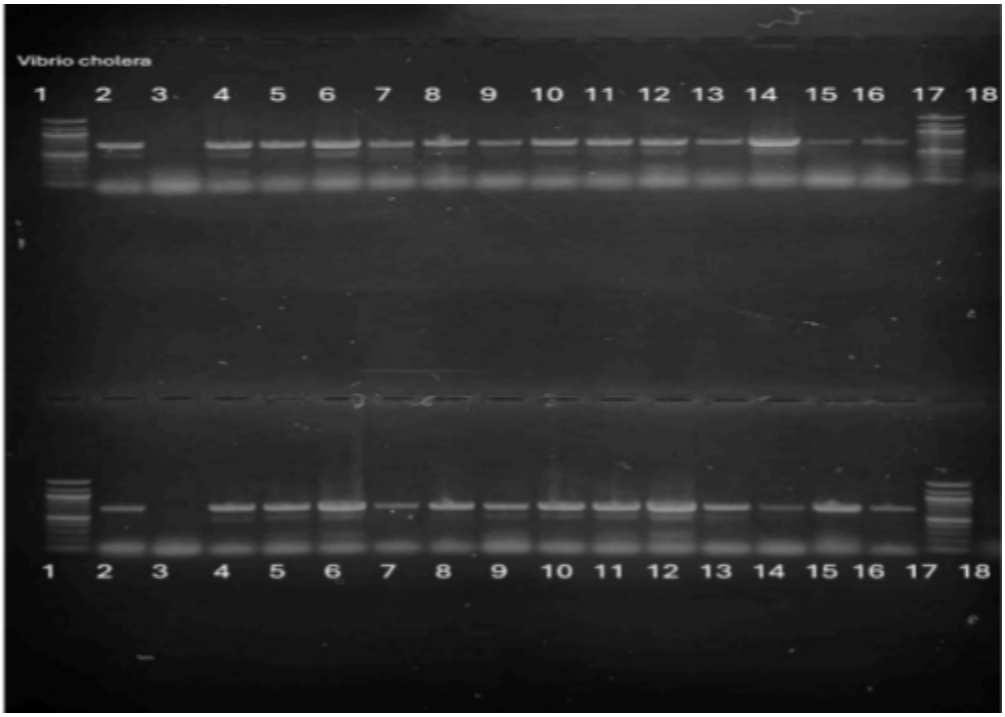


Figure 16: Agarose gel electrophoresis image showing PCR amplification results from genomic DNA of *Vibrio cholera* isolates.

The agarose gel electrophoresis image shows PCR amplification of *Vibrio cholerae* genomic DNA alongside a 100 bp DNA ladder used as a molecular size marker. The DNA ladders are loaded in wells 1 and 17 in both the upper and lower sets of wells. The ladder bands are clearly resolved and provide a reliable reference for estimating the size of amplified fragments. The expected amplicon size for the target *Vibrio* DNA fragment is approximately 200–300 base pairs. In the gel, strong and distinct bands are observed in wells 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 in the top set, and wells 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 in the bottom set, indicating positive detection of *Vibrio* genomic DNA in those samples. In contrast, well 3 in the top set and well 3 in the bottom set show negative amplification, as no visible band is present or

only extremely faint signal is detected. Overall, these results confirm the observation that 28 out of the 30 test samples produced strong positive amplification for *Vibrio*.

3.3: Antibiotic Susceptibility Results and Analysis:

E. coli

Table 7: Antibiotic Susceptibility Result of *E.coli* January

CODE	AMP 10	ATM 30	C 30	CPM 30	DO 30	E15	IPM 10	CN 10	LE 5	LZD 30	VA 30	AZM 30	K 30	FEP 30
GJ1E1	8.5	0	24.5	0	0	8.5	24	19.5	22.5	0	10	20	-	-
GJ1E2	0	26	26.5	21.5	16.5	0	27	17.5	27	0	0	13.5	-	-
GJ1E3	17.5	29.5	24	23.5	17	8.5	27.5	16.5	30.5	0	0	12	-	-
GJ1E4	7.5	26.5	23.5	21	15.5	5.5	25	16.5	15	0	0	10	-	-
GJ1E5	10	27.5	25.5	20.5	17	0	23	18	29.5	0	0	13	-	-
GJ1E8	0	28.5	22	21.5	10	0	28.5	20	0	0	10	0	-	-
DJ1E1	20	33	24.5	28	19.5	0	8.5	18.5	33	0	0	10.5	-	-
DJ1E2	0	11	23	0	10	9.5	24.5	17	18	0	0	17	-	-
DJ1E3	15	23.5	22	19	14.5	6.5	20	15	23	0	0	16	-	-
DJ1E4	10	27.5	25.5	20.5	17	0	23	18	29.5	0	0	16.5	-	-
DJ1E5	15	27.5	21.5	22.5	12	0	22	18	28	28	0	15.5	-	-
DJ1E6	10	26	22.5	19.5	15.5	0	21.5	17	24	0	0	10.5	-	-
DJ1E7	0	30	23.5	23.5	17	0	24	16.5	17	0	0	11	-	-
DJ1E8	8	29	26	22.5	16	0	24	17.5	24.5	0	0	9.5	-	-
GJ2E1	0	33	24	-	7	0	25	22	-	-	0	-	24	35
GJ2E2	0	10	22	0	14	0	27	16	16.5	0	0	10.5	-	-

GJ2E3	0	28.5	25.5	24	19	0	27	15	27	0	0	0	-	-
GJ2E4	21	30	24	24.5	22.5	9.5	29.5	17	30	-	10	14	-	-
GJ2E5	0	26	21	21	10	0	26	17	20	0	9	0	-	-
GJ2E6	0	29.5	25.5	21.4	22	0	30	17	28.5	0	0	12	-	-
GJ2E8	8	28	21	23	21	0	29.5	17	27	26.5	0	11	-	-
GJ2E9	22	30	24.5	25	20	11	28	17	29	-	9	19.5	-	-
GJ2E10	22	30	26	22	20	8.5	26	18.5	27	0	0	10	-	-
GJ2E12	0	28.5	25	22.5	19.5	0	23.5	17.5	28.5	0	0	11	-	-
GJ2E13	17	26.5	24	22	28	10	26	19.5	27	-	8	12	-	-
GJ2E14	17.5	30	25	25	13.5	11	29	20	20	0	10	22	-	-
DJ2E1	0	24	20	-	25	0	22	21	-	-	0	-	23	28
DJ2E2	0	27.5	27	21	19	0	25	16.5	24	0	9	17	-	-
DJ2E3	9	10	21	21	16	0	29	16.5	27.5	0	0	10	-	-
DJ2E7	10.5	27	23.5	27.5	17	0	28	17.5	25.5	0	0	11	-	-

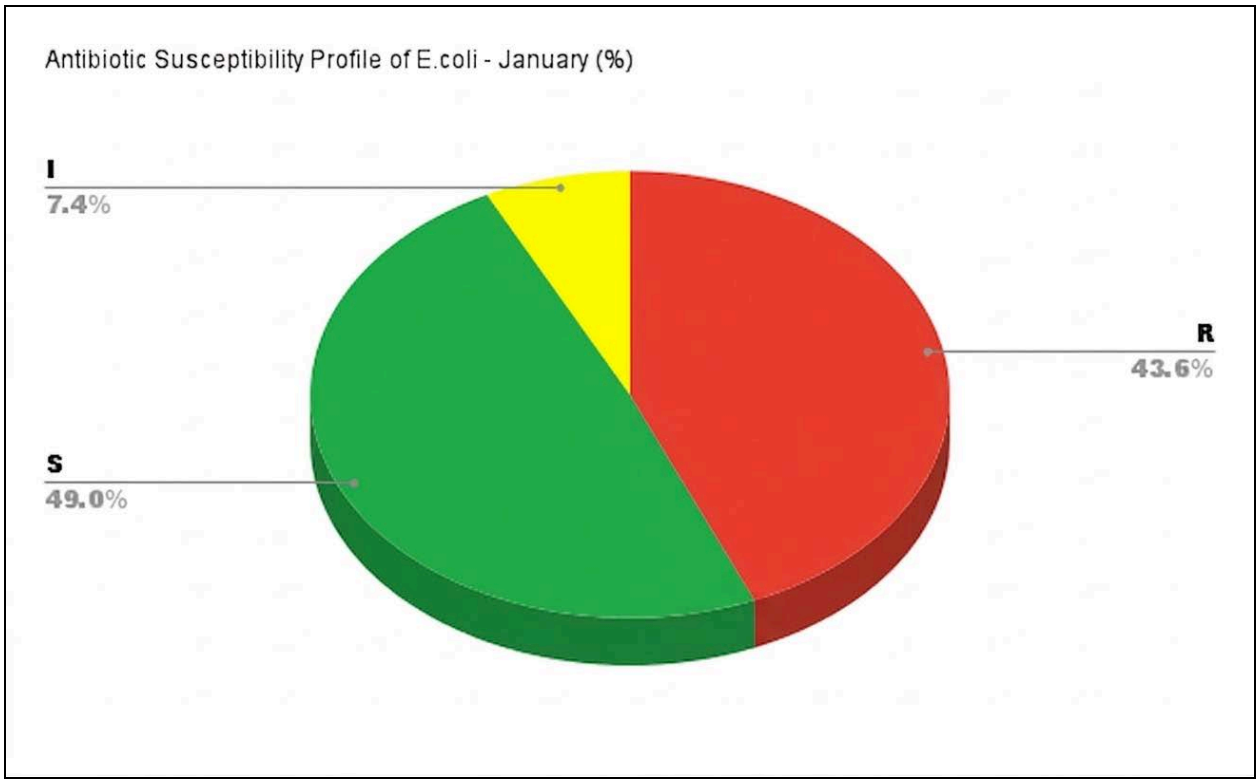


Figure 17: Antibiotic Susceptibility Profile of *E.coli* - January (%).

The pie chart illustrates the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in January. It shows that 43.6% of the isolates were resistant, 7.4% were intermediate, and 49.0% were susceptible to the 14 antibiotics tested.

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

Code	AMP10	ATM30	C30	DO30	E15	IPM10	CN10	K30	FEP30	VA30	AZM30	LE5	LZD30	CPM30
GF1E1	12	29	25	10.5	0	26	19	20	31	0	-	-	-	-
GF1E2	0	21	25	11.5	0	28.5	20	20	23.5	0	-	-	-	-
GF1E3	20	30	25	12	0	31	20	20	32	0	-	-	-	-
GF1E4	19	30	22.5	12	0	27.5	20.5	21	32	0	-	-	-	-
GF1E5	11	28	25	20	0	22	20	18	33	0	-	-	-	-
GF1E6	20	29	25	20	9	28	16.5	19.5	30	0	-	-	-	-
GF1E7	18	28	25	20	8	26.5	20	16.5	27	0	-	-	-	-
GF1E8	0	30	25.5	19.5	10	30	18	20	31	0	-	-	-	-
GF1E9	0	10	24	12	0	27	19	12	12	0	-	-	-	-

GF1E10	0	27.5	22	20	0	25	23	17.5	29	0	-	-	-	-
GF1E11	17	30	25.5	10	0	26	16.5	17.5	30	0	-	-	-	-
GF1E12	18.5	30	25	18	9.5	26.5	20	19	30	0	-	-	-	-
GF1E13	8.5	30	26.5	26	0	26	18	20	27	0	-	-	-	-
GF1E14	11	27	25.5	11	10	25	18	19	30	0	-	-	-	-
GF1E15	0	28.5	0	10	0	26.5	0	0	29	0	-	-	-	-
GF1E16	18	29	23.5	12.5	10	27	20	20	30	0	-	-	-	-
GF1E17	0	30	18	8	0	23	18	15	32.5	0	-	-	-	-
GF1E18	12	31	26	20	10	27	20	22	33	0	-	-	-	-
GF1E19	6	31	19	22	12	30	19	22	28	0	-	-	-	-
GF1E20	8	30	23	15	8.5	25	18	19.5	30	0	-	-	-	-
GF1E21	0	30	13	10	9	29	17	17	30	0	-	-	-	-
GF1E22	11	30	10.5	17.5	8	30	20	20	27	0	-	-	-	-
GF1E23	11	33	24.5	8	0	26.5	18.5	20	31	0	-	-	-	-
GF1E24	11	31	28	19	8	28	17.5	20	28	0	-	-	-	-
GF1E25	0	15	25	18	10	30	21	20.5	20	0	-	-	-	-
GF1E26	0	0	0	0	0	0	0	0	0	0	-	-	-	-
GF1E27	6	35	29	8	10	27.5	22	27	33	0	-	-	-	-
GF1E28	0	30	22	17	0	22.5	17	17	30	0	-	-	-	-
GF1E29	0	27	21	0	0	25	20	21	33	0	-	-	-	-
GF1E30	0	33.5	19.5	8	0	22	19	20	34	0	-	-	-	-
DF1E1	12	20	21.5	8	10	25	20	21	28.5	0	-	-	-	-
GF2E1	8.5	15	14	11	0	15	10	-	-	4	6.5	15	0	12
GF2E2	0	30	29	9	0	29	19	20	32	0	-	-	-	-
GF2E3	20	30.5	27	7	0	29	19	21	33	0	-	-	-	-
GF2E4	0	32	21	9	0	27	18	21.5	34	0	-	-	-	-
GF2E5	11	29	29	10	0	37	19	19	31.5	0	-	-	-	-
GF2E6	4.5	13	10	10	0	14.5	8.5	-	-	0	5	12.5	0	12
GF2E7	8.5	13.5	12	9.5	0	12	9	-	-	0	5	12.5	0	10.5
GF2E9	16	33	28	9	0	31	21.5	21	34	0	-	-	-	-

GF2E10	10	15	15	11	0	15	9	-	-	5	6	13.5	0	12.5
DF2E12	4	14	13	11	0	13	14	-	-	5	0	15	0	13

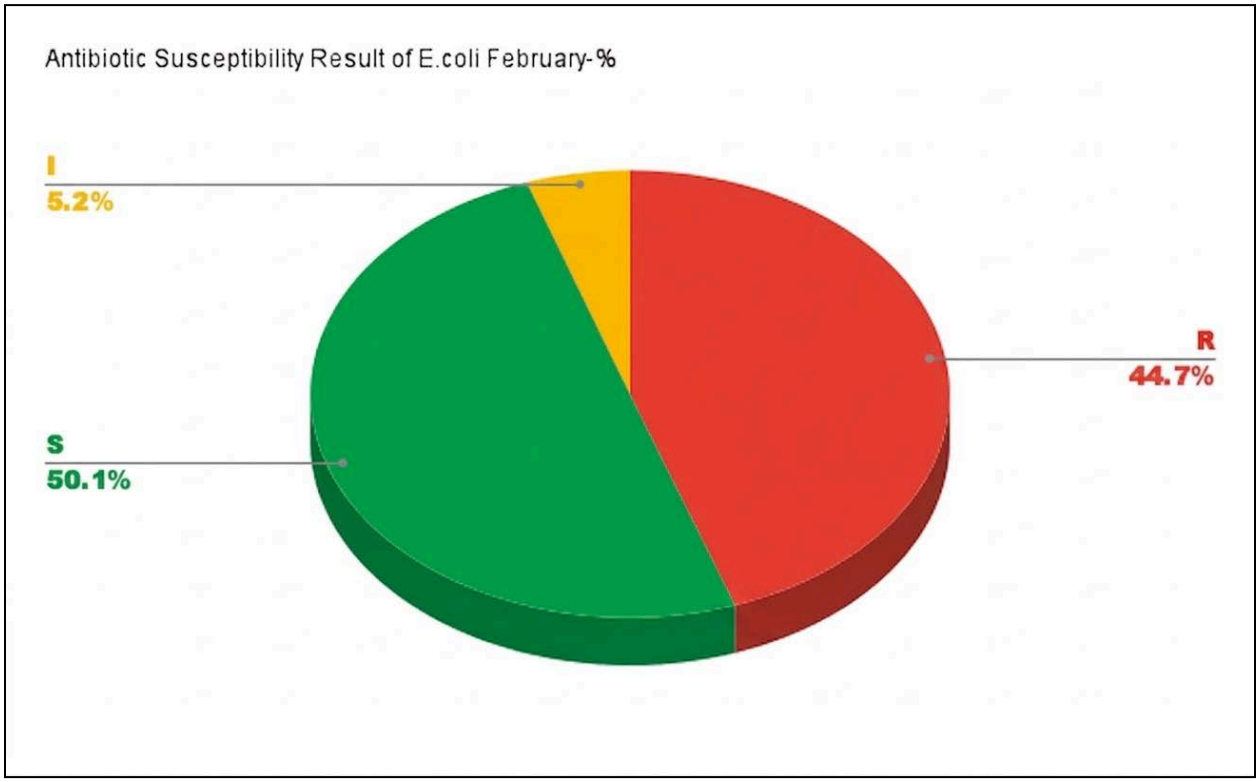


Figure 18: Antibiotic Susceptibility Profile of *E.coli* - February (%).

