Correlation Between Plasmid Presence & Antibiotic Resistance: In Enteric Bacteria; Isolated From Different Water Bodies Inside Dhaka City

By

Tasnim Tabassum
22136021

Aurchi Sarker
22136065

Kamrun Nahar Asha
22136070

A thesis report submitted to the Department of Biotechnology in partial fulfillment of the requirement for the degree of
Bachelor of Science in Biotechnology

Department of Biotechnology
School of Life Science
BRAC University
February 19, 2026

Declaration

It is hereby declared that

1. The thesis report submitted is our original work while completing my 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 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:

_____

Tasnim Tabassum

Student ID: 22136021

_____

Aurchi Sarker

Student ID: 22136065

_____

Kamrun Nahar Asha

Student ID: 22136070

Approval

The thesis titled “Correlation Between Plasmid Presence & Antibiotic Resistance: In Enteric Bacteria; Isolated From Different Water Bodies Inside Dhaka City” submitted by

1. Tasnim Tabassum (22136021)
2. Aurchi Sarker (22136065)
3. Kamrun Nahar Asha (22136070)

Spring 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology in 19th February, 2026.

Examining Committee:

Supervisor: (Member)

Iftekhar Bin Naser, PhD

Associate Professor and Chairperson

Department of Biotechnology

BRAC University

Departmental Head: (Chair)

Iftekhar Bin Naser, PhD

Associate Professor and Chairperson

Department of Biotechnology

BRAC University

Ethics Statements

This study was carried out in compliance with ethical norms and protocols for the environment along with microbiological research. The study included the collection and examination of water samples from Hatirjheel, Curzon Hall and Ramna Lake in Dhaka Metropolitan City. This study included no subjects from humans or animals and so did not need approval from the institution's review board. All methods of collecting samples were done in a way that would cause the least amount of harm to the environment. During collection, transit, and laboratory processes, all safety rules were observed to protect researchers and keep the environment safe. The study did not involve genetic modification or the introduction of biological agents into the environment. The information from this study was exclusively utilized for research and teaching. The findings and conclusions were clearly stated, and the data were not made up or fabricated. This investigation was conducted in compliance with the concepts of scientific integrity, responsibility towards the environment, and data protection.

Acknowledgement

First and foremost, we are greatly indebted to our Almighty who gave us strength, perseverance, and resolution of wit to pursue and accomplish our thesis successfully. His blessings helped us to complete our role with dedication and submit this work with commitment and being sincere.

We are extremely grateful to our respected supervisor, **Dr. Iftekhar Bin Naser,** Associate Professor and Chairperson, Department of Biotechnology, School of life sciences, BRAC University. We also earnestly recognize him as the Chairperson who gave us the chance to perform our research in the thesis laboratory. His wise counseling, long period of working, constructive feedback and unending encouragement played a crucial role in all the phases of this study. His mentoring facilitated the quality of this work, as well as our analytical and research abilities. This thesis would have not been completed successfully without his supervision and constant support.

We also want to express our warmest gratitude to the lab officers, lab attendants and the research assistant **Fariya Akter**, who greatly helped us with the invaluable support, technical assistance and all-time cooperation during the experimental part of our work. They were helpful and their professional dedication played a significant role in ensuring seamless completion of our work in the laboratory.

Lastly, we like to express our deepest gratitude to everyone who helped us in this course of studies directly or indirectly. Their support and collaboration have been immensely valued.

Table of contents

List of Tables

List of Figures

List of Acronyms

Acronyms	Meaning
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Gene
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide Triphosphate
EMB agar	Eosin Methylene Blue
EtBr	Ethidium Bromide
HGT	Horizontal Gene Transfer Multidrug Resistant
MCT	Micro Centrifuge Tube
MDR	Multiple Drug Resistant
MHA	Mueller Hinton Agar
Nf water	Nuclease free water
PCI	Phenol-Chloroform-Isoamyl Alcohol
PCR	Polymerase Chain Reaction
SS agar	Salmonella-Shigella (agar)
TAE Buffer	Tris-Acetate-EDTA buffer
TCBS agar	Thiosulfate-Citrate-Bile Salts-Sucrose (agar)
TE Buffer	Tris-EDTA buffer
UV	Ultraviolet
XDR	Extensively Drug Resistant

Abstract

Antimicrobial resistance (AMR) is an endemic issue of public health in the world, and there is increasing evidence that surface water bodies are significant environmental reservoirs of antibiotic-resistant bacteria. This study has analyzed the relationship between the occurrence of plasmids and antibiotic resistance in the pathogenic enteric bacteria that develop in surface water sources in Dhaka Metropolitan City. To identify the contribution of anthropogenic interference to the waters of three urban water bodies, namely, Hatirjheel, Curzon Hall, and Ramna Lake, water samples were taken from January to June 2025, and with varying degrees of anthropogenic impact. Three clinically and ecologically important bacteria, i. e. *Escherichia coli, Salmonella* spp., and *Vibrio cholerae*, were the subject of the investigation. The selective and differential media (EMB, TCBS, and SS agar) were used to perform isolation, and antibiotic susceptibility testing was conducted to identify the profiles of resistance. Molecular validation was done by polymerase chain reaction (PCR) and plasmid isolation done to assess genetic basis of resistance. PCR amplicons and plasmid DNA were analyzed utilizing agarose gel electrophoresis, which allowed performing the correlation analysis between the plasmid carriage and the observed resistance patterns. The results showed that the prevalence of PCR-confirmed antibiotic-resisting isolates and plasmid-bearing isolates were very high in Hatirjheel and then in Ramna Lake, but relatively low in Curzon Hall. There was a close correlation existing between the presence of plasmids and the presence of multidrug resistance thereby highlighting the importance of plasmid-mediated horizontal gene transfer in water.

Key Words: Antimicrobial resistance (AMR); Plasmid-mediated resistance; Surface water contamination; Dhaka Metropolitan City; Hatirjheel, Curzon Hall, Ramna Lake, *Escherichia coli*; *Salmonella* spp.; *Vibrio cholerae*; PCR identification; Antibiotic Susceptibility Test (AST); Horizontal gene transfer (HGT); Multidrug Resistance (MDR); Environmental Reservoirs; Agarose gel electrophoresis; Antibiotic Resistant Bacteria (ARB); Seasonal variation; Public health risk.

Chapter 1

Introduction

Antibiotic resistance has become one of the most urgent concerns of the global public health of the twenty-first century that endangers the effective prevention and treatment of bacterial infections. The emergence of new antibiotic-resistant bacteria (ARB) threatens the world with a rapid expansion because the causes of the problem are interlinked complicated with clinical, agricultural and environmental factors, where the burden is disproportionately faced by low- and middle-income countries due to poor regulatory frameworks, unreasonable antibiotic prescriptions, and insufficient surveillance systems. Over the past years, the focus on the environmental aspect of antibiotic resistance has grown, specifically surface water bodies which act as hot spots and vectors of resistant bacteria and antibiotic resistance genes. Unmanaged sewage, industrial effluents, hospital wastes and agricultural runoffs continuously enter aquatic environments with antibiotics, resistant microbes, and mobile genetic components and thus the anthropogenic contribution of these micro-pollutants to environmental and clinical resistance dynamics is directed.

Water bodies on the surface, like rivers, lakes, canals and urban ponds offer optimal environments to the maintenance and spread of antibiotic resistance. These systems store antibiotic residues and pollutants which have a selective pressure, which selects resistant bacteria and increases their survival. Meanwhile, the dense and diverse bacterial populations within aquatic systems enhance horizontal gene transfer during their interactions especially via plasmids which are also the most effective in transferring resistance genes across the bacterial species and genera. Specific seasonal processes like rain, flood and low flow during the dry season also play a role in affecting microbial distributions and pollutant concentration, which further complicate resistance based dynamics in monsoon dominated areas. In turn, surface waters have become known as so-called hotspots of emergence and amplification of plasmid-mediated antibiotic resistance, and with direct effects to the welfare of civil society because of human contact to them in the form of household use, recreation, irrigation, and food production.

Bangladesh is one of the most vulnerable ones in this context because it has high population density, large surface water network, rapid urbanisation and insufficient wastewater treatment. Livelihoods, agriculture, fisheries, and domestic activities are supported by the surface water

system of the country, which is in turn massively stressed due to an untreated municipal sewage, industrial release, solid waste disposal, and agricultural effluents. Rivers, lakes and canals in large urban areas like Dhaka Metropolitan City are turning out to be sources of chemical and microbial pollution. Several research findings have reported that the important water quality parameters often exceed the acceptable levels and therefore most of the surface waters are not safe without treatment even to be used directly by human beings. Such contaminated waters contain large numbers of pathogenic and antibiotic-resistant bacteria, which exacerbates the risk of waterborne diseases and spreads resistance to the environment. The seasonal floods also redischarge pollutants throughout the floodplains and the limited dry-season flows concentrate the pollutant formations, irregular yet stable resistance-enhancing environments.

Enteric pathogens e.g. *Escherichia coli, Salmonella* spp. and *Vibrio cholerae* are of the greatest concern because of their high affinity or connection to human disease and resistance to antibiotics amongst microorganisms in polluted surface waters. They are frequently found in urban surface water receiving untreated sewage and are frequently known to be of human and animal fecal origin. Notably, they are also known to harbor plasmids that contain several antibiotic resistance genes and as a result, the process of acquisition and dissemination of multidrug-resistant (MDR), as well as, extensively drug-resistant (XDR) phenotypes would occur rapidly.Plasmid-mediated resistance poses a particularly serious problem since it permits the passage of the resistance features across the bacterial populations on a horizontal level making evolution much more accelerated than it would be in case of chromosomal mutations only. In aquatic ecosystems prone to anthropogenic pollution, selective demands due to antibiotics, heavy metals and other pollutants only contribute to the increase of plasmid persistence and transfer, which justify the ability of surface water to serve as a reservoir of mobile elements of resistance.

Although the role of the environment in antibiotic resistance has increasingly been identified, little data exists on the relationship between plasmid carriage and resistance patterns in urban

surface waters in Bangladesh.The association between the prevalence of plasmids with antibiotic resistance in the various water systems and seasons is important in clarifying the environmental mechanisms that relate pollution, ecology of microbes, and risk to human health. In that regard, the Dhaka Metropolitan City offers a topical and risky environment, in which water bodies of varying anthropogenic effect are located in a highly populated urban environment. A study of pathogenic bacteria of sampled lakes in Dhaka during different seasons can provide significant knowledge about the influence of environmental change and human pressure on the development of plasmid-mediated resistance. This type of evidence is vital in enhancing environmental surveillance, policy intervention of One Health, and reducing the rising threat of antibiotic resistance associated with the urban surface

Chapter 2

Literature Review

2.1 Water bodies as sampling site

The water bodies make the right sampling place to investigate antibiotic-resistant bacteria since they portray environmental reservoirs where the resistant bacteria, antibiotic residues, and resistance genes may survive and spread (Kusi et al., 2022; Bartley et al., 2019). Agricultural runoff, untreated sewage, discharge of industries, pharmaceutical waste, and wastewater treatment systems without entire removal of antimicrobial contaminants pollute rivers, lakes, groundwater, and wastewater (Santiago-Rodriguez et al., 2013; Silva et al., 2021). Such conditions favor selective pressure that favours the survival and proliferation of resistant bacteria. The surrounding human action, as well as such natural processes as rainfall and climate, also influence the water quality by enhancing the pollutant load and transporting bacteria throughout water (Kim and An, 2015). It aligns with the facts that a higher anthropogenic impact is linked to an increased prevalence and dispersal of antibiotic-resistant bacteria in water bodies (Na et al., 2018), and that antibiotic effluents of treated humans or animals are also related to resistance emergence (Megantara et al., 2023). As there is a possibility of exposure via water use, water bodies are of significance to monitor the health of the people (Meradji et al., 2025).

2.2 Overview of water bodies and surface water in Bangladesh

The country of Bangladesh is blessed with a vast chain of water bodies, which are overridden by surface water bodies that are central to ecology, livelihoods, agriculture, and the health of the population (Rahman et al., 2022). The surface water system consists of rivers, canals, floodplains, haors, baors, beels, lakes, and wetlands, and over 700 rivers are present in the Ganges-Brahmaputra-Meghna (GBM) basin, which is one of the largest systems in the world (Pasha et al., 2023). They are used to support fisheries, irrigation, inland navigation, and domestic usage and also sustained, rich biodiversity and natural flood control throughout the deltaic landscape (Parvin et al., 2022). A rapid urbanisation, industrial effluents, untreated domestic sewage, agricultural effluents, and careless dumping of solid waste has however caused dire water quality conditions in surface water, especially in and around large urban areas like

Dhaka where rivers, lakes and canals are becoming sinkholes of chemical and microbial pollution (Uddin et al., 2021). Seasonality also affects the conditions of surface water, since monsoon flooding disperses the contaminants throughout floodplains, and lower dry-season streams increase the levels of contaminants (Parvin et al., 2022). This means that in most cases, surface water bodies are not clean enough to be directly used by humans without treatment, which is a cause of great concern to environmental sustainability and spreads of water borne diseases in Bangladesh.

2.3 The current situation of surface water in Bangladesh

Water bodies in Bangladesh, especially the inland waters in Dhaka, are in a very poor condition at the moment, and rivers, lakes, and canals are characterized by extensive chemical and microbial pollution (Islam et al., 2019). The investigation of urban rivers suggests that the majority of surface water sources cannot be used as a drinking source without advanced treatment since such important indicators as dissolved oxygen, biochemical oxygen demand (BOD), heavy metals, and faecal coliforms are constantly above acceptable drinking-water standards (Islam et al., 2019; Khan et al., 2023). Pathogenic bacteria thrive well in such polluted water bodies, and continuous exposure to pollutants and antimicrobial residues may encourage the plasmid acquisition and antibiotic resistance. These waters have a high microbial load and environmental stress that enhances the probability of horizontal gene transfer between pathogenic bacteria via plasmid, which is a horizontal gene transfer method. It is also found that these rivers have a high concentration of toxic metals and a low level of dissolved oxygen, which is an indicator of severe urban and industrial discharge pollution (Research based on molecular toxicity measurements, 2019). The aggravating state of pollution is mainly caused by the rapid urbanisation process, uncontrolled industrialisation, direct dumping of un-treated sewage, poor solid waste management and the growing land-use pressure, which has increased over the past decades as a result of population growth and laxity in the enforcement of regulations. The effects of this growing pollution are especially significant since it reduces the quality of aquatic life, disrupts fisheries, and constrains the provision of safe water, and boosts dependence on other water supplies, thus compromising environmental sustainability and urban resilience. Despite the existence of surface water management policies and action plans, the lack of quality improvement in water quality has been hindered by poor implementation, poor wastewater

treatment capacity, and low monitoring. Its public health impact is great, since surface water contamination is a key factor in the distribution of waterborne diseases, including diarrhoeas, cholera, and typhoid, which become particularly common in highly populated and flood-prone cities where humans are exposed to contaminated water most often (Bilal et al., 2023).

2.4 Selection of sampling sites

In our thesis project, we have chosen three water sources in the Dhaka Metropolitan City, these include Hatirjheel, Ramna Lake, and Curzon Hall. Hatirjheel, Ramna Lake and Curzon Hall Lake were chosen based on their differences in environmental environment, anthropogenic stress as well as human exposure, which offer a good ecological gradient in studying plasmid-mediated resistance.

Hatirjheel is a very urbanised and hydrologically stressed lake receiving untreated sewage, urban runoff as well as mixed waste discharges. According to the literature on the topic, the severe levels of microbial contamination, the high loads of the pathogenic bacteria, and the constant surpass of the most important physicochemical parameters are reported, and the strong seasonal variability is an indication of chronic pollution stress (Miah et al., 2017; Alam, 2017; Rakib et al., 2024; Noor, 2022). The conditions are conducive to the survival and transmission of plasmid resistance to antibiotics.

Ramna Lake is a controlled leisure water body that has a moderate anthropogenic impact on it through public use and runoff. It is less exposed to water quality instability, but periodic loss has been reported, which is a manifestation of long-term but less intensive environmental pressure (Islam et al., 2015).

Curzon Hall Lake falls in a zone with academic and hospital proximity, with these activities and clinical proximity adding to the contribution of chemicals and microbes. Water quality measurements show variability due to a human presence and wastewater inflow, which means its importance in the study of the environmental-clinical interface of plasmid-mediated resistance (Shahed, 2025).

2.5 Pathogenic Bacteria in Surface Water and Selection of Target Organisms

Complex microbial communities of beneficial as well as pathogenic bacteria live in surface water environments. Aquatic microorganisms that occur naturally serve a role in the ecology of the environment in terms of nutrient cycling, degrading organic matter, and sustaining biogeochemical balance. Nonetheless, the growing anthropogenic pressure has dramatically shifted the microbial community of surface waters causing proliferation of the pathogenic and antibiotic-resistant bacteria. Recent findings are that surface waters are important environmental reservoirs of human pathogens and antimicrobial resistance genes, especially in urbanisation impacted areas, wastewater release, and ineffective sanitation systems (Sharif et al., 2025).

Enteric bacteria that are present in human and animal faecal material are usually found in surface waters. They are *Escherichia coli*, *Salmonella spp, Vibrio cholerae*, *Shigella* spp, *Campylobacter* spp, *Enterococcus* spp, *Pseudomonas aeruginosa and Aeromonas* spp that are commonly found in rivers, lakes and other fresh water bodies that receive sewage that is not treated. Not only do such bacteria cause waterborne diseases, but also play a major role in the persistence and spread of antibiotic resistance in the environment. Plasmids and other mobile genetic elements mediate horizontal gene transfer between aquatic environments and allow resistance traits to be transferred between bacterial populations and species (Cabral, 2010). Considering these issues, *Salmonella* spp., *Vibrio cholerae*, and *Escherichia coli* will be included in the current study because they are common in contaminated water bodies, have a high impact on human health, and their presence is closely linked with plasmid-mediated antibiotic resistance (WHO, 2025).

2.5.1 Rationale Selection of *Salmonella* spp.

One of the greatest enteric pathogens that are linked with contaminated surface waters is *Salmonella* species. They are mostly found in water bodies due to faecal contamination and have been rampantly reported to occur in rivers and other freshwater bodies affected by human activities. It has been proved that *Salmonella* may survive a long time in surface water, especially under high-nutrition conditions, which increases the chances of human contact with water by using and consuming it (Mina et al., 2024).

The rationale of using *Salmonella* spp. in the present research is highly justified by their reported involvement in actions of anti-bacterial resistance and the ability to carry plasmids. Environmental *Salmonella* isolates are also found to be highly resistant to a variety of drugs, and the genes associated with resistance are often present in the transferable plasmids (Mina et al., 2024). These plasmids can encode resistance to the most popular antibiotics, and horizontal gene transfer can be achieved in aquatic microbial communities. Also, *Salmonella* has been identified as a valuable indicator organism that can be used to estimate the spread of antimicrobial resistance in the environment (Akinyemi et al., 2018). Thus, the study of plasmid in *Salmonella* found on the surface water plays a crucial role in the dynamics of this problem in the environment.

2.5.2 Rationale Selection of *Vibrio cholerae*

Vibrio cholerae is an aquatic bacterium which is highly adapted to the surface water. It lives and thrives in the presence of plankton, sediments, and biofilms so that it can continue to exist without the direct presence of faecal contamination. *V. cholerae* is a pathogenic agent of cholera, a serious waterborne infection that is still endemic in most regions of the world especially in areas where there is inadequate access to clean drinking water (Duodu et al, 2025).

V. cholerae has been included in the study because of its ecological importance and the emergence of this microorganism in antimicrobial resistance. Strains of *V. cholerae* in the environment have been demonstrated to contain plasmids and other mobile genetic components that enable the resistance to antibiotics and adaptive survivability under water bodies (Amalina et al. 2019). The surface waters can offer the best environment where genetic exchange can occur between *V. cholerae* and other bacteria to enhance the possibility of spreading the resistance genes. In addition, *V. cholerae* has been reported to develop resistance to widely used antibiotics making it challenging to control the disease when there is an outbreak of cholera (Duodu et al, 2025). These attributes render *V. cholerae* a very important organism in the study of plasmid-mediated resistance in surface water habitats.

2.5.3 Rationale Selection of Escherichia coli

The *E. coli* is extensively used as a primary indicator of faecal contamination in the quality of the water. Its appearance in the surface water is the sign of direct anthropogenic impact as well as insufficient sanitation. Although most *E. coli* variants are not pathogenic, pathogenic variants cause numerous gastrointestinal and extraintestinal infections, which present a serious health issue to the population (Saima et al., 2023).

The choice of *E. coli* is further justifiable by the fact that it plays a central role in the distribution of antibiotic resistance in the environment. Plasmids with the resistance to various classes of antibiotics, including b-lactams, sulfonamides, and aminoglycosides, are often present in environmental *E. coli* isolates (Hossain et al., 2023). *E. coli* is an important reservoir and carrier of resistance genes in aquatic microbial communities because of its great genetic plasticity and horizontal gene transfer ability.

2.6 Antibiotic resistance in Bangladesh and why it is increasing

Antibiotic resistance is an issue of significant threats to the health and health care systems of the population and has become a serious and increasing health problem in Bangladesh. Antibiotic resistance is the ability of bacteria to survive and reproduce despite an exposure to antibiotics, and can happen through genetic mutations, horizontal gene transfer, and altered gene expression that allow bacteria to evade or modify or inactivate the antimicrobial agents (Hassan & Nohor, 2025). Poor awareness, weak regulatory systems, irrational antibiotic use and poor surveillance systems among developed countries such as Bangladesh are the ones that imbalance their differences in health (Okeke et al., 1999). The National Antimicrobial Resistance Surveillance of Bangladesh (2016-2023) indicates the alarming statistics of the past five years 11% of the growth of antibiotic resistance and the significant deterioration in the effectiveness of commonly used antibiotics, namely, 71% in 2016 and 18% in 2023 (Hassan and Nohor, 2025). Additionally, extensive systematic review of 46 studies demonstrated extensive resistance of major bacterial pathogens such as *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas* spp. in different areas of Bangladesh, revealing the intensity and national scope of the issue (Ahmed et al., 2019).

In Bangladesh, the high rate of increase in the problem of antibiotic resistance is highly influenced by the high misuse and overuse of antibiotics in the human and animal sectors. Research has found that about 83% of doctors prescribe antibiotics without a proper diagnostic examination, usually in the self-limiting diseases colds and fever, which encourages unreasonable use (Biswas et al., 2014). Secondly, the uncontrolled over-the-counter sale of antibiotics promotes self-medication and approximately one-third of people take antibiotics without medical advice to help treat minor conditions (Biswas et al., 2014). The problem is also worsened by poor treatment adherence because approximately 60% of patients stop taking antibiotics too soon, which leads to higher risks of reinfections and development of resistance (Hossain et al., 2023). Another common use of antibiotics in the agricultural industry is the use of antibiotics in poultry farming without the oversight of a veterinarian, usually as growth factors, despite the presence of rules, including the Animal Feed Act (Tasmim et al., 2023). To worsen these problems, there is no strong national surveillance, and the resistance data can be only found in 6 of the 64 districts in Bangladesh (Ahmed et al., 2019). The lack of awareness, attitudes, and practices towards antibiotic use among different groups of the population is also widely spreading, and it is only through joint efforts by the government, education, and increased surveillance that the effects of antibiotic resistance can be positively changed (Islam et al., 2024).

2.7 Overview of Antibiotic Susceptibility Test

Antimicrobial susceptibility testing (AST) is an important laboratory technique that is known to assess the resistance or susceptibility of bacterial isolates against various antimicrobial agents. The most frequently used phenotypic AST techniques are the Kirby-Bauer disk diffusion assay, broth microdilution and E-test where the result is interpreted by reference to internationally agreed guidelines, such as Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. AST enables the classification of bacterial isolates as susceptible, intermediate and resistant and yields crucial information on the characterization of resistance patterns in environmental samples. AST is extensively applied in research on urban water bodies to determine the abundance of antimicrobial-resistant pathogenic bacteria, which indicates contamination of the environment, misuse of antibiotics, and poor health of people exposed to polluted water resources (Gajic et al., 2022; CLSI, 2024).

The classification of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria is based on the results of AST. Based on the internationally recognized definitions, MDR is defined as the non-susceptibility to at least one agent in three or more classes, whereas XDR is considered as non-susceptibility to all but one or two antimicrobial classes (Magiorakos et al., 2012). MDR bacteria have also been reported to be high in surface water, wastewater, and recreational water bodies, especially among enteric pathogens, including *Escherichia coli*, *Salmonella* spp., and *Vibrio cholerae* (WHO, 2025). The prevalence of MDR and XDR phenotypes in waterborne bacteria underscores the significance of aquatic environment as a substantial site of antimicrobial resistance and the significance of continued surveillance through standard AST methods in eliminating the risks to the health of the population in highly populated metropolitan regions (Sharif et al., 2025).

2.8 Plasmids and Their Impact in Surface Water Bacteria

Plasmids are self-replicating DNA molecules that are extrachromosomal and are typically present in bacteria that live in the surface waters of rivers, lakes, canals, and city ponds (Shintani et al., 2015). The sewage discharge, the stormwater runoff, the industrial effluents, and the human activity provide incessant microbial input to surface waters into numerous ecological niches, where the high diversity of bacteria and the exchange of genes thrives (Rodriguez-Beltran et al., 2021). Under these conditions, plasmids are vital in the adaptation of microbes because they harbor accessory genes that improve the survival of bacteria in the changing environmental conditions, such as alterations in nutrient availability and chemical stressors (Shintani et al., 2015). Experiments have also indicated that bacteria collected in surface waters often carry several plasmids of different sizes, which indicate the dynamism of the aquatic microbial community and the selective pressures which exist in urban water bodies (Fiamenghi et al., 2025).

Genetic plasticity and evolutionary potential of aquatic microbial communities are also significantly caused by the presence of plasmids in surface water bacteria (Rodriguez-Beltran et al., 2021). Plasmids promote the horizontal gene transfer among indigenous aquatic bacteria and those that are introduced by humans or animals and bring about a rapid genetic diversification in the surface water ecosystems (Shintani et al., 2015). Environmental plasmid research has shown

that the abundance and variety of plasmids are greatly affected by the water quality metrics and anthropogenic pollution and surface waters are significant sources of mobile genetic elements (Fiamenghi et al., 2025). Surface water bodies are tightly linked with urban activity in a densely populated city like Dhaka Metropolitan City, where the presence of plasmids in pathogenic bacteria is important to the persistence, adaptability, and ecological fitness of microbes in the urban aquatic environment (Rodriguez-Beltran et al., 2021).

2.9 Plasmid driven Antibiotic Resistance Spread through Horizontal Gene Transfer (HGT)

Plasmids are prime examples of mobile genetic elements that play a crucial role in the transmission and diversification of antibiotic resistance in the bacterial population as they harbor antibiotic resistance genes (ARGs) and allow their transfer between different bacterial hosts. ARGs may be located in the accessory genomic regions of plasmids which include integrons and transposons forming mosaic genetic elements that, in turn, enhance the process of acquiring resistance factors in one replicon (Castaneda-Barba et al., 2023). The broad-host-range plasmids, which can replicate in a wide range of bacterial species, have been regularly discovered in environmental, animal, and clinical isolates, which proves that they are centrally involved in the transmission of resistance determinants across the ecological boundaries (Sun et al., 2019). As an illustration, b-lactam as well as carbapenem antibiotic resistance and that of last-resort drugs like colistin have been transmitted in plasmids in Enterobacteriaceae and other Gram-negative bacteria, which have major effects on the clinical care of bacterial infections (Castaneda-Barba et al., 2023; Rozwandowicz, 2018). To enhance this, environmental research also indicates that ARG-containing plasmids are common to aquatic ecosystems, livestock farms, and soils, which indicates that the environmental reservoirs are significant contributors to the global resistome (Sun et al., 2019).

Antibiotic resistance transmission is solely catalyzed by the horizontal gene transfer (HGT) processes, which is mediated by plasmids, particularly conjugation. Horizontal transfer and the mobility of ARGs initiated by cell division allow plasmids to transfer ARGs not only horizontally (between chemologically unrelated bacterial species or strains), but also laterally (between closely related bacterial species or strains) to rapidly restructure resistance profiles in

microbial communities (Sun et al., 2019; Wachino et al., 2025). Complex transfer machinery in conjugative plasmids enables possible transfer of ARGs even to cells of a different taxonomic group direct DNA transfer by donor to recipient cell, and mobilizable plasmids can be transferred via other conjugation systems of individual plasmids (Ramirez et al., 2014). The study has indicated that environmental stresses and selective pressures such as exposure to antibiotics and pollutants can increase the transfer rate and persistence of plasmids in water and other environmental conditions contributing to the importance of HGT in resistance prevolution (Li and Zhang, 2023). Also, ARGs cannot remain in a fixed state, inter-plasmid recombination and transposon can make ARGs shuffle in different plasmids or be integrated into new genetic environments which complicates resistance dynamics (Rozwandowicz, 2018). In general, the role of HGT mediated by plasmids continues to be a highly important mechanism that facilitates the spread and sustenance of antibiotic resistance on the global scale.

2.10 Rising Threat of Plasmid mediated Antibiotic Resistance

The antibiotic resistance carried in plasmids is becoming a menace and is considered a significant public-health and environmental issue due to the fact that plasmids are extremely efficient horizontal-gene transfer (HGT) vectors of antibiotic resistance genes (ARGs) known to rapidly disseminate the resistance trait across bacterial species, genera and ecological infrastructures (Bennett, 2008; Smillie et al., 2010). In contrast to chromosomal resistance, vertically inherited, and relatively slow to spread, plasmids, in particular, conjugative ones, can also transfer multiple ARGs in a single genetic event, which is usually multidrug resistance (Partridge et al., 2018). Anthropogenic activities enhance this process considerably, particularly in urban settings, where the intensive application of the antibiotics in human healthcare, livestock production, aquaculture among many others leads to the ongoing introduction of antibiotics and resistant bacteria into natural environments (Rizzo et al., 2013). The use of sub-inhibitory levels of antibiotics, heavy metals and disinfectants among other chemical pollutants in aquatic environment are selective pressure factors that prefer plasmids harboring bacteria as well as triggering HGT and persistence of plasmids in microbial communities (Zhang et al., 2009). City surface waters that take untreated or insufficiently treated sewage and animal hospital effluents can be seen as ecological hotspots in which not only human and animal origin bacteria but also environmentally associated bacteria have a high concentration in them, leading

to a high opportunity to exchange plasmids and ARGs (Rizzo et al., 2013). This is especially the case with densely populated mega cities like Dhaka, whose rivers, lakes, and dumping of solid waste are greatly affected by domestic sewage, industrial effluents, and dumping of solid waste, all of which have provided the best environments to support the maintenance and dissemination of plasmid-borne resistance genes. Recent international studies have shown that ARG abundance on conjugative plasmids has increased significantly over the last 20 years, which illustrates the continuous increase in significance of the environmental reservoirs such as surface waters in connecting anthropogenic pollution and the clinical antibiotic resistance crisis (Wang et al., 2023). The plasmid-mediated resistance in the water context, therefore, is an important conduit by which environmental pollution by itself can lead to the development of hard-to-solve infections, which are threatening the lives of the population, water safety, and the sustainability of antibiotics

2.11 Emerging Threats of Antibiotic Resistant Bacteria Worldwide

Antibiotic-resistant bacteria (ARB) has become one of the most important global subjects of human health worldwide as it negatively affects the treatment and management of infectious diseases, morbidity, mortality, and healthcare expenditures. The ARB scourge leads to a higher burden in low- and middle-income countries (LMICs), in which the already established health system difficulties in limited surveillance capability, weak policy and legislative frameworks, ineffective antibiotic stewardship, and massive misuse of antibiotics make the spread and effects of resistance worse and more severe (Prestinaci et al., 2015; World Health Organization [WHO], 2023). In developing nations, the risks are much more dangerous because of the lack of appropriate awareness, unregulated access to antibiotics, a weak system of prescription regulations, and ineffective infection control, as well as resistance trends are worse than in high-income conditions (Hasan and Nohor, 2025). The recent estimates worldwide indicate that bacterial antimicrobial resistance directly caused 1.27 million deaths in 2019 and almost 5 million deaths in total, which identifies the issue as an enormous problem in the entire world (Murray et al., 2022). Monitoring data reveal that there is alarming levels of resistance in typical pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* in both human medicine, agricultural and animal production due to the overuse of antibiotics (WHO, 2023). Unless the globe acts in a coordinated way that incorporates

antimicrobial stewardship, efficient monitoring, regulatory oversight, and the implementation of One Health considering human, animal, and environmental health, the further increase in ARB is poised to take away decades of achievements in controlling infectious diseases on the planet (Hasan and Nohor, 2025; Murray et al., 2022).

2.12 Objectives of the Study

The main aim of the research was to examine the correlation between plasmid presence and antibiotic resistance in pathogenic bacteria isolated in the chosen surface water bodies in Dhaka Metropolitan City in relation to different seasons. To do this the investigation was narrow to the isolation and identification of *Escherichia coli*, *Salmonella* spp., and *Vibrio cholerae* in place of Hatirjheel, Ramna lake and Curzon hall lake which were water bodies of increasing levels of anthropogenic impact. Thereafter, antibiotic susceptibility testing was conducted to identify the resistance profiles and categorize the isolates as multidrug resistant (MDR) and extensively drug resistant (XDR) based on the CLSI (2024) and internationally recommended standard. Besides, the plasmid presence in the individual isolated pathogenic bacteria was also identified and evaluated to gain insight into how it was distributed between sites and seasons of sampling. In addition, the paper has also looked at the relationship that exists between the presence of the plasmid and the pattern of antibiotic resistance that was observed and in particular how this correlation changed across the seasons. In addition, the different seasonal changes in bacterial prevalence, resistance profile and plasmid distribution were compared among the sampled water bodies to estimate the interaction between environmental variability and anthropogenic pressure. In general, the research had attempted to come up with comprehensive evidence on how seasonal processes contribute to the development of plasmid-mediated antimicrobial resistance in urban surface waters and elucidate the public health implications that may have to Dhaka Metropolitan City.

Chapter 3

Materials and Methods

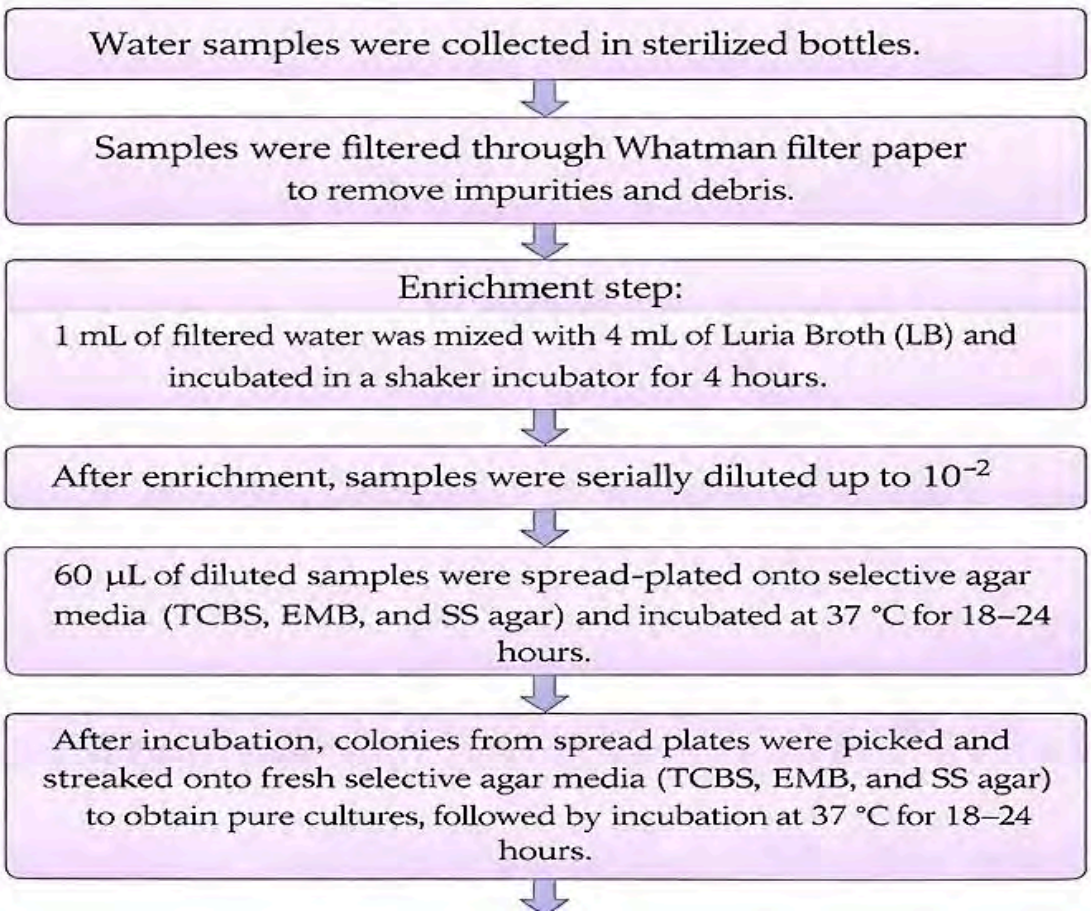
3.1 Study Place

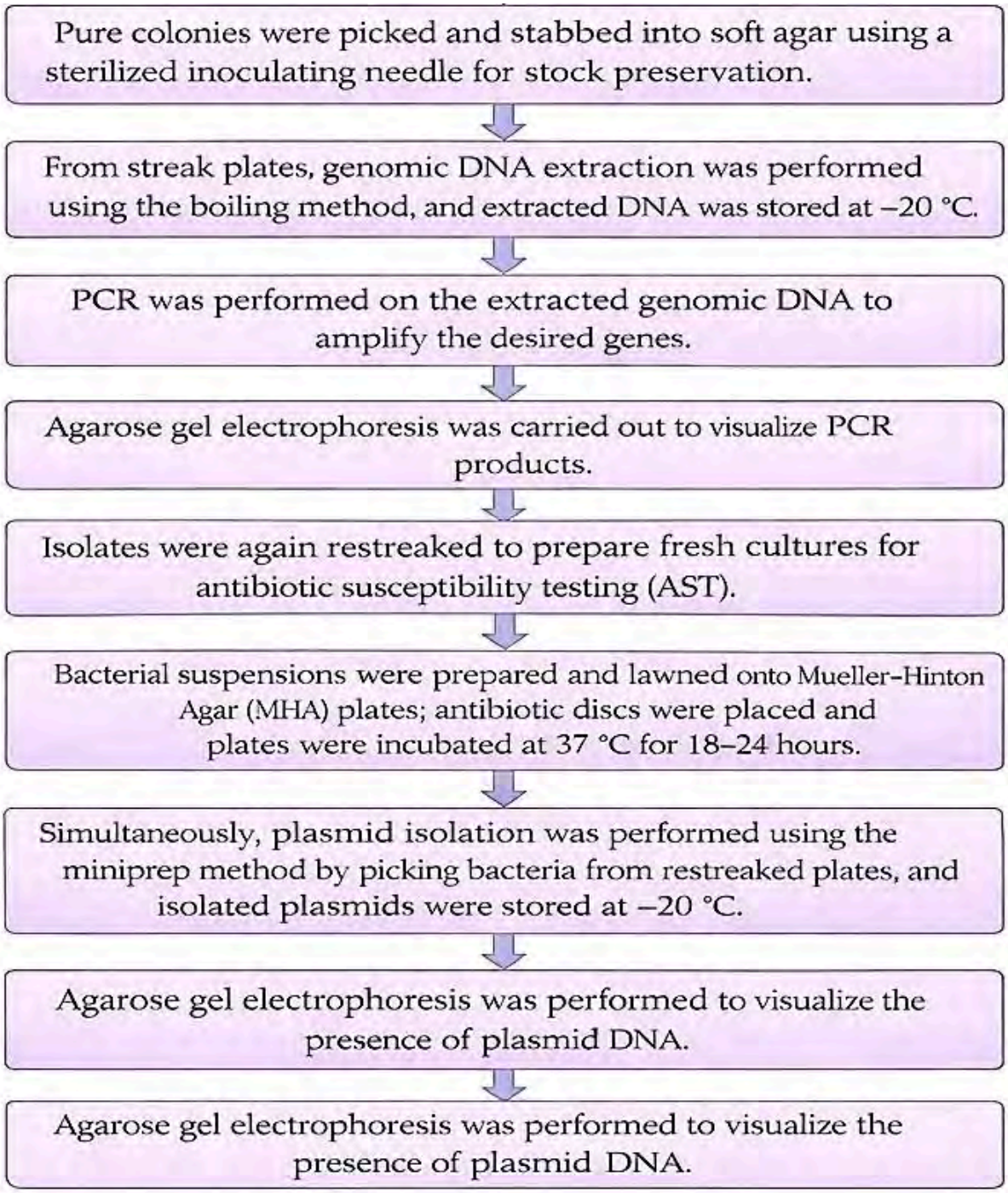
The laboratory experiments for the research were conducted in the Biotechnology, and Molecular Biology Laboratory of the Department of Mathematics and Natural Sciences at Brac University.

3.2 Study Duration

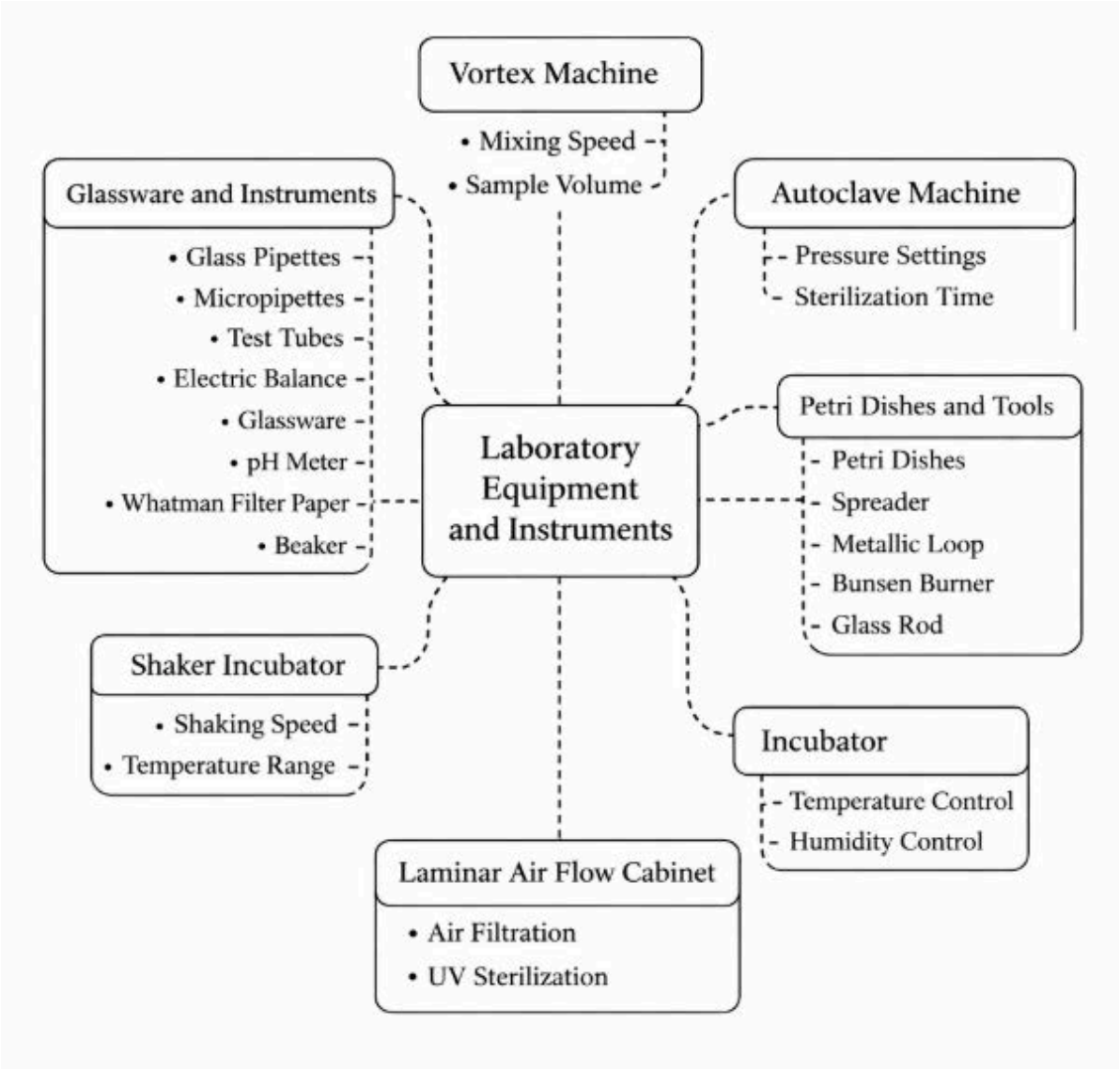
The study project spanned from January 2025 to June 2025.

3.3 Experimental Workflow




Pure colonies were picked and stabbed into soft agar using a sterilized inoculating needle for stock preservation.
From streak plates, genomic DNA extraction was performed using the boiling method, and extracted DNA was stored at –20 °C.
PCR was performed on the extracted genomic DNA to amplify the desired genes.
Agarose gel electrophoresis was carried out to visualize PCR products.
Isolates were again restreaked to prepare fresh cultures for antibiotic susceptibility testing (AST).
Bacterial suspensions were prepared and lawned onto Mueller-Hinton Agar (MHA) plates; antibiotic discs were placed and plates were incubated at 37 °C for 18–24 hours.
Simultaneously, plasmid isolation was performed using the miniprep method by picking bacteria from restreaked plates, and isolated plasmids were stored at –20 °C.
Agarose gel electrophoresis was performed to visualize the presence of plasmid DNA.
Agarose gel electrophoresis was performed to visualize the presence of plasmid DNA.

3.4 Types of Equipment



3.5 Water Sample Collection and Processing

In this study, water samples have been collected from three sources of water in Dhaka city namely Hatirjheel, Curjon Hall and Ramna Lake. These locations are highly impacted by human activity and, therefore, may be contaminated with possible knock-on effects on microbial diversity and the profile of antibiotic resistance.

The samples were collected in pre-labeled, sterile sampling bottles in a manner that prevents contamination. Upon collection, the bottles were put directly into an insulated ice box whereby

the water was kept at a constant temperature throughout transportation. This is necessary to preserve the integrity of the microbes in the samples and uncertainty of changing the bacterial populations. Once the samples were taken to the Laboratory at BRAC University they had to be analyzed immediately in order to have reliable microbiological information. This timely treatment is critical in capturing the true condition of the water bodies and eliminating any form of threat of degradation or pollution that may distort outcomes.

3.6 Collection of Bacteria

In order to begin with bacterial collection of the water samples, a filtration process was conducted on the water using 0.4 mm Whatman filter paper, which is effective in removing the particulate matter and impurities. After filtration, 1 ml of the filtered sample was placed in a sterile autoclaved Falcon tube and 4 ml of LB (Luria Broth) was added to enable the enrichment of bacteria. This mix was then left to incubate in a shaker incubator at 37°C for 4 hours and this provided the best environment to facilitate the growth of bacteria. After incubation, a dilution was carried out sequentially to dilute the bacteria and conduct viable count measurements. In particular 100 µl of the enriched culture was inoculated into 900 µl of sterile LB medium, and serial dilutions of the culture were performed until the dilution factor of 10^{-2} had been achieved. Also, 60 µl of the undiluted (raw) sample was inoculated directly in selective culture media, where the preferred bacteria species would grow. This selective plating and enrichment will increase the chances of isolating various bacteria in the water samples thus adding a more integrated microbiological analysis.

3.7 Culture Media Used for Bacterial Isolation

The choice of culture media is determined by the bacterial groups to be studied, the aim of the research and availability of laboratory facilities.Selective and differential media are needed to be effective in isolation and identification of different bacteria given that they have different nutritional and physiological needs. Eosin Methylene Blue (EMB) agar, Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar and *Salmonella-Shigella* (SS) agar were used in the current study as selective and differentiating agar. After serial dilution, samples were inoculated into these media by the spread plate method and incubated at 37^{o}C for 18-24 hours. Bacterial colonies were

further subcultured on the same selective media through streak plate technique after incubation and followed by the identification and further down stream analysis.

3.7.1 *Salmonella Shigella* (SS) Agar

Salmonella-Shigella (SS) agar is a selective and differentiation medium which isolates *Salmonella* and selected *Shigella* species of clinical and non-clinical samples. It is a form of deoxycholate citrate agar. The SS agar has bile salts and brilliant green in order to prevent Gram-positive bacteria and the majority of coliforms. These inhibitors do not affect *Salmonella* and *Shigella*, therefore, allowing them to grow. The medium identifies enteric organisms with the use of lactose and neutral red pH indicator (Aryal, n.d.). Lactose-fermenting bacteria generate acid thus giving red colonies whereas non-fermenters such as *Salmonella* and *Shigella* give the colorless colonies. The ferrous nitrate (or ferric citrate) on the agar will react with the generation of hydrogen sulfide (H 2 S); the colonies of the organism will offer black centers to the hydrogen sulfide-producing organisms such as *Salmonella* (BabioMed, n.d.). *Salmonella Typhi, Salmonella Typhimurium, Salmonella Enteritidis*, and *Shigella flexneri* are some of the organisms found to grow on SS agar. To make SS agar, 63 g of SS agar powder is mixed in one litre of distilled water, boiled, and transferred into sterile Petri plates; autoclaving is not essential (Himedia Laboratories, n.d.). Incubation is generally done for a period of 18-24 hours at 37°C.

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

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar is a selective and differential culture medium that is widely utilized in isolation and presumptive identification of *Vibrio* species, especially in detection of *Vibrio cholerae*, the causative agent of cholera (Aryal, n.d.). The high alkaline pH of the medium, selective components of sodium citrate and bile salts, inhibit the growth of most Enterobacteriaceae and Gram-positive bacteria and thus promote the growth of Vibrio organisms in the mixed samples (Himedia Laboratories, n.d.). The primary fermentable carbohydrate is added, which is sucrose, and differentiation is made based on the fermenting ability of each bacterium: *Vibrio cholerae* ferments sucrose to give yellow colonies because of acid generation, and non-sucrose-fermenting Vibrio species give green or blue-green colonies instead (Aryal, n.d.). It contains sodium chloride to fulfil the growth needs of halophilic *Vibrio* species. Due to its high degree of selectivity and visible colonies, TCBS agar finds extensive application in

clinical diagnostics and food microbiology as well as the surveillance of the environment in the detection and growth of *Vibrio cholerae* and associated pathogens. The organisms that are supported by TCBS agar include *Vibrio cholerae, Vibrio fluvialis, Vibrio parahaemolyticus*, and *Vibrio vulnificus*. TCBS agar is prepared by adding the required 84.22g of the TCBS powder to one litre of distilled water, boiled, and poured into sterile Petri plates, which are not to be autoclaved (Himedia Laboratories, n.d.).

3.7.3 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar is a selective and differential media that has been mainly used in the identification of Gram-negative bacteria and in particular in the Enterobacteriaceae family. It is selective and contains the dyes of eosin and methylene blue, which prevent the development of the majority of Gram-positive bacteria. EMB agar divides the fermentative and non-fermentative bacteria in regards to lactose fermenting. Lactose fermenters generate acid, which results in an increase in the dye absorption, which generates a dark purple or black colony (Aryal, n.d.). The presence of bacteria in the EMB agar is usually characterized by a metallic green sheen because of the high production of acid and dye precipitation. The fermentation by non-lactose fermenters produces colorless colonies such as *Salmonella* and *Shigella*. The indicator dyes, peptone, lactose, and dipotassium phosphate are present in EMB agar. Sucrose is also another source of carbohydrate in some formulations. It is applied in medical laboratories to quicken the process of identifying enteric bacteria and tested in water quality to identify coliforms and fecal coliforms.EMB agar is used to propagate *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis,* and *Salmonella Typhimurium*. EMB agar is prepared by adding approximately 35.96 g EMB agar powder to one liter of distilled water then boiled and followed by autoclaving. It must be cooled and swirled and then poured into sterile Petri plates to allow dye to oxidize and be suspended (Himedia Laboratories, n.d.).

3.8 Bacterial Inoculation Methods

Inoculation methods are laboratory processes that help to inoculate microorganisms into the culture medium to grow and be analysed. In the study, a serial dilution was conducted to obtain bacterial samples at the required concentration. This was followed by inoculation of the samples on the culture medium using spread plate method, which allowed the growth of samples evenly

on the surface. The visualization of individual bacterial colonies was enabled with ease by this method. The streak plate technique was subsequently used in order to isolate individual strains, which was useful in separating individual colonies of the mixed culture. This step-by-step method was fundamental to precise identification and investigation of involved bacterial strains (Sanders, 2012).

3.8.1 Spread Plate Method

The spread plate method is an essential method in microbiology, and it is mostly applied to determine the count of viable microorganisms in liquids. This technique is by spreading a known amount of sample evenly onto a solidified medium of culture. A successful spread plate would have discrete colonies after a period of incubation and these colonies should be uniformly spread on the surface of the medium.This is a critical technique that not only supports the isolation of microorganisms but also allows a researcher to measure the population in units of colony-forming units per milliliter (CFU/mL), which is a good measure of the viability of the microorganisms (Dahal, 2022).

