Assessment of Antibiotic Resistance Profiles and Pathogenic Traits of *Escherichia coli* Isolates in Dhaka City's Municipal Water Supply

By

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of
MSc. In Biotechnology

Biotechnology
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.
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Approval

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Ethics Statement

This study involved the collection and analysis of household drinking water samples from Dhaka City to assess microbial contamination and antibiotic resistance. Ethical approval for the study was obtained from the appropriate institutional review board Institutional Review Board of BRAC University. The study did not involve human participants, personal data, or direct contact with individuals. Water samples were collected from publicly accessible taps or with the informed consent of the household owners. The purpose of the study was clearly communicated to participants, and no personal or identifying information was recorded. All procedures were conducted in accordance with ethical guidelines and relevant regulations to ensure integrity, safety, and respect for the communities involved.

The findings of this research are intended to contribute to public health improvements, with the ultimate goal of promoting safe drinking water practices and informing policy recommendations.

Abstract/ Executive Summary

Antimicrobial resistance (AMR) in *Escherichia coli* is a growing global threat, with significant public health implications. This study evaluated municipal household water in Dhaka North, Bangladesh, revealing fecal contamination in 78.26% of samples, and detecting *E.coli* in 76%. Total 20% isolates showed pathogenic genes, among them rbfE was prevalent. Molecular analysis identified CTX-M-15 as the most prevalent ESBL gene (54.29%) with the presence of other ESBL gene such as shv,tem and most prevalent carbapenem-resistant genes such as blaVIM in 19.12% of isolates. Multidrug resistance was observed in 34.2% of *E.coli* isolates and XDR observed in 5.8% isolates, complicating treatment options. These findings underscore the urgent need for enhanced water treatment systems and routine monitoring to mitigate the spread of multidrug-resistant pathogens in urban water supplies.

Keywords: *Escherichia coli*, antimicrobial resistance, extended-spectrum beta-lactamase, carbapenem resistance, fecal contamination, public health, Dhaka water safety, seasonal variation, multidrug resistance, environmental microbiology.

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List of Acronyms

AMR	Antimicrobial Resistance
ESBL	Extended-Spectrum Beta-Lactamases
ETEC	Enterotoxigenic *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
UTI	Urinary Tract Infection
MNEC	Meningitis-associated *Escherichia coli*
ExPEC	Extraintestinal Pathogenic *Escherichia coli*
NDM	New Delhi Metallo-β-lactamase
KPC	Klebsiella Pneumoniae Carbapenemase
IMP	Imipenemase
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
GLASS	Global Antimicrobial Resistance and Use Surveillance System
PCR	Polymerase Chain Reaction
CFU	Colony Forming Units
STEC	Shiga Toxin-producing *Escherichia coli*

HUS	Hemolytic Uremic Syndrome
PAI	Pathogenicity Island
EPEC	Enteropathogenic *Escherichia coli*

Chapter 1 Introduction

1.1 Background

Antimicrobial resistance (AMR), particularly in connection to *Escherichia coli* and other bacterial infections, is one of today's most pressing global health issues. The World Health Organization (WHO) has recognized AMR as a serious danger to global public health, directly attributing about 1.27 million fatalities to bacterial AMR in 2019, with an additional 4.95 million deaths related to it (Antimicrobial Resistance | WHO Research Project Collaboration | OHT, n.d.). According to the WHO's 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, 42% of *E.coli* bacteria exhibited resistance to third generation cephalosporins, highlighting resistance rates among prevalent infections. More than 50% of individuals with UTIs in certain areas are no longer sensitive to therapy due to *E.coli*'s resistance to fluoroquinolone medicines(Antimicrobial Resistance, n.d.).According to a CDC research, approximately 2.8 million antimicrobial-resistant health issues and over 35,000 fatalities occur each year in the United States alone(CDC, 2024).According to projections, AMR may cause 10 million deaths annually by 2050 if present trends continue, with substantial economic consequences that might result in yearly GDP losses of between $1 trillion and $3.4 trillion(Antimicrobial Resistance, n.d.),(CDC, 2024) .Extended Spectrum β-Lactamases (ESBLs) production is one of the most familiar procedures seen in Enterobacteriaceae including Klebsiella pneumoniae and *Escherichia coli* to achieve resistance against β-lactam antibiotics(Paterson et al., 2000). Extended-spectrum beta-lactamases (ESBLs) and carbapenemases are key method by which *Escherichia coli* (*E.coli*) acquires resistance to betalactam antibiotics, particularly carbapenems, which are frequently used as last resort therapies for multidrug-resistant infections. The rise of ESBL-producing and carbapenem-resistant *E.coli* bacteria has generated concerns due to their connection to higher morbidity and death rates in affected individuals. The prevalence of ESBL-producing *E.coli* has been

increasing worldwide, with research showing that these bacteria can also be resistant to other antibiotic classes, complicating options for treatment. ESBL synthesis is frequently plasmid-mediated, allowing for easy horizontal gene transfer among bacterial populations. This contributes to the rapid spread of resistance genes in both clinical and community settings (Bagaya et al., 2023). The most prevalent ESBL gene in *E.coli* isolated from environmental sources, such as soil and water, is the CTX-M group, specifically CTX-M-15 and CTX-M-1. According to studies, because of their greater resistance profiles and capacity for horizontal gene transfer across bacterial populations, CTX-M types have basically replaced previous ESBL kinds like TEM and SHV. CTX-M-15 is commonly found in both clinical and environmental isolates, highlighting the worldwide characteristics of this pattern (Ribeiro et al., 2024). The TEM and SHV families of ESBLs continue to be important contributors to resistance in environmental *E.coli*, while being less common than CTX-M. For instance, research has revealed that blaTEM genes are still present in a variety of environmental samples, especially in areas where aquaculture and agriculture use antibiotics extensively (Ribeiro et al., 2024). The prevalence of certain ESBL genes varies greatly among regions. For example, investigations done in South Asia, notably Bangladesh and India, found significant rates of CTX-M-15 and CTX-M-1 in both clinical and environmental isolates (Bagaya et al., 2023). Environmental reservoirs, including wastewater treatment plants and agricultural runoff, have been identified as key areas for the spread of ESBL-producing *E.coli*. A comprehensive analysis indicated that the pooled prevalence of ESBL-producing *E.coli* from environmental sources was around 39%, indicating a considerable risk of transmission to people via contaminated water supplies or food products (Bagaya et al., 2023; Ribeiro et al., 2024). *E.coli* is the most extensively used indicator organism for assessing the microbiological quality of water and food. It is also a leading cause of diarrhoea and other enteric diseases (Qadri et al., 2005). Furthermore, uropathogenic *E.coli* (UPEC) is the causative agent of urinary tract infections (UTIs), the most prevalent extraintestinal *E.coli* infection. Another increasingly

frequent source of extraintestinal infections is the pathotype known as meningitis-associated *E.coli* (MNEC), which causes sepsis and meningitis. ExPEC is a new term for the *E.coli* pathotypes linked to extraintestinal infections (Russo & Johnson, 2000). In developing countries, antibiotic resistance to enteric pathogens is of particular concern as it is considered to be one of the most significant challenges to treat infectious diseases. In Bangladesh, next to rotavirus, the second most prominent causative agent of diarrheal infections is pathogenic *E.coli*(Talukder et al., 2006).Several studies have reported the isolation of pathogenic as well as resistant *E.coli* from environmental samples, which can participate in horizontal gene transfer of their resistance genes containing plasmids and lead to outbreaks (Fenlon et al., 2000).A major public health problem in both developed and developing countries is the emergence and transmission of antibiotic-resistant bacteria through water systems, especially Extended-Spectrum Beta-Lactamase (ESBL) and carbapenem-producing *Escherichia coli*. For these resistant organisms, water is an essential environmental reservoir and a means of transmission, which promotes their persistence and the spread throughout populations of humans and animals (J. Wang et al., 2015). The transmission mechanisms of these resistant bacteria in water systems are diverse, including complex interaction between bacterial survival strategies and environmental factors. Horizontal gene transfer, which is especially common in aquatic environments, is critical for the transmission of resistance genes among bacterial populations. (Marathe et al. 2017) found that water habitats are optimal for the exchange of genetic material, especially resistance-carrying plasmids, across diverse bacterial species. The existence of biofilms, which protect bacterial colonies and increase their survival rates, further facilitates genetic exchange (Marathe et al., 2017). The introduction of resistant bacteria into water sources is mostly caused by human activity. High quantities of microbes resistant to antibiotics are introduced into environmental water systems by hospital effluents, especially in areas with insufficient wastewater treatment facilities. This issue is made worse by agricultural practices including the release of animal faeces and the use of tainted irrigation water. Urban

wastewater systems can serve as pathways for the spread of resistant bacteria, particularly in places with outdated or inadequate infrastructure (Zarfel et al., 2017). Environmental variables include seasonal changes, the consequences of climate change, and extreme weather events can overwhelm current water management systems, making the impact of these human causes even worse. Pathogenic *Escherichia coli* (*E.coli*) strains carry a variety of virulence genes that may readily be transmitted through water, causing serious health risks associated with intestinal disorders. Among them, the eae gene, which is associated with enteropathogenic *E.coli* (EPEC), is found in environmental isolates and is required for the bacteria to attach to and infiltrate intestinal epithelial cells. Furthermore, the eaeA, stx1, and stx2 genes are important virulence factors in pathogenic *Escherichia coli* (*E.coli*), contributing considerably to their pathogenicity and the health risks associated with intestinal illnesses. The eaeA gene, present in both enteropathogenic *E.coli* (EPEC) and enterohemorrhagic *E.coli* (EHEC), is important for intimate attachment to epithelial cells, which enables the creation of attaching and effacing lesions that disturb normal intestinal function (Nataro & Kaper, 1998).These virulence genes are abundant in diverse environmental conditions, including water sources polluted with faeces, allowing for horizontal gene transfer across bacterial populations via mobile genetic elements such as plasmids and bacteriophages (Carlos et al., 2011).Because these pathogenic strains can contaminate drinking water and agricultural goods, causing outbreaks of gastrointestinal disorders, their transmission through water systems poses serious dangers to public health (Persad & LeJeune, 2014).In addition another serotype of *E.coli*, *Escherichia coli* O157:H7 is a pathogenic strain that can cause severe gastrointestinal disorders such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). The virulence of *E.coli* O157:H7 is mostly due to genes such as fliCH7, which encodes the H7 flagellar antigen, and rfbO157, which synthesizes the O157 lipopolysaccharide antigen. These genes improve the bacterium's capacity to attach to intestinal cells and avoid immune responses(Bagaya et al., 2023).The presence of *E.coli* O157:H7 in municipal water systems is alarming, as studies have shown that this disease may

persist in many water sources, including drinking and recreational water, for lengthy durations, particularly at lower temperatures(G. Wang & Doyle, 1998).Outbreaks related with untreated irrigation water have been observed, emphasizing the necessity for severe water quality monitoring and management methods to limit the spread of this disease and associated health concerns(Engda et al., 2023).Extended-spectrum betalactamase (ESBL)-producing strains of *Escherichia coli* O157:H7 can induce infections with rather severe clinical symptoms, but they can also produce asymptomatic occurrences in apparently healthy people. A considerable percentage of ESBL-producing *E.coli* strains, for example, may be identified from asymptomatic carriers, especially in community settings, where they might not exhibit any obvious symptoms but yet have the capacity to produce infections under specific circumstances, according to research(Carlos et al., 2011).Furthermore, *E.coli* O157:H7 is often found in municipal water systems, where it can persist and be transmitted through contaminated drinking water or recreational water sources, leading to outbreaks of gastrointestinal diseases(Shiga Toxin–Producing *Escherichia coli* O157:H7 Illness Outbreak Associated with Untreated, Pressurized, Municipal Irrigation Water — Utah, 2023 | MMWR, n.d.).These pathogenic strains' existence in water systems emphasizes how important it is to maintain water quality well and implement public health initiatives to reduce the risk of transmission and shield susceptible groups from possible health issues. All of these characteristics make ESBL-producing *E.coli* a model indicator for enteric bacteria that are resistant to antibiotics and spread across the fecal-oral pathway. Effective steps have been made to combat antimicrobial resistance, including the discovery of novel antibiotics and the restriction and regulation of antibiotic usage (O'Neill, 2016). To effectively address the presence of ESBL-producing *Escherichia coli* (*E.coli*) and other pathogenic strains in municipal water supplies in Bangladesh, particularly in Dhaka, the Dhaka Water Supply and Sewerage Authority (WASA) can implement several strategic measures. Dhaka Water and Sanitation Authority should invest in updating and expanding water treatment facilities to include advanced methods such as