The pie chart summarizes the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in February. It indicates that 44.7% of the isolates were resistant, 5.2% were intermediate, and 50.1% were susceptible to the 14 antibiotics tested.

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

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	E15	AMP10	VA30
GM1E1	28	25	15	16	25	17	17	0	0	0
GM1E2	29	26	20	16	25	10.5	16	0	0	0
GM1E4	30	0	30	0	26	18	17	0	0	0
GM1E5	29	24	29	16	25	17	17	0	0	0
DM1E1	30	20.5	31.5	17.5	25.5	18.5	21	0	13.5	0
DM1E2	29	24	32	0	23.5	15	17	7	0	0

DM1E3	30	26	27	15	25	16	16.5	0	0	0
DM1E4	30	22	32	7	25	20	18.5	0	9	0
DM1E5	30	24	16	14	26	17	15	0	0	0
GM2E6	29	24	18	20	23	17	17	0	0	0
GM2E7	30	26	19	19	26	15	17	0	0	0
GM2E8	21.5	23.5	25	11	27.5	19	22.5	0	0	0
GM2E9	31.5	21	29	9	27	19	21	0	0	0
GM2E10	28	24	29	24	20	16	16.5	0	0	0
DM2E1	28.5	20	30	17.5	24.5	19	21	0	0	0
DM2E2	30	27	32	17	25	17	19	0	0	0
DM2E3	30	27	17	14	26	18	17.3	0	0	0
DM2E4	27	26	28	7	25	21	22	7	0	0
DM2E5	29	26	33	16	26	19	17	0	0	0

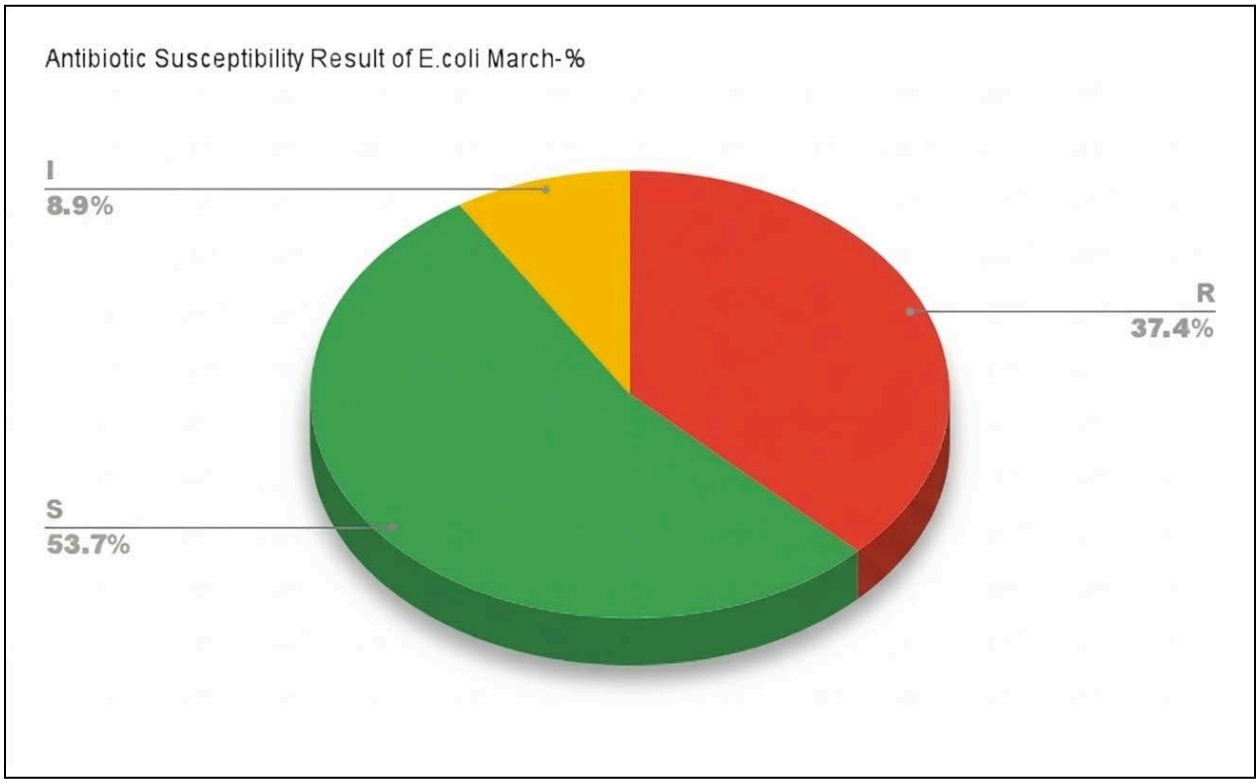


Figure 19: Antibiotic Susceptibility Result of *E.coli* March-%

The pie chart summarizes the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in March. It shows that 37.4% of the isolates were resistant, 8.9% were intermediate, and 53.7% were susceptible to the 10 antibiotics tested.

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

Code	AMP10	ATM30	C30	FEP30	DO30	E15	IPM10	CN10	K30	VA30
GA1E1	18	30	25	30	18	10	26	20	23.5	0
GA1E2	0	32	0	33	0	0	24	20	20	0
GA1E3	12	32	24	31	8	0	27	20	21	0
GA1E4	10	32	22	32	9	9	28	21.5	21	0
GA1E5	0	38	26	34.5	18	10	30	20	23.5	0
GA1E6	0	30.5	20.5	33	20	0	28	17.5	20	0
GA1E7	12	33	22	32	9	0	28	19	20	0
DA1E9	0	27	24.5	30	17	0	23.5	20	23	0
DA1E10	16	30	21	30	17	0	22.5	20	18	0
GA2E1	12	22	25	31	9	0	21	19	21	0
GA2E2	0	35	30	35.5	23	0	30	14	22.5	0
GA2E3	20	35	26	32.5	20	0	30	23	25	0
GA2E5	0	35.5	0	34.5	12	11.5	30	22	23	0
GA2E9	12	20	20	22	9	0	17	18	22	0
DA2E6	0	0	25	31	12	0	16	0	11	0
DA2E7	0	24.5	0	32.5	12	0	16	21	20	0
DA2E8	8	31.5	24	30	17	0	23.5	20	23	0
DA2E9	0	30	0	35	15	13.5	35	23.5	23	0
DA2E10	11	32	26	34	13	11	31	19.5	23	0

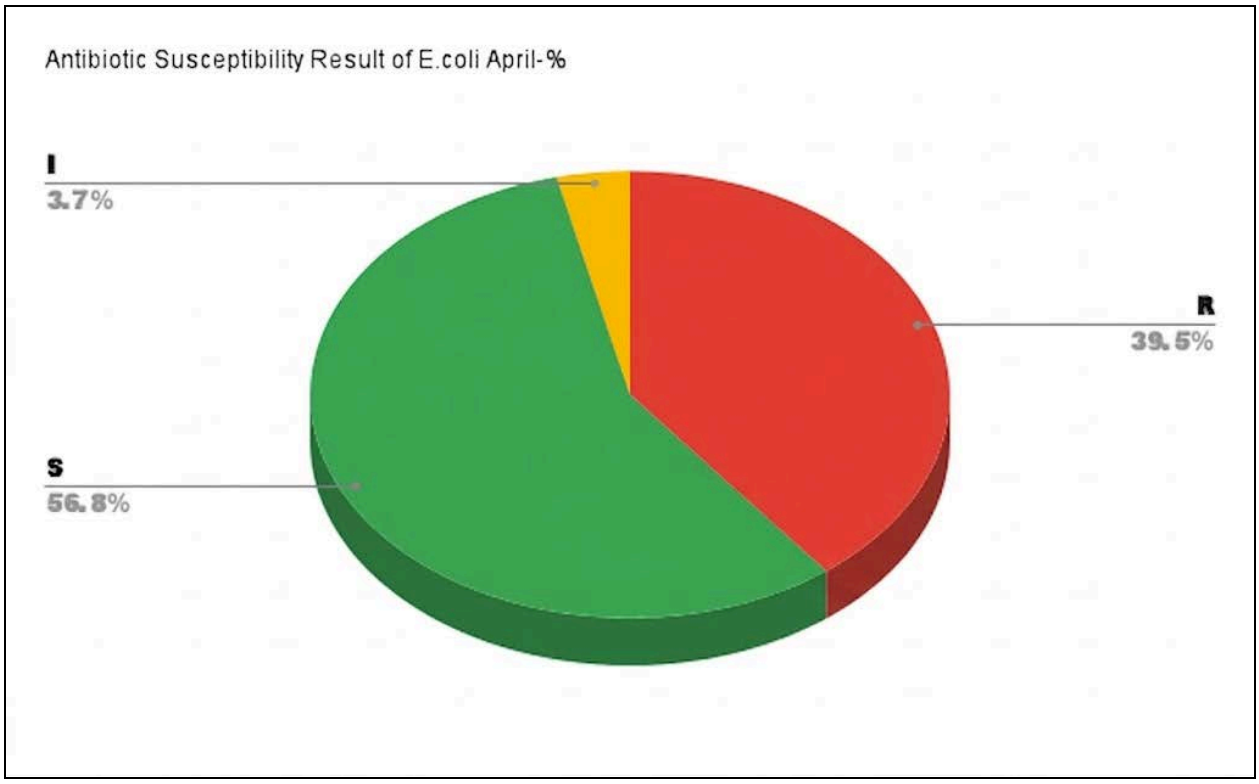


Figure 20: Antibiotic Susceptibility Result of *E.coli* April-%

The pie chart summarizes the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in April. It indicates that 39.5% of the isolates were resistant, 3.7% were intermediate, and 56.8% were susceptible to the 10 antibiotics tested.

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

Code	ATM 30	C30	FEP30	DO30	E15	IPM10	CN10	AMP10	K30	VA30
GMY1E1	30	28	30	19	0	29	20	10	21	0
GMY1E2	35	22	33.5	20	13	32	21	9	23	0
GMY1E3	31	17	31	12	0	27.5	19	0	20	0
GMY1E4	21	22	32	20	11	32	20	9	21	0
GMY1E5	20	22	23	20	0	24	20	0	19	0
GMY1E6	29	27	31	20	0	31.5	20	0	20	0
DMY1E1	32	0	30	11	0	20	17	0	20	0
DMY1E2	31	0	34	13	0	27	20	0	22	0
DMY1E3	30	0	30	10	0	30	17	0	19	0
DMY1E4	25	23	27	11	0	30	18	0	20	0

DMY1E5	28	21	26	10	10	25	18	0	20	0
DMY1E6	30	23	30	10	10	28	20	0	20	0
DMY1E7	19	29	22	14	0	27	21	0	20	0
GMY2E1	35	25	32	17	0	30	16	17	20	0
GMY2E2	30	30	32	18	0	30	17	17	17	0
GMY2E3	10	30	20	10	0	22	20	0	23	0
GMY2E4	28	24	30	12	9	22	20	17	21	0
GMY2E5	15	30	30	12	0	26	20	0	20	0
DMY2E6	21	21	24	19	0	30	19	0	22	0
DMY2E7	30	26	30	10	0	21	16	10	20	0
DMY2E8	10	28	17	10	0	29	17	0	0	0
DMY2E9	30	26	35	10	0	27	20	22	19	0
DMY2E10	30	26	30	16	0	16	18	0	16	0

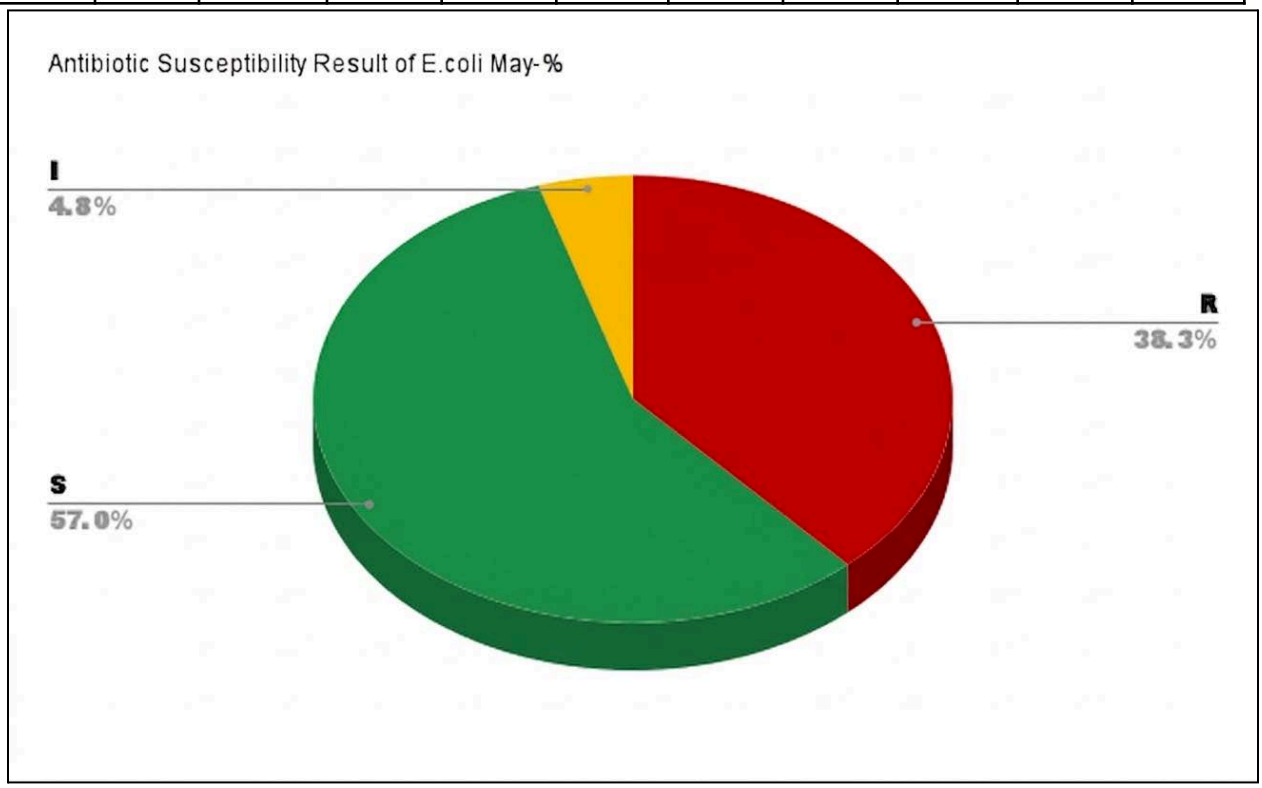


Figure 21: Antibiotic Susceptibility Result of *E.coli* May-%

The pie chart summarizes the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in May. It indicates that 38.3% of the isolates were resistant, 4.8% were intermediate, and 57.0% were susceptible to the 10 antibiotics tested.