In this research study, a water sample was combined with the Luria-Bertani (LB) medium so that the microbes could grow well. Serial dilution was carried out before plating to adjust the concentration of the sample. This was aimed at obtaining a microbial count of 20 to 300 CFU/mL. Although 20-200 CFU/mL is widely mentioned as the number of colonies, different sources recommend a wider range, some going as high as 30-300 CFU/mL. Generally, a range of 25-250 CFU/mL is said to be normal in the assessment of accurate colony count (Wise, 2006).

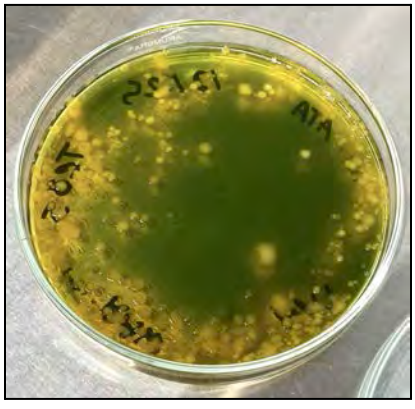


Figure 1: CFU/ml was counted following the spread plate method at 10^{-2} dilution

In order to carry out the spread plate technique, a 80 µl sample that had been diluted was transferred to the middle of a solid agar plate using a pipette. The sample was then spread evenly across the surface of the medium using a sterile spreader, that would allow the colony to form properly but never overcrowded, otherwise that would hinder proper counting. Such a technique is paramount in reproducibility because even distribution is a key factor to separation and colony growth. The plates were incubated with a temperature of 37°C and a duration of 18 -24 hours, which is best suited to the growth of most of the mesophilic bacteria. Following the incubation, the plates were studied to determine the number of colonies and these were recorded. The findings would offer useful information in regard to the microbial load of the initial water sample and would be significant in terms of the diversity of the microbes and the health implications that might arise due to the source of water being investigated.

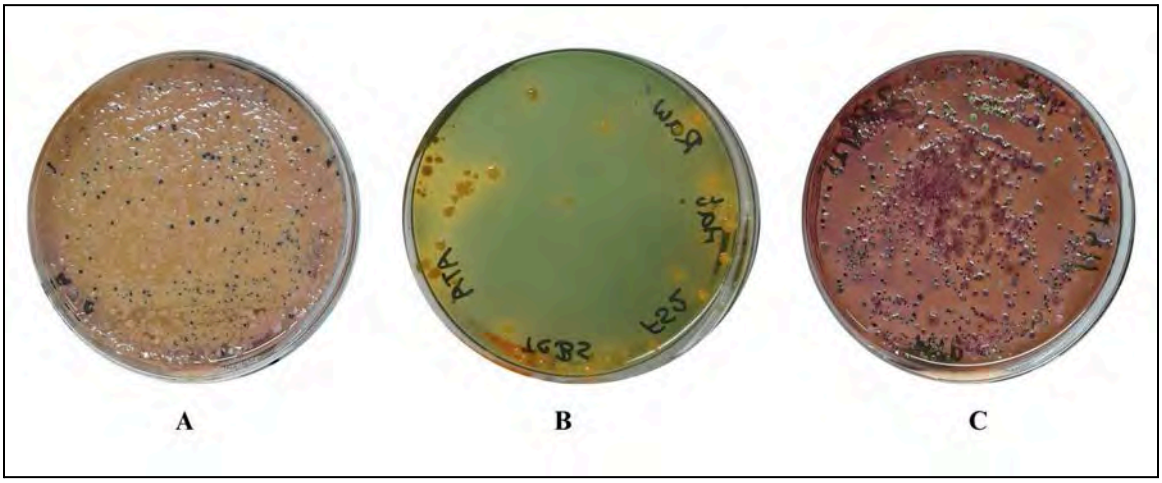


Figure 2: Colonies observed on (A) SS agar, (B) TCBS agar and, (C) EMB agar after the spread plate method

3.8.2 Streak plate method

Streak plate technique is one of the widely used microbiological procedures aiming to acquire pure cultures of bacteria and well-isolated colonies of mixed or congested samples. This method operates on the principle that the bacterial cells become successively diluted on the surface of a solid agar medium by a method of systematic streaking. At every consecutive streak, the amount of transferred bacteria cells reduces, and the single cells can become separated spatially. These single cells then multiply to grow in incubation producing discrete colonies. In a sample that has one bacterial species, the streaked colonies tend to give uniform colonies when there is only one

bacterial species but this produces colonies of varying morphologies in the event of having more than one bacterial species, thus allowing isolation and differentiation of type of bacterium (Dahal, 2025).

In the research, colonies that were acquired through the method of the spread plate were used to isolate them further through the streak plate procedure. A bacterium colony on a sterile solid agar plate was transferred to one side by using a sterile inoculating loop forming an initial inoculum. This loop was then streaked around the agar surface methodically in repeated streaks in accordance with set streaking patterns. The process guaranteed gradual decrease in bacteria level, successfully spreading the cells throughout the agar medium, and reduced overlap of growing colonies. After the inoculation, incubation of the streaked plates was done at 37°C to enable growth of bacteria.In the incubation, the isolated bacterial cells in the terminal regions of the streaks increased to give distinct, individual colonies that were distinct with each other. These pure colonies were taken as isolated colonies and could be further analyzed. The effective isolation of discrete colonies permitted a thorough analysis of colony morphology and facilitated the further identification and characterization of the bacterial isolates (Tankeshwar, 2016).

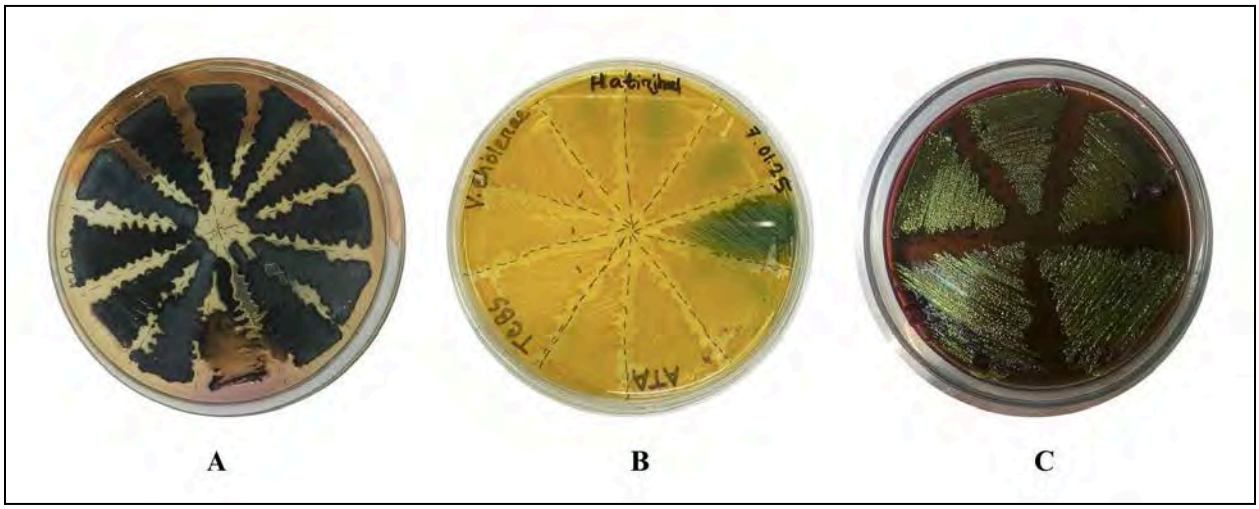


Figure 3: Colonies observed on (A) SS agar, (B) TCBS agar and, (C) EMB agar after the streak plate method

3.9 Sample Stocking

Sample stocking was done to keep the isolated bacterial cultures alive for a long time and to make sure they would be available for future sub-culturing, biochemical characterisation, antibiotic susceptibility testing, along with molecular analysis. When samples are properly stocked, the chances regarding contamination, genetic variation, alongside loss of viability with repeated handling are lower. To do this, nutritional agar with a lower agar concentration (0.3–0.5% w/v) was used to make soft agar medium. This made the medium semi-solid, which helped bacteria stay alive while keeping diffusion and dehydration to a minimum. The medium was made sterile by autoclaving it at 121 °C around 15 minutes. After being sterilized, the soft agar that was melted was cooled to around 45–50 °C and placed in sterile 1ml MCT (Micro Centrifuge Tubes) without any contamination. The tubes were put up and let to harden into soft agar depths. To keep track of each stored sample, it was clearly labeled with an isolation code, source, and date. The cultures that were in stock were kept in the right conditions until they were needed for further experiments.

The stab needle technique was used to put bacterial isolates to a soft agar medium so that samples could be stored properly (Xu et al, 2010) . This method is very good for keeping clean cultures since it cuts down on surface contamination and lets bacteria grow in a controlled way in the agar matrix. A sterile inoculating stab needle was utilized to select a well-isolated colony off a newly developed agar plate without getting any germs on it. The needle was then put straight into the soft agar medium. For this investigation, three stab lines were made within each agar deep by putting the stab needle in three different places in the same tube. Each stab went straight down, almost to the bottom of the agar. The needle was then gently pulled back along the same route to prevent disrupting the surface of the media.With three stab lines, the bacteria were well-preserved, and the danger of losing the culture was lower if a single growth line stopped working. After inoculation, the soft agar MCT tubes were allowed to persist in the right conditions for a short time to make sure they were growing. Along the stab lines, bacteria were growing, which showed that the samples had been properly stocked.

3.9.1 Sample Labeling

The three sources of surface water that were sampled in Dhaka metropolitan city included: Hatirjheel, Curzon hall and Ramna Lake. Samples sampling at each of the sites was done twice a month with a duration of six months, which generated 12 sampling events, and 36 water samples in total. The research was carried out from January to June. Isolated colonies of bacteria to facilitate systematic identification and traceability were labeled through the use of a standard coding system which relied on the sampling site, bacteria species, month of isolation and the sample number.

Description of Sample Labeling Code

Bacterial Species:

S = *Salmonella* spp., V = *Vibrio cholerae* , E = *Escherichia coli*

Month of Collection :

Ja = January, F = February, Ma = March , Ap = April , M = May and Jn = June

Sampling Site:

H = Hatirjheel, C = Curzon Hall and R = Ramna Lake

Sample Number:

S1 = 1st Sample of the month

S2 = 2nd Sample of the month

Example of Label Interpretation:

EApHS1 represents *E.coli* isolated from Hatirjheel and collected in April, Sample-1.

3.10 Possible Identifications

The identification of isolated bacterial colonies took place using a combination of culture features and molecular techniques. At first, the presumptive identification of bacterial isolates had been determined on the shape of the colonies seen on selective and differential media. Colony color, size, shape, margin, elevation, along with growth pattern were all looked at closely and compared to conventional reference descriptions. These first findings facilitated tentative distinction among the target species and informed the selection of isolates to undergo further validation. Molecular approaches were then used to provide accurate and dependable identification. DNA-based identification was chosen because it is very precise and sensitive, which means it can confirm the type of bacteria at the genetic level. To achieve this, genomic

DNA was taken out of the bacterial isolates and utilized as a template for molecular investigations like polymerase chain reaction (PCR).

3.10.1 DNA Isolation through boiling method

The boiling approach was used to get genomic DNA from bacterial isolates. This is a simple, quick, and cheap way to do molecular identification on a regular basis. This approach works by breaking apart bacterial cells with heat, which lets genomic DNA escape into the surrounding fluid. A loopful of newly produced bacterial culture was first aseptically placed in a sterile microcentrifuge tube with 500 µL of TE buffer. To make sure that the bacterial cells were evenly spread out, the suspension was stirred well by vortexing. Then, the tube was spun in a centrifuge at 13,000 rpm for 10 minutes. After that, the supernatant had been carefully thrown away to get rid of any leftover media parts. Then, 500 µL of TE buffer was introduced to the pellet of bacteria. The suspension was vortexed appropriately again and spun in a centrifuge at 13,000 rpm for 10 minutes. After centrifugation, the supernatant was thrown away again to make sure the bacterial cells were thoroughly washed. After cleaning, TE buffer was added to the pellet, along with the tube was put in a boiling water bath at 100 °C for 10 minutes to break apart the bacterial cells and liberate genomic DNA. After boiling, the tubes were put in a -20 degrees for 10 minutes (cold shock) to help the cell debris settle and keep the DNA stable.
After freezing, the sample was spun again at 13,000 rpm for 10 minutes. The clear supernatant with the isolated genomic DNA was gently moved to a fresh sterile microcentrifuge tube preventing damaging the cell debris. The isolated DNA was ultimately preserved at –20 °C for additional usage as a template for PCR-based molecular identification.

3.10.2 PCR

In molecular biology, studying a particular gene or DNA region directly from an environmental or clinical sample is generally difficult due to the often low quantity of target DNA present. Most downstream tests, such gel electrophoresis, sequencing, or gene confirmation, need a measurable and usable amount of DNA. Polymerase Chain Reaction (PCR) is a common method that gets around this problem by selectively amplifying a certain region of DNA. In summary, PCR is a regulated way to replicate DNA molecules that make a lot of identical fragments of DNA in a

short amount of time (Khehra et al, 2023) . To get around this problem, Polymerase Chain Reaction (PCR) is used as a dependable way to make copies of a particular DNA segment that are large enough to be seen. A thermally stable DNA polymerase, dNTPs, Mg^{2+}, along with buffer are used to amplify a chosen target area in this approach. A thermocycler carefully regulates the temperature variations needed for each reaction step. PCR works well because it is both specific and sensitive (National Laboratory of Enteric Pathogens, Bureau of Microbiology, Laboratory Centre for Disease Control, 1991). Specificity is mostly due to the primer binding to the complementary target sequence. Sensitivity, on the other hand, comes from recurring cycles that keep doubling the target DNA. Because of this, PCR is often used to validate the identification of bacteria, find certain flag genes, and make sure that a target fragment is present before doing other tests like agarose gel electrophoresis.

Steps of PCR Procedure

Initial Denaturation:

The first step in PCR amplification is denaturation, which means heating the reaction mixture enough to split double-stranded DNA into single strands and get the polymerase ready to work well. To make sure that the strands were completely separated before cycling, the mixture was maintained at 95°C for 5 minutes at the commencement of this investigation. After this step of preparation, the reaction went through rounds of amplification over and over again.

Denaturation:

At the beginning of each amplification cycle, the mixture was heated up again to a high temperature. This destroyed the hydrogen bonds between the complementary DNA bases, producing single-stranded DNA templates. In this case, denaturation was done by keeping the reaction at 95°C for 30 seconds in each cycle. This made sure that the DNA strands were split over and over again so that the primers could bind.

Annealing:

After denaturation, the temperature was lowered such that the forward and reverse primers could bind to their complementary target sequences on the single-stranded DNA. The annealing condition was changed depending on the organism being amplified since primer binding is sensitive to temperature (Ishii and Fukui, 2001). This process lasted for 1 minute. Annealing was done at 57°C for *E. coli*, 60°C for *Vibrio cholerae* , and 53.2°C for *Salmonella*. This kept the primer attachment specific and the amplification efficiency high.

Extension (Elongation):

After the primers were joined, the temperature was raised to help the DNA polymerase work best so that a new complementary strand could be made from each primer. In this work, the extension step was done by keeping the reaction at 72°C for 1 minute (Lopez and Zepka, 2019) . This lets the polymerase add nucleotides in the 5′→3′ direction and make the amplified DNA product.

Final Extension:

After all of the amplification cycles were done, there was one more extension phase to make sure that any DNA strands that were only half produced in the last cycle were completely synthesized. To do this, the reaction was kept at 72°C for 5 minutes, resulting in the PCR products more complete and even.

Final Hold:

At the conclusion of the amplification process, the reaction was kept at a low temperature to keep the amplified DNA stable until it was taken out of the thermocycler. The products were maintained at 4°C for short-term handling, and the amplified samples were held at −20°C until downstream analysis for longer-term storage.

Table 1: PCR components and their function

Characteristics	**Template DNA**	**Primers**	**DNA polymerase**	**Mg^{2+}**	**Buffer**	**dNTPs**
Function	Provides the target sequence to be amplified	Binds to complementary target sites and initiates amplification	Produces new DNA strands by extending from primers	Acts as a cofactor required for polymerase activity	Maintains suitable pH and ionic conditions for the reaction	Supplies nucleotide building blocks for new strand synthesis
Length	N/A	Typically, 18–25 nucleotides	N/A	N/A	N/A	N/A

Requirement	Must be separated into single strands during denaturation	Must be complementary to the target region for specific binding	Requires the correct working temperature for efficient amplification	Required to support enzyme function and primer-template interaction	Ensures a stable chemical environment for the reaction	Essential for formation of newly synthesized DNA strands

Equipment for PCR

Thermal Cycler (PCR Machine): The PCR process encompasses to change temperatures several times (cycling) so that it can denature, anneal, and extend. For dependable amplification, a thermal cycler makes sure that temperature changes are exact and that holding durations are steady.

PCR Tubes: The tubes used for PCR are designed to quickly transmit heat and stay stable as the temperature changes. Their structure helps keep the reaction going and keeps evaporation to a minimum.

Preparing the PCR Reaction: The reagents were mixed in the right amounts for each tube, and then the extracted DNA template was added to make more copies.

Table 2: Reaction Mixture of PCR and Their Amounts

Component	**Master mix**	**Nuclease-free water**	**Forward primer**	**Reverse primer**	**Isolated DNA**	**Total**
Volume per PCR tube	6 µL	2 µL	0.5 µL	0.5 µL	3 µL	12 µL

Preparation for each sample:

There were 9 µL of PCR mixture (Master mix + nuclease-free water + forward primer + reverse primer) and 3 µL of extracted DNA in each PCR tube. This made a total of 12 µL.

Negative control: To make sure there was no contamination, 3 µL of nuclease-free water was utilized as opposed to DNA template and mixed with 9 µL of PCR mixture (Total 12 µL).

The manner in which to Use a PCR Machine

The PCR run started using the thermocycler interface, and then the temperature and time settings were made according to the PCR software (Garibyan and Avashia, 2013). The reaction tubes were put into the thermocycler; then, the number of cycles was changed, and the PCR run was begun to start the process of making more copies.

Cycle of Heat

The PCR cycling protocol started with a denaturation phase, then repeated cycles of denaturation, annealing (depending on the primer/target), and extension. At the conclusion of the cycle, there was a last extension phase, and then the amplified products were stored at a low temperature to keep them safe.

Primer Condition

Table 3: Primer sequence of three different bacteria

Target Organism	Gene	Primer (5′→3′)	Size Bp	Reference
Salmonella spp.	*InvA*	**F:** 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′ **R:** 5′-TCATCGCACCGTCAAAGGAACC-3'	484	Malorny et al., 2003
V. cholerae	*ompW*	**F**: 5′-ATAATGGCTCACCAAGAAGG-3′ **R**: 5′-TTAGAACTTATAACCACC-3′	592	Ranjbar et al, 2016
E. coli	*16S rRNA*	**F:** 5′-GACCTCGGTTTAGTTCACAGA-3′ **R:** 5′-CACACGCTGACGCTGACCA-3′	585	Parvin et al., 2020

3.10.3 Agarose Gel Electrophoresis

To make sure that the amplified DNA fragments were there after PCR and to get a rough idea of their size, we used agarose gel electrophoresis to separate DNA molecules by length. This method works because DNA has a phosphate backbone that gives it a negative charge overall. So, when an electric field is turned on, DNA pieces move through the gel matrix toward the positive electrode. The agarose gel creates a network of holes, which lets smaller pieces move quicker and further while bigger pieces move more slowly. This is how size-based separation works. This approach is quite common in molecular biology because it lets you quickly see PCR products, helps you check the specificity of the amplification, and helps you find any contamination or non-specific bands. As a confirmation step before interpreting PCR results, agarose gel electrophoresis has been used in this work. The fundamental reagents and materials consisted:

- **Agarose powder:** Used to prepare the gel matrix for DNA separation.
- **Distilled water:** Used to dissolve agarose and prepare the gel solution.
- **TAE buffer (50×):** Used (after dilution) for preparing the gel solution.
- **Running buffer TAE (1×):** Used in the electrophoresis tank to allow DNA migration and maintain pH/ionic strength.
- **PCR products:** Loaded into the gel wells for separation and band detection.
- **Ethidium bromide (EtBr):** Used as an intercalating dye to stain DNA for visualization.
- **Visualization system:** DNA bands were observed using a UV transilluminator or a gel documentation system.

Importance of the tools and chemicals utilized for agarose gel electrophoresis

Agarose powder: This is what makes up the gel matrix. The amount of agarose in a gel influences the size of the pores. A gel with around 1% agarose is usually good for separating PCR results. The agarose network works like a molecular sieve, letting pieces of DNA separate depending on their length (Lee et al ,2021)

Distilled water: Distilled water is used as the solvent for making gel to keep it clean and to keep the ionic conditions stable.

TAE buffer (50×): TAE keeps the pH steady throughout electrophoresis and supplies the ions needed for current flow. It's very important to utilize the right concentration since a buffer that is too concentrated will make more heat, while a buffer that is too dilute can lower conductivity and influence the quality of band separation.

Running buffer (TAE 1×): The gel must be in the running buffer while electrophoresis is going on. This buffer keeps the electrical circuit working, stops the gel from drying up, and makes sure that DNA moves quickly.

Casting tray: This is where the melted agarose stays until it hardens into a gel slab. The size of the tray dictates the amount of the gel, which in turn impacts where the wells are placed and how many samples may be run at once.

Combs: When the gel hardens, combs are put in it to make wells. The wells let you load PCR products in an orderly way. Utilizing one or two combs lets you hold more samples and controls.

Electrophoresis chamber and power supply: The electrophoresis chamber holds the gel and buffer, whereas the power source gives it a regulated voltage. A consistent voltage is necessary to keep the gel from becoming too hot and to make sure that DNA separation can be repeated.

Micropipettes (2-20µL): Micropipettes (2-20µL) as well as sterile tips are necessary for putting products from PCR into wells in the right way. Carefully loading keeps samples from becoming mixed up and keeps wells from breaking, which may happen when samples are mixed.

Ethidium bromide (EtBr): EtBr fits into DNA and glows under UV light, which lets you see the bands. EtBr is dangerous, therefore you need to wear gloves when you handle it, and you need to observe lab safety rules when you throw away trash.

Gel documentation system/UV transilluminator: Required to see, take pictures of, and record DNA bands so that results may be understood and kept.

3.10.3a Agarose Gel Preparation

The preparation of agarose gel was done on the basis of standard gel electrophoresis apparatus and regular laboratory protocols to get uniform and reliable separation of DNA.

Runview gel electrophoresis machine (the casting tray can hold 100 mL)

The casting tray for the Runview gel electrophoresis device could accommodate 100 mL of gel. To make this gel, 100 mL was needed: 2 mL of 50× TAE buffer, 1 g of agarose powder, along with 98 mL of distilled water. The solution was combined well and then heated until the agarose was completely dissolved and the solution was clear and uniform. This showed that the agarose had entirely melted.The melted agarose solution was allowed to cool after heating until it was safe to touch, which meant that it had to cool sufficiently such that the conical container was able to be handled comfortably via bare hands. At this point, cooling is very important since adding EtBr at too high of a temperature might create more vapor and make it harder to handle safely. After the gel solution cooled to a temperature that was safer to handle, 4 µL of ethidium bromide (EtBr) was incorporated and stirred in gently to make sure the stain was spread evenly throughout the gel. After staining, the solution was gently poured into the casting tray. Prior to pouring, one or two combs had previously been placed on the tray in the right way to make wells. After that, the gel was left alone until it was entirely firm, which made sure that the wells formed smoothly and that the gel didn't become too thick in any places.

3.10.3b Agarose Gel Electrophoresis Analysis for PCR Products

After the agarose gel had completely set, the casting tray with the gel was put into the electrophoresis chamber. Then, the tank was filled with a TAE running buffer (1×) until it reached the level of the buffer that was shown on the device. The gel was completely immersed to make sure that the current flowed evenly and that the DNA moved over the gel surface in a steady way. Using a micropipette, the PCR products had been placed into the right wells. When loading, extra care was taken to make sure that the pipette tip was not going to break or change the shape of the wells. This might cause samples to leak or mix between wells. To keep track of isolates, controls, as well as duplicates accurately, samples were put into the right wells in an ordered way. After loading, electrophoresis was carried out in two different ways, depending on the equipment utilized. In the case of Gel Electrophoresis apparatus, the power source for the typical gel electrophoresis setup was adjusted to 90 volts, and the gel was run for 40 minutes to make sure that the PCR products moved and separated properly without becoming too hot or changing shape.

After the run was over, the gel was carefully taken off and put on a UV transilluminator. EtBr fluorescence was used to see the bands, and the fact that there were different bands showed that the amplification worked (Priyashantha and Umashankar, 2021). We utilized the locations of the bands to estimate the size of the product and check the specificity of the amplification. The gel photos were taken so that they could be used as evidence and included in the findings part of the thesis report.

3.11 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is referred to as a laboratory test that is done to ensure the potential of certain antimicrobial agents to suppress the growth of microorganisms, which ultimately leads to categorizing bacteria as susceptible, intermediate or resistant to certain antibiotics (Bayot & Bragg, 2024).

Antibiotic susceptibility testing was done to determine the antimicrobial resistance profiles of the identified bacterial isolates as well as to identify their sensitivity profiles to major antibiotics used. The Kirby-Bauer disc diffusion test was used to achieve the test and is one of the standardized and universally accepted methods of antimicrobial susceptibility testing of clinical and environmental isolates of bacteria. All the operations were conducted according to the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI, 2024) to guarantee reliability, reproducibility, and accuracy of the findings.

This technique is anchored on the capacity of an antibiotic to penetrate through solid agar media and suppress the growth of bacteria. The degree of bacterial inhibition is in terms of the diameter of the clear zone around each antibiotic disc which corresponds to susceptibility of the organism to the specific antibiotic.

3.11.1 Bacterial Suspension Preparation

A standardized bacterial suspension is a very important preliminary procedure in the testing of antibiotic susceptibility. Proper standardization guarantees a consistent bacterial density which can only be reproducible and comparable when it comes to the inhibition zone diameters. The effect of the concentration of the inoculum can be quite large on the antimicrobial diffusion and can result in false interpretation of the susceptibility results. Thus, the rigorous observance of

standardized practices of inoculum preparation was followed in the course of research (CLSI, 2024).

Procedure:

Firstly, from freshly growing agar plate incubated at 37ºC for 18-24 hours well-isolated bacterial colonies collected.

Then morphologically similar colonies were moved using a sterile inoculating loop to sterile 2-3 mL normal saline solution (0.9% NaCl).

Further, the suspension was mixed by vortexing carefully in order to have uniform distribution of the bacterial cells.

However, the turbidity of the suspension was adjusted at the desired level and compared to the 0.5 McFarland turbidity standard to obtain a standardized bacteria concentration.

Then the prepared standardized bacterial suspension was used within 15 minutes after preparation to ensure that cell density does not change and the tests remain reliable.

3.11.2 Mueller-Hinton Agar

Mueller-Hinton agar (MHA) was the reference medium in the test of antibiotic susceptibility because the medium has a good reproducibility, consistent composition, and optimum capacity to allow diffusion of antimicrobials. The medium also favors proliferation of most non-fastidious bacteria and does not affect the activity of most popular antibiotics, thus, it is the recommended medium when carrying out disc diffusion tests (Wilkins et al., 1972). MHA plates were made ready and to a depth that was permissible to solidify. The plates were checked with regard to excess moisture on the surface before inoculation, and, where needed, were dried at room temperature using the air. A sterile cotton swab was subsequently used to inoculate the surface of the agar using the standardized bacterial suspension. The swab was dipped into the suspension and to leave the excess amount the swab was pressed against the inner wall of the tube, the agar was streaked by four times along with a round swab to the edges to make the surface uniformly inoculated. The plates were left to stand without disturbance for 3-5 minutes after inoculation in order to allow the excess moisture to be absorbed before the antibiotic discs were placed.

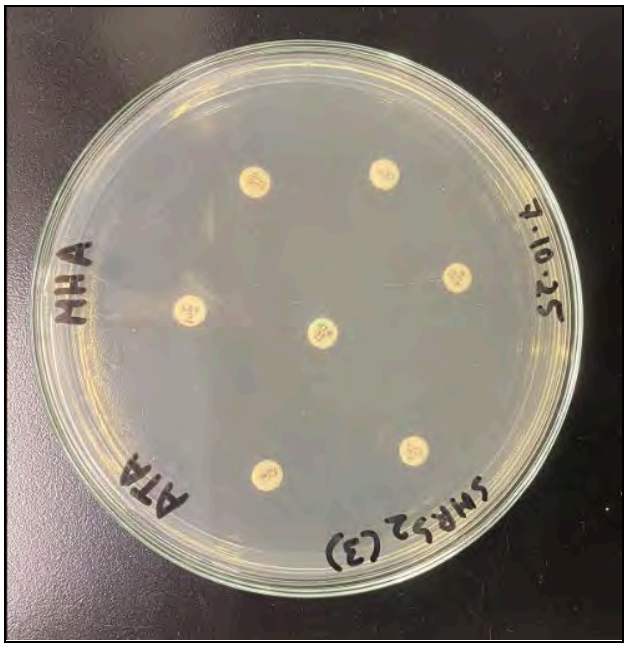


Figure 4: A fresh sterile MHA plate with few antibiotics before 18-24 hours.

3.11.3 List of Antibiotics

A set of antibiotics belonging to various antimicrobial classes was picked to determine multidrug resistance patterns across the bacteria isolates. The antibiotics of the given study were:

Antibiotic group	Antibiotic Name	Code	Disk Con cen-t ratio n (µg)	Type of Species	Diameter of zone inhibition		
					Resistant (R) (mm)	Intermediate (I) (mm)	Susceptible (S) (mm)
Penicillin	Ampicillin	AMP	10	Enterobactericia *V. cholerae*	≤13	14-16	≥17
Monobactams	Aztreonam	ATM	30	Enterobactericia *V. cholerae*	≤17	18-20	≥21
Amphenicol	Chloramphenicol	C	30	Enterobactericia *V. cholerae*	≤12	13-17	≥18
				Enterobactericia	≤12	15-17	≥18

Cephalosporin – 4th gen	Cefepime	FEP	30	*V. cholerae*	≤18	19-24	≥25
Cephalosporin – 3rd gen	Cefixime	CFM	5	Enterobactericia	≤15	16-18	≥19
				V. cholerae	≤17	18-20	≥21
Tetracycline	Doxycycline	DO	30	Enterobactericia	≤10	11-13	≥14
				V. cholerae	≤11	12-14	≥15
Macrolide	Erythromycin	E	15	Enterobactericia	-	-	-
				V. cholerae	≤11	14-22	≥23
Carbapenem	Imipenem	IPM	10	Enterobactericia	≤13	14-15	≥16
				V. cholerae	≤19	20-22	≥23
Aminoglycoside	Gentamicin	GEN	10	Enterobactericia	≤12	13-14	≥15
				V. cholerae			
Aminoglycoside	Kanamycin	K	30	Enterobactericia	≤13	14-17	≥18
				V. cholerae			
Fluoroquinolone	Levofloxacin	LEV	5	Enterobactericia	≤13	14-16	≥17
				V. cholerae			
Oxazolidinone	Linezolid	LZ	30	Enterobactericia	-	-	-
				V. cholerae	-	-	-
Glycopeptide	Vancomycin	VA	30	Enterobactericia	-	-	-
				V. cholerae	≤14	15-16	≥17

The agar surface was inoculated with the microbes and sterile forceps were aseptically used to place the antibiotic discs on the surface of the agar ensuring that there was proper space between the discs to avoid overlapping of the zone of inhibitions. The plates were then incubated at a 37ºC temperature during a period of 18-24 hours.

Incubation was followed by measurement of the diameter of each inhibition zone in millimeters with a ruler or a caliper. The findings were categorized as being susceptible, intermediate or resistant based on CLSI interpretive criteria (CLSI, 2024).

3.12 Plasmid Isolation

Plasmid isolation refers to the isolation process of Plasmid DNA in the bacterial cells by depleting chromosomal DNA, proteins, RNA and other cellular materials. Plasmids are small, circular, and double-stranded DNA molecules, which do not replicate along with the bacterial chromosome and usually possess genes related to antibiotic resistance, virulence and metabolic processes. Plasmid DNA isolation is a basic procedure in the field of molecular biology especially in genetic characterization and study of bacterial isolates (Takashashi & Nagano, 1984).

3.12.1 Plasmid Isolation via Miniprep method

In the current research, plasmid DNA was isolated with the help of a miniprep technique based on phenol:chloroform:isoamyl alcohol (PCI), which has remained a very solid method of purifying plasmids of bacterial cells (Kodackattumannil et al., 2023; Taha et al., 2023). The recent reviews of plasmid extraction protocols also highlight that the method choice is important to maximize the yield and purity of the plasmid when using downstream molecular processes, such as sequencing and plasmid profiling (Kruasuwan et al., 2024). More recent developments in miniprep technique, such as the use of devices to aid in the mixing of bacterial samples and the optimization of conditions to achieve the best results in lysis, have also contributed to the increased effectiveness and quality of plasmid DNA isolation in recent research (AMB Express, 2022).

3.12.2 Solution needed for plasmid isolation

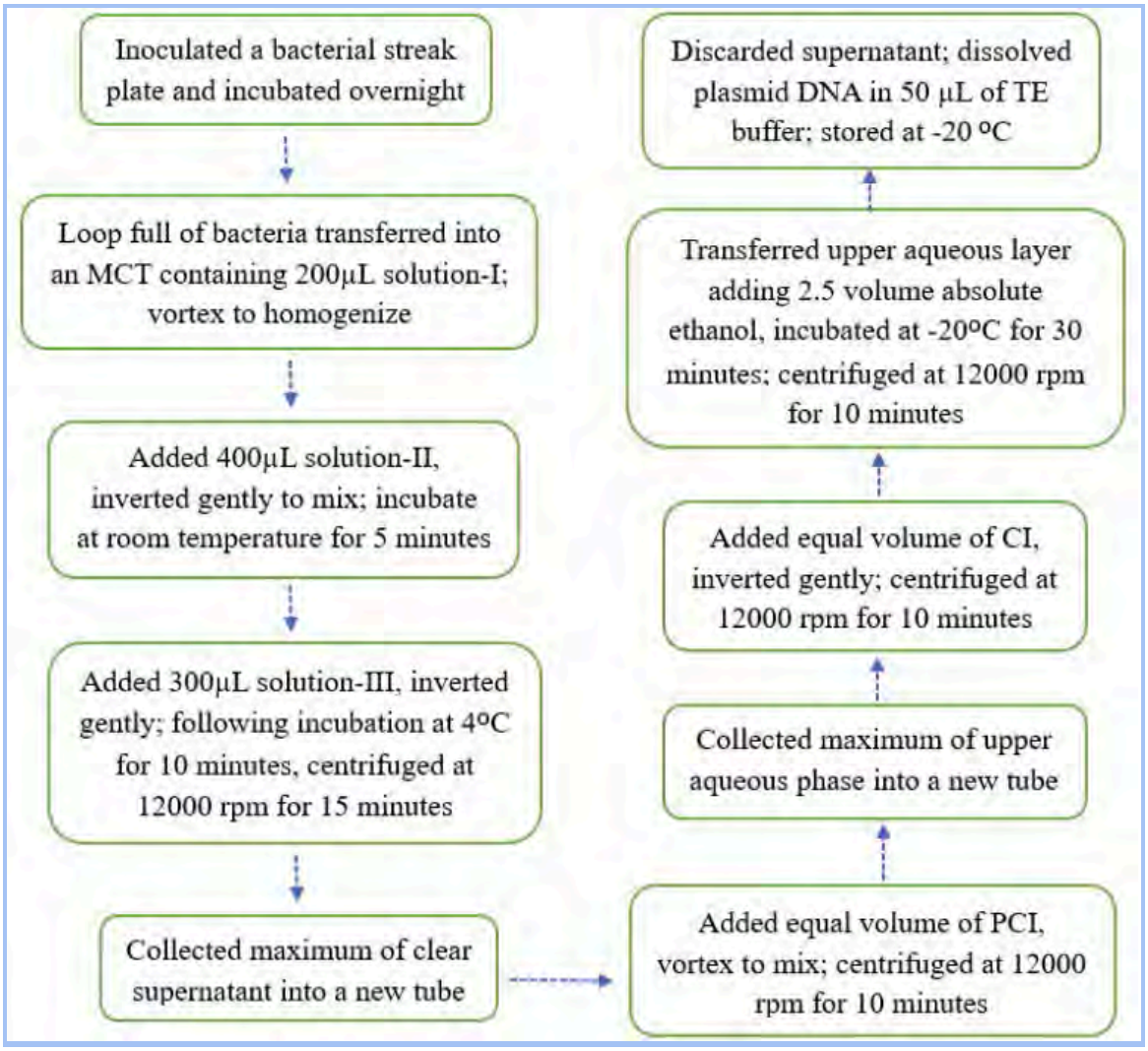
Three specific solutions were used during plasmid isolation, each playing a distinct role in cell disruption, DNA separation, and purification.

Table 4: Solutions for plasmid isolation

Solution-I (High-concentration buffer): It is made of 1 M glucose, 1 M Tris-HCl and 0.5 M EDTA with pH set to 8.0.	Solution II (Lysis buffer): It consisted of 0.2 N sodium hydroxide (NaOH) and 1% sodium dodecyl sulfate (SDS).	Solution III (Neutralization solution): It has 5 M potassium acetate with a pH of 5.2 and was used to neutralize the alkaline lysate.
Function: Glucose keeps the osmotic pressure constant, preventing the early lysis of cells, and allowing the disruption of bacterial cells to be controlled. Tris -HCl was used to buffer the pH during the process and this is vital in ensuring the integrity of the DNA. Divavalent metal ions (Mg2+ and Ca2+) were chelated by EDTA and these are cofactors of DNases, therefore, EDTA inhibited the action of the nuclease and prevented the degradation of plasmid DNA. (Salwan & Sharma, 2023).	Function: SDS disrupted the bacterial cell membrane and denatured cellular proteins by solubilizing lipids. NaOH created an alkaline environment that denatured both chromosomal and plasmid DNA by separating the hydrogen bonds between DNA strands. At this stage, cellular components including proteins and chromosomal DNA were disrupted, allowing effective release of plasmid DNA into the solution. (Salwan & Sharma, 2023; Kodackattumannil et al., 2023).	Function: Upon addition, potassium ions interacted with SDS to form an insoluble potassium dodecyl sulfate complex, which precipitated along with denatured proteins and chromosomal DNA. Due to its small circular and supercoiled nature, plasmid DNA rapidly renatured and remained soluble in the aqueous phase, while large chromosomal DNA aggregated and precipitated. This selective precipitation enabled efficient separation of plasmid DNA from cellular debris. (Salwan & Sharma, 2023)

3.12.3 Plasmid isolation procedure

Using a flowchart showing the procedure of plasmid isolation:



3.13 Plasmid Visualization by Gel Electrophoresis

Once the plasmid DNA is isolated, the quality, purity and the approximate size of the plasmid can be determined through agarose gel electrophoresis. Gel electrophoresis uses size and conformation to separate the DNA molecules, which makes it possible to see the plasmid DNA bands in a clear manner. Nicked, supercoiled, and linear types of plasmid DNA will migrate differently through an agarose matrix, and this will give details concerning purity and integrity of plasmids. This is the important step to ensure successful extraction of the plasmid prior to

subsequent downstream uses of the plasmid like cloning or PCR analysis (Hahn, 2025; Goyon et al., 2023).

3.13.1 Sample Preparation

To perform electrophoresis, the plasmid DNA was isolated and combined with an appropriate loading dye (e.g., bromophenol blue) in a ratio that could facilitate the desires of the sample and visualization. The loading dye introduces color that allows one to follow the movement of DNA and enhances the density of the samples, preventing the separation of the DNA as it floats out of the wells. The plasmid DNA (~8-9µL) was usually combined with 1-2µL loading dye and carefully pipetted to ensure the mixture is mixed. Samples were also prepared in advance and stored on ice before loading to avoid degradation (Asad et al., 2023).

3.13.2 Gel Electrophoresis Analysis

The TAE buffer (Tris-acetate-EDTA) used to make a 1% agarose gel, which offers a stable ionic environment to the development of DNA migration. An intercalating dye like ethidium bromide or GelRed was placed in the gel to enable the visualization of DNA bands in the UV light. The gel was pre-run a couple of minutes before the sample loading to stabilize the agarose matrix and promote homogenous movement of DNA molecules (Asad et al., 2023).

Each sample of plasmid DNA was mixed with loading dye and loaded into different wells of the gel carefully. A DNA ladder with a known size of fragments was loaded together with the samples to act as a reference to estimate the size of the plasmid. The electrophoresis process was run at 100V between 40-45minutes where the DNA molecules would separate on the basis of their size and conformation (Hahn, 2025).

Following the electrophoresis, the gel was viewed in a UV transilluminator and the bands of the plasmid DNA were recorded. The strength and location of the bands were revealing: clear, high intensities of bands was a sign of high yield and purity of the DNA, the migration patterns were determined by distinguishing between supercoiled plasmids and relaxed or linear ones, and the fact that smeared or weak bands indicated some degradation process or contaminants. This test established the isolation of plasmid DNA and its structural integrity basis along with the efficiency of the extraction and purification process (Goyon et al., 2023; Hahn, 2025).

Chapter 4

Results

4.1 Results of PCR

4.1.1 Results of PCR for *Salmonella* Spp.

4.1.1a Gel Electrophoresis Result

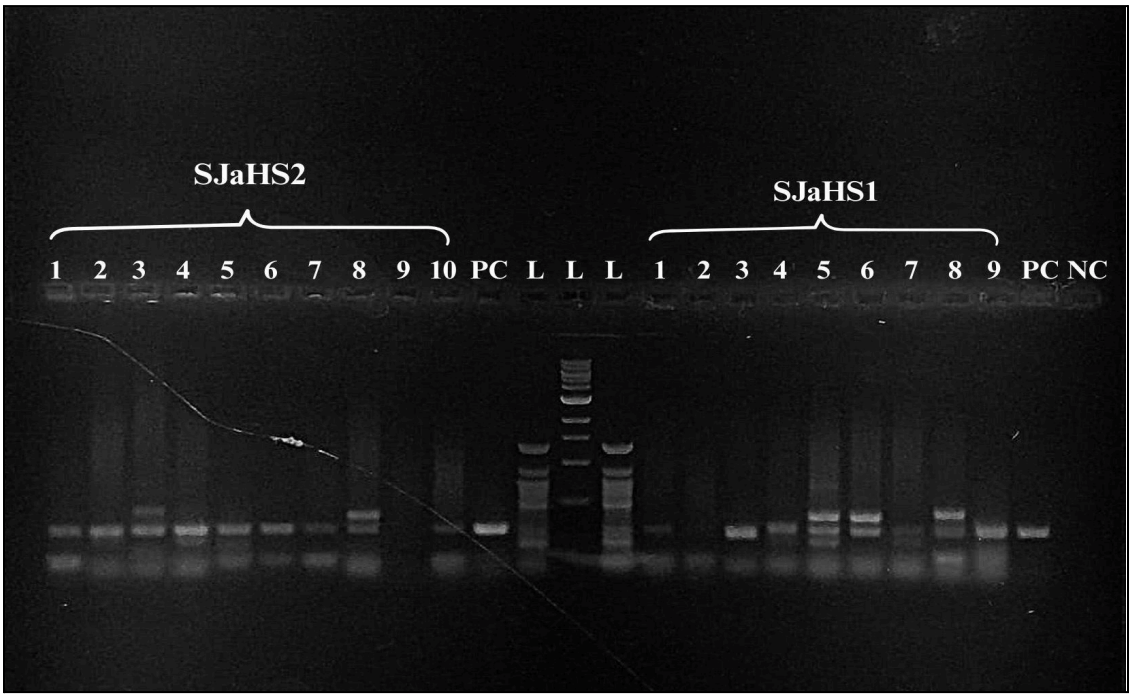


Figure 5: Agarose gel electrophoresis displays PCR amplification profiles of *Salmonella* isolates collected in January from Hatirjheel.

The figure shows the PCR-based identification of *Salmonella* spp. in samples of January from Hatirjheel. SJaHS1 (Right panel) showed a total of 9 PCR products (lanes 1–9), with clear amplification of lanes 1 and 3–9 to indicate the presence of *Salmonella*, and a negative result on lane 2. SJaHS2 (Left panel) had 10 PCR products (lane 1-10), whereas lanes 1-8 and lane 10 demonstrated clear amplification bands, and in lane 9 no amplification was observed and was declared as negative. The given PCR assay was specific as the positive control (PC) exhibited the expected amplicon with no band being obtained in the negative control (NC). The identity of the amplified products was also confirmed by the presence of 1 kb and 50 bp DNA ladders that

confirmed that the bands matched the size of the DNA amplicons expected to result in *Salmonella*-specific products.

4.1.1b Table of PCR Results

Table 5: PCR detection results and positivity rates of *Salmonella* spp. in Hatirjheel samples on the basis of month

Sampling site	Month	No. of stock	No. of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
Hatirjheel	January S1	9	9	8	8	1	88.9
	January S2	10	10	9	9	1	90
	February S1	16	16	12	12	4	75
	February S2	16	16	14	14	2	87.5
	March S1	7	7	1	1	6	14.3
	March S2	9	9	3	3	6	33.3
	April S1	17	17	15	15	2	88.2
	April S2	15	15	2	2	13	13.3
	May S1	14	14	13	13	1	92.8
	May S2	12	12	10	10	2	83.3
	June S1	16	16	16	16	0	100
	June S2	12	12	11	11	1	91.7
Total			**154**		**114**		

Table 6: PCR detection results and positivity rates of *Salmonella* spp. in Curzon Hall samples on the basis of month

Sampling site	Month	No. of stock	Number of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
Curzon Hall	January S1	0	-	-	-	-	0
	January S2	0	-	-	-	-	0
	February S1	3	3	2	2	1	66.7
	February S2	0	-	-	-	-	0
	March S1	0	-	-	-	-	0
	March S2	11	11	0	0	11	0
	April S1	1	1	0	0	1	0
	April S2	9	9	0	0	9	0
	May S1	2	2	2	2	0	100
	May S2	12	12	10	10	2	83.3
	June S1	2	2	2	2	0	100
	June S2	0	-	-	-	-	0
Total			**40**		**16**		

Table 7: PCR detection results and positivity rates of *Salmonella* spp. in Ramna Lake samples on the basis of month

Sampling site	Month	No. of stock	Number of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
	January S1	0	0	-	-	-	0

Ramna Lake	January S2	0	0	-	-	-	0
	February S1	8	8	3	3	5	37.5
	February S2	0	0	-	-	-	0
	March S1	0	0	-	-	-	0
	March S2	0	0	-	-	-	0
	April S1	0	0	-	-	-	0
	April S2	4	4	0	0	4	0
	May S1	15	15	14	14	1	93.33
	May S2	3	3	3	3	0	100
	June 1	0	0	-	-	-	0
	June 2	12	12	12	12	0	100
Total			**42**		**32**		

4.1.1c Pie Chart of the PCR Positive Results

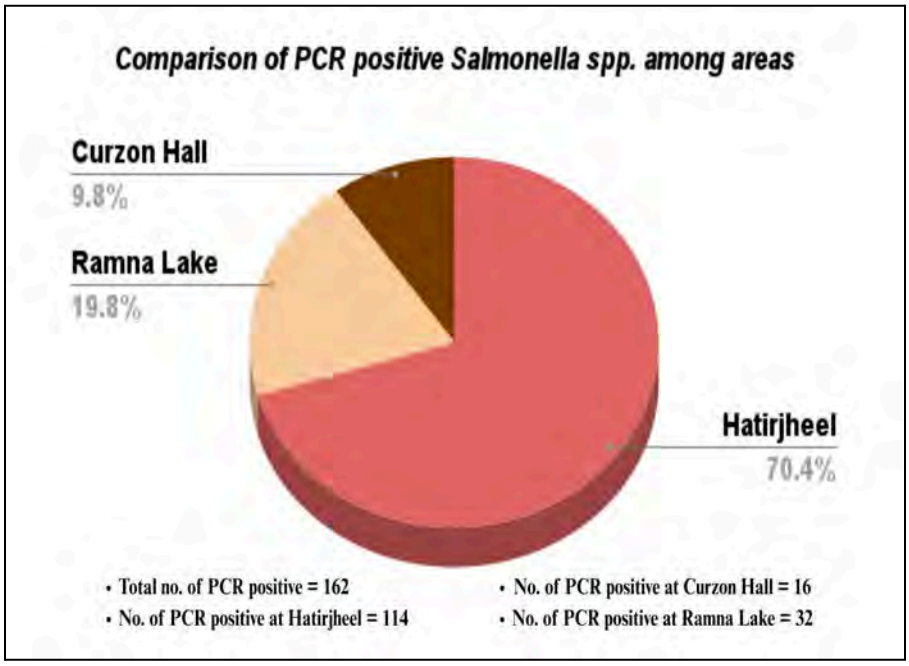
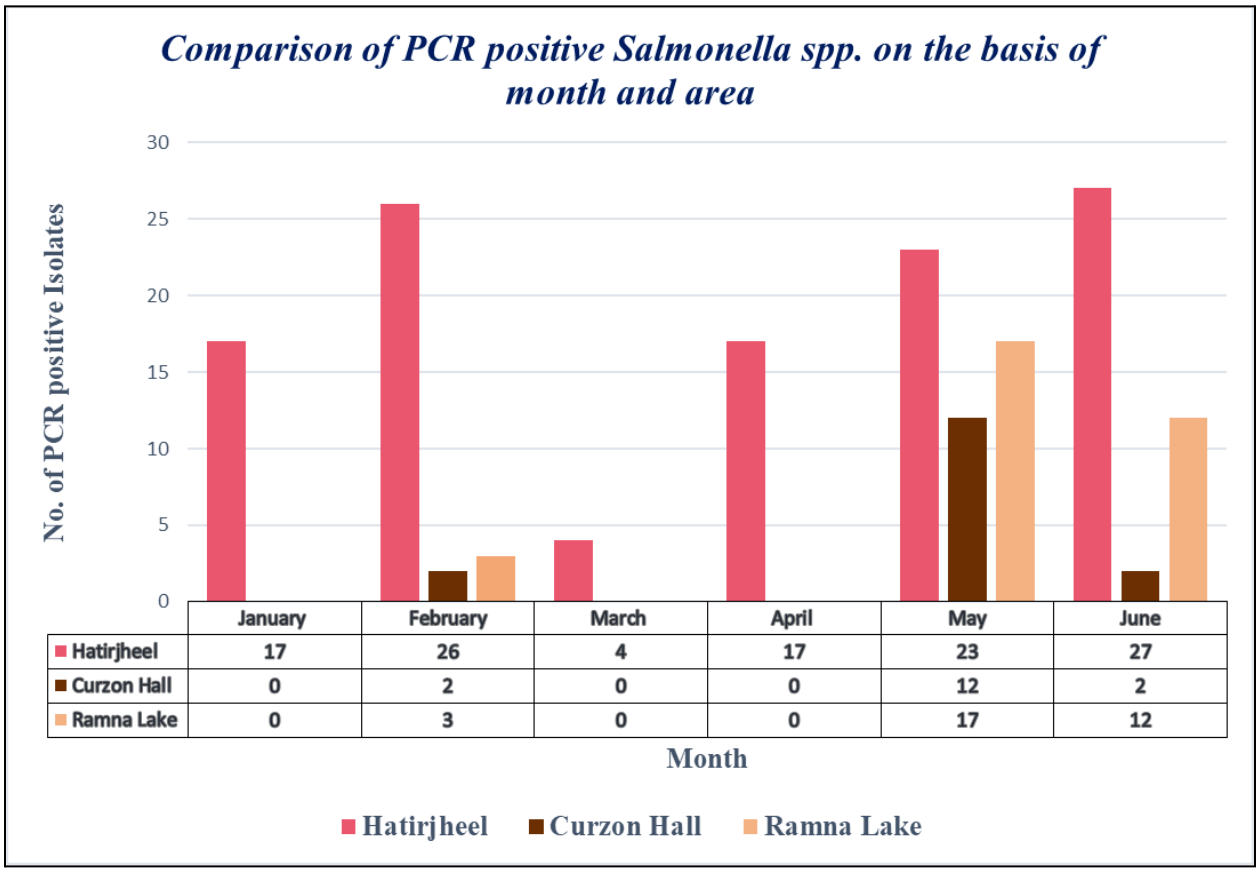


Figure 6: Comparative distribution of PCR positive *Salmonella* spp. isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

The figure represents the spread of PCR-positive isolates of *Salmonella* spp. in the three areas of sampling: Hatirjheel, Curzon Hall, and Ramna Lake. Among 162 PCR-positive isolates, the maximum number of the ones were found in Hatirjheel which contributed 114 isolates and 70.4% of the total. Comparatively, Ramna Lake had registered 32 PCR-positive isolates that comprised 19.8% of the total. Curzon Hall 16 PCR-positive isolates were identified but only 9.8% of the total.

