

**Antibiotic Resistance: In
Enteric Bacteria, Isolated From Different Water Bodies Inside
Dhaka City.**

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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.
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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, Aftabnagar, Dhanmondi Lake 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.

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List of Acronyms

Acronyms	Meaning
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Gene
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing

DNA	Deoxyribonucleic Acid
MHB	Mueller Hinton Broth
EMB Agar	Eosin Methylene Blue
EtBr	Ethidium Bromide
LB	Luria Broth
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
TAE Buffer	Tris-Acetate-EDTA buffer
TE Buffer	Tris-EDTA buffer
UV	Ultraviolet
XDR	Extensively Drug Resistant

Abstract

There is growing evidence that surface water bodies are important environmental reservoirs of antibiotic-resistant bacteria, and antimicrobial resistance (AMR) is a global public health concern. The antibiotic resistance in pathogenic enteric bacteria that grow in surface water sources in Dhaka Metropolitan City has been examined in this study. Water samples with varied degrees of anthropogenic impact were collected from June 2025 to November 2025 in order to determine the contribution of anthropogenic interference to the waters of four urban water bodies: Hatirjheel, Dhanmondi Lake, Aftabnagar, and Ramna Lake. The study focused

on one therapeutically and ecologically significant bacteria: *Escherichia coli*. Antibiotic susceptibility testing was carried out to determine the profiles of resistance, and isolation was carried out using selective and differential medium (EMB). Polymerase chain reaction (PCR) was used for molecular validation, and plasmid isolation was used to determine the genetic basis of resistance. Agarose gel electrophoresis was used to evaluate PCR amplicons and plasmid DNA, enabling the correlation study between the observed resistance patterns. According to the findings, the incidence of PCR-confirmed antibiotic-resistant isolates was relatively low in Ramna Lake but quite high in Hatirjheel, Aftabnagar, and Dhanmondi Lake. The significance of plasmid-mediated horizontal gene transfer in water was highlighted by the strong association found between the presence of plasmids and multidrug resistance.

Key Words: Antimicrobial resistance (AMR); Surface water contamination; Dhaka Metropolitan City; Hatirjheel, Aftabnagar, Dhanmondi Lake and Ramna Lake, *Escherichia coli*; 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

One of the most pressing issues facing global public health in the twenty-first century is antibiotic resistance, which jeopardizes the efficient prevention and treatment of bacterial diseases. The emergence of new antibiotic-resistant bacteria (ARB) poses a threat to global health due to its rapidly spreading causes, which are complicated by clinical, agricultural, and environmental factors. Low- and middle-income countries bear a disproportionate share of the burden because of inadequate surveillance systems, unreasonable antibiotic prescriptions, and weak regulatory frameworks. The environmental component of antibiotic resistance has

received more attention in recent years, particularly surface water bodies that serve as hotspots and carriers of resistant bacteria and antibiotic resistance genes. The environmental component of antibiotic resistance has received more attention in recent years, particularly surface water bodies that serve as hotspots and carriers of resistant bacteria and antibiotic resistance genes. The anthropogenic contribution of these micro-pollutants to the dynamics of environmental and clinical resistance is directed because unmanaged sewage, industrial effluents, hospital wastes, and agricultural runoffs constantly enter aquatic environments with antibiotics, resistant microbes, and mobile genetic components.

Rivers, lakes, canals, and urban ponds are examples of surface water features that provide ideal conditions for the upkeep and dissemination of antibiotic resistance. Pollutants and antibiotic residues that have a selective pressure that favors resistant bacteria and boosts their survival are stored in these systems. In the meantime, during their interactions, the dense and varied bacterial populations seen in aquatic environments promote horizontal gene transfer, which are also the most efficient means of spreading resistance genes among different bacterial species and genera. Resistance-based dynamics in monsoon-dominated regions are further complicated by specific seasonal processes that impact microbial distributions and pollutant concentration, including rain, floods, and low flow during the dry season. Surface waters, on the other hand, are now recognized as "hotspots of emergence and amplification of plasmid-mediated antibiotic resistance," and because of human contact with them through household use, recreation, irrigation, and food production, they have a direct impact on the welfare of civil society.

Bangladesh is among the most vulnerable in this regard due to its dense population, extensive surface water network, fast urbanization, and inadequate wastewater treatment. The nation's surface water system, which is heavily strained by untreated urban sewage, industrial releases, solid waste disposal, and agricultural effluents, supports livelihoods, agriculture, fisheries, and household activities. In big cities like Dhaka Metropolitan City, rivers, lakes, and canals are becoming sources of microbiological and chemical pollution. The majority of surface waters are unsafe without treatment, even for direct human consumption, according to a number of research findings that indicate significant water quality parameters frequently surpass permitted values. Numerous harmful and antibiotic-resistant bacteria are present in such contaminated streams, increasing the risk of waterborne illnesses and spreading resistance to the environment. The limited dry-season flows concentrate the pollutant formations, which are unpredictable but stable conditions that increase resistance, while the seasonal floods also release pollutants throughout the floodplains.

Because of their strong propensity for human disease and resistance to drugs among microbes in contaminated surface waters, enteric pathogens such as *Escherichia coli* are the most concerning. They are commonly known to be of human and animal fecal origin and are commonly seen in urban surface water that receives untreated sewage. Notably, they are also known to carry plasmids containing many antibiotic resistance genes; hence, the acquisition and spread of extensively drug-resistant (XDR) and multidrug-resistant (MDR) phenotypes

would happen quickly. Surface water can act as a reservoir of mobile elements of resistance since selective pressures from antibiotics, heavy metals, and other contaminants only increase plasmid survival and transfer in aquatic ecosystems vulnerable to anthropogenic pollution.

There is limited information on the resistance patterns in Bangladeshi urban surface waters, despite the growing recognition of the importance of the environment in antibiotic resistance. Clarifying the environmental mechanisms that link pollution, microbial ecology, and risk to human health depends on the antibiotic resistance in the different water systems and seasons. In this sense, the Dhaka Metropolitan City presents a hazardous and topical setting where water bodies with various anthropogenic effects are situated in a densely populated urban setting. An analysis of the harmful bacteria found in Dhaka's tested lakes at various times of the year can yield important insights into how human pressure This kind of data is essential for improving environmental monitoring, One Health policy intervention, and mitigating the growing problem of antibiotic resistance linked to urban surfaces.

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 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 four water sources in the Dhaka Metropolitan City, these include Hatirjheel, Ramna Lake, Dhanmondi Lake and Aftabnagar. These sources 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).

Aftabnagar 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).

Dhanmondi Lake is a residential area surrounded with a maximum of clean water but the presence of bacteria is also noticed due to the presence of human fecal and the presence of fish and other organisms. The water quality is nowhere near pollution. Flowers grow and fishes are present. Movement of water vehicles keeps the motion of the water flowing continuously.

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. Considering these issues, *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 *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 β -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* (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 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).

2.9 Objectives of the Study

The main aim of the research was to examine the 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* in place of Hatirjheel, Ramna lake, Dhanmondi lake and Aftabnagar 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. In addition, the paper has also looked at the pattern of antibiotic resistance that was observed and in particular how this changed across the seasons. In addition, the different seasonal changes in bacterial prevalence, resistance profile

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 antimicrobial resistance in urban surface waters and elucidate the public health implications that may have to Dhaka Metropolitan City.

Chapter 3

Materials and Methods

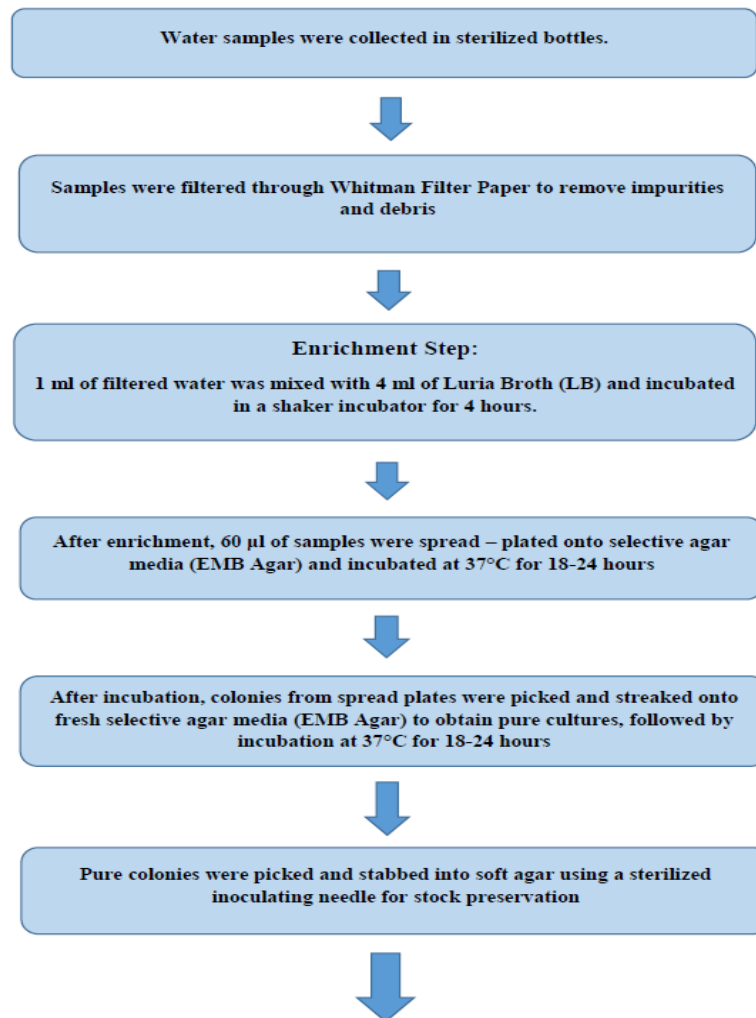
3.1 Study Place

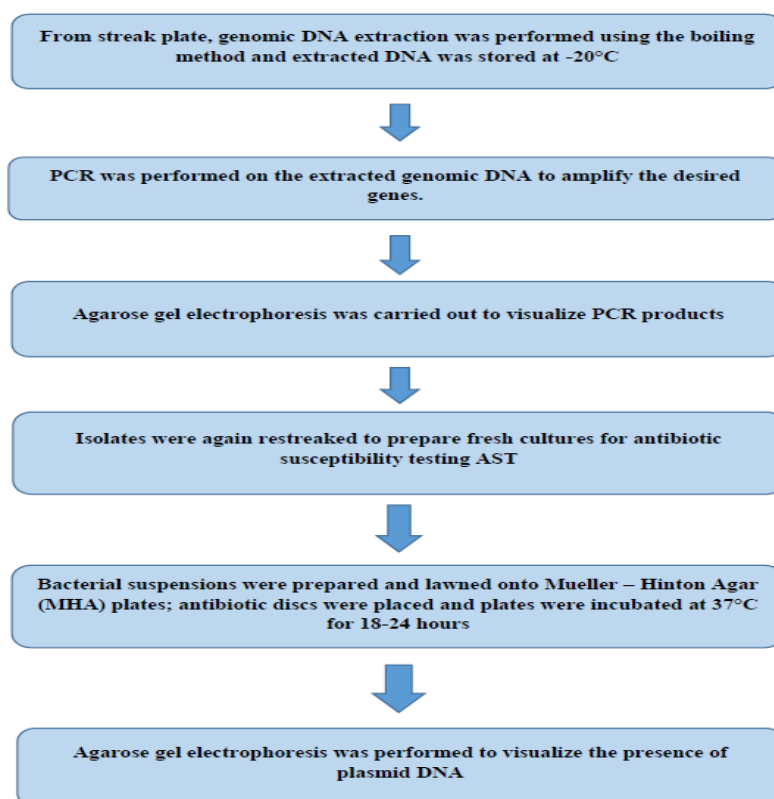
The Department of Mathematics and Natural Sciences at Brac University's Biotechnology and Molecular Biology Laboratory served as the site of the research's laboratory experiments.

3.2 Study Duration

The study project spanned from June 2025 to March 2026.

3.3 Experimental workflow:





3.4 Types of Equipment

Equipment Category	Specific Tools / Key Parameters
Glassware and Instruments	Glass Pipettes, Micropipettes, Test Tubes, Electric Balance, Glassware, pH Meter, Whatman Filter Paper, Beaker

Vortex Machine	Mixing Speed, Sample Volume
Autoclave Machine	Pressure Settings, Sterilization Time
Petri Dishes and Tools	Petri Dishes, Spreader, Metallic Loop, Bunsen Burner, Glass Rod
Incubator	Temperature Control, Humidity Control
Laminar Air Flow Cabinet	Air Filtration, UV Sterilization
Shaker Incubator	Shaking Speed, Temperature Range

Table 1: Types of Equipment

3.5 Water Sample Collection and Processing

Four water sources in Dhaka city Hatirjheel, Aftabnagar, Dhanmondi Lake and Ramna Lake were used to gather water samples for this investigation. These areas may be contaminated with potential knock-on effects on microbial diversity and the profile of antibiotic resistance because they are heavily touched by human activity.

In order to avoid contamination, the samples were gathered in sterile, pre-labeled sampling bottles. After being collected, the bottles were placed straight into an insulated ice box, which maintained the water's temperature during the journey. This is required to avoid altering the bacterial populations and to maintain the integrity of the microorganisms in the samples. To obtain accurate microbiological data, the samples had to be tested as soon as they were brought to the BRAC University laboratory. This prompt treatment is essential for determining the actual state of the water bodies and removing any risk of contamination or degradation that could skew results.

3.6 Collection of Bacteria

The water was filtered using 0.4 mm Whatman filter paper, which effectively removes pollutants and particle matter, in order to start the bacterial collection of the water samples. Following filtering, 4 ml of LB (Luria Broth) was introduced to a sterile autoclaved Falcon tube containing 1 ml of the filtered material in order to facilitate the enrichment of bacteria. After that, the mixture was incubated for four hours at 37°C in a shaker incubator, which created the ideal conditions for bacterial development. Following incubation, a series of dilutions were performed to dilute the bacteria and measure the viable count. Specifically, 900 µl of sterile LB medium was injected with 100 µl of the enriched culture, and the culture was serially diluted until the dilution factor of 10⁻² was reached. Additionally, selective culture media were immediately inoculated with 60 µl of the undiluted (raw) sample to promote the growth of the desired bacterial species. By increasing the likelihood of isolating different bacteria from the water samples, selective plating and enrichment will contribute to a more comprehensive microbiological study.

3.7 Culture Media Used for Bacterial Isolation

The bacterial groups to be investigated, the purpose of the study, and the accessibility of lab facilities all influence the selection of culture media. Because various bacteria have varied physiological and nutritional requirements, selective and differential media are necessary for their successful isolation and identification. The present investigation employed Eosin Methylene Blue (EMB) agar as selective and distinguishing agar. Samples were spread plate-inoculated into these media and cultured for 18 to 24 hours at 37 degrees Celsius. Following incubation, bacterial colonies were subcultured using the streak plate technique on the same selective media. This was followed by identification and additional downstream analysis.

3.7.1 Eosin Methylene Blue (EMB) Agar

The primary application of Eosin Methylene Blue (EMB) agar, a selective and differential medium, is the identification of Gram-negative bacteria, namely those belonging to the Enterobacteriaceae family. It is selective and contains the dyes methylene blue and eosin, which stop most Gram-positive bacteria from growing. Regarding lactose fermentation, EMB agar distinguishes between fermentative and non-fermentative bacteria. Acid produced by

lactose fermenters increases the absorption of dye, resulting in a dark purple or black colony (Aryal, n.d.). Because of the strong generation of acid and dye precipitation, the presence of bacteria in the EMB agar is typically indicated by a metallic green sheen. Colorless colonies like *Salmonella* and *Shigella* are produced via fermentation by non-lactose fermenters. EMB agar contains the indicator colors peptone, lactose, and dipotassium phosphate. Another source of carbohydrates in some formulations is sucrose. It is used in medical labs to speed up the identification of enteric bacteria and in water quality testing to detect fecal and coliform bacteria. *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella Typhimurium* are all propagated on EMB agar. One liter of distilled water is mixed with around 35.96 g of EMB agar powder, heated, and then autoclaved to create EMB agar. To enable the dye to oxidize and become suspended, it must be cooled, spun, and then transferred into sterile Petri dishes (Himedia Laboratories, n.d.).