membrane filtration and ultraviolet disinfection. These approaches are more efficient in eradicating resistant bacteria and diseases than typical chlorination procedures (Talukdar et al., 2013a). Establishing a comprehensive water quality monitoring system is crucial. This system should regularly test for the presence of ESBLproducing *E.coli* and other pathogens in municipal water supplies. Collaborating with local health authorities and research institutions can enhance the effectiveness of these monitoring efforts (Mahmud et al., 2020). Waterborne diseases are a leading cause of morbidity and mortality in developing countries, particularly in regions like Bangladesh, where access to safe drinking water remains a significant challenge. According to the World Health Organization (WHO), over 3.4 million people die each year due to waterborne infections, making these diseases one of the most prevalent causes of illness and death worldwide (WHO, 2020). These diseases affect vulnerable groups the most, especially children under five, who face a higher risk of severe illness from diarrheal diseases. In Bangladesh, outbreaks of waterborne diseases are frequently linked to contaminated drinking water sources, inadequate sanitation facilities, and poor hygiene practices. The country's fast urban growth and rising population have worsened the problem, putting more strain on water supply systems. A study conducted in Dhaka revealed that approximately 38% of household water samples analyzed were contaminated with fecal coliform bacteria, exceeding the safe threshold of 100 colony-forming units per milliliter (CFU/ml) (Talukdar et al., 2013b). Furthermore, *Escherichia coli* was isolated from 63% of these samples, indicating a significant risk of gastrointestinal infections among the population. The presence of pathogenic *E.coli* in drinking water is particularly concerning because it indicates fecal contamination within the water supply system. This contamination often results from inadequate sewage treatment processes and poor sanitation practices, which are prevalent in many urban areas of Bangladesh. For instance, a study conducted in the Rohingya refugee camps found that 52% of drinking water samples contained extended-spectrum beta-lactamase (ESBL)-producing *E.coli*, highlighting the urgent need for continuous monitoring and

management of water quality (Mahmud et al., 2020). The presence of antibiotic-resistant strains further complicates treatment options for affected individuals and poses a significant public health threat. Despite the importance of this concerns, there is a notable lack of comprehensive studies focused on assessing the prevalence and impact of pathogenic *E.coli* in household water systems across Bangladesh. Most available research has concentrated on specific outbreaks or isolated communities rather than providing a holistic view of the situation across urban settings like Dhaka. This knowledge gap emphasizes the necessity for routine evaluations and monitoring initiatives to inform public health policies and focuses at ensuring safe drinking water. Moreover, while some studies have documented the prevalence of fecal contamination in household water supplies, there is limited information regarding the specific strains of *E.coli* present in these environments and their associated virulence factors. Understanding the genetic diversity and resistance profiles of these strains is crucial for developing effective interventions to mitigate health risks associated with contaminated household water. Seasonal monitoring of household water supplies is an important yet under-researched area in environmental microbiology, especially in relation to the spread of antibiotic-resistant bacteria. Although the public health risks are significant, there has been limited research on how water sources in households change across different seasons. This lack of comprehensive monitoring creates major gaps in understanding of microbial communities and the spread of resistance genes in the environment. To address these knowledge gaps, this study will conduct a comprehensive assessment of residence water quality in Dhaka City, with an emphasis on the prevalence of pathogenic *E.coli* strains and their virulence characteristics. This project, which will use microbiological and molecular techniques, will give important details on the prevalence and spread of distinct *E.coli* pathotypes in municipal water sources.

Furthermore, this study will assist in ensuring that households have access to safe and clean drinking water by developing a framework for frequent monitoring and evaluation of home

water quality. Such activities are critical for understanding public health policies and treatments aimed to protect community health. This study aims to identify contamination sources and better understand the behavior of pathogenic *E.coli* in urban water systems, which will lead to more effective water management practices that reduce health risks. In conclusion, addressing the issue of pathogenic *E.coli* in municipal water is critical for improving public health in Bangladesh. The findings will not only close existing gaps in knowledge but also provide important insights for policymakers and health officials focused on improving water safety in urban areas.

1.2 Research Objective

1.2.1 General Objective

To investigate fecal coliforms and *E.coli* from household municipal water supply, furthermore focusing on identifying and confirming of pathogenic *E.coli* through PCR. The study will assess these pathogens' antibiotic resistance and genetic variability, identifying *E.coli* O157:H7, EPEC, EIEC, STEC emphasizing the detection of extended-spectrum betalactamase (ESBL) production and carbapenem-resistant genes. Additionally, it will examine the overall household water quality of Dhaka City.

1.2.2 Specific Objective:

- To isolate Fecal Coliforms from Household municipal water supply.
- To isolate *E.coli* from water supply.
- Cultural confirmation of *E.coli* from TBX, UTI, MacConkey medium.
- Biochemical Confirmation of *E.coli* by MIU, MR,VP ,Catalase, Oxidase test.
- Molecular Detection of *E.coli* by 16srRNA genes through PCR.
- Molecular Detection of EIEC by Ipah gene, STEC by stx1,stx2 gene, EPEC by ease gene, *E.coli* O157:H7 by flicH7,rfbE gene through PCR.
- Determination of antibiotic susceptibility profile of the *E.coli*.
- Molecular detection of antibiotic resistant ESBL producing *E.coli* by CTX, SHV, TEM gene through PCR, and Carbapenem producing *E.coli* through NDM,KPC,IMP gene through PCR.

Chapter 2 Literature Review

2.1 The Organism

Escherichia coli

2.1.1 Taxonomy

Domain: Bacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma proteobacteria

Order: Enterobacterial

Family: Enterobacteriaceae

Genus: Escherichia

Species: *Escherichia coli*

2.1.2 Historical Background of *E.coli*

The bacterium *Escherichia coli* (*E.coli*) was first identified by Theodor Escherichia, a German bacteriologist, in 1885. Initially named Bacterium coli commune, this organism is commonly found in the human intestinal tract and plays a crucial role in gut health. The size of *E.coli* is approximately two microns in length and one micron in width. The name *Escherichia coli* was officially adopted in 1958 to honor its discoverer (Kuhnert et al., 2000). Theodor Escherichia's research focused on the intestinal bacteria of infants, leading to significant insights into the role of these microorganisms in human physiology and pathology. His work laid the foundation for

future studies on gut microbiota and its impact on health (Engda et al., 2023). Over the years, *E.coli* has been recognized not only as a harmless inhabitant of the intestines but also as a versatile pathogen capable of causing various diseases, including gastroenteritis and urinary tract infections. The evolution of pathogenic strains of *E.coli*, particularly those producing Shiga toxin (such as O157:H7), has raised public health concerns due to their association with severe foodborne outbreaks. This dual nature of *E.coli*—as both a beneficial gut microbe and a potential pathogen—has made it a focal point for microbiological research and public health policy. The evolution of pathogenic strains of *E.coli*, particularly those producing Shiga toxin (such as O157:H7), has raised public health concerns due to their association with severe foodborne outbreaks. This dual nature of *E.coli*—as both a beneficial gut microbe and a potential pathogen—has made it a focal point for microbiological research and public health policy.

2.1.3 General Characteristics of *E.coli*

Escherichia coli (*E.coli*) is a member of the Enterobacteriaceae family, characterized as a rod-shaped, gram-negative bacterium that is a facultative anaerobe and capable of fermenting lactose. Measuring approximately 1-2 µm in length and 0.1-0.5 µm in diameter, *E.coli* thrives optimally at a temperature of 37°C (98.6°F). This organism is typically motile in liquid environments due to the presence of peritrichous flagella. Most *E.coli* strains ferment lactose, producing gas in the process, and they are methyl-red positive, indicating acid production from glucose fermentation. About 10% of *E.coli* strains are classified as late lactose fermenters, and some are non-lactose fermenters altogether. *E.coli* produces indole and exhibits a negative Voges-Proskauer reaction while failing to utilize citrate as the sole carbon source. Isolates from this genus generally do not produce extracellular DNase, phenylalanine deaminase, or urease, and they do not grow in media containing inositol or potassium cyanide (KCN). Typical gas production is observed from glucose, indole, lysine, arabinose, mannitol,

ortho-nitrophenyl galactoside (ONPG), trehalose, and xylose(Schaechter, 2004). Historically, *E.coli* was considered the sole species within the genus Escherichia, but four additional species have since been identified: E. fergusonii, E. vulneris, and E. hermannii, all isolated from human sources (Scheutz & Strockbine, 2015).Despite these additions, *E.coli* remains the predominant species, accounting for over 99% of isolates from the genus. This bacterium is commonly found in the large intestine of vertebrates as part of the normal flora and is widely distributed in nature, including soil and surface water, as well as animal and human feces. It is estimated that up to 10^8 *E.coli* cells can be present per gram of fecal material.

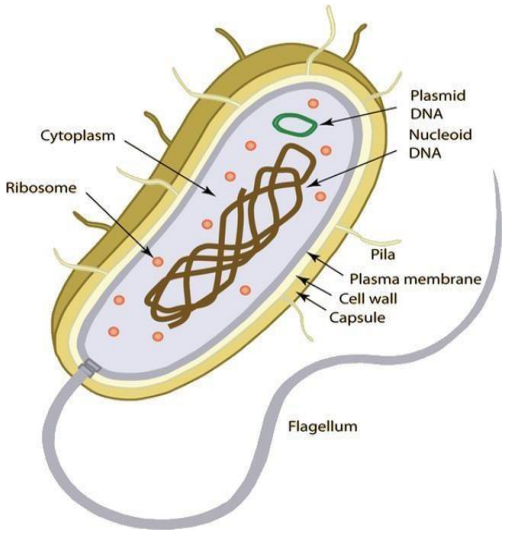

Figure 1: Basic structure of *E.coli*

2.1.4 Physiochemical properties of *E.coli*

E.coli is a facultative anaerobe, which means that it can survive in both aerobic and anaerobic environments. *E.coli* demonstrates a remarkable capacity to grow across a broad temperature range, typically from approximately 15°C to 48°C, with optimal growth occurring between 37°C and 42°C. The optimum growing temperature is about 37°C (98.6°F), which corresponds to to the average human body temperature, allowing it to survive in the gastrointestinal tract This mesophilic bacterium can thrive within a pH range of about 5.5 to 8.0, with the best growth conditions found at neutral pH levels (Raghubeer & Matches,

1990). Interestingly, certain diarrheagenic strains of *E.coli* can tolerate exposure to acidic conditions as low as pH 2.0. This acid shock mimics the transit through the stomach and triggers the expression of specific genes associated with survival and pathogenesis It is capable of reducing nitrates to nitrites. It produces acid and gas (mainly H2 and CO2) at the time of growth on glucose or other carbohydrates via fermentation. Traditional biochemical tests reveal that *E.coli* is positive for indole production and the methyl red test, indicating its ability to ferment glucose with acid production (VM). In contrast, most strains of *E.coli* are negative for oxidase, citrate, urease, and hydrogen sulfide production (Raghubeer & Matches, 1990) .*E.coli* demonstrates a remarkable capacity to grow across a broad temperature range, typically from approximately 15°C to 48°C, with optimal growth occurring between 37°C and 42°C (Wikipedia, 2024). This mesophilic bacterium can thrive within a pH range of about 5.5 to 8.0, with the best growth conditions found at neutral pH levels (Raghubeer & Matches, 1990). Interestingly, certain diarrheagenic strains of *E.coli* can tolerate exposure to acidic conditions as low as pH 2.0. This acid shock mimics the transit through the stomach and triggers the expression of specific genes associated with survival and pathogenesis (Xu et al., 2020).