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

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	AMP10	E15	VA30
GJN1E6	31	27	30	19	26	17	20	0	0	0
GJN1E7	30	25	30	19	28	20	20	0	0	0
GJN1E8	30	20	33	20	28	20	20	16	0	0
GJN1E9	31	23	32	19.5	22	20	21	0	0	0
GJN1E10	21	23	29	13	30	20	24	0	0	0
DJN1E1	31	25	30	19	30	20	20	0	7	0
DJN1E2	25	24	32	14	23	19	21	8	0	0
DJN1E3	31	23	30	11	24	22	19.5	0	6	0
DJN1E4	30	26	30	19	26	16	18	0	0	0
DJN1E5	30	28	26	11	26	20	17	0	0	0
GJN2E1	33	20	32	20	20	12	15	0	0	0
GJN2E2	30	25	30	10	25	18	20	20	0	0
GJN2E3	28	25	25	10	25	16	20	0	0	0
GJN2E4	11	0	23	10	24	18	20	0	0	0
GJN2E5	26	30	30	16	24	16	18	0	0	0
DJN2E6	32	25	30	20	27	16	19	9	0	0
DJN2E7	31	18	20	16	30	12	20	10	0	0
DJN2E8	30	20	31	19	25	19	19	0	0	0
DJN2E9	32	25	30	21	30	20	20	0	0	0
DJN2E10	30	25	30	18	29	17	19	0	0	0

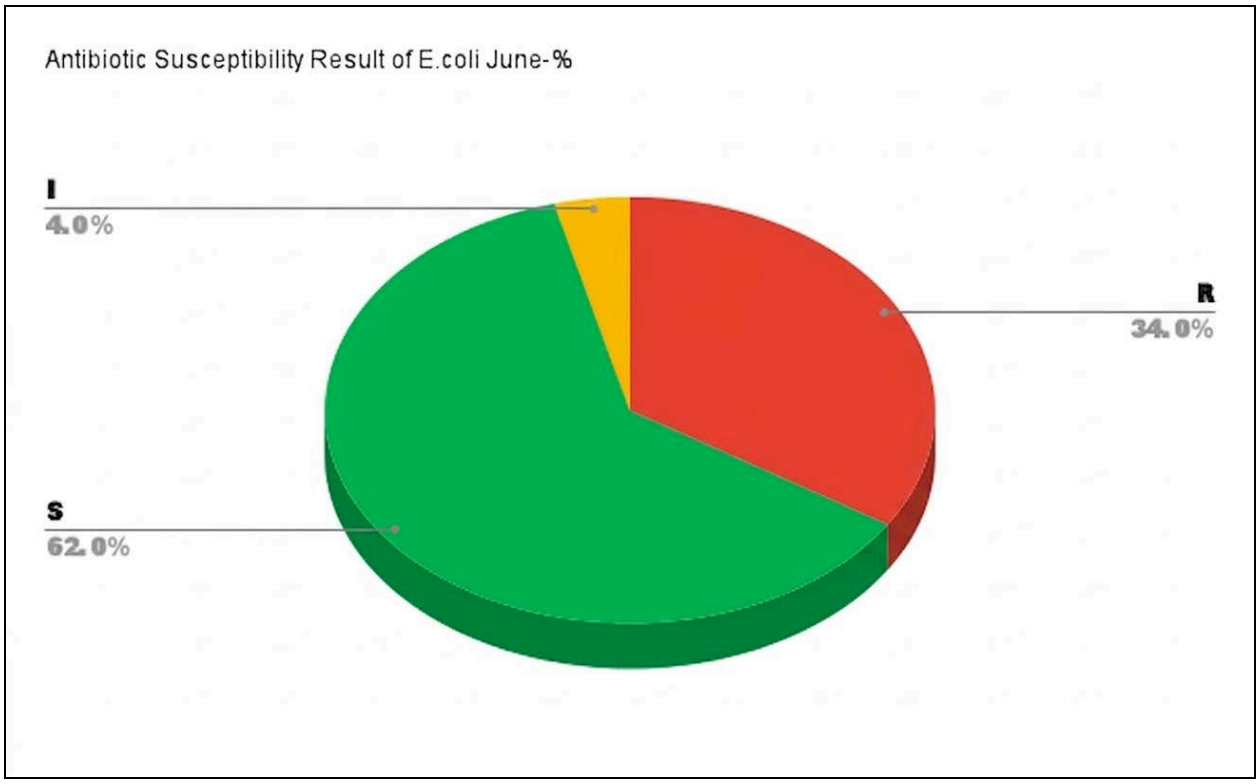


Figure 22: Antibiotic Susceptibility Result of *E.coli* June-%

The pie chart summarizes the antibiotic susceptibility pattern of *E. coli* isolates recovered from Dhanmondi and Gulshan Lakes in June. It indicates that 34.0% of the isolates were resistant, 4.0% were intermediate, and 62.0% were susceptible to the 10 antibiotics tested.

General Trend of *Escherichia coli*:

- High susceptibility to Carbapenems (Imipenem): Imipenem (IPM) consistently showed large zones of inhibition (typically >20mm), indicating nearly 100% susceptibility across the dataset.
- Very high resistance to Ampicillin (AMP): The samples showed extremely high resistance to Ampicillin, with almost every sample recording a result of 'R' (often with 0mm zone diameter) throughout the entire dataset.
- Gentamicin (CN) performed well: This aminoglycoside maintained moderate to high susceptibility levels across most sample batches (GJ, DJ, GF, etc.), making it a reliable agent in this dataset.

- Doxycycline (DO) showed low performance: There was a very high percentage of resistance (%R) to Doxycycline, with most samples showing small or zero zone diameters.
- Levofloxacin (LEV) showed excellent susceptibility: The fluoroquinolone Levofloxacin remained highly active against the majority of *E. coli* isolates, with consistent 'Susceptible' results.

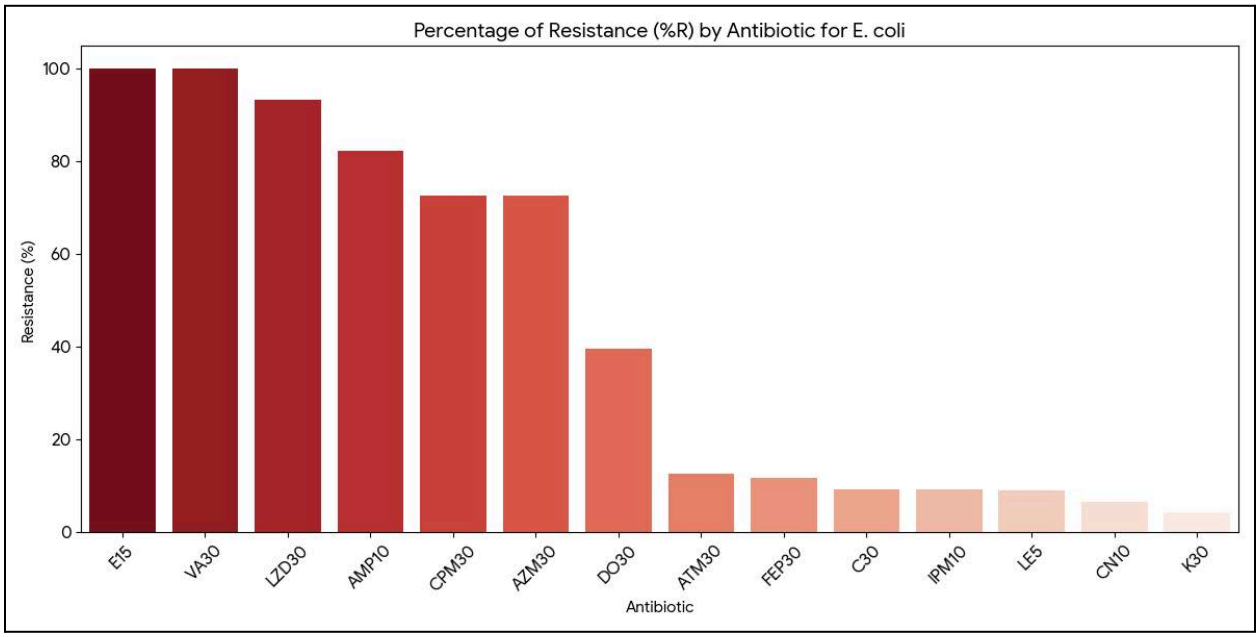


Figure 23- Chart comparing the resistance percentages (%R) for the tested antibiotics against the *E. coli* samples.

Observations:

- Azithromycin (AZM) susceptibility was variable: The results were inconsistent; while many samples were susceptible, there was a notable number of Intermediate (I) and Resistant (R) results compared to other antibiotics.
- Chloramphenicol (C30) remained reasonably active: The majority of samples were sensitive to Chloramphenicol (S), showing it retained fair activity against these isolates.
- Vancomycin (VA) and Linezolid (LZD) have no clinical utility: As expected for *E. coli* (a Gram-negative bacterium), these antibiotics showed nearly 0% activity (consistently 'R' with 0-10mm zones).

- Cephalosporins (Cefepime/FEP) and Monobactams (Aztreonam): These showed variable but generally good activity, particularly in specific batches (e.g., the 'GF' series showed strong susceptibility to Cefepime and Aztreonam, whereas the 'GJ' series showed more resistance).

Salmonella spp.

Table 13: Antibiotic Susceptibility Result of *Salmonella spp.* January.

Code	ATM30	C30	FEP30	DO30	IPM10	CN10	K30	E15	VA30	AMP10	LE5	AZM30
GJ1S1	31	14.5	29.5	17	29	18	20	0	0	0	-	-
GJ1S2	17	0	27.5	6.5	19.5	19	18.5	0	0	0	-	-
GJ1S3	25.5	0	27.5	7	24	0	0	0	0	0	0	-
GJ1S4	26.5	24	27.5	15	23	18	18	8	0	18	23.5	12.5
DJ1S6	27	23.5	29	15.5	20	16	17	0	0	17	22.5	10.5
GJ2S1	26	24	27	9	23	20	19.5	0	0	17	22.5	-
GJ2S2	25.5	17.5	27.5	15.5	20	17	17	0	0	14.5	20.5	-
GJ2S3	23.5	19.5	25	10.5	20	20	10.5	0	0	0	20.5	-
GJ2S4	28	22.5	26.5	11.5	25	16	16.5	6	0	0	-	-
GJ2S5	27.5	24	28	15	24.5	16	17.5	0	0	0	-	-
GJ2S6	28	24	28.5	19	26	16	17	8.5	0	0	-	-
GJ2S7	26.5	23.5	26.5	15.5	22.5	19.5	16.5	0	0	19	23	-
GJ2S8	28	25.5	26	16.5	25	16	17	0	0	0	-	-
GJ2S11	28	23.5	30	15	20	15	18.5	0	0	0	-	-
GJ2S13	8.5	26.5	13.5	17	23	17.5	18.5	8.5	0	0	-	-
GJ2S14	24.5	0	30	10	23.5	0	11.5	0	0	0	-	-
GJ2S16	25	22	24.5	16.5	20	16	17	0	0	0	-	-
DJ2S1	27	25	30	17	29	19	19	0	0	0	-	-

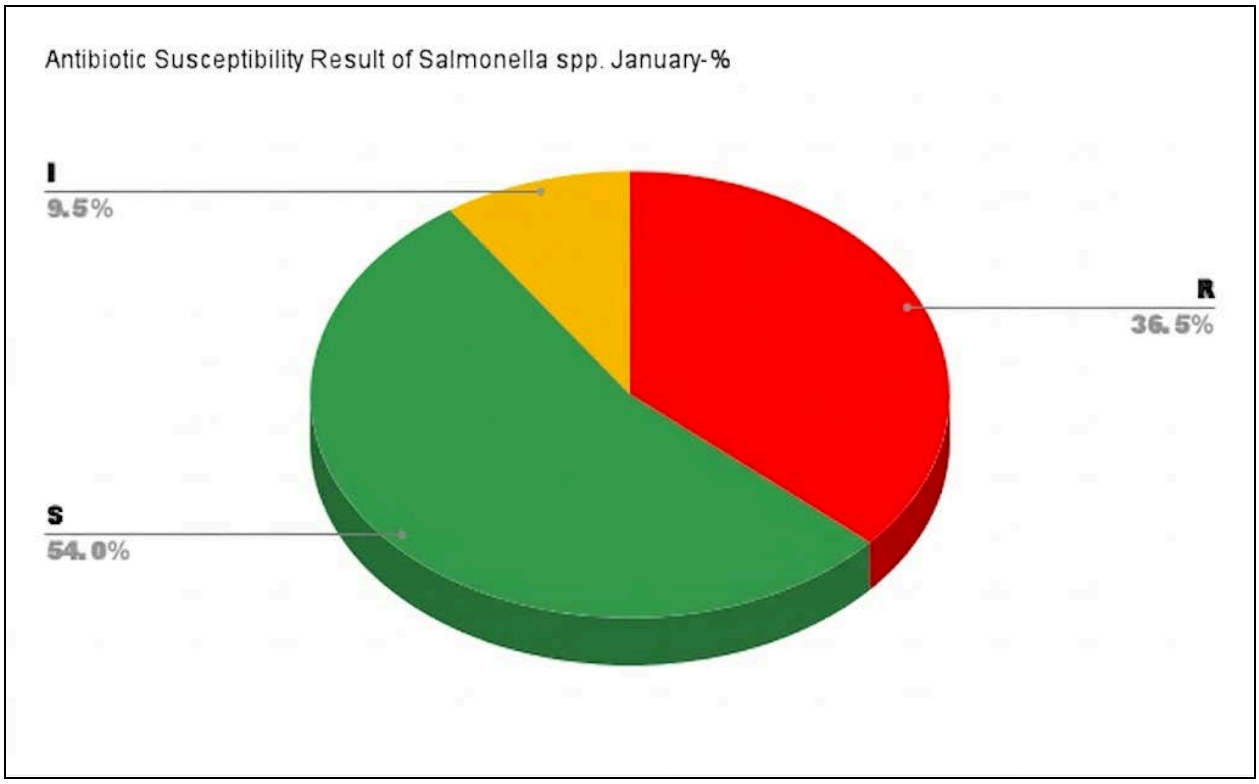


Figure 24- Antibiotic Susceptibility Result of *Salmonella spp.* January-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in January. It indicates that 36.5% of the isolates were resistant, 9.5% were intermediate, and 54.0% were susceptible to the 12 antibiotics tested.

Table 14- Antibiotic Susceptibility Result of *Salmonella spp.* February.