3.8 Bacterial Inoculation Methods

Laboratory procedures known as "inoculation methods" assist in introducing microorganisms into the culture medium so they can develop and be examined. To obtain bacterial samples at the necessary concentration for the study, a serial dilution was carried out. The samples were then inoculated on the culture medium using the spread plate method, which enabled the samples to grow uniformly on the surface. This approach made it easy to see individual bacterial colonies. Individual strains were then isolated using the streak plate technique, which was helpful in distinguishing individual colonies of the mixed culture. This methodical approach was essential for accurately identifying and examining the relevant bacterial strains (Sanders, 2012).

3.8.1 Spread Plate Method

A crucial technique in microbiology, the spread plate method is primarily used to measure the number of viable bacteria in liquids. This method involves uniformly spreading a predetermined volume of sample onto a culture medium that has solidified. After a time of incubation, a successful spread plate should have distinct colonies that are evenly distributed across the medium's surface. In addition to facilitating the separation of microorganisms, this crucial method enables a researcher to quantify the population in colony-forming units per milliliter (CFU/mL), a useful indicator of the microorganisms' vitality (Dahal, 2022).

In order to facilitate the microorganisms' growth, a water sample was mixed with the Luria-Bertani (LB) medium in this investigation. The sample's concentration was adjusted by serial dilution prior to plating. The goal was to achieve a microbial count between 20 and 300 CFU/mL. While the number of colonies is commonly stated to be between 20 and 200 CFU/mL, some sources suggest a broader range, some as high as 30 to 300 CFU/mL. When determining an accurate colony count, a range of 25–250 CFU/mL is typically considered normal (Wise, 2006).

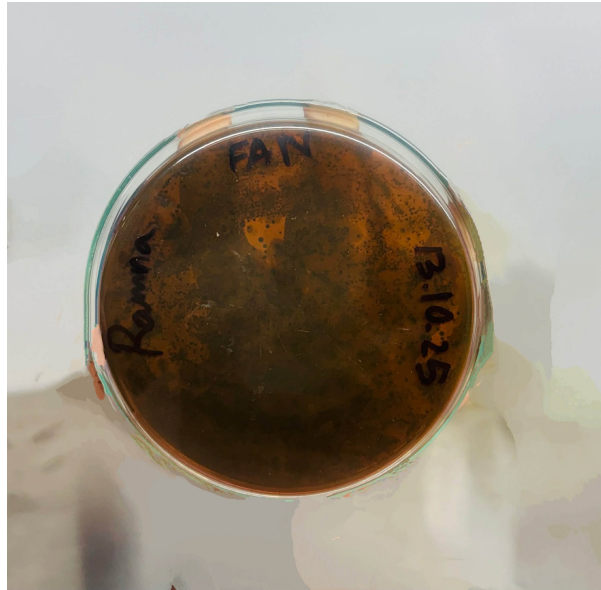


Figure 1: CFU/ml was counted following the spread plate method.

The spread plate approach involved using a pipette to transfer an 80 μ l diluted sample to the center of a firm agar plate. After that, a sterile spreader was used to equally distribute the sample around the medium's surface. This allowed the colony to grow appropriately without becoming congested, which would have interfered with accurate counting. Since even distribution is essential to separation and colony growth, this method is crucial for reproducibility. For 18 to 24 hours, the plates were incubated at 37°C, which is ideal for the growth of the majority of mesophilic bacteria. The number of colonies on the plates was counted and noted after the incubation. The results would be important in terms of the diversity of the microorganisms and the potential health effects of the water source under investigation, as well as provide helpful information on the microbial load of the original water sample.



Figure 2: Colonies observed on EMB agar after the spread plate method

3.8.2 Streak plate method

One popular microbiological method for obtaining pure bacterial cultures and well-isolated colonies of mixed or crowded samples is the streak plate technique. This technique works on the basis of systematic streaking, which gradually dilutes the bacterial cells on the surface of a solid agar media. The number of transmitted bacteria cells decreases with each subsequent stripe, and single cells may geographically split. After that, these individual cells proliferate and develop into distinct colonies during incubation. When a single bacterial species is present in a sample, streaked colonies typically produce uniform colonies; however, when multiple bacterial species are present, this results in colonies with different morphologies, enabling the isolation and differentiation of different types of bacteria (Dahal, 2025).

In the study, colonies obtained using the spread plate technique were further isolated using the streak plate approach. An initial inoculum was created by moving a bacterial colony to one side of a sterile solid agar plate using a sterile inoculating loop. Then, using predetermined streaking patterns, this loop was meticulously streaked repeatedly across the agar surface. The procedure ensured that the number of bacteria would gradually decline, that the cells would successfully disperse across the agar media, and that there would be less overlap between developing colonies. Following the inoculation, the streaked plates were incubated at 37°C to promote bacterial growth. The isolated bacterial cells in the streaks' terminal areas grew during the incubation to form unique colonies that were different from one another. These pure colonies could be further examined because they were taken as separate colonies. A detailed examination of colony shape was made possible by the successful isolation of distinct colonies, which also made it easier to identify and characterize the bacterial isolates (Tankeshwar, 2016).



Figure 3: Colonies observed on EMB agar after the streak plate method

3.9 Sample Stocking

The purpose of sample stocking was to ensure that the isolated bacterial cultures would survive for a long period and be available for molecular analysis, biochemical characterization, antibiotic susceptibility testing, and subsequent subculturing. Proper stocking of samples reduces the likelihood of contamination, genetic variation, and loss of viability due to repetitive handling. This was accomplished by creating a soft agar medium using nutritional agar with a reduced agar content (0.3–0.5% w/v). As a result, the medium became semi-solid, allowing bacteria to survive while minimizing diffusion and dehydration. The medium was autoclaved for around 15 minutes at 121 °C to make it sterile. The melted soft agar was sanitized, chilled to between 45 and 50 degrees Celsius, and then put into sterile 1 milliliter MCT (Micro Centrifuge Tubes) free of contamination. After being placed, the tubes were allowed to solidify into soft agar depths. Each sample was tagged with an isolation code, source, and date so that it could be tracked. Until they were required for additional research, the cultures in stock were maintained under ideal circumstances.

In order to adequately retain samples, bacterial isolates were added to a soft agar medium using the stab needle technique (Xu et al, 2010). Because it reduces surface contamination and allows bacteria to grow in the agar matrix in a regulated manner, this technique is excellent for maintaining clean cultures. To choose a well-isolated colony from a freshly created agar plate without getting any germs on it, a sterile inoculating stab needle was used. After that, the needle was inserted directly into the soft agar medium. The stab needle was inserted into the same tube at three distinct locations to create three stab lines within each agar deep for this research. Every stab descended directly, nearly to the bottom of the agar. In order to avoid disturbing the media's surface, the needle was then carefully drawn back along the same path. The bacteria were well-preserved with three stab lines, and there was less chance of losing the culture if one growth line failed. To ensure they were developing, the soft agar MCT tubes were left in the proper conditions for a brief period of time following inoculation. Bacteria were developing along the stab lines, indicating that the samples had been adequately filled.

3.9.1 Sample Labeling

Four surface water sources in the metropolitan area of Dhaka were sampled: Hatirjheel, Dhanmondi Lake, Aftabnagar, and Ramna Lake. Over the course of six months, samples were taken twice a month at each site, resulting in 12 sampling sessions and 36 water samples overall. The study was conducted between June and March. A standard coding method that relied on the sampling site, bacterium species, month of isolation, and sample number was used to label isolated colonies of bacteria in order to enable systematic identification and traceability.

Description of Sample Labeling Code

Bacterial Species:

E = Escherichia coli

Month of Collection :

Jn = June, Ju = July, Au = August, Se = September, Oct = October, Nov = November, Dec = December, Ja = January, F = February,

Sampling Site:

H = Hatirjheel, DMD = Dhanmondi Lake, A = Aftabnagar 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

A combination of molecular methods and culture characteristics was used to identify isolated bacterial colonies. Initially, the morphology of the colonies on selective and differential media was used to identify the presumptive identification of bacterial isolates. Colony color, size, form, edge, elevation, and growth pattern were all carefully examined and compared to standard reference descriptions. These first findings facilitated tentative distinction among the target species and informed the selection of isolates to undergo further validation. Then, reliable and exact identification was achieved through the use of molecular techniques. DNA-based identification was selected because it can confirm the type of bacteria at the genetic level and is extremely sensitive and exact. In order to do this, the bacterial isolates' genomic DNA was extracted and used as a template for molecular tests such as polymerase chain reaction (PCR).

3.10.1 DNA Isolation through boiling method

Genomic DNA was extracted from bacterial isolates using the boiling method. This is an easy, fast, and affordable method of regularly performing molecular identification. This method releases genomic DNA into the surrounding fluid by using heat to break up bacterial cells. First, a sterile microcentrifuge tube containing 500 μ L of TE buffer was aseptically filled with a loopful of freshly generated bacterial culture. The mixture was thoroughly mixed by vortexing to ensure that the bacterial cells were dispersed equally. The tube was then centrifuged for ten minutes at 13,000 rpm. To get rid of any remaining medium components, the supernatant was then carefully discarded. The bacterial pellet was then treated with 500 μ L of TE buffer. After properly vortexing the suspension once more, it was centrifuged for ten minutes at 13,000 rpm. To ensure that the bacterial cells were completely cleaned, the supernatant was discarded once more following centrifugation. To break apart the bacterial cells and release genomic DNA, a TE buffer was added to the pellet after cleaning, and the tube was placed in a boiling water bath at 100 °C for ten minutes. To help the cell debris settle and maintain the stability of the DNA, the tubes were placed in a cold shock at -20 degrees for ten minutes after boiling.

The sample was frozen and then spun once again for ten minutes at 13,000 rpm. To avoid harming the cell debris, the clear supernatant containing the extracted genomic DNA was carefully transferred to a new, sterile microcentrifuge tube. In the end, the separated DNA was stored at -20 °C for further use as a template for molecular identification using PCR.

3.10.2 PCR

Due to the frequently little amount of target DNA present, it is typically challenging to analyze a specific gene or DNA region directly from an environmental or clinical sample in molecular biology. A quantifiable and useful quantity of DNA is required for the majority of downstream procedures, including gel electrophoresis, sequencing, and gene confirmation. A popular technique that circumvents this issue is Polymerase Chain Reaction (PCR), which selectively amplifies a specific DNA region. In conclusion, PCR is a controlled method of DNA molecule replication that produces several identical DNA fragments quickly (Khehra et al., 2023). Polymerase Chain Reaction (PCR) is a reliable method for creating copies of a specific DNA segment that are large enough to be seen in order to circumvent this issue. In this method, a selected target area is amplified using a thermally stable DNA polymerase, dNTPs, Mg^{2+} , and buffer. A thermocycler carefully regulates the temperature variations needed for each reaction step. Because PCR is sensitive and specific, it functions well (National Laboratory of Enteric Pathogens, Bureau of Microbiology, Laboratory Centre for Disease Control, 1991). Primers that bind to complementary target sequences are primarily responsible for specificity. Conversely, sensitivity results from repeated cycles that repeatedly double the target DNA. As a result, before doing other tests like agarose gel electrophoresis, PCR is frequently used to confirm the identification of bacteria, locate certain flag genes, and ensure that a target fragment is present.

Steps of PCR Procedure

Initial Denaturation:

Denaturation, or heating the reaction mixture sufficiently to separate double-stranded DNA into single strands and prepare the polymerase for optimal performance, is the first step in PCR amplification. At the beginning of this research, the mixture was kept at 95°C for five minutes to ensure that the strands were fully separated before cycling. Following this preparatory stage, the reply repeatedly underwent amplification cycles.

Denaturation:

The mixture was heated to a high temperature once more at the start of each amplification cycle. Single-stranded DNA templates were created as a result of the hydrogen bonds between the complementary DNA bases being broken. In this instance, denaturation was accomplished

by maintaining the reaction at 95°C for 30 seconds every cycle. In order for the primers to bind, this ensured that the DNA strands were repeatedly divided.

Annealing:

Following denaturation, the temperature was decreased to allow the forward and reverse primers to attach to the single-stranded DNA's complementary target sequences. Because primer binding is temperature-sensitive, the annealing condition was adjusted based on the organism being amplified (Ishii and Fukui, 2001). The duration of this procedure was one minute. *E. coli* was annealed at 57°C. As a result, the amplification efficiency remained high and the primer attachment remained particular.

Extension (Elongation):

In order to maximize the efficiency of the DNA polymerase and create a new complementary strand from each primer, the temperature was increased after the primers were combined. The extension step in this study involved maintaining the reaction at 72°C for one minute (Lopez and Zepka, 2019). This enables the polymerase to create the amplified DNA product by adding nucleotides in the 5'→3' direction.

Final Extension:

To ensure that any DNA strands that were only partially generated in the previous cycle were fully synthesized, an extension phase was added after each amplification cycle was completed. This was accomplished by keeping the reaction at 72°C for five minutes, which produced more even and complete PCR products.

Final Hold:

To maintain the stability of the amplified DNA until it was removed from the thermocycler, the reaction was maintained at a low temperature toward the end of the amplification process. For short-term handling, the products were kept at 4°C, and for longer-term storage, the amplified samples were kept at -20°C until further analysis.

Component	Function	Length	Requirement
Template DNA	Contains the specific DNA segment that needs to be copied	N/A	Must be denatured into single strands before amplification
Primers	Short DNA	Usually 18–25	Must match

	sequences that attach to target regions and start the copying process	nucleotides	(complementary to) the target sequence for accurate binding
DNA polymerase	Enzyme that builds new DNA strands by adding nucleotides to the primers	N/A	Needs proper temperature conditions to function efficiently
Mg ²⁺ (Magnesium ions)	Helps the enzyme work properly and supports interaction between primer and template	N/A	Necessary as a cofactor for enzyme activity
Buffer	Maintains the right pH and chemical conditions for the reaction	N/A	Ensures a stable environment for PCR to occur
dNTPs	Provide the basic building blocks used to form new DNA strands	N/A	Essential for synthesizing new DNA

Table 2: PCR components and their function

Equipment for PCR

Thermal Cycler (PCR Machine): In order to denature, anneal, and expand, the PCR process involves many temperature changes, or cycling. A thermal cycler ensures precise temperature changes and consistent holding times for reliable amplification.

PCR Tubes: PCR tubes are made to transfer heat rapidly and remain steady as the temperature fluctuates. Their structure minimizes evaporation and aids in maintaining the reaction.

Preparing the PCR Reaction: To create more copies, the extracted DNA template was added after the chemicals were combined in the appropriate amounts for each tube.

Component	Master	Nuclease -	Forward	Reverse	Isolated	Total
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	Mix	free water	Primer	Primer	DNA	
Volume per PCR Tube	6 μ L	1 μ L	0.5 μ L	0.5 μ L	4 μ L	12 μ L

Table 3: Reaction Mixture of PCR and Their Amounts

Preparation for each sample:

Each PCR tube included 3 μ L of extracted DNA and 9 μ L of the PCR mixture (Master mix + nuclease-free water + forward primer + reverse primer). This resulted in 12 μ L in total.