4.1.1d Column Chart of the PCR Positive Results



	January	February	March	April	May	June
Hatirjheel	17	26	4	17	23	27
Curzon Hall	0	2	0	0	12	2
Ramna Lake	0	3	0	0	17	12

Figure 7: A monthly comparison of PCR-positive *Salmonella* spp. isolates in three sampling sites: Haltirjheel, Curzon Hall, and Ramna Lake.

The figure shows the distribution of PCR-positive *Salmonella* spp. isolates on a monthly and area basis at Hatirjheel, Curzon Hall and Ramna lake between January to June. Over the period of study, Hatirjheel recorded the maximum number of PCR positive isolates with a minimum of 17 isolates in January and a maximum of 27 isolates in June. Curzon Hall had extremely low and

irregular detection with 0 isolates in January, March and April and only 2 isolates in February and June with an increased number of 12 isolates being detected in May. The isolates of Ramna lake did not show any PCR-positive results during January to April but suddenly reached 17 isolates and 12 isolates correspondingly in May and June respectively. Overall, Hatirjheel was the most contaminated site, whereas Ramna Lake was clearly seasonal and Curzon Hall varying in its occurrence.

4.1.2 Results of PCR for *Vibrio cholerae*

4.1.2a Gel Electrophoresis Result

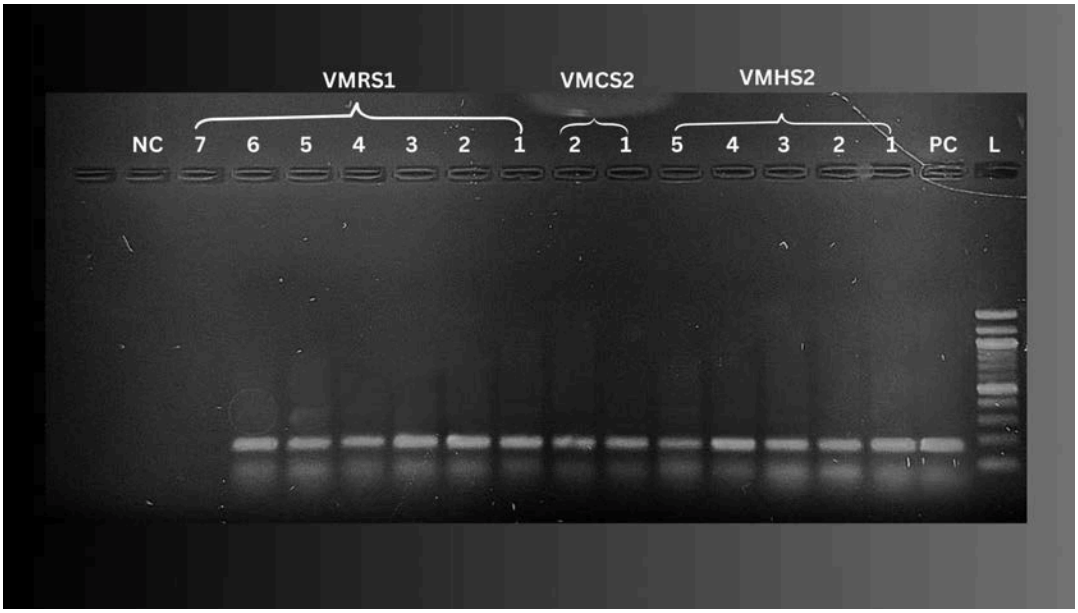


Figure 8: Agarose gel electrophoresis displays PCR amplification profiles of *Vibrio cholerae* isolates collected in May from Hatirjheel, Curzon Hall and Ramna Lake

The gel electrophoresis picture indicates the PCR amplification pattern of *Vibrio cholerae* isolates in May in the locations of Hatirjheel, Curzon Hall and Ramna Lake, which indicates the presence or absence of the target DNA. On the right, the gel has the 1 kb DNA marker (L), then the positive control (PC, a clinical strain (C6706)) which presents the amplified band as expected confirming the PCR test. VMHS2 (1-5) lanes were used to denote May Hatirjheel Sample-2, and all of them had distinctly amplified bands, indicating a PCR positive result. This is succeeded by VMCS2 (1-2) of May Curzon Hall Sample-2 in which both lanes also had clear

bands, which proves the existence of *V. cholerae* DNA.The VMRS1 (1-7) is then a representation of May Ramna Lake Sample-1 whereby the amplification bands were clear in lanes 1-6, which is a PCR-positive result, whereas the value in lane 7 was negative, which is a PCR-negative result despite loading. The negative control (NC) did not demonstrate any band which proved that there was no contamination. In general, the gel affirms successful PCR amplification in the majority of *V. cholerae* isolates in all three of the sites, and one negative isolate was observed in VMRS1 (7).

4.1.2b Table of PCR Results

Table 8: PCR detection results and positivity rates of *Vibrio cholerae* in Hatirjheel samples on the basis of month

Sampling site	Month	No. of stock	Number of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
Hatirjheel	January S1	9	9	2	2	7	22.22
	January S2	11	11	6	6	5	54.54
	February S1	3	3	0	0	3	0
	February S2	23	23	12	12	11	52.17
	March S1	4	4	1	1	3	25
	March S2	0	-	-	-	-	0
	April S1	14	14	10	10	4	71.42
	April S2	0	0	-	-	-	0
	May S1	3	3	1	1	2	33.33
	May S2	12	12	5	5	7	41.67
	June S1	10	10	2	2	8	20
	June S2	14	14	3	3	11	21.42
Total			**103**		**42**		

Table 9: PCR detection results and positivity rates of *Vibrio cholerae* in Curzon Hall samples on the basis of month

Sampling site	Month	No. of stock	Number of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
Curzon Hall	January S1	1	1	1	1	0	100
	January S2	0	-	-	-	-	0
	February S1	0	-	-	-	-	0
	February S2	0	-	-	-	-	0
	March S1	0	-	-	-	-	0
	March S2	0	-	-	-	-	0
	April S1	0	-	-	-	-	0
	April S2	0	-	-	-	-	0
	May S1	1	1	0	0	1	0
	May S2	6	6	2	2	4	33.33
	June S1	4	4	0	0	4	0
	June S2	1	1	0	0	1	0
Total			**13**		**3**		

Table 10: PCR detection results and positivity rates of *Vibrio cholerae* in Ramna Lake samples on the basis of month

Sampling site	Month	No. of stock	Number of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%)
	January S1	0	-	-	-	-	0

Ramna Lake	January S2	0	-	-	-	-	0
	February S1	0	-	-	-	-	0
	February S2	1	1	1	1	0	100
	March S1	0	-	-	-	-	0
	March S2	0	-	-	-	-	0
	April S1	1	1	0	0	1	0
	April S2	0	-	-	-	-	0
	May S1	12	12	6	6	6	50
	May S2	11	11	6	6	5	54.55
	June S1	0	-	-	-	-	0
	June S2	2	2	1	1	1	50
Total			**27**		**14**		

4.1.2c Pie Chart of the PCR Positive Results

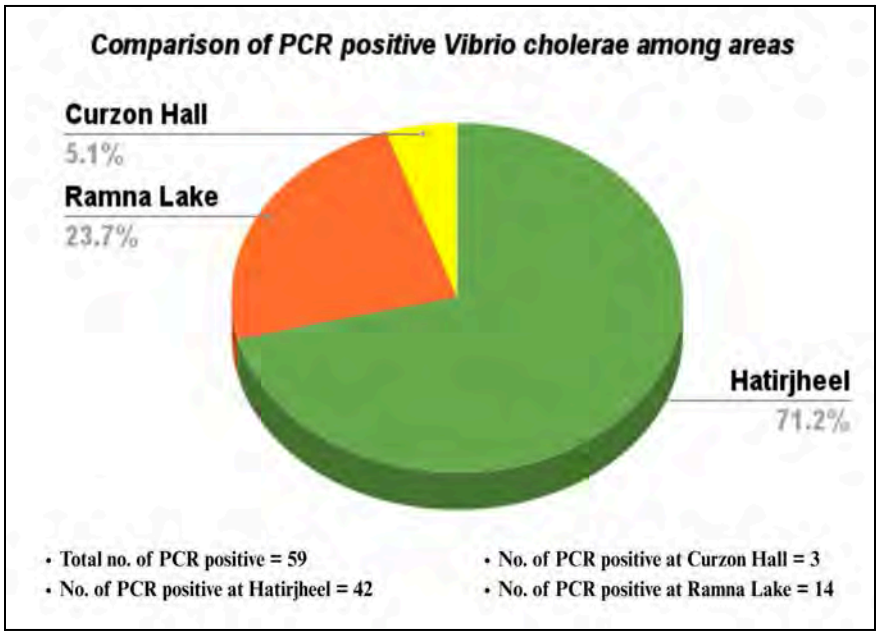


Figure 9: Comparative distribution of PCR positive *Vibrio cholerae* isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

Here, individual PCR *Vibrio cholerae* positive samples were observed to be 59 with the highest number being in Hatirjheel which comprised 42.712% of the total positive samples. The occurrence was moderate at Ramna Lake, as 14 PCR-positive samples were obtained, corresponding to 23.7%.. On the contrary, the Curzon Hall had 3 positive samples, including 5.1 percent, which represents a rather low prevalence. In general, it can be observed that the distribution indicated that Hatirjheel harbors most of the PCR- positive *Vibrio cholerae i*solates whereas Ramna Lake and Curzon Hall were found at intermediate and minimal levels respectively.

4.1.2d Column Chart of the PCR Positive Results

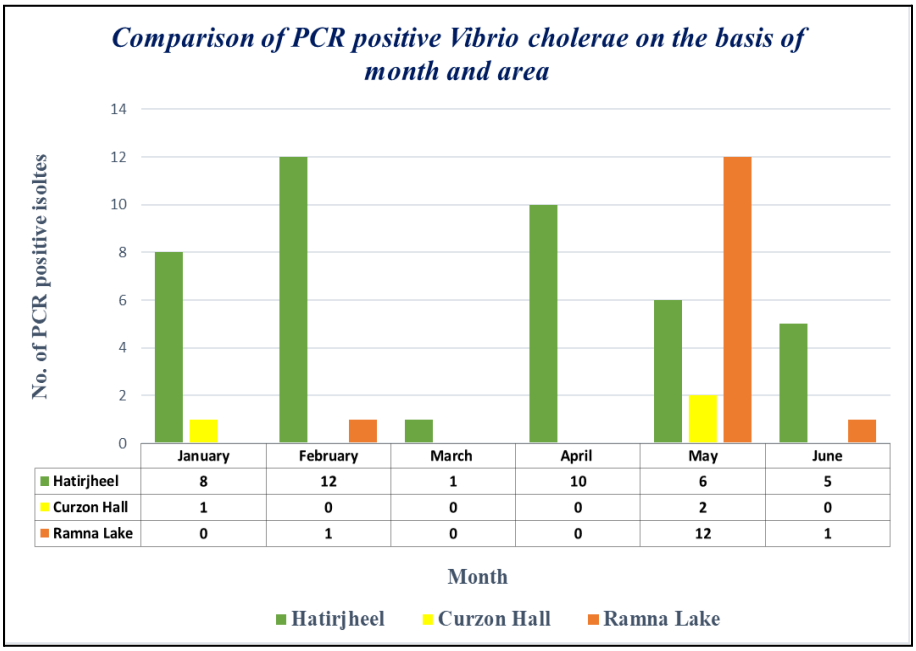


Figure 10: A monthly comparison of PCR-positive *Vibrio cholerae* isolates in three sampling sites: Haltirjheel, Curzon Hall, and Ramna Lake.

The bar graph shows how many PCR positive *Vibrio cholerae* isolates were found each month from January to June in three places: Hatirjheel, Ramna Lake, and Curzon Hall. Throughout the months, Hatirjheel always had the most PCR-positive isolates. In February, there were 12 isolates, and in April, there were 10 isolates, which was a clear peak. In March, the overall count of isolates from Hatirjheel went down (1 isolation), but it went back up in May (6 isolates) and stayed around the same in June (5 isolates). On the other hand, Curzon Hall had the fewest

PCR-positive isolates overall, with only one in January and two in May. It was negative for the remainder of the month. Ramna Lake has a distinct pattern: there was one isolation in February, then a big jump to 12 isolates in May, and then 1 isolate in June. This suggests that May is the month with the most isolates in case of Ramna Lake. This analysis shows that Hatirjheel is the predominant place where *Vibrio cholerae* contamination occurs, while Ramna Lake saw a clear rise in May. During the research period, Curzon Hall had the fewest *Vibrio cholera*e isolates.

4.1.3 Results of PCR for *Escherichia coli*

4.1.3a Gel Electrophoresis Result

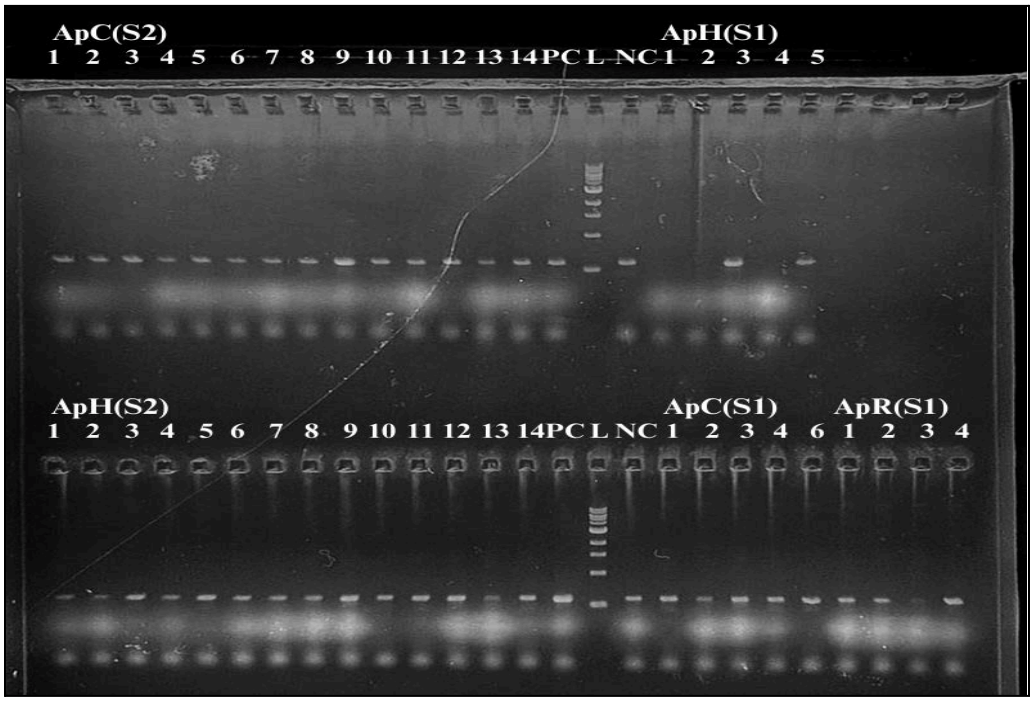


Figure 11: Agarose gel electrophoresis displays PCR amplification profiles of *E.coli* isolates collected in April from Hatirjheel, Curzon hall and Ramna Lake

The upper row of the gel displays lanes 1-14 that correspond to the *E.coli* samples of April Curzon Hall (Sample-2) and all 14 samples had definite amplification bands which means that the samples were PCR-positive. These lanes are followed by positive control (PC), 1 kb DNA ladder, negative control(NC) and five samples of April Hatirjheel (Sample-1). In these five samples, lanes 1, 2 and 4 have no amplification band, which means that they are PCR-negative, whereas the other two samples were PCR-positive. In this case, PCR-negative indicates that the

target gene amplification band is absent, indicating that the target gene was not visualized in such samples indicating that these are not *E. coli* species.

The first 14 lanes of the lower row are the *E. coli* isolates of Hatirjheel (Sample-2) and the amplification bands were all visible, which validates the presence of PCR-positive results. These lanes are preceded by the positive control (PC), 1 kb DNA ladder and negative control (NC). In both of the negative controls, an amplification band was observed probably because of the contamination present in the nuclease free water. After the control, five samples of April Curzon Hall(Sample-1) were loaded because the fifth sample had no green fluorescence hence not used. PCR amplification was positive in all the four loaded samples. Lastly, it has four samples in the April Ramna Lake (Sample-1) and all of them showed a band, which indicated PCR-positive samples.

All in all, it was found that PCR amplification was present in most of the *E. coli* isolates in all 3 sampling areas as only a few samples had negative results because of the lack of any amplification of the target genes.

4.1.3b Table of PCR Results

Table 11: PCR detection results and positivity rates of *Escherichia coli* from Hatirjheel samples on the basis of month

Sampling site	Month	No. of stock	No of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%) in comparison of no. of stock
	January S1	11	11	11	11	0	100
	January S2	12	12	10	10	2	83.33
	February S1	16	16	8	8	8	50
	February S2	16	16	16	16	0	100
	March S1	12	12	12	12	0	85.71
	March S2	12	12	12	12	0	85.71
	April S1	5	5	2	2	3	28.57

Hatirjheel	April S2	14	14	14	14	0	100
	May S1	11	11	7	7	4	58.33
	May S2	12	12	12	12	0	100
	June S1	11	11	11	11	0	100
	June S2	11	11	9	9	2	75
Total			**143**		**124**		

Table 12: PCR detection results and positivity rates of *Escherichia coli* from Curzon Hall samples on the basis of month

Sampling site	**Month**	**No. of stock**	**No of samples loaded in gel**	**Band observed in gel**	**PCR positive**	**PCR negative**	**Positivity (%) in comparison of no. of stock**
Curzon Hall	January S1	13	13	12	12	1	92.31
	January S2	0	-	-	-	-	0
	February S1	6	6	4	4	2	66.67
	February S2	0	-	-	-	-	0
	March S1	0	-	-	-	-	0
	March S2	7	7	7	7	0	100
	April S1	5	5	5	5	0	83.33
	April S2	14	14	14	14	0	100
	May S1	3	3	3	3	0	27.27
	May S2	12	12	11	11	1	91.67
	June S1	13	13	13	13	0	100
	June S2	11	11	4	4	7	33.33
Total			**84**		**73**		

Table 13: PCR detection results and positivity rates of *Escherichia coli* from Ramna Lake samples on the basis of month

Sampling site	Month	No. of stock	No of samples loaded in gel	Band observed in gel	PCR positive	PCR negative	Positivity (%) in comparison of no. of stock
Ramna Lake	January S1	0	-	-	-	-	0
	January S2	0	-	-	-	-	0
	February S1	9	9	9	9	0	90
	February S2	10	10	10	10	0	76.92
	March S1	1	1	1	1	0	100
	March S2	0	-	-	-	-	0
	April S1	4	4	4	4	0	100
	April S2	0	-	-	-	-	0
	May S1	12	12	11	11	1	91.67
	May S2	11	11	11	11	0	91.67
	June S1	0	-	-	-	-	0
	June S2	11	11	11	11	0	91.67
Total			**58**		**57**		

4.1.3c Pie Chart of the PCR Positive Results

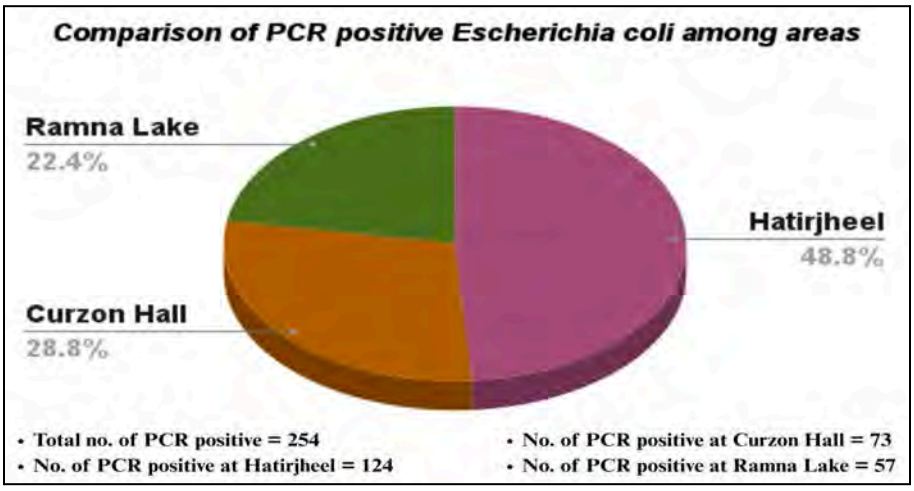


Figure 12: Comparative distribution of PCR positive *E. coli* isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

According to the pie chart, there were 254 PCR positive can and isolates of *Escherichia coli* which were collected throughout study sites. The highest percentage of these isolates is Hatirjheel of 48.8 (124 isolates), much higher than the rest of the locations. Curzon Hall then comes with 28.8% (73 isolates), then Ramna Lake has the lowest share with 22.4% (57 isolates). This space distribution indicates that Hatirjheel is a major environmental reservoir of these particular genetic markers than the other city water bodies.

4.1.3d Column Chart of the PCR Positive Results

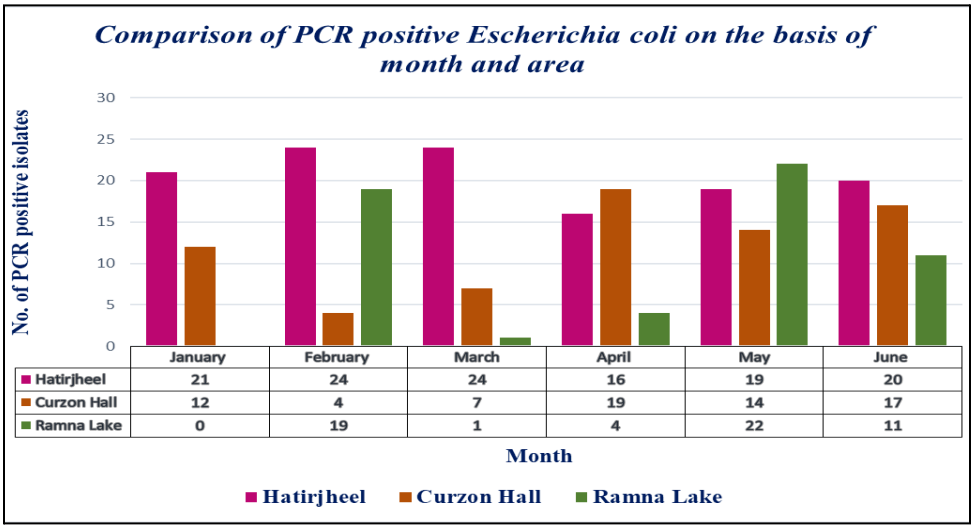


Figure 13: A monthly comparison of PCR-positive *E.coli* isolates in three sampling sites: Haltirjheel, Curzon Hall, and Ramna Lake.

The monthly distribution of the PCR-positive *E. coli* indicates a variation in the data during the six months of study and as a result, 254 positive isolates were obtained. Hatirjheel was the most stable in presentation with an overall monthly range of 16 to 24; it was highest in February and March (24 isolates each) and lowest in April (16 isolates each). On the contrary, Ramna Lake was highly variable with zero isolates in January and maximum study-wide at 22 isolates in May. Curzon Hall was the most active in April with 19 isolates which decreased considerably to its lowest point of 4 in February. All these findings indicate that the prevalence of bacteria changes with the seasons, but the higher concentration of PCR-positive samples is always observed in Hatirjheel than other urban waters in the first half of the year.

4.1.4 PCR Positive Results Based on Bacterial Species

Table 14: Comparison of PCR-positive samples based on bacterial species

Bacterial Species	**Places**			**Total**
	Hatirjheel	**Curzon Hall**	**Ramna Lake**	
Salmonella spp.	114	16	32	162
Vibrio cholerae	42	3	14	59
Escherichia coli	124	73	57	254
				475

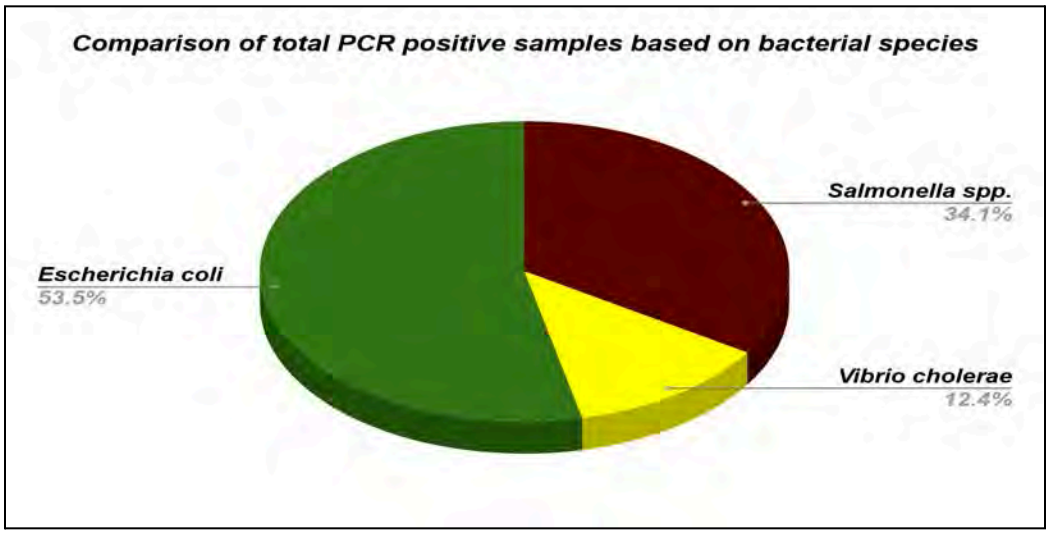


Figure 14: Comparison of total PCR-positives bacterial samples among three bacterial species

The table and figure demonstrate that *Escherichia coli* makes up the biggest part of the sum of all PCR-positive samples, with 53.5% (254) of the isolates found being *Escherichia coli* . This demonstrates more than half of the confirmed PCR bacterial presence is attributable to *E. coli*, which shows that it is the most common type of bacteria in the study. *Salmonella* spp. accounts for the second-largest proportion, providing 34.1% (162) of the overall PCR-positive samples, indicating a significant yet relatively lesser prevalence compared to *E. coli* . *Vibrio cholerae*, on the other hand, makes up the smallest part of the distribution, making up only 12.4% (59) of the total PCR- confirmed isolates. The pie chart reveals that *Escherichia coli* is clearly the most common PCR-positive isolate, afterwards *Salmonella* spp. *Vibrio cholerae* is far less common in the samples that were analyzed.

4.2 Results of Antibiotic Susceptibility Test (AST)

4.2.1 AST Results of *Salmonella* spp.

4.2.1a AST Results From Hatirjheel (January)

Table 15: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel collected in January.

Sample ID	AMP 10	ATM 30	C 30	FEP30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
SJaHS1 (1)	0 (R)	32.5 (S)	19.5 (S)	32 (S)	33 (S)	17.5 (S)	0 (R)	25 (S)	22.5 (S)	26 (S)	28 (S)	0 (R)	0 (R)
SJaHS1 (3)	0 (R)	32 (S)	29.5 (S)	34 (S)	22 (S)	20.5 (S)	0 (R)	30.5 (S)	20.5 (S)	22 (S)	27.5 (S)	0 (R)	0 (R)
SJaHS1 (4)	0 (R)	31.5 (S)	27 (S)	33.5 (S)	23.5 (S)	20.5 (S)	0 (R)	31.5 (S)	20 (S)	21 (S)	30 (S)	0 (R)	0 (R)
SJaHS1 (6)	0 (R)	30 (S)	25 (S)	31 (S)	20 (S)	20 (S)	0 (R)	26.5 (S)	18.5 (S)	21 (S)	22.5 (S)	0 (R)	0 (R)
SJaHS1 (7)	20 (S)	29 (S)	30 (S)	30.5 (S)	17.5 (I)	21 (S)	0 (R)	29 (S)	21 (S)	24 (S)	29 (S)	0 (R)	0 (R)
SJaHS1 (8)	0 (R)	32 (S)	22 (S)	30 (S)	22.5 (S)	15.5 (S)	0 (R)	26 (S)	19.5 (S)	23 (S)	34 (S)	0 (R)	0 (R)
SJaHS2 (1)	0 (R)	34 (S)	27 (S)	30 (S)	30 (S)	17 (S)	0 (R)	24 (S)	20 (S)	20 (S)	30 (S)	0 (R)	0 (R)

SJaHS2 (2)	23 (S)	32 (S)	30 (S)	36 (S)	18 (I)	20 (S)	0 (R)	28 (S)	20 (S)	18 (S)	18 (S)	0 (R)	0 (R)
SJaHS2 (3)	18 (S)	30 (S)	25 (S)	32 (S)	22 (S)	12 (I)	0 (R)	27 (S)	19 (S)	19 (S)	26 (S)	0 (R)	0 (R)
SJaHS2 (5)	16 (I)	28.5 (S)	24 (S)	32 (S)	20 (S)	15 (S)	0 (R)	24 (S)	15 (S)	15 (I)	25 (S)	0 (R)	0 (R)
SJaHS2 (6)	0 (R)	38 (S)	35 (S)	35 (S)	34 (S)	27 (S)	0 (R)	20 (S)	16.5 (S)	18 (S)	30 (S)	0 (R)	0 (R)
SJaHS2 (8)	17 (S)	28 (S)	23 (S)	31 (S)	20 (S)	19 (S)	0 (R)	27.5 (S)	19 (S)	17.5 (I)	24 (S)	0 (R)	0 (R)
SJaHS2 (10)	0 (R)	38 (S)	31 (S)	34 (S)	32 (S)	25 (S)	0 (R)	22 (S)	20 (S)	21.5 (S)	25 (S)	0 (R)	0 (R)

According to the table, 13 *Salmonella* isolates were collected in January and subjected to antibiotic susceptibility tests, all of which were collected from Hatirjheel. Of these isolates, none of them exhibited MDR properties, since they lacked some patterns of resistance other than that of ampicillin (penicillins).

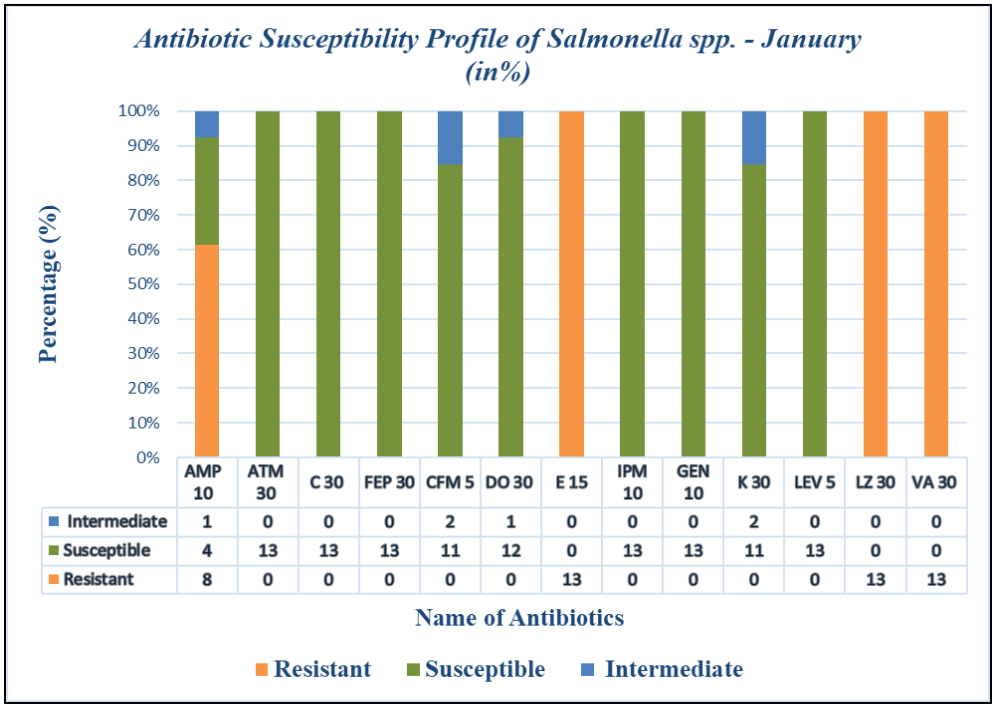


Figure 15: Antibiotic susceptibility profile of *Salmonella* spp. isolates in January in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure illustrates that the isolates of *Salmonella* in January exhibited a high percentage of resistance to ampicillin (61.5%), whereas erythromycin, linezolid, and vancomycin were not determined due to their intrinsic resistance in Gram-negative bacteria. Most of the isolates had high sensitivity levels to aztreonam (100%), chloramphenicol (100%), cefepime (100%), cefixime (84.6%), doxycycline (92.3%), imipenem (100%), gentamicin (100%), kanamycin (84.6%), and levofloxacin (100%). An observable rate of isolates had an intermediate resistance to cefixime (15.4%) and to kanamycin (15.4%), which showed the possibility of emerging resistance to both these antibiotics.

4.2.1b AST Results From Hatirjheel, Curzon Hall and Ramna Lake (February)

Table 16: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel, Ramna Lake and Curzon Hall collected in February.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
SFHS1 (1)	0 R	31S	20S	30S	22.5S	18 S	0R	27.5S	17 S	19 S	0R	0 R	0R
SFHS1 (2)	0 R	30 S	26.5S	30S	18 I	0 R	0 R	0 R	12R	18 S	20S	0 R	0R
SFHS1 (3)	0 R	30 S	25 S	31 S	19 S	17 S	0 R	0 R	18 S	20 S	20S	0 R	0R
SFHS1 (4)	0R	35S	27S	28 S	13 R	11 I	0 R	26 S	18 S	11R	19S	0 R	0R
SFHS1 (5)	0 R	38 S	35 S	34 S	34 S	26 S	0 R	20 S	18 S	20S	28S	0 R	0 R
SFHS1 (6)	18 S	32 S	27 S	31 S	19 S	18 S	0 R	29 S	17 S	29S	25 S	0 R	0 R
SFHS1 (7)	0 R	30 S	27 S	29 S	19 S	17 S	0 R	29 S	12 R	20S	20 S	0 R	0 R
SFHS1 (8)	0 R	31 S	26 S	30 S	22 S	18 S	0 R	28 S	17 S	29S	19 S	0 R	0 R
SFHS1 (9)	12 R	33 S	30 S	33.5S	0 R	19 S	0 R	29 S	0 R	29S	25 S	0 R	0 R
SFHS1 (10)	0 R	32 S	25 S	31 S	18 I	18 S	0 R	27.5S	18 S	19S	20S	0 R	0 R

SFHS1 (11)	0 R	38 S	30 S	33 S	33 S	26 S	0 R	27 S	17 S	20S	25 S	0 R	0 R
SFHS1 (12)	0 R	35 S	26.5S	33.5S	30 S	25.5S	0 R	26 S	18 S	11R	0 R	0 R	0 R
SFCS1 (1)	0 R	29 S	24.5S	29 S	20 S	13.5 I	0 R	22 S	16 S	16 I	26 S	0 R	0 R
SFCS1 (2)	0 R	23.5S	0 R	15 I	15 R	9 R	0 R	0 R	16 S	17I	0 R	0 R	0 R
SFRS1 (2)	0 R	38 S	35 S	34 S	33 S	20 S	0 R	19 S	19 S	19S	36 S	0 R	0 R
SFRS1 (4)	0 R	26 S	22 S	25 S	16 I	11 I	0 R	22 S	15 S	12R	20 S	0 R	0 R
SFRS1 (5)	0R	27.5S	27 S	27 S	19 S	17 S	0 R	24 S	16 S	16I	23 S	0 R	0 R
SFHS2 (1)	0 R	34 S	27 S	30 S	30 S	17 S	0 R	24 S	20 S	20 S	30 S	0 R	0 R
SFHS2 (2)	23 S	32 S	30 S	36 S	18 I	20 S	0 R	28 S	20 S	18 S	18 S	0 R	0 R
SFHS2 (3)	18 S	30 S	25 S	32 S	22 S	12 I	0 R	27 S	19 S	19 S	26 S	0 R	0 R
SFHS2 (4)	16 I	28.5 S	24 S	32 S	20 S	15 S	0 R	24 S	15 S	15 I	25 S	0 R	0 R
SFHS2 (5)	0 R	38 S	35 S	35 S	34 S	27 S	0 R	20 S	16.5 S	18 S	30 S	0 R	0 R
SFHS2 (6)	17 S	28 S	23 S	31 S	20 S	19 S	0 R	27.5 S	19 S	17.5 I	24 S	0 R	0 R
SFHS2 (7)	0 R	38 S	31 S	34 S	32 S	25 S	0 R	22 S	20 S	21.5 S	25 S	0 R	0 R
SFHS2 (8)	0 R	37.5 S	34 S	36 S	35 S	25 S	0 R	23 S	20 S	22.5 S	28 S	0 R	0 R
SFHS2 (9)	0 R	34 S	30 S	32 S	33 S	20 S	0 R	24 S	19 S	20 S	28 S	0 R	0 R
SFHS2 (10)	0 R	35 S	28 S	33 S	31 S	20 S	0 R	25 S	19 S	18.5 S	30 S	0 R	0 R
SFHS2 (11)	0 R	36 S	27 S	35 S	34 S	19 S	0 R	27 S	22 S	22 S	31 S	0 R	0 R
SFHS2 (12)	0 R	37 S	33 S	32 S	30 S	21 S	0 R	30 S	18.5 S	23 S	28 S	0 R	0 R

SFHS2 (13)	0 R	35 S	32 S	33 S	29 S	20 S	0 R	28 S	19 S	21 S	29 S	0 R	0 R
SFHS2 (14)	0 R	32 S	31 S	34 S	27 S	22 S	0 R	24 S	20 S	22 S	32 S	0 R	0 R

The table indicates that 31 *Salmonella* isolates were collected in February and subjected to antibiotic susceptibility tests, whereby 26 were isolated at Hatirjheel and the remaining 2 and 3 at Curzon Hall and Ramna Lake respectively. Among these isolates, 4 Hatirjheel isolates (SFHS1(2,4,9,12)) and 1 Curzon Hall isolate (SFCS1(2)) were found to be multiple drug resistant mostly to ampicillin (penicillins), chloramphenicol (amphenicols), doxycycline (tetracyclines), levofloxacin (fluoroquinolones), imipenem (carbapenems), gentamicin and kanamycin (aminoglycosides) and cefixime (cephalosporins) and thus were categorised as MDR.

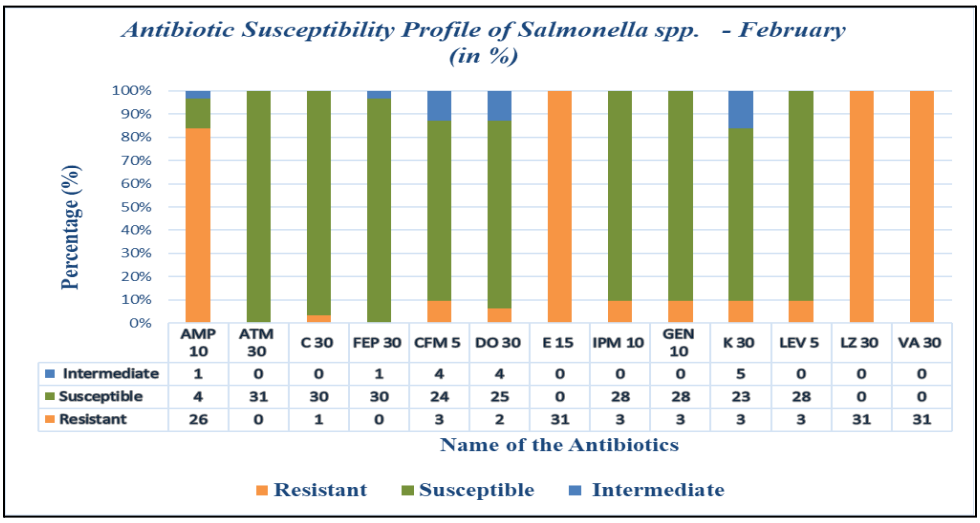


Figure 16: Antibiotic susceptibility profile of *Salmonella* spp. isolates in February in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure indicates that the *Salmonella* isolates in February had a high rate of ampicillin resistance (83.9%), whereas erythromycin, linezolid, and vancomycin were not included in the determination of resistance types because they are inherently resistant antibiotics for Gram-negative bacteria. The majority of them were highly sensitive to aztreonam (100%), chloramphenicol (96.8%), cefepime (96.8%), cefixime (77.4%), doxycycline (80.6%), imipenem (90.3%), gentamicin (90.3%), kanamycin (90.3%) and levofloxacin (90.3%). But a significant

proportion of isolates were observed to be intermediate to cefixime (12.9%), doxycycline (12.9%), and streptococcal kanamycin (16.1%), implying the potential shift to resistance.

4.2.1c AST Results From Hatirjheel (March)

Table 17: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel collected in March.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM5	DO30	E15	IPM 10	GEN 10	K30	LEV 5	LZ 30	VA30
SMaHS1(2)	21.5 S	29.5 S	29 S	30 S	25.5 S	21 S	0 R	27 S	19 S	22 S	33 S	0 R	0 R
SMaHS2(1)	0 R	31 S	25.6 S	30 S	19 S	20 S	8 R	23 S	20 S	20 S	33 S	0 R	0 R
SMaHS2(2)	0 R	29 S	26 S	30 S	19 S	19 S	0 R	23 S	18 S	20 S	32 S	0 R	0 R
SMaHS2(4)	0 R	31.5 S	26.5 S	28.5 S	20 S	16.7 S	0 R	25 S	19 S	20 S	24 S	0 R	0 R

According to the table, 4 *Salmonella* isolates were collected in March and subjected to antibiotic susceptibility tests, all of which were collected from Hatirjheel. Of these isolates, none of them exhibited MDR properties, since they lacked some patterns of resistance other than that of ampicillin (penicillins).

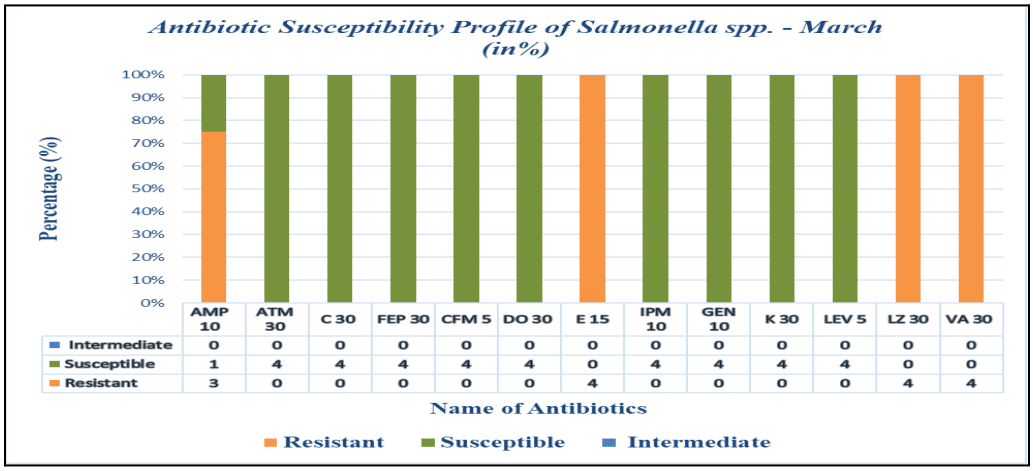


Figure 17: Antibiotic susceptibility profile of *Salmonella* spp. isolates in March in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure indicates that in March, the *Salmonella* isolates exhibited significant resistance to ampicillin (75%), and erythromycin, linezolid, and vancomycin were not considered resistance types because of intrinsic resistance levels in Gram-negative bacteria. These isolates demonstrated 100% susceptibility to aztreonam, chloramphenicol, cefepime and cefixime, doxycycline, imipenem, gentamicin, kanamycin and levofloxacin. None of the antibiotics had any intermediate level of susceptibility, indicating a general pattern of stability in the susceptibility profile of the isolates in March.

4.2.1d AST Results From Hatirjheel (April)

Table 18: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel collected in April.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM10	GEN 10	K 30	LEV 5	LZ30	VA30
SApHS1 (1)	0 (R)	35 (S)	25 (S)	31 (S)	25 (S)	20 (S)	0 (R)	20 (S)	20 (S)	22 (S)	26 (S)	0 (R)	12 (R)
SApHS1 (3)	0 (R)	30 (S)	23 (S)	33 (S)	19 (S)	17 (S)	0 (R)	26 (S)	20 (S)	21 (S)	30 (S)	0 (R)	0 (R)
SApHS1 (4)	0 (R)	33 (S)	27 (S)	33 (S)	21 (S)	20 (S)	0 (R)	30 (S)	19 (S)	22 (S)	33 (S)	0 (R)	0 (R)
SApHS1 (5)	0 (R)	29 (S)	25 (S)	31 (S)	19 (S)	17 (S)	0 (R)	25 (S)	20 (S)	21 (S)	28.5(S)	0 (R)	0 (R)
SApHS1 (6)	0 (R)	35 (S)	23 (S)	30 (S)	20 (S)	17 (S)	0 (R)	25 (S)	20 (S)	22 (S)	29 (S)	0 (R)	0 (R)
SApHS1 (7)	0 (R)	35 (S)	24.5 (S)	36 (S)	25 (S)	20 (S)	0 (R)	24.5 (S)	20 (S)	20 (S)	27 (S)	0 (R)	0 (R)
SApHS1 (8)	0 (R)	34 (S)	31 (S)	36 (S)	24 (S)	20 (S)	0 (R)	23.5 (S)	20 (S)	20 (S)	28 (S)	0 (R)	0 (R)
SApHS1 (9)	0 (R)	35 (S)	30 (S)	35 (S)	28 (S)	17 (S)	0 (R)	23 (S)	21.5 (S)	23 (S)	25 (S)	0 (R)	0 (R)
SApHS1 (10)	0 (R)	34 (S)	26 (S)	34 (S)	37.5 (S)	20 (S)	0 (R)	24 (S)	25 (S)	26 (S)	28 (S)	0 (R)	0 (R)
SApHS1 (12)	0 (R)	13 (R)	26 (S)	20 (S)	0 (R)	14 (S)	0 (R)	25 (S)	19 (S)	14 (I)	25 (S)	0 (R)	0 (R)
SApHS1 (13)	0 (R)	30 (S)	27 (S)	34 (S)	22 (S)	13 (I)	0 (R)	20 (S)	19 (S)	19 (S)	22 (S)	0 (R)	0 (R)

SApHS1 (14)	0 (R)	30 (S)	24 (S)	32 (S)	19 (S)	18 (S)	0 (R)	24 (S)	19 (S)	17 (I)	22 (S)	0 (R)	0 (R)
SApHS1 (15)	10 (R)	32 (S)	26 (S)	34 (S)	21 (S)	18 (S)	0 (R)	27 (S)	17 (S)	19 (S)	28 (S)	0 (R)	0 (R)
SApHS1 (16)	10 (R)	33 (S)	27 (S)	31 (S)	23 (S)	20 (S)	0 (R)	22 (S)	19 (S)	22 (S)	29 (S)	0 (R)	0 (R)
SApHS1 (17)	0 (R)	30 (S)	25 (S)	33 (S)	20 (S)	20 (S)	0 (R)	28 (S)	20 (S)	28 (S)	29 (S)	0 (R)	0 (R)
SApHS2 (1)	0 (R)	27 (S)	17.5 (I)	30 (S)	19 (S)	0 (R)	0 (R)	25.5 (S)	16.5 (S)	16.5 (I)	28.5 (S)	0 (R)	0 (R)
SApHS2 (7)	0 (R)	31.5 (S)	26.5 (S)	31 (S)	21 (S)	15.5 (S)	0 (R)	25 (S)	15.5 (S)	15.5 (I)	20.5 (S)	0 (R)	0 (R)

The table shows that 17 *Salmonella* isolates were collected in April and subjected to antibiotic susceptibility tests, all of which were collected from Hatirjheel. Out of these Hatirjheel isolates, 1 (SApHS1(12)) isolate was detected to be multiple drug resistant (MDR) since it was resistant to ampicillin (penicillins), aztreonam (monobactams), and cefixime (cephalosporins).

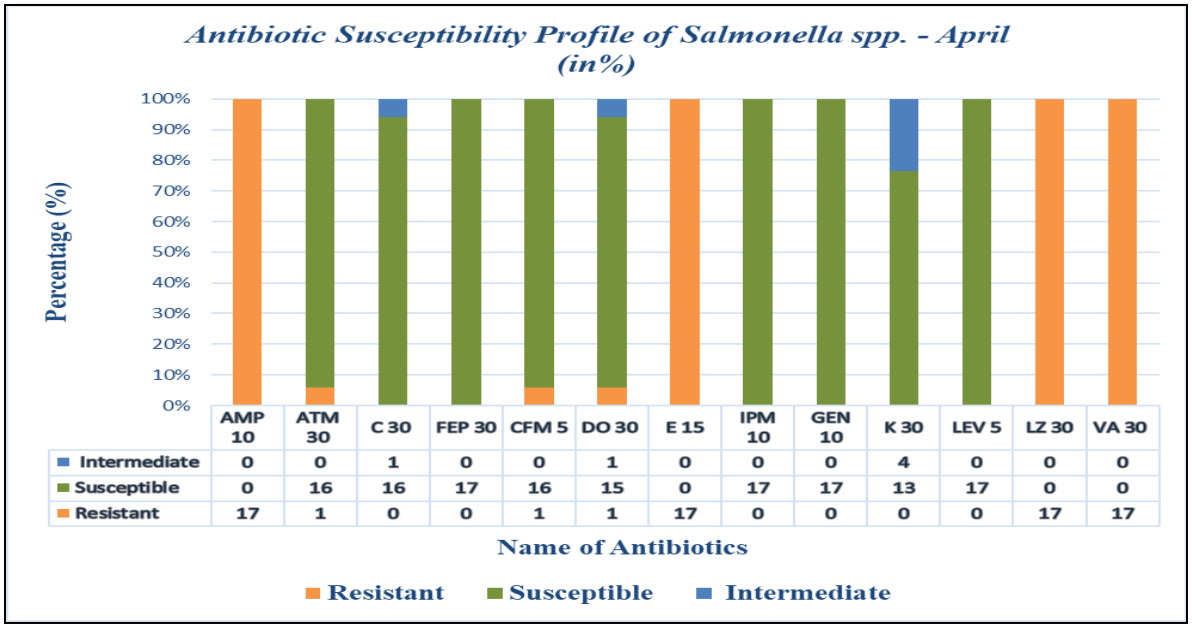


Figure 18: Antibiotic susceptibility profile of *Salmonella* spp. isolates in April in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure indicates that in April, the *Salmonella* isolates were completely resistant to ampicillin (100%), whereas erythromycin (E), linezolid (LZ), and vancomycin (VA) have not found their

place in resistance type determination since they are considered intrinsically resistant antibiotics in Gram-negative bacteria. Most of the isolates were highly susceptible to aztreonam (94.1%), chloramphenicol (94.1%), cefepime (100%), cefixime (94.1%), doxycycline (88.2%), imipenem (100%), gentamicin (100%), kanamycin (76.5%), and levofloxacin (100%), which showed that there was an overall good sensitivity to these antibiotics. The percentage of isolates that exhibited intermediate susceptibility to chloramphenicol (5.9%), doxycycline (5.9%), and kanamycin (23.5%) indicated a possibility of early indication of lower susceptibility, especially to kanamycin.