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	E15	AMP10	VA30
GF1S2	30	25	33	20	22	19	20	0	0	0
DF1S1	31	27	30	25	20	21	20	0	0	0
DF1S2	30	23	30	20	28	16.5	23.5	0	0	0
DF1S4	32	28	35	23	22	21	23	0	0	0
DF1S5	30	20	30	20	23	18	21	0	0	0
DF1S8	29	23.5	27	20	22	18	25	0	0	0
DF1S9	35	25	35	20	25	20	20	0	0	0
DF1S13	23	20	32	23	25	20	21	0	0	0

GF2S1	28	21	30	20	26	20	22	0	10	0
GF2S2	31	20	31	21	25	18	8	0	0	0

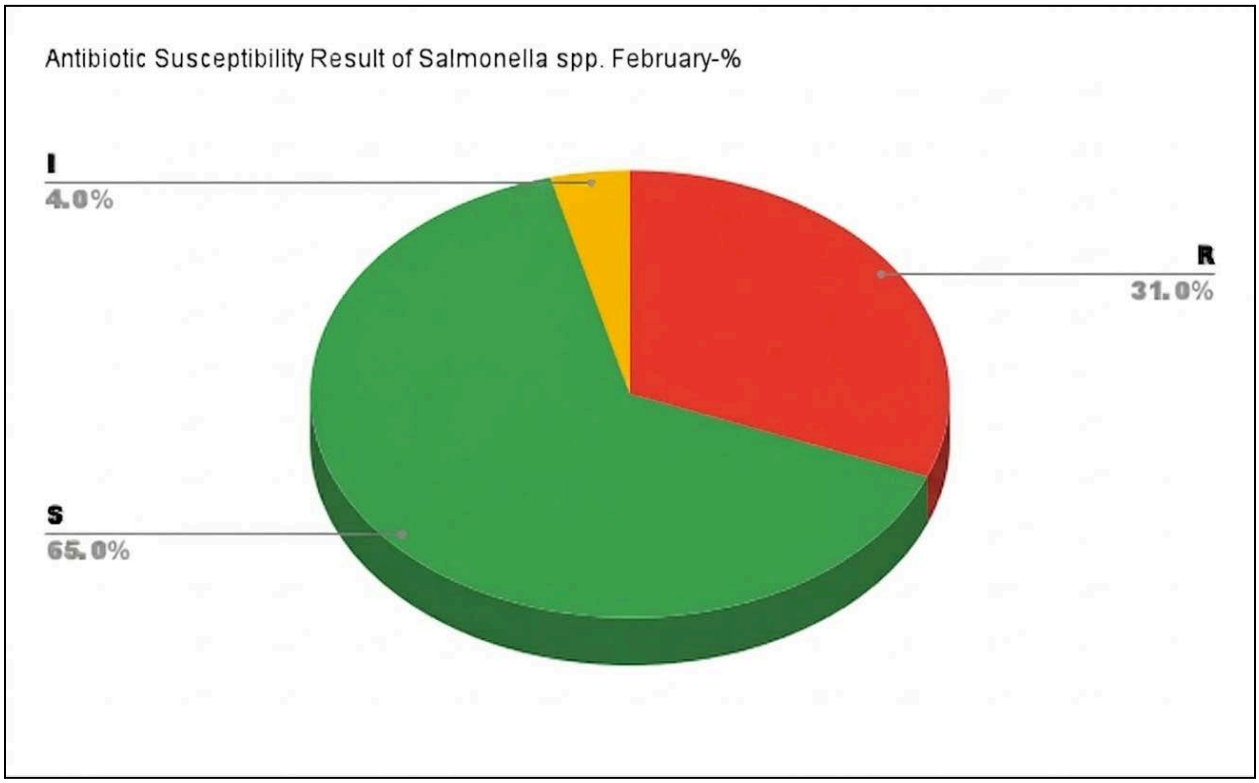


Figure 25- Antibiotic Susceptibility Result of *Salmonella spp.* February-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in February. It indicates that 31.0% of the isolates were resistant, 4.0% were intermediate, and 65.0% were susceptible to the 10 antibiotics tested.

Table 15- Antibiotic Susceptibility Result of *Salmonella spp.* March.

Code	ATM 30	C30	FEP30	DO30	E15	IPM10	CN10	K30	AMP10	VA30
GM1S15	29	24	34	19.5	0	25.5	17	20	0	0
GM1S16	38	30	37	20	0	30	20	22	0	0
GM1S17	35	24	36	20	0	30	17	25	0	0
GM1S18	11	22	15	11	0	22	16	17	0	0
GM1S19	25	27.5	37.5	20	0	30	23	22	0	0

GM1S22	36	25	40	18	0	25	18	20	0	0
DM1S3	38.5	23	35.5	26	0	30	21	21	13	0
DM1S4	28	35	37	30	0	35	21	21	0	0
DM1S5	31.5	25	32	20	12	24.5	21	21	7	0
DM1S6	33	24	35	19	0	24.5	20	22	0	0
DM1S7	30	25	33	0	0	25	15	20	0	0
DM1S8	33.5	26	35	20	0	29.5	19.5	21	0	0
DM1S9	35	20	40	20	0	31	20	23	0	0
DM1S10	32	24.5	33	19.5	0	29	20	19.5	0	0
DM1S11	25	30	35	20	16	20	20	21	0	0
DM1S13	32	26	36	26	0	32	22	24	0	0
DM1S14	25	30	35	20	0	25	20	20	0	0
DM1S20	27	20	30	16	7	29	18	20	0	0
DM1S21	27	26	37	23	0	28	20	20	0	0
DM2S1	35	28	37	26	0	36	20	20	0	0
DM2S2	37	30	37	27	0	30	20	22	0	0
DM2S3	20	20	38	20	0	21	20	20	0	0
DM2S4	30	24	35	20	0	17	15	20	0	0
DM2S5	37	27	38	25	0	30	20	20	17	0
DM2S6	30	27	35	30	0	31	23	20	0	0
DM2S7	27	26	36	25	0	32	22	23	0	0
DM2S8	38	25	37	25	0	32	20	21	15	0
GM2S1	35	25	35	20	0	28	20	20	0	0

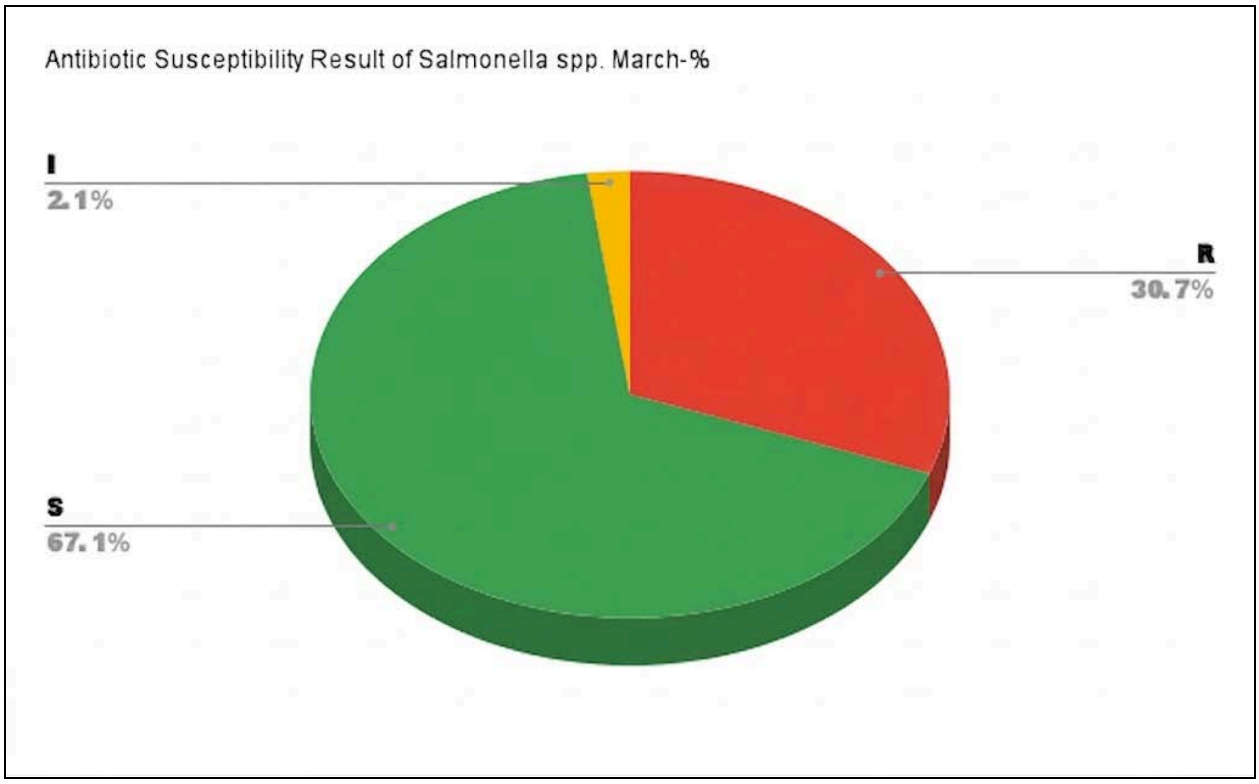


Figure 26- Antibiotic Susceptibility Result of *Salmonella spp.* March-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in March. It indicates that 30.7% of the isolates were resistant, 2.1% were intermediate, and 67.1% were susceptible to the 10 antibiotics tested.

Table 16- Antibiotic Susceptibility Result of *Salmonella spp.* April.

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	AMP10	E15	VA30
GA1X1	27	23	32	20	26	20	22	0	0	0
GA1X2	25	0	30	0	20	16	16	0	0	0
GA1X3	32	24.5	36	21	30	21	20	0	0	0
GA1X4	30	25	32	20	27.5	20	22	0	0	0
GA1X5	30	24	32.5	21	24	22	22	0	0	0
GA1X6	32	25.5	35	21.5	23	19	20	0	0	0
GA1X7	16	25	20	10	30	20	0	0	0	0
GA1X8	32	26	36	22	25	22	22	0	0	0
GA1X9	31	26	35.5	22	23	21	20	0	0	0

GA1X10	35	30	35	12	30	20	22	0	10	0
GA1X11	30	15	30	0	20	13	20	0	0	0
GA1X12	33	28.5	29.5	21	23.5	20	21	0	0	0
GA2X1	27	0	28	10	24	17	0	0	0	0
GA2X2	35	0	35	10	29	20	20	0	0	0
GA2X3	28	28	30	20	28.5	20	21	0	0	0
GA2X5	33	23.5	36	20	23	21	18.5	0	0	0
GA2X6	30	0	31	10	25	18	20	0	0	0
GA2X7	32	26	33	18	30	20	20	20	11	0
GA2X8	30	26.5	35	21	24	20	21.5	0	0	0
GA2X9	30	25.5	32	20	29	21	21	0	0	0

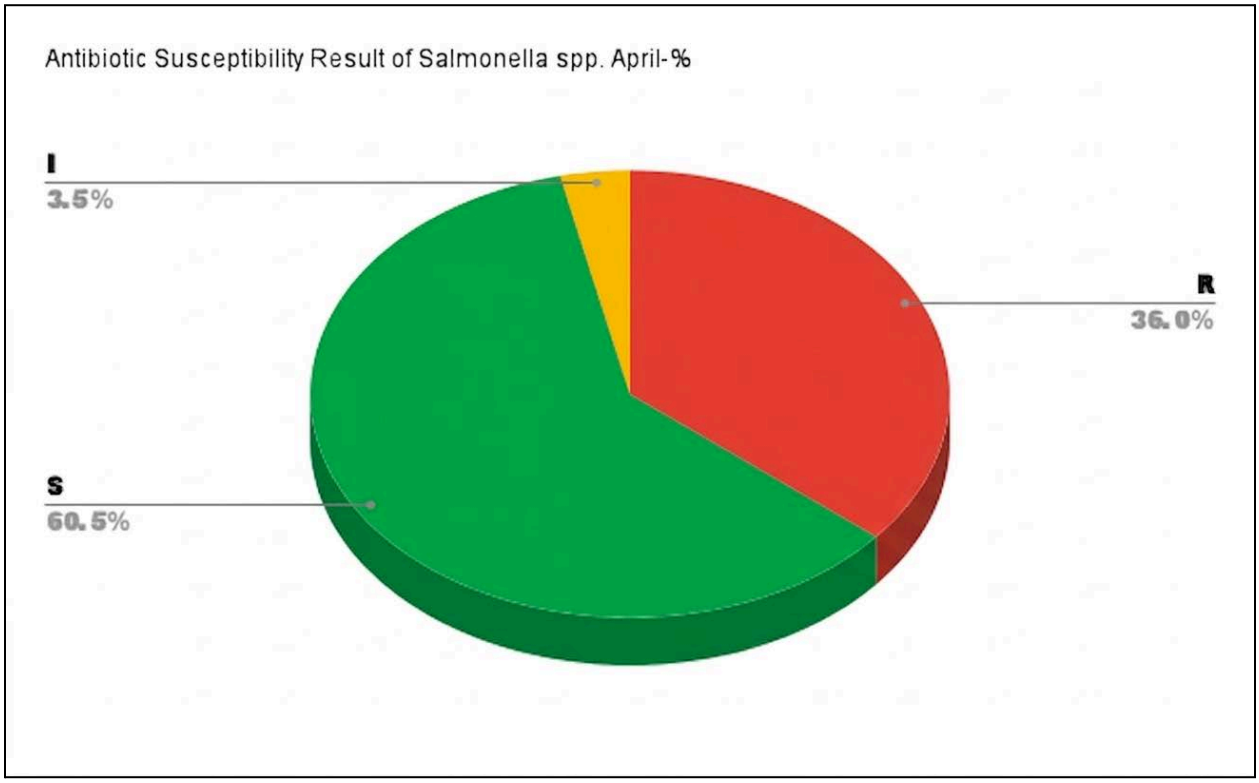


Figure 27- Antibiotic Susceptibility Result of *Salmonella spp.*April-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in April. It indicates that 36.0% of the isolates were resistant, 3.5% were intermediate, and 60.5% were susceptible to the 10 antibiotics tested.

Table 17- Antibiotic Susceptibility Result of *Salmonella spp.* May.

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	E15	AMP10	VA30
GMY1S1	30	35	0	0	20	17	12	0	0	0
GMY1S2	29.5	24.5	30	19.5	18	18.5	16	0	0	0
GMY1S3	29.5	25.5	34.5	21	23.5	20	20	0	0	0
GMY1S4	24	25	30	18	22	17.5	15	0	0	0
GMY1S5	30	25	35	20	26	21	21	0	0	0
GMY1S6	36	26	32.5	22	28	20	22	0	0	0
GMY1S7	20	0	24.5	15	24	20	25	0	0	0
GMY1S8	31	24	28.5	21.5	26.5	20	20.5	0	0	0
GMY1S9	27.5	26.5	34	21	25	20	19.5	0	0	0
GMY1S10	31	28	35	20	26.5	20	21	0	0	0
GMY1S11	33.5	27.5	28	22	25	21.5	20	0	0	0
GMY1S12	32	24.5	30	0	20	10	17	0	0	0
DMY2S1	32	24.5	25	18	27.5	20	18	0	0	0
DMY2S2	27	29	31	19.5	22.5	19.5	21	0	0	0
DMY2S3	28	30	28.5	19.5	25.5	17	18	0	0	0
DMY2S4	30	27	31.5	20	25	17.5	18.5	0	0	0
DMY2S5	35	27	35	0	28	20	26	0	0	0
GMY2S6	35	27	35	0	30	20	26	0	0	0
GMY2S7	36	25	34	0	25	20	23	0	0	0
GMY2S8	23	26.5	28	0	24	19	22	0	0	0
GMY2S9	34	26	35	0	25	20	24	0	0	0
GMY2S10	36	30	34.5	0	26	20	22	0	24	0

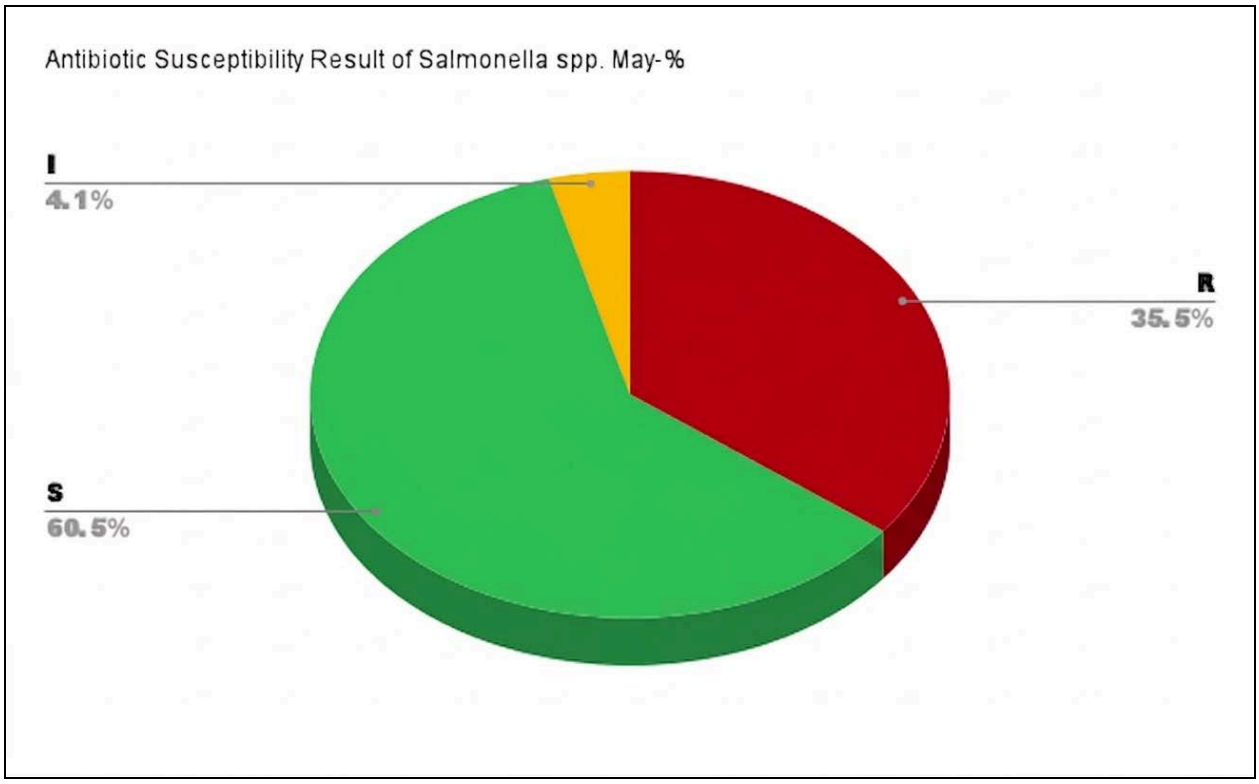


Figure 28- Antibiotic Susceptibility Result of *Salmonella spp.* May-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in May. It indicates that 35.5% of the isolates were resistant, 4.1% were intermediate, and 60.5% were susceptible to the 10 antibiotics tested.