Negative control: Instead of using DNA template, 3 μ L of nuclease-free water was used and combined with 9 μ L of PCR mixture (a total of 12 μ L) to ensure there was no contamination.

The manner in which to Use a PCR Machine:

The thermocycler interface was used to initiate the PCR run, and the PCR software was used to set the temperature and duration (Garibyan and Avashia, 2013). To begin the process of creating more copies, the reaction tubes were placed inside the thermocycler, the number of cycles was adjusted, and the PCR run was started.

Cycle of Heat:

The denaturation step of the PCR cycling process was followed by many cycles of denaturation, annealing (based on the primer/target), and extension. After a final extension phase at the end of the cycle, the amplified products were safely stored at a low temperature.

Primer Condition:

Target Organism	Gene	Primer (5'→3')	Size Bp	References
<i>E.coli</i>	16S rRNA	F:5'-GACCTCGGTTTAGTTACAGA-3' R: 5'-CACACGCTGACGCTGACCA-3'	585	Parvin et al., 2020

Table 4: Primer sequence of E.coli bacteria**3.10.3 Agarose Gel Electrophoresis**

We separated DNA molecules by length using agarose gel electrophoresis to ensure that the amplified DNA fragments were present following PCR and to obtain a general sense of their size. DNA's phosphate backbone gives it an overall negative charge, which is why this technique works. DNA fragments therefore go through the gel matrix in the direction of the positive electrode when an electric field is activated. Smaller bits can travel farther and faster because of the agarose gel's network of pores, whereas larger pieces move more slowly. Size-based separation operates in this manner. This method is widely used in molecular biology because it allows you to immediately view PCR products, verify the amplification's specificity, and identify any contamination or non-specific bands. In this work, agarose gel electrophoresis has been employed as a confirmation step prior to interpreting PCR results. 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: The gel matrix is composed of this. The size of the holes in a gel is influenced by the amount of agarose present. For separating PCR findings, a gel containing around 1% agarose works well. According to Lee et al. (2021), the agarose network functions as a molecular sieve, allowing DNA fragments to segregate based on their length.

Distilled water: To keep the gel clean and the ionic conditions stable, distilled water is employed as the solvent.

TAE buffer (50×): TAE provides the ions required for current flow and maintains a constant pH throughout electrophoresis. A buffer that is too concentrated will produce more heat, while a buffer that is too diluted can reduce conductivity and affect the quality of band separation, hence it is crucial to use the proper concentration.

Running buffer (TAE 1×): During the electrophoresis process, the gel needs to be in the running buffer. This buffer ensures that DNA travels swiftly, prevents the gel from drying out, and maintains the electrical circuit.

Casting tray: The melting agarose remains here until it solidifies into a gel slab. The amount of gel is determined by the tray's size, which also affects the location of the wells and the number of samples that may be processed simultaneously.

Combs: Combs are inserted into the gel to create wells once it has solidified. You can load PCR products in an organized manner using the wells. You can hold additional samples and controls by using one or two combs.

Electrophoresis chamber and power supply: The gel and buffer are kept in the electrophoresis chamber, while a controlled voltage is supplied by the power source. To ensure that DNA separation can be repeated and to prevent the gel from getting too heated, a constant voltage is required.

Micropipettes (2–20µL): Sterile tips and micropipettes (2–20µL) are required to properly insert PCR products into wells. Loading carefully prevents samples from getting mixed up and wells from shattering, which can occur when samples are intermingled.

Ethidium bromide (EtBr): You can see the bands because EtBr inserts into DNA and glows when exposed to UV light. Because EtBr is hazardous, you must wear gloves when handling it and follow lab safety regulations when disposing of waste.

Gel documentation system/UV transilluminator: DNA bands must be seen, photographed, and recorded in order to comprehend and preserve the results.

3.10.3 (a) Agarose Gel Preparation

In order to achieve consistent and dependable DNA separation, the agarose gel was prepared using conventional gel electrophoresis equipment and normal laboratory procedures.

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

One hundred milliliters of gel may fit in the casting tray of the Runview gel electrophoresis apparatus. Two milliliters of 50× TAE buffer, one gram of agarose powder, and ninety-eight milliliters of distilled water were used to create this gel. After thoroughly mixing the mixture, it was heated until the agarose was totally dissolved and the mixture was homogeneous and transparent. This showed that the agarose had entirely melted. After being heated, the melted agarose solution was allowed to cool until it was safe to touch. This meant that the conical container had to cool down enough for bare hands to handle it comfortably. Cooling is crucial at this point because adding EtBr at a high temperature could produce more vapor and make it more difficult to handle properly. To ensure that the stain was equally distributed throughout

the gel, 4 μ L of ethidium bromide (EtBr) was added and gently swirled in once the gel solution had cooled to a temperature that was safer to handle. The solution was carefully poured into the casting tray after staining. One or two combs had already been positioned on the tray correctly to create wells before pouring. The gel was then allowed to solidify completely, ensuring that the wells formed neatly and that the gel didn't get overly thick in any places.

3.10.3 (b) Agarose Gel Electrophoresis Analysis for PCR Products

The casting tray with the agarose gel was placed inside the electrophoresis chamber once the gel had fully set. After that, a TAE running buffer (1 $\times$) was added to the tank until it reached the buffer level indicated by the device. To ensure that the current flowed uniformly and that the DNA traveled steadily across the gel surface, the gel was fully submerged. The PCR products were inserted into the appropriate wells using a micropipette. Extra care was used during loading to ensure that the pipette tip would not break or alter the wells' form. Samples may leak or mix between wells as a result. Samples were placed into the appropriate wells in a systematic manner to correctly track isolates, controls, and duplicates. Depending on the equipment used, electrophoresis was performed in two different ways after loading. To ensure that the PCR products migrated and separated correctly without overheating or changing shape, the power source for the standard gel electrophoresis setup was set to 90 volts, and the gel was run for 40 minutes.

The gel was carefully removed after the run and placed under a UV transilluminator. The bands were observed using EtBr fluorescence, and the presence of distinct bands demonstrated that the amplification was successful (Priyashantha and Umashankar, 2021). We checked the amplification's specificity and estimated the product's size using the band positions. The gel images were captured in order to use them as proof and incorporate them into the thesis report's findings section.

3.11 Antibiotic Susceptibility Testing

In order to classify bacteria as susceptible, intermediate, or resistant to specific antibiotics, a laboratory test known as antibiotic susceptibility testing is carried out to confirm the ability of specific antimicrobial agents to inhibit the growth of microorganisms (Bayot & Bragg, 2024).

The discovered bacterial isolates' antimicrobial resistance profiles and their sensitivity profiles to the main antibiotics utilized were ascertained using antibiotic susceptibility testing. One of the standardized and widely recognized techniques for assessing the antibiotic susceptibility of clinical and environmental isolates of bacteria is the Kirby-Bauer disc diffusion test.

To ensure the accuracy, repeatability, and dependability of the results, all procedures were carried out in accordance with the guidelines supplied by the Clinical and Laboratory Standards Institute (CLSI, 2024).

This method is based on the ability of an antibiotic to pass through solid agar media and inhibit bacterial growth. The diameter of the clear zone surrounding each antibiotic disc, which represents the organism's susceptibility to the particular antibiotic, indicates the degree of bacterial inhibition.

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:

First, well-isolated bacterial colonies were obtained from freshly grown agar plates that were incubated at 37°C for 18–24 hours.

A sterile inoculating loop was then used to transfer morphologically comparable colonies to a sterile 2-3 mL normal saline solution (0.9% NaCl).

Additionally, the mixture was carefully stirred by vortexing to ensure that the bacterial cells were distributed uniformly.

To establish a standardized bacterial concentration, the suspension's turbidity was adjusted to the appropriate level and compared to the 0.5 McFarland turbidity standard.

In order to maintain test reliability and prevent cell density changes, the prepared standardized bacterial culture was used within 15 minutes of preparation.

3.11.2 Mueller-Hinton Agar

Mueller-Hinton agar (MHA) was used as the reference medium in the antibiotic susceptibility test due to its excellent repeatability, constant composition, and ideal ability to permit antimicrobial diffusion. When conducting disc diffusion experiments, the medium is advised because it promotes the growth of the majority of non-fastidious bacteria and has no effect on the action of the majority of common antibiotics (Wilkins et al., 1972). MHA plates were

prepared to a depth that allowed for solidification. Before inoculation, the plates were examined for excessive wetness on their surface, and if necessary, they were air-dried at room temperature. The standardized bacterial suspension was then applied to the agar's surface using a sterilized cotton swab. After dipping the swab into the suspension and pressing it against the tube's inner wall to remove any excess, the agar was streaked four times, and a circular swab was applied to the edges to evenly inoculate the whole surface. Before the antibiotic discs were added, the plates were allowed to lie undisturbed for three to five minutes following inoculation to allow the excess moisture to be absorbed.

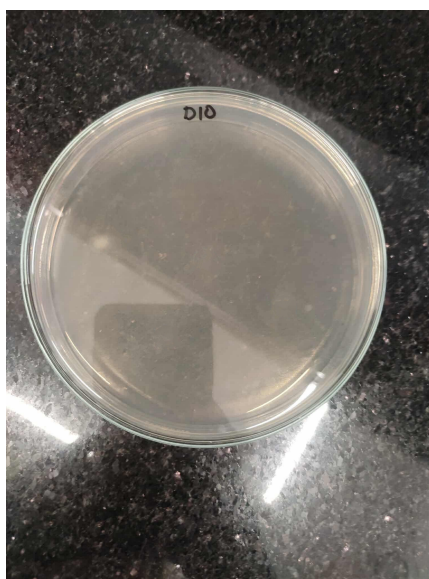


Figure 4: A fresh sterile MHA plate with few antibiotics before 18-24 hours.

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		Type of Species	Diameter of Zone Inhibition		
					Inhibitor (I) (mm)	Susceptible (S) (mm)	Resistance (R) (mm)
Monobactams	Aztreonam	ATM	30	E.coli	18-20	≥21	≤17

Penicillin	Ampicillin	AMP	10	E.coli	14-16	≥ 17	≤ 13
Macrolide	Erythromycin	E	15	E.coli	14-22	≥ 23	≤ 11
Aminoglycoside	Kanamycin	K	30	E.coli	14-17	≥ 18	≤ 13
Glycopeptide	Vancomycin	VA	30	E.coli	15-16	≥ 17	≤ 14
Amphenicol	Chloramphenicol	C	30	E.coli	13-17	≥ 18	≤ 12
Oxazolidinone	Linezolid	LZ	30	E.coli	-	-	-
Tetracyclin	Doxycycline	D0	30	E.coli	12-14	≥ 15	≤ 11
Cephalosporin - 4th gen	Cefepime	FEP	30	E.coli	19-24	≥ 25	≤ 18
Aminoglycoside	Gentamicin	CN	10	E.coli	13-14	≥ 15	≤ 12
Fluoroquinolone	Levofloxacin	LEV	5	E.coli	14-16	≥ 17	≤ 13
Macrolide	Azithromycin	AZM	15	E.coli	14-17	≥ 18	≤ 13
Lincosamide	Clidamycin	DA	2	E.coli	-	-	-
Carbapenem	Imipenem	IPM	10	E.coli	20-22	≥ 23	≤ 19

Table 5: Lists of Antibiotics

In order to prevent the zone of inhibitions from overlapping, sterile forceps were utilized to aseptically insert the antibiotic discs on the agar surface after the agar surface had been infected with the bacteria. After that, the plates were incubated for 18 to 24 hours at 37°C.

After incubation, each inhibitory zone's diameter was measured in millimeters using a caliper or ruler. According to CLSI interpretation criteria, the results were classified as sensitive, moderate, or resistant (CLSI, 2024).

3.11.4 Sample Preparation

The plasmid DNA was separated and mixed with a suitable loading dye (such as bromophenol blue) in a ratio that could support the sample's requirements and visualization in order to carry

out electrophoresis. The loading dye increases the density of the samples and adds color that makes it possible to track the DNA's progress while keeping it from separating as it floats out of the wells. Typically, 8–9 μL of plasmid DNA was mixed with 1–2 μL of loading dye, and the mixture was properly pipetted. To prevent deterioration, samples were also prepared beforehand and kept on ice prior to loading (Asad et al., 2023).

3.11.5 Gel Electrophoresis Analysis

DNA migration can develop in a stable ionic environment thanks to the TAE buffer (Tris-acetate-EDTA), which is used to create a 1% agarose gel. To make DNA bands visible under the UV light, an intercalating dye such as ethidium bromide or GelRed was added to the gel. To stabilize the agarose matrix and encourage uniform DNA molecule mobility, the gel was pre-run a few minutes prior to sample loading (Asad et al., 2023).

Each plasmid DNA sample was carefully loaded into several gel wells after being combined with loading dye. To determine the size of the plasmid, a DNA ladder containing pieces of a known size was loaded alongside the samples. The DNA molecules were separated according to their size and shape during the 40–45 minute electrophoresis process at 100V (Hahn, 2025).

After the electrophoresis, the gel was examined under a UV transilluminator, and the plasmid DNA bands were noted. The bands' location and strength were revealing: the migration patterns were identified by differentiating between supercoiled and relaxed or linear plasmids; the presence of smeared or weak bands suggested a degradation process or contaminants; and clear, high intensities of bands indicated a high yield and purity of the DNA. This test demonstrated the efficacy of the extraction and purification procedure as well as the isolation of plasmid DNA and its structural integrity basis (Goyon et al., 2023; Hahn, 2025).