2.1.5 Role as normal flora

E.coli typically colonizes the gastrointestinal tract of most warm-blooded animals within hours or days after birth, often introduced through food, water, or contact with caregivers. In the large intestine, it attaches to the mucus layer. In humans, the colonization of the bowel generally occurs within 40 hours of birth, with the bacteria adhering to the mucus lining the large intestine. It is the primary facultative organism of the human gastrointestinal tract (Ogundare & Onifade, 2009). The human colon sustains an incredibly dense microbial population, ranging from 10^12 to 10^14 organisms per gram of feces, forming a

wellbalanced ecosystem. The commensal microbiota, comprising over 400 species, coexists harmoniously with the human intestine (Hooper & Gordon, 2001). *E.coli* supports the prevention of pathogenic bacteria colonization by occupying ecological niches within the gastrointestinal tract, promoting competitive exclusion (FAQ, 2011). These microorganisms perform various functions that benefit the host. For instance, they produce enzymes that break down molecules indigestible by vertebrates, synthesize small amounts of vitamins for absorption into the bloodstream, and inhibit the colonization of harmful bacteria. In the large intestine, *E.coli* contributes similar benefits, aiding in waste processing, vitamin K synthesis, and nutrient absorption. Healthy individuals often harbor a diverse array of non-pathogenic, commensal *E.coli* strains from different serotypes, which can be isolated from feces. These strains are widely shed into the environment, potentially contaminating foods such as meat, vegetables, fruits, and their products, as well as surface and groundwater. However, such contamination typically does not pose significant risks to human health.

2.1.6 *E.coli* as a model organism

Escherichia coli, or *E.coli*, has been used extensively as a model organism in scientific studies because of a number of beneficial traits that make it easier to examine fundamental biological processes. Its simplicity as a single-celled organism, which enables researchers to manipulate and develop it without the ethical issues connected with multicellular species, is one of the main reasons for its selection (Ogundare & Onifade, 2009). This species is often used in microbiological research due to its ease of access, generally high virulence, and potential for rapid growth. On the majority of laboratory medium, this organism grows easily at 37°C and forms large colonies during an overnight incubation period. Our current understanding of DNA replication, fundamental molecular processes, and gene expression control is largely derived from research on *E.coli*. Furthermore, until these phenomena were experimentally created and investigated in the bacterium, many biological processes (such

genetic transformation) that commonly occur in bacterial species remained unclear. *E.coli* provides an excellent platform for researching gene function and regulation because of its well-understood genomic structure and ease of genetic manipulation. The K-12 strain of *Escherichia coli*, for example, has played a significant role in important molecular biology discoveries, such as the understanding of the genetic code and processes of gene expression (FAQ, 2011). Furthermore, *E.coli* serves as a host for recombinant DNA technology; researchers can insert genes from other organisms into *E.coli* to produce proteins such as insulin, which has revolutionized biotechnology and medicine. *E.coli* is a flexible model for researching metabolic processes and ecological interactions because of its capacity to adapt to different growth conditions and flourish in a variety of habitats (eLife, 2015). Its significance for studying human health and disease is further highlighted by the fact that it is a normal component of the flora in the human gut. Overall, *E.coli*'s reputation as an excellent model organism in scientific study has been established by its adaptability, quick growth, simplicity of manipulation, and important contributions to our understanding of biology.

2.1.7 *E.coli* as an indicator of fecal contamination:

Escherichia coli (*E.coli*) is widely recognized as a major fecal indicator organism due to its strong link with fecal pollution and capacity to detect the presence of potentially harmful pathogens in water sources. *E.coli* is a member of the coliform group of bacteria, and its presence in water indicates recent fecal contamination by warm-blooded animals, including humans(F. M. Khan & Gupta, 2020).Unlike other coliforms, *E.coli* is more specific for fecal contamination, making it a reliable marker for assessing water quality and safety. Detecting disease-causing germs and pathogens in water may be costly and sometimes dangerous. Pathogen screening takes significant amounts of water and can be challenging to grow and isolate in the lab. *E.coli* bacteria are effective indicators of fecal contamination due to their longevity, abundance, and ease of collection and culture. *E.coli* can persist in aquatic

environments for several weeks, making it an effective tool for long-term monitoring of water sources (F. M. Khan & Gupta, 2020). Monitoring the microbiological quality of drinking water heavily relies on analyzing indicator bacteria such as coliforms, *E.coli*, and Pseudomonas aeruginosa. As a member of the fecal coliform group, *E.coli* is a more precise indicator of fecal contamination compared to other fecal coliforms. It is the dominant facultative anaerobe in the human colon's normal flora (Odonkor & Ampofo, 2013).*E.coli* naturally resides in the large intestines of warm-blooded animals, and while it generally does not survive well outside this environment, its presence in environmental samples, food, or water typically signals recent fecal contamination or inadequate sanitation practices(Brüssow et al., 2004).*E.coli* is widely used as an indicator organism to detect unsafe levels of fecal contamination in food and water. It is considered more specific than general fecal coliform tests, as the latter may also detect thermotolerant, non-fecal coliform bacteria. The test recommended by the United States Environmental Protection Agency (EPA) identifies *E.coli* by confirming the absence of a particular enzyme that distinguishes it from other thermotolerant coliforms. This method effectively differentiates *E.coli* from non-fecal coliforms. The population of *E.coli* in samples depends on the degree of fecal contamination, poor hygiene practices, and storage conditions. While the presence of *E.coli* in food or water does not directly indicate pathogenic microorganisms, it signifies an elevated risk of other fecal-borne pathogens, such as Salmonella spp. or hepatitis A virus, which can pose serious health risks (Program, 2024).It’s role as an indicator organism is further supported by the simplicity and affordability of detection methods, which deliver quick results to guide public health decisions on water safety . As a result, tracking *E.coli* levels in drinking and recreational water is crucial for preventing waterborne illnesses and safeguarding public health.

	WHO	**US EPA**
Number of *E.coli* **acceptable**	<1 CFU/100mL Water	<1 CFU/100mL Water

Table 1: Acceptable level of fecal coliforms by various agencies

2.1.8 Serology

Serology is the scientific study of serum and other bodily fluids, with a primary focus on diagnosing and identifying antibodies or antigens found within these fluids. *Escherichia coli* (*E.coli**) identification and characterization depend significantly on serology, especially when it comes to distinguishing pathogenic strains from non-pathogenic ones using their unique antigens. The somatic (O), flagellar (H), and capsular (K) antigens of *E.coli* are used to classify the organism into different serotypes, which are crucial for epidemiological research and determining the pathogenic potential of the organism (Kaper et al., 2004a). Pathogenic microorganisms have limited antigen patterns and phylogenetic groups. *E.coli* has about 150 antigenically distinct O-antigens. According to (Whitfield and Roberts,1999), K type capsular material can take two or four forms based on morphological, biochemical, and genetic parameters. There are around 80 capsular polysaccharides, each with its own chemical and serological characteristics. Many *E.coli* isolates share a slime layer called colonic acid extracellular polysaccharide, which can co-express with certain K-type capsules. There are 53 H-antigen specificities found in *E.coli*.

2.1.9 Variability in *E.coli*: commensals to pathogenic

E.coli, typically a harmless inhabitant of the human intestinal flora, can act as an opportunistic pathogen under certain conditions. Generally, these commensal strains coexist peacefully with their host and only pose a risk in rare instances, such as in individuals with weakened immune systems who cannot keep these bacteria contained within their natural habitat. Additionally, *E.coli* can become problematic following after a traumatic break in the natural barriers between the gut and other normally sterile sites of the body or after surgical interventions. In some cases, these bacteria may contribute to mixed infections when primary pathogens compromise local defenses. However, certain strains of *E.coli* have evolved into primary pathogens with a potential ability to cause disease. These pathogenic strains are classified into various pathotypes, each associated with specific diseases and mechanisms of action (Kaper et al., 2004a). Commensal strains of *Escherichia coli* (*E.coli*) generally lack the specialized virulence factors found in pathogenic strains, whether intestinal or extraintestinal, and are typically harmless to their host. These commensal bacteria are part of the normal gut flora and can provide benefits, such as synthesizing vitamin K2 (Suvarna et al., 1998) and inhibiting the growth of pathogenic bacteria within the intestine (Hudault et al., 2001). In contrast, pathogenic strains of *E.coli* are infrequently found in the fecal flora of healthy individuals; they are primarily obligate pathogens that can lead to gastroenteritis or colitis when ingested by a susceptible host (Johnson, 1991). Pathogenic *E.coli* is broadly categorized into several pathotypes, including enterotoxigenic *E.coli* (ETEC), enteropathogenic *E.coli* (EPEC), Shiga toxin-producing *E.coli* (STEC or EHEC), enteroinvasive *E.coli* (EIEC), enteroaggregative *E.coli* (EAEC), and diffusely adherent *E.coli* (DAEC), with each pathotype associated with distinct diseases. Extra-intestinal infections (EIs) caused by *Escherichia coli* (*E.coli*) are prevalent across all age groups and can affect nearly any organ or anatomical site. Common types of EIs include urinary tract infections (UTIs), meningitis—particularly in

neonates and post-neurosurgery patients—various intraabdominal infections, pneumonia (especially in hospitalized or institutionalized individuals), infections associated with intravascular devices, osteomyelitis, and soft tissue infections, which typically occur when the tissue is compromised. Additionally, bacteremia may accompany infections at any of these sites. The spectrum of diseases caused by *Escherichia coli* (*E.coli*) is largely due to the acquisition of specific virulence genes, which are often located on plasmids, bacteriophages, or within unique DNA segments known as pathogenicity islands (PAIs). These PAIs are typically absent in commensal strains of *E.coli* and are believed to have been transferred horizontally, integrating into the *E.coli* chromosome through mechanisms such as bacteriophage or plasmid integration and (Kaper et al., 2004b) Pathogenicity islands contribute significantly to the bacterium's ability to cause a wide range of diseases by providing the necessary genetic tools for virulence. For example, research has shown that pathogenic strains possess a mosaic genome structure with numerous genes that facilitate their adaptation to different environments and enhance their pathogenic potential (Kaper et al., 2004c).The presence of these virulence factors allows pathogenic *E.coli* to effectively colonize host tissues, evade immune responses, and cause damage, leading to various clinical syndromes (Kaper et al.,2004d)such as gastroenteritis, urinary tract infections (UTIs), and sepsis.

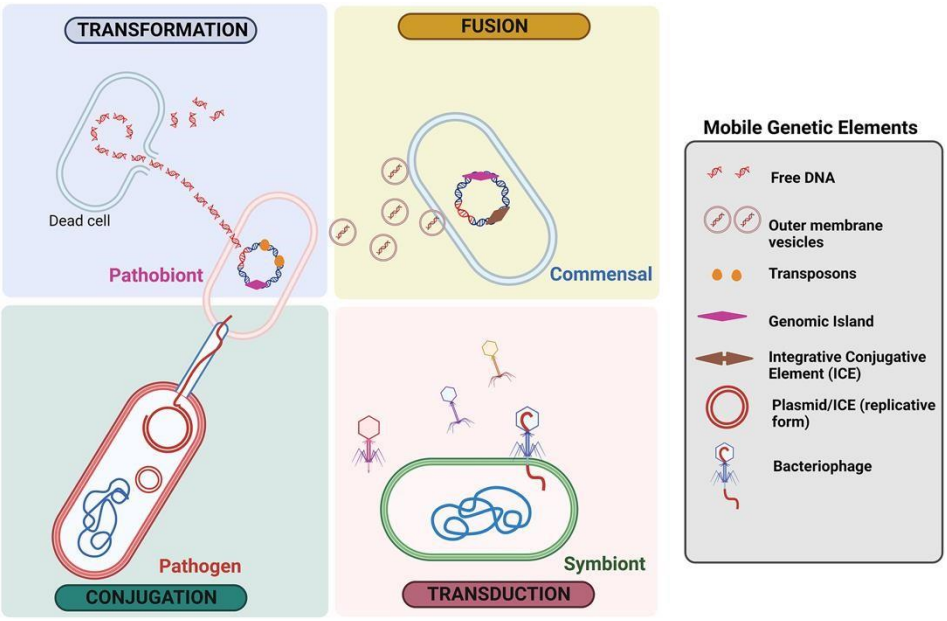

Figure 2: How commensal *E.coli* transform in pathogen[Toxin-linked mobile genetic elements in major enteric bacterial pathogens]