Table 18- Antibiotic Susceptibility Result of *Salmonella spp.* June.

Code	ATM 30	C30	FEP30	DO30	IPM10	CN10	K30	AMP10	E15	VA30
GJN1S1	31	26.5	32	22	27	20	22.5	0	0	0
GJN1S2	29	23	30	19.5	25	17	20	0	0	0
GJN1S3	32	25	32	0	23.5	20	20	20	0	0
GJN1S4	30	28	32	15	24	20	17	0	0	0
GJN1S5	29	26.5	31	20	21.5	20	16	0	0	0
GJN1S6	32	27	36	22	28	19.5	20	0	0	0
GJN1S7	30	24	33.5	18.5	28	0	10	9	0	0
GJN1S8	30	22	33	19	25	19	20	0	0	0
GJN1S9	23	28	35	20	27.5	21	22	0	0	0
GJN1S10	32	29.5	36	9	27.5	20	23	0	0	0

GJN2S1	28	23.5	29	16.5	25	17	17	0	0	0
GJN2S2	30	25.5	30	21	30	19	20	0	0	0
GJN2S3	31	23	35	18	25	17	0	10	0	0
GJN2S4	17	0	16.5	10	30	20	18	0	10	0
GJN2S5	20	0	25	0	24	18	0	0	0	0
DJN2S6	34	29	34	0	29	23	10	0	0	0
DJN2S7	35	27	35	23	27.5	20	22	9	0	0
DJN2S8	30	20	31	16	24	18	0	10	0	0
DJN2S9	33	27	30	0	28	-	0	0	0	0
DJN2S10	31	0	32	0	25	19	0	21	0	0

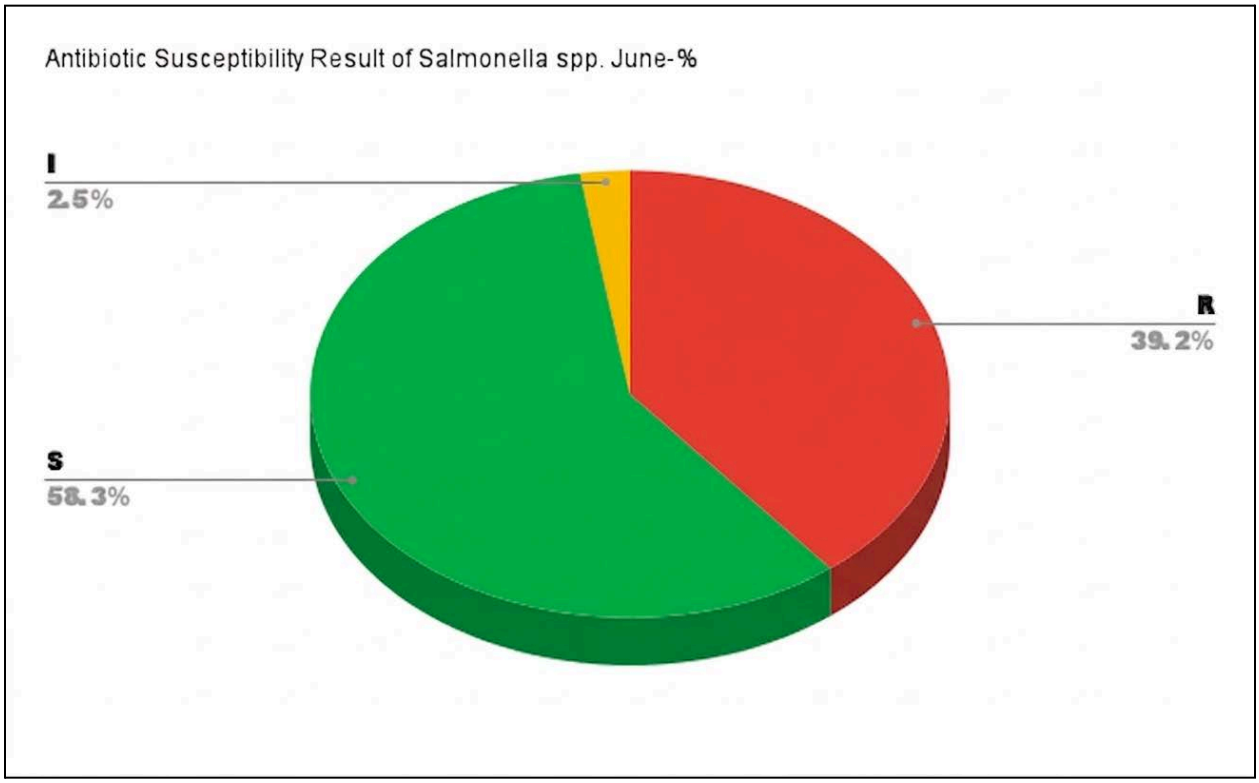


Figure 29- Antibiotic Susceptibility Result of *Salmonella spp.* June-%

The pie chart summarizes the antibiotic susceptibility pattern of *Salmonella spp*. isolates recovered from Dhanmondi and Gulshan Lakes in June. It indicates that 39.2% of the isolates were resistant, 2.5% were intermediate, and 58.3% were susceptible to the 10 antibiotics tested.

General Trend of *Salmonella*:

- High susceptibility to Carbapenems (IMP), Cephalosporins (FEP), and Monobactams (ATM): These classes maintained excellent efficacy throughout the study period. Cefepime and Aztreonam consistently showed >88% susceptibility across all months.
- Very high resistance to Ampicillin (AMP): Resistance was consistently high, ranging from ~72% in January to nearly 100% in the February–June period.
- Gentamicin (CN) was highly effective: This aminoglycoside maintained superior susceptibility levels (90–100%) consistently from January through June without significant decline.
- Doxycycline (DO) showed variable performance: It was highly effective in February and March (>90% susceptibility) but showed reduced efficacy (~65% susceptibility) in January and the April–June period.
- Kanamycin (K) fluctuated significantly: It showed excellent susceptibility in the spring months (February–May) but dropped to lower performance levels (~45–50%) in January and June.

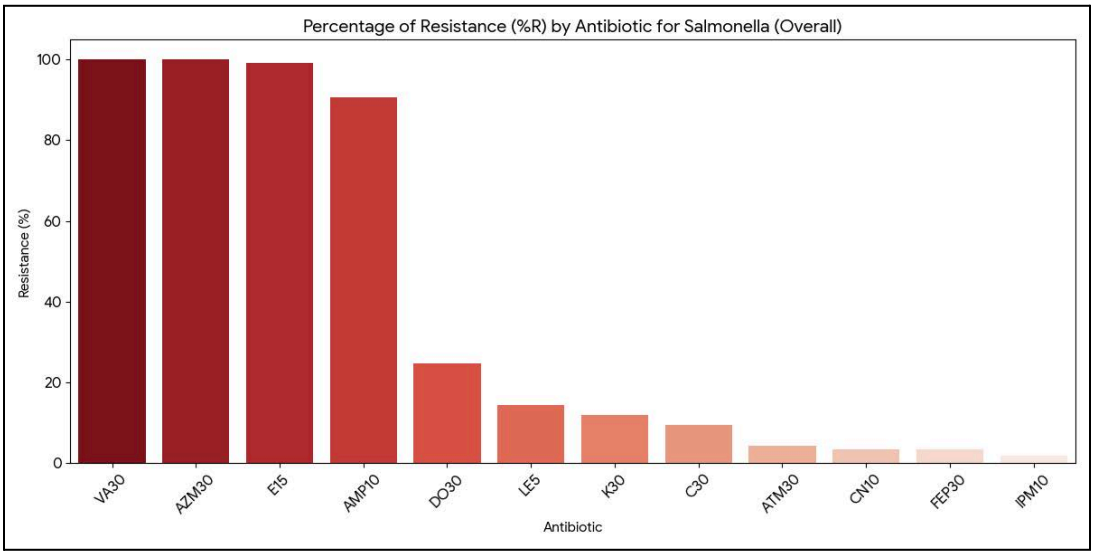


Figure 30: Chart comparing the resistance percentages (%R) for the tested antibiotics against the *Salmonella spp.* Samples.

Observations:

- Vancomycin (VA) and Erythromycin (E): As expected for *Salmonella,* these agents showed 100% resistance across all months and have no clinical utility.

- Chloramphenicol (C30): Remained reasonably active throughout the months, with susceptibility peaking at 100% in February/March and remaining above 70% in other months.
- Levofloxacin (LEV): Data for this agent was limited to January, where it showed good susceptibility (~85%), but trends for subsequent months were not available in this dataset.

Vibrio spp.

Table 19- Antibiotic Susceptibility Result of *Vibrio spp.* January.

Code	ATM 30	C30	FEP 30	DO 30	E 15	IPM 10	CN 10	K30	L E5	AMP 10	LZ D3 0	CPM 30	OX1	VA30
GJ1T1	29.5	28	30	24	12	28	20	17	27	0	-	25	-	0
GJ1T2	29	25	32.5	18	9	-	18	14	29	0	0	-	0	0
GJ1T3	35	12	37	0	16	32	22	21	29	0	-	-	-	0
GJ1T4	39	22	40	21.5	12	-	21	22	32	0	-	-	-	0
GJ1T5	32	28	35	7	14	-	21	23	32	0	-	-	-	0
GJ1T6	45	36	41	27	0	-	24	25	32	0	-	-	-	0
GJ1T7	43.5	35	39	26	0	-	22	23	30	0	-	-	-	0
GJ1T8	45	0	38	0	0	-	15	21	28 .5	0	-	-	-	0
GJ1T9	44.5	32	40	27	17	-	21	22	33	0	-	-	-	0
GJ1T10	40	0	39.5	0	0	-	23	22	25	0	-	-	-	0
GJ2T1	25	30	29	17	10	24	18	17	30	0	-	-	-	0
GJ2T3	34	27	37	20	14	30.5	21. 5	20	38	0	-	-	-	0
GJ2T4	31.5	28.5	33	19	11	25.5	20	20	32	0	-	-	-	0
GJ2T5	37	0	40	0	0	25	16	19.5	24	0	-	-	-	0
GJ2T6	35.5	28	35	19	12	25	20	24	39 .5	0	-	-	-	0
GJ2T7	36	6	37	0	0	24.5	14	17	22	0	-	-	-	0
GJ2T8	37	28	37.5	21	13 .5	29	22	25	33	0	-	-	-	0
GJ2T9	35	7	37	0	0	20	12	22	21	0	-	-	-	0
GJ2T10	42	26	39.5	18	16	19	21	19	28 .5	0	-	-	-	0

DJ2T1	32	8.5	30	0	0	-	19	20	28	11	0	20	0	0
DJ2T2	28	28	30	15	10	22	18	23	28	0	-	-	-	0
DJ2T3	39	7	36	0	0	25	15	25	24	0	-	-	-	0
DJ2T7	47	35	36	27	15	20	25	20	45	0	-	-	-	0
DJ2T11	29	7	30	0	0	-	15.5	18.5	25	18.5	0	17	0	0
DJ2T12	39.5	25.5	37	17	12	32	19	23	37	18	-	-	-	0
DJ2T13	35	8	36.5	0	0	26.5	13	22	17	0	-	-	-	0
DJ2T14	35	22	32.5	20	9	28	20	23	35.5	17	-	-	-	0

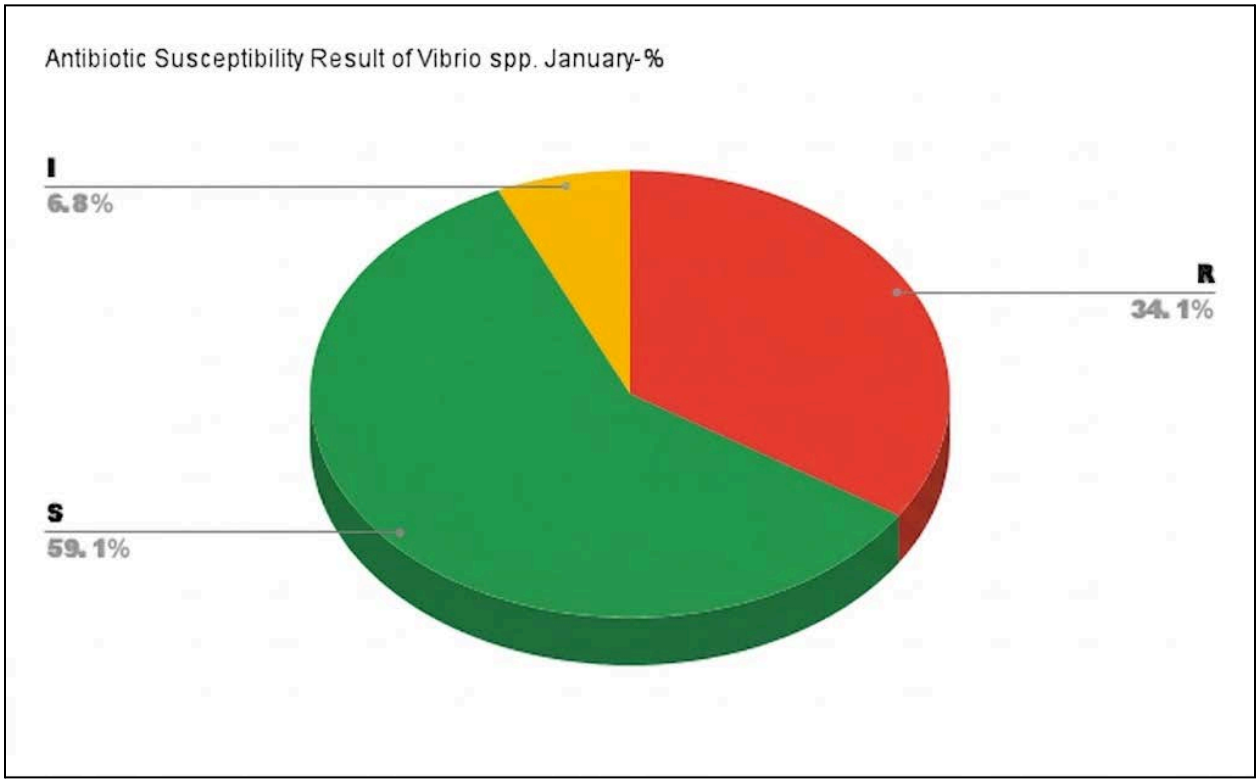


Figure 31- Antibiotic Susceptibility Result of *Vibrio spp.* January-%

The pie chart summarizes the antibiotic susceptibility pattern of *Vibrio spp*. isolates recovered from Dhanmondi and Gulshan Lakes in January. It indicates that 34.1% of the isolates were resistant, 6.8% were intermediate, and 59.1% were susceptible to the 14 antibiotics tested.