4.2.1e AST Results From Hatirjheel, Curzon Hall and Ramna Lake (May)

Table 19: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel, Ramna Lake and Curzon Hall collected in May.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
SMHS1(1)	0 R	38.5 S	27 S	35 S	22 S	24.5 S	0 R	28 S	20 S	25 S	35.5 S	0 R	0 R
SMHS1(2)	0 R	21 S	31 S	35 S	0 R	14 S	0 R	24 S	20 S	23 S	25 S	0 R	0 R
SMHS1(3)	0 R	14.5 R	28.5 S	14 R	0 R	17.5 S	0 R	26 S	20 S	0 R	32.5 S	0 R	0 R
SMHS1(4)	0 R	18 I	27 S	20 S	0 R	12.5 S	0 R	23 S	12 R	0 R	36 S	0 R	0 R
SMHS1(5)	0 R	32.5 S	28 S	28 S	24.5 S	13 I	0 R	29 S	20 S	25 S	37 S	0 R	0 R
SMHS1(6)	0 R	19 I	30 S	30 S	20 S	13 I	0 R	25 S	20 S	26.5 S	33 S	0 R	0 R
SMHS1(7)	16 I	35 S	26 S	38 S	0 R	29 S	20 R	18 S	24 S	0 R	0 R	0 R	0 R
SMHS1(8)	0 R	36 S	31 S	40 S	0 R	38 S	23 R	21 S	30 S	0 R	0 R	0 R	0 R
SMHS1(9)	0 R	37 S	25 S	40 S	0 R	35 S	23 R	26 S	30 S	0 R	0 R	0 R	0 R
SMHS1(10)	20 S	38 S	28 S	38 S	0 R	35 S	21 R	20 S	30 S	0 R	0 R	0 R	0 R

SMHS1(11)	0 R	40 S	26 S	38 S	0 R	35 S	21 R	20 S	31 S	0 R	0 R	0 R	0 R
SMHS1(12)	0 R	35 S	27 S	38 S	0 R	30 S	8 R	20 S	26 S	0 R	0 R	0 R	0 R
SMHS1(13)	16 I	35 S	25 S	37 S	0 R	32 S	20 R	21 S	32 S	0 R	0 R	0 R	0 R
SMRS1(1)	0 R	30 S	25 S	32.5 S	13 R	17.5 S	0 R	25 S	17 S	19 S	27.5 S	0 R	0 R
SMRS1(2)	0 R	19 I	27 S	21 S	31.5 S	10 R	0 R	22.5 S	19 S	20 S	28 S	0 R	0 R
SMRS1(3)	0 R	17 R	26 S	19 S	10 R	10 R	0 R	26 S	18 S	20 S	24 S	0 R	0 R
SMRS1(4)	0 R	20 I	25 S	20 S	10 R	17.5 S	0 R	25 S	17 S	20 S	24 S	0 R	0 R
SMRS1(5)	0 R	30 S	32 S	32 S	0 R	10 R	0 R	25 S	19 S	19 S	25 S	0 R	0 R
SMRS1(6)	0 R	33 S	0 R	34.5 S	24 S	10 R	0 R	26 S	19 S	21 S	30 S	0 R	0 R
SMRS1(7)	0 R	31 S	26.5 S	32 S	20 S	0 R	0 R	27 S	23 S	0 R	29 S	0 R	0 R
SMRS1(8)	0 R	35 S	29 S	35 S	21 S	15 S	0 R	25 S	21 S	23.5 S	18 S	0 R	0 R
SMRS1(9)	0 R	22 S	24 S	31 S	18 I	0 R	0 R	25 S	24.5 S	0 R	14 I	0 R	0 R
SMRS1(10)	0 R	30 S	27 S	33 S	19 S	16.5 S	0 R	26 S	23.5 S	21 S	28.5 S	0 R	0 R
SMRS1(11)	0 R	32 S	29 S	33 S	25 S	19 S	0 R	27.5 S	24 S	21 S	30 S	0 R	0 R
SMRS1(12)	17 S	30 S	31 S	33 S	15 R	18.5 S	0 R	24 S	25 S	21.5 S	28.5 S	0 R	0 R
SMRS1(13)	0 R	35 S	26 S	33 S	22 S	13.5 S	0 R	23.5 S	20S	20 S	29 S	0 R	0 R
SMRS1(14)	0 R	18 I	29 S	19 S	11 R	15 S	0 R	22 S	19 S	19 S	25 S	0 R	0 R
SMCS1(1)	0 R	31 S	0 R	34 S	22 S	12 R	0 R	25 S	0 R	14 I	25 S	0 R	0 R
SMCS1(2)	0 R	32.5 S	0 R	32 S	16 I	10 R	0 R	33.5 S	0 R	17 I	23.5 S	0 R	0 R
SMHS2(1)	10 R	29S	0 R	30 S	21 S	0 R	8 R	22 S	24 S	18 S	23 S	0 R	0 R

SMHS2(2)	0 R	31 S	27.5 S	33 S	21 S	17 S	0 R	23.5 S	20 S	16 I	30 S	0 R	0 R
SMHS2(3)	0 R	34 S	26.5 S	30 S	25 S	0 R	0 R	25 S	23 S	15 I	24 S	0 R	0 R
SMHS2(4)	0 R	32 S	27 S	32 S	28.5 S	10 R	0 R	26 S	21 S	17 I	27 S	0 R	0 R
SMHS2(5)	0 R	30 S	21.5 S	29 S	15 R	19 S	0 R	24 S	20 S	16 I	29 S	0 R	0 R
SMHS2(6)	0 R	15 R	15 S	17 I	0 R	0 R	0 R	24.5 S	18 S	17 I	21.5 S	0 R	0 R
SMHS2(7)	10 R	32.5 S	29 S	30 S	26 S	19 S	0 R	21.5 S	20 S	19 S	26 S	0 R	0 R
SMHS2(8)	0 R	35 S	30 S	30 S	27 S	15 S	0 R	20 S	20 S	19 S	18 S	0 R	0 R
SMHS2(9)	0 R	30 S	0 R	30 S	15 R	0 R	0 R	27 S	20 S	0 R	0 R	0 R	0 R
SMHS2(10)	0 R	22 S	27 S	13 R	0 R	10 R	0 R	30 S	20 S	16 I	22 S	0 R	0 R
SMCS2(1)	13.5 S	29.5 S	31.5 S	33.5 S	17.5 I	21 S	0 R	28 S	21.5 S	18 S	10 R	0 R	0 R
SMCS2(2)	0 R	31.5 S	0 R	34.5 S	18.5 I	9 R	0 R	29 S	21.5 S	0 R	0 R	0 R	0 R
SMCS2(3)	0 R	33 S	28.5 S	33 S	22 S	18 S	0 R	29 S	19.5 S	21 S	30.5 S	0 R	0 R
SMCS2(4)	0 R	28 S	0 R	34 S	15 R	0 R	0 R	19 S	20 S	0 R	0 R	0 R	0 R
SMCS2(5)	0 R	30 S	0 R	31.5 S	17.5 I	0 R	0 R	27 S	20 S	0 R	0 R	0 R	0 R
SMCS2(6)	0 R	30 S	0 R	31.5 S	18.5 I	0 R	0 R	22.5 S	20 S	0 R	0 R	0 R	0 R
SMCS2(7)	0 R	29 S	0 R	34 S	17.5 I	0 R	0 R	27 S	0 R	13 R	18.5 S	0 R	0 R
SMCS2(8)	0 R	31 S	30 S	31.5 S	18.5 I	0 R	0 R	26 S	30 S	20 S	0 R	0 R	0 R
SMCS2(9)	0 R	35.5 S	29 S	30 S	29 S	0 R	0 R	20 S	19 S	17 I	30 S	0 R	0 R
SMCS2(10)	0 R	25.5 S	0 R	30 S	17 I	0 R	0 R	25 S	20 S	0 R	0 R	0 R	0 R
SMRS2(1)	0 R	32 S	0 R	31 S	12.5R	0 R	0 R	26 S	19 S	17 I	29 S	0 R	0 R

SMRS2(2)	0 R	30 S	0 R	20 S	15 R	0 R	0 R	27 S	17 S	17 I	26 S	0 R	0 R
SMRS2(3)	0 R	35 S	0 R	26 S	33 S	0 R	0 R	23 S	0 R	0 R	25 S	0 R	0 R

The table indicates that 52 *Salmonella* isolates were collected in May and subjected to antibiotic susceptibility tests in which 23 of them were collected in Hatirjheel, 12 in Curzon Hall and 17 in Ramna lake. Among the isolates of Hatirjheel, 13 isolates (SMHS1 (3,4,7,8,9,10,11,12,13) and SMHS2 (1,6,9,10)) were identified to be multiple drug resistant (MDR) mostly to ampicillin (penicillins), aztreonam (monobactams), chloramphenicol (amphenicols), doxycycline (tetracyclines), levofloxacin (fluoroquinolones), gentamicin. Moreover, 7 Ramna Lake isolates (SMRS1(3,5,6,7), SMRS2(1,2,3), SMCS1(1,2), SMCS2(2,4,5,6,7,8,10)) were also categorized as MDR.

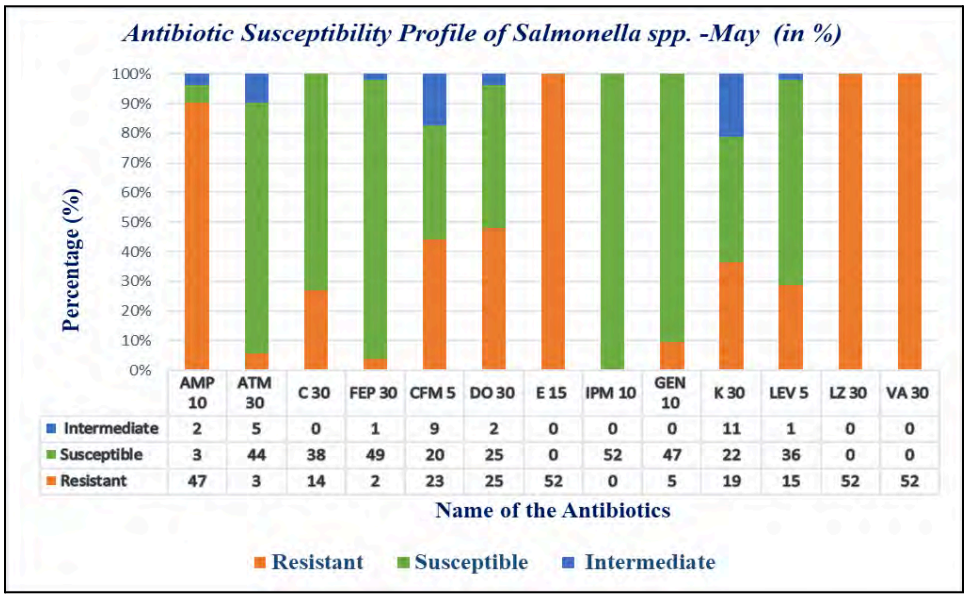


Figure 19: Antibiotic susceptibility profile of *Salmonella* spp. isolates in May in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure indicates that the *Salmonella* isolates recorded high rates of resistance to ampicillin (90.4%), and the use of erythromycin (E), linezolid (LZ) and vancomycin (VA) did not contribute to the determination of their resistance type since they are considered to be intrinsically resistant antibiotics to Gram-negative bacteria. Most of those isolated were highly sensitive to aztreonam (84.6%), chloramphenicol (73.1%), cefepime (94.2%), imipenem (100%),

gentamicin (90.4%), and levofloxacin (69.2%), though levofloxacin resistance was detected in 28.8% of the isolates. The rate of resistance was found to be very high against cefixime (44.2%) and doxycycline (48.1%), whereas the intermediate susceptibility to kanamycin (21.2%), cefixime (17.3%) and aztreonam (9.6%) was also high implying possible shift towards resistance of these antibiotics.

4.2.1f AST Results From Hatirjheel, Curzon Hall and Ramna Lake (June)

Table 20: The pattern of antibiotic susceptibility of *Salmonella* spp. isolates at Hatirjheel, Ramna Lake and Curzon Hall collected in June.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
SJnHS1 (1)	0 R	34 S	22 S	34 S	24.5 S	0 R	0 R	26.5 S	21 S	20 S	30 S	0 R	0 R
SJnHS1 (2)	0 R	29 S	0 R	28 S	17 I	10 R	0 R	28 S	19 S	18 S	29 S	0 R	0 R
SJnHS1 (3)	0 R	28.5 S	26 S	33 S	0 R	16 S	0 R	27 S	19 S	16 I	25 S	0 R	0 R
SJnHS1 (4)	0 R	20 I	0 R	21.5 S	0 R	10 R	0 R	29 S	20 S	17.5 I	27 S	0 R	0 R
SJnHS1 (5)	22 S	30 S	26 S	36.5 S	22 S	17 S	0 R	29 S	20 S	17 I	29 S	0 R	0 R
SJnHS1 (6)	0 R	30 S	25 S	32 S	20 S	15 S	0 R	21.5 S	19 S	17 I	26 S	0 R	0 R
SJnHS1 (7)	0 R	11 R	28 S	16 I	0 R	11 I	0 R	30 S	20 S	17 I	23 S	0 R	0 R
SJnHS1 (8)	0 R	20 I	27 S	25 S	11 R	0 R	0 R	24 S	27 S	17 I	23 S	0 R	0 R
SJnHS1 (9)	0 R	15 R	28.5 S	18 S	0 R	12.5 I	0 R	29 S	18 S	17 I	27 S	0 R	0 R
SJnHS1 (10)	0 R	0 R	20.5 S	0 R	0 R	10 R	0 R	28 S	22.5 S	20 S	26 S	0 R	0 R
SJnHS1 (11)	0 R	31.5 S	22 S	33 S	19.5 S	17 S	0 R	28 S	18 S	0 R	0 R	0 R	0 R
SJnHS1 (12)	0 R	16.5 R	32 S	20 S	11 R	13 I	0 R	36.5 S	21.5 S	22 S	25 S	0 R	0 R
SJnHS1 (13)	0 R	35 S	29 S	33 S	31 S	22 S	0 R	28 S	20 S	20 S	28 S	0 R	0 R
SJnHS1 (14)	0 R	10 R	32.5 S	15 I	0 R	13 I	0 R	31 S	16 S	0 R	24 S	0 R	0 R
SJnHS1 (15)	0 R	35 S	14 I	34 S	33 S	30 S	0 R	23 S	19 S	18 S	30 S	0 R	0 R
SJnHS1 (16)	0 R	37 S	0 R	32 S	20 S	11 I	0 R	30 S	0 R	0 R	15 I	0 R	0 R

SJnCS1 (1)	0 R	27 S	24.5 S	32 S	16 I	17 S	0 R	26 S	20 S	21 S	35 S	0 R	0 R
SJnCS1 (2)	0 R	33 S	27 S	34 S	22 S	20 S	0 R	23 S	16 S	10 R	32 S	0 R	0 R
SJnHS2 (1)	0 R	29 S	28.5 S	33.5 S	20 S	22 S	10 R	23 S	21 S	15 I	20 S	0 R	0 R
SJnHS2 (2)	0 R	32 S	29 S	30 S	29 S	17 S	10 R	25 S	20 S	15 I	27 S	0 R	0 R
SJnHS2 (3)	0 R	33 S	25.5 S	32.5 S	18 I	18 S	10 R	22.5 S	18.5 S	15 I	30 S	0 R	0 R
SJnHS2 (4)	0 R	28 S	27 S	30 S	17 I	17 S	10 R	23 S	20 S	15 I	34 S	0 R	0 R
SJnHS2 (5)	0 R	30 S	28 S	30 S	29 S	17 S	9 R	23 S	18 S	15 I	32 S	0 R	0 R
SJnHS2 (6)	0 R	31 S	26 S	32 S	17 I	18 S	9 R	26 S	23.5 S	15 I	26 S	0 R	0 R
SJnHS2 (7)	0 R	27 S	22.5 S	27.5 S	17 I	15 S	0 R	21.5 S	23 S	16 I	26 S	0 R	0 R
SJnHS2 (8)	0 R	30 S	28.5 S	27 S	20 S	16 S	10 R	25 S	20 S	16 I	27 S	0 R	0 R
SJnHS2 (9)	0 R	29 S	23.5 S	31.5 S	17 I	17 S	0 R	21 S	16 S	10 R	27 S	0 R	0 R
SJnHS2 (10)	0 R	32 S	26 S	30.5 S	17 I	13 I	9 R	23 S	21 S	15 I	25 S	0 R	0 R
SJnHS2 (11)	0 R	30 S	22 S	27 S	19 S	16 S	0 R	23 S	21 S	16.5 I	31 S	0 R	0 R
SJnRS2 (1)	0 R	28.5 S	26.5 S	31 S	16 I	16 S	0 R	26 S	22.5 S	19 S	34.5 S	0 R	0 R
SJnRS2 (2)	0 R	31.5 S	26.5 S	32 S	17 I	18 S	0 R	20 S	27 S	15.5 I	30 S	0 R	0 R
SJnRS2 (3)	0 R	30 S	25 S	28 S	20 S	12 I	0 R	20 S	23 S	17 I	32 S	0 R	0 R
SJnRS2 (4)	0 R	28 S	23.5 S	27 S	17 I	14 S	0 R	20 S	21 S	15 I	32 S	0 R	0 R
SJnRS2 (5)	0 R	31 S	35 S	30 S	35 S	12 I	0 R	22 S	23 S	17 I	30 S	18 R	10 R
SJnRS2 (6)	0 R	37.5 S	25 S	32 S	33 S	19 S	9 R	20.5 S	25 S	16 I	35 S	0 R	13 R
SJnRS2 (7)	0 R	30 S	30 S	33 S	19 S	9 R	10 R	29 S	29 S	14 I	26 S	0 R	0 R
SJnRS2 (8)	0 R	31.5 S	23 S	34.5 S	18 I	19 S	0 R	28 S	20 S	13 R	20.5 S	0 R	0 R
SJnRS2 (9)	0 R	28 S	25 S	23 S	42 S	15 S	0 R	24.5 S	20 S	15 I	33 S	0 R	0 R
SJnRS2 (10)	0 R	37 S	30 S	30 S	32 S	20 S	0 R	23 S	23.5 S	20 S	35 S	12 R	0 R
SJnRS2 (11)	0 R	33 S	33 S	32 S	34 S	17 S	10 R	20 S	20 S	15 I	27 S	15 R	11 R
SJnRS2 (12)	0 R	33 S	23.5 S	31 S	19.5 S	0 R	0 R	20 S	20.5 S	16 I	26 S	0 R	0 R

As indicated in the table, 41 *Salmonella* isolates were collected in June and antibiotic susceptibility tests were performed, with 27 being collected at Hatirjheel, 2 at Curzon Hall, and 12 at Ramna Lake. Among these isolates, 10 Hatirjheel isolates (SJnHS1(2,4,7,8,9,10,11,12,14,16)) were identified as multiple drug resistant especially to ampicillin (penicillins), aztreonam (monobactams), chloramphenicol (amphenicols), doxycycline (tetracyclines), levofloxacin (fluoroquinolones), gentamicin and kanamycin (aminoglycosides) and cefixime (cephalosporins) and thus were categorised as MDR.

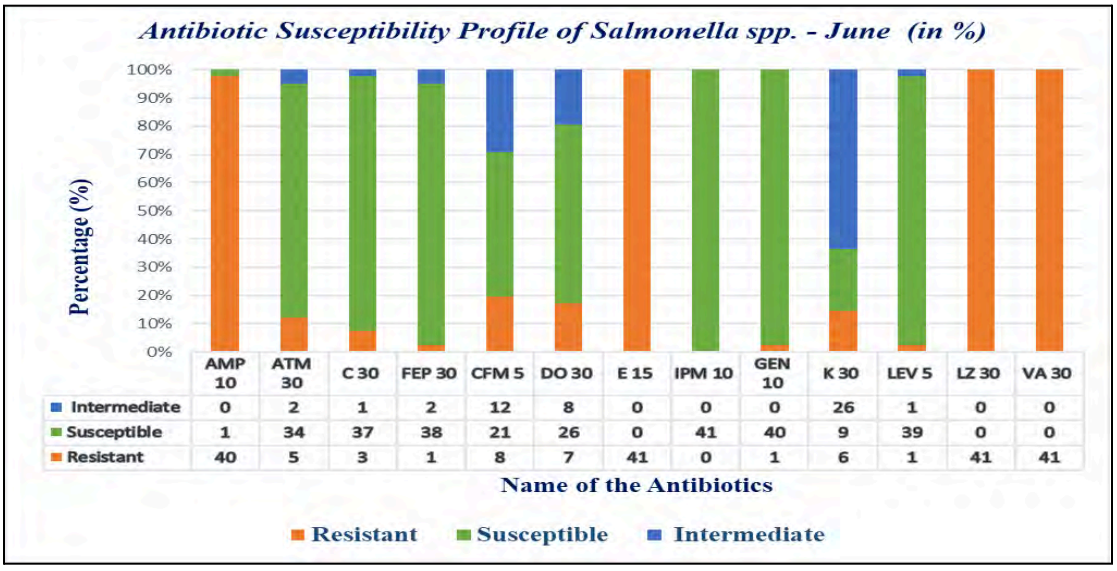


Figure 20: Antibiotic susceptibility profile of *Salmonella* spp. isolates in June in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure indicates that in June the Salmonella isolates exhibited a very high percentage of ampicillin resistance (97.6%), and erythromycin (E), linezolid (LZ), and vancomycin (VA) were not identified with resistance type types, as these are regarded as intrinsically resistant Gram-negative bacteria antibiotics. Most of the isolates were highly sensitive to aztreonam (82.9%), chloramphenicol (90.2%), cefepime (92.7%), imipenem (100%), gentamicin (97.6%) and levofloxacin (95.1) with very little resistance to these antibiotics. Moderate susceptibility to cefixime (19.5% and doxycycline (17.1%), and a significant proportion of the isolates had

intermediate susceptibility to cefixime (29.3%), doxycycline (19.5%), and kanamycin (63.4%) were observed thus showing a risk of increasing resistance mostly in the case of kanamycin.

4.2.2 AST Results of *Vibrio cholerae*.

4.2.2a AST Results From Hatirjheel and Curzon Hall (January)

Table 21 : The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel, and Curzon Hall collected in January .

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
VJaHS1 (1)	0 R	39 S	29 S	36 S	35 S	22 S	12 R	30 S	20 S	21 S	27 S	9 R	0 R
VJaHS1 (2)	0 R	39 S	30 S	35 S	35 S	24 S	13 R	34 S	20 S	22 S	26 S	10 R	0 R
VJaCS1 (1)	0 R	40 S	31 S	39 S	22 S	26 S	17 I	30 S	23 S	25 S	27 S	13 R	0 R
VJaHS2 (1)	0 R	34 S	30 S	30 S	29 S	20 S	13 R	28 S	19 S	20 S	22 S	0 R	0 R
VJaHS2 (2)	0 R	30 S	30 S	28 S	28 S	21 S	11 R	30 S	20 S	20 S	24 S	0 R	0 R
VJaHS2 (3)	0 R	24 S	30 S	28 S	29 S	21 S	13 R	15 R	17 S	20 S	20 S	20 R	0 R
VJaHS2 (4)	0 R	35 S	29 S	33 S	29 S	19 S	12 R	30 S	21 S	22 S	24 S	0 R	0 R
VJaHS2 (5)	0 R	35 S	31 S	31 S	30 S	21 S	12 R	30 S	21 S	21 S	25 S	0 R	0 R
VJaHS2 (6)	0 R	25 S	29 S	33 S	28 S	19 S	12 R	16 R	17 S	22 S	28 S	0 R	0 R

The table indicates that 9 *Vibrio cholerae* isolates were studied in January, 8 of them were gathered at Hatirjheel and one at Curzon Hall. Each of the nine isolates such as VJaHS1 (1, 2), VJaCS1 (1) and VJaHS2 (1-6) was resistant to ampicillin, although all were susceptible to most of the other antibiotics that were tested. Also, there were two isolates, VJaHS2 (3) and VJaHS2 (6), which were resistant to imipenem (carbapenem antibiotic).

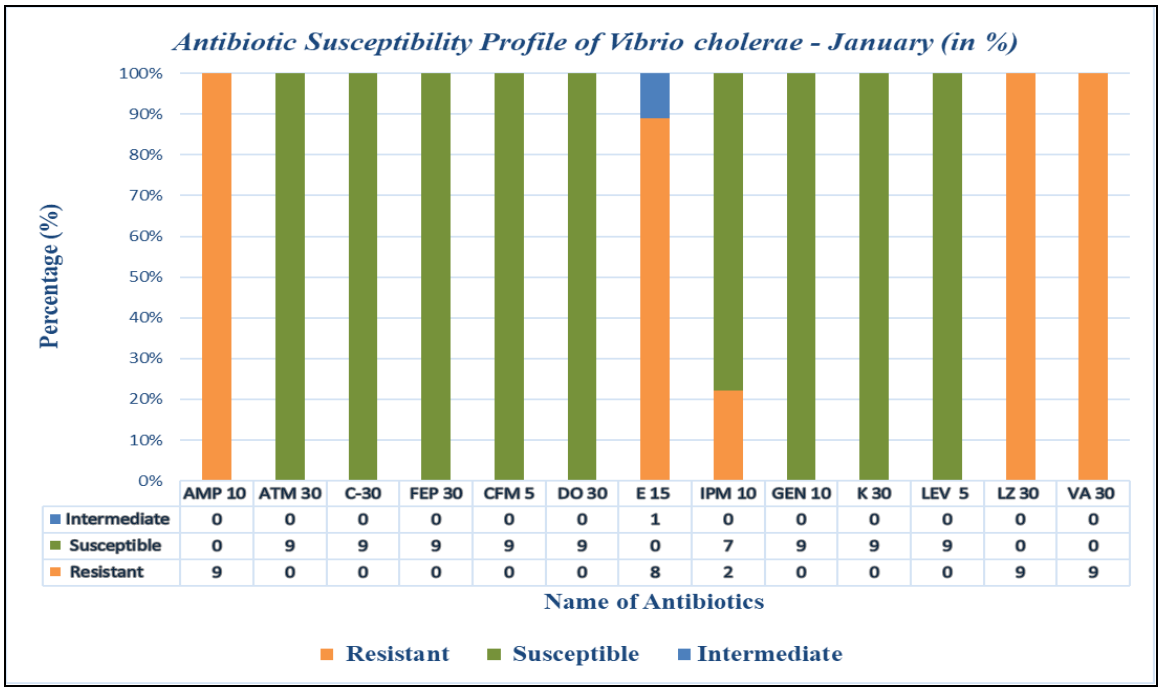


Figure 21: Antibiotic susceptibility profile of *Vibrio cholerae* isolates in January in terms of resistant, intermediate and susceptible reactions to various antibiotics.

As can be seen in the figure, the *Vibrio cholerae* isolates were resistant to some select antibiotics in January; though, it was mainly susceptible to the others. Ampicillin, linezolid and vancomycin were resisted by 100 percent of the isolates with 100 percent giving resilient reactions to the presented drugs. That is to be expected because linezolid and vancomycin are already resistant to Gram-negative bacilli, and the fact that ampicillin is generally resistant is the explanation of its decreasing use because of the ample and long-lasting usage. Also, there was a high resistance to erythromycin with a little percentage of respondents remaining sensitive. Contrastingly, the isolates were highly susceptible (100 percent) to aztreonam, chloramphenicol, cefepime, cefixime, doxycycline, imipenem, gentamicin, kanamycin, and levofloxacin thus showing the high anti-bacterial performance of these drugs. There were intermediate responses of erythromycin and most of the other antibiotics portrayed no intermediate response. The general susceptibility profile *Vibrio cholerae* isolates in January were also mostly sensitive to most of the common antibiotics that were used, with resistance primarily to a limited number of antibiotics

4.2.2b AST Results From Hatirjheel and Ramna (February)

Table 22 : The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel and Ramna Lake collected in February

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
VFHS2 (1)	0 R	30 S	30 S	30 S	25 S	13 R	14 I	25 S	16 S	19 S	20 S	0 R	0 R
VFHS2 (2)	0 R	32 S	30 S	32 S	29 S	20 S	14 I	22 I	20 S	19 S	28 S	0 R	0 R
VFHS2 (3)	0 R	37 S	30 S	28 S	30 S	22 S	13 R	24 S	20 S	20 S	28 S	0 R	0 R
VFHS2 (4)	0 R	30 S	31 S	30 S	32 S	20 S	12 R	24 S	18 S	21 S	26 S	0 R	0 R
VFHS2 (5)	0 R	35 S	32 S	33 S	33 S	21 S	12 R	25 S	19 S	20 S	20 S	0 R	0 R
VFHS2 (6)	0 R	35 S	30 S	37 S	31 S	19 S	12 R	25 S	19 S	20 S	24 S	0 R	0 R
VFHS2 (7)	0 R	37 S	32 S	27 S	0 R	22 S	12 R	15 R	19 S	20 S	28 S	0 R	0 R
VFHS2 (8)	0 R	37 S	30 S	36 S	31 S	22 S	14 I	20 I	18 S	18 S	24 S	0 R	0 R
VFHS2 (9)	0 R	36 S	33 S	30 S	30 S	23 S	17 I	22 I	18 S	19 S	20 S	0 R	0 R
VFHS2 (10)	0 R	37 S	32 S	35 S	29 S	21 S	12 R	25 S	20 S	18 S	28 S	0 R	0 R
VFHS2 (11)	0 R	37 S	30 S	37 S	32 S	22 S	12 R	20 I	18 S	19 S	24 S	0 R	0 R
VFHS2 (12)	0 R	32 S	30 S	36 S	32 S	20 S	14 I	24 S	20 S	18 S	28 S	0 R	0 R
VFRS2 (1)	0 R	37 S	29 S	34 S	31 S	23 S	12 R	20 I	20 S	21 S	28 S	12 R	0 R

The table demonstrates that 13 *Vibrio cholerae* isolates were identified in February, namely, 12 isolates of Hatirjheel (VFHS2: 1-12) and one isolate of Ramna lake VFRS2(1). There was a one-dimensional resistance of all isolates to ampicillin, a penicillin class antibiotic . Besides, VFHS2 (1) was resistant to doxycycline (DO), an antibiotic of the tetracycline group, and VFHS2 (7) was resistant to cefixime (CFM), a cephalosporin-group antibiotic and also resistant to Imipenem (IPM; carbapenems), , in addition to ampicillin resistance . This resistance to three

classes of antibiotic (Ampicillin,Cefixime and Imipenem) evidently makes VFHS2 (7) an MDR (Multidrug Resistant isolate). In antibiotic E (15), VFHS2(2), VfHS2(5), VFHS2(7), and VFHS2(11), there is resistance, whereas VFHS2(1), VfHS2(3), VfHS2(4), VfHS2(6), VfHS2(8), VfHS2(9), VfHS2(10), VfHS2(12), and VFRS2(1) are susceptible. According to these findings, 1 isolate was categorized as MDR because of the resistance profile against three different classes of antibiotics.

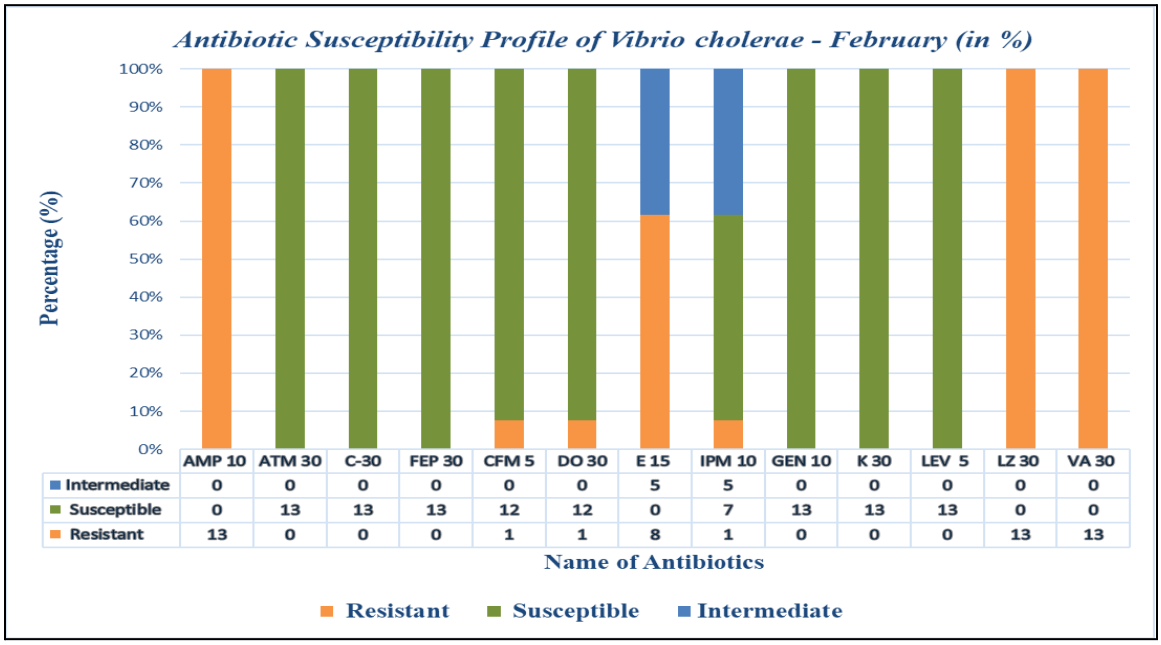


Figure 22: Antibiotic susceptibility profile of *Vibrio cholerae* isolates in February in terms of resistant, intermediate and susceptible reactions to various antibiotics.

As indicated in the figure, the *Vibrio cholerae* isolates in the month of February were highly resistant to a number of antibiotics, and that majority of the isolates was very susceptible to various antimicrobial agents employed. Complete resistance to ampicillin (penicillin-class antibiotic) was observed, and 100% resistance was observed in all the isolates. A small proportion of isolates were resistant to doxycycline (DO), a tetracycline-class antibiotic and cefixime (CFM), a cephalosporin-class antibiotic. In line with the expectations, 100 percent resistance was noted against linezolid and vancomycin which in itself are ineffective against Gram-negative bacteriophages. The resistance of erythromycin was great, and the majority of isolates were resistant, and only some of them demonstrated a middle degree of susceptibility,

which indicates a weakening effect of this medication. On the other hand, most of the isolates exhibited high susceptibility rates (almost 100%) to aztreonam, chloramphenicol, cefepime, imipenem, gentamicin, kanamycin and levofloxacin which demonstrates that these drugs can have an antibacterial effect. Only erythromycin and imipenem had an intermediate response, none of the other antibiotics showed an intermediate response. Altogether, the susceptibility profile shows that the *V. cholerae* isolates in February were also mostly susceptible to widely-used antibiotics with the resistance mostly restricted to antibiotics that cannot attack Gram-negative bacteria or were poorly utilized

4.2.2c AST Results From Hatirjheel (March)

Table 23: The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel collected in March

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
VMaHS1 (1)	0 R	35 S	31 S	32 S	22 S	21 S	20 I	20 I	18 S	20 S	23 S	0 R	0 R

The table shows that there was just one *Vibrio cholerae* sample obtained in March, which was one strain coming from Hatirjheel (VMaHS1(1)). This bacterium was resistant to ampicillin, which is an antibiotic that belongs to the penicillin category.

4.2.2d AST Results From Hatirjheel (April)

Table 24 : The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel collected in April.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ30	VA 30
VApHS1 (1)	0 R	38 S	20 S	29 S	28 S	27 S	20 I	22 I	19 S	18 S	22 S	0 R	0 R
VApHS1 (2)	0 R	37 S	30 S	20 I	27 S	23 S	10 R	24 S	18 S	18 S	23 S	0 R	0 R
VApHS1 (3)	0 R	39 S	29 S	32 S	28 S	24 S	8 R	24 S	18 S	20 S	23 S	0 R	0 R

VApHS1 (5)	0 R	16 I	0 R	20 I	0 R	23 S	20 I	15 R	0 R	0 R	21 S	12 R	15 I
VApHS1 (6)	0 R	38 S	26 S	33 S	29 S	29 S	11 R	22 I	20 S	23 S	28 S	0 R	0 R
VApHS1 (7)	0 R	34 S	0 R	27 S	32 S	30 S	0 R	25 S	21 S	22 S	23 S	0 R	0 R
VApHS1 (8)	0 R	39 S	0 R	35 S	30 S	23 S	9 R	30 S	19 S	21 S	22 S	0 R	0 R
VApHS1 (9)	0 R	40 S	15 R	32 S	32 S	0 R	0 R	20 I	22 S	20 S	22 S	0 R	0 R
VApHS1 (10)	0 R	34 S	0 R	35 S	28 S	15 R	8 R	30 S	20 S	23 S	21 S	0 R	0 R

In April, the number of *Vibrio cholerae* isolates studied according to the table were nine, in Hatirjheel, VApHS1 (1, 2, 3, 5, 6, 7, 8, 9 and 10). All isolates showed the same resistance to ampicillin, which is an antibiotic of the penicillin-class. Moreover, VApHS1 (7, 8, 9 and 10) demonstrated chloramphenicol resistance C-30 -a phenicol-class antibiotic, and ampicillin resistance. One of these, the ampicillin and chloramphenicol alone-resistant. Notably, VApHS1 (5) was the most resistant with the resistance to chloramphenicol (phenicols), cefixime (CFM; cephalosporins), imipenem (IPM; carbapenems), and Gentamicin and Kanamycin both of aminoglycoside class planes and to ampicillin resistance. In antibiotic E (15), there is an intermediate range between VaPHS1(1) and VaPHS1(5) with VApHS1(2), VApHS1(3), VApHS1(6), VApHS1(7), VApHS1(8), VApHS1(9), and VApHS1(10) resistant with no completely sensitive isolates.This resistance to various classes of antibiotic evidently makes VApHS1 (5) an MDR isolate. Moreover, both VApHS1 (9 and 10) were resistant to doxycycline (DO) an antibiotic of the tetracycline group, along with ampicillin and chloramphenicol and thus categorized as multidrug-resistant (MDR).

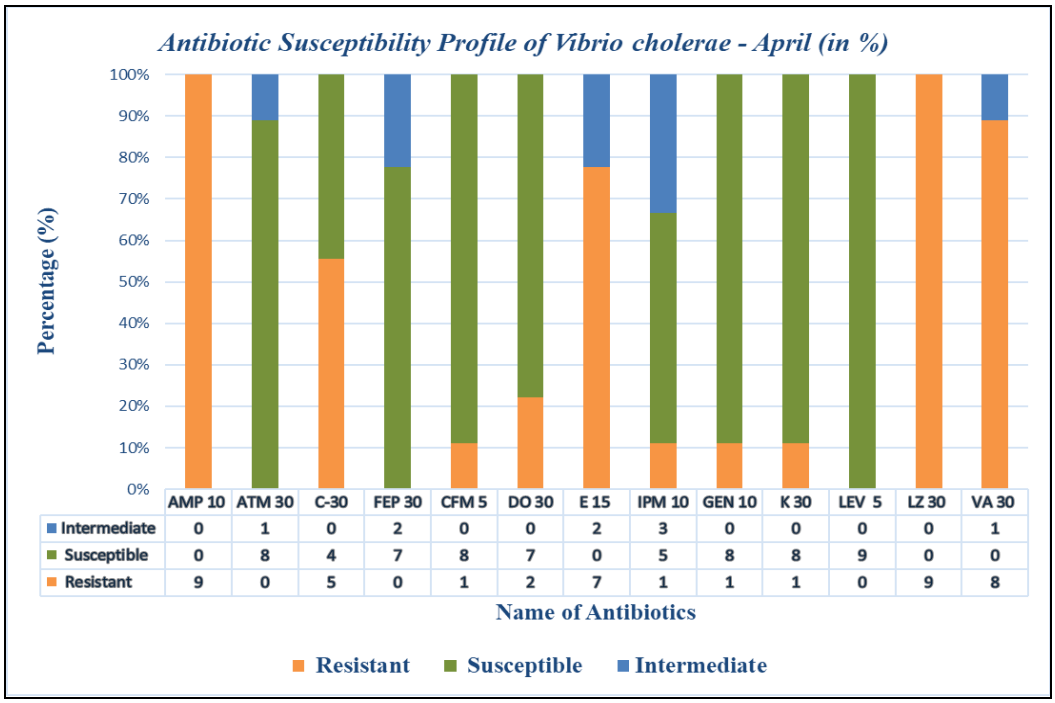


Figure 23: Antibiotic susceptibility profile of *Vibrio cholerae* isolates in April in terms of resistant, intermediate and susceptible reactions to various antibiotics.

According to the figure, the *Vibrio cholerae* isolates were resistant to few antibiotics in the month of April and were very susceptible to most of the antimicrobial agents tested. The ampicillin (AMP) was totally resistant and all the nine isolates (100 percent) were resistant. The level of resistance exhibited by linezolid (LZ) and vancomycin (VA) were also very high, which concurs with their inactivity to Gram-negative bacteria, including *V. cholerae*. Chloramphenicol (C-30) had moderate resistance with five resistant and four susceptible isolates whereas doxycycline (DO) exhibited low resistance with only two resistant isolates and the rest were susceptible.Conversely, the majority of isolates were sensitive to aztreonam (ATM), cefepime (FEP), cefixime (CFM), imipenem (IPM), gentamicin (GEN), kanamycin (K), and levofloxacin (LEV) and so were very susceptible to the agents in the month of April. Intermediate levels of response were only detected in a small percentage of isolates of ATM, FEP, erythromycin (E15), and IPM with most antibiotics showing no intermediate patterns. In general, the April susceptibility model depicts that the *V. cholerae* isolates are largely susceptible to most of the clinically relevant antibiotics, with susceptibility largely restricted to ampicillin and antibiotics which are inherently ineffective against Gram-negative bacteria.

4.2.2e AST Results From Hatirjheel, Curzon Hall Ramna (May)

Table 25: The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel, Ramna Lake and Curzon Hall collected in May

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
VMHS1 (1)	0 R	41 S	27 S	38 S	15 R	10 R	23 S	20 I	23 S	25 S	28 S	0 R	0 R
VMRS1 (1)	0 R	30 S	35 S	26 S	30 S	27 S	20 I	20 I	19 S	21 S	26 S	0 R	0 R
VMRS1 (2)	0 R	28 S	35 S	29 S	28 S	30 S	23 S	18 R	18 S	22 S	33 S	0 R	0 R
VMRS1 (3)	0 R	30 S	32 S	35 S	29 S	30 S	20 I	20 I	20 S	21 S	29 S	0 R	0 R
VMRS1 (4)	0 R	30 S	31 S	30 S	30 S	27 S	20 I	21 I	20 S	20 S	30 S	0 R	0 R
VMRS1 (5)	0 R	28 S	32 S	29 S	34 S	27 S	23 S	19 R	19 S	22 S	26 S	0 R	0 R
VMRS1 (6)	17 S	32 S	31 S	34 S	34 S	30 S	22 I	21 I	20 S	21 S	18 S	20 R	0 R
VMHS2 (1)	0 R	41 S	35 S	37 S	32 S	26 S	23 S	25 S	15 S	20 S	28 S	0 R	0 R
VMHS2 (2)	0 R	30 S	31 S	29 S	0 R	22 S	12 R	24 S	18 S	20 S	20 S	0 R	0 R
VMHS2 (3)	0 R	29 S	30 S	29 S	29 S	22 S	15 I	24 S	16 S	19 S	33 S	12 R	0 R
VMHS2 (4)	0 R	37 S	30 S	38 S	32 S	22 S	13 R	25 S	18 S	21 S	28 S	0 R	0 R
VMHS2 (5)	0 R	30 S	31 S	16 R	0 R	19 S	13 R	16 S	20 S	20 S	20 S	0 R	0 R
VMRS2 (1)	0 R	40 S	27 S	38 S	23 S	25 S	11 R	20 I	22 S	15 I	35 S	0 R	0 R
VMRS2 (2)	0 R	37 S	27 S	27 S	17 R	22 S	15 I	20 I	21 S	21 S	30 S	0 R	0 R
VMRS2 (3)	0 R	37 S	30 S	40 S	35 S	24 S	13 R	24 S	16 S	19 S	33 S	0 R	0 R
VMRS2 (4)	0 R	39 S	27 S	37 S	30 S	22 S	17 I	20 I	18 S	18 S	30 S	0 R	0 R
VMRS2 (5)	0 R	37 S	28 S	38 S	33 S	23 S	13 R	24 S	22 S	21 S	34 S	0 R	0 R

VMRS2 (6)	0 R	40 S	28 S	29 S	29 S	24 S	15 I	21 I	21 S	21 S	33 S	0 R	0 R
VMCS2 (1)	0 R	23 S	35 S	29 S	15 R	27 S	0 R	31 S	20 S	20 S	24 S	13 R	0 R
VMCS2 (2)	0 R	18 I	31 S	23 S	12 R	22 S	0 R	28 S	16 S	17 I	23 S	12 R	0R

As can be seen in the table, 20 *Vibrio cholerae* isolates obtained in May in Hatirjheel, Ramna Lake and Curzon Hall were analysed and these include VMHS1 (1); VMRS1 (1-6); VMHS2 (1-5); VMRS2 (1-6); and VMCS2 (1-2). Of this number, 19 were ampicillin-resistant (penicillin group), but VMRS1 (6) was excluded from antibiotic resistant profiling. VMHS1(1) (Resistant to ampicillin, doxycycline(tetracycline) and cefixime(cephalosporins) at Hatirjheel was regarded as MDR(Multi drug resistance). VMRS1 (2), and VMRS1 (5) in Ramna Lake resisted ampicillin and imipenem (carbapenems). In E (15), the pattern of susceptibility is VMHS1(1), VMRS1(2), VMRS1(5) and VMHS2(1): the pattern of intermediate response is VMRS1(1), VMRS1(3), VMRS1(4), VMRS1(6), VMHS2(3), VMRS2(2), VMRS2(4), VMRS2(6); and the VMHS2(2), VMHS2(4), VMHS2(5), VMRS2(1), VMRS2(3), VMRS2(5). Moreover, VMHS2 (2, 4), VMRS2 (2), and VMCS2 (1, 2) were found to be resistant to cefixime and VMHS2 (5) was found to be resistant to both cefixime and cefepime (cephalosporins) .

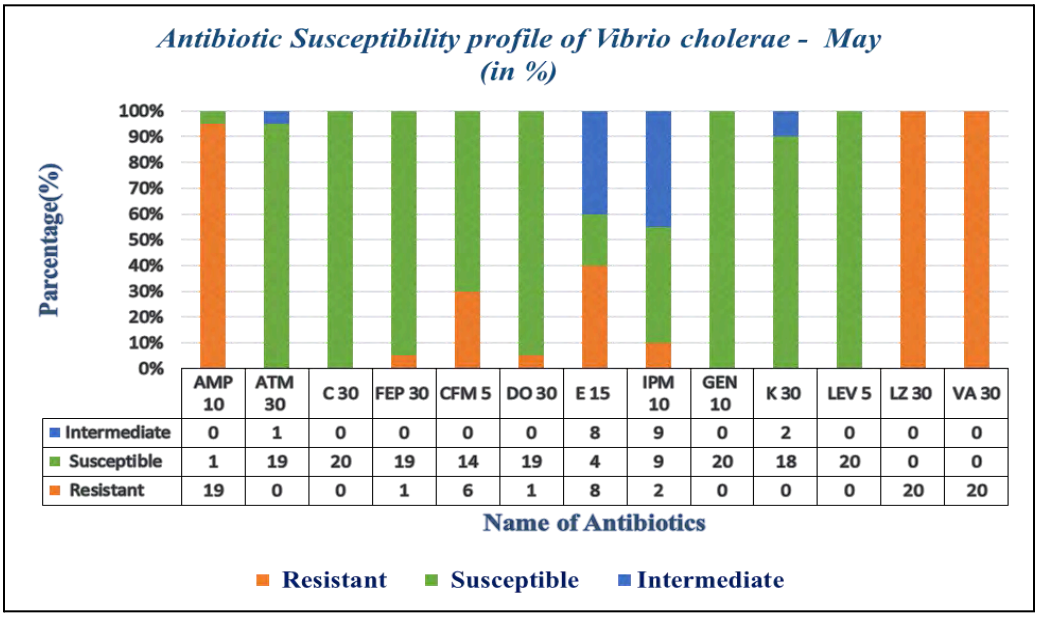


Figure 24: Antibiotic susceptibility profile of *Vibrio cholerae* isolates in May in terms of resistant, intermediate and susceptible reactions to various antibiotics.

The figure depicts the profile of the isolates of *Vibrio cholerae i*n May in terms of resistance, susceptibility, and intermediate to all the tested antibiotics. Ampicillin (AMP) demonstrated exceedingly high resistance with 95% of the isolates resistant and only 5% susceptible whereas linezolid (LZ) and vancomycin (VA) had 100% resistance in line with their inherent low efficacy in the Gram-negative bacteria like V. cholerae. Erythromycin (E15) gave the following mixed results, 40 percent resistant, 20 percent susceptible , and 40 percent intermediate Imipenem (IPM) had a resistance of 10 percent , susceptibility of 45percent , and a significantly high intermediate response level of 45 percent. With cefixime (CFM), 30% resistance and 70% susceptibility were seen and with cefepime (FEP) 5 percent resistance and 95 percent susceptibility . All high susceptibility observed was on Aztreonam (ATM), chloramphenicol (C-30), doxycycline (DO), gentamicin (GEN), kanamycin (K), and levofloxacin (LEV), and there was minimal or no resistance to them. In general, the profile of May shows a high susceptibility to most antibiotics had undergone testing, and significant resistance to ampicillin and intrinsic resistance to linezolid and vancomycin, and an intermediate response was particularly prominent with regard to the erythromycin and imipenem.

4.2.2f AST Results From Hatirjheel and Ramna (June)

Table 26: The pattern of antibiotic susceptibility of *Vibrio cholerae* isolates at Hatirjheel and Ramna Lake collected in June

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
VJnHS1 (1)	0 R	0 R	30 S	13 R	30 S	20 S	0 R	30 S	16 S	14 I	24 S	0 R	0 R
VJnHS1 (2)	0 R	0 R	32 S	15 R	30 S	22 S	0 R	29 S	18 S	20 S	29 S	0 R	0 R
VJnHS2 (1)	0 R	30 S	29 S	32 S	33 S	20 S	18 I	28 S	18 S	20 S	28 S	0 R	0 R
VJnHS2 (2)	0 R	29 S	31 S	32 S	32 S	22 S	17 I	16 R	20 S	22 S	29 S	0 R	0 R
VJnHS2 (3)	10 R	35 S	30 S	32 S	30 S	22 S	18 I	30 S	17 S	20 S	28 S	0 R	0 R
VJnRS2 (1)	0 R	15 R	25 S	17 R	0 R	19 S	0 R	27 S	18 S	19 S	24 S	0 R	0 R

As the table indicates, six *Vibrio cholerae* isolates were analysed in June, five of which were discovered in Hatirjheel (VJnHS1 (1, 2) and VJnHS2 (1, 2, 3)) and one in the Ramna Lake (VJnRS2 (1)). All the isolates were resistant to ampicillin which is a penicillin-class antibiotic. Besides this, VJnHS1 (1) and VJnHS1 (2) demonstrated aztreonam (ATM) resistance, which is a monobactam, and cefepime (FEP), which is a cephalosporin-like resistance as well as ampicillin resistance and were classified as multidrug-resistant (MDR). Also, VJnHS1(1), VJnHS1(2) and VJnRS2(1) were resistant to E(15). In the same manner, VJnRS2 (1) was resistant to aztreonam, cefepime, and cefixime (CFM), which are cephalosporins, and also belongs to the category of MDR.

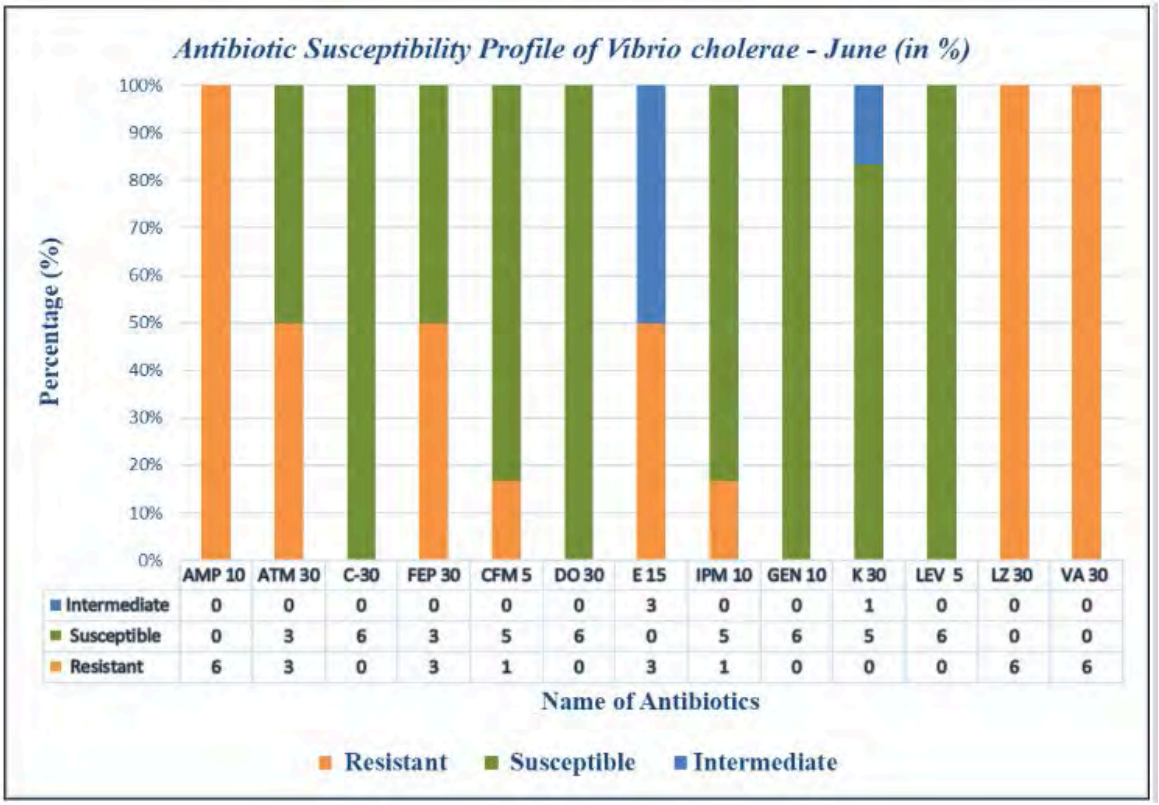


Figure 25: Antibiotic susceptibility profile of *Vibrio cholerae* isolates in June in terms of resistant, intermediate and susceptible reactions to various antibiotics.

As demonstrated in the figure, *Vibrio cholerae* isolates were resistant to few antibiotics in June but were otherwise highly susceptible to most of the tested agents. Ampicillin (AMP), linezolid (LZ), and vancomycin (VA) completely resisted, all the isolates gave a resistant reaction to the

drugs; linezolid and vancomycin are inherently ineffective against Gram-negative bacteria like *V. cholerae.* Aztreonam (ATM) and cefepime (FEP) were found to have moderate resistance with 50 percent resistance each and cefixime (CFM) low resistance (16.7) and with most of the isolates showing susceptibility. On the other hand, chloramphenicol (C-30), doxycycline (DO), imipenem (IPM), gentamicin (GEN), kanamycin (K) and levofloxacin (LEV), demonstrated high susceptibility (approximately 83-100%). Intermediate responses were minimal and only were primarily monitored to erythromycin (E15) and imipenem (IPM), and the majority of other antibiotics did not exhibit any intermediate pattern.