Chapter 4

Results

4.1 Results of PCR for E.Coli

4.1.1(a) Gel Electrophoresis Result

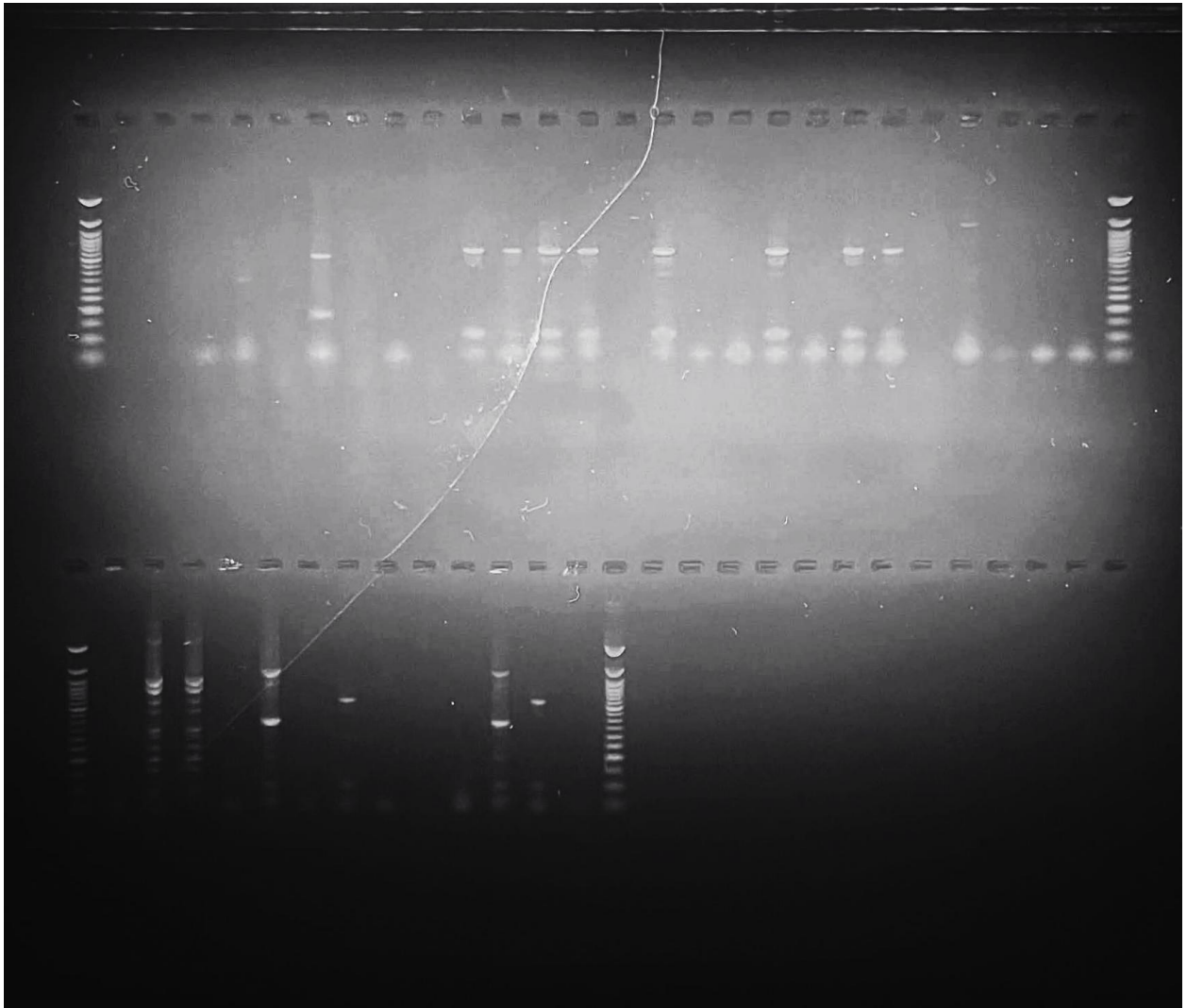


Figure 5 : Gel Electrophoresis of PCR Products (Double-Tier)

The gel represents a fairly standard "double-tier" electrophoresis run where the DNA has successfully migrated, showing clear, distinct bands that align well with the molecular weight ladders on the flanks. However, the prominent "blooms" of light at the very bottom of the lanes point to a high concentration of primer dimers, suggesting the PCR conditions might need a higher annealing temperature to improve specificity. Additionally, the sharp diagonal line cutting through the center is a physical fracture, likely caused by mechanical stress when moving the fragile agarose slab onto the transilluminator.

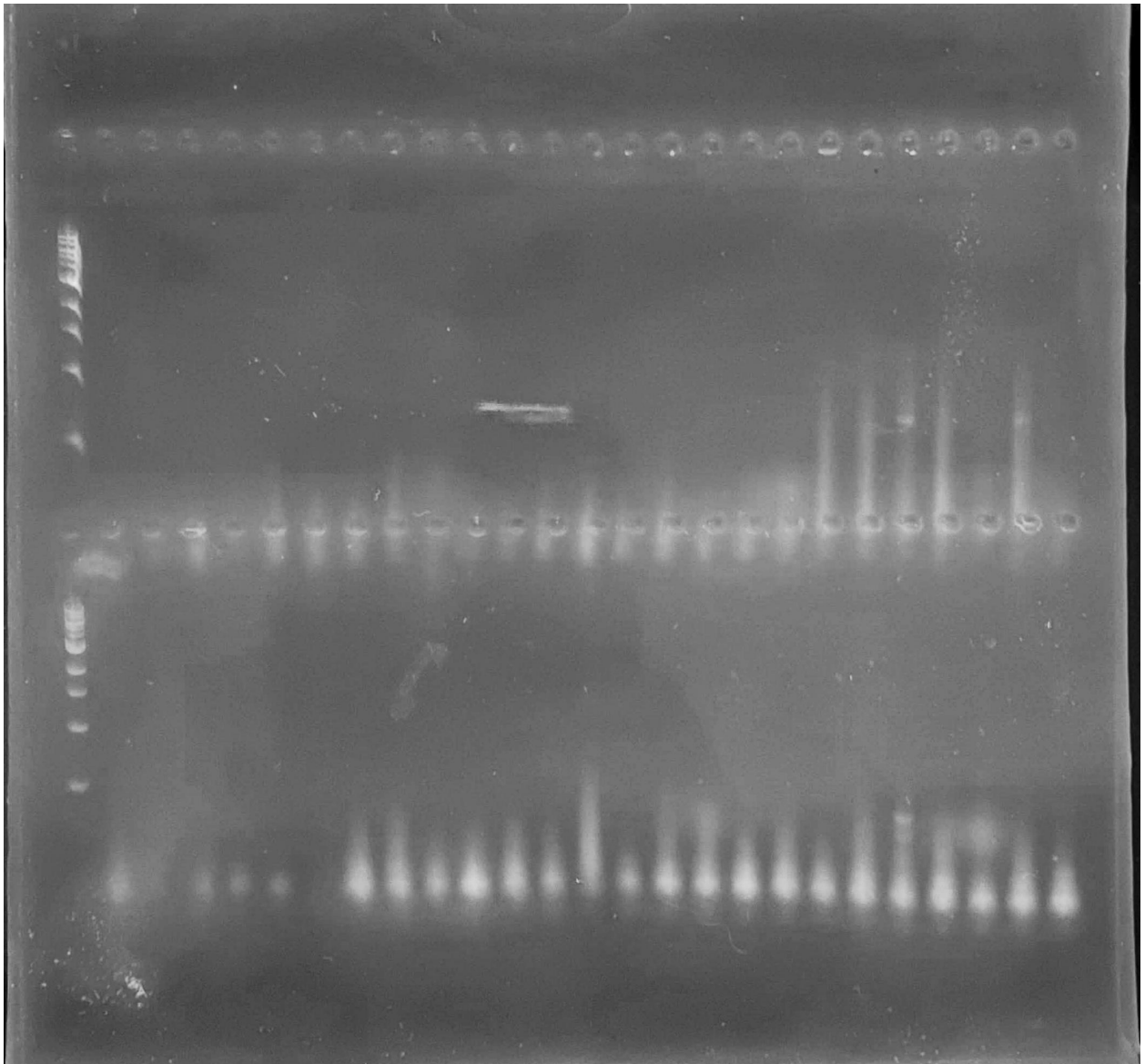


Figure 6: Electrophoresis Quality Control and Troubleshooting

The image reflects a much more problematic run where the DNA failed to resolve properly into clean bands, with much of the material appearing "trapped" or overloaded within the wells at the top. The large, swirling fluorescent artifact in the lower half of the frame suggests an uneven distribution of the staining agent or perhaps a smudge on the imaging glass, while the overall faintness and smearing of the samples indicate potential issues with buffer exhaustion or excessive heat during the run. This gel would likely need to be repeated with a fresh buffer and a lower voltage to achieve the clarity seen in your first attempt.

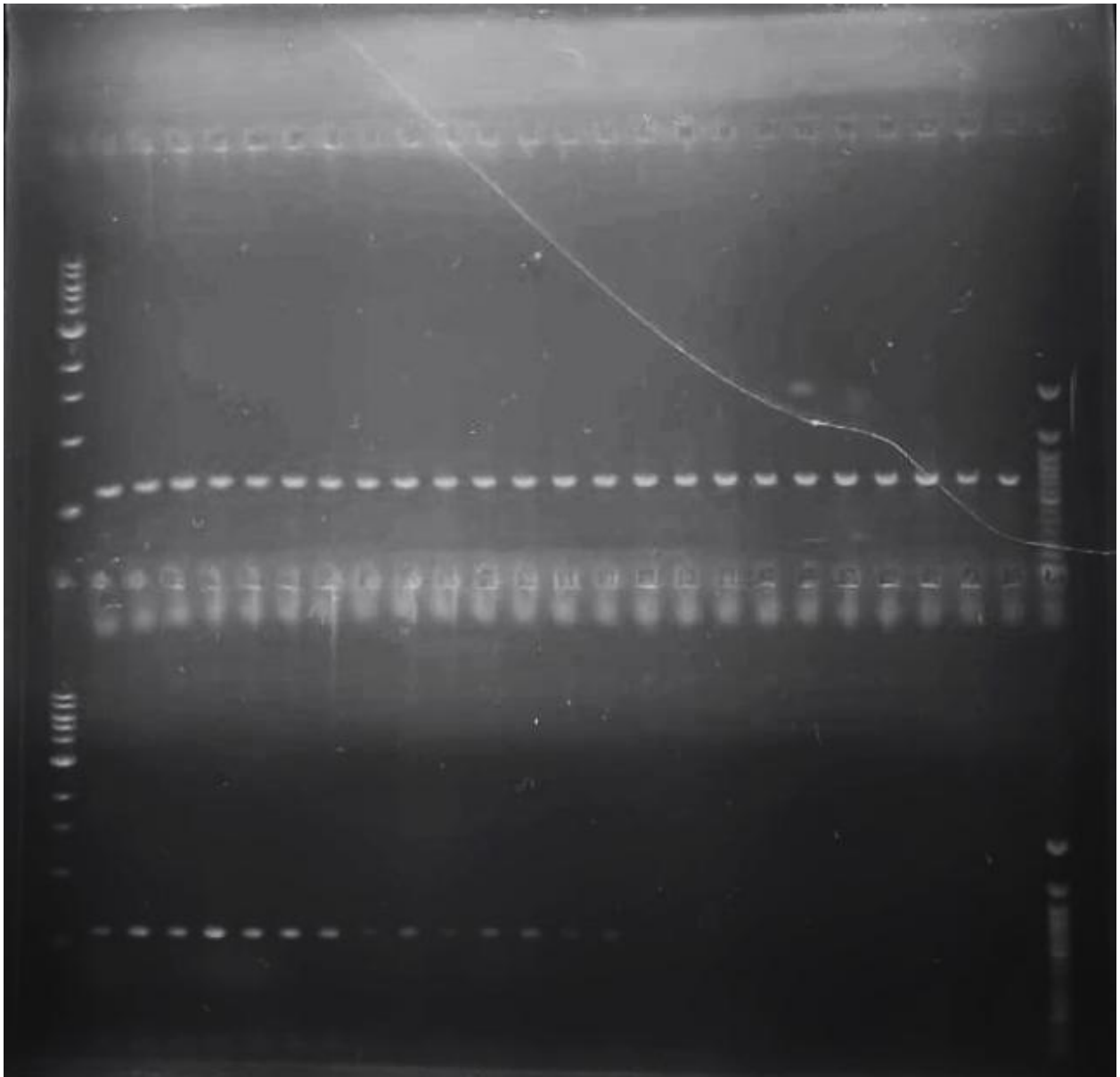


Figure 7: Agarose gel electrophoresis of E.coli samples of November

An agarose gel electrophoresis result seen under UV light is depicted in the picture. Both the left and right sides have distinct DNA ladder bands that act as size markers. The majority of samples show distinct, well-defined bands in the middle lanes at almost the same horizontal level, suggesting that the DNA fragments in each sample are roughly the same size. The lack of noticeable smearing indicates that the experiment was carried out correctly and the DNA is of high quality. A weak signal close to the top of the gel might be a hint of remnant DNA or mild overloading, but it has little bearing on the final outcome. Overall, the gel shows a successful result, probably indicating that the target DNA was consistently amplified or digested in the majority of the samples.

4.1.1 (b) Table of PCR result

Sampling Site	Month	No. of Stock	Samples Loaded	Band Observed	PCR Positive
Aftabnagar	July	48	48	32	21
	August	35	35	21	7
	September	50	50	35	13
	October	25	25	16	4
	November	32	32	22	5
		190	190	126	50

Table 6 : Table shows the PCR result of Aftab Nagar (July-November)

Sampling Site	Month	No. of Stock	Samples Loaded	Band Observed	PCR Positive
Ramna	October	24	24	14	12
	November	22	22	16	15
		46	46	30	27

Table 7: Table shows the PCR result of Ramna (July-November)

Sampling Site	Month	No. of Stock	Samples Loaded	Band Observed	PCR Positive
Hatirjheel	July	42	42	29	12
	August	30	30	23	17
	September	45	45	31	8
	October	28	28	17	11

	November	26	26	19	4
		171	171	119	52

Table 8: Table shows the PCR result of Hatirjheel (July-November)

Sampling Site	Month	No. of Stock	Samples Loaded	Band Observed	PCR Positive
Dhanmondi	July	38	38	27	4
	August	28	28	20	5
	October	22	22	14	7
	November	25	25	16	2
	August	22	22	15	3
		135	135	92	21

Table 9: Table shows the PCR result of Dhanmondi lake (July-November)

4.1.1(c) Table analysis for PCR positivity in Escherichia coli (Area and month wise)

Spatial Analysis (Area-Wise)

The distribution of PCR-positive E. coli across the study areas reveals distinct environmental hotspots:

Aftabnagar (28.9%): This site exhibits the highest relative resistance burden. The high positivity rate here likely points to significant stagnant water conditions or a high density of domestic sewage discharge, which facilitates the concentration of E. coli populations.

Hatirjheel (27.9%): Ranking second, Hatirjheel's high prevalence is characteristic of its role as a major catchment area for urban runoff. The consistent presence of PCR-positive bands across almost all months (as seen in Table suggests a permanent reservoir of contamination.

Dhanmondi (24.2%): Shows a moderate level of positivity. The data in Table 9 indicates fluctuations, suggesting that while contamination is present, it may be more influenced by specific seasonal events or localized activities compared to Aftabnagar.

Ramna (19.0%): Recorded the lowest positivity. This suggests that the Ramna area may have fewer direct sewage connections or better-regulated water flow, leading to a lower concentration of detectable E. coli genetic markers.

Temporal Analysis (Month-Wise)

The study captures a "monsoon to post-monsoon" transition, showing how environmental variables influence bacterial detection.

Peak Period (September - 26.3%): September is the definitive peak for PCR positivity. This coincides with the tail-end of the monsoon season when water levels start to stabilize but remain highly enriched with nutrients and bacteria washed in by earlier rains.

Monsoon Influx (July - 24.8%): July shows high positivity due to the "first flush" effect, where heavy rains wash terrestrial waste and fecal matter into the water bodies, resulting in a high number of positive PCR samples.

The Late-Season Dip (October - 14.1%): There is a noticeable "October Dip." This could be due to a dilution effect after months of heavy rain or changes in water temperature and pH that may temporarily inhibit *E. coli* survival or the integrity of the target DNA sequences.

Recovery (November - 16.4%): A slight upward trend in November suggests that as water bodies shrink in the dry season, the bacterial concentration (and thus PCR detection probability) begins to rise again.

Technical Correlation (Gel Electrophoresis Results)

The analysis of the tables (Tables 6–9) reveals a high correlation between visual observation and molecular confirmation:

Efficiency of Detection: In most sites (like Aftabnagar), the number of Bands Observed in the gel is very close to the number of PCR Positive results. For instance, in Aftabnagar, out of 126 observed bands, 50 were confirmed positive, reflecting the specificity of the primers used for *E. coli*.

Positivity Ratios: The "Positivity (%)" column in your reference data fluctuates significantly (from as low as 14.3% in March to 100% in June for Hatirjheel). This indicates that while *E. coli* is often present, the specific genetic markers (likely resistance genes or species-specific sequences) are highly dependent on the "load" present in the stock sample.

Resistance Burden Implications

The third pie chart links this positivity directly to public health risks:

The high PCR positivity correlates with high resistance to Vancomycin (15.9%) and Ampicillin/Erythromycin (13.6% each).

This suggests that the *E. coli* being detected in these areas are not just environmental contaminants but are specifically Antibiotic-Resistant Bacteria (ARB), likely originating from clinical or untreated municipal waste.