*There were no visible growth of *Vibrio spp* was seen for February hence there were no stock bacteria to be used for AST.*

Table 20- Antibiotic Susceptibility Result of *Vibrio spp.* March.

Code	ATM 30	C30	FEP 30	CP M30	DO30	CN10	K30	LE5	E15	LZD3 0	OX1	AMP 10	VA30
DM1T1	32.5	24.5	32.5	19.5	15	18.5	10	21.5	0	0	0	0	0
DM1T2	29.5	30	30	18.5	18.5	20	17.5	25	0	0	0	0	0
GM1T1	29	25	30	17	15	16	16	32	0	0	0	0	0
GM1T2	30	25	29	19.5	16	18	18.5	31	6	0	0	0	0
GM1T3	40.5	35.5	38	24	28	21	19	30	23	17	0	0	0
GM1T4	27	20.5	29.5	18.5	15	19.5	15	26.5	0	0	0	0	0
GM1T5	25.5	25	28.5	15	16.5	17	17	21	0	0	0	0	0
GM1T6	30.5	28.5	30	21	12	18	17.5	30	0	0	0	0	0
GM1T7	29	29	27	19	15.5	19	16	29	0	0	0	0	0
GM2T1	27	29	30	16	20	19	16	28	10	0	0	10	0
GM2T2	29	24	29.5	18	17.5	20	20	30	11	0	0	17.5	0
GM2T3	25	26	27	21	18	18	19	25	10	0	0	0	0
GM2T4	25	22	26	15	15	14	18	20	0	0	0	0	0
GM2T5	26	24	27	18	20	19	15	24	0	0	0	0	0
DM2T1	35	22	32	21	0	18	18	24	0	11	0	0	0
DM2T2	28.5	27.5	30	21	21	19	12.5	24	0	0	0	0	0
DM2T3	25.5	28.5	30	18	18	17.5	18.5	30	0	0	0	15	0
DM2T4	30	28	31	20	20	17.5	15	31	0	0	0	17	0
DM2T5	27	20	25	14	15	13	18	23	0	0	0	0	0

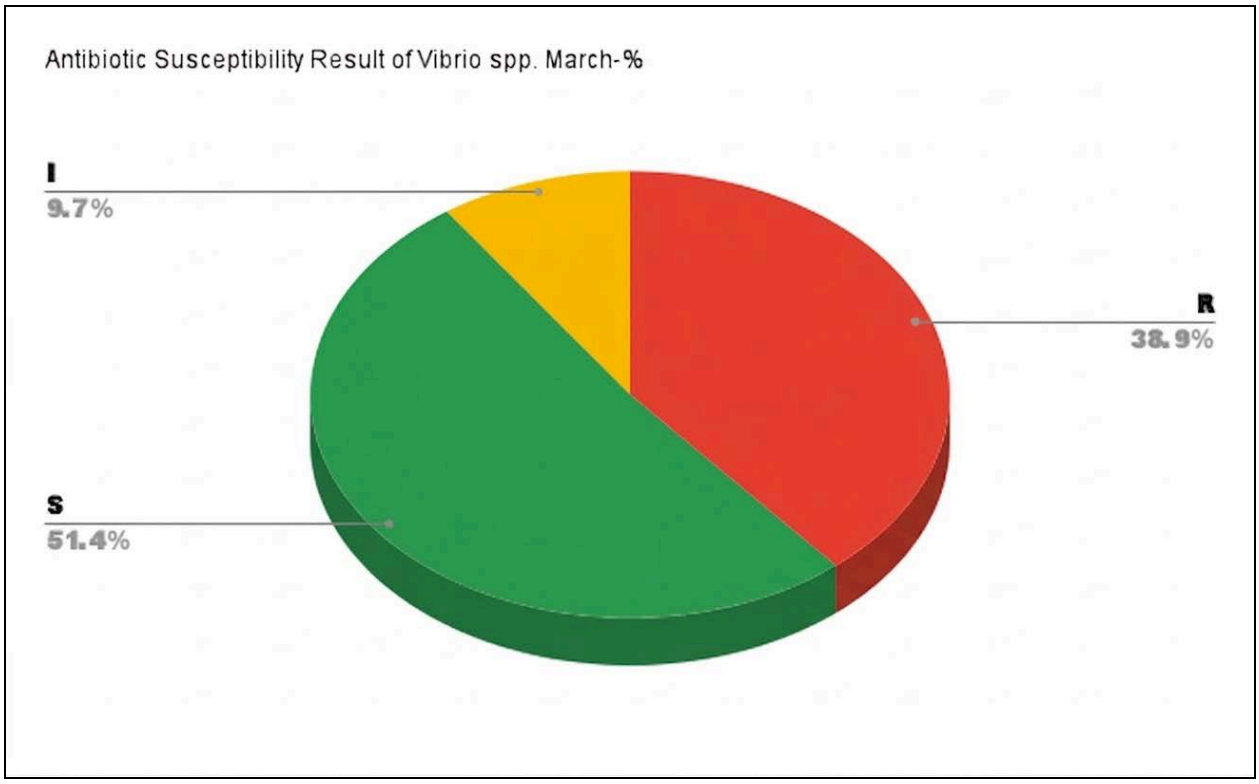


Figure 32- Antibiotic Susceptibility Result of *Vibrio spp.* March-%

The pie chart summarizes the antibiotic susceptibility pattern of *Vibrio spp*. isolates recovered from Dhanmondi and Gulshan Lakes in March. It indicates that 38.9% of the isolates were resistant, 9.7% were intermediate, and 51.4% were susceptible to the 13 antibiotics tested.

Table 21- Antibiotic Susceptibility Result of *Vibrio spp.* April.

Column 1	AMP10	ATM30	C30	FEP30	CPM30	DO30	E15	CN10	K30	LE5	LZD30	OX1	VA30
GAIT1	10	30	28	32	20	13	0	15	20	28	0	0	0
GAIT2	0	30	25	30	23	12	0	0	20	24	0	0	0
GAIT3	0	19	31	20	9	18	0	20	21	23	0	0	13
GAIT4	10	29	22	29.5	24	0	0	15	19	23	0	0	0

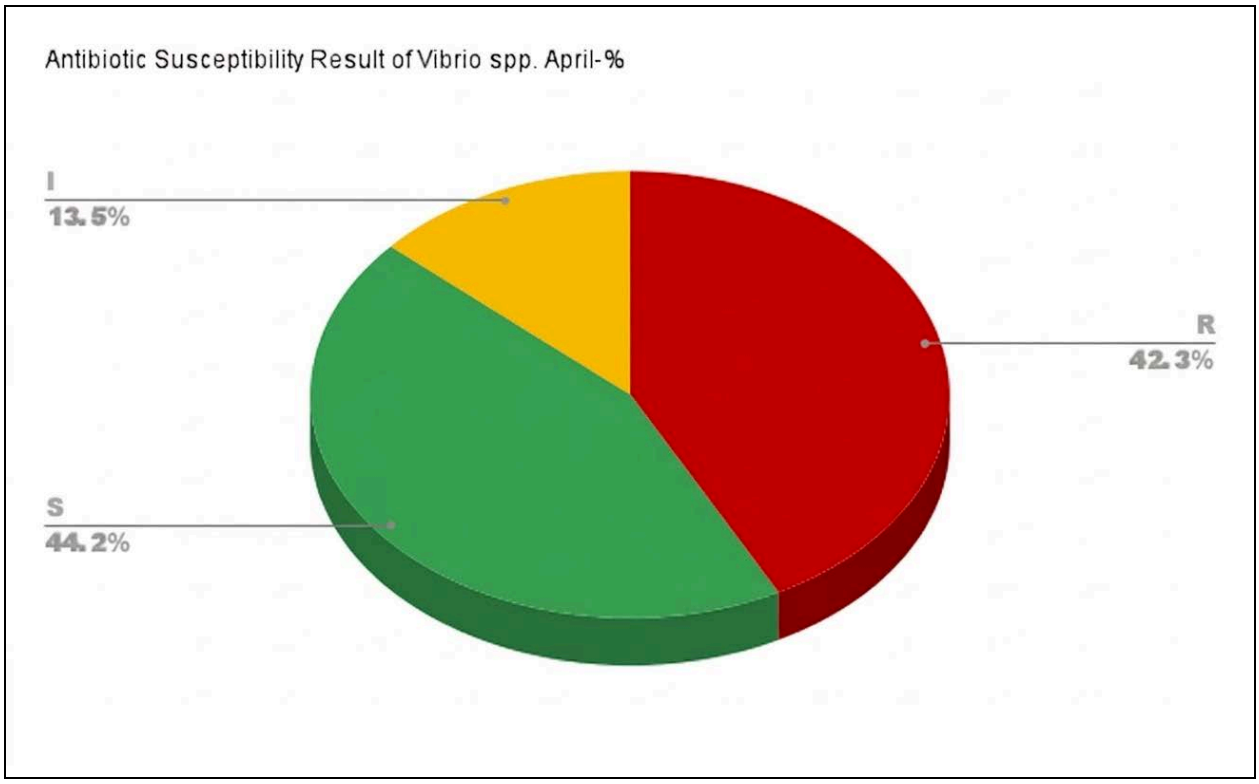


Figure 33- Antibiotic Susceptibility Result of *Vibrio spp.* April-%

The pie chart summarizes the antibiotic susceptibility pattern of *Vibrio spp*. isolates recovered from Dhanmondi and Gulshan Lakes in April. It indicates that 42.3% of the isolates were resistant, 13.5% were intermediate, and 44.2% were susceptible to the 13 antibiotics tested.

Table 22- Antibiotic Susceptibility Result of *Vibrio spp.* May.

Code	AMP10	ATM30	C30	FEP30	CPM30	DO30	E15	CN10	K30	LE5	LZD30	OX1	VA30
GMY1T1	16	31	25	30	20	0	0	19	12	23	0	0	0
GMY1T2	0	29	23	30	21	21	10	18	11	29	0	0	0
GMY1T3	0	18	21	29	18	0	0	16	19	22.5	0	0	0
GMY1T4	0	32	24	31	21	20	0	20	11.5	28	0	0	0
GMY1T5	0	36	21	35	21	23	0	20	18	25	0	0	0
GMY1T	0	28	25	28	20	20	0	16	10	20	0	0	0

6													
DMY1T1	0	35	30	30	18	20	16	18	17	33	11	0	9
DMY1T3	0	32	32	30	21	23	12	19	19	30	0	0	12
DMY1T4	0	35	29	30	20	22	14	18	19	27.5	9	0	13
DMY1T5	0	36	31	35	24	23	15	18	17	35	0	0	9
DMY1T6	0	32	29	30	21	23	14	19	20	30	10	0	9
GMY2T1	0	34	21	32	12	15	0	18	20	21.5	0	0	0
GMY2T3	0	30	14.5	29	29	0	0	17.5	20	27.5	0	0	0
GMY2T4	0	31	12	32	25	0	0	20	31	24	0	0	0
GMY2T5	0	32	21	31	21	20	0	18	32	25	0	0	0
DMY2T6	0	30	19	33	20	19	0	20	20	26	0	0	0
DMY2T7	0	30	24	31	25	12	14	19	24.5	31	0	0	0
DMY2T8	11	30	10	28	24	0	0	18.5	19	32	0	0	0
DMY2T9	0	35	29.5	32	24	24	13	21.5	24	33	11	0	0
DMY2T10	22	38	32	30	26	21	15	20	23	37	11	0	0

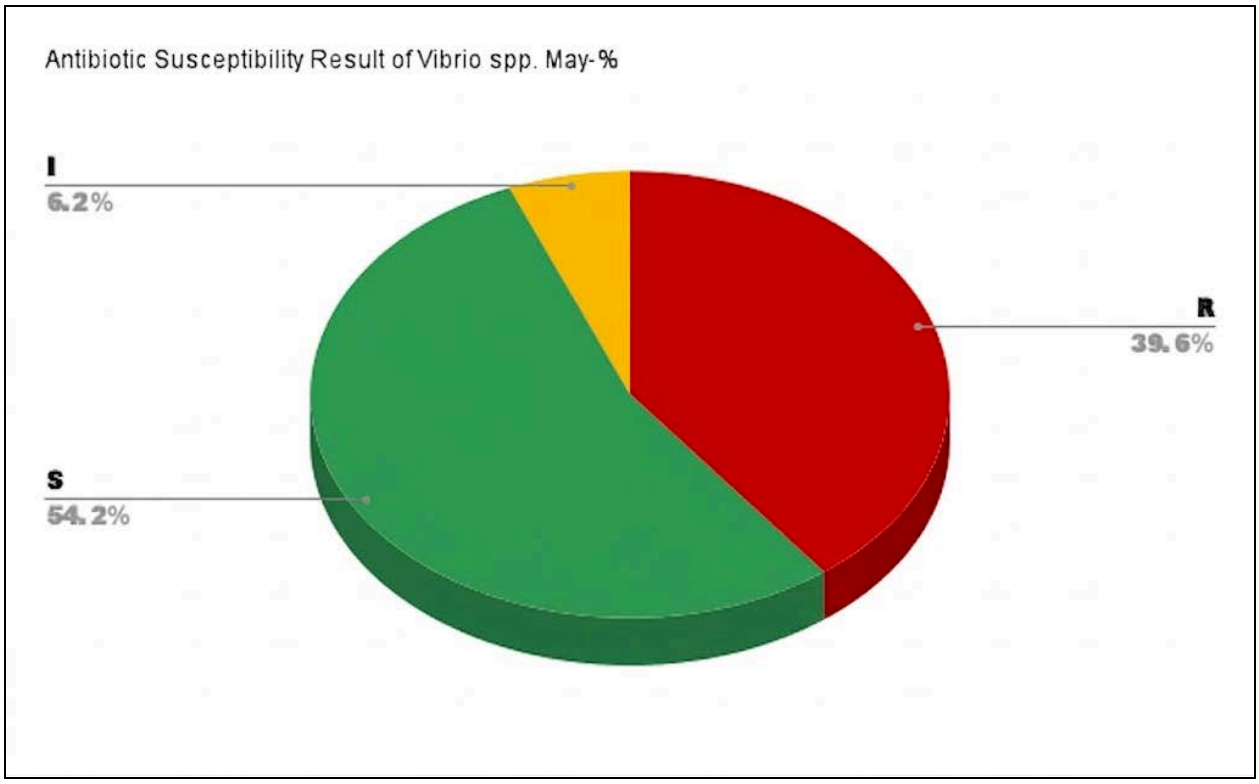


Figure 34- Antibiotic Susceptibility Result of *Vibrio spp.* May-%

The pie chart summarizes the antibiotic susceptibility pattern of *Vibrio spp*. isolates recovered from Dhanmondi and Gulshan Lakes in May. It indicates that 39.6% of the isolates were resistant, 6.2% were intermediate, and 54.2% were susceptible to the 13 antibiotics tested.

Table 23- Antibiotic Susceptibility Result of *Vibrio spp.* June.