4.2.3 AST Results of *Escherichia coli*

4.2.3a AST Results From Hatirjheel and Curzon Hall (January)

Table 27: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel and Curzon Hall collected in January.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K30	LEV5	LZ 30	VA 30
EJaHS1(1)	0 R	33 S	29.5 S	34 S	22.5 S	20 S	0 R	30 S	23.5 S	0 R	35 S	0 R	0 R
EJaHS1(2)	0 R	32 S	26 S	35 S	23 S	20 S	0 R	30 S	23 S	0 R	36 S	0 R	0 R
EJaHS1(3)	0 R	35 S	23.5 S	33.5 S	23.5 S	20 S	0 R	33.5 S	22.5 S	0 R	34.5 S	0 R	0 R
EJaHS1(4)	0 R	34 S	25 S	34 S	24 S	20 S	0 R	33 S	15 S	0 R	35 S	0 R	0 R
EJaHS1(5)	0 R	32.5 S	22 S	35.7 S	25 S	10 R	0 R	32 S	22 S	0 R	20 S	0 R	0 R
EJaHS1(6)	0 R	36.5 S	10 R	36 S	25 S	10 R	0 R	33 S	23 S	0 R	13 R	0 R	0 R
EJaHS1(7)	0 R	33.5 S	26.75 S	35 S	22.5 S	10 R	0 R	32.5 S	26 S	0 R	22 S	0 R	0 R
EJaHS1(8)	0 R	32 S	29 S	34 S	25 S	10 R	0 R	32 S	27 S	20 S	23 S	0 R	0 R
EJaHS1(9)	0 R	36.7 S	25.75 S	36 S	25 S	20 S	0 R	33 S	20 S	0 R	20 S	0 R	0 R
EJaHS1(10)	0 R	35 S	10 R	35 S	25 S	10 R	0 R	33 S	23 S	0 R	15 R	0 R	0 R

EJaHS1(11)	0 R	34.7 S	29.5 S	35 S	24.5 S	20 S	0 R	33.5 S	22 S	0 R	23 S	0 R	0 R
EJaCS1(1)	0 R	30 S	32.5 S	33.5 S	0 R	20 S	10 R	27 S	20 S	24 S	25 S	0 R	0 R
EJaCS1(2)	0 R	31 S	31 S	34 S	0 R	22 S	0 R	29 S	20 S	22 S	26 S	0 R	0 R
EJaCS1(3)	0 R	33 S	30 S	31 S	0 R	25 S	0 R	30 S	23 S	24 S	24 S	0 R	0 R
EJaCS1(4)	0 R	34.5 S	29.5 S	30 S	0 R	22.5 S	0 R	31.5 S	20 S	21 S	23.5 S	0 R	0 R
EJaCS1(5)	0 R	35 S	31.5 S	33 S	0 R	21 S	0 R	30 S	21 S	20 S	30 S	0 R	0 R
EJaCS1(6)	0 R	31.5 S	32 S	32 S	0 R	22 S	0 R	32.5 S	23 S	22 S	22 S	0 R	0 R
EJaCS1(7)	0 R	33 S	30 S	34 S	0 R	24 S	10 R	31 S	23 S	24 S	26.5 S	0 R	0 R
EJaCS1(8)	0 R	30 S	30 S	34.5 S	0 R	23 S	0 R	32 S	21.5 S	23 S	20 S	0 R	0 R
EJaCS1(9)	0 R	30 S	30 S	35 S	0 R	22 S	10 R	30 S	20 S	21 S	24 S	0 R	0 R
EJaCS1(10)	0 R	35 S	33 S	36 S	0 R	27 S	0 R	37 S	25 S	25 S	30 S	0 R	0 R
EJaCS1(11)	0 R	31 S	33 S	33 S	0 R	23 S	0 R	30 S	20 S	20 S	24 S	0 R	0 R
EJaCS1(12)	0 R	31.5 S	33.5 S	30 S	0 R	27.5 S	0 R	31.5 S	20 S	22 S	23 S	0 R	0 R
EJaHS2(1)	19 S	32 S	28 S	34 S	25 S	10 R	10 R	34 S	25 S	20 S	28 S	0 R	0 R
EJaHS2(2)	0 R	30 S	24 S	16 I	0 R	20 S	0 R	30 S	25 S	21 S	28 S	0 R	0 R
EJaHS2(3)	0 R	0 R	26 S	0 R	0 R	13 I	0 R	24 S	25 S	20 S	0 R	0 R	0 R
EJaHS2(4)	0 R	30 S	22 S	32 S	24 S	10 R	0 R	30 S	24 S	16 I	26 S	0 R	0 R
EJaHS2(6)	0 R	22 S	26 S	25 S	14 R	11 I	0 R	30 S	27 S	18 S	30 S	0 R	0 R
EJaHS2(7)	10 R	30 S	28 S	32 S	24 S	20 S	0 R	29 S	26 S	18 S	30 S	0 R	0 R
EJaHS2(9)	0 R	15 R	28 S	31 S	25 S	19 S	0 R	25 S	20 S	15 I	25 S	0 R	0 R
EJaHS2(10)	0 R	29 S	30 S	32 S	24 S	22 S	0 R	28 S	24 S	27 S	28 S	0 R	0 R

EJaHS2(11)	0 R	28 S	30 S	30 S	28 S	9 R	0 R	25 S	22 S	16 I	26 S	0 R	0 R
EJaHS2(12)	0 R	30 S	26 S	30 S	22 S	14 S	10 R	29 S	24 S	19 S	32 S	0 R	0 R

A total of 33 *Escherichia coli* were picked in January and were tested on antibiotic susceptibility tests of which 21 were Hatirjheel and 12 were Curzon Hall. Out of the Hatirjheel isolates, from sample-1, 4 isolates (EJaHS1(5,6,7,10)) and from sample-2, 1 isolate (EJaHS2(3)) were found to be multiple drug resistant, especially for β-lactam antibiotics, amphenicol, tetracyclines, fluoroquinolones, and aminoglycosides and thus were classified as MDR. In the same way, out of the isolates of the Curzon Hall, no isolates exhibited MDR. In general, among the 33 isolates, only 5 bacteria from both of the sides expressed MDR in January.

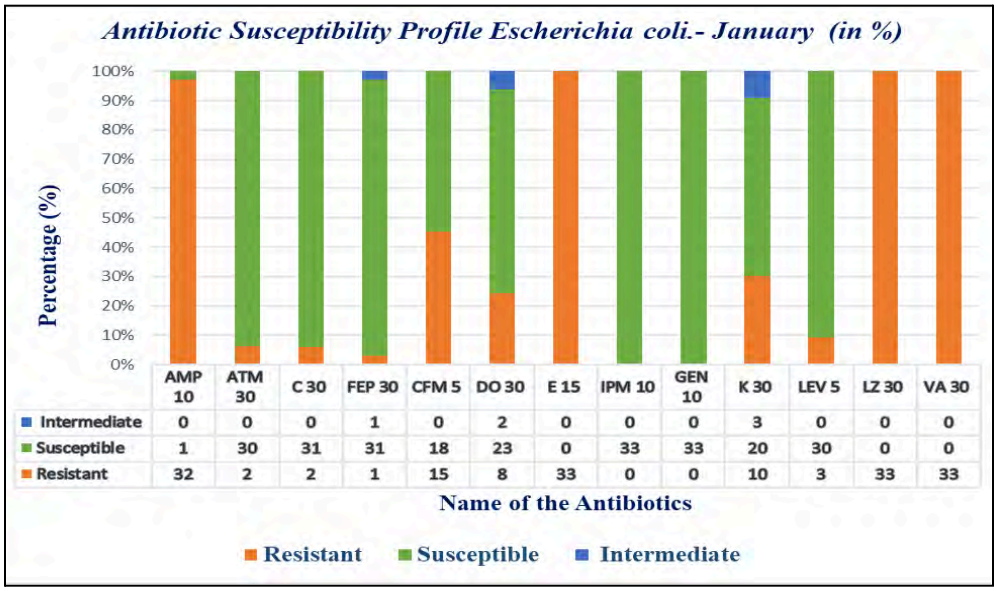


Figure 26: Antibiotic susceptibility profile of *E.coli* isolates in January in terms of resistant, intermediate and susceptible reactions to various antibiotics.

In January, the *E. coli* isolates were highly resistant to a limited number of antibiotics with almost all the isolates being resistant to ampicillin (97% not susceptible), and being resistant to erythromycin, linezolid, and vancomycin since they are intrinsic resistant antibiotics especially to the gram negative bacteria. The majority of isolates were highly sensitive to aztreonam, chloramphenicol, cefepime, imipenem, gentamicin, and levofloxacin, and moderately sensitive to cefixime, doxycycline and kanamycin.

4.2.3b AST Results From Hatirjheel, Curzon Hall and Ramna Lake (February)

Table 28: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel, Curzon Hall and Ramna Lake collected in February

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
EFHS1(1)	0 R	33 S	30 S	32 S	25 S	20 S	0 R	30 S	21 S	19 S	33 S	0 R	0 R
EFHS1(2)	20 S	32 S	33 S	33 S	25 S	21 S	0 R	30 S	20 S	18 S	37 S	0 R	0 R
EFHS1(3)	0 R	10 R	33 S	33 S	25 S	21 S	0 R	25 S	19 S	22 S	30 S	0 R	0 R
EFHS1(4)	0 R	32 S	18 S	24 S	20 S	15 S	0 R	27 S	23 S	27 S	26.5 S	0 R	0 R
EFHS1(5)	0 R	31 S	14 I	18 S	22 S	17 S	0 R	28 S	26 S	20 S	25 S	0 R	0 R
EFHS1(6)	20 S	32 S	14 I	14 R	0 R	10 R	0 R	28 S	22 S	18 S	28 S	0 R	0 R
EFHS1(7)	0 R	30 S	30 S	30 S	27 S	10 R	0 R	30 S	20 S	22 S	30 S	0 R	0 R
EFHS1(8)	17 S	0 R	32 S	32 S	25 S	10 R	0 R	27 S	20 S	20 S	26 S	0 R	0 R
EFCS1(1)	21 S	31 S	27 S	32 S	23 S	14 S	0 R	29 S	18.5 S	18 S	23.5 S	0 R	0 R
EFCS1(2)	0 R	35 S	30 S	35 S	26 S	17 S	0 R	31 S	17 S	20 S	32 S	0 R	0 R
EFCS1(3)	0 R	26 S	0 R	32 S	10 R	11 I	0 R	26 S	18 S	24 S	0 R	0 R	0 R
EFCS1(4)	0 R	32 S	26 S	32 S	21 S	12 I	0 R	28 S	18 S	21 S	0 R	0 R	0 R
EFRS1(1)	0 R	41 S	30 S	35 S	27.5 S	15 S	0 R	31 S	20 S	20 S	30 S	0 R	0 R
EFRS1(2)	0 R	33 S	31 S	33 S	25 S	18 S	0 R	33 S	23 S	24 S	28.5 S	0 R	0 R
EFRS1(3)	0 R	31 S	29 S	32.5 S	24 S	13 I	0 R	29 S	22 S	22.5 S	23.5 S	0 R	0 R
EFRS1(4)	0 R	28 S	27 S	34 S	23 S	13 I	0 R	26 S	20 S	20 S	31 S	0 R	0 R
EFRS1(5)	0 R	35 S	31.5 S	35 S	25 S	17.5 S	0 R	27.5 S	27 S	21.5 S	32.5 S	0 R	0 R

EFRS1(6)	0 R	31 S	33 S	36.5 S	22 S	20 S	0 R	26 S	25 S	18.5 S	35 S	0 R	0 R
EFRS1(7)	0 R	32 S	29 S	31.3 S	26 S	14 S	0 R	30 S	23 S	21.5 S	33 S	0 R	0 R
EFRS1(8)	0 R	28.5 S	34.5 S	33 S	21.5 S	17 S	0 R	31.5 S	21.5 S	24 S	27.5 S	0 R	0 R
EFRS1(9)	0 R	32.5 S	31.5 S	34.5 S	23.5 S	16.5 S	0 R	32.5 S	25 S	26.5 S	31.5 S	0 R	0 R
EFHS2(1)	19 S	30 S	20 S	32 S	21 S	20 S	10 R	27 S	19 S	20 S	35 S	0 R	0 R
EFHS2(2)	20 S	31 S	24 S	31 S	22 S	20 S	11 R	30 S	20 S	19 S	35 S	0 R	0 R
EFHS2(3)	0 R	0 R	27 S	11 R	0 R	22 S	0 R	31 S	19 S	12 R	31 S	0 R	0 R
EFHS2(5)	15 I	34 S	0 R	31 S	26 S	0 R	0 R	32 S	20 S	17 S	11 R	0 R	0 R
EFHS2(6)	0 R	30 S	22 S	32 S	22 S	12 I	9 R	29 S	20 S	20 S	8 R	0 R	0 R
EFHS2(7)	22 S	32 S	23 S	34 S	24 S	22 S	10 R	31 S	21 S	21 S	33 S	0 R	0 R
EFHS2(8)	0 R	23 S	24 S	29 S	21 S	11 I	0 R	23 S	19 S	21 S	27 S	0 R	0 R
EFHS2(10)	0 R	25 S	24 S	31 S	22 S	11 I	0 R	23 S	19 S	20 S	30 S	0 R	0 R
EFHS2(11)	19 S	31 S	26 S	31 S	23 S	20 S	0 R	27 S	19 S	19 S	33 S	0 R	0 R
EFHS2(13)	0 R	12 R	13 I	14 R	0 R	18 S	0 R	29 S	19 S	20 S	22 S	0 R	0 R
EFHS2(15)	19 S	30 S	24 S	32 S	23 S	14 S	10 R	30 S	18 S	20 S	35 S	0 R	0 R
EFHS2(16)	0 R	20 R	23 S	19 S	10 R	19 S	0 R	22 S	19 S	18 S	25 S	0 R	0 R
EFRS2(1)	0 R	33 S	29 S	34 S	25 S	12 I	0 R	30 S	18 S	19 S	10 R	0 R	0 R
EFRS2(2)	0 R	34 S	28 S	35 S	25 S	18 S	0 R	31 S	20 S	20 S	29 S	0 R	0 R
EFRS2(3)	0 R	34 S	30 S	36 S	25 S	11 I	0 R	30 S	18 S	19 S	0 R	0 R	0 R
EFRS2(4)	19 S	30 S	26 S	30 S	23 S	13 I	0 R	27.5 S	18 S	17 I	0 R	0 R	0 R
EFRS2(5)	0 R	32 S	26.5 S	32.5 S	25 S	12 I	0 R	29 S	20 S	18 S	27 S	0 R	0 R

EFRS2(6)	0 R	28 S	29 S	34 S	26 S	12 I	0 R	28 S	20 S	21 S	27 S	0 R	0 R
EFRS2(7)	0 R	31 S	29.5 S	31 S	25 S	13 I	0 R	30 S	0 R	0 R	23 S	0 R	0 R
EFRS2(8)	0 R	13 R	27.5 S	14 R	0 R	21 S	0 R	28 S	22 S	23.5 S	30 S	0 R	0 R
EFRS2(9)	0 R	27.5 S	25 S	33 S	20 S	11 I	0 R	19 S	19 S	23 S	32 S	0 R	0 R
EFRS2(10)	0 R	30 S	10 R	30 S	20 S	10 R	0 R	26 S	19 S	19 S	0 R	0 R	0 R

A total of 43 *Escherichia coli* were picked in February and were tested on antibiotic susceptibility tests of which 20 were from Hatirjheel, 4 from Curzon Hall and 19 from Ramna Lake. Out of the Hatirjheel isolates, from sample-1 where 8 isolates were examined none of them were MDR as resistancy showed mainly to different β-lactam for different isolates while from sample-2, 3 isolates (EFHS2(3,13,16)) were found to be multiple drug resistant, especially for β-lactam antibiotics. In consideration of Ramna Lake, 2 bacteria (EFRS2(8,10)) were found as MDR out of 10 isolates from sample-2. In the same way, out of the 4 isolates of the Curzon Hall, 1 isolate (EFCS1(3)) was exhibiting MDR. In general, from the 43 isolates there were only 6 bacteria expressing MDR in February.

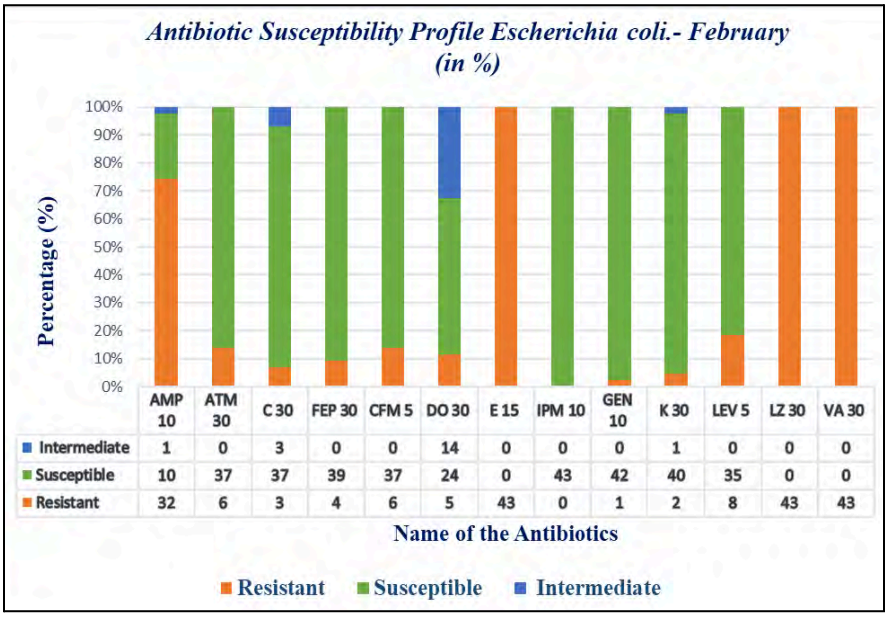


Figure 27: Antibiotic susceptibility profile of *E.coli* isolates in February in terms of resistant, intermediate and susceptible reactions to various antibiotics

In February, the *E. coli* isolates were highly resistant to ampicillin (74.4%) and these three (E, LZ and VA) were excluded from the resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria. The majority of isolates were highly sensitive to aztreonam, chloramphenicol, cefepime, cefixime (13.95% exhibits resistancy) imipenem, gentamicin, kenamycin, and levofloxacin (18.60% gave resistancy), and moderately sensitive (55.81% isolates) to doxycycline while (33.56%) isolates exhibited intermediate suggesting that there is chance of resistance. In general, from the 43 isolates there were only 6 bacteria expressing MDR in February and some of the bacteria is XDR while some were exceptional.

4.2.3c AST Results From Hatirjheel, Curzon Hall and Ramna Lake (March)

Table 29: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel, Curzon Hall and Ramna Lake collected in March

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
EMaHS1(1)	0 R	30 S	25 S	31 S	22 S	11 I	8 R	24 S	20 S	22 S	29 S	0 R	0 R
EMaHS1(2)	0 R	27 S	25 S	30 S	25 S	18 S	8 R	25 S	20 S	21 S	30 S	0 R	0 R
EMaHS1(3)	0 R	29 S	25 S	24 S	10 R	0 R	0 R	28 S	20 S	22 S	0 R	0 R	0 R
EMaHS1(4)	0 R	36 S	28 S	31 S	24 S	10 R	0 R	25 S	19 S	20 S	25 S	0 R	0 R
EMaHS1(5)	0 R	30 S	26 S	33 S	22 S	17 S	9 R	28 S	21 S	20 S	30 S	0 R	0 R
EMaHS1(6)	0 R	15 R	28 S	15 I	0 R	17 S	10 R	26 S	20 S	20 S	25 S	0 R	0 R
EMaHS1(7)	0 R	26 S	25 S	30 S	22 S	11 I	9 R	24 S	21 S	21 S	24 S	0 R	0 R
EMaHS1(8)	0 R	27 S	26 S	30 S	22 S	15 S	10 R	28 S	20 S	22 S	28 S	0 R	0 R
EMaHS1(9)	0 R	30 S	25 S	30 S	24 S	11 I	8 R	25 S	20 S	21 S	29 S	0 R	0 R
EMaHS1(10)	0 R	15 R	25 S	15 I	11 R	10 R	10 R	24 S	22 S	21 S	30 S	0 R	0 R

EMaHS1(11)	0 R	29 S	28 S	29 S	0 R	18 S	0 R	28 S	20 S	22 S	29 S	0 R	0 R
EMaHS1(12)	0 R	30 S	27 S	30 S	25 S	17 S	8 R	27 S	21 S	20 S	25 S	0 R	0 R
EMaRS1(1)	0 R	32 S	28 S	33 S	25 S	12 I	8 R	29 S	20 S	0 R	11 R	0 R	0 R
EMaHS2(1)	20 S	34 S	25 S	35 S	28 S	20 S	9 R	30 S	20 S	21 S	33 S	0 R	0 R
EMaHS2(2)	20 S	30 S	27 S	30 S	24 S	19 S	10 R	27 S	22 S	22 S	30 S	0 R	0 R
EMaHS2(3)	0 R	15 R	29 S	17 I	0 R	23 S	0 R	30 S	20 S	21 S	28 S	0 R	0 R
EMaHS2(4)	0 R	28 S	25 S	33 S	0 R	14 S	0 R	26 S	18 S	19 S	25 S	0 R	0 R
EMaHS2(5)	22 S	34 S	26 S	33 S	23 S	12 I	0 R	31 S	21 S	19 S	26 S	0 R	0 R
EMaHS2(6)	20 S	35 S	25 S	31 S	25 S	20 S	0 R	32 S	22 S	21 S	34 S	0 R	0 R
EMaHS2(7)	0 R	33 S	25 S	32 S	25 S	11 I	0 R	30 S	20 S	16 I	30 S	0 R	0 R
EMaHS2(8)	10 R	30 S	27 S	32 S	25 S	14 S	10 R	26 S	17 S	19 S	26 S	0 R	0 R
EMaHS2(9)	0 R	20 I	26 S	22 S	12 R	18 S	0 R	30 S	19 S	22 S	28 S	0 R	0 R
EMaHS2(10)	0 R	31 S	0 R	31 S	26 S	10 R	0 R	0 R	17 S	17 I	10 R	0 R	0 R
EMaHS2(11)	20 S	29 S	30 S	32 S	0 R	20 S	0 R	24 S	0 R	21 S	25 S	0 R	0 R
EMaHS2(12)	0 R	31 S	29 S	30 S	24 S	23 S	0 R	30 S	19 S	21 S	28 S	0 R	0 R
EMaCS2(1)	0 R	36 S	29 S	33 S	44 S	18 S	0 R	31 S	20 S	21 S	32 S	0 R	0 R
EMaCS2(2)	20 S	37 S	29 S	36 S	33 S	17 S	9 R	33 S	20 S	22 S	35 S	0 R	0 R
EMaCS2(3)	21 S	36 S	28 S	39 S	32 S	18 S	0 R	33 S	19 S	20 S	33 S	0 R	0 R
EMaCS2(4)	0 R	39 S	30 S	35 S	34 S	17 S	10 R	31 S	21 S	22 S	32 S	0 R	0 R
EMaCS2(5)	0 R	37 S	31 S	36 S	32 S	17 S	0 R	30 S	20 S	20 S	32 S	0 R	0 R
EMaCS2(6)	23 S	36 S	30 S	35 S	32 S	18 S	10 R	31 S	20 S	21 S	31 S	0 R	0 R

EMaCS2(7)	23 S	39 S	29 S	40 S	34 S	18 S	10 R	32 S	21 S	19 S	32 S	0 R	0 R

A total of 32 *Escherichia coli* were analyzed in March on antibiotic susceptibility tests of which 24 were from Hatirjheel, 7 from Curzon Hall and 1 from Ramna Lake. Out of the Hatirjheel isolates, from sample-1, 3 isolates (EMaHS1(3,6,10)) were identified as MDR mainly to β-lactam and tetracyclin. On the other hand, EMaHS2(3,6,10) isolates indicated MDR, others were excluded. From EMaRS1, no isolate is found as MDR and same for the isolates of the Curzon Hall. In conclusion, March indicates 6 MDR in total.

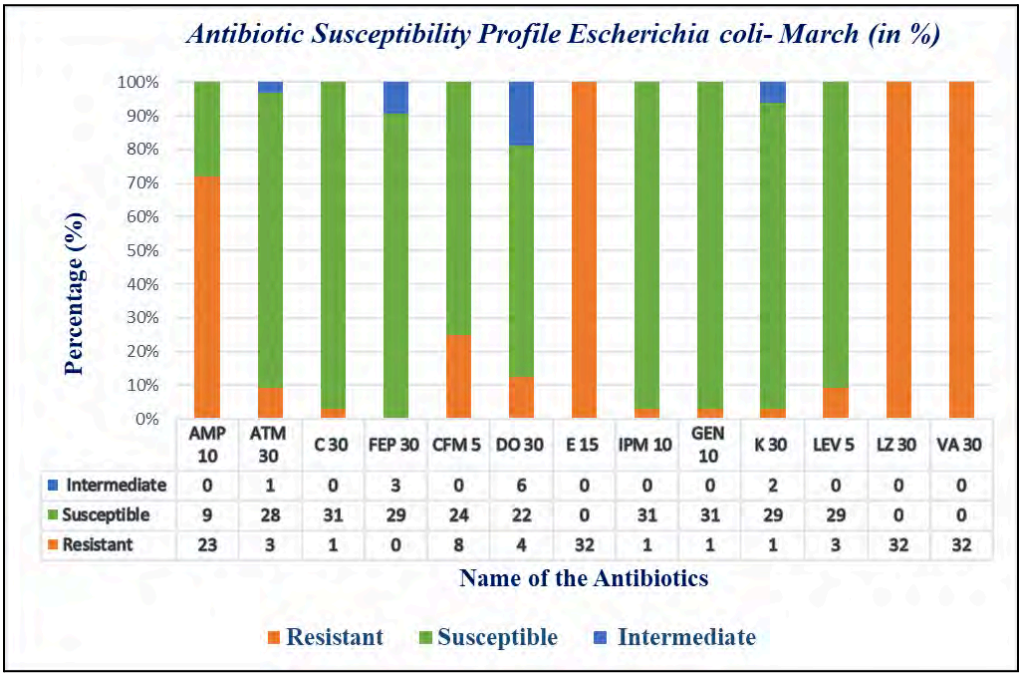


Figure 28: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel, Curzon Hall and Ramna Lake collected in April

In March, the *E. coli* isolates were moderately resistant to ampicillin (71.88%) and the percentage of susceptible is 28.12% and these three (E, LZ and VA) were excluded from the resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria. The majority of isolates were highly sensitive to aztreonam, chloramphenicol, cefepime, imipenem, gentamicin, kenamycin, and levofloxacin, and moderately sensitive to cefixime (still 25% exhibits resistancy), doxycycline with 68.75% sensitivity, 18.75% intermediate isolates along with 12.5% resistant isolates

4.2.3d AST Results From Hatirjheel, Curzon Hall and Ramna Lake (April)

Table 30: Antibiotic susceptibility patterns of *E. coli* isolates collected from Hatirjheel, Curzon Hall and Ramna Lake in April.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
EApHS1(1)	0 R	0 R	25 S	19.5 S	0 R	23 S	0 R	30 S	22.5 S	24.5 S	22 S	0 R	0 R
EApHS1(2)	0 R	32 S	22 S	36 S	23 S	20 S	0 R	32 S	23 S	21 S	30 S	0 R	0 R
EApHS1(3)	0 R	33 S	21.5 S	30 S	32 S	18.5 S	0 R	26 S	24.5 S	20 S	32 S	0 R	0 R
EApHS1(4)	0 R	31 S	0 R	31.5 S	0 R	0 R	0 R	31.5 S	23 S	23 S	35 S	0 R	0 R
EApHS1(5)	0 R	35 S	21.5 S	32 S	25 S	22 S	0 R	34 S	30 S	22 S	28 S	0 R	0 R
EApHS1(6)	0 R	30 S	23 S	33 S	26 S	21 S	0 R	33.5 S	31.5 S	25 S	31.5 S	0 R	0 R
EApCS1(1)	20 S	34 S	28 S	38.5 S	24 S	23 S	0 R	26 S	24 S	23.5 S	39 S	0 R	0 R
EApCS1(2)	0 R	33 S	26.5 S	34 S	0 R	20 S	0 R	29.5 S	23.5 S	24 S	33 S	0 R	0 R
EApCS1(3)	0 R	32 S	29.5 S	36.5 S	20 S	21.5 S	0 R	24.5 S	20 S	21.5 S	37.5 S	0 R	0 R
EApCS1(4)	0 R	34 S	25 S	40 S	0 R	0 R	0 R	31 S	21.5 S	20 S	31 S	0 R	0 R
EApRS1(1)	0 R	32 S	0 R	35 S	0 R	15 S	0 R	30 S	24 S	0 R	27 S	0 R	0 R
EApRS1(2)	0 R	33 S	30 S	32 S	15.5 R	18.5 S	0 R	32 S	26 S	22 S	25 S	0 R	0 R
EApRS1(3)	0 R	30.5 S	33 S	36.5 S	0 R	21 S	0 R	33.5 S	19.5 S	20.5 S	24.5 S	0 R	0 R
EApRS1(4)	0 R	19.5 I	32 S	20 S	0 R	25 S	0 R	28.5 S	22 S	24.5 S	32.5 S	0 R	0 R
EApHS2(2)	25 S	36 S	28 S	33 S	30.5 S	28 S	0 R	34.5 S	22.5 S	23.5 S	36 S	0 R	0 R
EApHS2(3)	26 S	39.5 S	31 S	36 S	37.5 S	0 R	0 R	34 S	24.5 S	24 S	29 S	0 R	0 R
EApHS2(4)	23 S	35 S	30 S	33 S	33 S	23 S	0 R	30 S	23 S	22 S	30 S	0 R	0 R

EApHS2(5)	28.5 S	33 S	32 S	32 S	32 S	24 S	0 R	33 S	25.5 S	22.5 S	31 S	0 R	0 R
EApHS2(6)	0 R	37 S	31 S	34 S	30 S	20 S	0 R	28.5 S	24 S	24 S	28 S	0 R	0 R
EApHS2(7)	24.5 S	32 S	35 S	30 S	36 S	19 S	0 R	30 S	27 S	27 S	34.5 S	0 R	0 R
EApHS2(8)	0 R	31 S	37 S	32 S	35 S	17 S	0 R	29 S	21 S	25.5 S	33 S	0 R	0 R
EApHS2(9)	0 R	35 S	30 S	33.5 S	32.5 S	24 S	0 R	32 S	20.5 S	21.5 S	31.5 S	0 R	0 R
EApHS2(10)	0 R	30 S	31 S	36.5 S	31 S	21 S	0 R	34.5 S	26.5 S	20.5 S	30 S	0 R	0 R
EApHS2(11)	0 R	29 S	30.5 S	31.5 S	38 S	23 S	0 R	33 S	21 S	23 S	27.5 S	0 R	0 R
EApHS2(12)	26 S	38 S	30 S	37 S	35 S	0 R	0 R	34 S	24 S	24.5 S	28.5 S	0 R	0 R
EApHS2(13)	20 S	37 S	28 S	38 S	38 S	29 S	0 R	33 S	20 S	25 S	42 S	0 R	0 R
EApCS2(1)	20 S	34 S	33.5 S	39.5 S	32 S	26 S	0 R	22.5 S	26.5 S	25.5 S	37.5 S	0 R	0 R
EApCS2(2)	18 S	30 S	34.5 S	35.5 S	36 S	28 S	0 R	20 S	30.5 S	27.5 S	30 S	0 R	0 R
EApCS2(3)	22.5 S	39.5 S	28 S	40 S	29 S	23.5 S	0 R	37 S	24 S	23.5 S	39 S	0 R	0 R
EApCS2(4)	23 S	34 S	32 S	38 S	25.5 S	22 S	0 R	32.5 S	29 S	29 S	38 S	0 R	0 R
EApCS2(5)	25 S	34 S	22 S	34 S	30 S	26 S	0 R	33 S	28 S	27 S	34.5 S	0 R	0 R
EApCS2(6)	20 S	36 S	24 S	30.5 S	32 S	20.5 S	0 R	34 S	26.5 S	25.5 S	33 S	0 R	0 R
EApCS2(7)	15.5 I	33 S	27.5 S	32 S	36.5 S	24.5 S	0 R	37 S	23 S	21.5 S	36 S	0 R	0 R
EApCS2(8)	0 R	35.5 S	25.5 S	36.5 S	37 S	30 S	0 R	32 S	22.5 S	20 S	32 S	0 R	0 R
EApCS2(9)	24 S	40 S	30 S	42 S	30 S	25 S	0 R	35 S	21 S	22.5 S	40 S	0 R	0 R
EApCS2(10)	0 R	38.5 S	23 S	36.5 S	33 S	20 S	0 R	30 S	25 S	26 S	36 S	0 R	0 R
EApCS2(12)	35 S	42 S	18 S	32 S	30 S	25 S	10 R	42 S	30 S	30 S	41 S	0 R	0 R
EApCS2(13)	0 R	0 R	27 S	0 R	0 R	23 S	0 R	28.5 S	22.5 S	29.5 S	26 S	0 R	0 R

All together 38 *Escherichia coli* were subjected to AST in April of which 18 were from Hatirjheel, 16 from Curzon Hall and 4 from Ramna Lake. In Hatirjheel sample-1, 2 isolates (EApHS1(1, 4)) were identified as MDR mainly to β-lactams tetracycline and chloramphenicol. Among 4 isolates from Curzon Hall, EApCS1(4) was MDR. From Curzon Hall sample-2, isolate (13) was MDR. Additionally, 2 isolates (1,3) from sample 1 were MDR. So, April data showed 6 MDR in total across all sampling sites.

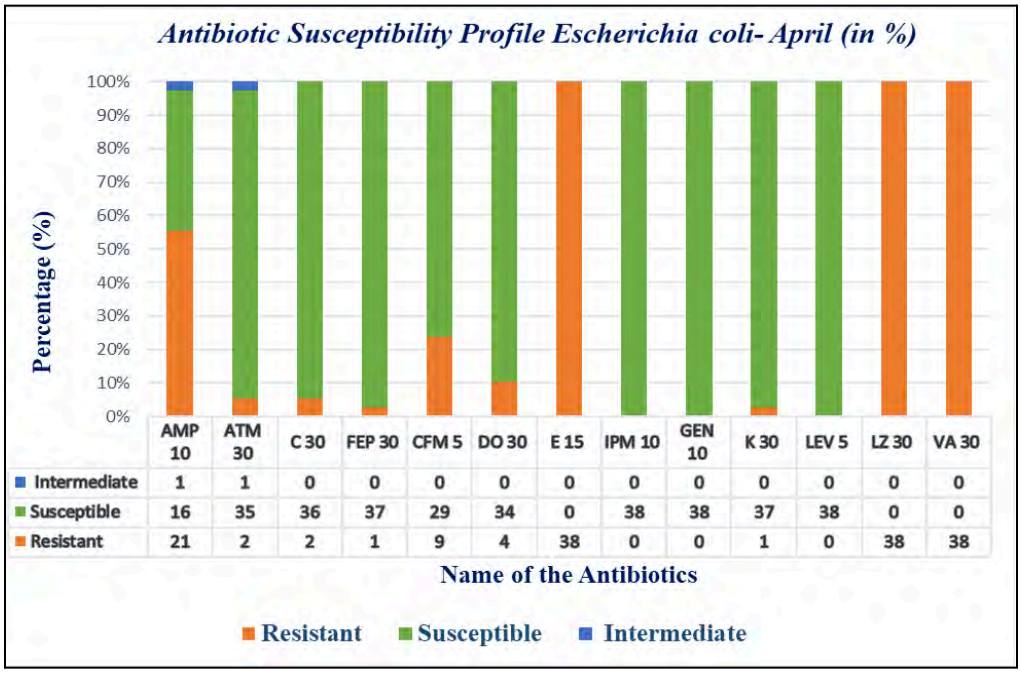


Figure 29: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel, Curzon Hall and Ramna Lake collected in May

The *E. coli* isolates of April showed moderately resistant (55.26%) and 42.11% susceptible to ampicillin while these three (E, LZ and VA) excluded from the resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria. The majority of isolates were highly sensitive to aztreonam, chloramphenicol, cefepime, imipenem, gentamicin, kenamycin, and levofloxacin, and moderately sensitive to cefixime (still 23.68% exhibits resistancy) with low sensitivity (10.53%) to doxycycline.

4.2.3e AST Results From Hatirjheel, Curzon Hall and Ramna Lake (May)

Table 31: Antibiotic susceptibility patterns of *E. coli* isolates collected from Hatirjheel, Curzon Hall and Ramna Lake in May.

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
EMHS1(1)	0 R	20 I	25 S	32 S	12 R	20 S	0 R	24 S	20 S	20 S	30 S	0 R	0 R
EMHS1(2)	0 R	35 S	26 S	36 S	27 S	10 R	0 R	32 S	21 S	21 S	25 S	0 R	0 R
EMHS1(3)	0 R	16 R	28 S	19 S	0 R	23 S	9 R	31 S	20 S	21 S	28 S	0 R	0 R
EMHS1(4)	0 R	37 S	0 R	35 S	30 S	12 I	10 R	31 S	19 S	20 S	12 R	0 R	0 R
EMHS1(5)	0 R	31 S	25 S	36 S	0 R	0 R	0 R	26 S	20 S	20 S	25 S	0 R	0 R
EMHS1(6)	20 S	30 S	25 S	34 S	25 S	20 S	10 R	30 S	19 S	20 S	30 S	0 R	0 R
EMHS1(7)	0 R	30 S	26 S	32 S	25 S	11 I	0 R	27 S	18 S	17 I	20 S	0 R	0 R
EMHS1(8)	0 R	26 S	21 S	33 S	29 S	18 S	0 R	29 S	19 S	20 S	27 S	0 R	0 R
EMHS1(9)	0 R	30 S	28 S	31 S	16 I	21 S	10 R	27 S	19 S	20 S	25 S	0 R	0 R
EMHS1(10)	10 R	30 S	30 S	12 R	28 S	20 S	10 R	23 S	19 S	20 S	29 S	0 R	0 R`
EMRS1(1)	0 R	23.5 S	23.7 S	24.5 S	15.5 I	15 S	0 R	31 S	20 S	22.5 S	25.2 S	0 R	0 R
EMRS1(2)	0 R	19.2 I	21.7 S	20.7 S	0 R	11 I	0 R	30 S	18 S	18.7 S	15 I	0 R	0 R
EMRS1(3)	0 R	15 R	29 S	16.5 I	0 R	22.2 S	0 R	30 S	20 S	21 S	28 S	0 R	0 R
EMRS1(4)	0 R	28.7 S	24.5 S	33 S	0 R	13.2 I	0 R	25.7 S	18.7 S	19 S	25 S	0 R	0 R
EMRS1(5)	22 S	34 S	25.7 S	33 S	23.5 S	12 I	0 R	31 S	21.2 S	19.7 S	26.5 S	0 R	0 R
EMRS1(6)	21 S	35 S	25 S	31 S	25 S	20 S	0 R	32 S	22 S	21.5 S	34.5 S	0 R	0 R
EMRS1(7)	0 R	31 S	25 S	32 S	25 S	11.5 I	0 R	30 S	19.7 S	16 I	30 S	0 R	0 R

EMRS1(8)	10 R	30 S	27 S	31 S	24.5 S	14 S	10 R	26.5 S	17 S	19 S	26 S	0 R	0 R
EMRS1(9)	0 R	20 I	26 S	22.5 S	12 R	18 S	0 R	30 S	19 S	22.5 S	28 S	0 R	0 R
EMRS1(10)	0 R	31 S	0 R	31 S	26.5 S	10 R	0 R	0 R	17.5 S	17 I	10 R	0 R	0 R
EMRS1(11)	0 R	29 S	30 S	31.5 S	0 R	20 S	0 R	24 S	0 R	21 S	25 S	0 R	0 R
EMCS1(1)	18 S	32 S	26 S	32 S	23 S	20 S	0 R	29 S	17 S	18 S	28 S	0 R	0 R
EMCS1(2)	19 S	32 S	29 S	34 S	23 S	22 S	0 R	30 S	19 S	19 S	33 S	0 R	0 R
EMCS1(3)	18 S	35 S	29 S	36 S	25 S	15 S	0 R	34 S	20 S	21 S	32 S	0 R	0 R
EMHS2(1)	21 S	34 S	28 S	36 S	26 S	14 S	0 R	31 S	22 S	21 S	38 S	0 R	0 R
EMHS2(2)	0 R	16 R	23 S	32 S	26 S	11 I	0 R	24 S	17 S	20 S	28 S	0 R	0 R
EMHS2(3)	19 S	31 S	27 S	32 S	21 S	0 R	0 R	26 S	20 S	20 S	32 S	0 R	0 R
EMHS2(4)	0 R	15 R	28 S	15 I	0 R	21 S	0 R	29 S	23 S	22 S	15 I	0 R	0 R
EMHS2(5)	20 S	30 S	27 S	20 S	26 S	14 S	0 R	25 S	20 S	20 S	27 S	0 R	0 R
EMHS2(6)	0 R	15 R	15 I	20 S	17 I	11 I	0 R	29 S	22 S	15 I	22 S	0 R	0 R
EMHS2(7)	10 R	29 S	28 S	35 S	21 S	11 I	0 R	24 S	20 S	21 S	30 S	0 R	0 R
EMHS2(8)	0 R	31 S	23 S	36 S	22 S	20 S	0 R	24 S	17 S	20 S	27 S	0 R	0 R
EMHS2(9)	10 R	29 S	24 S	30 S	26 S	16 S	0 R	24 S	17 S	20 S	25 S	0 R	0 R
EMHS2(10)	0 R	29 S	27 S	35 S	26 S	16 S	0 R	26 S	19 S	15 I	27 S	0 R	0 R
EMHS2(11)	0 R	31 S	27 S	26 S	22 S	16 S	0 R	24 S	22 S	21 S	28 S	0 R	0 R
EMHS2(12)	0 R	15 R	17 I	32 S	21 S	16 S	0 R	26 S	20 S	20 S	30 S	0 R	0 R
EMRS2(1)	0 R	29 S	23 S	28 S	22 S	14 S	0 R	25 S	16 S	17 I	27 S	0 R	0 R

EMRS2(2)	9 R	30 S	26 S	30 S	25 S	17 S	0 R	28 S	15 S	17 I	27 S	0 R	0 R
EMRS2(3)	0 R	30 S	23 S	27 S	26 S	16 S	0 R	25 S	17 S	16 I	27 S	0 R	0 R
EMRS2(4)	0 R	29 S	24 S	25 S	24 S	16 S	0 R	25 S	17 S	17 I	27 S	0 R	0 R
EMRS2(5)	0 R	30 S	27 S	30 S	24 S	17 S	0 R	26 S	17 S	18 S	26 S	0 R	0 R
EMRS2(6)	0 R	29 S	24 S	30 S	26 S	16 S	0 R	25 S	16 S	16 I	27 S	0 R	0 R
EMRS2(7)	0 R	26 S	26 S	30 S	25 S	16 S	0 R	26 S	16 S	17 I	26 S	0 R	0 R
EMRS2(8)	0 R	28 S	29 S	30 S	26 S	15 S	0 R	26 S	16 S	17 I	27 S	0 R	0 R
EMRS2(9)	0 R	26 S	21 S	28 S	23 S	12 I	9 R	20 S	16 S	17 I	24 S	0 R	0 R
EMRS2(11)	0 R	26 S	24 S	25 S	23 S	15 S	0 R	24 S	15 S	16 I	24 S	0 R	0 R
EMCS2(1)	0 R	31 S	25 S	31 S	26 S	17 S	0 R	28 S	21 S	22 S	25 S	0 R	0 R
EMCS2(2)	0 R	31 S	26 S	32 S	22 S	18 S	0 R	25 S	20 S	20 S	24 S	0 R	0 R
EMCS2(3)	0 R	36 S	0 R	39 S	20 S	0 R	0 R	29 S	21 S	0 R	0 R	0 R	0 R
EMCS2(4)	10 R	31 S	23 S	32 S	23 S	16 S	0 R	28 S	20 S	22 S	27 S	0 R	0 R
EMCS2(5)	0 R	31 S	24 S	31 S	23 S	17 S	0 R	29 S	20 S	21 S	25 S	0 R	0 R
EMCS2(6)	20 S	31 S	31 S	32 S	23 S	21 S	0 R	29 S	22 S	22 S	32 S	0 R	0 R
EMCS2(7)	11 R	33 S	24 S	32 S	26 S	16 S	0 R	29 S	22 S	22 S	27 S	0 R	0 R
EMCS2(8)	0 R	30 S	26 S	31 S	28 S	17 S	0 R	26 S	20 S	20 S	27 S	0 R	0 R
EMCS2(9)	11 R	31 S	23 S	39 S	21 S	17 S	0 R	28 S	21 S	20 S	24 S	0 R	0 R
EMCS2(10)	0 R	36 S	26 S	31 S	22 S	18 S	0 R	28 S	21 S	22 S	24 S	0 R	0 R
EMCS2(11)	17 S	30 S	26 S	31 S	21 S	20 S	10 R	26 S	23 S	22 S	32 S	0 R	0 R

EMCS2(12)	0 R	30 S	26 S	31 S	22 S	17 S	0 R	29 S	20 S	22 S	32 S	0 R	0 R

A total of 58 *Escherichia coli* were analyzed in May on antibiotic susceptibility tests of which 22 were from Hatirjheel, 15 from Curzon Hall and 11 from Ramna Lake. Out of the Hatirjheel isolates, from sample-1, 3 isolates (3,4,5) were identified as MDR. On the other hand, EMHS2(4,6) isolates indicated MDR. From Ramna Lake sample-1, 3 isolates (3,10,11) were MDR while no isolates from sample-2 were MDR. In Curzon Hall sample-1, all the 3 isolates were excluded from MDR/XDR categorization while in sample 2, only isolate (3) was MDR. Overall, May exhibited the highest burden of both MDR, particularly in Hatirjheel and Ramna Lake in total 9 were MDR.

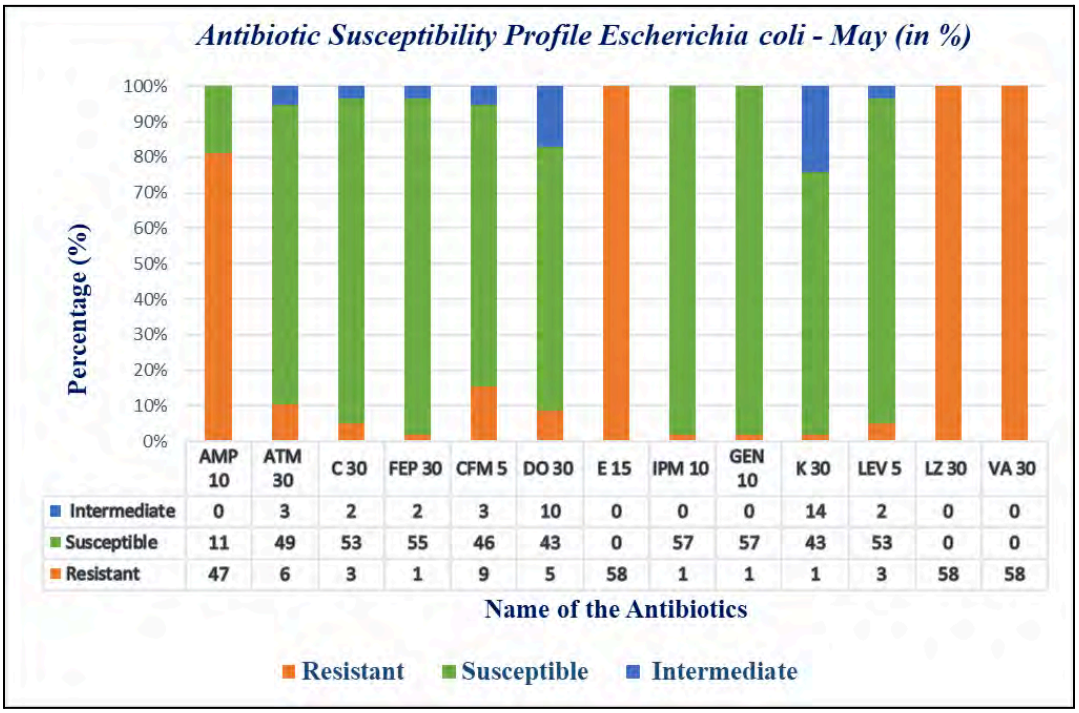


Figure 30: Antibiotic susceptibility profile of *E.coli* isolates in May in terms of resistant, intermediate and susceptible reactions to various antibiotics

In May, the *E. coli* isolates were highly resistant to ampicillin (81.03%) and the percentage of susceptible is 18.97% and these three (E, LZ and VA) were excluded from the resistance type The majority of isolates were highly sensitive to aztreonam (with 10.34% resistant isolates), chloramphenicol, cefepime, cefixime (still 15.52% exhibits resistancy) imipenem, gentamicin,

and levofloxacin while moderately sensitive to doxycycline with 74.12% sensitivity with 10 resistant isolates and 24.14% intermediate along with 74.12% susceptible isolates for kenamycin.