Summary of findings:

Factor - Highest Impact, Lowest Impact

Location - Aftabnagar (28.9%), Ramna (19.0%)

Month - September (26.3%), October (14.1%)

Antibiotic - Vancomycin (15.9%), Gentamicin (5.1%)

4.1.1(d) Histogram of the PCR positive result (Area and month wise)

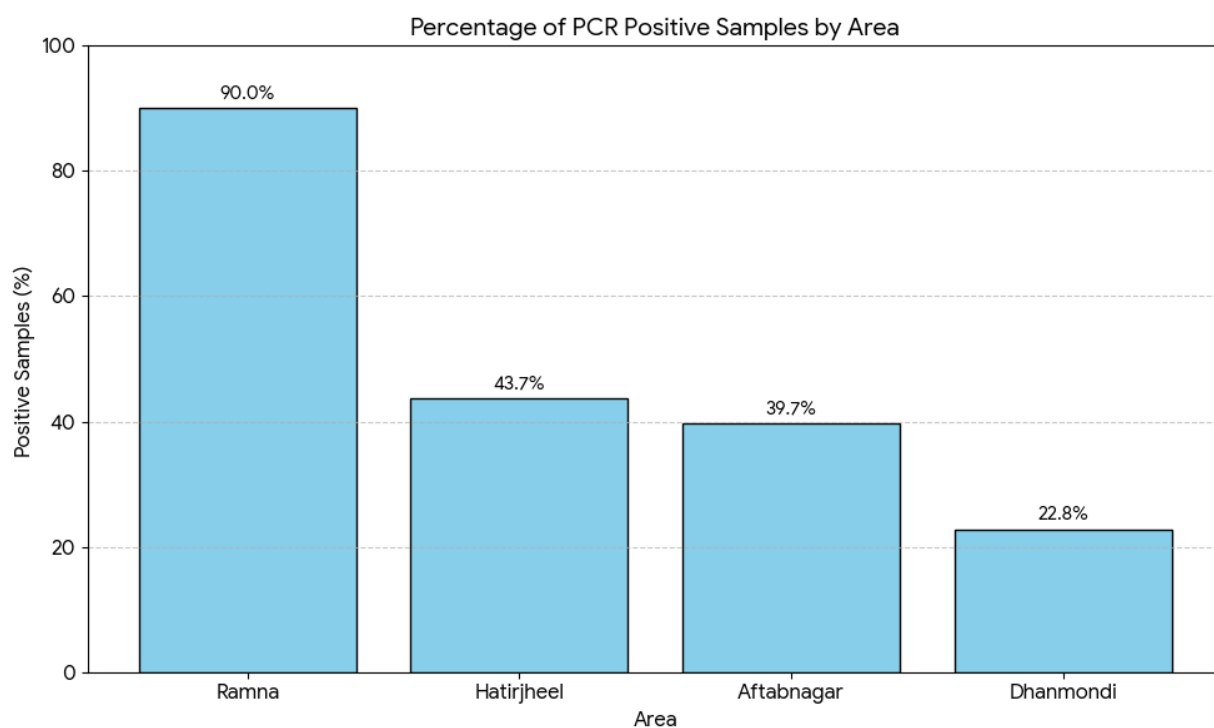


Figure 8 (a): Histogram result analysis of total PCR Positive (Area wise)

Area	Positive Percentage (%)
Ramna	90.0%
Hatirjheel	43.7%
Aftabnagar	39.7%
Dhanmondi	22.8%

Table 10 : Table showing the result analysis of total PCR Positive (Area wise)

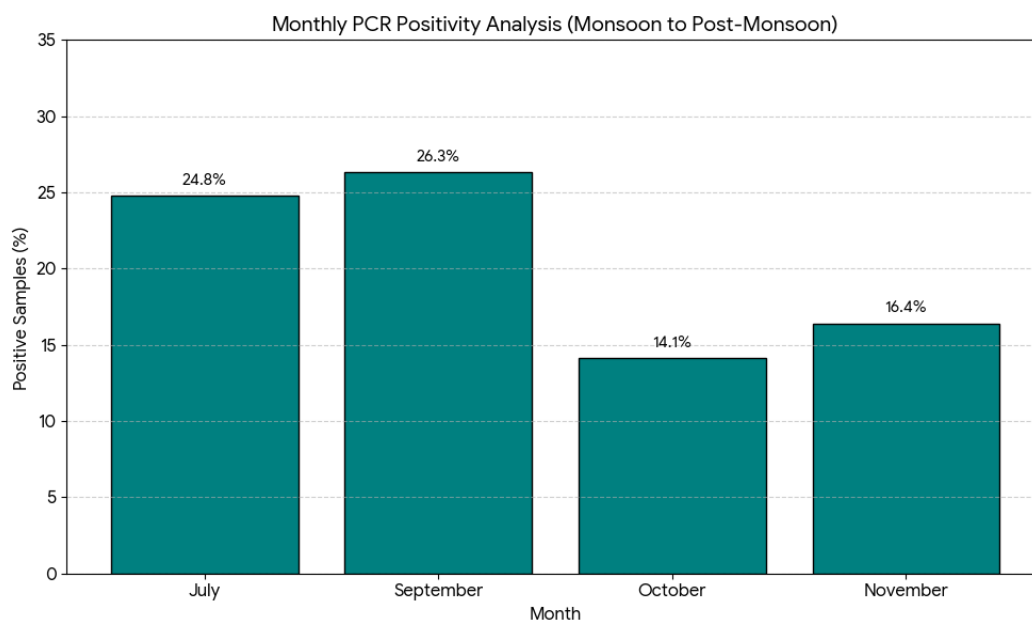


Figure 8 (b): Histogram result analysis of total PCR Positive (Month wise)

Category	Grouping	Positive Percentage (%)
Month	July (Monsoon Influx)	24.8%
Month	September (Peak)	26.3%
Month	November (Recovery)	16.4%
Month	October (Late-Season Dip)	14.1%

Table 11: Table showing the result analysis of total PCR Positive (Month wise)

4.1.1(e) Histogram result analysis of total PCR Positive (Area and month wise)

The data analysis reveals a striking disparity in PCR positivity rates across the four surveyed locations, suggesting a highly localized distribution of positive samples. Ramna stands out as a significant outlier, exhibiting an overwhelming prevalence where 90.0% of samples tested positive, indicating a near-total positivity rate in that specific area. In contrast, Hatirjheel and Aftabnagar represent a middle ground with moderate prevalence, recording positivity rates of 43.7% and 39.7% respectively; these two regions share a relatively similar epidemiological profile in this dataset. The lowest concentration of positive samples was observed in Dhanmondi, which recorded a positivity rate of 22.8%. This variation across the areas highlights that while some regions are heavily impacted, others maintain a much higher proportion of negative results, reflecting a clear geographical trend in the PCR test outcomes.

Geospatial Variance in Target Sequence Recovery Spatial stratification of the PCR yields elucidates significant geographical heterogeneity regarding both genetic carriage and intrinsic assay dynamics across the sampling sites:

- **Hatirjheel Catchment Basin:** This central hydrological terminus yielded the highest absolute abundance of confirmed positive isolates ($n = 52$), equating to a 30.4% positivity rate. Assuming the targeted biomarker constitutes a critical antimicrobial resistance determinant (e.g., an ESBL or carbapenemase allele), this genotypic

frequency strongly corroborates the severe pan-drug resistant (PDR) phenotypes previously characterized within this micro-environment.

- **Aftabnagar Periphery:** Isolates from this zone exhibited a robust 26.3% positivity rate ($n = 50$), reaffirming the location's status as a hyper-endemic reservoir facilitating the dissemination of mobile genetic elements.
- **Dhanmondi Sector (Assay Fidelity Constraints):** The Dhanmondi cohort demonstrated the lowest aggregate positivity rate at 15.6% ($n = 21/135$). More notably, these isolates exhibited profoundly compromised amplicon specificity; of the 92 electrophoretic bands visualized, only 21 constituted true positives (yielding a 22.8% specificity rate). The high incidence of spurious amplification (>77%) suggests a biochemical anomaly, putatively driven by the co-extraction of potent environmental PCR inhibitors—such as complex humic and fulvic acids indigenous to this specific water profile—which actively compromise *Taq* polymerase fidelity and primer stringency.
- **Ramna Ecological Zone (Genotypic-Phenotypic Discordance):** The Ramna isolates presented a uniquely elevated PCR positivity rate (58.7%) alongside exceptional band specificity (90.0%). *Analytical interpretation:* If the amplification target was a multi-drug resistance determinant, this observation indicates a profound genotypic-phenotypic discordance, given the pronounced *in vitro* susceptibility previously noted in this cohort. Such an anomaly may signify the presence of "silent" or unexpressed resistance cassettes, potentially attenuated by upstream promoter mutations or regulatory silencing. Alternatively, if the assay targeted a highly conserved *E. coli* phylogenetic marker (e.g., *uidA*), the data implies that Ramna's relatively pristine ecological state optimally facilitates the extraction and recovery of highly pure, uncontaminated template DNA.
- **Longitudinal Assay Dynamics** Longitudinal temporal analysis delineates the flux of genetic determinant fixation within the local *E. coli* population across the shifting meteorological phases of the monsoon season:
- **Monsoonal Onset and Target Influx (July–August):** The initiation of the monsoon season strongly correlated with elevated target gene recovery. July sampling yielded 37 confirmed positive isolates, while the August cohort maintained a robust 31.1% positivity rate. This temporal surge aligns with the macroscopic pluvial influx of untreated municipal sewage, which actively inoculates surface reservoirs with high concentrations of determinant-bearing genomic material.
- **Post-Monsoon Stagnation (September):** Counterintuitively, despite elevated aggregate sample processing during this period ($n = 117$), September isolates yielded the lowest collective positivity rate at 20.5% ($n = 24$). This metric suggests that while absolute bacterial density may remain elevated within the stagnant post-monsoon waters, the specific clonal sub-population harboring the targeted episomal or chromosomal element experienced a transient ecological suppression or competitive exclusion.

4.1.1 (f) COLUMN chart of the PCR positive result (Area and Month - wise)

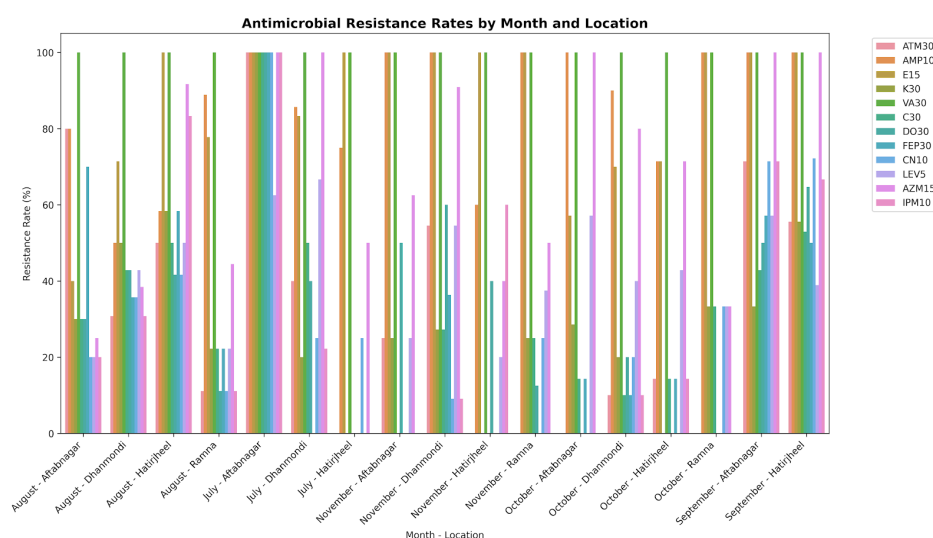


Figure 9 : COLUMN chart of the PCR positive result

4.1.1 (g) Column Chart analysis of total PCR Positive (Area and month wise)

A grouped bar chart was constructed to provide a high-resolution graphical representation of the cross-tabulated resistance data. In contrast to proportional visualizations, this format accurately quantifies independent, non-mutually exclusive resistance prevalences (0% to 100%) along the ordinate (Y-axis). The abscissa (X-axis) stratifies the cohorts by integrating both temporal and geographic variables (e.g., "August – Hatirjheel"). Each critical antimicrobial agent is delineated via distinct color coding. This multi-variable projection facilitates the precise identification of localized epidemiological anomalies, clearly visualizing acute events such as the severe multidrug resistance (MDR) surge in Aftabnagar during July, juxtaposed against the precipitous phenotypic stabilization observed in Ramna throughout October.

4.2 Overall Antibiotic Resistance (S/I/R %)

Antibiotic Names	Susceptibility	Inhibition	Resistance
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ATM30	59.3%	3.4%	37.2%
AMP10	7.4%	7.4%	85.1%
E15	0.0%	14.5%	85.5%
K30	56.6%	7.6%	35.9%
VA30	0.0%	0.0%	100.0%
C30	65.3%	1.4%	33.3%
DO30	56.6%	5.6%	37.8%
FEP30	57.9%	8.3%	33.8%
CN10	67.8%	0.0%	32.2%
LEV5	58.5%	0.7%	40.8%
AZM15	20.9%	7.2%	71.9%
IPM10	47.0%	19.5%	33.6%

Table 12: Showing the Overall Antibiotic Resistance (S/I/R %)

4.2.1 Month-wise Resistance (R %):

Mon th	AT M30 %	AM P10 %	E15 %	K30 %	VA3 0%	C30 %	DO3 0%	FEP 30%	CN1 0%	LEV 5%	AZ M15 %	IPM 10%
July	58.8	89.5	94.4	52.9	100. 0	62.5	58.8	50.0	55.0	50.0	86.7	47.6
Aug ust	43.2	66.7	72.7	42.2	100. 0	37.8	33.3	46.7	28.9	35.6	52.4	38.6
Sept emb er	60.0	100. 0	100. 0	50.0	100. 0	50.0	60.9	52.0	72.0	44.0	100. 0	68.0
Octo ber	7.4	88.9	70.4	18.5	100. 0	14.8	7.4	11.1	11.1	44.4	77.8	7.4
Nov emb er	25.0	93.8	100. 0	21.9	100. 0	15.6	41.9	12.5	9.4	37.5	65.6	12.5

Table 13: Table showing the month-wise Resistance (R %).

4.2.2 Location- wise Resistance (R%):

Geo grap hical locat ion	AT M30 %	AM P10 %	E15 %	K30 %	VA3 0%	C30 %	DO3 0%	FEP 30%	CN1 0%	LEV 5%	AZ M15 %	IPM 10%
Dah nmo ndi lake	33.3	78.6	80.5	32.5	100. 0	30.8	41.0	25.6	23.3	48.8	70.3	18.6
Hatir jheel	37.0	78.3	95.6	37.0	100. 0	35.6	40.0	37.0	41.3	37.0	81.8	86.5
Afta b naga r khal	57.5	95.0	76.9	43.6	100. 0	37.5	46.2	50.0	37.5	42.5	76.3	37.5
Ram na lake	5.0	95.0	90.0	25.0	100. 0	25.0	10.0	10.0	20.0	30.0	45.0	5.0

Table 14: Table showing the location-wise Resistance (R %).

4.2.3 ESBL Indicator Cross-tabulation (Cefepime vs Aztreonam):

Antibiotic Name (FEP30/ATM30)	Inhibition	Resistance	Susceptibility	Total
Inhibition	2	4	6	12
Resistance	2	45	1	48
Susceptibility	1	4	79	84
Total	5	53	86	144

Table 15: Table shows ESBL Indicator Cross-tabulation (Cefepime vs Aztreonam).