Code	AT M30	C30	FEP30	CPM30	DO30	E15	CN10	K30	LE5	LZD30	OX1	VA30	AMP10
GJN1T3	35	23	30	15	22	0	20	20	30	0	0	0	0
GJN1T4	30	25	31.5	17	20	0	21	20	24	0	0	0	0
GJN1T5	34	29	31	12	22	17	19	20	23	0	0	0	0
GJN1T6	28	27	30	19	18	12	17	21	21	0	0	0	0
DJN2T1	35	31	29	24	20	0	17	20	32	9	0	0	0
GJN2T2	28	27	27.5	23	17	10	17	19.5	25	0	0	0	10
GJN2T3	31	16	20	0	16	8	16	21	27	0	0	0	10

GJN2T4	33	16	37	25	11	0	20	21	28	0	0	0	10
GJN2T5	32	24	30	22	16	0	18	21	23	0	0	0	0
GJN2T6	25.5	25	32	24	18	9	18.5	14	28	0	0	0	0
GJN2T7	0	17	32	25	23	14	19	19	26	0	0	0	0
GJN2T8	30	20	31.5	24	20	0	21	20	25	0	0	0	0
GJN2T9	24	24	22	13	15	11	17	18	24.5	0	0	0	6
GJN2T10	31	20	33	20	16	0	21	19	25	0	0	0	10

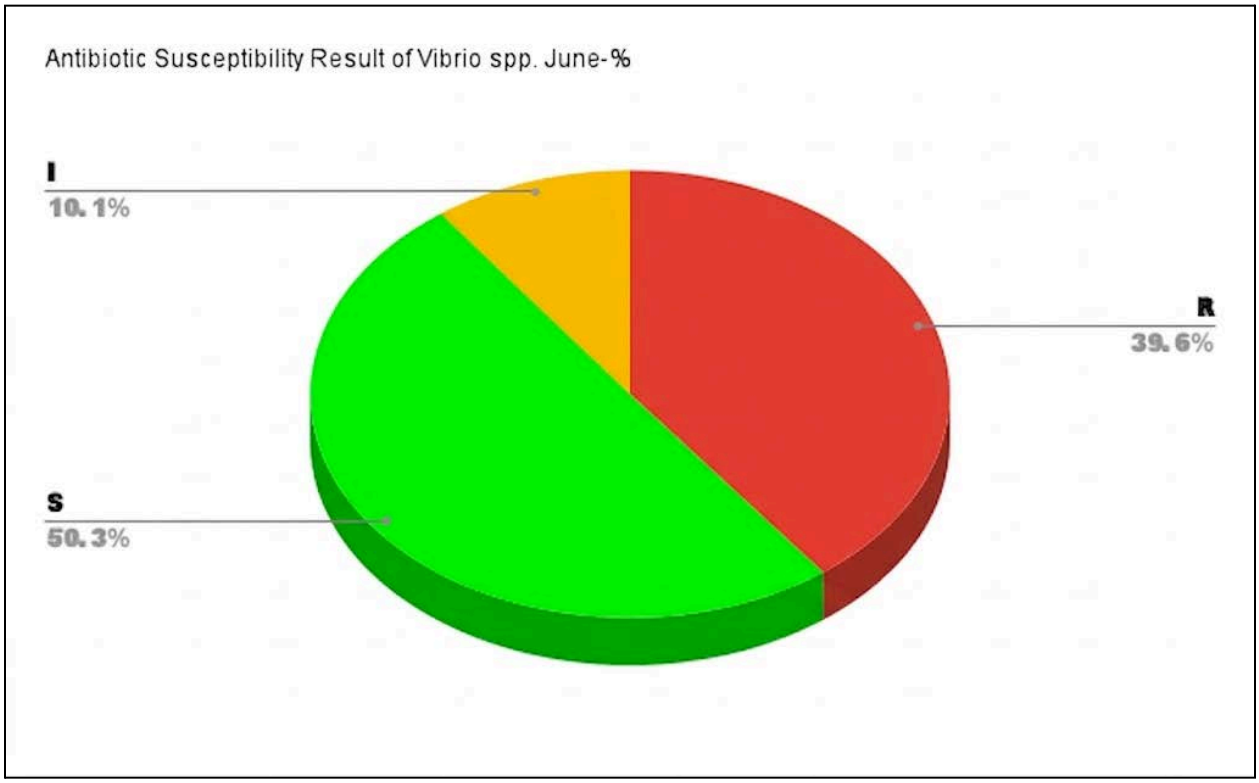


Figure 35- Antibiotic Susceptibility Result of *Vibrio spp.* June-%

The pie chart summarizes the antibiotic susceptibility pattern of *Vibrio spp*. isolates recovered from Dhanmondi and Gulshan Lakes in June. It indicates that 39.6% of the isolates were resistant, 10.1% were intermediate, and 50.3% were susceptible to the 13 antibiotics tested.

General Trend of *Vibrio*:

- High susceptibility to Cephalosporins (FEP) and Monobactams (ATM): Cefepime (FEP) and Aztreonam (ATM) showed excellent efficacy, with only ~1.2% resistance and >96% susceptibility.

- Excellent susceptibility to Levofloxacin (LEV): This fluoroquinolone was the most effective agent tested, with 0% resistance and 100% susceptibility across all samples.
- Very high resistance to Ampicillin (AMP): Similar to the other bacteria analyzed, *Vibrio* isolates showed high resistance to Ampicillin (~79.5%).
- Gentamicin (CN) and Kanamycin (K) were effective: These aminoglycosides showed low resistance levels (~7.2%), with good susceptibility rates (>70%).
- Doxycycline (DO) showed moderate performance: Resistance was moderate at ~26.5%, with susceptibility around 62.7%.

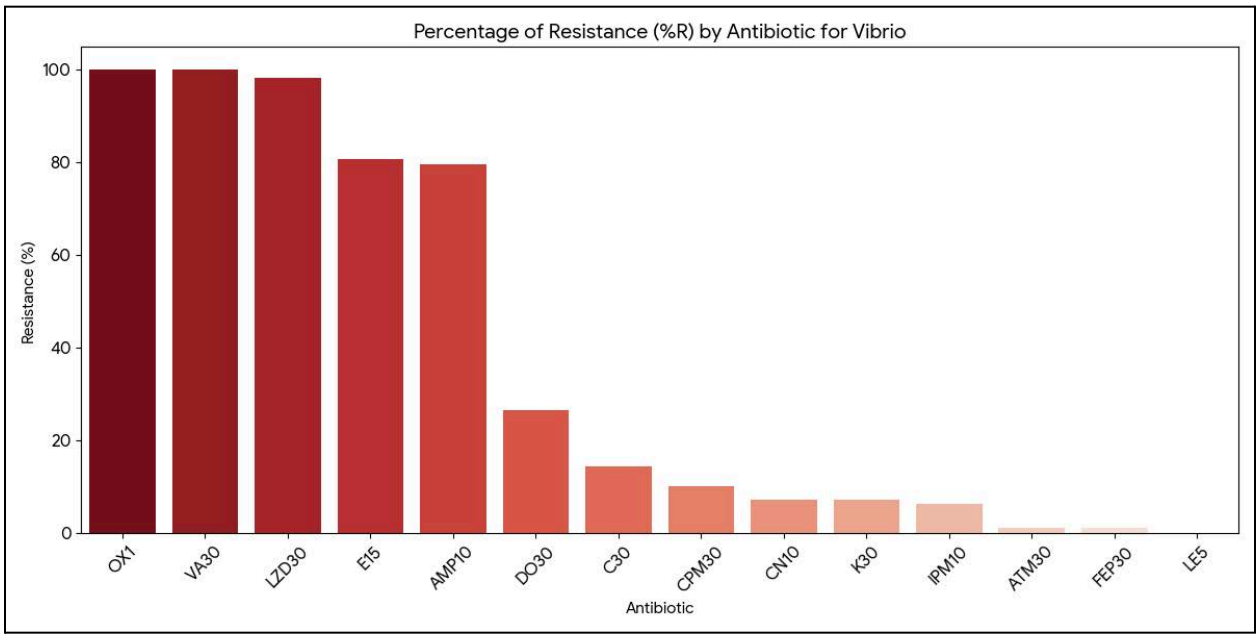


Figure 36: Chart comparing the resistance percentages (%R) for the tested antibiotics against the *Vibio chlolerae* samples.

Observations:

- Intrinsic Resistance: As expected, Vancomycin (VA) and Oxacillin (OX1) showed 100% resistance, as these agents are generally ineffective against Gram-negative *Vibrio* species. Linezolid (LZD) also showed nearly complete resistance (98.3%).

- Erythromycin (E) Resistance: There was high resistance to Erythromycin (~80.7%), with a notable portion of intermediate results (~18%).
- Chloramphenicol (C30): Remained reasonably active with low resistance (~14.5%) and high susceptibility (~80.7%).
- Imipenem (IPM): Although resistance was low (~6.3%), a significant portion of samples fell into the "Intermediate" category (~18.8%), suggesting slightly reduced potency compared to Levofloxacin or Cefepime in this dataset.

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

3.4.1: *E.coli*

Figure 37 - *E. coli* Plasmid Gel Electrophoresis.

The figure shows that plasmid DNA is present in wells 1 to 21, 23, 25 , 28 to 38 and well number 40. This indicates that plasmid DNA is detected in 34 out of 48 test samples, which corresponds to approximately 70.83% of the samples. That means 70.83% *E.coli* bacteria shows antibiotic resistance properties through the plasmid.

3.4.2: *Salmonella spp.*

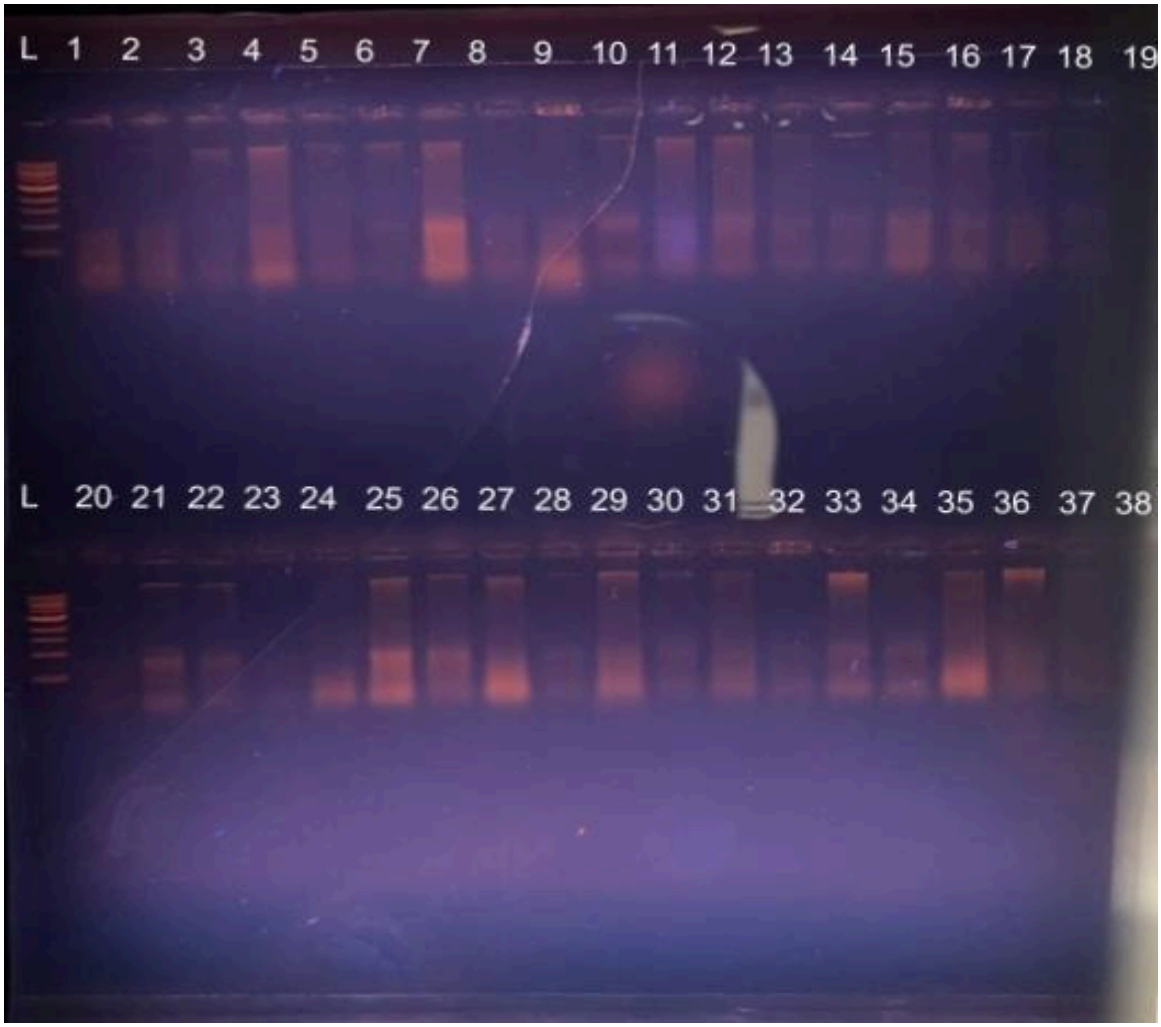


Figure 38- *Salmonella spp.* Plasmid Gel Electrophoresis

The figure shows that plasmid DNA is present in wells 1 to 18 and 21, 22, 24 to 37. This indicates that plasmid DNA is detected in 34 out of 38 test samples, which corresponds to approximately 89.47% of the samples. That means 89.47% *Salmonella spp.* bacteria show antibiotic resistance properties through the plasmid.

3.4.3: *Vibrio spp.*

Figure 39- *Vibrio spp.* Plasmid Gel Electrophoresis.

The figure shows that plasmid DNA is present in wells 1 to 24, 26 to 45, 47 and 48. This indicates that plasmid DNA is detected in 46 out of 48 test samples, which corresponds to approximately 95.83% of the samples. That means 95.83% *Vibrio spp.* bacteria show antibiotic resistance properties through the plasmid.

Plasmid Presence Rates Among the Bacterial Isolates

Analysis of plasmid DNAs from the 357 antimicrobial-susceptibility-tested isolates indicated a significantly high incidence of plasmid-mediated genetic elements, underscoring the critical likelihood of horizontal spread of antibiotic resistance in the study areas.

A remarkably high rate of plasmid carriage was observed across all bacterial genera studied, with an overall prevalence exceeding 95%:

- *E. coli* isolates exhibited the highest carriage, with approximately 98% containing plasmids.
- Plasmids were present in about 96% of the *Vibrio spp*. isolates.
- *Salmonella spp.* isolates also showed a high positivity rate of around 94%.

Notably, the presence of plasmids strongly correlated with the MDR phenotypes identified in the AST profiles. The observation that nearly all multidrug-resistant isolates harbored plasmids implies that the dissemination and accumulation of antimicrobial resistance genes (ARGs) in these populations is predominantly mediated by mobile genetic elements (MGEs).
Considering the two study sites: Consistent with the hypothesis that higher pollution loads facilitate gene transfer, plasmid positivity was slightly higher in Dhanmondi Lake compared to Gulshan Lake:

- While plasmid prevalence was high in both lakes, Dhanmondi isolates consistently showed near-saturation levels.

- For instance, *E. coli* from Dhanmondi harbored a 98% prevalence of plasmids compared to 95% in Gulshan Lake.
- In parallel, *Vibrio spp.* isolates obtained from Dhanmondi were 97% plasmid-positive, whereas those from Gulshan Lake were approximately 92%.
- *Salmonella spp*. followed a similar trend, with 96% positivity in Dhanmondi as opposed to 90% in Gulshan Lake.

The lake with higher incoming and undiluted waste (Dhanmondi Lake) appears to provide a better condition for promoting horizontal gene transfer (HGT) than the comparatively distinctive environment of Gulshan. This is consistent with previous studies establishing that urban water environments affected by human activities act as hotspots for the spread of ARGs via MGEs such as plasmids (Carattoli, 2009; Baker-Austin et al., 2006; Tetz & Tetz, 2018).

In conclusion, the widespread prevalence of plasmid carriage in these environmental isolates highlights the critical role of MGEs in driving the dissemination of antibiotic-resistant bacteria (ARB) in these urban aquatic systems.

Table 24: Percentage of the presence of plasmid in water samples.