2.1.10 Clinical characteristics and epidemiology

2.1.10.1 Epidemiology

Escherichia coli (*E.coli*) are important bacteria that can cause various diseases, particularly gastrointestinal issues. They are responsible for a significant percentage of urinary tract infections (UTIs), accounting for 85-95% of cases, and they contribute to 60-70% of hospitalacquired pneumonia and 17-37% of nosocomial bacteremia in Europe and North America [*Escherichia coli* (*E.coli*) are important bacteria that can cause various diseases, particularly gastrointestinal issues. They are responsible for a significant percentage of urinary tract infections (UTIs), accounting for 85-95% of cases, and they contribute to 60-70% of hospitalacquired pneumonia and 17-37% of nosocomial bacteremia in Europe and North America. Additionally, *E.coli* is a leading cause of neonatal meningitis and is involved in abdominal, pelvic, and surgical site infections (Russo & Johnson, 2000). Between 2000 and 2002, *E.coli* was identified as the third most common pathogen in clinical specimens

from intensive care unit patients in Canada, representing 13% of the pathogens isolated and being the most common Gram-negative pathogen (Fluit, A.C., et al. (2000). Treatment for diarrheal infections caused by *E.coli* typically involves fluid and electrolyte replacement, while extraintestinal infections are treated with antibiotics. However, the rising rates of antimicrobial resistance in *E.coli* are making it increasingly difficult to treat these infections effectively (Howard et al., 2014). Additionally, *E.coli* is a leading cause of neonatal meningitis and is involved in abdominal, pelvic, and surgical site infections *Escherichia coli* (*E.coli*) are important bacteria that can cause various diseases, particularly gastrointestinal issues. They are responsible for a significant percentage of urinary tract infections (UTIs), accounting for 85-95% of cases, and they contribute to 60-70% of hospital-acquired pneumonia and 17-37% of nosocomial bacteremia in Europe and North America (Russo and Johnson, 2003). Additionally, *E.coli* is a leading cause of neonatal meningitis and is involved in abdominal, pelvic, and surgical site infections (Russo and Johnson, 2003). Between 2000 and 2002, *E.coli* was identified as the third most common pathogen in clinical specimens from intensive care unit patients in Canada, representing 13% of the pathogens isolated and being the most common Gram-negative pathogen [Fluit, A.C., et al. (2000). "The Role of *Escherichia coli* in Nosocomial Infections." Clinical Infectious Diseases, 30(4), 682-683.] Treatment for diarrheal infections caused by *E.coli* typically involves fluid and electrolyte replacement, while extraintestinal infections are treated with antibiotics. However, the rising rates of antimicrobial resistance in *E.coli* are making it increasingly difficult to treat these infections effectively (Howard et al., 2014). Between 2000 and 2002, *E.coli* was identified as the third most common pathogen in clinical specimens from intensive care unit patients in Canada, representing 13% of the pathogens isolated and being the most common Gramnegative pathogen [https://academic.oup.com/cid/article-abstract/30/3/454/598905.]. Treatment for diarrheal infections caused by *E.coli* typically involves fluid and electrolyte replacement, while extraintestinal infections are treated with antibiotics. However, the rising

rates of antimicrobial resistance in *E.coli* are making it increasingly difficult to treat these infections effectively (Howard et al., 2014).

2.1.10.1.1 Enterohemorrhagic *E.coli* (EHEC)

In the United States, there were 463 reported cases of *E.coli* O157:H7 (0.97 per 100,000 people) and 521 cases of non-O157 STEC (1.10 per 100,000) in 2011, according to Food Net Centers for Disease Control and Prevention (Engda et al., 2023). In Canada, the National Enteric Surveillance Program (NESP) reported an incidence of 1.18 cases per 100,000 for *E.coli* O157:H7 in 2010. Meanwhile, in Europe, the overall incidence of STEC was reported at 0.75 cases per 100,000 in the European Union in 2009.Infections caused by Shiga toxinproducing *Escherichia coli* (STEC) can vary from mild watery diarrhea to severe bloody diarrhea, known as hemorrhagic colitis, and they pose a risk for developing hemolytic uremic syndrome (HUS). Both *E.coli* O157 and non-O157 STEC can present similar symptoms, but the severity of the disease may differ among serotypes. The incubation period for *E.coli* O157:H7 is typically around three days (Tarr, 1995) The initial symptom is usually diarrhea, which may be accompanied by fever, abdominal cramps, or vomiting (Tarr, 1995). Patients may develop hemorrhagic colitis a few days after the onset of diarrhea. Generally, *E.coli* O157:H7 is associated with higher rates of bloody diarrhea compared to non-O157 STEC. Diarrhea caused by *E.coli* O157:H7 usually resolves within about a week, while diarrhea from non-O157 STEC may last longer (X. Wang et al., 2013). Hemolytic uremic syndrome (HUS) is characterized by thrombocytopenia, hemolytic anemia, and acute renal failure, typically developing between 5 to 13 days after the onset of diarrhea. Long-term complications can occur in 20 to 40% of patients with HUS and may include cardiac issues, gastrointestinal problems, neurological disorders, hypertension, chronic kidney disease, changes in cognition and behavior, and diabetes mellitus. These complications have been discussed in detail in recent literature (Spinale et al., 2013).

2.1.10.1.2 Enteropathogenic *E.coli* (EPEC)

The prevalence of enteropathogenic *Escherichia coli* (EPEC) infections varies across different studies due to factors such as the populations studied, age distributions, and diagnostic methods used](Buuck et al., n.d.).Geographic location and socioeconomic status also play a role in the epidemiology of EPEC-related diarrhea(Boschi-Pinto et al., 2008).The observed resistance in adults and older children may be linked to the loss of specific receptors for aEPEC as individuals age or develop immunity (Croxen et al., 2013).Significant outbreaks of EPEC have not been reported. Symptoms of EPEC infection include abdominal cramps, nausea, vomiting, and diarrhea. The infection is usually self-limiting, with complications being rare; however, symptoms such as anorexia, malaise, fatigue, and muscle cramps may accompany the illness.

2.1.10.1.3 Enterotoxigenic *E.coli* (ETEC)

Enterotoxigenic *Escherichia coli* (ETEC) infections are a significant cause of diarrhea, particularly in infants, with studies indicating that ST-positive ETEC strains are major contributors to infantile diarrhea, while LT-only positive strains are less common (Croxen et al., 2013). It is estimated that there are nearly 840 million cases of ETEC annually in developing countries, with about 280 million of these cases occurring in children aged 0 to 4 years. Additionally, approximately 46.6 million children under the age of 4 are believed to be asymptomatic carriers of ETEC (Darrah et al., 2014). The largest reported ETEC outbreak in the United States occurred in Illinois in 1998, where serotype 06:H16 (LT/ST) was isolated from several patients. Although only 120 cases were officially documented, it was estimated that around 3,300 individuals may have fallen ill due to food prepared by a delicatessen over a few days. Symptoms of ETEC infection typically include mild to severe watery diarrhea (usually non-bloody), along with headaches, fever, abdominal cramps, nausea, and vomiting.

The onset of diarrhea is usually rapid, with an incubation period as short as 5 hours but generally averaging between 1 and 2 days after ingestion. Diarrhea typically lasts about 3 to 5 days but can persist for more than a week (McGovern et al., 2002). Those who experience traveler's diarrhea may have a five-fold increased risk of developing post-infectious irritable bowel syndrome (Stermer et al., 2006).Enteroinvasive *Escherichia coli* (EIEC) infections are often underreported in epidemiological studies due to their milder symptoms compared to other types of *E.coli*. Additionally, EIEC strains that closely resemble Shigella may be misidentified. Recent health surveillance programs in the United States, Canada, and Europe have not reported any EIEC cases (Pacheco-Gil et al., 2006). However, a notable outbreak occurred in Texas in 1985, affecting 370 individuals (Hedberg et al., 1997). Symptoms of EIEC infection typically include mild, self-limiting watery diarrhea, though in rare instances, it can lead to symptoms similar to shigellosis. Complications are uncommon (Hedberg et al., 1997). The clinical presentation of shigellosis can vary but generally includes mild watery diarrhea, fatigue, malaise, fever, and loss of appetite. As the disease progresses, patients may experience abdominal cramps and stools that contain blood and mucus, along with dehydration.

2.1.10.1.4 Enter invasive *E.coli* (EIEC)

Enteroinvasive Escherichia coli (EIEC) infections are often underreported in epidemiological studies due to their milder symptoms compared to other types of *E.coli*. Additionally, EIEC strains that closely resemble Shigella may be misidentified. Recent health surveillance programs in the United States, Canada, and Europe have not reported any EIEC cases. However, a notable outbreak occurred in Texas in 1985, affecting 370 individuals (Gordillo, M., et al.,1992). Symptoms of EIEC infection typically include mild, self-limiting watery diarrhea, though in rare instances, it can lead to symptoms similar to shigellosis. Complications are uncommon (Gordillo, M., et al.,1992). The clinical presentation of

shigellosis can vary but generally includes mild watery diarrhea, fatigue, malaise, fever, and loss of appetite. As the disease progresses, patients may experience abdominal cramps and stools that contain blood and mucus, along with dehydration.

2.1.10.1.5 Enteroaggregative *E.coli* (EAEC)

In 2011, a significant outbreak of enteroaggregative *Escherichia coli* (EAEC) in Germany affected 4,321 previously healthy individuals from 16 countries, resulting in over 900 cases of hemolytic uremic syndrome (HUS) and more than 50 deaths(Grad et al., 2012).The strain responsible for this outbreak was identified as *E.coli* O104:H4, which is a hybrid pathogen containing virulence genes from both typical enter adhesive *E.coli* (EAEC) strains and Shiga toxin-producing *E.coli* (STEC).Symptoms of EAEC infection typically include watery diarrhea that may contain mucus, along with low-grade fever, nausea, vomiting, and abdominal pain. In some cases, bloody stools can occur. While EAEC infections are usually self-limiting, they can lead to chronic inflammation that damages the intestinal lining and impairs nutrient absorption (Castro-Rosas et al., 2012) Additionally, individuals who experience acute gastroenteritis are at a significantly increased risk of developing postinfectious irritable bowel syndrome

2.2 Transmission of *E.coli*

Pathogenic strains of *Escherichia coli* (*E.coli*), such as Shiga toxin-producing *E.coli* (STEC) and Enteropathogenic *E.coli* (EPEC), are transmitted primarily via the fecal-oral route, often through contaminated food, water, or direct contact with infected individuals or animals. The bacteria can survive on contaminated surfaces for a short time, facilitating indirect transmission through contaminated utensils or household items(Jaro et al., 2023)

2.2.1 Transmission of *E.coli* through water system

Access to clean and treated water is standard in Europe and North America, but in many developing nations, adequate water and sanitation are far from universal, making waterborne diseases prevalent. Over 2.5 billion individuals lack improved sanitation, and diarrheal diseases claim the lives of more than 1.5 million children annually (Fenwick, 2006). According to the World Health Organization, water-related illnesses kill more than 5 million people each year. More than half of them are microbial intestinal illnesses, with cholera topping the list. In general, consuming water polluted with human or animal feces poses the biggest microbiological danger. Wastewater discharges in fresh waterways and coastal seawaters are the primary source of fecal bacteria, including diseases. [WHO (World Health Organization). Guidelines for Drinking-water Quality, Incorporating 1st and 2nd Addenda, Volume 1, Recommendations, 3rd ed; WHO: Geneva, Switzerland, 2008. [Google Scholar] (Fenwick, 2006). The spread of *Escherichia coli* through water systems poses a serious public health risk, especially with the rise of antibiotic-resistant strains. In Edo State, Nigeria, 41.7% of surface water samples contained ESBL-producing *E.coli*, which were completely resistant to beta-lactam antibiotics like ceftriaxone and ceftazidime but fully sensitive to carbapenems such as ertapenem and imipenem (Beshiru et al., 2024). Similarly, a study in Canada found that people using drinking water contaminated with resistant *E.coli* had a 1.26 times higher chance of carrying these strains than those with clean water sources (Anastasi et al., 2012).An outbreak of *E.coli* O157:H7 in Wyoming linked to unchlorinated water contaminated with fecal matter further highlighted the dangers of poor water treatment(Olsen et al., 2002).These findings stress the importance of improving water quality and monitoring systems to reduce the risks of antibiotic resistance in waterborne *E.coli*(Hasan et al., 2022).