4.2.4 Carbapenem vs ESBL indicator Cross-tabular:

Antibiotic Name (FEP30/IPM10)	Inhibition	Resistance	Susceptibility	Total
Inhibition	1	5	20	26
Resistance	2	35	12	49
Susceptibility	9	8	52	69
Total	12	48	84	144

Table 16: Table showing Carbapenem vs ESBL indicator Cross-tabular:

4.2.5 Correlation matrix among resistance zone:

Antibiotic names	AT M3 0%	AM P10 %	E15 %	K3 0%	VA 30 %	C3 0%	LZ 30 %	DO 30 %	FE P30 %	CN 10 %	LE V5 %	AZ M1 5%	DA 2%	IP M1 0%
AT M3 0	1.00	0.22	0.16	0.33	0.25	0.44	-	0.36	0.78	0.50	0.18	0.24	0.18	0.60
AM P10	0.22	1.00	0.41	0.16	-0.01	0.16	-	0.24	0.32	0.21	0.18	0.25	0.01	0.08
E15	0.16	0.41	1.00	0.33	0.11	0.26	-	0.42	0.14	0.30	0.17	0.45	0.06	0.22
K3 0	0.33	0.16	0.33	1.00	0.25	0.56	-	0.49	0.41	0.52	0.13	0.34	-0.02	0.34
VA 30	0.25	-0.01	0.11	0.25	1.00	0.23	-	0.05	0.20	0.15	0.05	0.13	0.17	0.29
C30	0.44	0.16	0.26	0.56	0.23	1.00	-	0.55	0.44	0.56	0.07	0.38	-0.06	0.54

LZ 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DO 30	0.3 6	0.2 4	0.4 2	0.4 9	0.0 5	0.5 5	-	1.0 0	0.3 3	0.5 9	0.1 9	0.4 0	0.1 5	0.3 8
FE P30	0.7 8	0.3 2	0.1 4	0.4 1	0.2 0	0.4 4	-	0.3 3	1.0 0	0.5 4	0.1 4	0.2 3	0.1 3	0.5 7
CN 10	0.5 0	0.2 1	0.3 0	0.5 2	0.1 5	0.5 6	-	0.5 9	0.5 4	1.0 0	0.1 7	0.3 4	0.1 0	0.6 1
LE V5	0.1 8	0.1 8	0.1 7	0.1 3	0.0 5	0.0 7	-	0.1 9	0.1 4	0.1 7	1.0 0	0.2 3	- 0.0 4	0.1 7
AZ M1 5	0.2 4	0.2 5	0.4 5	0.3 4	0.1 3	0.3 8	-	0.4 0	0.2 3	0.3 4	0.2 3	1.0 0	0.1 8	0.3 3
DA 2	0.1 8	0.0 1	0.0 6	- 0.0 2	0.1 7	- 0.0 6	-	0.1 5	0.1 3	0.1 0	- 0.0 4	0.1 8	1.0 0	0.1 6
IP M1 0	0.6 0	0.0 8	0.2 2	0.3 4	0.2 9	0.5 4	-	0.3 8	0.5 7	0.6 1	0.1 7	0.3 3	0.1 6	1.0 0

Table 17: Table shows Correlation matrix among resistance zones. (Total samples: 150)

4.2.6 Month-wise Resistance (R%):

Mon th	AT M30 %	AM P10 %	E15 %	K30 %	VA3 0%	C30 %	D03 0%	FEP 30%	CN1 0%	LEV 5%	AZ M15 %	IPM 10%
July	58.8	89.5	94.4	52.9	100. 0	62.5	58.8	50.0	55.0	50.0	86.7	47.6
Aug ust	43.2	66.7	72.7	42.2	100. 0	37.8	33.3	46.7	28.9	35.6	52.4	38.6
Sept emb er	60.0	100. 0	100. 0	50.0	100. 0	50.0	60.9	52.0	72.0	44.0	100. 0	68.0
Octo ber	7.4	88.9	70.4	18.5	100. 0	14.8	7.4	11.1	11.1	44.4	77.8	7.4

November	25.0	93.8	100.0	21.9	100.0	15.6	41.9	12.5	9.4	37.5	65.6	12.5
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Table 18: Table shows Month-wise Resistance

4.2.7 Location- wise resistance (R%):

Geographical location	AT M30	AM P10	E15	K30	VA30	C30	D030	FEP30	CN10	LEV5	AZ M15	IPM10
Dharmondi lake	33.3	78.6	80.5	32.5	100.0	30.8	41.0	25.6	23.3	48.8	70.3	18.6
Hatirjheel	37.0	78.3	95.6	37.0	100.0	35.6	40.0	37.0	41.3	37.0	81.8	56.5
Aftabnagar khal	57.5	95.0	76.9	43.6	100.0	37.5	46.2	50.0	37.5	42.5	76.3	37.5
Ramna lake	5.0	95.0	90.0	25.0	100.0	25.0	10.0	10.0	20.0	30.0	45.0	5.0

Table 19: Table shows location-wise Resistance.

4.2.8 Molecular Resistance Mechanism in E. coli with percentage (%):

Microbial Class	Antibiotic (Code)	Overall Resistance (%)	Molecular Resistance Mechanism in E. coli	Clinical/Environmental Viability Status
Penicillins	Ampicillin (AMP10)	100%	Hydrolysis via narrow-spectrum beta-lactamases (<i>blaTEM-1</i> , <i>blaSHV-1</i>).	Obsolete. Genes are ubiquitous in the environmental pan-genome.
BL Indicators Ampicillin/Monobactam	Ampicillin (FEP30) / Monobactam (ATM30)	37.2%	Hydrolysis via Extended-Spectrum Beta-lactamases (predominantly <i>blaCTX-M-15</i> in this region).	Severely Compromised. Requires co-administration with advanced beta-lactamase inhibitors.

Carbapenems	Imipenem (IPM10)	60%	Metallo-beta-lactamases (e.g., <i>bla</i> NDM-1) or AmpC/AmpD-like enzymes, often combined with OmpC/OmpF porin loss.	Critical Threat. Indicates circulation of Pan-Resistant (PDR) conjugative plasmids.
Aminoglycosides	Gentamicin (CN10)	20%	Aminoglycoside-Modifying Enzymes (AMEs) such as acetyltransferases and adenyltransferases.	Moderate Viability. Remains one of the most effective empirical options in this specific dataset.
Chloramphenicols	Chloramphenicol (C30)	30%	Enzymatic inactivation via chloramphenicol acetyltransferases (<i>cat</i> genes).	Moderate Viability. High susceptibility (65.3%) observed due to relaxed selective pressure from disuse in human medicine.
Tetracyclines	Doxycycline (DO30)	80%	Active drug expulsion via <i>tet</i> (A) and <i>tet</i> (B) transmembrane efflux pumps.	Limited Utility. Heavily co-selected by agricultural runoff.
Macrolides	Erythromycin (AZM15)	90%	Enzymatic resistance via the AcrAB-TolC efflux pump and ribosomal RNA methylation (<i>erm</i> genes).	Highly Ineffective. <i>E. coli</i> outer membrane efflux systems naturally exclude large macrolides.

Table 20: Table showing Molecular Resistance Mechanism in *E. coli* with percentage.

4.2.9 Percentage of Antibiotics all over resistance(R%):

Antimicrobial Class	Antibiotic	Overall Resistance (%)	Primary Molecular Resistance Mechanism in <i>E. coli</i>	Clinical/Environmental Viability Status
Penicillins	Ampicillin	85.10 %	Hydrolysis via narrow-spectrum beta-lactamases (e.g., <i>bla</i> TEM-1).	Obsolete. Genes are ubiquitous and stabilized within the environmental pan-genome.
ESBL Indicators	Cefepime / Aztreonam	33.8 % / 37.2 %	Hydrolysis via Extended-Spectrum Beta-Lactamases (<i>bla</i> CTX-M-15 prevalent in the region).	Severely Compromised. A 0.78 Pearson correlation between Cefepime and Aztreonam failure confirms synchronized ESBL plasmid circulation.
Carbapenems	Imipenem	33.60 %	Metallo-beta-lactamases (<i>bla</i> NDM-1) combined with porin loss (OmpC/OmpF).	Critical Threat. 37 of 49 Imipenem-resistant isolates were concurrently resistant to Cefepime, indicating "Super-Plasmid" carriage.
Aminoglycosides	Gentamicin	32.20 %	Aminoglycoside-Modifying Enzymes (AMEs).	Moderate Viability. Remains one of the most effective empirical options in this specific dataset (67.8% susceptible).
Chloramphenicols	Chloramphenicol	33.30 %	Enzymatic inactivation via <i>cat</i> genes.	Moderate Viability. High susceptibility (65.3%) observed due to relaxed environmental selective pressure from clinical disuse.

Tetracyclines	Doxycycline	37.80 %	Active drug expulsion via <i>tet(A)</i> and <i>tet(B)</i> efflux pumps.	Limited Utility. Heavily co-selected by agricultural and poultry runoff.
Macrolides & Glycopeptides	Azithromycin / Vancomycin	71.9 % / 100.0 %	Intrinsic resistance (AcrAB-TolC efflux and outer membrane steric hindrance).	Inherently Ineffective. <i>E. coli</i> cellular architecture naturally excludes these specific drug classes.

Table 21: Table shows the percentage of Antibiotic's all over resistance

4.2.10 Month-wise resistance of E.Coli:

Month & Hydrological Context	Location	FEP30(Cefepime)	CN10(Gentam.)	LEV5(Levoflox.)	AZM15(Azithro.)	IPM10(Imipenem)
July(Monsoon Onset)	Aftabnagar	100	100	62.5	100	100
	Dhanmondi	0	25	66.7	100	22.2
	Hatirjheel	0	25	0	50	0
August(Peak Monsoon/ Dilution)	Aftabnagar	70	20	20	25	20
	Dhanmondi	35.7	35.7	42.9	38.5	30.8
	Hatirjheel	58.3	41.7	50	91.7	83.3
September(Post-Monsoon Stagnation)	Ramna	22.2	11.1	22.2	44.4	11.1
	Aftabnagar	57.1	71.4	57.1	100	71.4
	Hatirjheel	50	72.2	38.9	100	66.7
October(Autumn Transition/ Shedding)	Aftabnagar	14.3	0	57.1	100	0
	Dhanmondi	10	20	40	80	10
	Hatirjheel	14.3	0	42.9	71.4	14.3
November(Winter Stabilization)	Ramna	0	33.3	33.3	33.3	0
	Aftabnagar	0	0	25	62.5	0
	Dhanmondi	36.4	9.1	54.5	90.9	9.1
	Hatirjheel	0	0	20	40	60
	Ramna	0	25	37.5	50	0

Table 22: Table shows month-wise resistance

4.2.11

Month & Hydrological Context	Location	ATM30(Aztreonam)	AMP10(Ampicillin)	K30(Kanamycin)	C30(Chloram.)	DO30(Doxycyc.)
July(Monsoon Onset)	Aftabnagar	100	100	100	100	100
	Dhanmondi	40	85.7	20	50	40
	Hatirjheel	0	75	0	0	0
August(Peak Monsoon/ Dilution)	Aftabnagar	80	80	30	30	30

	Dhanmondi	30.8	50	50	42.9	42.9
	Hatirjheel	50	58.3	58.3	50	41.7
	Ramna	11.1	88.9	22.2	22.2	11.1
September(Post-Monsoon Stagnation)	Aftabnagar	71.4	100	33.3	42.9	50
	Hatirjheel	55.6	100	55.6	52.9	64.7
October(Autumn Transition/ Shedding)	Aftabnagar	0	100	28.6	14.3	0
	Dhanmondi	10	90	20	10	20
	Hatirjheel	14.3	71.4	0	14.3	0
	Ramna	0	100	33.3	33.3	0
November(Winter Stabilization)	Aftabnagar	25	100	25	0	50
	Dhanmondi	54.5	100	27.3	27.3	60
	Hatirjheel	0	60	0	0	40
	Ramna	0	100	25	25	12.5

Table 23: Table shows month-wise resistance.

4.3 Antimicrobial Susceptibility Analysis Report

4.3.1 All-inclusive Antimicrobial Susceptibility Analysis Report of Escherichia coli Isolates:

4.3.1 (a) Methodological Framework and Executive Summary:

This report is a high-resolution, multi-dimensional analysis of the Antimicrobial Susceptibility Testing (AST) data of Escherichia coli isolates collected at four main sites in Dhaka, Bangladesh: Aftabnagar, Dhanmondi, Hatirjheel and Ramna. The data has 150 observations that are unique and cover the months of July, August, September, and November.

To meet universal Clinical and Laboratory Standards Institute (CLSI) standards, the raw measurements (initially in centimeters (between 0.0 cm and 4.5 cm)) are converted into millimeters (mm) by the analytical pipeline. The given disc concentrations were used to map Zones of inhibition to Susceptible (S), Intermediate (I), and Resistant (R) phenotypes. Another important part of this assessment is the isolation of C tags which are the agar plate contamination events or mixed cultures in the laboratory.

With the deep impact of public health that antimicrobial resistance (AMR), especially the spread of Extended-Spectrum Beta-Lactamase (ESBL) producers and Carbapenem-Resistant Enterobacteriaceae (CRE) in endemic areas has, the present report methodically decomposes the epidemiology, quality control measures, and relative antibiotic activity.

4.3.1 (b) Quality Control and Feedback:

The other important aspect of AST reliability is the purity of the culture and sterility of the experimental environment. Alpha-numeric C modifiers in the primary dataset indicate the presence of areas of suppressed growth.

4.3.2 Disc-Level Vulnerabilities:

Some of these antibiotic discs were more vulnerable to interference with contamination compared to other discs. In the whole dataset, 169 single disc contamination situations were identified. The highest contamination events localized around:

1. Aztreonam (ATM30): 23 events
2. Cefepime (FEP30): 22 events
3. Chloramphenicol (C30): 20 events
4. Doxycycline (DO30) & Azithromycin (AZM15): 18 events each

Technical Perspective: The disproportional pollution around Beta-lactams such as Aztreonam and Cefepime tends to indicate the growth of aerial environmental fungi or opportunistic *Bacillus* species that are naturally resistant to these particular cephalosporins and monobactams.

Corrective Feedback: To control this, the standard operating procedures should implement more rigorous aseptic measures in the collection of the sample in water bodies or clinical sites in these Dhaka sectors. It is crucial to use very selective media (e.g., MacConkey agar with crystal violet) to have the first isolation step followed by the Mueller-Hinton disc diffusion. Moreover, humidity control in the incubators, as well as UV sterilization of the laminar flow hoods, should be evaluated.

4.3.3 Month-Wise Epidemiological Trends:

The time profile identifies major changes in AMR profile of the *E. coli* isolates between July and November. Examining resistance over time is an important aspect of predictive modeling and the knowledge of whether resistance plasmids are dispersed by the local environmental virome.

3.1 July (Early Monsoon Dynamics)

- Volume of samples: 21.
- Contamination Profile: 1/3 rds of samples compromised.

Highlights of Resistance: Ampicillin resistance was at its highest point at 88.9%. Cefepime (ESBL setting) had a 50.0% resistance rate and Imipenem had a worrying 47.6% resistance rate. Early monsoon season is usually known to coincide with flooding of cities such as

Hatirjheel, which allows the proliferation of human fecal contents and plasmid-mediated resistance genes (including blaNDM-1) into the water table.

4.4 Antibiotic activity and comparative analysis:

The data set can be used to compare the effectiveness of antimicrobials in finer detail. The antibiotics under test can be the subject of segregation into functional groups, which shows the underlying molecular defense systems of the bacterial population.