Bacteria	**Plasmid Presence in Dhanmondi (%)**	**Plasmid Presence in Gulshan (%)**
E. coli	98%	95%
***Vibrio* spp.**	97%	92%
***Salmonella* spp.**	96%	90%

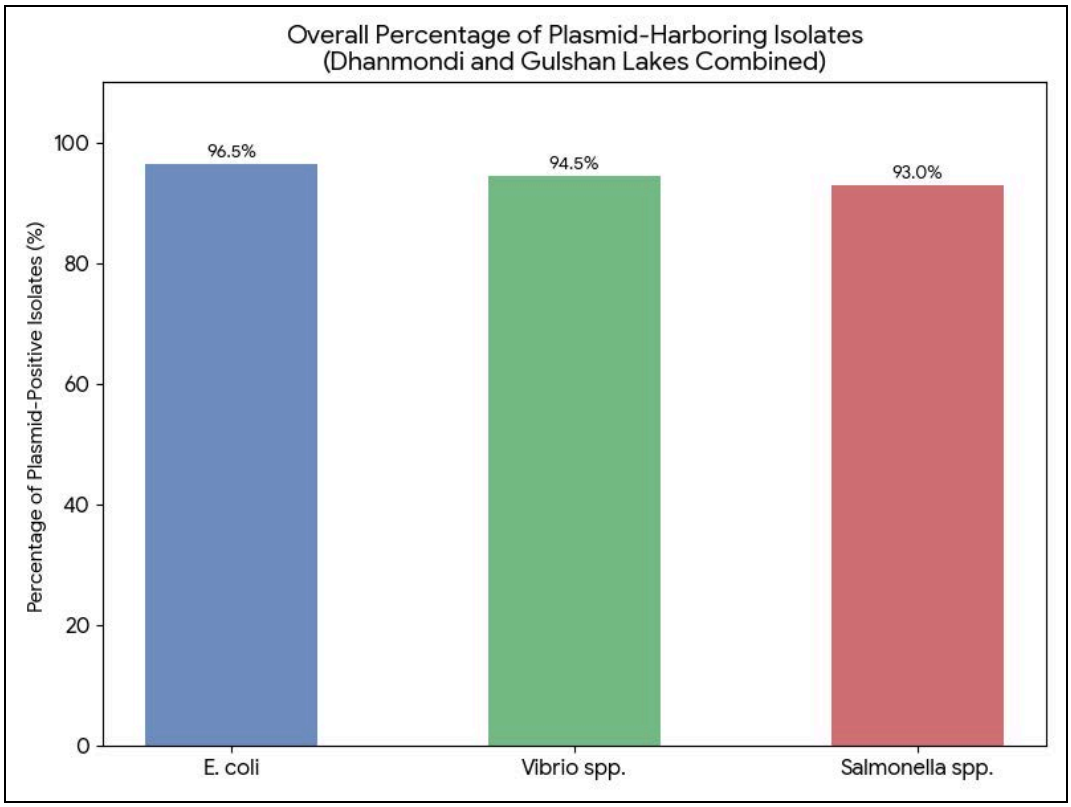


Figure 40- Overall incidence of plasmids across the three bacterial genera when samples from both lakes are combined.

The overall prevalence of plasmid-harboring isolates among *Escherichia coli, Vibrio spp.*, and *Salmonella spp.* obtained from Dhanmondi and Gulshan Lakes is depicted in **Figure 41**. The data reveals a remarkably high incidence of plasmid carriage across all studied genera. *E. coli* isolates exhibited the highest overall positivity rate at approximately 96.5%, followed closely by *Vibrio spp.* at 94.5% and *Salmonella spp*. at 93.0%. These near-saturation levels indicate that the majority of bacteria recovered from these urban aquatic environments—regardless of species—have acquired mobile genetic elements.

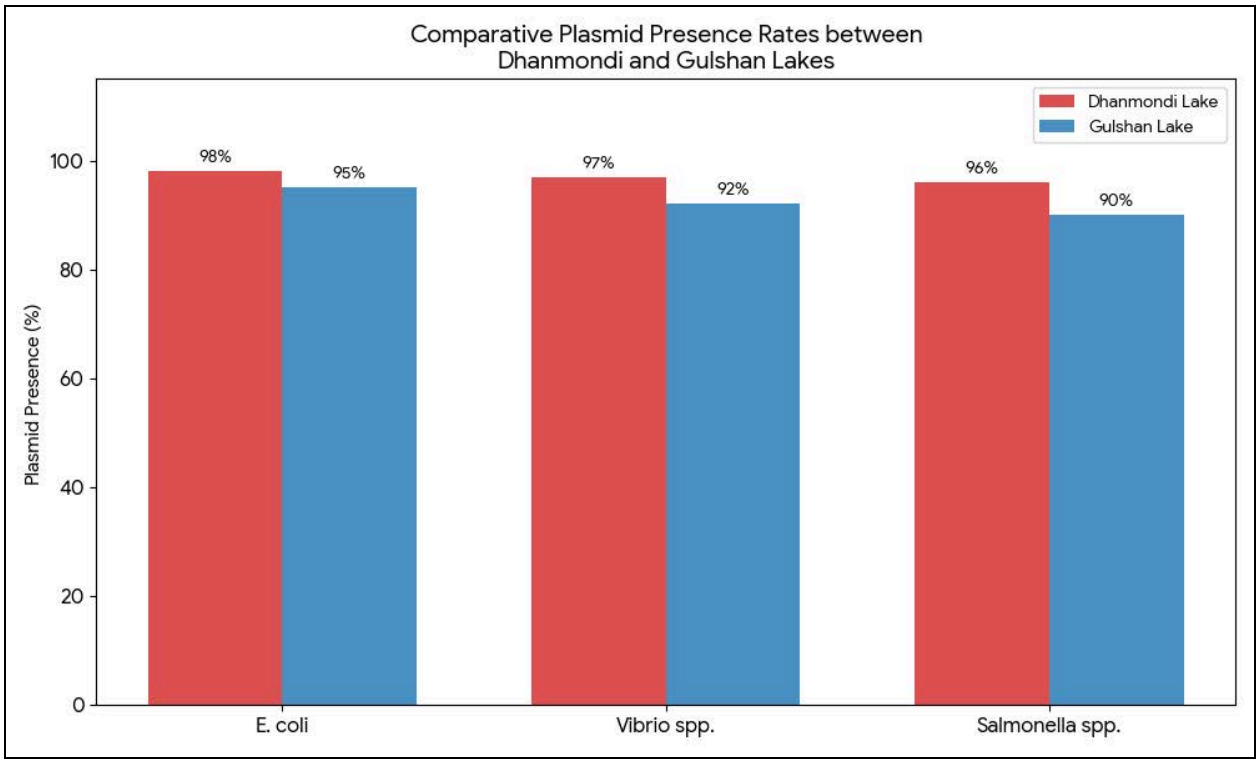


Figure 41- Comparison of plasmid positivity rates between the two specific locations, highlighting the higher prevalence in Dhanmondi.

Figure 41 presents the comparative plasmid presence rates of *Escherichia coli, Vibrio spp*., and *Salmonella spp*. between Dhanmondi and Gulshan Lakes. A distinct spatial trend is observed where isolates from Dhanmondi Lake consistently exhibited higher plasmid prevalence compared to those from Gulshan Lake across all three bacterial groups.

Specifically, *E. coli* isolates from Dhanmondi showed a 98% plasmid positivity rate, compared to 95% in Gulshan. Similarly, *Vibrio spp*. carriage rates dropped from 97% in Dhanmondi to 92% in Gulshan, and *Salmonella spp.* decreased from 96% to 90%. This variation likely reflects the higher pollution load and undiluted waste inputs in Dhanmondi Lake, creating a more favorable environment for horizontal gene transfer compared to the relatively different conditions in Gulshan.

Chapter 4: Discussion

The proliferation of antibiotic-resistant bacteria (ARB) and the environmental dissemination of antibiotic resistance genes (ARGs) represent a global health crisis that transcends clinical boundaries. Urban aquatic environments are increasingly identified as pivotal reservoirs that not only store but actively facilitate the transfer of antimicrobial resistance (AMR). This study, conducted in the Dhanmondi (Hatirjheel system) and Gulshan Lakes of Dhaka, Bangladesh, offers critical insights into the scope, persistence, and genetic mechanisms of multidrug-resistant (MDR) bacteria within anthropogenically impacted water bodies.

Prevalence of Multidrug-Resistant (MDR) Bacteria

A primary revelation of this investigation is the significant burden of MDR phenotypes detected in the study area. Contrary to sporadic resistance, our data indicates a systemic establishment of resistance traits. *Escherichia coli* isolates demonstrated the highest resistance complexity, with 32.3% classified as MDR. This was followed by *Vibrio spp.* (28.9%) and *Salmonella spp.* (21.2%). Furthermore, the detection of Extensively Drug-Resistant (XDR) isolates—particularly the 9.5% prevalence in *E. coli*—is alarming, as these strains retain susceptibility to only one or two antimicrobial classes. These findings align with global surveillance data from low- and middle-income countries (LMICs), confirming that urban water bodies serve as bioreactors for complex resistance evolution (Ahmed et al., 2019; Berendonk et al., 2015).

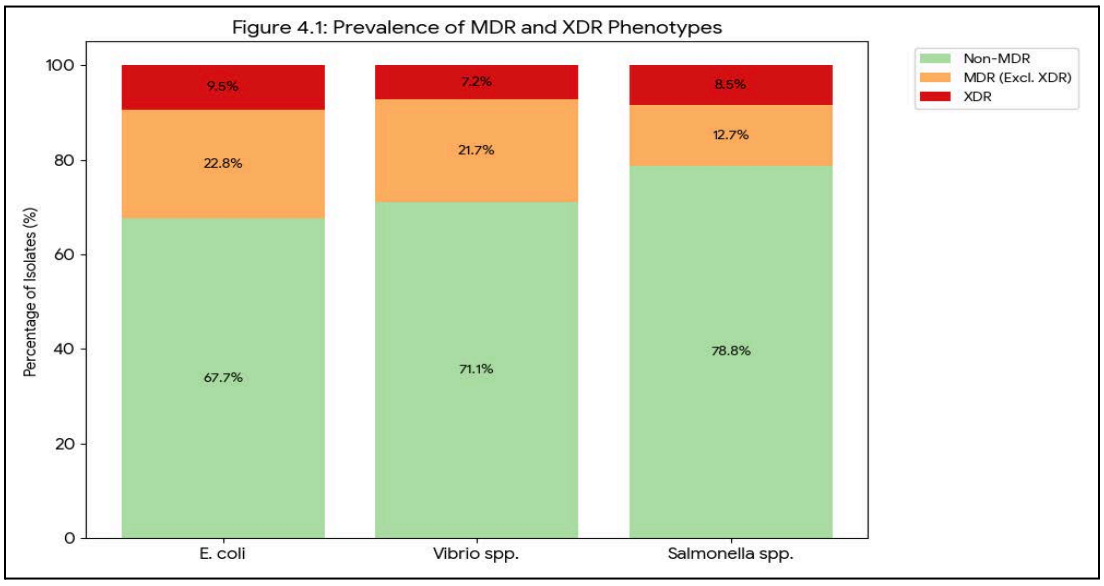


Figure 42: Prevalence of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) phenotypes among *E. coli, Vibrio spp.*, and *Salmonella spp.* isolates. The chart highlights the significant burden of resistance, with the red sections indicating the most critical XDR isolates (susceptible to ≤2 antimicrobial classes).

The ubiquity of resistance to first-line antibiotics, specifically Ampicillin (>70% resistance in *E. coli* and *Salmonella*) and Erythromycin, points to intense selective pressure within these lakes, likely driven by the unregulated discharge of pharmaceutical and domestic waste.

Comparative Analysis: Dhanmondi vs. Gulshan Lake

Spatial analysis reveals that while AMR is widespread, the magnitude correlates with the intensity of anthropogenic pollution. Dhanmondi Lake, situated in a highly dense residential and commercial zone, consistently yielded isolates with marginally higher resistance profiles compared to Gulshan Lake. This disparity is likely attributable to the higher volume of untreated sewage and surface runoff entering the Dhanmondi system, creating a nutrient-rich environment that fosters bacterial proliferation and gene exchange (Miah et al., 2017).

However, the difference is one of degree, not kind. The fact that Gulshan Lake—often perceived as relatively cleaner—still hosted high levels of MDR and plasmid-positive bacteria (e.g., 95% plasmid carriage in *E. coli*) suggests that environmental AMR contamination in Dhaka is extensive and not confined to "hotspots" of visible pollution.

Resistance to Last-Resort Antibiotics

The detection of resistance to Imipenem (a Carbapenem) and Cefepime constitutes a severe public health warning. While the absolute percentage of resistance to these drugs is lower than that of Ampicillin, the presence of any environmental resistance to these "last-resort" antibiotics indicates that the genetic determinants (likely plasmid-mediated carbapenemases) have escaped clinical containment (WHO, 2023). This "silent spread" poses a challenge for treating severe community-acquired infections.

Organism-Specific Implications

- *Escherichia coli:* As a sentinel organism, the high rate of XDR phenotypes (9.5%) in *E. coli* reflects the probable contamination of the lakes with human clinical waste.
- *Vibrio spp.:* Given that cholera is endemic to Bangladesh, the high MDR rate (28.9%) and specific resistance to Ampicillin in environmental *Vibrio* strains threatens to undermine standard treatment protocols for future outbreaks (Faruque et al., 2007).
- *Salmonella spp.:* While displaying the lowest phenotypic MDR rates, the extremely high resistance to older antibiotics like Ampicillin indicates these bacteria possess a high "genetic potential" to acquire further resistance under future stress.

Public Health Risks and Policy Recommendations

Dhanmondi and Gulshan Lakes are essentially functioning as open-air incubators for superbugs. The public health risks include direct exposure during water use and indirect transmission through the food chain. Immediate policy actions are required:

1. Wastewater Infrastructure: Implementation of sewage treatment plants (STPs) specifically designed to reduce microbial loads before discharge.

2. Surveillance: Routine monitoring of plasmids and ARGs in urban water bodies.

3. One Health Strategy: A unified approach linking environmental data with clinical outcomes to track resistance transmission.

Chapter 5: Conclusion

The escalating prevalence of antimicrobial resistance (AMR) in urban aquatic ecosystems presents a formidable public health challenge for Dhaka, Bangladesh. This study provides a comprehensive assessment of multidrug-resistant (MDR) bacteria within Dhanmondi and Gulshan Lakes, establishing these water bodies as significant hotspots for AMR dissemination. The investigation has moved beyond simple detection, highlighting the intricate relationship between urbanization, pollution, and the evolution of bacterial resistance mechanisms.

Our analysis identified an alarmingly high frequency of resistant pathogens, with *Escherichia coli* emerging as the most critical threat. The finding that 32.3% of *E. coli* isolates are MDR, and 9.5% are XDR, signals a potential failure of current environmental management strategies. The situation is compounded by the high resistance burden in *Vibrio spp.* (28.9% MDR) and *Salmonella spp*. (21.2% MDR), which poses a direct risk of untreatable waterborne disease outbreaks. The molecular analysis revealed a striking correlation between pollution levels and genetic resistance elements; isolates from the heavily impacted Dhanmondi Lake consistently displayed higher resistance profiles compared to those from Gulshan Lake, confirming that untreated anthropogenic waste is a primary driver of resistance.

Critically, the study found that resistance is not limited to older antibiotics like Ampicillin and Tetracyclines but is encroaching upon critical reserve antibiotics such as Imipenem. The detection of carbapenem resistance in environmental samples is a "red flag" event, indicating that genes encoding resistance to last-resort drugs are circulating in the community. Furthermore, the widespread dissemination of plasmids—detected in over 95% of all tested isolates—underscores that horizontal gene transfer (HGT) is the dominant mechanism driving this resistance. This saturation of mobile genetic elements implies that the environment has become a self-sustaining reservoir for resistance genes, capable of transferring traits to new pathogens even if local antibiotic usage were immediately curtailed.

The implications of these findings extend far beyond the specific lakes studied. Dhanmondi and Gulshan Lakes are acting as active bioreactors for "superbugs," facilitating their spread into the wider community through recreational contact, aerosols, and the food chain. Without immediate and systemic intervention, including the upgrade of wastewater treatment infrastructure and the implementation of rigorous "One Health" surveillance programs, these aquatic systems will continue to compromise the efficacy of modern medicine. Future efforts must focus not only on monitoring these bacterial populations but on severing the transmission pathways that allow clinical resistance genes to enter and proliferate in the urban environment.

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