2.3 Antibiotic resistance in *E.coli* in Water:

Antimicrobial resistance (AMR) poses a significant threat to the treatment of infectious diseases and is one of the most pressing public health issues of the 21st century (Blair et al., 2015). Antibiotic resistance not only complicates the treatment of bacterial infections but also diminishes the effectiveness of existing antimicrobial drugs. The rise of antimicrobialresistant Gram-negative bacteria, in particular, has outpaced the development of new antimicrobial classes (Patterson). While the use of antimicrobials is a key driver of resistance, the spread of resistant bacteria and resistance genes also plays a major role in the growing prevalence of resistance (Founou et al., 2017). Studies have highlighted that human-to-human transmission is a significant contributor to resistance, with risk factors such as hospitalization, attending daycare, or living with someone carrying resistant bacteria (Chen et al., 2021). Additionally, transmission through contaminated food or water has been suggested as another potential route (Chen et al., 2021). Antimicrobial resistance among entomopathogens, including *E.coli* has been reported to be increasing in recent years. From a study named “Prevalence of Bacterial Contamination with Antibiotic Resistant and Enterotoxigenic Fecal Coliforms in Treated Drinking Water" revealed that 36% of *E.coli* isolates were multidrugresistant (MDR), with 26% of these producing extended-spectrum beta-lactamase (ESBL), indicating significant resistance to commonly used antibiotics. Additionally, the study identified virulence factors associated with enterotoxigenic *E.coli* (ETEC), which is linked to diarrheal diseases, and detected quinolone resistance genes in some isolates. These results highlight a critical public health concern regarding the safety of treated drinking water and underscore the need for improved monitoring and intervention strategies to mitigate the risks posed by antibiotic-resistant bacteria in water supplies. The increasing issue of antibiotic resistance, particularly in developing countries, leads to situations where no effective antibiotic treatments are available for infections caused by entomopathogens, especially among

children. A notable example is the emergence of New Delhi Metallo β-lactamase (NDM)-producing organisms, which render many antibiotics ineffective (Moellering, 2010). This novel carbapenemase, along with other resistance factors, is often carried by mobile genetic elements such as plasmids or transposons. Horizontal gene transfer (HGT) plays a crucial role in the spread of these antibiotic resistance traits from one bacterium to another, exacerbating the public health crisis associated with antimicrobial resistance. Generic *E.coli* are often used to study antimicrobial resistance because they are common gut bacteria in humans and animals, easy to grow, and can pick up and keep resistance genes from other organisms. Detecting *E.coli* in water suggests fecal contamination and the possible presence of harmful microorganisms. This becomes especially concerning when the *E.coli* are resistant to multiple antibiotics and can cause diseases in people who drink the contaminated water. Research from other countries has also found antibiotic-resistant bacteria in water sources like rivers, ponds, and lakes. (Hu et al., 2008) Recent studies show that surface waters worldwide, both in developing and developed countries, are major reservoirs of multi-drugresistant pathogens. This is due to factors like excessive use of antimicrobials in medicine and farming, human activities near water sources, animal waste, municipal and industrial pollution, stormwater runoff, and faulty septic systems (Hu et al., 2008). Moreover, the spread of ESBL and carbapenemase (CARBase) enzymes, which confer resistance to vital βlactam antibiotics, is a major concern. Most *E.coli* infections are waterborne, as surface water is heavily contaminated with this bacterium. Contributing factors include poor sanitation,

Chapter 3

Materials and Methods

This study was carried out at the BRAC University under Mathematical and Natural Sciences Department. The entire study methodology is briefly given below.

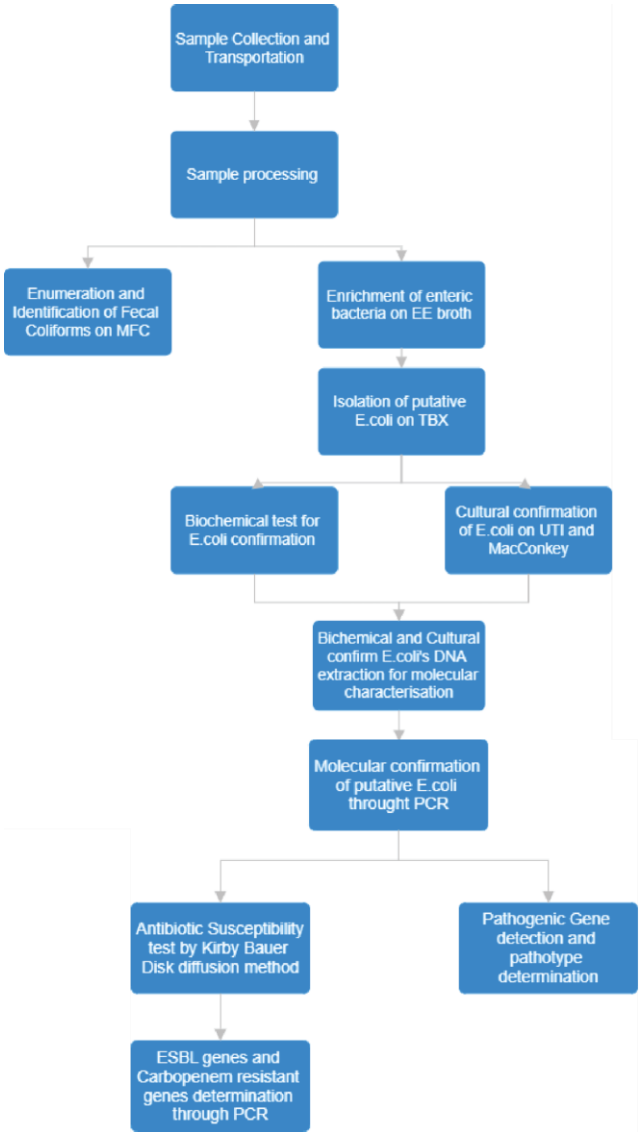

Table 2: Work Flow

3.1 Sampling Sites and Sample Collection

This study aimed to investigate the municipal water supply in household water across North Dhaka City. A total of 92 water samples were collected from various locations, including Farmgate, Tejgaon, Indira Road, Banani, Mohakhali, Gulshan, Bashabo, Banasree, Rampura, Badda, Bashundhara, Nadda, Uttara, Mirpur, Mohammadpur, Bailey Road, Motijheel, Malibagh, Aftabnagar. Sampling was conducted between May 2024 to November 2024.

Approximately 500 ml of water was collected for each sample in sterile 500 ml plastic bottles (NALGENE, USA), which were appropriately labeled. After collection, the samples were transported in an insulated box with sufficient ice packs to maintain a temperature range of 4°C to 10°C, ensuring the cold chain was preserved. All samples were processed following standard procedures within 6-8 Hours of collection.

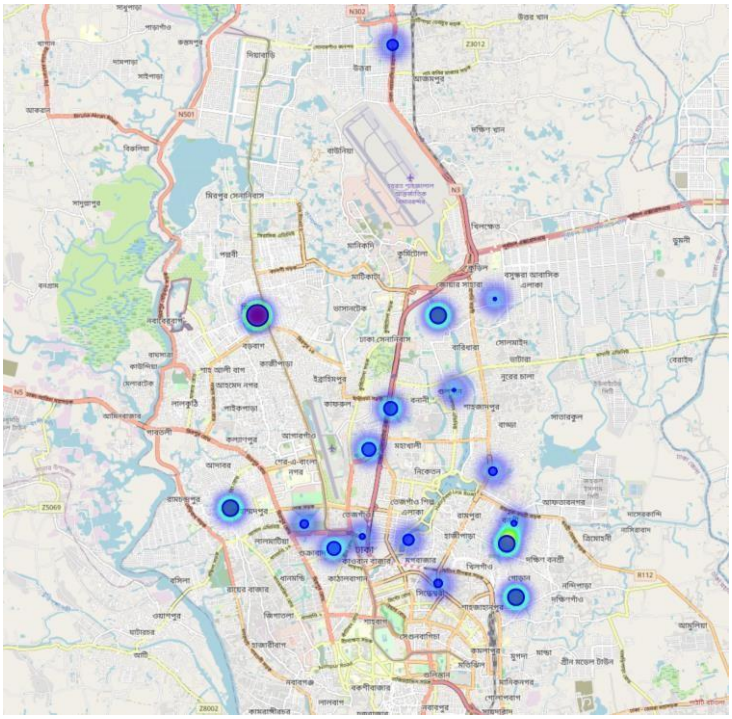

Figure 3: Mapping of Sample collection

3.2 Sample Processing

For the analysis, water samples were filtered using a 0.22 µm membrane filter (Sartorius Stedim, Goettingen, Germany). From each water sample, 100 ml was filtered. The membrane filter was then carefully placed, face-up, onto an M-FC agar plate. Simultaneously, another membrane filter from the same sample was transferred into EE broth contained in a Falcon tube. The M-FC agar plate was incubated at 44°C for 24 hours to detect and enumerate fecal coliforms. Meanwhile, the filter placed in the EE broth was incubated at 37°C for 24 hours to enrich *E.coli* from the water sample.

Figure 4: Sample processing of *E.coli* through membrane filtration

3.2.1 Detection and Enumeration of Fecal Coliforms

After 24 hours of incubation at 44°C, blue colonies appearing on the M-FC agar plate were identified as fecal coliforms. The colony- forming units (CFU) were then calculated and expressed as CFU/ml.

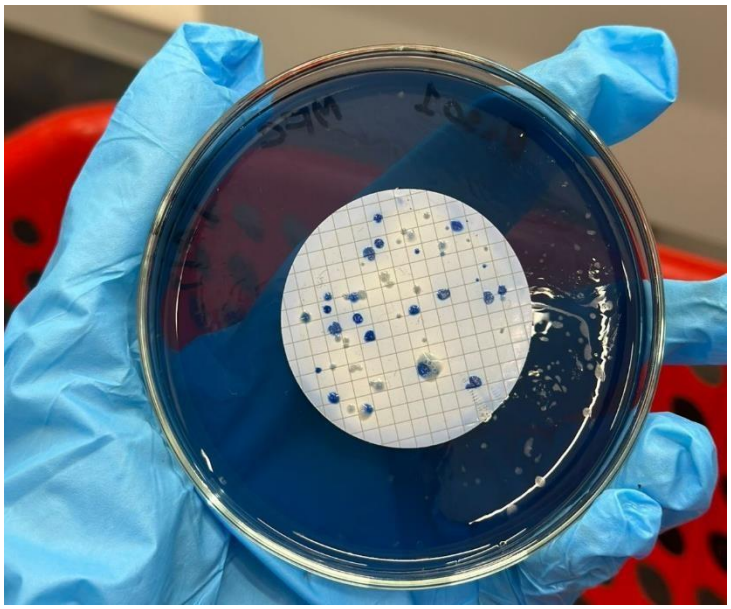
Figure 5: Enumeration and identification of Fecal Coliform on MFC

3.2.2: Isolation and identification of *E.coli* on selective media

Following enrichment in EE broth for 25 hours at 37°C, the cultured broth was serially diluted up to 10^{-6}. Aliquots from each dilution were spread onto TBX agar media (HiMedia) and incubated at 37°C for 18–24 hours. After incubation, colonies exhibiting a green-blue coloration were identified as putative *E.coli*.

To confirm the identification, single green-blue colonies were randomly selected and streaked onto UTI medium and MacConkey agar. These plates were then incubated at 37°C for 18–24 hours. Distinctive colony morphologies were observed: purple colonies on UTI medium and pink, dry, non-mucoid colonies on MacConkey agar, indicating presumptive *E.coli*.