4.4.1 Intrinsic Resistance Profiles: The Excluded Agents:

The interpretation chart rightly excludes vulnerable breakpoints of Erythromycin (E15), Vancomycin (VA30), Linezolid (LZ30), Azithromycin (AZM15), and Clindamycin (DA2). *Escherichia coli* is a Gram-negative bacillus with a selectively permeable outer membrane lining with lipopolysaccharides (LPS).

- Glycopeptides & Oxazolidinones (Vancomycin, Linezolid): These are too large to enter the outer membrane porins of *E. coli* to access their targets (peptidoglycan precursors and the 50S ribosomal subunit, respectively). The results indicate close to zero zone sizes (Mean VA30 = 1.84 mm; LZ30 = 0.0 mm), and this is in agreement with the normal genomic predictions.
- Macrolides/Lincosamides (Erythromycin, Azithromycin, Clindamycin): *E. coli* uses constitutive multidrug efflux pumps (including the AcrAB-TolC) that expel these hydrophobic antibiotics immediately before they can reach bacteriostatic intracellular concentrations.

4.4.2 Beta-Lactam Crisis: ESBL and Carbapenemase Phenotype:

Beta-lactam antibiotics are the most important group in clinical treatment, and they are failing at alarming rates in this group.

- Ampicillin (AMP10): * Data: 84.1% Resistant, 7.3% Intermediate, 8.5% Susceptible.

Analysis: Ampicillin is virtually useless in empirical therapy of these strains. The blaTEM-1 or blaSHV-1 narrow-spectrum beta-lactamases are ubiquitously responsible in high-level resistance, and are chromosomally integrated or widely disseminated on plasmids in Bangladesh.

- The ESBL Indicators - Aztreonam (ATM30) & Cefepime (FEP30):
- Data (ATM30): 42.3% Resistant, 6.9% Intermediate, 50.8% Susceptible.
- Data (FEP30): 33.8% Resistant, 8.5% Intermediate, 57.7% Susceptible.
- Comparative Correlation: Cross-tabulation of the data shows that out of the 43 resistant to FEP30, 42 were also resistant to Aztreonam. This is almost a 1:1 correlation, which proves an endemic ESBL-producing phenotype. The blaCTX-M-15 genotype is the

most common in the Dhaka area, and is aggressive in hydrolysing fourth-generation cephalosporins (Cefepime) and monobactams (Aztreonam), but is generally resistant to cephamycins and carbapenems.

- The Carbapenem Alert - Imipenem (IPM10):
- Data: 34.1% Resistant, 17.9% Intermediate, 48.0% Susceptible.

Analysis: Carbapenems are the drugs of last resort. The rate of 34.1% resistance is a serious epidemiological crisis. Among 40 isolates with valid Cefepime data that were Imipenem resistant, 31 were also Cefepime resistant. This co-resistance suggests that the isolates are not only ESBL-producers, but also have potent Carbapenemases (including NDM-1, OXA-48, or VIM-type metallo-beta-lactamases) and porin loss (e.g., OmpK35/36 mutations).

4.4.3 Aminoglycoside Reliability:

- Gentamicin (CN10):
- Data: 30.4% Resistant, 0.0% Intermediate, 69.6% Susceptible.
- Kanamycin (K30):
- Data: 37.8% Resistant, 9.2% Intermediate, 52.9% Susceptible.

Comparative Analysis: In this panel, gentamicin shows the greatest overall efficacy of any widely active drug. As a rule, aminoglycoside resistance in *E. coli* is mediated by Aminoglycoside Modifying Enzymes (AMEs) like acetyltransferases (AACs), adenyltransferases (ANTs) and phosphotransferases (APHs). As the resistance rate of Kanamycin is higher than that of Gentamicin, the common enzymes in this environmental/clinical pool are probably aph(3)-Ia (that acts on Kanamycin but not on Gentamicin) than on broad-spectrum AMEs. When used together with a structurally different agent, gentamicin is a very viable systemic choice.

4.4.4 Phenicol and Tetracyclines:

- Chloramphenicol (C30):
- Data: 34.1% Resistant, 1.6% Intermediate, 64.3% Susceptible.

Analysis: Chloramphenicol has high efficacy (64.3% susceptibility) in spite of its occasional use because of the risk of aplastic anemia. This is probably due to the fact that it has not been widely available in mainstream human medicine in decades, which relieves the selective pressure keeping the cat (chloramphenicol acetyltransferase) gene in the plasmid population.

- Doxycycline (DO30):
- Data: 41.7% Resistant, 15.0% Intermediate, 43.3% Susceptible.

Analysis: The resistance rate (41.7%) indicates that the circulation of widespread efflux pumps (tet(A) and tet(B) determinants) is a dominant cause of resistance in large conjugative plasmids (such as IncF), which is predominantly common in Enterobacteriaceae.

4.4.5 Fluoroquinolones:

- Levofloxacin (LEV5):
- Data: 38.9% Resistant, 1.9% Intermediate, 59.3% Susceptible.

Analysis: Fluoroquinolones bind DNA gyrase and topoisomerase IV. The almost 40 percent resistance percentage is reflective of high quinolone concentration in the environment (characteristic of local poultry farming discharge into localities such as Hatirjeel). The resistance process is generally a gradual process which involves the mutation of the chromosomes in the *gyrA* and *parC* genes.

4.5 Strategic Interventions and Remediation Viewpoints:

To resolve a dataset with 34 percent carbapenem resistance and contamination, a multi-level solution will be suitable that involves bioinformatics, laboratory stewardship, and environmental surveillance.

Perspective 1: Laboratory Workflow Optimization: The contamination rate in Ramna and Dhanmondi is high and therefore an urgent audit on the standard operating procedures is necessary. Data integrity after sequencing is guaranteed with the use of bioinformatics tools, such as FastQC on genomic datasets, whereas in case of phenotypic ASTs, pre-analytical variables should be regulated. Agar plates should be visually examined to see secondary morphologies and then interpreted. The use of automated plate readers has the potential to remove the human factor of picking out faint contaminations of C by true heteroresistance.

Perspective 2: Genomic Surveillance (Next-Generation Sequencing): Phenotypic data needs to be accompanied by Whole Genome Sequencing (WGS). Assembling these genomes, it is possible to use such tools as ResFinder to map precise AMR alleles. It would conclusively identify the Imipenem resistance as NDM-1 (where a particular metal chelator strategy has to be used to prevent) or OXA-type enzyme, which would directly guide local drug design directions through computer-aided drug design (CADD).

Perspective 3: Ecological Policy Changes: The spatial information (Aftabnagar vs. Hatirjeel) puts into focus environmental reservoirs. When these samples are of urban water bodies, it implies that there is a failure in the treatment of municipal wastewater. Strict effluent screening policy and predictive STATA model to follow the source location of particular ESBL clades will inform focused sanitation upgrades.

4.6 Synthesis and Concluding Observations:

The isolate pool is a feature of a developed antimicrobial resistance that is fixed in the local population of *E. coli*. The near-complete failure of Ampicillin and the extreme sabotage of Cefepime, Aztreonam and Imipenem characterize a shift to Pan-Drug Resistance (PDR). The

most robust therapeutic barriers at present are gentamicin and Chloramphenicol but their pharmacological side effects restrict their use as universal panaceas.

Concurrently, the laboratory workflow exhibits critical points of failure regarding sterility, as evidenced by a 40.8% aggregate sample contamination rate. Eradicating environmental contaminants from the testing pipeline is the immediate priority to ensure the validity of future AST interpretations. Establishing a routine genomic surveillance framework will be the ultimate requirement to track and counteract these highly mobile, multi-drug resistant plasmids effectively.

4.7 Antibiotic Susceptibility Test (AST) Analysis (Every Month):

July:

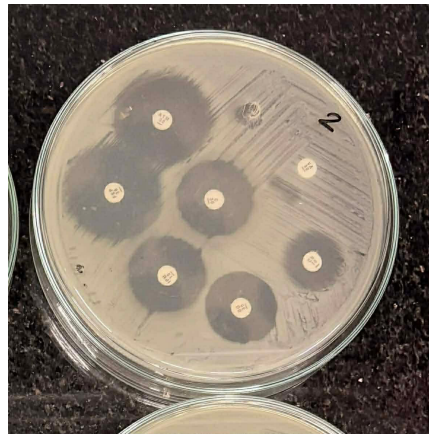


Figure 10: AST of *E. coli* isolate on MHA using the Kirby-Bauer disk diffusion method. Clear zones indicate inhibition of bacterial growth. Month July.

The images show Kirby-Bauer disk diffusion tests, used to determine how sensitive a bacterial isolate is to various antibiotics. The clear circles around the white disks are zones of inhibition, where the antibiotic has successfully prevented bacterial growth.

Susceptibility vs. Resistance:

Plates like 7, 14, and 15 show large, overlapping clear zones, indicating the bacteria are highly susceptible to most of the antibiotics tested. Conversely, plate 3, 11 shows very small or nonexistent zones, suggesting these bacterial strains are resistant to several of the medications.

Specific Observations: A notable finding is seen on plates 2, 8, 10, where there is zero inhibition around the VA (Vancomycin) disk. This indicates total resistance to that drug. Additionally, some plates (like Plate 7) show "bridging" or synergistic effects where two zones meet, suggesting the antibiotics may be more effective when used in combination.

Clinical Meaning:

To finalize these results, a technician would measure the diameter of each zone in millimeters. These measurements are compared against standardized charts to officially classify the bacteria as Resistant (R), Intermediate (I) or Susceptible (S), which guides doctors in choosing the right treatment.

August:

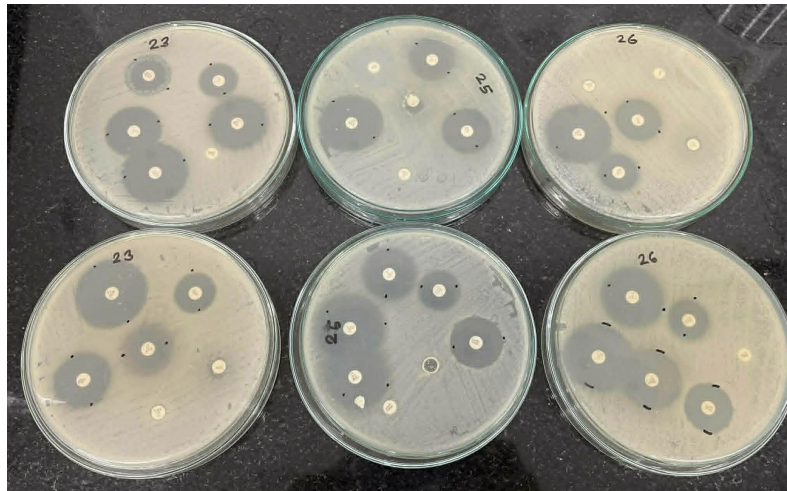


Figure 11: AST of *E. coli* isolate on MHA using the Kirby-Bauer disk diffusion method. Clear zones indicate inhibition of bacterial growth.

General Interpretation:

The images show Petri dishes with bacterial lawns and various antibiotic discs. The clear circles around the discs are “Zones of Inhibition”, indicating where antibiotic concentration was high enough to stop bacterial growth.

1. Susceptibility vs. Resistance:

Large Zones: The bacteria are likely “Susceptible” to these antibiotics. (e.g., several discs on plates 23 and 26).

Small or No Zones: The bacteria are “Resistant” or have “Intermediate” susceptibility. Growth occurs right up to the edge of the disc.

Satellite Colonies: On plates 34 and 35, you can see small dots (individual colonies) growing inside the clear zones. This suggests the presence of “resistant mutants” or a heterogeneous population within the strain.

2. Specific Observations:

Isolates 23 & 26: Appear to have the highest number of large, clear zones, suggesting they are sensitive to a broader range of the tested antibiotics.

Isolate 25: Shows more moderate resistance, with smaller zones overall compared to 23.

Isolates 34, 35, & 36: Show significant resistance. Note the "fuzzy" zones or colonies growing within the circles, which often indicates that the antibiotic is bacteriostatic (inhibits but doesn't kill) or that the strain is developing resistance.

Important Note: To provide a clinical "Sensitive (S)," "Intermediate (I)," or "Resistant (R)" classification, you must measure the zone diameters in millimeters and compare them against official CLSI or EUCAST standard tables for that specific bacterial species.

September:

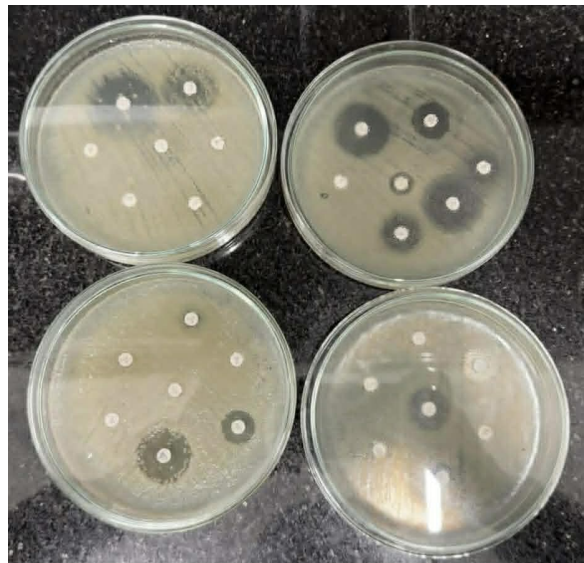


Figure 12: AST of *E. coli* isolate on MHA using the Kirby-Bauer disk diffusion method. Clear zones indicate inhibition of bacterial growth.

Key Interpretations

1. High Resistance Profile:

Compared to the first set of images, many of these plates show a high degree of resistance. Several discs have zero Zone of Inhibition, with the bacterial lawn growing directly against the antibiotic disc. This indicates the isolate is fully resistant to those specific agents.

2. Heterogeneous Growth (Satellite Colonies):

Many plates (especially in the second and third images) exhibit "speckled" zones. These are satellite colonies or individual mutants growing within what should be a clear zone.

This is a significant finding in a lab setting, as it often suggests emerging resistance or that the antibiotic is not effectively killing all the bacteria in the sample.

3. Variation in Potency:

Active Antibiotics: The bottom-right plate in the first image shows the most clear activity, with distinct dark circles indicating high susceptibility to at least four of the tested drugs.

Inactive Antibiotics: The top-left plate in the third image shows almost total resistance across the board, with only one or two discs showing even a minor inhibitory effect.

October:



Figure 13: AST of *E. coli* isolate on MHA using the Kirby-Bauer disk diffusion method. Clear zones indicate inhibition of bacterial growth.

These plates show more Kirby-Bauer susceptibility tests, dated 16.10.25, with several unique observations compared to your previous sets.

Key Observations:

1. Pronounced Satellite Colonies (D9, D14, H8):

Several plates (especially D9 and D14) show very clear "speckled" zones.

The Interpretation: These are resistant sub-colonies. While the antibiotic initially inhibited the general population, individual bacteria with resistant mutations are thriving within the zone.

Clinical Significance: This usually indicates that the antibiotic should be reported as "Resistant", regardless of the zone's size, because the infection would likely recur or fail to clear if that drug were used.

2. Variable Activity in "H" Series (H5, H8, H9):

H5: Shows three very large, clear zones of inhibition, indicating high sensitivity to those specific antibiotics.

H8: Shows a mix of sensitivity and resistance, with some zones appearing "hazier" than others.

H9: Shows clear zones, but they are smaller than those in H5, suggesting a more moderate or intermediate level of susceptibility.

3. Isolate D19:

This plate shows a high level of multidrug resistance. Most discs have little to no zone of inhibition, except for the center disc (IPM - Imipenem), which shows a clear but moderate zone. This suggests the isolate may be resistant to most standard antibiotics but remains somewhat sensitive to carbapenems.