4.2.3f AST Results From Hatirjheel, Curzon Hall and Ramna Lake (June)

Table 32: The pattern of antibiotic susceptibility of *E. coli* isolates at Hatirjheel, Curzon Hall and Ramna Lake collected in June

Sample ID	AMP 10	ATM 30	C 30	FEP 30	CFM 5	DO 30	E 15	IPM 10	GEN 10	K 30	LEV 5	LZ 30	VA 30
EJnHS1(1)	0 R	40 S	0 R	40 S	30 S	10 R	0 R	30 S	0 R	18 S	18 S	0 R	0 R
EJnHS1(2)	0 R	37 S	0 R	42 S	34 S	15 S	0 R	32 S	22 S	15.5 S	20 S	0 R	0 R
EJnHS1(3)	0 R	54 S	0 R	48 S	42 S	12 I	22 R	46 S	27 S	28 S	28 S	0 R	0 R
EJnHS1(4)	22 S	39 S	28 S	34 S	38.5 S	24 S	0 R	33.5 S	25 S	23.5 S	26.5 S	0 R	0 R
EJnHS1(5)	25 S	42 S	30 S	32 S	33.5 S	22.5 S	0 R	30 S	20.5 S	24 S	30 S	0 R	0 R
EJnHS1(6)	24 S	40 S	34 S	38 S	44 S	23 S	13 R	37 S	23 S	22.5 S	35 S	0 R	0 R
EJnHS1(7)	25 S	35 S	30 S	41.5 S	39 S	26.5 S	0 R	35.5 S	19 S	20.5 S	32 S	0 R	0 R
EJnHS1(8)	27 S	50 S	29 S	48 S	35 S	30 S	0 R	39 S	25 S	28 S	30 S	0 R	0 R
EJnHS1(9)	27 S	48 S	30 S	45 S	42 S	26 S	16 R	40 S	24 S	26 S	46 S	0 R	0 R
EJnHS1(10)	21.5 S	35 S	32 S	35 S	40 S	27.5 S	10 R	42 S	26 S	17.5 S	40 S	0 R	0 R
EJnHS1(11)	20 S	37.5 S	33 S	39.5 S	36 S	25 S	0 R	38.5 S	23 S	25.5 S	38 S	0 R	0 R
EJnCS1(1)	0 R	33 S	24.5 S	36.5 S	22 S	21 S	0 R	24 S	20 S	10 R	29.5 S	0 R	0 R
EJnCS1(2)	0 R	35 S	20 S	38 S	23 S	22 S	0 R	26.5 S	20 S	11 R	27 S	0 R	0 R
EJnCS1(3)	0 R	40 S	20 S	37 S	22 S	20 S	11 R	27 S	16 S	10 R	28 S	0 R	0 R
EJnCS1(4)	0 R	33.5 S	26.5 S	32.5 S	20.5 S	24 S	10 R	30 S	18.5 S	10 R	30 S	0 R	0 R

EJnCS1(5)	0 R	37 S	20 S	40 S	22 S	21 S	10 R	26 S	18 S	10 R	32 S	0 R	0 R
EJnCS1(6)	0 R	32 S	15 I	37 S	21 S	16 S	10 R	23 S	18 S	11 R	24 S	0 R	0 R
EJnCS1(7)	0 R	31.5 S	15.5 I	37.5 S	24 S	20 S	18.5 R	30.5 S	15.5 S	13.5 R	26.5 S	0 R	0 R
EJnCS1(8)	0 R	40 S	18 S	40 S	19 S	22 S	13 R	28 S	19 S	10 R	28 S	0 R	0 R
EJnCS1(9)	0 R	42 S	22.5 S	38.5 S	20 S	23 S	20 R	29.5 S	23.5 S	18 S	30 S	0 R	0 R
EJnCS1(10)	0 R	33.5 S	22 S	33.5 S	23.5 S	25 S	22 R	31 S	22 S	12.5 R	33 S	0 R	0 R
EJnCS1(11)	0 R	35 S	21 S	30 S	15.5 R	18.5 S	13.5 R	33 S	27.5 S	11 R	32 S	0 R	0 R
EJnCS1(12)	0 R	32 S	20.5 S	32.5 S	18 I	15 S	19.5 R	30 S	24 S	10 R	35.5 S	0 R	0 R
EJnCS1(13)	0 R	34.5 S	19 S	36.5 S	25.5 S	20.5 S	22 R	26.5 S	20 S	10 R	30 S	0 R	0 R
EJnHS2(1)	15 I	10 R	25 S	14 R	22 S	0 R	10 R	23 S	16 S	20 S	24 S	0 R	0 R
EJnHS2(2)	0 R	30 S	0 R	35 S	25 S	10 R	0 R	32 S	21 S	0 R	22 S	0 R	0 R
EJnHS2(3)	20 S	33 S	25 S	33 S	25 S	20 S	8 R	33 S	20 S	22 S	35 S	0 R	0 R
EJnHS2(4)	18 S	34 S	26 S	32 S	25 S	13 I	10 R	31 S	20 S	20 S	25 S	0 R	0 R
EJnHS2(5)	0 R	30 S	25 S	33 S	15 R	17 S	0 R	29 S	19 S	19 S	31 S	0 R	0 R
EJnHS2(6)	21 S	33 S	27 S	31 S	25 S	12 I	10 R	25 S	19 S	20 S	25 S	0 R	0 R
EJnHS2(8)	0 R	31 S	25 S	20 S	25 S	13 I	0 R	32 S	20 S	20 S	23 S	0 R	0 R
EJnRS2(1)	18 S	29 S	25 S	30 S	17 I	18 S	0 R	25 S	17 S	20 S	20 S	0 R	0 R
EJnRS2(2)	15 I	28 S	25 S	30 S	18 I	18 S	0 R	25 S	18 S	18 S	26 S	0 R	0 R
EJnRS2(3)	18 S	30 S	20 S	30 S	18 I	18 S	0 R	25 S	17 S	20 S	25 S	0 R	0 R
EJnRS2(4)	15 I	31 S	22 S	30 S	17 I	17 S	0 R	25 S	18 S	20 S	27 S	0 R	0 R
EJnRS2(5)	15 I	30 S	24 S	30 S	17 I	18 S	0 R	26 S	18 S	20 S	25 S	0 R	0 R

EJnRS2(6)	18 S	21 S	25 S	30 S	17 I	18 S	0 R	26 S	20 S	20 S	21 S	0 R	0 R
EJnRS2(7)	0 R	29 S	22 S	32 S	17 I	17 S	0 R	25 S	18 S	17 I	27 S	0 R	0 R
EJnRS2(8)	17 S	29 S	23 S	32 S	18 I	19 S	0 R	27 S	16 S	18 S	25 S	0 R	0 R
EJnRS2(9)	16 I	31 S	25 S	32 S	20 S	20 S	0 R	26 S	17 S	18 S	26 S	0 R	0 R
EJnRS2(10)	0 R	0 R	26 S	0 R	0 R	0 R	0 R	27 S	0 R	21 S	0 R	0 R	0 R
EJnCS2(2)	17 S	33 S	27 S	34 S	24 S	12 I	0 R	34 S	23 S	21 S	18 S	0 R	0 R
EJnCS2(3)	0 R	33 S	27 S	33 S	24 S	11 I	0 R	29 S	19 S	20 S	18 S	0 R	0 R
EJnCS2(7)	0 R	30 S	27 S	34 S	21 S	12 I	0 R	29 S	18 S	20 S	17 S	0 R	0 R

During June, 44 *Escherichia coli* were analyzed in May on antibiotic susceptibility tests of which 18 were from Hatirjheel, 16 from Curzon Hall and 10 from Ramna Lake. Out of the Hatirjheel isolates, from sample-1, the first isolates identified as MDR. In sample-2, isolates (1,2) exhibited MDR. From Ramna Lake sample-2, single MDR (isolate 10) found. In Curzon Hall sample-1, all the isolates were excluded from MDR/XDR categorization except isolate 3 which was MDR while in sample 2 none showed MDR. From 44 isolates, 7 were MDR across all areas.

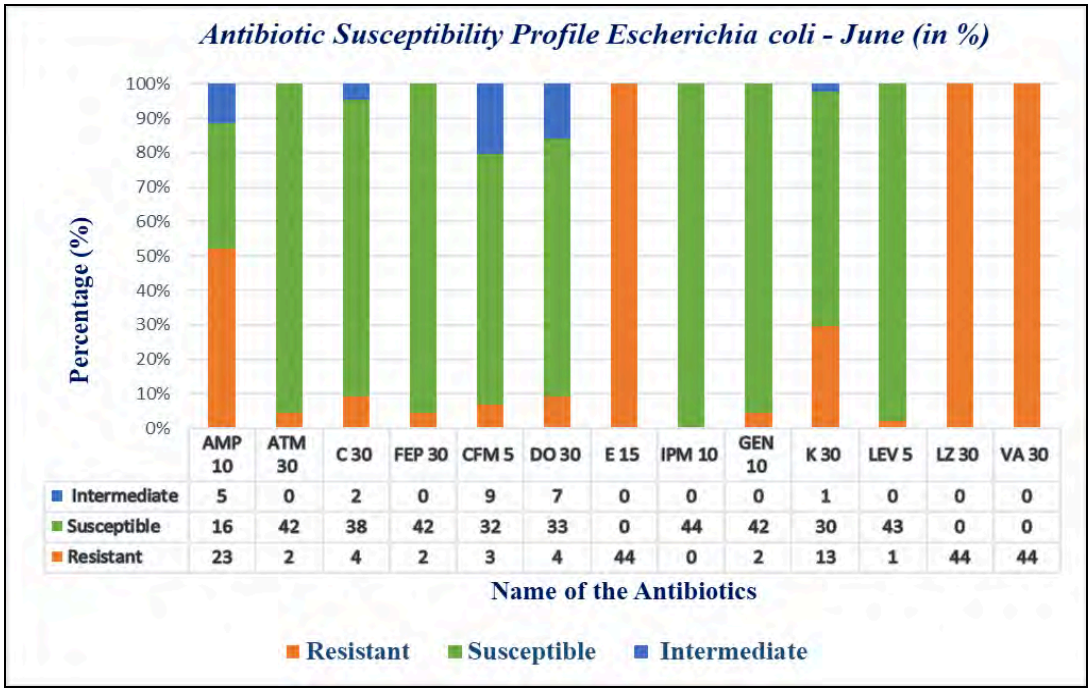


Figure 31:Antibiotic susceptibility profile of *E.coli* isolates in June in terms of resistant, intermediate and susceptible reactions to various antibiotics

In June, the *E. coli* isolates were moderately resistant to ampicillin (52.27%) and the percentage of susceptible is 36.377% with 5 intermediate isolates while these three (E, LZ and VA) were excluded from the determining resistance type. The majority of isolates were highly sensitive to aztreonam, chloramphenicol, cefepime, cefixime (with 20.45% intermediate isolates), doxycycline (with 15.91% intermediate isolates), imipenem, gentamicin, and levofloxacin while moderately sensitive (68.18%) to kenamycin with 29.55% resistant isolates.

4.2.4 Results of Resistance Types of Bacterial Isolates

4.2.4a Total No. of MDR Isolates for *Salmonella* spp.

Table 33: Total number of types of resistance (MDR) isolates among Hatirjheel, Curzon Hall and Ramna Lake for *Salmonella spp.*

Species	Area	Month	Type of Resistance (MDR)
		January	0

***Salmonella* spp.**	Hatirjheel	February	4
		March	0
		April	1
		May	13
		June	10
	Total		**28**
	Curzon Hall	January	-
		February	1
		March	-
		April	-
		May	9
		June	0
	Total		**10**
	Rama Lake	January	-
		February	0
		March	-
		April	-
		May	7
		June	0
	Total		**7**
Total			**45**

4.2.4b Total No. of MDR Isolates for *Vibrio cholerae*

Table 34: Total number of types of resistance (MDR) isolates among Hatirjheel, Curzon Hall and Ramna Lake for *Vibrio cholerae*.

Species	Area	Month	Type of Resistance (MDR)
Vibrio cholerae	Hatirjheel	January	0
		February	1
		March	0
		April	3
		May	1
		June	2
	Total		**7**
	Curzon Hall	January	-
		February	-
		March	-
		April	-
		May	0
		June	-
	Total		**0**
	Rama Lake	January	-
		February	0
		March	-
		April	-
		May	0
		June	1

	Total		**1**
Total			**8**

4.2.4c Total No. of MDR Isolates for *Escherichia coli*

Table 35: Total number of types of resistance (MDR) isolates among Hatirjheel, Curzon Hall and Ramna Lake for *Escherichia coli*.

Species	**Area**	**Month**	**Type of Resistance (MDR)**
Escherichia coli	Hatirjheel	January	5
		February	3
		March	6
		April	2
		May	5
		June	3
	Total		**24**
	Curzon Hall	January	0
		February	1
		March	0
		April	2
		May	3
		June	3
	Total		**9**
	Rama Lake	January	-
		February	2
		March	0
		April	2

		May	3
		June	1
	Total		**8**
Total			**41**

4.2.5 Results of Total No. of MDR Based on Bacterial Species

Table 36: Total No. of MDR on the basis of bacterial species

Bacterial Species	**Total No. of MDR**			**Total**
	Hatirjheel	**Curzon Hall**	**Ramna Lake**	
Salmonella spp.	28	10	7	**45**
Vibrio cholerae	7	0	1	**8**
Escherichia coli	24	9	8	**41**
Total				**94**

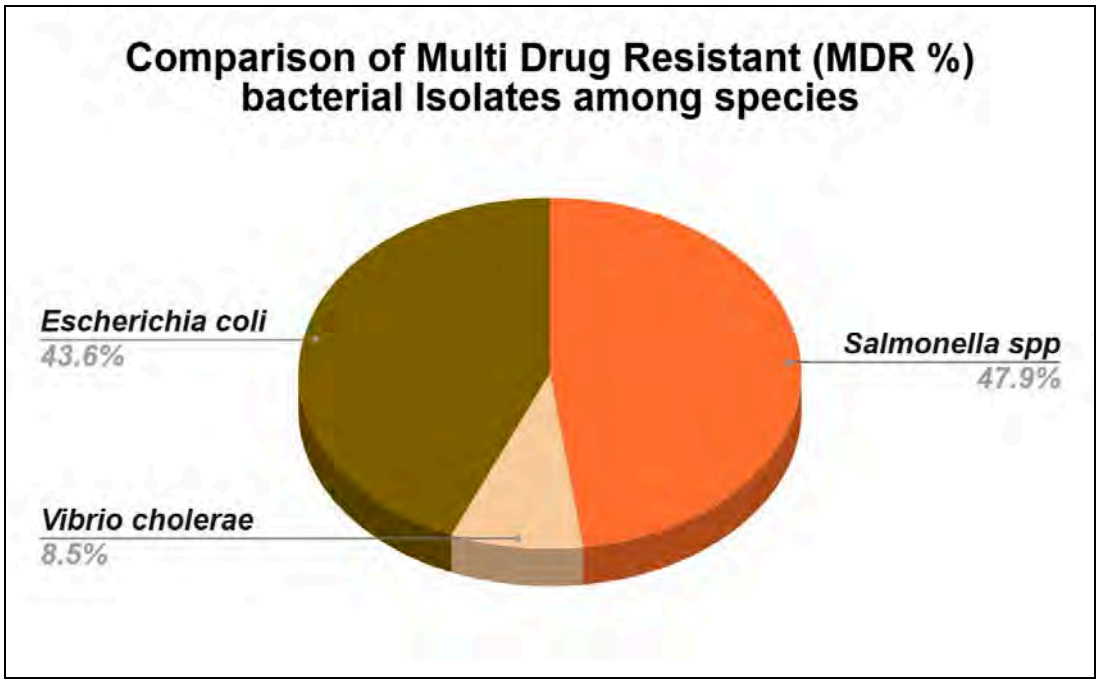


Figure 32: Comparative distribution of Multi Drug Resistant bacterial isolates among species

As indicated in the table (36) and figure (32), 94 multidrug-resistant (MDR) isolates were observed in the investigated bacterial species. *Escherichia coli* (45) and *Vibrio cholerae* (much less) had the same number of MDR isolates as *Salmonella* spp. (45), and *Escherichia coli* was the most frequently found (41). Hatirjheel was the location that consistently obtained a high number of MDR isolates of all species as opposed to Curzon Hall and Ramna Lake. This has shown in the MDR pie chart that Salmonella spp. caters the highest percentage percentage (47.9%), then *E. coli* and *V. cholerae* (43.6% and 8.5%, respectively).

4.3 Results of Plasmid

4.3.1 Results of Plasmid for *Salmonella* Spp.

4.3.1a Gel Electrophoresis Result

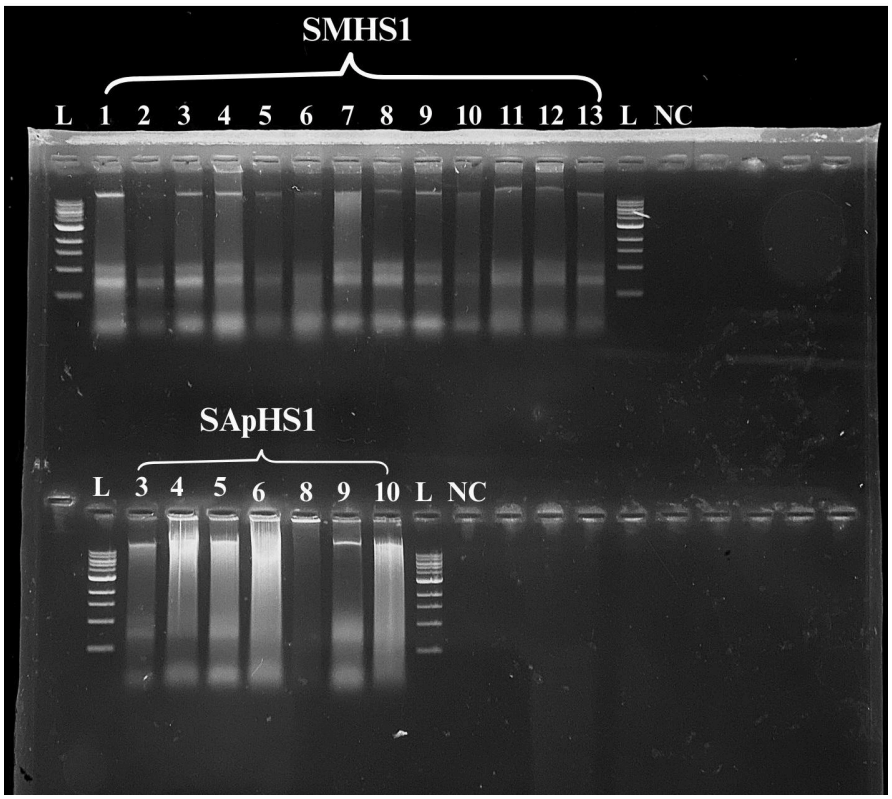


Figure 33: Agarose gel electrophoresis displays plasmid profiles of *Salmonella spp.* isolates collected in April (lower panel) and May (upper panel) from Hatirjheel.

This figure shows plasmid profiling of *Salmonella* isolates found in Hatirjheel. In the May Hatirjheel (SMHS1), 13 plasmid products (lane 1-13) were analyzed, and plasmid bands were clear across the gel, meaning that all the 13 samples were plasmid positive. Conversely, in the April Hatirjheel gel (SApHS1), 7 plasmid products (lanes 3, 4, 5, 6, 8, 9, and 10) were tested, with lane 8 having no visible plasmid band, whereas the rest of the lanes displayed separate bands, giving 6 plasmid-positive and 1 plasmid-negative sample. The 1 kb DNA ladder (L) used to indicate molecular size and the absence of the bands in the negative control (NC) shows the specificity of the plasmid detection and eliminates the presence of contamination possibilities.

4.3.1b Table of Plasmid Results

Table 37: The monthly distribution and plasmid positivity of *Salmonella* spp. isolates at Hatirjheel according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
Hatirjheel	January S1	6	6	6	0	100
	January S2	7	7	7	0	100
	February S1	12	11	11	1	91.7
	February S2	14	11	11	3	78.6
	March S1	1	1	1	0	100
	March S2	3	3	3	0	100
	April S1	15	14	14	1	93.3
	April S2	2	2	2	0	100
	May S1	13	13	13	0	100
	May S2	10	10	10	0	100
	June S1	16	13	13	3	81.3
	June S2	11	7	7	4	63.6
Total		**110**		**98**		

Table 38: The monthly distribution and plasmid positivity of *Salmonella* spp. isolates at Curzon Hall according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
	January S1	0	-	-	-	0

Curzon Hall	January S2	0	-	-	-	0
	February S1	2	2	2	0	100
	February S2	0	-	-	-	0
	March S1	0	-	-	-	0
	March S2	0	-	-	-	0
	April S1	0	-	-	-	0
	April S2	0	-	-	-	0
	May S1	2	2	2	0	100
	May S2	10	10	10	0	100
	June S1	2	2	2	0	100
	June S2	0	-	-	-	0
Total		**16**		**16**		

Table 39: The monthly distribution and plasmid positivity of *Salmonella* spp. isolates at Ramna Lake according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
Ramna Lake	January S1	0	-	-	-	0
	January S2	0	-	-	-	0
	February S1	3	3	3	0	100
	February S2	0	-	-	-	0
	March S1	0	-	-	-	0
	March S2	0	-	-	-	0
	April S1	0	-	-	-	0
	April S2	0	-	-	-	0

	May S1	14	12	12	2	85.7
	May S2	3	3	3	0	100
	June S1	0	-	-	-	0
	June S2	12	9	9	3	75
Total		**32**		**27**		

4.3.1c Pie Chart of the Plasmid Positive Results

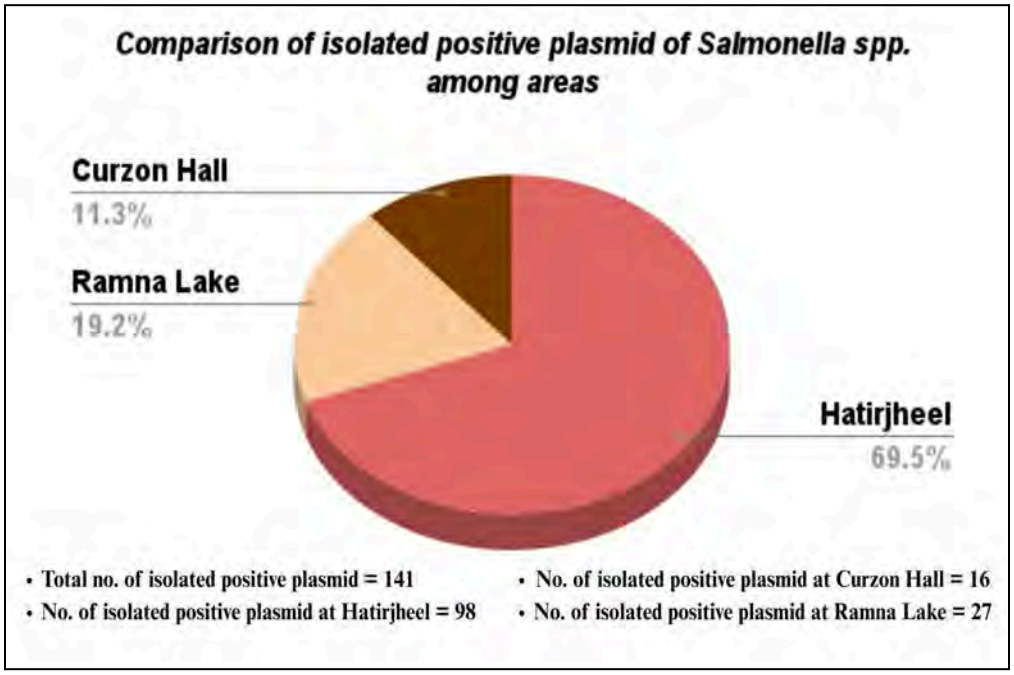


Figure 34: Comparative distribution of plasmid-positive *Salmonella* spp. isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

This figure represents the distribution of plasmid-positive *Salmonella* spp. isolates among three sampling sites: Hatirjheel, Ramna Lake, and Curzon Hall. Among 141 plasmid-positive isolates, the higher number was provided by Hatirjheel with 98 isolates (69.5%). Ramna Lake on the other hand, contributed 27 isolates (19.2%), with a relatively low number of plasmid-positive isolates. The lowest results were in Curzon Hall (16 isolates, 11.3%). The figure in general illustrates the significant hotspots of plasmid-carrying *Salmonella* spp. are Hatirjheel, with much lower rates in Ramna Lake and Curzon Hall.

4.3.1d Column Chart of the Plasmid Positive Results

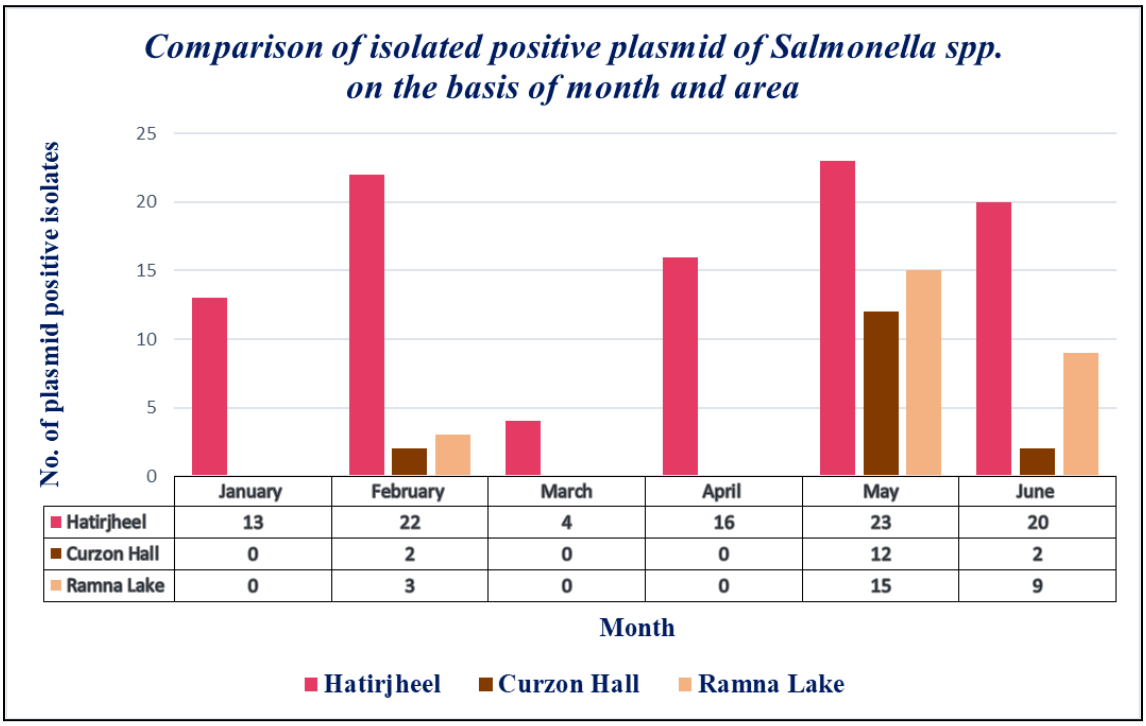


Figure 35: A monthly comparison of plasmid-positive *Salmonella* spp. isolates in three sampling areas: Haltirjheel, Curzon Hall, and Ramna Lake.

The figure illustrates the distribution of plasmid-positive *Salmonella* spp. isolates in the month and area from January to June in Hatirjheel, Curzon Hall and Ramna Lake. In general, Hatirjheel continuously presented the highest count of plasmid-positive *Salmonella* isolates over the study period with 13 isolates in January and highest counts of 23 isolates in May, and later, 20 isolates in June. Low and inconsistent detection was recorded in Curzon Hall, where there were no isolates in January, March and April, but high in May (12 isolates) and low in February and June (2 isolates). Ramna Lake demonstrated high monthly variation as there were no isolates during the months from January to April, a high volume of May (15 isolates), which was followed by the decline in June (9 isolates), indicating this is a seasonal phenomenon.

4.3.2 Results of Plasmid for *Vibrio cholerae*

4.3.2a Gel Electrophoresis Result of *Vibrio cholerae*

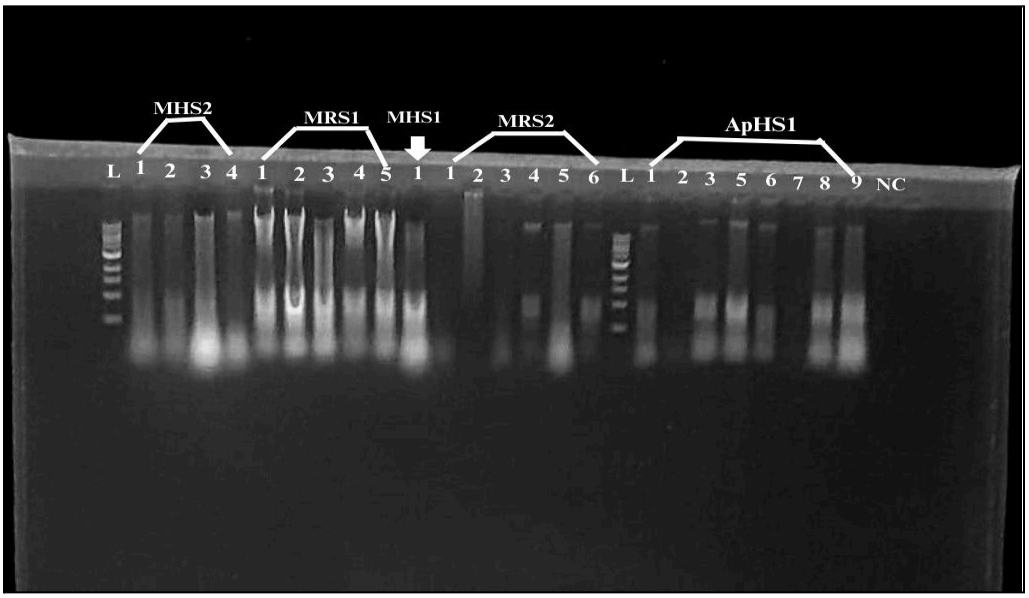


Figure 36: Agarose gel electrophoresis displays plasmid profiles of *Vibrio cholerae* isolates collected in May and April from Hatirjheel and Ramna Lake

Here, Agarose gel electrophoresis displays the plasmid profiles associated with *Vibrio cholerae* isolated from samples, with the initial lane (L) featuring a 1kb DNA ladder for size comparison. The first four lanes (1–4) in the first panel, from the left to the right, show plasmid samples coming from May Hatirjheel Sample-2. Plasmid positive was confirmed by the fact that all four plasmid samples (1, 2, 3, and 4) all had different bands. Next, five plasmid samples coming from May Ramna Lake Sample-1 (lanes 1, 2, 3, 4, and 5) were loaded. The gel electrophoresis analysis showed that all five samples had unambiguous plasmid-positive bands. After that, a single plasmid sample from May Hatirjheel Sample-1 (MHS1in lane 6) was loaded and showed plasmid-positive results. After that, six plasmid samples from May Ramna Lake Sample-2 (lanes 1, 2, 3, 4, 5, and 6) were tested, and all of them had plasmid-positive bands. Before the following set of samples, a 1kb ladder was reloaded. In the second panel, 9 plasmid isolates from April Hatirjheel Sample-1 were put into lanes 1–9. Lane 7 from April Hatirjheel Sample-1 (7) did not display any plasmid bands, which means that the result was plasmid-negative. But all of the other samples (lanes 1, 2, 3, 4, 5, 6, 8, and 9) had plasmid-positive bands, which means that these samples are also plasmid-positive.

4.3.2b Table of Plasmid Results

Table 40: The monthly distribution and plasmid positivity of *Vibrio cholerae* isolates at Hatirjheel according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
Hatirjheel	January S1	2	2	2	0	100
	January S2	6	6	6	0	100
	February S1	0	-	-	-	0
	February S2	12	12	12	0	100
	March S1	1	1	1	0	100
	March S2	0	-	-	-	0
	April S1	9	8	8	1	88.89
	April S2	0	-	-	-	0
	May S1	1	1	1	0	100
	May S2	5	5	5	0	100
	June S1	2	2	2	0	100
	June S2	3	3	3	0	100
Total		**41**		**40**		

Table 41: The monthly distribution and plasmid positivity of *Vibrio cholerae* isolates at Curzon Hall according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
	January S1	1	1	1	0	100

Curzon Hall	January S2	0	-	-	0	0
	February S1	0	-	-	-	0
	February S2	0	-	-	-	0
	March S1	0	-	-	-	0
	March S2	0	-	-	-	0
	April S1	0	-	-	-	0
	April S2	0	-	-	-	0
	May S1	0	-	-	-	0
	May S2	2	2	2	0	100
	June S1	0	-	-	-	0
	June S2	0	-	-	-	0
Total		**3**		**3**		

Table 42: The monthly distribution and plasmid positivity of *Vibrio cholerae* isolates at Ramna Lake according to the analysis of gel electrophoresis.

Sampling site	**Month**	**No. of isolated plasmid**	**Band observed in gel**	**Plasmid positive**	**Plasmid negative**	**Positivity (%)**
Ramna Lake	January S1	0	-	-	-	0
	January S2	0	-	-	-	0
	February S1	0	-	-	-	0
	February S2	1	1	1	0	100
	March S1	0	-	-	-	0
	March S2	0	-	-	-	0
	April S1	0	-	-	-	0
	April S2	0	-	-	-	0

	May S1	6	6	6	0	100
	May S2	6	6	6	0	100
	June S1	0	-	-	-	0
	June S2	1	1	1	0	100
Total		**14**		**14**		

4.3.2c Pie Chart of the Plasmid Positive Results

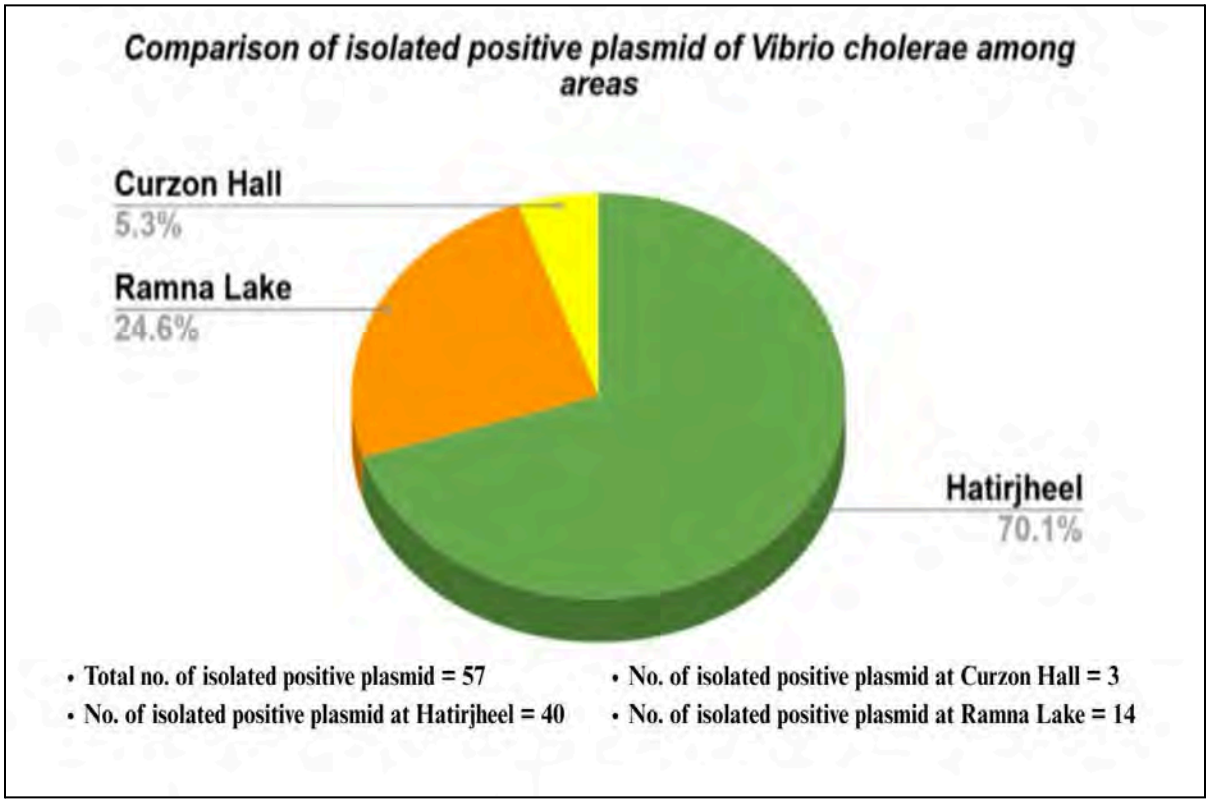


Figure 37: Comparative distribution of plasmid-positive *Vibrio cholerae* isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

The chart shows that Hatirjheel had the most plasmid- positive *Vibrio cholerae* isolates, making up 70.1% of all the isolates. Ramna Lake came next with 24.6%, and Curzon Hall had the lowest percentage at 5.3%. Overall, Hatirjheel is the most likely place for the plasmid-positive *Vibrio cholerae* to be found, compared to the other places.

4.3.2d Column Chart of the Plasmid Positive Results

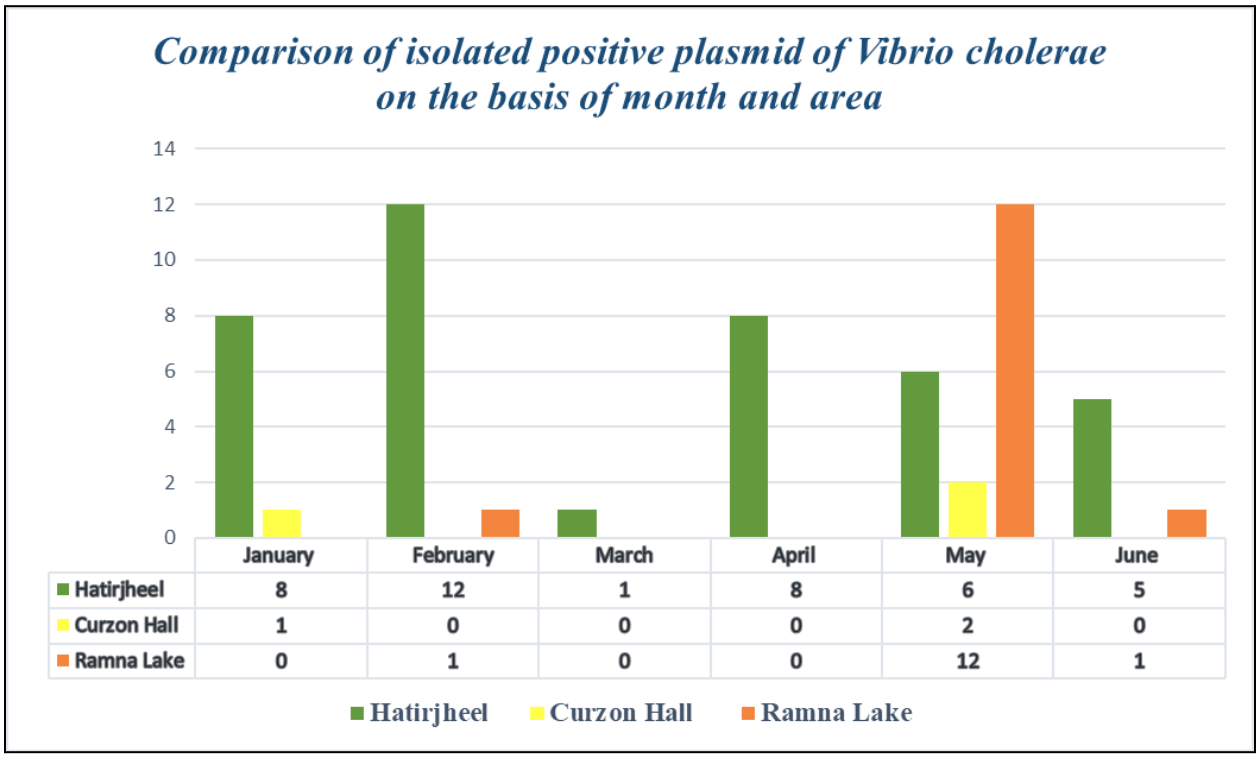


Figure 38: A monthly comparison of plasmid-positive *Vibrio cholerae* isolates in three sampling areas: Haltirjheel, Curzon Hall, and Ramna Lake.

The figure shows how plasmid-positive *Vibrio cholerae* isolates were spread out throughout six months (January to June) and three places: Hatirjheel, Ramna Lake, and Curzon Hall. There were consistently the most plasmid-positive isolates in Hatirjheel, with the most in February (12 isolates) along with May (6 isolates). In February, Curzon Hall had only one isolate, and in April, it had two. This was the lowest rate of plasmid isolation. Ramna Lake showed considerable plasmid-positive isolation, alongside a clear peak in May (12 isolates). The data shows that *Vibrio cholerae* is more common in the spring as well as the beginning summer, especially in bodies of water including Hatirjheel in addition to Ramna Lake.

4.3.3 Results of Plasmid for *Escherichia coli*

4.3.3a Gel Electrophoresis Results

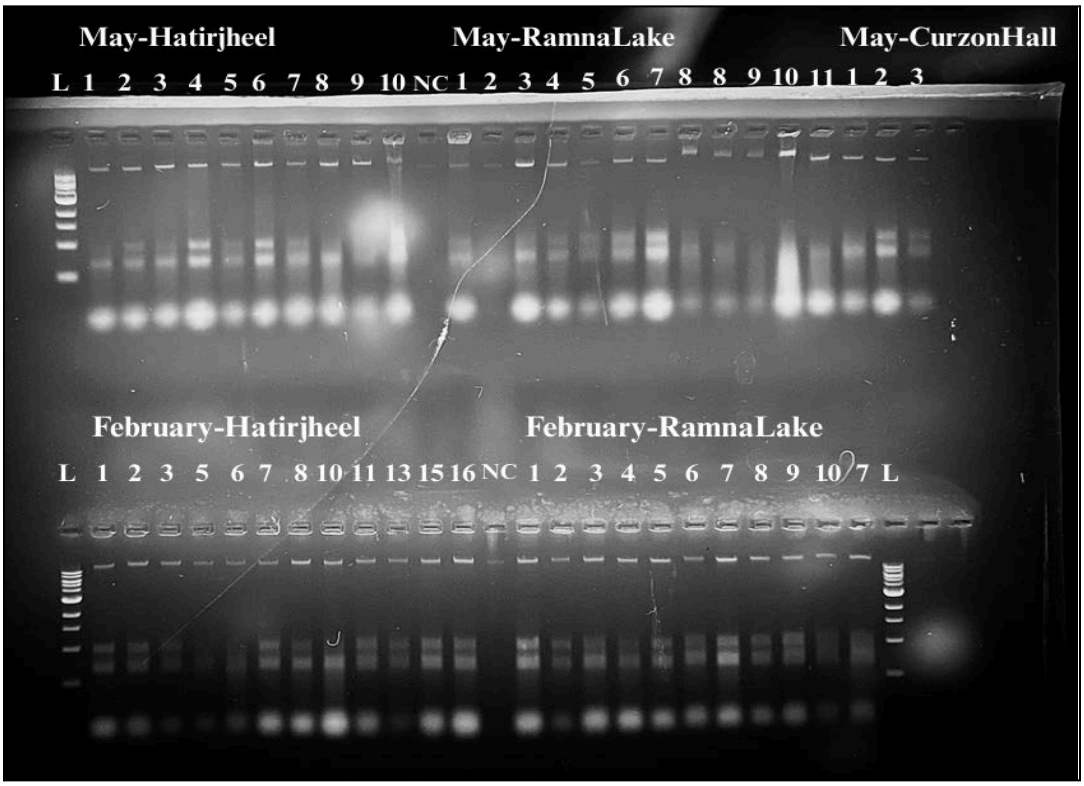


Figure 39: Agarose gel electrophoresis displays plasmid profiles of *E. coli* isolates among three sampling areas: Hatirjheel, Curzon Hall, and Ramna Lake

The comparative distribution of plasmid-positive. isolates In the May-Hatirjheel samples, 10 plasmid isolates were tested and clear plasmid bands were seen in each of the lanes which means that 100% of the isolates were plasmid-positive. In May-Ramna Lake samples, 11 isolates were analyzed. Plasmid DNA was present in all 11 lanes and confirmed that all the isolates harbored plasmid DNA. On the same note, in the case of the May- Curzon Hall samples, 3 samples were taken through plasmid analysis and all three samples had visible plasmid bands, implying total plasmid positivity.

In the case of the February-Hatirjheel collection, 16 isolates were re-streak from the stock, and 12 of them were chosen to analyze the plasmid. Results of all 12 isolates showed positive plasmid bands despite relatively weak visualization of bands in some of the lanes. In the example

of February-Ramna Lake, 10 isolates were examined, all of which produced clear plasmid bands with the result of 100 plasmid positivity.

The molecular size marker was 1 kb DNA which was three times inserted to determine the approximate size of plasmid bands. Negative control (NC) did not have any visible bands, which verified that no contamination was present, and testified to the effectiveness of the plasmid extraction and electrophoresis process. Nonetheless, due to technical error, isolate 8 of the May-Ramna Lake, and isolate 7 of the February-Ramna Lake was loaded twice.

4.3.3b Table of Plasmid Results

Table 43: The monthly distribution and plasmid positivity of *Escherichia coli* isolates at Hatirjheel according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
Hatirjheel	January S1	11	11	11	0	100
	January S2	10	10	10	0	100
	February S1	8	8	8	0	100
	February S2	12	12	12	0	100
	March S1	12	12	12	0	100
	March S2	12	12	12	0	100
	April S1	6	6	6	0	100
	April S2	12	12	12	0	100
	May S1	10	10	10	0	100
	May S2	12	10	10	2	83.33
	June S1	11	11	11	0	100
	June S2	7	7	7	0	100
Total		**123**		**121**		

Table 44: The monthly distribution and plasmid positivity of *Escherichia coli* isolates at Curzon Hall according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
Curzon Hall	January S1	12	12	12	0	100
	January S2	0	-	-	-	0
	February S1	4	4	4	0	100
	February S2	0	-	-	-	0
	March S1	0	-	-	-	0
	March S2	7	4	4	3	57.14
	April S1	4	4	4	0	100
	April S2	12	12	12	0	100
	May S1	3	3	3	0	100
	May S2	12	11	11	1	91.67
	June S1	13	13	13	0	100
	June S2	3	3	3	0	100
Total		**70**		**66**		

Table 45: The monthly distribution and plasmid positivity of *Escherichia coli* isolates at Ramna Lake according to the analysis of gel electrophoresis.

Sampling site	Month	No. of isolated plasmid	Band observed in gel	Plasmid positive	Plasmid negative	Positivity (%)
	January S1	0	-	-	-	0
	January S2	0	-	-	-	0

Ramna Lake	February S1	9	8	8	1	88.89
	February S2	10	10	10	0	100
	March S1	1	1	1	0	100
	March S2	0	-	-	-	0
	April S1	4	4	4	0	100
	April S2	0	-	-	-	0
	May S1	11	11	11	0	100
	May S2	10	10	10	0	100
	June S1	0	-	-	-	0
	June S2	10	10	10	0	100
Total		**55**		**54**		

4.3.3c Pie Chart of the Plasmid Positive Results

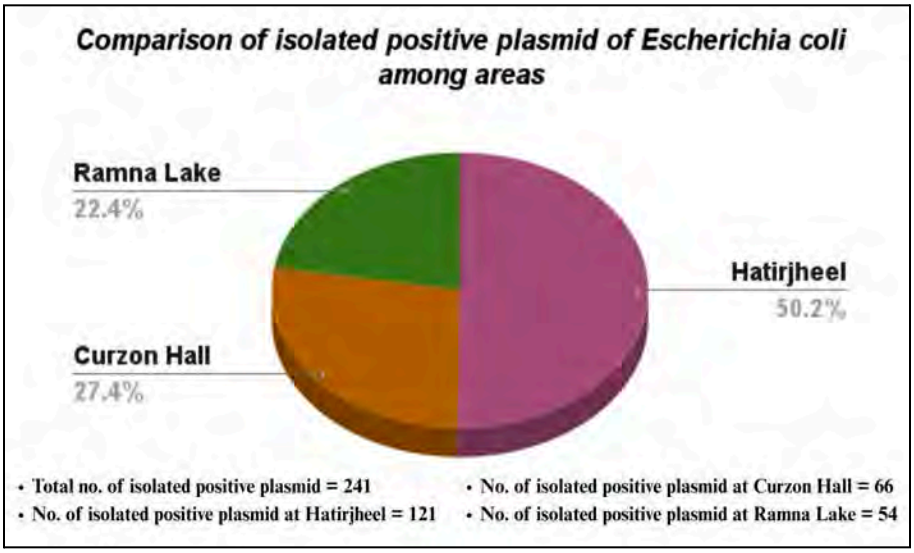


Figure 40: Comparative distribution of plasmid-positive *E. coli* isolates in three areas of sampling: Hatirjheel, Curzon Hall and Ramna Lake.

The distribution of the plasmid-positive *Escherichia coli* isolates in the area of Hatirjheel, Ramna Lake, and Curzon Hall is represented by the figure. Out of the total 241 plasmid-positive isolates, the largest number of isolates was obtained in Hatirjheel that added 121 isolates (50.2%). This suggests that *almost 50% of the isolates* of the *E. coli* that harbors plasmids were a result of this region. Comparatively, Curzon Hall registered 66 plasmid positive isolates (27.4) that indicated a moderate distribution of strains across the sites examined. Ramna Lake had the lowest number of plasmid-positive isolates which were 54 (22.4%). In general, this figure is an indication that Hatirjheel had a relatively greater prevalence of plasmid-carrying *E. coli* than Ramna Lake and Curzon Hall.

4.3.d Column Chart of the Plasmid Positive Results

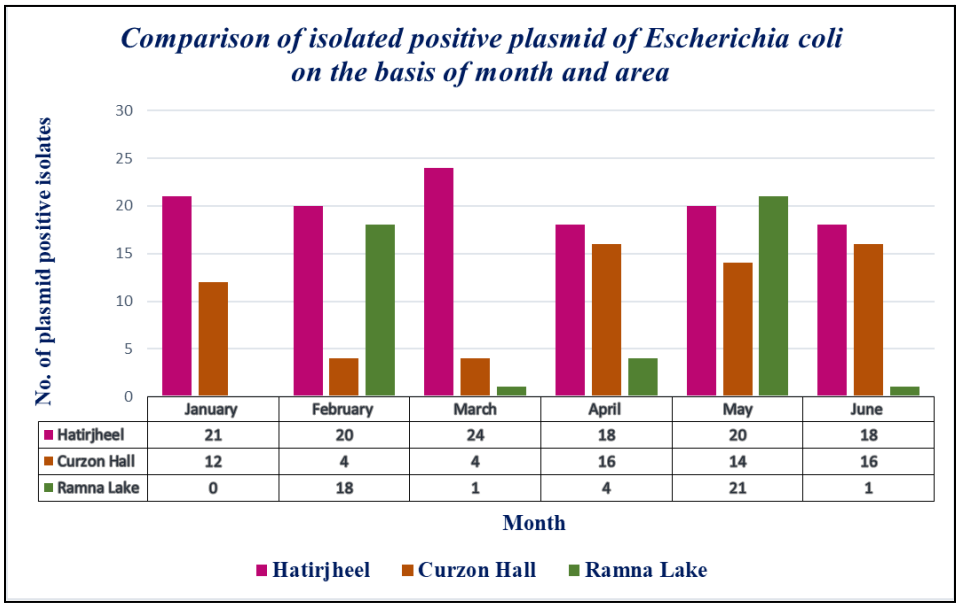


Figure 41: A monthly comparison of plasmid-positive *Escherichia coli* isolates in three sampling areas: Haltirjheel, Curzon Hall, and Ramna Lake.

The figure represents the monthly and local distribution of plasmid-positive *Escherichia coli* isolates that were obtained during the period of January to June in Hatirjheel, Curzon Hall and Ramna Lake.

Hatirjheel had a consistently higher figure of the number of plasmid-positive isolates than the other two sampling sites with the figures ranging between 18 and 24 isolates per month. Hatirjheel had the highest number recorded in March (24 isolates).

Curzon Hall showed intermediate values of plasmid positive isolates during the period of study and the monthly values ranged from 4 to 16 isolates.

Ramna Lake had a significant difference in the amount of isolates on a monthly basis, with no isolates in the months of January and March, but much higher numbers in the months of February (18 isolates) and May (21 isolates).

In general, the key finding of the figure is that the prevalence of *E. coli*, which had plasmids, was maintained by Hatirjheel during the study period, but Ramna Lake showed high monthly variability and Curzon Hall was an intermediate between months.

4.3.4 Plasmid Positive Results Based on Bacterial Species

Table 46: Distribution of plasmid-positive isolates based on bacterial species

Bacterial Species	**Places**			**Total**
	Hatirjheel	**Curzon Hall**	**Ramna Lake**	
Salmonella spp.	98	16	27	141
Vibrio cholerae	40	3	14	57
Escherichia coli	121	66	54	241
Total				439

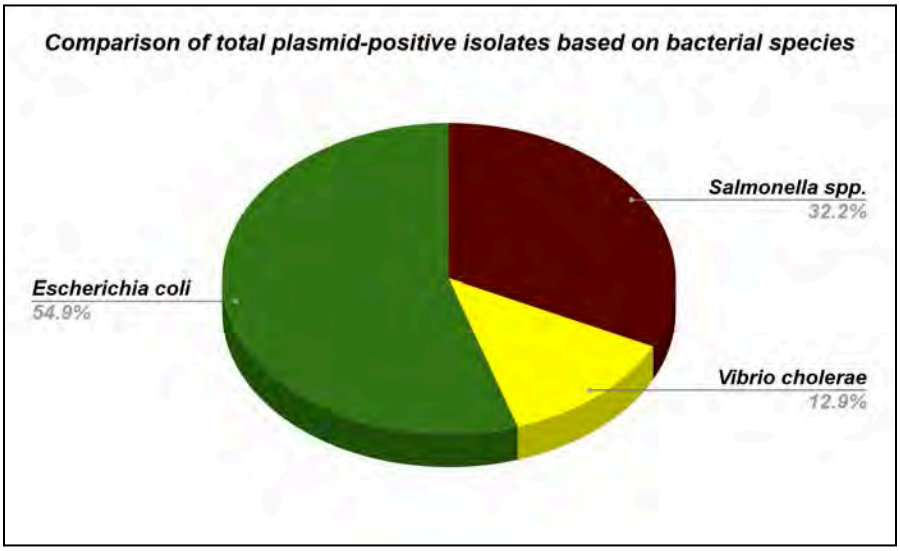


Figure 42: The comparison of plasmid-positive bacterial isolates among three bacterial species

The table and figure shows that the most common species within the plasmid-positive isolates is *E. coli* which counts over half of the total population 54.9% (241 isolates) with the vast majority being in all three sites though substantially lower in Ramna Lake (54) than the other two sites. *Salmonella spp.* comprises the second-largest proportion with a value of 32.2% (141 isolates) and is distributed across all the three locations but much lower in Curzon Hall (16). The smallest portion of the distribution for the *Vibrio cholerae* is about 12.9%, representing 57 isolates in total.

Chapter 5

Discussion

5.1 PCR analysis

5.1.1 Analysis of *Salmonella* spp.

5.1.1a Gel Electrophoresis analysis

In figure-5, the PCR detection of the *Salmonella* spp. in samples of January Hatirjheel revealed that a high prevalence of the pathogen is present in most test lanes, which indicates the existence of the expected amplicon (484-bp long) of *Salmonella* spp. Assay was directed towards *invA* gene, which is a highly conserved *Salmonella*-specific virulence gene and amplification of this fragment makes certain that the size of the amplicon was correctly verified (Malorny et al., 2003). 1 kb and 50 bp DNA ladders were used to mark the molecular size, as they are sure that the size of the amplicon was properly verified.

In SJaHS1, most PCR samples (lanes 1, 3-9) yielded the target band and only lane 2 did not amplify. In the same manner, in SJaHS2 the lanes 1-8 and lane 10 were definitely positive whereas lane 9 was negative. This frequency of detection ranks with earlier environmental PCR investigations, which document that PCR tests can identify *Salmonella* DNA in water and sediment with a great sensitivity (Touron et al., 2005). A positive control (PC) was previously known *Salmonella* spp. isolate, and formed a clear band at the same position, confirming the specificity of the primers and efficiency of the PCR, whereas no amplification was detected with the negative control (NC) confirming the specificity of the assays and ruling out contamination. Although the target bands have a distinct color, several non-specific amplification bands were observed in several lanes. These kinds of off-target bands are typical in environmental PCR experiments because of complicated DNA templates, secondary primer binding and differences in the sequences of different environmental isolates (Quaranta et al., 2007).