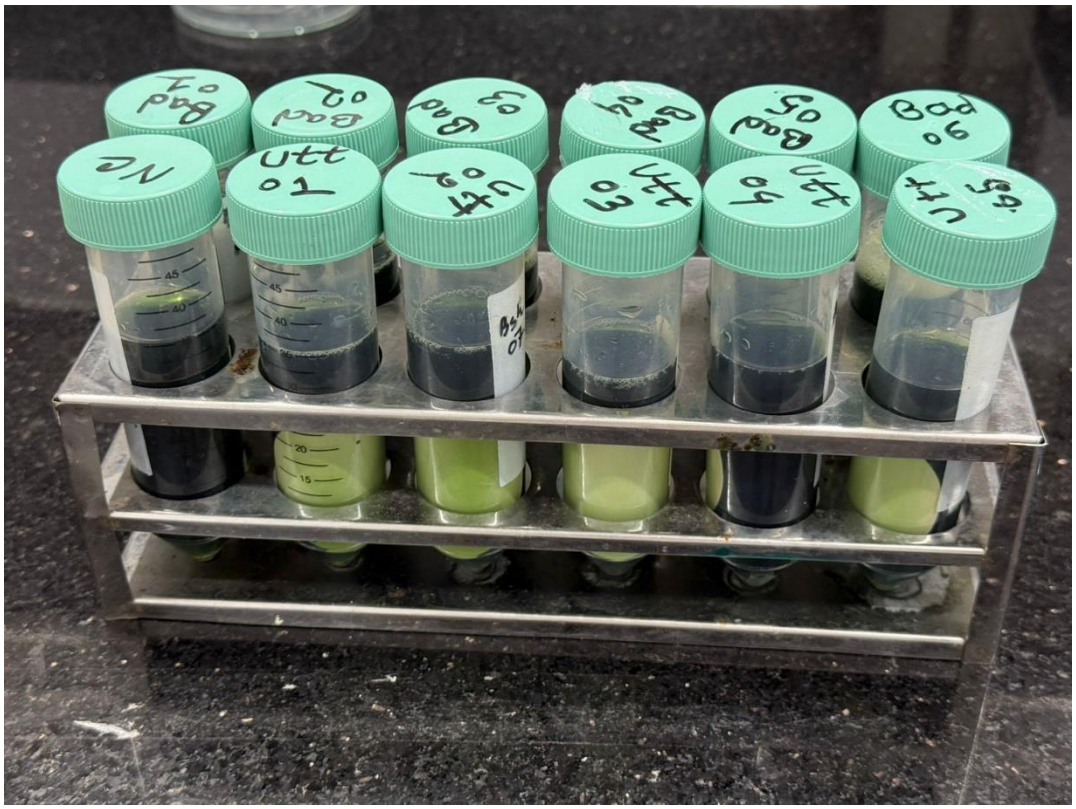

Figure 6: Enrichment of *E.coli* in EE broth

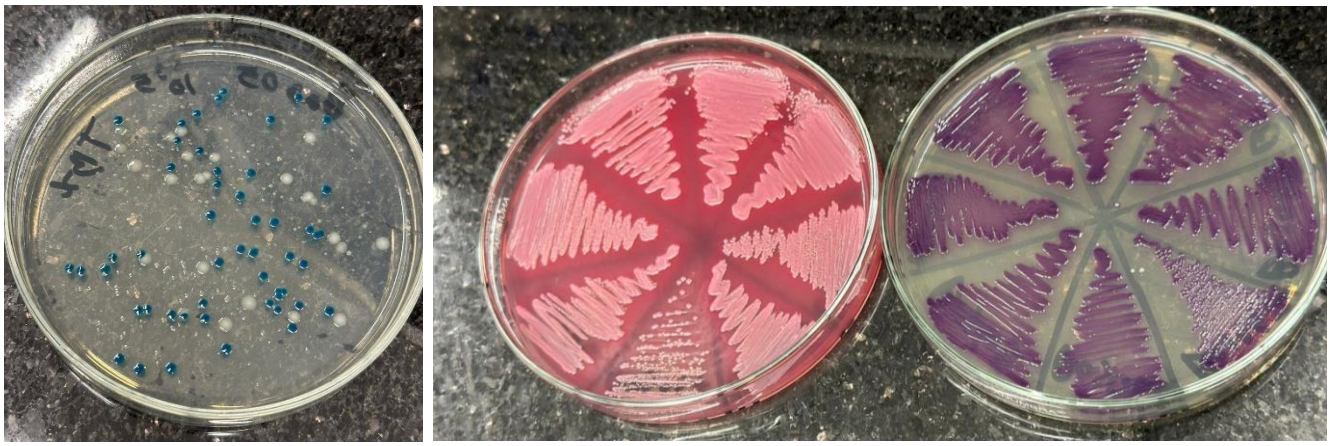

Figure 7: Cultural confirmation of *E.coli* on TBX, following by MacConkey and UTI

3.2.3 Biochemical confirmation

The isolates were further confirmed as *E.coli* through a series of biochemical tests, including the MIU (Motility, Indole, Urease) test, Catalase test, Oxidase test, Citrate utilization test, Methyl Red (MR) test, and Voges-Proskauer (VP) test.

Organism	**Catalase**	**Oxidase**	**Glucose Ferm**	**Sucrose Ferm**	**Lactose Ferm**	**Gas Prod.**	**H2S Prod.**
Escherichia coli	+ve	-ve	+ve	variable	+ve	+ve	-ve
	H2S Prod.	**Motility**	**Indole**	**Urease**	**MR**	**VP**	**Citrate utilization**
	-ve	+ve	+ve	-ve	+ve	-ve	-ve

Table 3 : Biochemical test results of *E.coli*

3.2.3.1 MIU test

The MIU (Motility, Indole, Urease) test is a biochemical multitest assay used to identify *Escherichia coli* and other gram-negative bacilli by assessing their motility, urease activity, and ability to produce indole.

Protocol: To prepare MIU agar, 1.0 g of casein enzymic hydrolysate, 0.1 g of dextrose, 0.5 g of sodium chloride, 10 mg of phenol red, and 0.2 g of agar were dissolved in 100 ml of distilled water. The solution was autoclaved at 121°C for 15 minutes. After cooling, sterile urea solution was aseptically added to the medium. The test organism was then inoculated by stabbing the medium to about one-third of its depth using a sterile inoculating needle. The inoculated medium was incubated at 35–37°C for 18–24 hours.

Interpretation: After incubation, motility was assessed by observing any spreading of growth beyond the stab line. A positive urease test was indicated by a color change from yellow to pink-red, signifying ammonia production. Indole production was confirmed by adding Kovac's reagent, with a positive result showing a pink-red ring forming at the surface of the medium. For *E.coli*, the motility test was positive, the indole test was positive, and the urease test was negative.

3.2.3.2 Catalase test

The catalase test is a biochemical procedure used to detect the presence of the catalase enzyme in bacteria, which decomposes hydrogen peroxide (H_2O_2) into water and oxygen. This test is particularly useful for distinguishing between catalase-positive bacteria, such as Staphylococcus and *Escherichia coli*, and catalase-negative bacteria, like Streptococcus (ASM, 2010).

Protocol: To perform the catalase test on *E.coli*, a small portion of a bacterial colony was placed on a glass slide or in a test tube, followed by the addition of a few drops of 3% hydrogen peroxide.

Interpretation: A positive result was indicated by the rapid formation of bubbles, signifying the release of oxygen gas. If no bubbles formed, the result was negative. *E.coli* showed a positive result(Aryal, 2015).

3.2.3.3 Oxidase test

The oxidase test is a biochemical procedure used to detect the presence of cytochrome c oxidase in bacteria, an enzyme involved in the electron transport chain. This test is particularly useful for differentiating between oxidase-negative Enterobacteriaceae, such as *Escherichia coli*, and oxidasepositive bacteria, like Pseudomonas aeruginosa.

Protocol: To perform the oxidase test on *E.coli*, a sterile swab was used to collect a sample from a well-isolated colony on an agar plate. The sample was then transferred to a clean glass slide or a piece of filter paper. Next, 2-3 drops of oxidase reagent (commonly 1% tetramethyl-p-phenylenediamine dihydrochloride) were added directly onto the sample.

Interpretation: If the smear turned dark purple within 10-30 seconds, the organism was considered oxidase-positive. If no color change occurred, the organism was oxidase-negative. *E.coli* showed an oxidase-negative result.

3.2.3.4 Citrate utilization test

The citrate utilization test is used to determine if bacteria can use sodium citrate as their only carbon source. This test is conducted using Simmons citrate agar, which contains sodium citrate, ammonium dihydrogen phosphate, and bromothymol blue as a pH indicator.

Protocol: To perform the citrate utilization test for *Escherichia coli*, Simmons citrate agar was prepared, containing sodium citrate as the sole carbon source, ammonium dihydrogen phosphate as the nitrogen source, and bromothymol blue as a pH indicator. A sterile inoculating needle was used to lightly inoculate the surface of the slant by touching the tip to a well-isolated 18-24-hour old colony of *E.coli*. The agar was not stabbed to maintain an aerobic environment. The inoculated slant was then incubated at 35-37°C for 18-24 hours, although some organisms might require up to seven days for growth. After incubation, the color change of the medium was observed.

Interpretation: A positive result was indicated by a color change from green to blue, signifying the organism's ability to utilize citrate and produce alkaline by-products, while no color change (the medium remaining green) indicated a negative result. *E.coli* showed negative results.

3.2.3.5 MR-VP test

The MR-VP test, consisting of the Methyl Red (MR) and Voges-Proskauer (VP) tests, is a set of biochemical assays used to differentiate bacteria based on their glucose fermentation pathways. The MR test detects the presence of mixed acids produced during glucose fermentation by adding methyl red, a pH indicator. If enough acid is produced to lower the pH below 4.4, the medium turns red, indicating a positive result for mixed acid fermentation. In contrast, the VP test focuses on detecting 2,3-butanediol, a fermentation product. After inoculating and incubating the broth, Barritt's reagents (α-naphthol and potassium hydroxide) are added; if acetoin is present, it reacts to produce a pink-red color, indicating a positive result for butanediol fermentation. *Escherichia coli* is typically methyl red positive (MR+) and Voges-Proskauer negative (VP−), reflecting its glucose fermentation pathway, which primarily produces mixed acids rather than butanediol

Protocol: To perform the MR-VP test for *Escherichia coli*, MR-VP broth was inoculated with a pure culture of *E.coli* and incubated at 35-37°C for 24-48 hours. After incubation, for the methyl red test, 2-3 drops of methyl red reagent were added to a portion of the broth.

Interpretation: A red color indicated a positive result, signifying mixed acid fermentation, while yellow indicated a negative result. For the Voges-Proskauer test, 1 mL of the broth was transferred to a separate tube, and 2-3 drops of Barritt's reagent A (α-naphthol) were added, followed by 2-3 drops of Barritt's reagent B (potassium hydroxide). The mixture was gently shaken and allowed to stand for 15-30 minutes. A pink-red color indicated a positive result, showing the presence of acetoin, while no color change indicated a negative result. Typically, *E.coli* was positive for the methyl red test (MR+) and negative for the Voges-Proskauer test. *E.coli* showed positive for MR test and negative for VP test.

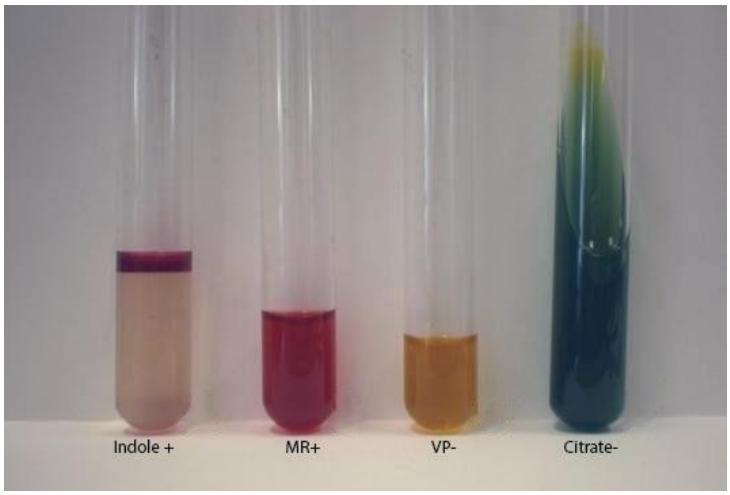

Figure 8: Biochemical test of putative *E.coli* isolates

3.2.4: Preparation of Stock Cultures for Further Analysis

For further analysis, two stock cultures were prepared: one cryo-glycerol stock, which was stored at 80°C, and one T1N1 stock, which was kept at room temperature.