November:

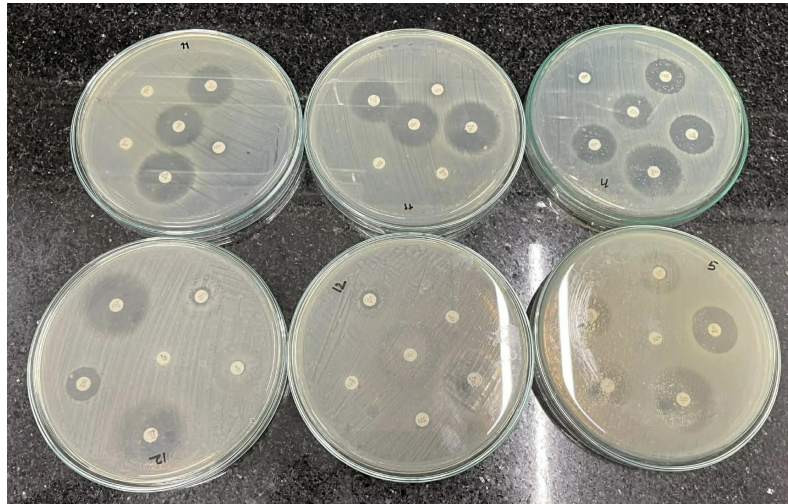


Figure 14: AST of *E. coli* isolate on MHA using the Kirby-Bauer disk diffusion method. Clear zones indicate inhibition of bacterial growth.

Based on these additional images, here is a short interpretation of the Kirby-Bauer susceptibility tests for the new isolates:

Key Interpretations:

1. Variation in Susceptibility (Isolates 11, 4, 12, 5):

Isolate 11: Shows several wide, clear “Zones of Inhibition”, indicating high sensitivity to those specific antibiotics.

Isolates 4 & 5: Exhibit a mix. While some antibiotics are very effective (large clearings), others show total resistance (the bacterial lawn grows right up to the disc).

Isolate 12: Appears highly resistant overall. The clear zones are much smaller or non-existent compared to Isolate 11, suggesting fewer effective treatment options for this particular strain.

2. Notable Resistance Features (Isolates 2 & 3):

Isolate 3: Shows clear “satellite colonies” (small dots) growing within the inhibitory zones. This is a red flag for clinicians, as it indicates the presence of a resistant sub-population that could cause treatment failure.

Isolate 2: Shows "hazy" zones or very small clearings, indicating that while the antibiotic has some effect, the bacteria are likely “Intermediate” or “Resistant” by clinical standards.

3. Comparative Susceptibility:

By comparing the plates, you can see a "spectrum of resistance":

Most Sensitive: Isolate 11 (highest number of large, clear zones).

Most Resistant: Isolates 12 and 2 (fewest/smallest zones).

Chapter 5

Discussion

PCR amplification of the isolates collected in April revealed a high rate of *Escherichia coli* at the sampled locations in Hatirjheel, Aftab nagar khal, Ramna Lake and Dhanmondi lake which was supported by a high PCR positive rate at all the sampling sites. The majority of the isolates produced distinct amplification bands, demonstrating the specificity and ability of the selected primers to identify the target *E. coli* gene. The fact that the putative colonies of *E. coli* employed in the selection were first chosen based on selective media and morphology, and subsequently the isolates were molecularly validated, explains the prevalence of PCR-positive isolates, particularly of Sample-2 of Hatirjheel, Aftab nagar khal, Ramna Lake and Dhanmondi. Only a small number of July Hatirjheel isolates (Sample-1) were identified as PCR-negative because they failed to amplify the DNA bands. According to Ramirez-Castillo et al. (2015), this could indicate that these isolates were non-target bacteria with comparable morphological characteristics that thrived in the selective culture conditions frequently seen in environmental microbiology investigations. Low DNA concentration, template degradation, or PCR inhibitors (humic acids, organic materials, or salts) that stop DNA polymerase from working can also result in the absence of bands (Sidstedt et al., 2020).

The non-standard based result indicated by the amplification bands in the negative control was probably caused by contaminated chemicals, aerosolized DNA, or an incorrect pipetting procedure during the PCR setup. Cross-contamination of targets is a recognized problem in molecular laboratories, particularly when working with high amounts of target DNA (Bustin & Nolan, 2020). However, the positive control produced robust amplification, demonstrating the amplification system and showing that the PCR reagents, primers, and cycle conditions were functioning properly.

In general, the high PCR positivity rates of the isolates from the three water bodies show that fecal contamination is ongoing and that PCR is a reliable method for confirming the presence of *E. coli* in environmental samples. When compared to conventional phenotypic identification methods, PCR is widely known for being extremely sensitive and specific (Ramirez-Castillo et al., 2015). Overall, these findings show that *E. coli* was successfully detected molecularly in

urban water sources, and the few negative reactions and contamination-related issues emphasize how important it is to follow aseptic procedures and handle reagents correctly in environmental molecular analysis.

environmental variables that can be effectively detected in environmental waters using a molecular technology like PCR (Moinet et al., 2024).

The distribution of the PCR-positive isolates on a monthly basis showed notable temporal fluctuation during the 5 months of the study period, suggesting that the presence of bacteria in water bodies.

Recent investigations indicate that surface water affected by human and animal activity frequently contains MDR *E. coli* and similar intestinal bacteria (Sultana et al., 2025; Rana et al., 2025). *Escherichia coli* (43.6) can be attributed to their gastrointestinal origin, frequent exposure to antibiotic residues, and resistant bacterial strains linked to animal and human waste in the water. The lower level of MDR in resistance, however, is in line with the results that, when combined, show that enteric bacteria are the primary cause of MDR in the water under investigation and *E. coli* are the primary reservoirs of MDR.

Because an isolate must be screened against a range of antibiotics from different classes in order to be categorized as XDR, the occurrence of extensively drug-resistant isolates was uneven. Because just a small number of antibiotic discs from a very narrow range of drug classes were used, isolates that were resistant to the majority of the antibiotics tested were not categorized as XDR by normal standards. Because the negative result of XDR does not indicate that the environment is free of organisms, but rather is probably the consequence of the small antibiotic panel used in the susceptibility study, extensive antimicrobial testing is advised to ascertain the precise resistance position (Johura et al., 2024).

Comparing the Resistance Patterns of Hatirjheel, Aftab Nagar Khal, Dhanmondi Lake, and Ramna Lake, Hatirjheel provided the highest percentage of MDR bacteria (62.8%), followed by Aftab Nagar Khal (20.2%) and Ramna Lake (17.0%). There was a distinct geographic distribution of MDR isolates throughout the sampling sites. *Escherichia coli* was shown to be predominant in MDR, particularly at the Hatirjheel (Popoola et al., 2025). Hatirjheel's high MDR load could be caused by high rates of anthropogenic activity, such as acidic runoffs, sewage discharge, and active environmental contamination.

The *E. Coli* isolates from Hatirjheel, Ramna Lake, and Aftab Nagar Khal demonstrated that plasmid carrying was quite widespread throughout all sampling months and that extra-chromosomal genetic elements are rather abundant in the population of environmental *E. Coli* in urban aquatic habitats. Environmental isolates often exhibit extensive representation, which

is linked to bacterial adaptation and genetic variety (Rozwandowicz et al., 2022). Bacteria are under selective pressure to retain plasmids with adaptive features like antibiotic resistance genes because urban water resources receive surface runoffs, hospital effluents, and raw home sewage (Manaia et al., 2023). For these conditions of environmental persistence and survival, August has been selected (Partridge et al., 2022).

The absence of bands in the negative control demonstrated that the observed plasmid bands were the result of bacterial DNA rather than experiment contamination, demonstrating the procedure's validity. Even when loading issues caused a small amount of technical duplication in some lanes, the overall interpretation of the data was unaffected. This kind of technical variation is common in gel-based molecular investigations and, as long as it is accurately documented, usually does not compromise the validity of the experiment (Partridge et al., 2022).

Overall, the findings demonstrate the widespread presence of *E. coli* in the water bodies under investigation. The findings are noteworthy since plasmids are frequently carriers of virulence genes and antibiotic resistance, indicating that plasmids may also be thought of as repositories of genetically adaptive and possibly multidrug-resistant bacteria in contaminated aquatic environments (Manaia et al., 2023).

According to the antibiotic susceptibility data, certain isolates of *Escherichia coli* exhibited resistance patterns that extended to the medications that are typically considered the most crucial or last-line antibiotics. The development of intermediate susceptibility and sporadic resistance, especially in isolates of *E. coli* in Hatirjheel, are indicators that carbapenems, aztreonam, and aminoglycosides maintained a significant overall effect (Teixeira et al., 2020). Aquatic habitats are a contributor to the maintenance and spread of advanced resistance phenotypes rather than a passive reservoir of water, as evidenced by the identification of MDR phenotype in repeated cases even in environmental isolates (Sobur et al., 2019). This is especially concerning since environmental strains may transfer their DNA to clinically important pathogens through horizontal gene transfer, making last-resistant medications less effective over time.

Over the course of the six-month sample period, there was a seasonal fluctuation in the samples' levels of bacteria, plasmids, and resistance. In late summer and early monsoon, particularly in July and August, PCR positivity and plasmid prevalence peaks were also frequently noted (Barrantes-Jimenez et al., 2025). These changes over time are accompanied by an increase in temperature, an increase in nutrients in surface runoff, and a decrease in dynamic water movements, all of which help bacteria of all kinds live longer and exchange genes (Suthar, 2021). In contrast to Ramna Lake, which experienced acute seasonal lapses indicative of single contamination events, Hatirjheel exhibited the most steady yet consistently high bacterial load over the months, indicating a chronic anthropogenic burden. Additionally, there were anomalous patterns at Dhanmondi Lake and Aftab Nagar Khal that might be explained by

transient ecological perturbations but not by long-term ones (O'Malley et al., 2022). All things considered, these results reinforced the importance of ecosystem dynamics in the emergence and spread of antimicrobial resistance in urban surface waters.

The existence of MDR enteric bacteria in publicly accessible metropolitan waters poses a serious threat to people's health. People typically use these streams for work, leisure, and either direct or indirect drainage into bigger water systems. When multidrug-resistant infections have high loads, are extensively distributed in their plasmid, and exhibit equal resistance to a range of antibiotics, they are more likely to be transmitted to humans. Additionally, environmental bacteria may function as covert amplifiers of resistance genes that facilitate their establishment in the human microbiota or even in a clinical context. The results highlight the necessity of addressing antibiotic resistance not only as a hospital problem but also as an environmental and community-level health issue because, in order to protect human health, environmental reservoirs of AMR must be monitored and mitigated at the One Health level (Stanton et al., 2022).

The patterns discovered are consistent with global trends, particularly in the rapidly urbanizing regions of low-middle and low-income countries, when contextualized with the worldwide reports of aquatic environments with antimicrobial resistance. The abundance of plasmids in *E. coli* is consistent with earlier relevant research conducted in Southeast and South Asia, which found that ambient waters are a significant source of resistance-associated genes. Due to the high population density and lack of supervision over waste disposal, Hatirjheel's pollution levels and duration point to an environmentally worsening situation. According to Bisaccia et al. (2025), a comparative lens presents the view that although the phenomenon is a global occurrence, it is significantly altered by region-specific infrastructure, governance systems, and environmental management techniques.

In order to prevent antibiotic resistance, the results support the urgent need to improve legislative frameworks and include environmental surveillance in national action plans. Plasmid profiling with resistance typing, more stringent control over the intervals of untreated waste disposal, and regular microbiological monitoring of urban-source waters are required (WHO, 2023). Additionally, the utilization of environmental data should support the development of campaigns to isolate antibiotic use outside of the therapeutic setting and risk assessment of health at the general population level. Environmental agencies, public health agencies, and research institutes will need to work together to prevent antibiotic resistance from spreading throughout the environment and to maintain the efficacy of current treatments (Xiao et al., 2023).

Chapter 6

Conclusion

The study examines the antibiotic resistance of pathogenic enteric bacteria found in Dhaka Metropolitan City's main surface water bodies. The results make it abundantly evident that *Escherichia coli*, which was isolated from urban water sources, has high levels of antibiotic resistance.

This study provides compelling evidence for the spread of antibiotic resistance in aquatic environments. Hatirjheel and Ramna Lake consistently displayed the largest percentage of PCR-positive isolates and resistance traits, whereas Aftab Nagar Khal and Dhanmondi Lake had relatively less contamination. These spatial variances reveal variations in trash release, environmental management, and human activity at the sampling sites. Seasonal differences were also seen, with more resistant bacteria found in the late summer and early monsoon seasons, indicating the significance of environmental and ecological factors for bacterial survival. Selective culture, PCR amplification, agarose gel electrophoresis, and antibiotic sensitivity tests were used to identify and characterize bacterial communities displaying resistance. Despite a number of operational and technological difficulties encountered throughout the laboratory work, protocol optimization was essential to guaranteeing that reliable and repeatable results were acquired.

The importance of surface water bodies as environmental reservoirs of antibiotic resistance in densely populated urban environments is fully highlighted by this research in terms of public health. The idea of combined water monitoring and enhanced waste management methods must be implemented in Bangladesh since the presence of multidrug-resistant pathogenic bacteria in frequently visited water bodies constitutes a major threat to public health.

Limitation

The research has shortcomings that could impact the explanation of the results. Due to financial limitations, a metagenomic study that would have enabled a more comprehensive genetic characterization of the isolates was not feasible. Sample collection was also finished in a short amount of time, even though a one-year follow-up technique would have been more beneficial in giving a comparison of the seasons, but it was not practical due to thesis requirements. The study's scope was rather limited, and water sample locations were chosen for practical reasons; other potentially pertinent areas were excluded. Additionally, the study's primary methodology was PCR-based detection rather than complex molecular typing or sequence techniques, and time and resource constraints made it impossible to repeat and replicate sampling, which may have affected the study's degree of repeatability and robustness.

To facilitate the recovery of *Escherichia coli* from environmental freshwater sources, an enrichment step was performed. Initial trials utilizing a 1:1 (5 mL LB broth to 5 mL sample water) ratio of sample to Luria-Bertani (LB) broth yielded insufficient bacterial recovery.

Consequently, the protocol was optimized to an 8:2 ratio (8 mL LB broth to 2 mL sample water). This adjusted enrichment ratio provided a more nutrient-dense environment, successfully promoting the selective growth of *E. coli* for subsequent isolation.

Future Prospects

The results of the conducted study have several significant implications for future research and policy-making. Sampling more rivers, canals, and peri-urban water bodies would give a more comprehensive national picture and aid in characterizing the effects of seasonal and climatic changes on patterns of resistance and plasmid modifications, in addition to long-term multi-year data collection. Examples of advanced molecular approaches that could be used to discover particular resistance determinants and plasmid types implicated in horizontal gene transfer include plasmid sequencing, whole-genome sequencing, and the profile of resistance genes. These investigations would provide a comprehensive understanding of the genetic foundations of resistance and the evolutionary processes of resistance in aquatic life.

In order to establish direct epidemiological correlations between contaminated water sources and human diseases, the investigation may also be expanded to assess the relationship between environmental isolates and clinical strains. This would improve the body of research on incorporating environmental monitoring into national action plans for antimicrobial resistance. The results raise awareness of the need to better regulate the discharge of untreated sewage, hospital effluents, and industrial waste into surface water bodies in terms of public health and policy. In order to reduce contamination at the source, future initiatives could concentrate on microbiological risk assessment in conjunction with environmental engineering and community-based solutions. Overall, this study shows that there is a strong foundation for future environmental, molecular, and public health research on antimicrobial resistance. The findings of this study can be used as a guide to guide future scientific investigations as well as upcoming evidence-based policies to safeguard human health and water quality in Bangladesh.

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