In general, the findings indicate a general occurrence of *Salmonella* in the samples of Hatirjheel and the use of PCR with the *invA* gene as the fast and sensitive method of monitoring the pathogen of the environment. PCR-based techniques tend to be more rapid and more frequently

detected as compared to traditional culture methods, particularly in sluggishly loaded matrices or heterogeneous populations of microbes (Kasturi and Drgon, 2017).

5.1.1b Table analysis for PCR positivity (Area and month wise)

Analysis of Monthly PCR Results of *Salmonella* spp. in Hatirjheel

According to table-5, a consistent high prevalence of *Salmonella* spp. was observed in the monthly PCR analysis of samples taken at Hatirjheel, representing constant contamination of the environment. Among 154 PCR samples, 114 samples (74.0%) were PCR-positive, which shows a high level of microbial loading in this water body. The highest level of detection was in January, 88.9% of them were positive at S1 and 90.0% were positive at S2, and in February the level of detection was also high (75.0% at S1 and 87.5% at S2). In March, positivity dropped to the minimum of 14.3% at S1 and 33.3% at S2, indicating that the environment is not favorable to work in temporarily. But in April an upward detection was recorded (88.2% at S1, 13.3% at S2) and this was also high in May (92.8% at S1, 83.3% at S2) and June, with S1 responding 100%. *Salmonella* is highly likely to survive and proliferate in such regularly high and persistent patterns of detection, which are typical of urban surface waters that are fed with untreated sewage, organic waste, and stormwater flow (Bell et al., 2021). Besides, slowing of water flow and nutrient build-up can also increase the retention and regeneration of bacteria in this ecosystem. The seasonal variation in March agrees with findings whereby seasonal environmental dilution and low levels of nutrients have the potential to keep bacteria undetected in a temporarily modifiable manner (Rocha et al., 2025). On balance, the numerical data validate that Hatirjheel is one of the chronic hot spots of contamination and long-term large-scale threats to people's health.

Analysis of Monthly PCR Results of Salmonella spp. in Curzon Hall

In table-6, PCR analysis of the samples of Curzon Hall proved a relative low and sporadic occurrence of *Salmonella* spp. over the time of the study. PCR-positive samples of PCR samples showed very low contamination, thus the site was not as contaminated as the others. There were no samples or detections made in January but in February, *Salmonella* was detected at only S1 (2 of the 3 samples) with 66.7% positivity and there were no positive S2 samples. Overall, there

was total undetection (0%) in March even after analysis of 11 samples in S2 whereas in April, it was undetected in both points of sampling. There was a sudden and a sharp increase in May and was 100% at S1 (2/2 samples), and 83.3% at S2, and again 100% at S1 in June, but no S2 samples were taken. This trend indicates that the contamination of *Salmonella* in Curzon Hall is episodic and must be related to temporal environmental factors like surface run-offs, or local wastewater sources (instead of the persistent sources of contamination) (Liu et al., 2018). The overall low detection facet implies that the environmental conditions in the background are less favorable to allow the long-term existence of bacteria. Nevertheless, the peaks occur under an episodic incidence, which means that contamination can grow very fast when good conditions occur. This variability is why it is especially important to initiate periodic monitoring despite the relatively low risks involved in a particular setting (Bell et al., 2021).

Analysis of Monthly PCR Results of *Salmonella* spp. in Ramna Lake

According to table-7, during the period of investigation, Ramna Lake had a moderate yet highly seasonal level of *Salmonella* spp. Among 42 PCR samples, the prevalence of PCR-positive samples (32) was 76.2 %, signifying an unusual contamination trend not existing across time. In January no samples were taken hence 0 percent detected whereas in February *Salmonella* was only detected in S1 only with 3 out of 8 samples (37.5) showing positive results and 0 showing negative results. No detection (0%) was observed at the two sites in March and April, which means that there was little contamination in these months. Conversely, there was a sharp rise in May, with 93.3% positivity at S1 and 100% positivity at S2 and high levels of detection also continued in June with S2 again registering 100% positivity and no samples were at S1. This marked seasonal trend can be explained by either prior research indicating that, in warmer months, stronger temperatures, more nutrient-toxic compounds and higher runoff can drive a great increase in Salmonella survival in surface waters (Cho et al., 2020). Use of recreation and added human occupations in the warmer seasons can also be a contributing factor to contamination. Ramna Lake may not seem to be a permanent reservoir but because of complete or underprivileged positivity that was observed in late summer and early monsoon, it is evident that there are specific periods when there is a high risk of patient health. This evidence demonstrates the use of seasonal-based surveillance strategies.

5.1.1c Pie chart analysis of total PCR Positive (Area wise)

In figure-6, the distribution of PCR-positive *Salmonella* spp. on an area-wise basis showed that there was a significant spatial variability of Salmonella spp. among the study sites. Among a total of 162 PCR-positive samples a total of 114 samples were positive in Hatirjheel (70.4%), 32 samples in Ramna Lake (19.8%), and 162 in Curzon Hall (9.8). The dominance of Hatirjheel with a majority of 2/3 of all positive samples is a clear indication that it is the centre of contamination. Ramna Lake also had a moderate contribution, with Curzon Hall making less than one-tenth of total positives. These spatial variations align with the prior information that urban water bodies that are subjected to severe anthropogenic load, discharge of untreated sewage, and stagnant water organisms permanently display much higher levels of *Salmonella* (Cho et al., 2020). Hydrological differences, input of waste, and immediate land utilization probably increase these disparities. In addition, long-term pollution indicates long-term impacts, as there has been persistent pollution in Hatirjheel. These findings indicate that environmental setting and the role played by human activity are critical in defining spatial contamination of *Salmonella*.

5.1.1d Column Chart analysis of total PCR Positive (Area and month wise)

In figure-7, the distribution of monthly and area-wise PCR-positive *Salmonella* spp. isolates showed clear temporal and spatial difference throughout the study sites. The maximum number of PCR-positive isolates were always found at Hatirjheel having 17 isolates in January, 26 isolates in February, 6 isolates in March, 15 isolates in April, 23 isolates in May, and the highest of 27 isolates in June indicating continuous contamination during the study time. Curzon hall had 0 positive isolates in January, 2 positives in February, 0 positives in April, a total of 12 positives in May and 2 positives in June, showing that there were no regular contamination events. Ramna Lake had 0 positives in January, 3 positives in February, 0 positives in March and 0 positives in April then the seasonal increase is clear with 17 positives in May and 12 positives in June. These statistical patterns indicate that in Hatirjheel, continuously surviving Salmonella's is under stable conditions, and in Curzon Hall and Ramna Lake, short-term environmental and seasonal drivers affect the conditions (Bell et al., 2021). Temperature and rainfall are climatic factors that would contribute significantly to the variations in a month. Patterns of human activity can also

contribute to contamination during the high months (Cho et al., 2020). All in all, the evidence highlights both the effect of environmental peculiarities of a site and the effect of time implications on the distribution of *Salmonella*.

5.1.2 Analysis of *Vibrio cholerae*

5.1.2a Gel Electrophoresis analysis

The figure 8 reflects an agarose gel electrophoresis product illustrating molecular identification of *Vibrio cholerae* in water samples taken in May in Hatirjeel, Curzon Hall, or Ramna Lake. PCR amplification was conducted based on specific primers to ompW gene; the gene of an outer membrane protein that is commonly deemed a species-specific biological protein marker of *V. cholerae*. The presence of clear amplification bands at a spot of about 592 bp that is related to the anticipated size of the ompW amplicon will be an indication of successful and specific amplification (Ranjbar et al, 2016). A molecular size marker (1 kb DNA ladder L) was used, and positive control (PC; clinical strain C6706) produced a specific band at the expected position, which validates specificity of the primer and efficacy of the PCR. The negative control (NC), which had nuclease-free water instead of template DNA, amplified not, meaning that it was not contaminated. The geographical location analysis further showed that the isolates of Hatirjheel (VMHS2; lanes 1-5) gave good and consistent 592 bp bands, which indicates that an environment is stable and could be loaded with anthropogenic activities and nutrient enrichment. The isolates (VMCS2; lanes 1-2) had detectable but relatively lower amplification, which suggests less prevalence. The presence of 592 bp at lanes 1-6 in Ramna Lake (VMRS1; lanes 1-7) indicates that the PCR was positive and the bacterial population was in active circulation, but the failure to amplify the 592 bp band in lane 7 indicates heterogeneity within the bacterial population. Altogether, the gel pattern indicates that throughout May *V. cholerae* was distributed most favorably and consistently across all the sampling sites followed by somewhat less in Curzon Hall and slightly higher in Ramna Lake and in Hatirjheel, which explains the significance of ompW-based molecular surveillance in monitoring the presence of this pathogen in the environment.

5.1.2b Table analysis for PCR positivity (Area and month wise)

Discussion of Results of Monthly PCR Results in the Analysis of *Vibrio cholerae* in Hatirjheel Samples.

According to table 8, the prevalence of *Vibrio cholerae* in Hatirjheel was high over the period of time and this indicates that the site was more polluted compared to the two others. Bacterial presence was evidenced by the consistent presence of bacteria in 42 out of 103 samples analysed with PCR. Detection in sampling points and months were not uniform with each other showing unequal spatial distributions. It was found to be moderate in January and only detected at S2 in February with heterogeneous spread. The detection levels were low but consistent in March, and a definite peak was observed in April, at which the highest positivity rate (71.42) was observed as evidence of favourable environmental support of the bacterial persistence. Even though May and June had relatively less positivity, the presence of *V. cholerae* continued to be detected in both locations suggesting that they are still circulating. The overall finding of the temporal variations and repetitive PCR positivity was an indication of a long-term environmental reservoir and possible risk to the population in Hatirjheel (Ferdous et al., 2018).

Discussion of Results of Monthly PCR Results in the Analysis of *Vibrio cholerae* in Curzon Hall Samples.

In table 9 , Curzon Hall samples depicted the fewest total *Vibrio cholerae* found at the three sites thereby showing a relatively small amount of contamination. Out of 13 samples undergone throughout the time of conducting the study, only 3 samples were positive by PCR. Identification was very intermittent and time bound. One sample of S1 was analysed and tested positive in January with a nominal positivity of 100% which is however not an accurate reflection of prevalence due to the very small sample size. Between February and April, there was no sampling and/or no PCR amplification, which showed the absence of the organism. In May, S1 revealed no positive results after processing the samples, but S2 showed moderate identification with a rate of positivity of 33.33%, which suggests that bacteria were present for a short time. Sample S1 and S2 June were homologically PCR-negative, and this pattern suggests the absence or reduction of *V. cholerae* towards the end of the sampling period. In general, the Curzon Hall

location had low and patchy detection, which suggests that the environmental burden of *V. cholerae* is relatively lower than in Hatirjheel and Ramna Lake (Righetto et al., 2015).

Discussion of Results of Monthly PCR Results in the Analysis of *Vibrio cholerae* in Ramna Lake Samples.

According to table 10, the data from Ramna Lake demonstrate that *Vibrio cholerae* is occasionally found at moderate levels, but only during certain times of the year. PCR positive is clearly clustered throughout time. There were 27 samples tested, and 14 of them were PCR-positive. This shows that the site was only moderately contaminated compared to the others. There was no detection in January as well as March, which means that there were very few or no bacteria present during those months. February demonstrated isolated detection at S2, with 100% positivity coming from a single sample. This shows that the occurrence is intermittent. Samples from April exhibited very little detection, resulting in only PCR-negative outcomes at S1. In May, there was a big spike, with both sample points showing high detection rates. S1 had a 50% positivity rate and S2 had a 54.55% positivity rate, which meant that conditions were good for bacteria to grow during this time. Detection decreased again in June with a single positive sample at S2 (50% of limited samples). In general, the *V. cholerae* detection in Ramna Lake showed definite seasonality, the highest being observed in late spring to early summer, which should be associated with environmental conditions like.

5.1.2c Pie chart analysis of total PCR Positive (Area wise)

Figure 9 shows the percentage of PCR-positive *Vibrio cholerae* isolates in Hatirjheel, Ramna Lake and Curzon Hall making a clear indication that there is a high degree of spatial variation which is attributed by environmental factors as well as the intensity of human activity. Hatirjheel had the largest percentage of positive (71.2%; 42 out of 59), thus it is considered one of the biggest hotspots. This increased prevalence must be related to high-density residential environments, uninterrupted human traffic, domestic discharge that remains untreated, industrial runoffs, and relatively stagnant water masses that favor the survival and maintenance of bacteria over time. Ramna Lake displayed a moderate rate of detection (23.7%; 14 out of 59), indicating that seasonal contamination was caused by recreational human action, urban runoff and nutrient enrichment, but relatively improved water management could restrain widespread accumulation

of bacteria. Curzon Hall on the other hand was the least positive (5.1%; 3 out of 59) which points to low input of sewage, less stagnant water areas, and a more controlled anthropogenic influence. On the whole, the comparison underlines the fact that environmental factors along with the type and the magnitude of human activity contribute significantly to the development of the spatial distribution and the possible risk of the *V. cholerae* to human health among the urban water bodies investigated.

5.1.2d Column Chart analysis of total PCR Positive (Area and month wise)

The figure 10 indicates evident month-wise and area-wise difference between the PCR-positive isolates of the *Vibrio cholerae* between the three areas at the same time throughout Hatirjheel, Ramna Lake, and Curzon Hall, which is indicative of dissimilar environmental conditions and human actions. Hatirjheel had always shown the best results with highest recovery in February (12 isolates) and April (10 isolates) indicating that the conditions were favourable like increased temperature, nutrient enrichment, stagnant water and sewage that favour bacterial endurance. Hatirjheel was positive even in months when overall negative was detected, which is a good indication of a steady environmental reservoir, and the minor drop in May and June can be ascribed to rain or higher water flow. Curzon Hall exhibited the least regularity pattern and the fewest occurrences, with only 1 and 2 isolates detected in January and May, respectively, possibly because of a low reason of sewage discharge, little amount of organic waste, and considerably regulated anthropogenic activity, which led to the reduced long-term bacterial survival. Conversely, the data of Ramna Lake were highly seasonal with no fertilizer detected during January, March, and April, a sporadic one in February (1 isolate) and a high peak in May (12 isolates), potentially associated with an increase in temperature and nutrient load and recreational activity, and then a sharp decrease in June (1 isolate). In total it can be highlighted that Hatirjheel was the most constantly in contamination, followed by the Ramna Lake, which showed moderate but seasonally dependent contamination, and Curzon Hall, which corresponded to a relatively low-risk area, indicating the prominence of local environmental determinants and the need to conduct site-specific monitoring of *V. cholerae* in the context of potential human health risks. In general, Hatirjheel was the most contaminated point in all months, Ramna Lake was medium in contamination and peak contamination was strong at certain seasons, and Curzon Hall was a relatively low-risk location. The lack of synchronisation of the peaks in the sites

points to the local environmental effect as opposed to homogenous seasonal drivers. These results show that the distribution of *V. cholerae* is influenced by complicated mechanisms between water quality, nutrient load, hydrology, and human activity that underscores the problem of site-specific monitoring and specific interventions in years of high risk.

5.1.3 Analysis of *E. coli*

5.1.3a Gel Electrophoresis analysis

A high PCR positivity rate at all the sampling sites confirmed a high rate of *Escherichia coli* at the sampled sites in Hatirjheel, Curzon Hall, and Ramna Lake as exhibited by PCR amplification of the isolates collected in April. The majority of the isolated provided clear amplification bands, which proved that the chosen primers were specific and capable of detecting the target *E. coli* gene. The abundance of PCR positive isolates, especially of the Sample-2 of Hatirjheel and Curzon Hall, can be explained by the fact that the presumptive colonies of *E. coli* used in the selection were previously selected on selective media and morphology, and then, the isolates were molecularly confirmed.

There were only a few isolates of April Hatirjheel (Sample-1) that did not amplify the DNA bands and were pronounced as PCR-negative. This could mean that these isolates were non-target bacteria of similar morphological features that survived under selective culture conditions that are regularly observed in environmental microbiology studies (Ramirez-Castillo et al., 2015). The absence of bands can also be caused by low DNA concentration, degradation of the template, or the inhibitors of PCR (humic acids, organic substances, or salts) which prevent the activity of DNA polymerase (Sidstedt et al., 2020).

The amplification bands found in the negative control indicates non-standard based result and was most likely a result of contamination during the PCR set up through aerosolised DNA, contaminated reagents or improper pipetting process. One of the known issues in molecular laboratories is the cross-contamination of targets, especially in cases of work with high concentrations of target DNA (Bustin & Nolan, 2020). Nevertheless, the positive control gave strong amplification, indicating that the PCR reagents, primers, and cycling conditions were working well and demonstrated the amplification system.

Generally, the high PCR positivity rates of the isolates of the three water bodies indicates continuous fecal contamination and the credibility of PCR as a confirmative technique of identifying *E. coli* in environmental samples. PCR is well known as highly specific and sensitive in comparison with traditional phenotypic identification techniques (Ramirez-Castillo et al., 2015). Overall, these results indicate the successful molecular detection of *E. coli* in urban water sources and the limited negative reaction and contamination-related problems highlight the necessity of observing aseptic precautions and the correct handling of reagents in the environmental molecular analysis.

5.1.3b Table analysis for PCR positivity in *Escherichia coli* (Area and month wise)

Interpretation of Hatirjheel Samples:

PCR analysis revealed that the *Escherichia coli* persisted in the course of the study in Hatirjheel. In a loaded sample of 143, the proportion of PCR-positive samples was 124, which means that the overall prevalence of contamination is large. The two sampling points recorded high rates of detection in January, with S1 being 100% positive and S2 being 83.33, indicating that the contamination occurred on a large scale during the initiation of the sampling. S2 was 100% positive and S1 dropped to 50% in February, which is a sign of spatial variation in contamination. Both S1 and S2 recorded high positivity levels in March (85.71%), which indicates fairly steady contamination.

S1 recorded a steep fall in April (28.57) but S2 was entirely positive (100%), meaning there was an uneven distribution of contamination in the site. The positivity of S1 and S2 was moderate to high at 58.33 and 100 percent, respectively. The contamination grew once again in June with S1 demonstrating 100% positivity and S2 demonstrating 75%. Altogether, Hatirjheel showed the continuous and extensive contamination of the *E. coli* with a significant temporal and spatial fluctuation, which indicates the constant fecal pollution and makes PCR a sensitive technique to detect the environment (Moinet et al., 2024).

Interpretation of Curzon Hall Samples:

At Curzon Hall samples, *E. coli* was found in intermediate but inconsistent concentrations, out of 84 loaded samples 73 being positive in the PCR test, meaning that the contamination is

intermittent. S1 (sample-1) was very positive (92.31 percent) in January and no samples were found in S2. In February, they were only detected in S1 with 66.67% while S2 recorded 100% positivity in March, which showed localised contamination, with no samples of S1.

S1 (83.33%) and S2 (100%) were very positive in April indicating that there was a favorable environment in which bacteria could survive. A significant decrease was observed in S1 (27.27%), whilst S2 did not change significantly (91.67%). S1 remained 100% positive and S2 fell hugely down to 33.33 in June. In general, Curzoon Hall had a high level of temporal and spatial variability in detecting *E. coli*, which is typical of urban water bodies that are subject to varying environmental and human-induced factors (Moinet et al., 2024).

Interpretation of Ramna Lake Samples:

Ramna Lake displayed overall contamination of *E. coli* which was seasonally variable. Out of 58 loaded samples, 57 were PCR positive, which means that the contamination levels are large. In January, no samples were found. The highest detection was recorded in February, S1 was 90% and S2 was 76.92%. S1 was 100% positive in March (S2 samples were not available).

On the same note, S1 was a 100% positive in April while S2 samples were not found. Both points of sampling were equally positive in May (91.67%), and S2 was still positive (91.67%) in June even though there were no samples from S1. In general, Ramna Lake showed a continued contamination with a significant seasonal fluctuation, indicating a long-term fecal pollution based on environmental and seasonal conditions, which are efficiently identified by a molecular technique like PCR in environmental waters (Moinet et al., 2024).

5.1.3c Pie chart analysis of total PCR Positive (Area wise)

The pie chart analysis showed obvious geographical differences in the occurrence of bacteria in the three sampling sites. Out of the total 254 PCR positive isolates the highest proportion was found in Hatirjheel (48.8, 124 isolates), Curzon Hall (28.8, 73 isolates) and Ramna Lake (22.4, 57 isolates). This distribution indicates that the targeted bacterial genetic marks are concentrated in Hatirjheel as a source of major environmental reservoirs.

The prevalence is higher in Hatirjheel and this could be related to increased exposure to human sources of contamination like domestic sewage drainage, surface runoff and frequent human activities. The urban lakes receiving inflows of wastewater are regularly fecally contaminated, which promotes the appearance of enteric bacteria in water sources (Ahmed et al., 2023). Conversely, Ramna Lake had a relatively lower PCR positivity, which might result in less direct wastewater addition, periodical cycling of water and regular maintenance activities that might curb microbial build-up.

Curzon Hall was characterized by a different trend as it had the highest activity in April (19 isolates) and reduced to four isolates in February. The irregular contamination events may create such fluctuations, or the laboratory drainage input may not be constant, or the human activity can be intermittent, but not sustained sources of pollution (Korajkic et al., 2024)

Taken together, the findings reveal that PCR-positive *E. coli* is not uniformly distributed per year but rather subject to seasonal changes, in terms of temperature, the pattern of rainfall, as well as anthropogenic factors. Nevertheless, these temporal variations did not render Hatirjheel less significant than the other locations in terms of being a constant source of PCR-positive isolates, which, in turn, supported the idea that it was a constant contamination hotspot in the urban water system (Ahmed et al., 2023).

5.1.3d Column Chart analysis of total PCR Positive (Area and month wise)

The distribution of the PCR-positive isolates on a monthly basis revealed significant temporal fluctuation within the six months of the study period and this implies that seasonal and environmental factors have an impact on the prevalence of bacteria in urban water systems. Even though 254 PCR-positive isolates were observed, their distribution per month across locations of sampling varied.

Hatirjheel had a steady detection in all months and PCR-positive isolates of 16-24. The highest number of peaks was observed in January and February (24 isolates each), and March, which implies that moderate temperatures, a decrease in water movement, and a stable environment during the late winter and early spring may promote the survival and persistence of bacteria. Such a minor decrease as that in April could be linked to higher levels of water disturbance or dilution influences, which are also known to affect the concentration of bacteria in the surface waters (Korajkic et al., 2024).

The greatest temporal change occurred in Ramna Lake whereby there were no PCR-positive isolates in January and maximum number (22) in May of all sites sampled. This growth can be associated with the elevation in temperature, availability of more nutrients and the build up of organic materials during the pre-monsoon season, which support the growth of bacteria. This fact can be attributed to the lack of isolates in January, which might be explained by the lower temperature and the increased dilution of contaminants in colder seasons (Ahmed et al., 2023).

The trend of Curzon Hall was fluctuating with the maximum number of the detection in April (19 isolates) and the minimum (4 isolates) in February. Such fluctuation can be a result of intermittent pollution through lab drainage systems, inconsistent human activities and intermittent pollution sources and not constant environmental pollution (Ahmed et al., 2023; Korajkic et al., 2024).

In general, the results show that the PCR positivity in urban water bodies is seasonal depending on the temperature, rainfall, and anthropogenic contributions. Despite these variations, Hatirjheel, nevertheless, produced the most PCR-positive isolates, which is why it can be proposed that it is a chronic contamination hot spot in the urban aquatic environment (Korajkic et al., 2024).

5.1.4 Table and Pie chart analysis of total PCR Positivity (Species wise)

The table and pie chart demonstrated the distribution of PCR-confirmed bacterial isolates by species in Hatirjheel, Curzon Hall and Ramna Lake. Four hundred and seventy five PCR-positive isolates were detected and the prevalence of these isolates varied across the bacterial species.

The largest proportion was *Escherichia coli* (53.5% or 254 isolates) of the total PCR-positive samples. It means that *E. coli* was the most consistently observed bacterium during the study period of time and is a widely used indicator of fecal pollution of water. Hatirjheel had the maximum number of *E. coli* isolates (124) and Curzon Hall (73) and Ramna Lake (57). *E. coli* is a common cause of fecal pollution in aquatic systems caused by humans and animals and is associated with its capacity to survive under a variety of water conditions (Sultana et al., 2025).

The second predominant bacterial group was *Salmonella* spp. which included 34.1% (162 isolates) of the total PCR-positive samples. The highest percentage of *Salmonella* isolates (114) was produced by Hatirjheel, then Ramna Lake (32), and Curzon Hall (16). *Salmonella* spp. in

surface water has been detected in urban environments, where wastewater will evidently be discharged and run-off that further promotes the spread of enteric pathogens and related health hazards afforded to the population (Siddique et al., 2024).

Vibrio cholerae was the least prevalent with 12.4% (59 isolates) of the total PCR positive samples. The majority of *V. cholerae* isolates were observed in Hatirjheel (42), then Ramna Lake (14) and Curzon Hall (3).Reduced occurrence rate of *V. cholerae* in environmental waters compared to fecal indicator bacteria is in line with the fact that its distribution in the environment is specific to certain ecological parameters and might not be evenly distributed across locations (Awere-Duodu et al., 2025).

Altogether, the findings verify that *E. coli* was the most prevalent bacterial type in all locations of study, then *Salmonella* spp. and *V. cholerae*. The results of the species distribution observed shows the impact of the pollutant intensity, environmental factors, and the sources of contamination on the prevalence of the microbes in the urban waters. These results demonstrate the need to conduct regular molecular analyses of urban waters to determine the level of contamination and mitigate the possible health hazards caused by pathogenic organisms on the population by water.

5.2 Antibiotic Susceptibility Test(AST) Analysis

5.2.1 Occurrence of Multidrug-Resistant (MDR) and Extensively Drug Resistance (XDR) Bacteria

The level of multidrug resistant (MDR) bacteria was highest in *Salmonella* spp. (47.9%), then in *Escherichia coli* (43.6) and *Vibrio cholerae* (8.5) species. The increased rate of MDR in *Salmonella* spp. and *E. coli* can be related to their enteric origin and common exposure to the antibiotic residues and resistant strains of bacteria associated with human and animal waste in the waters, as recent reports have indicated widespread MDR *E. coli* and associated enteric bacteria in surface water affected by human and animal activities (Sultana et al., 2025; Rana et al., 2025). On the other hand, the reduced level of MDR in *V. cholerae* indicates a relatively low level of multidrug resistance, and this is in agreement with the findings that *V. cholerae* has lower rates of resistance in comparison to other enteric organisms in the environment probably because of

ecological adaptation and selective pressure differences (Rana et al., 2025). When combined, these findings suggest that enteric bacteria are the primary cause of MDR in the water under investigation, and *Salmonella* spp. and *E. coli* are the primary reservoirs of multidrug resistance.

There was inconsistency in the prevalence of extensively drug-resistant (XDR) isolates since to be defined as XDR, the isolate has to be tested against a wide range of antibiotics representing more than one class. In the given study, only a small amount of antibiotic discs that represent rather a small variety of drug classes were used, thus even isolates with resistance to the majority of the antibiotics that were tested were not characterized as XDR based on the standard definitions. Thus, the negative finding of XDR does not mean that the environment is free of XDR organisms but instead, it is probably the result of the small antibiotic panel used in the susceptibility study, and therefore, extensive antimicrobial testing is advised to determine the accurate resistance position (Johura et al., 2024).

5.2.2 Comparison of Resistance Pattern: Hatirjheel, Curzon Hall and Ramna Lake

The distribution of MDR isolates among the sampling sites was clearly spatial with Hatirjheel supplying the greatest number of MDR bacteria (62.8%), followed by Curzon Hall (20.2%), and Ramna Lake (17.0%). The *Salmonella* spp. and *Escherichia coli* were found to be dominant at MDR, particularly at the Hatirjheel with the *Vibrio cholerae* being insignificant (Popoola et al., 2025). All these facts can be the reason for the high MDR load in Hatirjheel that is likely connected with the high rates of anthropogenic activity, i.e., the acidic runoffs, the discharge of sewage, and the active pollution of the environment. The outcome of this observation establishes Hatirjheel to be a large hot spot of MDR, yet the curzon hall and Ramna lake are also doing the same to a smaller extent which suggests that it is reservoirs of multidrug-resistant bacteria.

5.3 Plasmid analysis

5.3.1 Analysis of *Salmonella* spp.

5.3.1a Gel Electrophoresis analysis

The figure-33 shows plasmid profiling of *Salmonella* spp. isolates at Hatirjheel demonstrated that the frequency of plasmid carriage is very high with the majority of the isolates displaying

obvious and distinct plasmid bands on agarose gel electrophoresis. This implies that the plasmids are highly distributed in the environmental *Salmonella*, which justifies their contribution to the survival and adaptation of bacteria to environmental conditions (Carattoli, 2013). The possibility of having a plasmid-negative isolate helps indicate that there is some common, although not universal, carriage of the plasmid within the population. The difference in the size of the plasmid bands of the isolates suggests that there was a difference in size of the plasmid and a difference in the number of copies of the plasmid. The described diversity is inherent to environmental bacteria and is an indicator of active horizontal gene transfer that enables bacteria to gain new genetic characteristics, such as antimicrobial resistance and virulence factors (Rozwandowicz et al., 2018). It has been reported that urban water bodies serve as reservoirs of mobile genetic factors because of constancy in coming in touch with pollutants and antimicrobial remains, which establish a selection pressure to keep plasmids alive (Carattoli, 2013). The fact that no band is present in the negative control confirms that the plasmid extraction and electrophoresis processes were consistent and not contaminated. In general, the results indicate that ecologically, plasmid-mediated genetic diversity in environmental *Salmonella* is significant and that there is the probable danger to human health of plasmid-carrying pathogens in the surface waters.

5.3.2b Table analysis for Isolated Plasmid positivity (Area and month wise)

Interpretation of Results of Hatirjheel Samples

The table-37 demonstrates that the plasmid positive *Salmonella* spp. in Hatirjheel was invariably high, which pointed towards a highly contaminated water body having a chronically available environmental store of mobile genetic components that facilitates adaptability as well as the spread of antimicrobial resistance. Among 110 *Salmonella* isolates, 98 of them contained plasmids indicating extensive spread of plasmids throughout the bacterial population. It is consistent with environmental literature indicating that plasmids serve as carriers of resistance genes in waterborne bacteria as well as because they remain in polluted water bodies because of anthropogenic pollution and horizontal gene transfer processes (Mendoza-Guido et al., 2024). Both S1 and S2 showed a 100% plasmid positivity in January with gel electrophoresis showing visible bands which showed an early formation of plasmid bearing strains. February experienced a downward movement at S2 (78.6%) and S1 was high (91.7%), indicating the existence of space

heterogeneity at the source of localized pollution and streams. At both points again March was completely positive, indicating clearly stable maintenance of plasmids in the case of lower isolates. In April, high positivity was observed (S1: 93.3%, S2: 100%), and in May, at both locations, 100% plasmid positivity was observed, with the greatest manifestation of plasmid-carrying Salmonella. The month of June also demonstrated lower positivity and this was particularly low at S2 (63.6%), compared to S1 (81.3%) which could be as a result of season stressors, variations in water quality, or changes in the bacterial community. Such trends are in line with studies that show that environmental plasmids vary with pollution and other environmental stresses and as reservoirs of antimicrobial resistance determinants in aquatic environments (Barrantes et al., 2025).

Interpretation of Results of Curzon Hall Samples

According to table-38, Curzon Hall showed a low rate of *Salmonella* isolation but an absolute plasmid positivity of the initially detected isolates, showing that despite the rare occurrence of contamination events, any isolate that was observed was genetically prepared with plasmids. Throughout the study period, 16 isolates were obtained, all of which were positive with plasmids, which suggests a high level of interaction between the presence and carriage of plasmids in the *Salmonella*. In January, March or April no isolates were observed at S1 and S2 indicating that there was a low level of contamination or that there were unfavorable conditions that would support *Salmonella* survival. Only in February, plasmid-positive isolates were detected at S1 with 100 percent positivity, thus showing that contamination had occurred locally. Isolates were obtained in both S1 and S2, S2 providing more of the strains, and all were found to have plasmids, attributed to transient environmental or human effects. The isolates were once more only detected at S1 in June, with the result remaining 100% positive. This is in line with findings that show that the plasmid-based sources of resistance may last amid low prevalence of bacteria which is a reservoir of high risk genetic components in aquatic conditions even when bacterial recovery is low (Monte et al., 2025).

Interpretation of Results of Ramna Lake Samples

According to table-39, Ramna Lake established a moderate plasmid level of *Salmonella* isolates with seasonal and variable levels indicating oscillating environmental states. Among 32

recovered isolates 27 were plasmid positive indicating a high plasmid carriage. There were no isolates during January, March, or April in S1 and S2 indicating that the contamination was low during this time. Positive isolates of plasmids appeared in February found at S1 and were 100 percent positive, which suggested a small but genetically stable presence. May was characterized by a significant number of isolates made at both locations and a high level of positivity in site S1 (85.7%) and a 100% positivity in site S2 indicating good plasmid-bearing strain conditions. Isolates were only identified at S2 with low positivity (75%) in June reflecting a greater heterogeneity or partial loss of the plasmids. This temporal variability has been supported by ecological studies indicating that seasonal ecological factors such as rainfall, temperature and runoff can affect the occurrence and diversity of *Salmonella* in surface waters, which can consequently have an effect on the maintenance of plasmids and dissemination of resistance (Monte et al., 2025).

5.3.2c Pie chart analysis of total Isolated Plasmid positivity (Area wise)

The figure-34 shows a pie chart with distinct spatial variations on the plasmid carriage of the *Salmonella* spp., which is a significant predictor of genetic adaptability and antimicrobial resistance capability (Martínez, 2009). Among the 141 plasmid-positive isolates, the highest percentage was obtained with Hatirjheel (69.5% n = 98) and Ramna Lake (19.2% n = 27) and Curzon Hall (11.3% n = 16).

Hatirjheel predominantly has plasmid-positive isolates, which indicates that the environmental conditions in the area may be conditioned to favor a bacterial survival and plasmid maintenance, probably as a result of unremitting exposure to pollutants, antibiotics, heavy metal, and organic waste leading to such a selective pressure (Baquero et al., 2008). The plasmid stereotyping was moderate in Ramna Lake yet, possibly, due to urban drainage, leisure, and the seasonal enrichment of nutrients, this Lake was the factor that facilitated a potentially favorable condition in the transmission of plasmid-encoded phenotypes (Wellington et al., 2013).The lowest percentage was found in Curzon Hall, which was in line with the lowered percentage of bacterial recovery; nevertheless, the presence of plasmid in the all identified isolates shows that even low levels of contamination sites could have genetically prepared strains. On the whole, the city of Hatirjeel is a high-risk hotspot, Ramna Lake a moderate-risk area, and Curzon Hall a

comparatively low-risk zone, that is why environmental monitoring is needed based on the public-health applicability of plasmid-positive *Salmonella* (Martínez, 2009).

5.3.2d Column Chart analysis of total Isolated Plasmid positivity (Area and month wise)

The figure-35 shows a column chart that depicts significant spatial and temporal difference between plasmid-positive *Salmonella* isolates in the research locations. The highest numbers of isolates were registered in Hatirjheel, varying between 13 isolates in January to a maximum of 23 in May, where it was still detected in June (20 isolates), meaning that selective pressure was continuing to selectively favour retention of plasmid and horizontal gene transfer (Martínez, 2009). Curzon Hall had intermittent and inconsistent detection, with no plasmid-positive isolates in January, March, and April, restriction on detection in February and June (2 isolates each) however an intermittent increase in May (12 isolates), which indicated that there was no constant environmental persistence but sporadic contamination. There was a high seasonal variation in Ramna Lake, and it was not detected during January, March and April, and only a sudden surge of isolates was noted in May (15), then with a decrease in June (9) because it was probably affected by changes in seasonal temperature, nutrient availability and human activities. The findings taken together confirm the presence of Hatirjheel as a main source of Salmonella bearing plasmids, Ramna Lake as a seasonally-dependent moderate-risk premises, and Curzon Hall as a low-risk premise with sporadic presence, highlighting an issue of antimicrobial resistance genes distribution within the environment (Baquero et al., 2008).

5.3.2 Analysis of *Vibrio cholerae*

5.3.2a Gel Electrophoresis analysis

Figure 36 demonstrates agarose gel electrophoresis based plasmid profiling of *Vibrio cholerae* isolates in the *Vibrio cholerae* of Hatirjheel and Ramna lake in April and May. Plasmid DNA separation was done by size and visualized as a band, a 1 kb DNA ladder (L) was used to confirm the size estimation and precision in electrophoresis. In the May Hatirjheel Sample-2 (MHS2; lanes 1-4), there were clear plasmid bands in each lane, showing the presence of plasmids with difference in size/shape across the isolates. Equally, May Ramna Lake Sample-1 (MRS1; lanes 1-5) had all five plasmid isolates which had strong bands, indicating that plasmid

carriage was widespread. May Hatirjheel Sample-1 (MHS1) and May Ramna Lake Sample-2 (MRS2; lanes 1-6) also exhibited different plasmid bands in the entire isolates. One second 1 kb size marker was added before the analysis of the final cluster to be sure of the right size determination. Eight lanes of the April Hatirjheel Sample-1 (ApHS1; lanes 1-9) give visible plasmid bands, but no band appeared in lane 7, which indicated a plasmid-negative isolate. The negative control (NC) lane did not contain any bands, which proves that the procedure was correct and there was no contamination. It is evidenced by the presence of plasmid bands in most lanes that plasmid carriage is widespread among environmental *V. cholerae,* and that the presence of many different band locations-especially in Hatirjheel samples-is evidence of plasmid diversity, which in environmental *V. cholerae* are usually due to horizontal gene transfer and changing environmental pressures including pollutants or antimicrobial residues (Faruque et al., 2003). In general, this gel profile indicates that plasmids are common in these urban water systems, which could assist in increasing genetic plasticity and spreading virulence and antibiotic resistance genes in *V. cholerae*, making continuous monitoring of plasmids at the environmental reservoir level critical (Verma et al., 2019).

5.3.2b Table analysis for Isolated Plasmid positivity (Area and month wise)

Interpretation of monthly occurrence of Vibrio cholerae plasmid-positive in Hatirjheel:

The table 40 shows the distribution of Vibrio cholerae isolates and plasmid positivity in Hatirjheel on a monthly basis. Subsequent agarose gel electrophoresis showed the presence of high levels of plasmid carriage in all the *V. cholerae* isolates throughout the study period. Of the 41 isolates analysed, 40 appeared to be plasmid positive, suggesting an almost ubiquitous prevalence of plasmids. Complete plasmid positivity in January (S1 and S2), February (S2), March (S1), May (S1 and S2) and June (S1 and S2) was confirmed by distinctive plasmid bands. In April (S1), a slight deviation from this was observed with eight of nine isolates being plasmid positive. The detection of the plasmid containing isolates over several months and sampling sites appear to be associated with ongoing environmental pressure selecting for plasmid maintenance. Overall, these findings suggest Hatirjheel to represent a chronic environmental reservoir of plasmid-harboring of *V. cholerae* supported possibly by intense anthropogenic involvement, nutrient enrichment and stagnant water condition favouring long lasting genomic plasticity and adaptation (Vezzulli et al., 2010).

Interpretation of monthly occurrence of *Vibrio cholerae* plasmid-positive in Curzon Hall:

In contrast, Curzon Hall the infrequent and localized instance is indicated by the gel electrophoresis findings. The isolates were also only recovered three times during the sampling period; and only the plasmid-positive isolates of January S1 and May S2 were found. The other months and sampling points remained negative even though they indicated the absence of bacterial persistence. Otherwise, despite a 100% plasmid positivity among the few recoveries, high ratio of low recovery indicates that *V. cholerae* is not ecologically viable to survive and transfer plasmids (Given et al., 2022). According to these tables, low-impact and transient pattern occurs in Curzon Hall where the plasmid-carrying *V. cholerae* is only always incidental in the short-run, when favourable conditions are momentarily favourable, likely due to the low organic load, reduced sewage entry, and relatively controlled human activity.

Interpretation of monthly occurrence of Vibrio cholerae plasmid-positive in Ramna Lake:

In table 42, the distribution of the plasmid positive *Vibrio cholerae* isolates during the month in Ramna Lake showed the intermediate seasonally associated pattern. A total of 14 isolates were recovered all plasmid positive; however, isolation was limited to particular months. No plasmid-positive isolates were identified in January, March or April and S2 in February gave a single plasmid-positive isolate. A pronounced increase was found in May, specifically 6 plasmid-positive isolates for both S1 and S2, which suggests a peak time for proliferation of plasmid-bearing *V. cholerae*. An additional plasmid positive isolate was seen in June S2. These findings suggest that Ramna Lake favors plasmid-positive *V. cholerae* by seasonally favorable conditions as opposed to year-round persistence conditions and most likely the elevated temperature, greater nutrient supply, and increased human recreational use of Lake Ramna (Hanson et al., 2007).

Comparative analysis of all three locations indicates different plasmid-ecological trend i.e. Hatirjheel is a stable high pressure plasmid reservoir, Ramna Lake shows intermediate but seasonally enhanced plasmid occurrence and Curzon Hall shows low frequency and transient plasmid presence.

5.3.2c Pie chart analysis of Isolated Plasmid positivity (Area wise)

In figure 37, the pie chart represents the area wise distribution of positive isolates for plasmid positive *Vibrio cholerae* from three sampling sites. Out of 57 isolates, the highest number came from Hatirjheel (70.1% isolates, n = 40), Ramna Lake (24.6% isolates, n = 14) and Curzon Hall (5.3% isolates, n = 3) indicating that plasmid carrying *V. cholerae* were most abundant at Hatirjheel, followed by Ramna Lake and least abundant in Curzon Hall. The high prevalence at Hatirjheel implies the presence of environmental conditions which strongly favour maintenance and spread of these plasmids through an untreated sewage, industrial effluents and the urban runoff and intense human activity (Fouz et al., 2020). Ramna Lake had a moderate level of positive for plasmids, which means that there was some contamination as well as genetic exchange happening via things like recreational activities, adding nutrients seasonally, and runoff from the city. The lowest proportion was found by Curzon hall, in line with less anthropogenic pressure, but all recovered isolates contained plasmids, proving that even less affected environments can be inhabited by the genetically adaptable *V. cholerae*. Overall, Hatirjheel is a hotspot region of plasmid positive *V. cholerae,* Ramna Lake is a medium-risk site, and Curzon Hall is a low-risk site which clearly indicates the importance of properly targeted and location-specific environmental monitoring.

5.3.2d Column Chart analysis of Isolated Plasmid positivity (Area and month wise)

Figure 38 shows a comparison of plasmid-positive *Vibrio cholerae* isolates in January to June in Hatirjheel, Ramna Lake and Curzon Hall and shows definite temporal peaks and geographical variations. Hatirjheel reported the largest values with peaks in February (12 isolates) and April (8 isolates) that represent a positive environment like nutrient enrichment, water stagnation and anthropogenic input that favour bacteria survival and plasmid retention. Even though the numbers dropped in March (1 isolate) and May and June (6 and 5 isolates), plasmid-positive isolates had already remained, suggesting a rather appropriate environment. The suitable seasonal pattern of Ramna Lake showed there were no plasmid-positive isolates in January, three in March, two in February, and a drastic increase in May (12 isolates) indicating the intermittent environmental suitability of the lake depending on temperature, urban runoff, and recreational activity (Huq et al., 2005). Curzon Hall recorded the fewest instances, only twelve months

(January 1 isolate and May 2 isolates) reported plasmid-positive isolates and this was probably as a result of the low input of organic matter, greater drainage and less pools of water. All in all, the distribution of plasmid-carrying *V. cholerae* indicates that Hatirjheel is a consistent hot spot, Ramna Lake has seasonal patterns, and Curzon Hall is a low-risk location, suggesting that there is a necessity of specific attention to environmental distribution in time, space, and anthropogenic factors.

5.3.3 Analysis of *E. coli*

5.3.3a Gel Electrophoresis analysis

The plasmid profiling of *E. coli* isolates in Hatirjheel, Ramna Lake, and Curzon Hall revealed that plasmid carriage was quite extensive in all months of sampling, and therefore, extra-chromosomal genetic factors are highly prevalent in the population of environmental *E. coli* in urban aquatic environments. This extensive representation in plasmids is a common finding in environmental isolates and is linked to adaptation of bacteria and genetic variety (Rozwandowicz et al., 2022).

The plasmid positivity in May was high and the plasmid positivity was observed across all sites indicating that the plasmids could be associated with increased anthropogenic activities. The water resources in urban areas receive raw domestic sewage, hospital effluents, and surface runoffs, which provide a strong selective pressure to bacteria to retain plasmids with adaptive features, including antibiotic resistance genes (Manaia et al., 2023). These conditions of survival and environmental persistence are selective to favor bacterial survival.

On the same note, plasmid positivity was 100% in the month of February, though some of the isolates had weak plasmid bands. Low plasmid copy number, degradation of DNA during extraction process, or difference in growth density of bacteria before plasmid extraction, can also be a cause of reduced band intensity. Large or low-copy plasmids commonly exist in environmental bacteria, and often they yield weaker electrophoretic bands than the laboratory strains (Partridge et al., 2022).

The uniformity of the plasmid within the sampled times indicates that the presence of the plasmid is a fixed trait of environmental *E. coli* populations and not a random occurrence. This stability can take place through the horizontal gene transfer process like conjugation, which is

widely prevalent in the aquatic environment where microbial communities develop (Sheppard et al., 2023).

Plasmid size could be estimated by estimating the band position and the extent of variation in the intensity of the band with the help of the 1 kb DNA ladder, whereas the Iso-isolate variability of band size, position, and intensity was likely because of the difference in plasmid size, structural conformation (supercoiled, nicked, or linear), and copy number which is often reported in environmental plasmid studies (Rozwandowicz et al., 2022).

The negative control showed that there were no bands, which indicated that the observed plasmid bands were the product of bacterial DNA and not the contamination of the experiment, which proved the credibility of the procedure. As much as there was some slight technical duplication in some of the lanes owing to loading error, the same plasmid profiles in the duplicated samples revealed that these errors had no significant effect on the general interpretation of data. This type of technical difference is typical of gel-based molecular analyses and in most cases does not undermine experimental validity provided that such differences are correctly recorded (Partridge et al., 2022).

On the whole, the findings reveal widespread occurrence of plasmid-bearing *E. coli* in the water bodies under investigation. The findings are important, as in most cases, plasmids act as vectors of antibiotic resistance and virulence genes, which indicate that in contaminated aquatic environments, plasmids may also be viewed as reservoirs of genetically flexible and possibly multidrug-resistant bacteria (Manaia et al., 2023).

5.3.3b Table analysis for Isolated Plasmid positivity (Area and month wise)

Monthly Isolated Plasmid Positivity of *Escherichia coli* in Hatirjheel Results Interpretation:

As indicated in the table, plasmid positivity of *E. coli* isolates in Hatirjheel was high at all times during the study period, which suggests a large proportion of plasmid and high level of genetic adaptability in the circulating bacterial population. Among 41 *E. coli* isolates, 40 of them were plasmid-positive, indicating the widespread distribution of plasmids and the possible role in the formation of resistance to antimicrobials and survival in environments (Mendoza-Guido et al., 2024).

Both sampling sites (S1 and S2) in January exhibited 100% plasmid-positivity, which shows that the plasmid-bearing *E. coli* was in place at the beginning of the study. In February, S2 produced only plasmid-positive isolates, and S1 none, indicating that there was spatial heterogeneity in sources of contamination. The same trend was observed in March where the plasmid-positive isolates were only detected at S1 indicating that the plasmid-carrying strains can be maintained even when recovery varies. In April, there was a minor decrease in positivity in S1 (88.89%), and no isolates were found at S2, which could be caused by the effect of environmental stress or dilution typical of dynamic water bodies (Li et al., 2023). In May and June, the 100% positivity of both sampling points was again observed in the presence of isolates, showing that the plasmid carriage was stable with time. All in all, Hatirjheel was found to be consistently and widely plasmid positive with slight temporal and spatial fluctuations which supports its capacity to serve as a long-term environmental reservoir of antimicrobial resistance in plasmids.

Results Interpretation of Monthly Isolated Plasmid Positivity of *Escherichia coli* in Curzon Hall Samples Interpretation of Results:

Curzon Hall showed minimal *E. coli* recovery, however, all recovered isolates (3) were plasmid-positive which implies that when contamination had taken place it had been by plasmid-bearing strains. The plasmid-positive *E. coli* was only detected in January in S1 and none in S2. No isolates were obtained between February and April in either site, which suggests a low level of contamination or unfavorable environmental conditions that support the survival of bacteria. In May, only S2 had plasmid-positive isolates; none of the other sites had any. No plasmid-bearing isolates were obtained in June. This low rate of detection is together with a high rate of plasmid carriage indicating that even episodically introduced bacteria can carry plasmids that increase survival and resistance which underscores the significance of observing sites where overall bacterial recoveries are low (Hsu et al., 2025).

Results of Monthly Isolated Plasmid Positivity of *Escherichia coli* in the Ramna Lake Samples:

Ramna Lake was found to be moderately and seasonally variable with regard to plasmid positivity. The 14 recovered *E. coli* isolates were all plasmid-positive, suggesting standardised distribution of plasmids in the identified *E. coli* strains. In January, March and April, no isolates were identified that may indicate cases of contamination with reduced intensity or conditions. Localized and genetically stable contamination was observed in February with plasmid-positive

E. coli with 100% positivity at S2. Both the S1 and S2 produced several plasmid-positive isolates in May when the conditions were favorable to bacterial and plasmid growth. S2 was 100% positive in plasmids in June and S1 was negative. These yearly trends provide an idea that there are conditions in late spring and early summer that encourage plasmid-associated bacteria longevity (Rana et al., 2025). Although there are variations of recovery over time, consistent plasmid carriage by isolates shows that Ramna Lake may act as a source of plasmid mediated traits, such as antimicrobial resistance.

5.3.3c Pie chart analysis of total Isolated Plasmid positivity (Area wise)

The pie chart indicates that there are evident spatial variations in the plasmid-positive *E. coli* in the urban water bodies: Hatirjheel gave the greatest percentage (50.2), then Curzon Hall (27.4) and Ramna Lake (22.4). This implies that Hatirjheel is a significant reservoir of *E. coli* that carries plasmids.

To support plasmids with adaptive benefits such as resistance and stress tolerance, stronger anthropogenic stressors, including high-frequency release of untreated wastewater and surface runoff, can be associated with increased prevalence of plasmids in Hatirjheel (Tang et al., 2024). Horizontal gene transfer and plasmid survival is more likely to occur in environments that are repeatedly contaminated.

On the contrary, the positive response on the lower plasmid of Ramna Lake might be associated with comparatively smaller sewage contribution and more unsteady water environments that decrease the bacterial survival and plasmid maintenance. Curzon Hall was intermediate positive which can be attributed to the effect that pollution had only at times but not throughout.

On the whole, these tendencies show that environmental stress factors related to humans have a significant effect on plasmid distribution within urban aquatic *E. coli* communities, and the high prevalence of plasmids in Hatirjheel is concerning in terms of the spread of mobile genetic factors that cause antimicrobial resistance (Singh et al., 2023).

5.3.3d Column Chart analysis of Isolated Plasmid positivity (Area and month wise)

The monthly and geographical distribution of plasmid-positive *E. coli* showed unique time and spatial temporalities during a period of six months which indicated the role of the environmental conditions and sources of contamination.

Hatirjheel had the highest plasmid-positive rates (18-24 per month, highest in March) indicating that there is a stable environment, which promotes the persistence of plasmid-carrying bacteria. Here, the environment and constant flowing of nutrients and wastewater, as well as the absence of selective pressure, probably sustain the plasmids and the survival of bacteria, and the presence of selective forces encourages the maintenance of adaptive properties like antibiotic resistance (Tang et al., 2024).

Ramna Lake had a significant variability in month with no isolates in January and March and peaks in February and May. The temperature, precipitation, nutrient levels, and water dilution were probably seasonal and impacted the bacterial growth and plasmid stability, where warmer seasons offered good conditions (Chen et al., 2023).

The Curzon Hall demonstrated moderate and spotty positivity with plasmids (4-16 isolates per month), probably indicating the contamination with the drainage releases or human actions, but not a continuous contamination. Even though the count of the plasmid-carrying *E. coli* was lower and more fluctuated, it still persisted suggesting a possibility of horizontal gene transfer even at intermittent contamination (Li et al., 2023).

In general, the prevalence of plasmids in the environmental *E. coli* depends on both the sources of local contamination and seasonal environmental factors. Hatirjheel is a stable reservoir of mobile genetic elements whereas Ramna Lake and Curzon hall exemplify how varying environmental conditions can impact on the maintenance of plasmids.