3.2.4.1: Cryo-preservation with Glycerol

After confirmation, a single colony from the UTI agar and MacConkey agar plates was picked and cultured in 3 mL of LB broth for enrichment. The culture was incubated in a shaking incubator at 37°C for 18-24 hours, maintaining 120 rpm. A stock culture was then prepared by supplementing the enriched culture with 30% (v/v) glycerol (700 µL of enriched culture + 300 µL of glycerol), and it was stored at -80°C for further analysis.

3.2.4.2: T1N1 Stock

T1N1 medium was prepared and autoclaved. Then, 3 mL of T1N1 medium was poured into autoclaved vials. A single isolated colony from freshly grown overnight cultures on Na plates (which had been subculture from a presumptive single colony of *E.coli* from the UTI and MacConkey plates) was stabbed into the agar using a sterile needle. The vials were incubated at 37°C for 24-48 hours. After incubation, 300 µL of autoclaved paraffin oil was added, and the stock was kept at room temperature.

3.3 Molecular Identification of *E.coli* isolates

Fresh cultures were obtained for analysis using the PCR method, and DNA was extracted through the boiling technique. Following initial identification based on the colony morphology of the organisms, a species-specific PCR was conducted to confirm the identification of the isolates at the molecular level.

3.3.1: Total Cell DNA isolation

DNA extraction involves isolating DNA from cell membranes, proteins, and other biological components through physical or chemical methods. In this study, colony isolates from the T1N1 stock were streaked onto nutrient agar plates and incubated overnight at 37°C. If growth was insufficient, the samples were subculture onto fresh plates and incubated again. The boiling method was employed for DNA extraction.

Autoclaved microcentrifuge tubes (MCTs) were labeled with colony isolate numbers. Loopfuls of colonies from the subcultures were inoculated into 400 µL of TE buffer in the labeled tubes. The contents were vortexed to ensure thorough mixing. The tubes were then centrifuged at 13,000 rpm for 10 minutes, after which the supernatants were discarded. Another 400 µL of TE buffer was added to the remaining pellets, and the tubes were placed in a water bath at 100°C for 10 minutes. Following this, the samples were centrifuged again at 13,000 rpm for 5 minutes, and the supernatant was carefully transferred to new sterilized microcentrifuge tubes. The extracted DNA was stored at -20°C for further use.

3.3.2 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a highly efficient technique used for detecting bacterial isolates at the molecular level by amplifying specific genes under controlled conditions. In this study, samples were collected weekly from designated areas, and PCR was frequently utilized to identify *E.coli* by targeting the amplification of the 16S rRNA genes, ECO-1 and ECO-2.

The PCR assay was carried out in PCR tubes with a total reaction volume of 13 µL. The reaction mixture included 2 µL of DNA template, 6 µL of 2X Emerald PCR Master Mix (Takara Bio), 1 µL of each primer pair (10 µM), and 3 µL of nuclease-free water. Precise pipetting and brief centrifugation were performed to ensure thorough mixing and to prevent the formation of bubbles.

The PCR was conducted using an Applied Biosystems (Thermo Fisher) thermal cycler under a modified program. Details regarding the target gene, bacterial organism, primer sequences, PCR thermocycler annealing temperature, and amplicon sizes are provided in Table 4

Primer name	Sequence	Process	Temperature (°C)	time	Segment	Reference
ECO-1	5'-GACCTCGGTTTAGTTCACAGA-3'	Initial Denaturation	95 °C	7 min	Segment 1	(Conte et al., 2006)
		Denaturation	94°C	1 min	Segment 2 (35 cycle)	
ECO-2	5'-CACACGCTGACGCTGACCA-3'	Annealing	55°C	1 min		
		Extension	72°C	1 min		
		Final Extension	72°C	7 min	Segment 3	

Table 4: Primer sequence and PCR condition for 16srRNA gene (Band Size: 585bp) of *E.coli*

3.3.3: Electrophoretic Analysis of PCR-Amplified DNA

A 100 mL, 1.5% agarose gel solution was prepared by measuring 1.5 grams of agarose into a flask. To this, 2 mL of 50x TAE buffer and 98 mL of distilled water were added. The mixture was then heated in a microwave until the agarose completely dissolved and the solution became clear. After cooling, 4 µL of ethidium bromide (EtBr) was added. The solution was poured into a gel tray, and a comb was placed near one end. The gel was left undisturbed at room temperature for 10 minutes to solidify evenly. Once the gel solidified, the comb was carefully removed, and the gel tray was placed in an electrophoresis chamber, ensuring the gel was submerged in 1x TAE electrophoresis buffer. Following PCR, 4 µL of PCR products from each reaction was loaded onto the gel, along with a 100 bp ladder. The PCR products were separated in the 1.5% agarose gel using 1x TAE buffer at 100 V for 35 minutes. The gel was then visualized under UV light to detect the target gene.

3.4 Molecular Identification of pathogenic *E.coli*

Molecular identification of *Escherichia coli* was performed by screening for pathogenic genes using singleplex and multiplex PCR techniques. The serotyping of *E.coli* O157:H7 was conducted by targeting the flicH7 and rbfE genes. Screening for Shiga toxin-producing *E.coli* (STEC) involved the detection of the stx1 and stx2 genes. Enteroinvasive *E.coli* (EIEC) was identified through the presence of the IpaH gene, while Enteroadherent *E.coli* (EAEC) was screened using the CVC 432 gene. [Table 5]

Target gene	**Primer name**	**Primer sequence**	**product size**	**Reference**
fliC	FLICH7 F	F-5'-GCGCTGTCGAGTTCTATCGAGC-3'	625 bp	(Al-Ajmi et al., 2020)
	FLICH7 R	R-5'-CAACGGTGACTTTATCGCCATTCC-3'		
rfbE	O157AF	F-5'-AAGATTGCGCTGAAGCCTTTG-3'	497 bp	
	O157AR	R-5'-CATTGGCATCGTGTGGACAG-3'		
Stx(STEC/ shiga toxin)	stx1	F-5'-ATAAATCGCCATTCGTTGACTAC-3'	180 bp	(Paton & Paton, 1998)
		R-5'-CGGTCGCCGCACCAGGATTC-3'		
	stx2	F-5'-GGCACTGTCTGAAACTGCTCC-3'	255 bp	
		R-5'-TCGCCAGTTATCTGACATTCTG-3'		
IpaH(EIEC)	Ipah	F-5'-AGGTTAATCT-TTGCAGGGCT-3'	423 bp	(Akhter et al., 2011)
		R-5'-CAACAACCAGCTTACTGCCT-3'		
CVD 432 (EAEC)	CVD 432	F-5'-CTGGCGAAAGACTGTATCAT-3'	630 bp	(Aranda et al., 2004)
		R-5'-CAATGTATAGAAATCCGCTGTT-3'		

Table 5: List of primers used to detect pathogenic genes in this study

PCR reaction mixture for 1 sample of total 25 µl volume		
seriel no	**Thermo ScientificTM DreamTaqTM Green PCR Master Mix (2X)**	**12.5 µl**
1	Primer flicH7-F (10 pM/ µl)	0.5 µl
2	Primer flicH7-R (10 pM/ µl)	0.5 µl
3	Primer rbfE-F (10 pM/ µl)	0.5 µl
4	Primer rbfE-r (10 pM/ µl)	0.5 µl
5	Template DNA	2 µl
6	Nuclease free water	8.5 µl

Table 6: PCR mixture for flicH7 and rbfE gene by multiplex PCR for detection of *E.coli* O157:H7

Process	Temperature	Time	Segment
Initial denaturation	95 °C	2 min	Segment 1
denaturation	94 °C	30 s	Segment 2 (30 cycle)
Annealing	58 °C	15 s	
Extension	68 °C	1 min	
Final extension	68 °C	10 min	Segment 3

Table 7: PCR condition for flic and rbfE gene by multiplex PCR for detection of *E.coli* O157:H7

PCR reaction mixture for 1 sample of total 25 µl volume		
Serial no	**Thermo ScientificTM DreamTaqTM Green PCR Master Mix (2X)**	**12.5 µl**
1	Primer stx1-F (10 pM/ µl)	0.5 µl
2	Primer stx1-R (10 pM/ µl)	0.5 µl
3	Primer stx2-F (10 pM/ µl)	0.5 µl
4	Primer stx2-r (10 pM/ µl)	0.5 µl
5	Template DNA	2 µl
6	Nuclease free water	8.5 µl

Table 8: PCR mixture for STX1 and STX2 gene by multiplex PCR for detection of STEC

Process	Temperature	Time	Segment
Initial denaturation	95 °C	1 min	Segment 1
denaturation	94 °C	30 s	Segment 2 (35 cycle)
Anneling	60 °C [for initial 10 cycle] 65 °C[for last 25 cycle]	15 s	
Extenision	72 °C	1.5 min [for first 10 cycle] 2.5 min [for last 25 cycle]	
Final extension	**72°C**	10 min	Segment 3

Table 9: PCR condition for STX1 and STX2 gene by multiplex PCR for detection of STEC

PCR reaction mixture for 1 sample of total 13 µl volume		
seriel no	**Thermo ScientificTM DreamTaqTM Green PCR Master Mix (2X)**	**6.5 µl**
1	Primer IpaH-F (10 pM/ µl)	0.5 µl
2	Primer IpaH-R (10 pM/ µl)	0.5 µl
3	Template DNA	2 µl
4	Nuclease free water	3.5 µl

Table 10: PCR mixture for IpaH gene by PCR for detection of EIEC

Process	Temperature	Time	Segment
Initial denaturation	95 °C	3 min	Segment 1
denaturation	94 °C	1 min	Segment 2 (35 cycle)
Anneling	57 °C	1 min	
Extension	72 °C	1 min	
Final extension	72°C	10 min	Segment 3

Table 11: PCR condition for IpaH gene by multiplex PCR for detection of EIEC

PCR reaction mixture for 1 sample of total 13 µl volume		
Serial no	**Thermo ScientificTM DreamTaqTM Green PCR Master Mix (2X)**	**6.5 µl**
1	Primer IpaH-F (10 pM/ µl)	0.5 µl
2	Primer IpaH-R (10 pM/ µl)	0.5 µl
3	Template DNA	2 µl
4	Nuclease free water	3.5 µl

Table 12: PCR mixture for gene CVD 432 by PCR for detection of EAEC

Process	Temperature	Time	Segment
Initial denaturation	94°C	5 min	Segment 1
denaturation	94 °C	45 s	Segment 2 (40 cycle)
Annealing	54 °C	30 s	
Extension	72 °C	45 s	
Final extension	72°C	10 min	Segment 3

Table 13: PCR condition for CVD 432 gene by multiplex PCR for detection of EIEC

3.4.1: Electrophoretic Analysis of PCR-Amplified DNA (serotyping)

A 100 mL, 1.5% agarose gel solution was prepared by measuring 1.5 grams of agarose into a flask. To this, 2 mL of 50x TAE buffer and 98 mL of distilled water were added. The mixture was then heated in a microwave until the agarose completely dissolved and the solution became clear. After cooling, 4 µL of ethidium bromide (EtBr) was added. The solution was poured into a gel tray, and a comb was placed near one end. The gel was left undisturbed at room temperature for 10 minutes to solidify evenly.

Once the gel solidified, the comb was carefully removed, and the gel tray was placed in an electrophoresis chamber, ensuring the gel was submerged in 1x TAE electrophoresis buffer. Following PCR, 4 µL of PCR products from each reaction was loaded onto the gel, along with a 100 bp ladder. The PCR products were separated in the 1.5% agarose gel using 1x TAE buffer at 100 V for 40 minutes. The gel was then visualized under UV light to detect the target gene.

3.5: Antibiotic susceptibility testing

The antibiotic susceptibility patterns of *Escherichia coli* isolates were evaluated, derived from 70 positive samples out of 92 tested. From each *E.coli*-positive sample, two isolates were examined for antibiotic susceptibility testing (AST) using the standard Kirby-Bauer disk diffusion method, performed according to Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. A total of 20 antibiotic discs representing 11 antibiotic groups were selected to assess the susceptibility of the isolates. Interpretation of antimicrobial susceptibility patterns

was made of 20 antibiotic agents. For antimicrobial susceptibility testing commercially available antibiotic disks were used (Thermo Scientific™ Oxoid™). Following antibiotic disks were used: Ampicilllin (10µg), Ceftriaxone (30 µg), Ciprofloxacin(5 µg), Chloramphenicol (30 µg), Trimethoprim-sulfamethoxazole(25 µg), Gentamycin(10 µg), Meropenem(10 µg), Nalidixic acid (30V µg), Tetracycline(30 µg), Norfloxacin(10 µg), Imipenem(10 µg), Kanamycin(30 µg), Erythromycin(15 µg), Azetreonam (30 µg), Ceftazidime(30 µg), Piperacillin-tazobactam(110 µg), Amoxcillin-Clavulanic acid (20 µg), Colistin (10 µg), Cefepime.