5.3.4 Table and Pie chart analysis of Isolated Plasmid positivity (Species wise)

A comparison table and a figure (14) illustrate a comparative profile on the distribution pattern of plasmid positive bacterial isolates of three major bacterial species *Escherichia coli, Salmonella* spp and *Vibrio cholerae* across Hatirjeel, Curzon hall and Ramna lake. The results indicate the apparent trend of species dominance resulting in the dissimilarity of the plasmid carrying ability and the environmental adaptability of the bacteria of the studied group (Geurtsen e al., 2022). Overall, all the sampling sites identified 439 plasmid-positive isolates. *Escherichia coli* had the largest percentage of 54.9 (241 isolates) of the total population of plasmids. As per this finding, *E. coli* is the most genetically adaptable and environmentally resilient species in the studied water ecosystems. The highest prevalence of the plasmid-positive isolates of *E. coli* was found in Hatirjheel (121 isolates), Curzon Hall (66 isolates), and Ramna Lake (54 isolates). The

presence of *E. coli* with plasmids in all the three locations highlights the strong deal with fecal contamination and their ability to acquire and maintain plasmid in diverse environmental settings. The second-largest group of plasmid-positive isolates was *Salmonella* spp. which comprised 32.2 (141) of them. Hatirjheel registered most of such isolates (98) this time, by comparison Ramna Lake (27) and Curzon Hall (16) registered much lower. It is indicative of the fact that *Salmonella* spp. are not prevalent at the same rate as *E. coli*, but are an influential source of plasmid-bearing bacteria in general, and, most importantly, the most polluted environment (Hatirjheel). Plasmid positive *Salmonella* spp. are of particular concern because they are associated with food-borne diseases and antimicrobial resistance(Quiñones et al., 2024). *Vibrio cholerae* recorded the lowest level of percent plasmid-positive isolates at 12.9 (57 isolates) which represented the entire sample. Hatirjeel (40), Ramna Lake (14), and Curzon Hall (3) had *V. cholerae* isolates that were positive in plasmid. *V. cholerae* has a relatively lesser plasmid positivity (Mathieu-Denoncourt & Duperthuy, 2025), and this might have been the result of the ecological major factors of survival or acquisition proficiency of the plasmids in comparison to *E. coli* and *Salmonella* spp.

Overall, it can be stated that the greatest proportion of plasmids carriers is among *E. coli*, followed by *Salmonella* spp., and *V. cholerae* is the least important carrier. The dominance of plasmid positive *E. coli* is used to support the importance of this organism as a bioindicator of the occurrence of plasmid-mediated genetic phenotype (including antimicrobial resistance) in water.

5.4 Plasmid Distribution and Its Relationship to Antibiotic Resistant Profile

The analysis of antibiotic susceptibility testing (AST) and plasmid profiling demonstrated the correlation between the presence of antimicrobial resistance and plasmids. Amid the AST tests conducted on the Salmonella spp. isolates, it was found that there were 158 Salmonella bacteria among which 131 bacterial isolates had a resistant profile indicating a high prevalence of antimicrobial resistance. One of the most effective tools that contributed to this resistance was plasmid profiling since gel electrophoresis revealed that the majority of these resistant isolates had plasmids suggesting that the resistance genes were plasmid mediated and spread by the horizontal gene transfer (HGT) (plasmids are the main mediators of HGT and ARG spread in bacterial populations). The high prevalence rates of resistant Salmonella isolates are therefore an

indicator of the active role of plasmids in enhancing the expression of the MDR phenotype in this species (Salmonella has many antibiotic resistance genes which are plasmid-located and contribute to the transmission of resistance) (McMillan et al.,2020). This was also true with Vibrio cholerae. The prevalence of multiple antibiotic resistance was remarkable as 56 out of the 58 samples which went through the AST were resistant. Plasmid extraction and gel electrophoresis confirmed the presence of plasmids that confirmed that plasmid carriage was a significant process in the resistance in *V. cholerae (V. cholerae* commonly acquires mobile genetic elements, including plasmids, which carry antibiotic resistance determinants) (Das et al., 2020; De, 2021) . The nearly complete overlap of resistant phenotypes with plasmid positivity shows the efficiency in the exchange of genes that is offered by the plasmids to increase the MDR phenotype of the aquatic populations of *V. cholerae.* AST was done in Escherichia coli in 248 isolates and 202 were in resistant profiles which is the highest absolute number of resistant bacteria of the species being investigated. Gel electrophoresis analysis of these resistant E. coli cultures also showed a widespread prevalence of plasmids and should confirm the importance of plasmids as an important carrier of resistance gene (plasmid-encoded b-lactamase and other ARGs induce multidrug resistance in E. coli) in these cases (Ibekwe et al., 2021). E. coli is also known to be genetically flexible, so horizontal gene transfer via plasmids is likely to be involved in acquisition of multiple determinants of resistance, therefore, its domineering role in MDR phenotype in the study (horizontal transfer of ARGs via plasmids is a significant force of AMR dissemination)). It is likely that the site-specific effects of this study are observed due to the fact that not all three areas of the sampling take the same level of the anthropogenic impacts and the environmental pollution. Hatirjheel had the poorest burden of MDR because of continuous influx of untreated containment or partially treated sewage, urban run-offs, and other hospital and domestic effluent, which impose a strong and selective pressure on antibiotic resistance and harbor extensive viable micro-organisms capable of a horizontal gene transfer because of plasmids (environmental waters receiving anthropogenic waste are hotspots of resistant bacteria and horizontal gene exchange) (Alawi et al., 2022)). In its turn, the Curzon Hall presented slightly less levels of resistance that could be attributed to the fact that the degree of direct sewage input was lower and that the contaminants were dispensed and could not maintain any direct selective pressure and gene trading. The intermediate distribution of resistance was observed in Ramna Lake, and may be contaminated by humans on a seasonal basis, and

recreational activity, both of which maintains the load and distribution of the resistance periodically. Overall, these geographical differences imply that urbanisation level and the amount of waste are a prominent factor that predetermines the survival and increase of MDR bacteria in water. Overall, the correlation between high resistance levels and high rates of plasmid presence in the three bacteria species is indicative that horizontal gene transfer by plasmid makes a major contribution to the development of MDR in environmental bacteria (plasmid-mediated ARG transfer is a central mode in environmental AMR development).The distribution of plasmids within and between species and sites of sampling is directly proportional to the level of resistance that is being expressed, and this verifies that plasmid-rich environments are reservoirs of advanced antimicrobial resistance. The findings bring out the relevance of the environmental spreading of plasmids in sustaining and propagation of MDR bacteria beyond the clinical setting.

5.5 Advanced Resistance Patterns in Critical Therapeutic Antibiotics

The data of the antibiotic susceptibility indicate that partial groups of *Salmonella* spp., *Escherichia coli*, and *Vibrio cholerae* isolates had resistance patterns that extended to those drugs traditionally defined as the last-line or most important antibiotics. Carbapenems, aztreonam, and aminoglycosides retained considerable overall effect; however, the development of intermediate susceptibility and occasional resistance, particularly in isolates of Salmonella in Hatirjheel are pointers (Teixeira et al., 2020). Identification of MDR phenotype in repeated cases even in isolates of the environment indicates that aquatic ecosystems are not a passive reservoir of water, but a contributor to the maintenance and dissemination of sophisticated resistance phenotypes (Sobur et al., 2019). This is of particular concern because the environmental strains may give up their genes to clinically significant pathogens through horizontal gene transfer and therefore may render last-resistant medicines not as reliable in the long term.

5.6 Temporal Variability and Ecological Influences on Bacterial Prevalence

The number of bacteria present, number of plasmids present and strength of resistance showed a seasonal shift in the samples throughout the 6 months sample period. The PCR positivity and plasmid prevalence peaks were also regularly observed in late summer and early monsoon, especially in May and June (Barrantes-Jimenez et al., 2025). These transformations through time are coinciding with the rise in temperature, inflow of more nutrients in the surface runoff, and

decreased dynamic water movements, which contribute to the bacteria of all kinds living longer and exchanging genes, all of which are mentioned (Suthar,2021). Hatirjheel had the most consistent but always high bacterial load over the months, that is indicative of chronic anthropogenic burden, compared to Ramna Lake, which had acute seasonal lapses indicative of single events of contamination. There were also irregular patterns at Curzon Hall, which might be explained by short-term ecological disturbances but not by long-term ecological disruptions (O'Malley et al., 2022). In totality, these findings enforced the concern of ecosystem dynamics in the development of antimicrobial resistance spread in urban surface waters.

5.7 Implications for Human Exposure and Environmental Health Risk

The presence of MDR coupled with plasmid-carrying enteric bacteria in urban waters accessible to people is a major concern to the normal health of people. These streams are normally used by people as a way of entertainment, work, as well as draining into larger water systems, either directly or indirectly. Multidrug-resistant pathogens are more likely to be passed to humans when their loads are high, they are widely present in their plasmid, and they are equally resistant to a variety of antibiotics. Also, environmental bacteria can act as hidden amplifiers of resistance genes that help them to settle in human microbiota or simply in a clinical setting. The findings note the need to address antibiotic resistance not only as a hospital issue but as an environmental and community-level health issue since environmental reservoirs of AMR need to be addressed with both monitoring and mitigation at the One Health level to ensure the protection of human health (Stanton et al., 2022).

5.8 Putting the Results in the Context of Global Resistance Trends

Contextualised with the global reports of aquatic environments with antimicrobial resistance, the patterns found are in line with global trends especially within the fast urbanising areas of low-middle and low-income nations. The prevalence of the plasmids among the *E. coli* and *Salmonella* spp. follows the trend observed in other related studies in Southeast and South Asia whereby the ambient waters serve as an important reservoir of genes that relate to resistance. The size and duration of pollution in Hatirjheel suggest an environmentally worsened case due to the lack of control over garbage disposal along with high population density.Such a comparative lens puts forward the idea that the phenomenon alters greatly by the region-specific infrastructure,

governance structures, and environmental management practices, yet it is a global phenomenon (Bisaccia et al., 2025).

5.9 Strategic Guidelines for Environmental along with Antimicrobial Governance

The findings justify the existence of immediate need to strengthen policy frameworks, incorporating environmental surveillance in the national action plans to contain antimicrobial resistance. The frequent microbiological follow-up of urban-source waters, the use of plasmid profiling with resistance typing, and an increase in strict control over intervals of untreated waste disposal are needed (WHO, 2023). Besides, the use of environmental information ought to contribute to the general population level of risk assessment of health and the implementation of isolation of antibiotic use campaigns outside the clinical sphere. In order to prevent the environment-wide occurrence of antibiotic resistance and ensure that the existing therapies remain effective, the collaboration between environmental agencies, public health agencies, and research institutes will become relevant (Xiao et al., 2023).

Chapter 6

Conclusion

The study is a detailed research of the relationship between plasmid carrying and antibiotic resistance in pathogenic enteric bacteria obtained in the major surface water bodies of Dhaka Metropolitan City. It is clear in the findings that *Escherichia coli, Salmonella* spp. and *Vibrio cholerae* that had been isolated in the urban water sources possess significant concentrations of antibiotic resistance with a good percentage of the resistant isolates depicted by the presence of plasmids.

This finding is in a strong position of proving the hypotheses that the horizontal gene transfer of antimicrobial resistance through plasmids is essential in the spread of antimicrobial resistance in the aquatic environment. Hatirjheel along with Ramna Lake always displayed the highest percentage of PCR-positive isolates and resistance patterns, whereas the contamination was relatively lower in Curzon Hall. These spatial changes indicate disparities in human activity, waste release and environmental management about the sampling sites. Seasonal patterns were also observed where more resistant and plasmid carrying bacteria were found during the late summer and early monsoon seasons, indicating the role of environmental and ecological processes in the survival of bacteria and their gene transfer. A combination of the selective culture, testing of antibiotic susceptibility, isolation of plasmids, PCR amplification and agarose gel electrophoresis allowed to identify and characterize bacterial populations with resistance. Although there are a number of technical and procedural constraints that were experienced in the course of the laboratory work, protocol optimization was essential in making sure that legitimate and reproducible results were produced.

Comprehensively, this paper highlights the essence of surface water bodies as environmental reservoirs of plasmid-mediated antibiotic resistance in overcrowded urban environments in terms of public health. The occurrence of the multidrug-resistant pathogenic bacteria in the water bodies that are frequently visited by the people is a severe hazard to the community health and a pressing need arose to implement the concept of combined water monitoring and better waste management techniques in Bangladesh.

Limitation

The research has a few shortcomings which may be influenced when explaining the research results. The limited funding prevented carrying out a metagenomic analysis that would have allowed carrying out in more detail the genomic characterization of the isolates. Collection of samples was also done over a short duration, though a one-year long follow up strategy would have played a stronger role in giving a comparison of the seasons, but this was impossible as the thesis had deadlines to meet. The entire area of the research was very limited and water sampling locations were chosen according to the logistical convenience, that is, other potentially interested areas were not included. Additionally, PCR-based detection was used as the primary tool in the study without using advanced molecular typing or sequence methods, and resource and time limitation did not create the possibility of repeating and replicating sampling, potentially affecting the level of the robustness and repeatability of the study.

Future Prospects

The results of the conducted research lead to a number of significant opportunities in terms of future research and policy-making. Sampling of more rivers, canals and peri-urban water bodies in addition to long-term multi-year data collection would give a better national overview and help define the effect of seasonal and climatic changes on patterns of resistance and plasmid changes. Such advanced molecular methods as plasmid sequencing, whole-genome sequencing, and profile of resistance genes could be used to determine particular resistance determinants and types of plasmids that are involved with the transfer of genes horizontally. These analyses would give extensive knowledge about the genetic basis of resistant and evolutionary routes of resistance in aquatic life.

The study may also be extended to evaluate the connection between environmental isolates and clinical strains thus forming direct epidemiological correlations between polluted water sources and human infections. This would enhance the literature of integrating environmental monitoring in antimicrobial resistance national action plans. In terms of public health and policy, the findings create awareness of the necessity to improve the control of the release of untreated sewage, hospital effluents, and industrial waste into the surface water bodies. Future efforts

could be focused on microbiological risk assessment combined with environmental engineering and community-based responses to minimize contamination of its origin. Overall, this research confirms that future environmental, molecular and public health studies on antimicrobial resistance have a solid platform, and the results of this study can be used as a reference to inform future scientific research as well as forthcoming evidence-based policies in order to protect water quality and human health in Bangladesh.

References

Ahmed, I., Rabbi, M. B., & Sultana, S. (2019). Antibiotic resistance in Bangladesh: A systematic review. *International Journal of Infectious Diseases, 80*, 54–61. https://doi.org/10.1016/j.ijid.2018.12.017

Ahmed, W., Payyappat, S., Cassidy, M., Besley, C., & Power, K. (2023). Assessing fecal contamination in urban aquatic environments using molecular markers and environmental monitoring approaches. *Science of the Total Environment, 857*, 159543. https://doi.org/10.1016/j.scitotenv.2022.159543

Alam, M. S. (2014). Assessment of water quality of Hatirjheel Lake in Dhaka City. International Journal of Technology Enhancements and Emerging Engineering Research, 2(6). https://www.researchgate.net/publication/313672562

Alawi, M., Velasco Torrijos, T., & Walsh, F. (2022). Plasmid-mediated antimicrobial resistance in drinking water. *Environmental Advances*, 8, 100191. https://doi.org/10.1016/j.envadv.2022.100191

AMB Express. (2022). *A new device-mediated miniprep method improves plasmid DNA isolation quality and efficiency.* https://doi.org/10.1186/s13568-022-01360-7

Amalina, N. Z., Santha, S., Zulperi, D., Amal, M. N. A., Yusof, M. T., Zamri-Saad, M., & Ina-Salwany, M. Y. (2019). Prevalence, antimicrobial susceptibility and plasmid profiling of *Vibrio* spp. isolated from cultured groupers in Peninsular Malaysia. *BMC microbiology*, *19*(1), 251. https://doi.org/10.1186/s12866-019-1624-2

Antimicrobial resistant enteric bacteria are widely distributed among environmental water sources in Dhaka, Bangladesh. https://www.nature.com/articles/s41545-025-00447-5

Aryal, S. (2022). *EMB Agar- Composition, Principle, Preparation, Results, Uses.* Microbe Notes. https://microbenotes.com/eosin-methylene-blue-emb-agar/

Aryal, S. (2022). *Salmonella Shigella (SS) Agar- Composition, Principle, Uses, Preparation and Result Interpretation.* Microbiology Info.

https://microbiologyinfo.com/salmonella-shigella-ss-agar-composition-principle-uses-preparation-and-result-interpretation/

Aryal, S. (2022). *Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar- Composition, Principle, Uses, Preparation and Colony Morphology.* Microbiology Info. https://microbiologyinfo.com/thiosulfate-citrate-bile-salts-sucrose-tcbs-agar-composition-principle-uses-preparation-and-colony-morphology/

Aryal, S. (2025). *Streak Plate Method: Principle, Types & Step-by-Step Guide.* Microbe Notes. https://microbenotes.com/streak-plate-method-principle-methods-significance-limitations/

Asad, N., Smith, E., Shakya, S., Stegman, S., & Timmons, L. (2023). Sustainable methodologies for efficient gel electrophoresis and streamlined screening of difficult plasmids. *Methods and Protocols, 6*(2), 25.https://doi.org/10.3390/mps6020025

Awere-Duodu, A., Ntim, O. K., & Donkor, E. S. (2025). *Vibrio cholerae in water environments: A systematic review and meta-analysis. Environmental Microbiology Reports*, 17(3), e70103. https://doi.org/10.1111/1758-2229.70103

BabioMed. (n.d.). *SS Agar (Salmonella Shigella Agar).* https://www.babiomed.com/products/culture_medium/dehydrated_culture_medium/dehydrated_culture_media/ss_agar.html

Baquero, F., Martínez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current opinion in biotechnology*, *19*(3), 260–265. https://doi.org/10.1016/j.copbio.2008.05.006

Barrantes-Jiménez, K., Mendoza-Guido, B., Morales-Mora, E., Rivera-Montero, L., Montiel-Mora, J., Chacón-Jiménez, L., Rojas-Jiménez, K., & Arias-Andrés, M. (2025). Influence of Seasonality and Pollution on the Presence of Antibiotic Resistance Genes and Potentially Pathogenic Bacteria in a Tropical Urban River. *Antibiotics (Basel, Switzerland)*, *14*(8), 798. https://doi.org/10.3390/antibiotics14080798

Bartley P.S., Domitrovic T.N., Moretto V.T., Santos C.S., Ponce-Terashima R., Reis M.G., Barbosa L.M., Blanton R.E., Bonomo R.A., Perez F. Antibiotic Resistance in Enterobacteriaceae

from Surface Waters in Urban Brazil Highlights the Risks of Poor Sanitation. Am. J. Trop. Med. Hyg. 2019;100:1369–1377. doi: 10.4269/ajtmh.18-0726

Bayot ML, Bragg BN. Antimicrobial susceptibility testing. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 May 27. https://www.ncbi.nlm.nih.gov/books/NBK539714/

Bell, R. L., Kase, J. A., Harrison, L. M., Balan, K. V., Babu, U., Chen, Y., Macarisin, D., Kwon, H. J., Zheng, J., Stevens, E. L., Meng, J., & Brown, E. W. (2021). The Persistence of Bacterial Pathogens in Surface Water and Its Impact on Global Food Safety. *Pathogens (Basel, Switzerland)*, *10*(11), 1391. https://doi.org/10.3390/pathogens10111391

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology, 153*(S1), S347–S357. https://doi.org/10.1038/sj.bjp.0707607

Bilal, H., Li, X., Iqbal, M. S., Mu, Y., Tulcan, R. X. S., & Ghufran, M. A. (2023). Surface water quality, public health, and ecological risks in Bangladesh—a systematic review and meta-analysis over the last two decades. *Environmental Science and Pollution Research*, 30(40). https://www.researchgate.net/publication/372826840

Biswas, M., Roy, D. N., Tajmim, A., Rajib, S. S., Hossain, M., Farzana, F., & Yasmen, N. (2014). Prescription antibiotics for outpatients in Bangladesh: A cross-sectional health survey conducted in three cities. *Annals of Clinical Microbiology and Antimicrobials, 13*, 15. https://doi.org/10.1186/1476-0711-13-15

Bustin, S. A., & Nolan, T. (2020). RT-qPCR testing of SARS-CoV-2: A primer. *International Journal of Molecular Sciences, 21*(8), 3004. https://doi.org/10.3390/ijms21083004

Cabral, J. P. S. (2010). Water microbiology: Bacterial pathogens and water. *International Journal of Environmental Research and Public Health, 7*(10), 3657–3703. https://doi.org/10.3390/ijerph7103657

Carattoli A. (2013). Plasmids and the spread of resistance. *International journal of medical microbiology : IJMM*, *303*(6-7), 298–304. https://doi.org/10.1016/j.ijmm.2013.02.001

Chen, Y., Wang, X., & Li, J. (2023). Seasonal dynamics of plasmid-mediated antibiotic resistance in urban water systems. *Environmental Microbiology Reports, 15*(4), 450–460. https://doi.org/10.1111/1758-2229.13125

Cho, S., Jackson, C. R., & Frye, J. G. (2020). The prevalence and antimicrobial resistance phenotypes of Salmonella, Escherichia coli and Enterococcus sp. in surface water. *Letters in applied microbiology*, *71*(1), 3–25. https://doi.org/10.1111/lam.1330

Clinical and Laboratory Standards Institute (CLSI). *Antimicrobial Susceptibility Testing Standards*. https://clsi.org/standards-development/specialty-areas/antimicrobial-susceptibility-testin/

Dahal, P. (2025). *Spread Plate Method- Definition, Principle, Procedure, Uses*. Microbe Notes. https://microbenotes.com/spread-plate-technique/

Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: Understanding the ecology of resistance genes and mechanisms. *Vaccine*, 38(Suppl. 1), A83–A92. https://doi.org/10.1016/j.vaccine.2019.06.031

De, R. (2021). Mobile genetic elements of *Vibrio cholerae* and the evolution of its antimicrobial resistance. *Frontiers in Tropical Diseases*, 2, 691604. https://doi.org/10.3389/fitd.2021.691604

Ekenze, O. S., Ezeala-Adikaibe, B. A., Anyim, O., Onwuekwe, I., & Orjioke, C. (2018). Prevalence and pattern of migraine, tension-type headache and chronic daily headache among medical and nursing students in Enugu, South East Nigeria. *Health, 10*(6), 734–744. https://doi.org/10.4236/health.2018.106058

Faruque, Shah & Sack, David & Sack, R & Colwell, Rita & Takeda, Yoshifumi & Nair, G. (2003). Emergence and evolution of *Vibrio cholerae* O139. Proceedings of the National Academy of Sciences of the United States of America. 100. 1304-9. 10.1073/pnas.0337468100.https://www.researchgate.net/publication/8563430_Emergence_and_evolution_of_Vibrio_cholerae_O139/citation/download

Ferdous, J., Sultana, R., Rashid, R. B., Tasnimuzzaman, M., Nordland, A., Begum, A., & Jensen, P. K. M. (2018). A comparative analysis of *Vibrio cholerae* contamination in point-of-drinking and source water in a low-income urban community, Bangladesh. *Frontiers in Microbiology, 9*, 489. https://doi.org/10.3389/fmicb.2018.00489

Fouz, N., Pangesti, K. N. A., Yasir, M., Al-Malki, A. L., Azhar, E. I., Hill-Cawthorne, G. A., & Abd El Ghany, M. (2020). The contribution of wastewater to the transmission of antimicrobial resistance in the environment: Implications of mass gathering settings. *Tropical Medicine and Infectious Disease, 5*(1), 33. https://doi.org/10.3390/tropicalmed5010033 Garibyan, L., & Avashia, N. (2013). Polymerase chain reaction (PCR). *Journal of Investigative Dermatology*, 133(3), e6. https://doi.org/10.1038/jid.2013.1

Gajic, I., et al. (2022). *Antimicrobial susceptibility testing: A comprehensive review*. https://pmc.ncbi.nlm.nih.gov/articles/PMC9024665/

Given, C., Penttinen, R., & Jalasvuori, M. (2022). Plasmid viability depends on the ecological setting of hosts within a multiplasmid community. *Microbiology Spectrum, 10*(2), e00133-22. https://doi.org/10.1128/spectrum.00133-22

Goyon, A., Zhang, H., & Anspach, J. A. (2023). Separation of plasmid DNA topological forms by agarose gel electrophoresis. *Analytical Chemistry*. https://doi.org/10.1021/acs.analchem.3c02944

Hao, R., Li, X., Zhang, M., et al. (2025). Optimized plasmid extraction through controlled temperature, prolonged alkaline lysis, and gentle mixing. *Journal of Chromatography B, 1262*, 124646.https://doi.org/10.1016/j.jchromb.2025.124646

Hahn, M. B. (2025). Rapid quantitative analysis of double-stranded plasmid DNA with capillary gel electrophoresis for applications in quality control and radiation research. *Scientific Reports, 15*(1), 1068.https://doi.org/10.1038/s41598-025-85132-w

Hanson, K. C., Cooke, S. J., Suski, C. D., Niezgoda, G., Phelan, F. J. S., Tinline, R., & Philipp, D. P. (2007). Assessment of largemouth bass (*Micropterus salmoides*) behaviour and activity at multiple spatial and temporal scales utilizing a whole-lake telemetry array. *Hydrobiologia, 582*(1), 243–256. https://doi.org/10.1007/s10750-006-0549-6

Hassan, M. M., Nohor,N. (2023). *Perspective on antibiotic resistance in Bangladesh: A critical yet overlooked public health crisis*. Health Science Reports, 6(10), e70407. https://doi.org/10.1002/hsr2.70407

HiMedia Laboratories. (n.d.). *SS Agar (Salmonella Shigella Agar)*. HiMedia Labs. https://www.himedialabs.com/us/m108-ss-agar-salmonella-shigella-agar.html

HiMedia Laboratories. (n.d.). *TCBS Agar*. HiMedia Labs. https://www.himedialabs.com/us/m189-tcbs-agar.html

HiMedia Laboratories. (n.d.). *EMB Agar*. HiMedia Labs. https://www.himedialabs.com/us/m317-emb-agar.html

Hossain, M. J., Jabin, N., Ahmmed, F., Sultana, A., Rahman, S. M. A., & Islam, M. R. (2023). Irrational use of antibiotics and factors associated with antibiotic resistance: Findings from a cross-sectional study in Bangladesh. *Health Science Reports, 6*(8), e1465. https://pmc.ncbi.nlm.nih.gov/articles/PMC10375841/

Hossain, M. S., Chowdhury, A. I. A., Islam, M. R., Kirtunia, R., Abedin, M. F., Alam, M. A. U., Shampa, Murshed, S. B., Sakib, M. S., Alam, S., Mondal, M. S., & Mahmud, Z. H. (2025). Multidrug-resistant ESBL *E. coli* in urban surface waters and public health implications: A Case Study from Goranchatbari, Dhaka. *Heliyon*, *11*(3), e42219. https://doi.org/10.1016/j.heliyon.2025.e42219

Hsu, B.-M., Lee, C.-Y., & Wang, C.-L. (2025). Episodic contamination and genetic determinants of antimicrobial resistance in urban surface waters. *Environmental Science & Technology, 59*(3), 2254–2263. https://doi.org/10.1021/acs.est.4c06532

Huq, A., Sack, R. B., Nizam, A., Longini, I. M., Nair, G. B., Ali, A., Morris, J. G., Jr, Khan, M. N., Siddique, A. K., Yunus, M., Albert, M. J., Sack, D. A., & Colwell, R. R. (2005). Critical factors influencing the occurrence of Vibrio cholerae in the environment of Bangladesh. *Applied and environmental microbiology*, *71*(8), 4645–4654. https://doi.org/10.1128/AEM.71.8.4645-4654.2005

Ibekwe, A., Durso, L., Ducey, T. F., Oladeinde, A., Jackson, C. R., Frye, J. G., Dungan, R., Moorman, T., Brooks, J. P., Obayiuwana, A., Karathia, H., Fanelli, B., & Hasan, N. (2021). Diversity of Plasmids and Genes Encoding Resistance to Extended-Spectrum β-Lactamase in *Escherichia coli* from Different Animal Sources. *Microorganisms*, *9*(5), 1057. https://doi.org/10.3390/microorganisms9051057

Islam, M. W., Shahjahan, M., Azad, A. K., & Hossain, M. J. (2024). Factors contributing to antibiotic misuse among parents of school-going children in Dhaka City, Bangladesh. *Scientific Reports, 14*(1), 2318. https://doi.org/10.1038/s41598-024-52313-y

Islam, M. S., Afroz, R., & Mia, M. B. (2019). Investigation of surface water quality of the Buriganga river in Bangladesh: Laboratory and spatial analysis approaches. *Dhaka University Journal of Biological Sciences*, 28(2), 147–158. https://doi.org/10.3329/dujbs.v28i2.46501

Jacob-Lopes, E., & Zepka, L. Q. (Eds.). (2020). *Biotechnology and bioengineering*. BoD – Books on Demand. https://books.google.com.bd/books?hl=en&lr=&id=LkP8DwAAQBAJ&oi=fnd&pg=PA5&dq=pcr+steps+&ots=VYNhHj6JWO&sig=MnZejeyoG2K4AN4MWo2nzFip3sM&redir_esc=y#v=onepage&q=pcr%20steps&f=false

Jeroen Geurtsen, Mark de Been, Eveline Weerdenburg, Aldert Zomer, Alan McNally, Jan Poolman, Genomics and pathotypes of the many faces of *Escherichia coli*, *FEMS Microbiology Reviews*, Volume 46, Issue 6, November 2022, fuac031, https://doi.org/10.1093/femsre/fuac031

Johura, F.-T., Sultana, M., Sadique, A., Monira, S., Sack, D. A., Sack, R. B., Alam, M., & Chakraborty, S. (2024). The antimicrobial resistance of enterotoxigenic *Escherichia coli* from diarrheal patients and the environment in two geographically distinct rural areas in Bangladesh over the years. *Microorganisms, 12*(2), 301. https://doi.org/10.3390/microorganisms12020301

Kamal Pasha, A. B. M., Nur, M. S., Mozumder, S., & Parveen, M. (2023). Impact of river water quality on public health in perspective of Asian rivers: A case study of Buriganga River, Bangladesh. *Journal of Environmental & Earth Sciences*, 5(1), 1–16. https://doi.org/10.30564/jees.v5i1.5132

Khan, M. A. J. R., Tahnun, M. N., Saif, E., & Islam, M. S. (2023). Recent water quality assessment of Buriganga and Turag River. *Chemical Engineering Research Bulletin*, 23, 79–84. https://doi.org/10.3329/cerb.v23i10.78501

Kasturi, K. N., & Drgon, T. (2017). Real-Time PCR Method for Detection of Salmonella spp. in Environmental Samples. *Applied and environmental microbiology*, *83*(14), e00644-17. https://doi.org/10.1128/AEM.00644-17

Khehra, N., Padda, I. S., & Zubair, M. (2025). *Polymerase chain reaction (PCR)*. In StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK589663/

Kim, J. Y., & An, K. G. (2015). Integrated ecological river health assessments, based on water chemistry, physical habitat quality and biological integrity. *Water*, 7(11), 6378–6403. https://doi.org/10.3390/w7116378

Kodackattumannil, P. K., Shina Sasi, S., Saranya Krishnan, S., Lekshmi, G., Kottackal, M., & Amiri, K. M. A. (2023). Protocol for the high-quality plasmid isolation from different recalcitrant bacterial species: *Agrobacterium* spp., *Rhizobium* sp., and *Bacillus thuringiensis*. *Bio-Protocol, 13*(15), e4788.
https://doi.org/10.21769/BioProtoc.4788

Korajkic, A., McMinn, B. R., & Harwood, V. J. (2024). Relationships between microbial water quality indicators and environmental drivers in urban surface waters. *Water Research, 248*, 120783. https://doi.org/10.1016/j.watres.2023.120783

Kruasuwan, W., Sawatwong, P., Jenjaroenpun, P., et al. (2024). *Comparative evaluation of commercial DNA isolation approaches for nanopore-only bacterial genome assembly and plasmid recovery.* Scientific Reports, 14, 27672.
https://doi.org/10.1038/s41598-024-78066-2

Kusi J., Ojewole C.O., Ojewole A.E., Nwi-Mozu I. Antimicrobial Resistance Development Pathways in Surface Waters and Public Health Implications. Antibiotics. 2022;11:821.
https://www.mdpi.com/2079-6382/11/6/821

Li, H., Cheng, G., Zheng, J., & Yang, S. (2023). Seasonal and hydrological influences on bacterial communities and resistance determinants in urban rivers. *Science of the Total Environment, 914*, 136604. https://doi.org/10.1016/j.scitotenv.2023.136604

Li, H., Zhang, W., & Xu, L. (2023). Plasmid carriage in environmental *Escherichia coli*: Implications for horizontal gene transfer. *Microbial Ecology, 85*(3), 652–664. https://doi.org/10.1007/s00248-022-02145-9

Lee, P. Y., Costumbrado, J., Hsu, C. Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of visualized experiments : JoVE*, (62), 3923. https://doi.org/10.3791/3923

Liu, H., Whitehouse, C. A., & Li, B. (2018). Presence and Persistence of *Salmonella* in Water: The Impact on Microbial Quality of Water and Food Safety. *Frontiers in public health*, *6*, 159. https://doi.org/10.3389/fpubh.2018.00159

Magiorakos, A. P. et al. (2012). *Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions*. *https://www.sciencedirect.com/science/article/pii/S1198743X14616323*

Manaia, C. M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I. C., Zammit, I., & Nunes, O. C. (2023). Antibiotic resistance in urban aquatic environments: Global dissemination and environmental drivers. *Environmental International, 171*, 107685. https://doi.org/10.1016/j.envint.2022.107685

Martinez J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental pollution (Barking, Essex : 1987)*, *157*(11), 2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Mathieu-Denoncourt, A., & Duperthuy, M. (2025). Functional versatility of *Vibrio cholerae* outer membrane proteins. *Applied Microbiology*, 5(3), 64. https://doi.org/10.3390/applmicrobiol5030064

McMillan, E. A., Jackson, C. R., & Frye, J. G. (2020). Transferable plasmids of *Salmonella enterica* associated with antibiotic resistance genes. *Frontiers in Microbiology*, 11, 562181. https://doi.org/10.3389/fmicb.2020.562181

Megantara, I., Sylviana, N., Amira, P. A., Pradini, G. W., Krissanti, I., & Lesmana, R. (2023). Potential of waterbodies as a reservoir of *Escherichia coli* pathogens and the spread of antibiotic resistance in the Indonesian aquatic environment. *Journal of Water, Sanitation and Hygiene for Development*, 13(10), 776–792. https://doi.org/10.2166/washdev.2023.040

Mendoza-Guido, B., Barrantes, K., Rodríguez, C., Rojas-Jimenez, K., & Arias-Andres, M. (2024). The Impact of Urban Pollution on Plasmid-Mediated Resistance Acquisition in Enterobacteria from a Tropical River. *Antibiotics (Basel, Switzerland)*, *13*(11), 1089. https://doi.org/10.3390/antibiotics13111089

Meradji, S., Basher, N. S., Sassi, A., Ibrahim, N. A., Idres, T., & Touati, A. (2025). The Role of Water as a Reservoir for Antibiotic-Resistant Bacteria. *Antibiotics (Basel, Switzerland)*, *14*(8), 763. https://doi.org/10.3390/antibiotics14080763

Mendoza-Guido, B., Barrantes, K., Rodríguez, C., Rojas-Jimenez, K., & Arias-Andres, M. (2024). *The impact of urban pollution on plasmid-mediated resistance acquisition in enterobacteria from a tropical river.* Antibiotics, 13(11), 1089. https://doi.org/10.3390/antibiotics13111089

Miah, M. B., Majumder, A. K. M., & Latifa, G. A. (2016). Evaluation of microbial quality of the surface water of Hatirjheel in Dhaka City. Stamford Journal of Microbiology, 6(1), 30–33. https://www.researchgate.net/publication/319050273

Mina, S. A., Debnath, P., Hossain, A. K. M. Z., Hasan, M. Z., & Azad Chowdhury, A. M. M. (2024). Screening and identification of multiple antibiotic- resistant genes containing *Salmonella* Typhi from drinking water: A severe public health concern in Bangladesh. *Heliyon*, *10*(22), e40523. https://doi.org/10.1016/j.heliyon.2024.e40523

Moinet, M., et al. (2024). Qualitative detection of *Escherichia coli* in distributed drinking water using real-time reverse transcription PCR targeting 16S rRNA: Validation and practical experiences. *Water Research, 259*, 121843. https://doi.org/10.1016/j.watres.2024.121843

Momba, M. N., Malakate, V. K., & Theron, J. (2006). Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *Journal of water and health*, *4*(3), 289–296. https://www.researchgate.net/publication/6758227

Monte, D. F. M., de Lima Rocha, A. D., Lemos, M. L. P., de Lima, L. A., Cabrera, J. M., da Silva, N. J., Huang, X., Chen, Z., Brown, E. W., Allard, M. W., Bell, R. L., Toro, M., Meng, J., & de Oliveira, C. J. B. (2025). High Prevalence of Plasmid-Mediated Quinolone Resistance in Salmonella enterica Serovars Isolated From Surface Water. *Environmental microbiology*, *27*(7), e70140. https://doi.org/10.1111/1462-2920.70140

Murray, C. J. L., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet, 399(10325), 629–655. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02724-0/fulltext

Na, G., Lu, Z., Gao, H., Zhang, L., Li, Q., Li, R., Yang, F., Huo, C., & Yao, Z. (2018). The effect of environmental factors and migration dynamics on the prevalence of antibiotic-resistant *Escherichia coli* in estuary environments. *Scientific Reports*, 8, 1663. https://doi.org/10.1038/s41598-018-20077-x

National Laboratory of Enteric Pathogens, Bureau of Microbiology, Laboratory Centre for Disease Control (1991). The polymerase chain reaction: An overview and development of diagnostic PCR protocols at the LCDC. *The Canadian journal of infectious diseases = Journal canadien des maladies infectieuses*, *2*(2), 89–91. https://doi.org/10.1155/1991/580478

Noor, F. H. (2022). A study on surface water quality test of Hatirjheel lake in Dhaka, Bangladesh. *International Research Journal of Modernization in Engineering, Technology and Science*,4(7). https://www.researchgate.net/publication/363520449_A_study_on_surface_water_quality_test_of_Hatirjheel_lake_in_Dhaka_Bangladesh

Okeke, I. N., Lamikanra, A., & Edelman, R. (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases, 5*(1), 18–27. https://wwwnc.cdc.gov/eid/article/5/1/99-0103_article

Oliveira, S. D., Santos, L. R., Schuch, D. M. T., Silva, A. B., Salle, C. T. P., & Canal, C. W. (2002). Detection and identification of salmonellas from poultry-related samples by PCR. *Veterinary Microbiology*, 87(1), 25–35. https://doi.org/10.1016/S0378-1135(02)00028-7

O'Malley, K., McNamara, P. J., & McDonald, W. M. (2022). Seasonal and spatial patterns differ between intracellular and extracellular antibiotic resistance genes in urban stormwater runoff. *Environmental Science: Advances*, 1, 380–390. https://doi.org/10.1039/D2VA00065B

Parvin, F., Haque, M. M., & Tareq, S. M. (2022). *Recent status of water quality in Bangladesh: A systematic review*. *Environmental Challenges*, *6*, 100416. https://doi.org/10.1016/j.envc.2021.100416

Parvin, M. S., Talukder, S., Ali, M. Y., Chowdhury, E. H., Rahman, M. T., & Islam, M. T. (2020). Antimicrobial resistance pattern of *Escherichia coli* isolated from frozen chicken meat in Bangladesh. *Pathogens*, 9(6), 420. https://doi.org/10.3390/pathogens9060420

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2022). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews, 35*(2), e00116-21. https://doi.org/10.1128/cmr.00116-21

Popoola, B. M., Adeyemi, O. A., & Samson, O. J. (2025). *Antibiotic-resistant bacteria in tropical freshwater ecosystems: A review of occurrence, distribution and environmental implications.* The Microbe, 8, Article 100457. https://doi.org/10.1016/j.microb.2025.100457

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health, 109(7), 309–318. https://pmc.ncbi.nlm.nih.gov/articles/PMC4768623/

Priyashantha, A. K. H., & Umashankar, S. (2021). *Separation of DNA fragments using agarose gel electrophoresis; protocol, results, principle and possible errors to avoid.* In *Advances in Biotechnology and Bioscience* (Vol. 10, p. 145). AkiNik Publications. https://www.researchgate.net/publication/359781430_Separation_of_DNA_Fragments_using_Agarose_Gel_Electrophoresis_Protocol_Results_Principle_and_Possible_Errors_to_Avoid

Quaranta, G., Laurenti, P., Boccia, S., Amore, R., & Ricciardi, G. (2007). Real-time PCR detection of Salmonella spp. and Listeria monocytogenes in ready-to-eat foods: A comparison

between the biomolecular method and traditional microbiology. *Italian Journal of Public Health*, *5*(1). https://www.researchgate.net/profile/Stefania-Boccia/publication/282764037

Quiñones, B., Lee, B. G., Avilés Noriega, A., & Gorski, L. (2024). Plasmidome of *Salmonella enterica* serovar Infantis recovered from surface waters in a major agricultural region for leafy greens in California. *PLOS ONE*, 19(12), e0316466. https://doi.org/10.1371/journal.pone.0316466

Rahman, A., Jahan, S., Yildirim, G., Alim, M. A., Haque, M. M., Rahman, M. M., & Kausher, A. H. M. (2022). *A review and analysis of water research, development, and management in Bangladesh*. *Water*, *14*(12), 1834. https://doi.org/10.3390/w14121834

Rakib, M. R., Akther, M., Mondol, M. A. H., & Rahaman, M. S. (2023). Assessing the water quality of Hatirjheel Lake of Dhaka City, Bangladesh. *Present Environment and Sustainable Development*, 17(2), 249–262. https://doi.org/10.47743/pesd2023172018

Ranjbar, R., Naghoni, A., Afshar, D., Nikkhahi, F., & Mohammadi, M. (2016). Rapid molecular approach for simultaneous detection of *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae*. *Osong Public Health and Research Perspectives*, 7(6), 373–377. https://doi.org/10.1016/j.phrp.2016.10.002

Ramírez-Castillo, F. Y., Loera-Muro, A., Jacques, M., Garneau, P., Avelar-González, F. J., Harel, J., & Guerrero-Barrera, A. L. (2015). Waterborne pathogens: Detection methods and challenges. *Pathogens, 4*(2), 307–334. https://doi.org/10.3390/pathogens4020307

Rampley, C. P. N., Whitehead, P. G., Softley, L., Hossain, M. A., Jin, L., David, J., Shawal, S., Das, P., Thompson, I. P., Huang, W. E., Peters, R., Holdship, P., Hope, R., & Alabaster, G. (2020). River toxicity assessment using molecular biosensors: Heavy metal contamination in the Turag-Balu-Buriganga river systems, Dhaka, Bangladesh. *Science of the Total Environment*, 703, 134760. https://doi.org/10.1016/j.scitotenv.2019.134760

Rana, M. L., Ullah, M. A., Hoque, M. N., et al. (2025). Preliminary survey of biofilm forming, antibiotic resistant *Escherichia coli* in fishes from land based aquaculture systems and open water bodies in Bangladesh. *Scientific Reports, 15*, 7811. https://doi.org/10.1038/s41598-024-80536-6

Righetto, L., Zaman, R. U., Mahmud, Z. H., Bertuzzo, E., Mari, L., Casagrandi, R., Gatto, M., Islam, S., & Rinaldo, A. (2015). Detection of *Vibrio cholerae* O1 and O139 in environmental

waters of rural Bangladesh: A flow-cytometry-based field trial. *Epidemiology & Infection, 143*(11), 2330–2342. https://doi.org/10.1017/S0950268814003252

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the Total Environment, 447*, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., Mevius, D. J., & Hordijk, J. (2022). Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy, 77*(5), 1117–1132. https://doi.org/10.1093/jac/dkac024

Ishii, K., & Fukui, M. (2001). Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. *Applied and environmental microbiology*, *67*(8), 3753–3755. https://doi.org/10.1128/AEM.67.8.3753-3755.2001

Islam, M. S., Rehnuma, M., Tithi, S. S., Kabir, M. H., & Sarkar, L. (2015). Investigation of water quality parameters from Ramna, Crescent and Hatirjheel lakes in Dhaka City. *Journal of Environmental Science & Natural Resources, 8*(1), 1–5. https://www.researchgate.net/publication/389046050

Shahed, N. A. (2025). *Assessment of water quality in selected urban ponds of Dhaka: A case study of Shahidullah Hall, Jagannath Hall, Nababri, and Baldha Garden ponds* (Lab report). https://www.scribd.com/document/940825186/Lab-Report

Saima, S., Ferdous, J., Sultana, R., Rashid, R. B., Almeida, S., Begum, A., & Jensen, P. K. M. (2023). Detecting enteric pathogens in low-risk drinking water in Dhaka, Bangladesh: An assessment of the WHO water safety categories. *Tropical Medicine and Infectious Disease, 8*(6), 321. https://doi.org/10.3390/tropicalmed8060321

Salwan, R., & Sharma, V. (2023). 5.5.1 Plasmid isolation using alkaline lysis method. In *Laboratory Methods in Microbiology and Molecular Biology* (ScienceDirect Topics). https://www.sciencedirect.com/topics/immunology-and-microbiology/alkaline-lysis

Sanders E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of visualized experiments : JoVE*, (63), e3064. https://pmc.ncbi.nlm.nih.gov/articles/PMC4846335/

Santiago-Rodriguez T.M., Rivera J.I., Coradin M., Toranzos G.A. Antibiotic-Resistance and Virulence Genes in Enterococcus Isolated from Tropical Recreational Waters. J. Water Health. 2013;11:387–396. doi: 10.2166/wh.2013.005

Stanton, I. C., Bethel, A., Leonard, A. F. C., Gaze, W. H., & Garside, R. (2022). Existing evidence on antibiotic resistance exposure and transmission to humans from the environment: A systematic map. *Environmental Evidence*, 11, 8. https://doi.org/10.1186/s13750-022-00262-2

Sharif, N., Opu, R. R., Saha, T., Khan, A., Alzahrani, F. M., Alsuwat, M. A., Suárez, R. S. R., Villena, E. G., Alzahrani, K. J., & Dey, S. K. (2025). Antimicrobial resistant enteric bacteria are widely distributed among environmental water sources in Dhaka, Bangladesh. *npj Clean Water, 8*, Article 16. https://doi.org/10.1038/s41545-025-00447-5

Sheppard, A. E., Stoesser, N., & Wilson, D. J. (2023). Horizontal gene transfer and the evolution of antimicrobial resistance in environmental bacteria. *Nature Reviews Microbiology, 21*(9), 531–544. https://doi.org/10.1038/s41579-023-00874-2

Siddique, A. B., Munni, A., Hasan, M., et al. (2024). Investigation and detection of multiple antibiotic-resistant pathogenic bacteria in municipal wastewater of Dhaka city. *Discover Water, 4*, 52. https://doi.org/10.1007/s43832-024-00114-9

Sidstedt, M., Rådström, P., & Hedman, J. (2020). PCR inhibition in qPCR, dPCR and MPS—Mechanisms and solutions. *Analytical and Bioanalytical Chemistry, 412*, 2009–2023. https://doi.org/10.1007/s00216-020-02490-2

Singh, R., Gupta, S. K., & Singh, A. K. (2023). Plasmid-mediated resistance among *Escherichia coli* from water sources impacted by human activities. *Journal of Water and Health, 21*(8), 1234–1245. https://doi.org/10.2166/wh.2023.334

Smillie, C., Garcillán-Barcia, M. P., Francia, M. V., Rocha, E. P. C., & de la Cruz, F. (2010). Mobility of plasmids. Microbiology and Molecular Biology Reviews, 74(3), 434–452. https://doi.org/10.1128/MMBR.00020-10

Sobur, M. A., Ahmed, S., Islam, M. T., Sabuj, A. A. M., Sarker, R., Rahman, M. T., & Rahman, M. M. (2019). Antibiotic-resistant *Escherichia coli* and *Salmonella* spp. associated with dairy farm environments and their public health implications. *Veterinary World*, 12(10), 1680–1687. https://doi.org/10.14202/vetworld.2019.1680-1687

Sultana, S., Johura, F.-T., Lewis, J., Sadique, A., Sack, D. A., Alam, M., & Chakraborty, S. (2025). Occurrence of enterotoxigenic *Escherichia coli* in the aquatic environment and impact of

climatic factors. *Frontiers in Environmental Science, 13*, 1593899. https://doi.org/10.3389/fenvs.2025.1593899

Suthar, M. B. (2021). Climate change and antimicrobial resistance in aquatic ecosystems: Emerging interactions, ecological consequences, and public health implications. *Microbiology Archives: An International Journal*, 3(2), Article 01. https://doi.org/10.51470/MA.2021.3.2.01

Taha, A. M., Hammed, Z. N., & Kareem, A. M. (2023). *Extraction & purification of plasmid from some strains of Escherichia coli in different ways.* Journal for Research in Applied Sciences and Biotechnology, 2(2), 161–166. https://doi.org/10.55544/jrasb.2.2.23

Takashashi, S., & Nagano, Y. (1984). Rapid procedure for isolation of plasmid DNA and application to epidemiological analysis. *Journal of Clinical Microbiology, 20*(3), 614–620. https://pmc.ncbi.nlm.nih.gov/articles/PMC271394/

Tang, L., Liu, P., Wang, X., Yang, F., & Liu, D. (2024). Anthropogenic influence on the distribution of resistance plasmids in urban river systems. *Environmental Pollution, 326*, 121900. https://doi.org/10.1016/j.envpol.2024.121900

Tang, P., Zhao, H., & Chen, S. (2024). Anthropogenic impacts on plasmid prevalence and diversity in urban aquatic ecosystems. *Science of the Total Environment, 872*, 162776. https://doi.org/10.1016/j.scitotenv.2024.162776

Tankeshwar, A. (2016). *Streak Plate Method: Principle, Procedure, Uses.* Microbe Online. https://microbeonline.com/streak-plate-method-principle-purpose-procedure-results/

Tasmim, S. T., Hasan, M. M., Talukder, S., Mandal, A. K., Parvin, M. S., Ali, M. Y., Ehsan, M. A., & Islam, M. T. (2023). Sociodemographic determinants of use and misuse of antibiotics in commercial poultry farms in Bangladesh. *IJID regions*, *7*, 146–158. https://doi.org/10.1016/j.ijregi.2023.01.001

Teixeira, P., Tacão, M., Pureza, L., Gonçalves, J., Silva, A., Cruz-Schneider, M. P., & Henriques, I. (2020). Occurrence of carbapenemase-producing Enterobacteriaceae in a Portuguese river: *blaNDM*, *blaKPC* and *blaGES* among the detected genes. *Environmental Pollution*, 260, 113913. https://doi.org/10.1016/j.envpol.2020.113913

Touron, A., Berthe, T., Pawlak, B., & Petit, F. (2005). Detection of *Salmonella* in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. *Research in Microbiology*, *156*(4), 541-553. https://www.sciencedirect.com/science/article/pii/S0923250805000124

Uddin, M. J., Jeong, Y. K., & Lee, S. H. (2021). *Urban river pollution in Bangladesh during the last four decades*. *Science of the Total Environment*, *758*, 143825. https://doi.org/10.1016/j.scitotenv.2020.143825

Vanessa S., Ferreira E., Manageiro V., Reis L., Tejedor-Junco M.T., Sampaio A., Capelo J.L., Caniça M., Igrejas G., Poeta P. Distribution and Clonal Diversity of *Staphylococcus Aureus* and Other *Staphylococci* in Surface Waters: Detection of ST425-T742 and ST130-T843 Mec C-Positive MRSA Strains. Antibiotics. 2021;10:1416. doi: 10.3390/antibiotics10111416

Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T. S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., Kumar, D., Rana, P., Kumar, B., Maiti, T. K., Sharma, N. C., Bhadra, R. K., Mutreja, A., Nair, G. B., Ramamurthy, T., & Das, B. (2019). Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(13), 6226–6231. https://doi.org/10.1073/pnas.1900141116

Vezzulli, L., Pruzzo, C., Huq, A., & Colwell, R. R. (2010). *Environmental reservoirs of Vibrio cholerae and their role in cholera.* Environmental Microbiology Reports, 2(1), 27–33. https://doi.org/10.1111/j.1758-2229.2009.00128.x

Wang, X., et al. (2023). Global increase of antibiotic resistance genes in conjugative plasmids. *Microbiology Spectrum, 11*(2), e03912-22. https://pmc.ncbi.nlm.nih.gov/articles/PMC10100709/

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., & Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *The Lancet. Infectious diseases*, *13*(2), 155–165. https://doi.org/10.1016/S1473-3099(12)70317-1

Wilkins, T. D., Holdeman, L. V., Abramson, I. J., & Moore, W. E. C. (1972). Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. *Antimicrobial Agents and Chemotherapy, 1*(6), 451–459. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC444242/

Wise, K. (2016). *Preparing spread plate protocols.* American Society for Microbiology. https://asm.org/asm/media/protocol-images/preparing-spread-plates-protocols.pdf?ext=.pdf

World Health Organization (WHO). (2023). *Global antimicrobial resistance and use surveillance system (GLASS) report*. WHO https://www.who.int/initiatives/glass

Xu, J., Czinn, S. J., & Blanchard, T. G. (2010). Maintenance of *Helicobacter pylori* cultures in agar stabs. *Helicobacter*, *15*(5), 477–480. https://doi.org/10.1111/j.1523-5378.2010.00769.x

Zhang, X.-X., Zhang, T., & Fang, H. H. P. (2009). Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology, 82*(3), 397–414. https://www.researchgate.net/publication/23764846_Antibiotic_resistant_genes_in_water_environment