Figure 9: Antibiotic Susceptibility test by Kirby Bauer disk diffusion method

3.5.1: Procedure

The PCR-confirmed *E.coli* isolates were subculture onto nutrient agar plates and incubated overnight at 37°C to produce fresh bacterial growth. Loopfuls of pure bacterial colonies were suspended in 0.9% normal saline, and the turbidity of the suspension was adjusted to match the 0.5 McFarland standard. The prepared suspensions were evenly spread on Mueller-Hinton agar (MHA) plates using sterile cotton swabs to create bacterial lawns. Antibiotic discs were gently placed on the agar surface using sterile forceps to ensure proper diffusion w. The plates were labeled, stacked, and incubated at 37°C for 18–24 hours. The plates were then investigated and the diameter of clear zones due to growth inhibition was recorded in millimeter along with

6mm disc diameter. The antibiotics were tested at specific concentrations per disk [Table 14], and the critical values for each antibiotic are listed in [Table 14] Multidrug-resistant (MDR) strains were defined as those exhibiting resistance to at least one agent in three or more antibiotic categories(Basak et al., 2016).Extensively drug-resistant (XDR) strains were classified as isolates resistant to all but two or fewer antibiotics(Magiorakos et al., 2012).Commercially available antibiotic discs (Thermo Scientific™ Oxoid™) were used for testing. The detailed list of antibiotics, their respective groups, disc potencies, and interpretive criteria is presented in [Table 14]. This study provides insights into the susceptibility patterns of *E.coli* isolates and highlights their resistance levels to clinically significant antibiotics.

3.5.2 Interpretation

According to the information from the diameter of zone of inhibition for individual antibiotic agents, isolates were categorized as susceptible, intermediate or resistant as per CLSI guidelines [Table 14]

Groups	**Antibiotic Used**	**Sensitive**	**Interm ediate**	**Resistant**
Penicillin	Ampicillin	≥17	14-16	≤13
	Piperacillin-Tazobactam	≥21	18-20	≤17
	Amoxicillin-clavulanic acid	≥18	14-17	≤13
3G Cephalosporin	Ceftriaxone	≥23	20-22	≤19
	Ceftazidime	≥21	18-20	≤17
	Cefixime	≥19	16-18	≤15
4G Cephalosporin	Cefepime	≥25	19-24	≤18
Carbapenem	Imipenem	≥23	20-22	≤19
	Meropenem	≥23	20-22	≤19
Aminoglycoside	Kanamycin	≥18	14-17	≤13
	Gentamicin	≥15	13-14	≤12
Quinolone	Norfloxacin	≥17	13-16	≤12
	Nalidixic Acid	≥19	14-18	≤13
	Ciprofloxacin	≥31	21-30	≤20
Tetracycline	Tetracycline	≥15	12-14	≤11

Groups	Antibiotic Used	Sensitive	Intermediate	Resistant
Sulfonamide	Sulfamethoxazole-trimethoprim	≥16	11-15	≤10
Phenicol	Chloramphenicol	≥18	13-17	≤12
Monobactams	Aztreonam	≥21	18-20	≤17
Polymyxin	colistin	≥15	13-14	≤12
Macrolide	Erythromycin	≥21	14-20	≤13

Table 14: Zone of diameter interpretation for *E.coli*

3.5.3: PCR Amplification for Detecting Antibiotic resistant genes:

PCR analysis was conducted on *E.coli* PCR positive isolates to identify extended-spectrum beta-lactamase (ESBL) genes and carbapenem resistance genes. This molecular characterization aimed to classify *E.coli* isolates with multidrug resistance (MDR) and extensive drug resistance (XDR). The ESBL genes screened included *blaTEM*, *blaCTX*, and *blaSHV*. For carbapenem resistance, the genes *blaNDM*, *blaVIM*, and *blaKPC* were targeted.

Types of primers	Target Gene	Primer name	Sequence	Product Size	Reference
ESBL primers	Bla*TEM*	TEM-F	F-AAAATTCTTGAAGACG	1080bp	(Sharma et al., 2010)
		TEM-R	R- TTACCAATGCTTAATCA		
	Bla*CTX-M*	CTX-M-upper	F-ACGCTGTTGTTAGGAAGTG	759bp	(Altayb et al., 2020)
		CTX-M-lower	R-TTGAGGCTGGGTGAAGT		
	Bla*SHV*	SHV-F	F-TACCATGAGCGATAACAGCG	450bp	(Doosti et al., 2015)
		SHV-R	R-GATTTGCTGATTTCGCTCGG		
Carbapenam Resistant Gene primers	Bla*KPC*	KPC-F	F-CATTCAAGGGCTTTCTTGCTGC	498 bp	(Mushi et al., 2014)
		KPC-R	R-ACGACGGCATAGTCATTTGC		
	Bla*NDM*	NDM-F	F-GGTTTGGCGATCTGGTTTTC	624 bp	(Shoja et al., 2016a)
		NDM-R	R-CGGAATGGCTCATCACGATC		
	Bla*VIM*	VIM-F	F-GGTGTTTGGTCGCATATCGCAA	501bp	(Shoja et al., 2016b)
		VIM-R	R-ATTCAGCCAGATCGGCATCGGC		

Table 15: List of Primers used in ESBL gene detection

Process	Temperature	Time	Segment
Initial denaturation	95°C	3 min	Segment 1
denaturation	95 °C	30 s	Segment 2 (35 cycle)
Anneling	51 °C	30 s	
Extenision	78 °C	7 min	
Final extension	72°C	10 min	Segment 3

Table 16: PCR condition for *TEM* gene

Process	Temperature	Time	Segment
Initial denaturation	94°C	5 min	Segment 1
denaturation	94 °C	30 s	Segment 2 (35 cycle)
Annealing	58 °C	30 s	
Extension	72°C	30 s	
Final extension	72°C	7 min	Segment 3

Table 17: PCR condition for CTX-M gene

Process	Temperature	Time	Segment
Initial denaturation	94°C	5 min	Segment 1
denaturation	94 °C	30 s	Segment 2 (35 cycle)
Anneling	58 °C	30 s	
Extenision	72°C	30 s	
Final extension	72°C	7 min	Segment 3

Table 18: PCR condition for *SHV* gene

Process	Temperature	Time	Segment
Initial denaturation	95°C	5 min	Segment 1
denaturation	95°C	50 s	Segment 2 (35 cycle)
Anneling	57 °C	30 s	
Extenision	72°C	40 s	
Final extension	72°C	10 min	Segment 3

Table 19: PCR condition for *KPC* gene

Process	Temperature	Time	Segment
Initial denaturation	94°C	5 min	Segment 1
denaturation	94°C	30 s	Segment 2 (35 cycle)
Anneling	58 °C	30 s	
Extenision	72°C	30 s	
Final extension	72°C	7 min	Segment 3

Table 20: PCR condition for *NDM* gene

Process	Temperature	Time	Segment
Initial denaturation	95°C	5 min	Segment 1
denaturation	95°C	45 s	Segment 2 (35 cycle)
Annealing	60 °C	45 s	
Extension	72°C	1 min	
Final extension	72°C	10 min	Segment 3

Table 21: PCR condition for *VIM* gene

Chapter 4

Result

The study aimed to detect fecal coliforms in household water samples and isolate *Escherichia coli* (*E.coli*), a bacterium responsible for diarrhea and various extraintestinal infections such as urinary tract infections (UTIs) and meningitis when pathogenic. A total of 92 water samples were collected from areas in Dhaka North, including Uttara, Badda, Gulshan, Farmgate, Indira Road, Tejgaon, Mirpur, Bashundhara Residential Area, Nadda, Rampura, Aftabnagar, Banasree, Banani, Mohakhali, Bashabo, Mohammadpur, Malibagh, and Khilgaon. Molecular characterization was performed to determine the serotypes of the isolates based on their pathogenic traits.

4.1 Fecal coliform count in CFU/100ml

Following sample processing, 100 mL of water from each sample was filtered using membrane filtration with a 0.22 µm pore size filter. The filter paper was then placed on MFC agar plates and incubated at 44°C for 18-24 hours. After incubation, fecal coliforms were quantified and expressed in colony-forming units per milliliter (CFU/mL). Out of the 92 water samples tested,

72 (78.26%) [Figure:10] were found to be positive for fecal coliforms. Among the areas where water has been collected, most contamination by Fecal coliforms were found in Mohakhali and Nadda area.

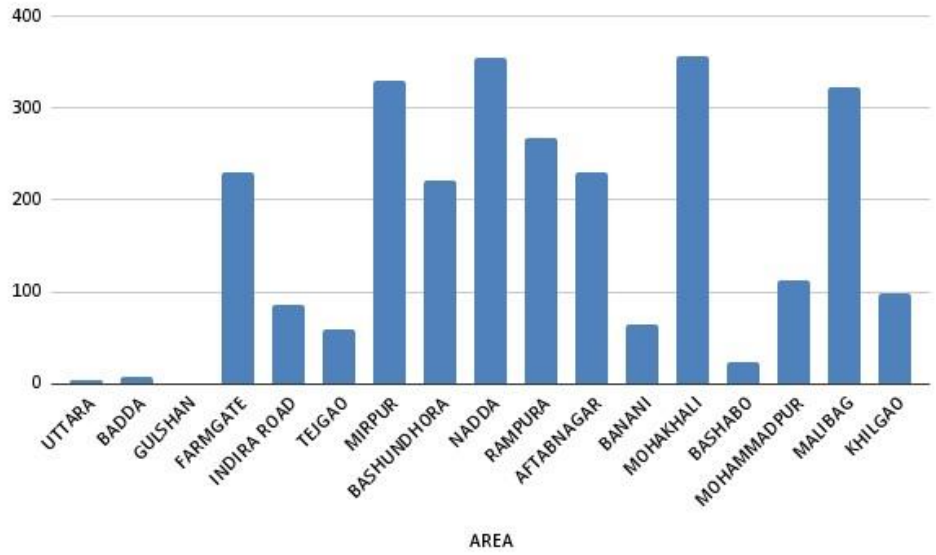

Figure 10: Prevalence of Fecal coliforms in areas of Dhaka North

The analysis of fecal coliform (FC) contamination in urban areas showed noticeable differences, with levels ranging from 0 CFU/100 ml in Gulshan to 356 CFU/100 ml in Malibagh. Areas like Gulshan and Badda met the WHO standard for safe water (0 CFU/100 ml), but others, such as Mirpur (335 CFU/100 ml), Mohakhali (328 CFU/100 ml), and Malibagh (356 CFU/100 ml), had contamination far above the safe limit. Mirpur also recorded the highest number of TNTC (Too Numerous to Count) cases (8 counts) [Table 22], showing significant health risks.

AREA	Median FC CFU/100ML	TNTC Count	Limit of contamination allowed by WHO
UTTARA	3.5	1	0 CFU/100ml
BADDA	8	0	
GULSHAN	0	0	
FARMGATE	230	3	
INDIRA ROAD	86	1	
TEJGAON	60	0	
MIRPUR	335	8	
BASHUNDHARA	221	2	
NADDA	314	1	
RAMPURA	256	2	
AFTABNAGAR	230	1	
AREA	**Median FC CFU/100ML**	**TNTC Count**	
BANANI	65	1	
MOHAKHALI	328	3	
BASHABO	24	1	
MOHAMMADPUR	112.5	3	
MALIBAG	356	3	
KHILGAON	98	2	

Table 22: Contamination Levels of Fecal Coliforms in Different Areas

4.2: Cultural detection of *E.coli*

Following sample processing, 100 mL of water from each sample was filtered using a membrane filter with a pore size of 0.22 µm. The filter paper was transferred into EE broth contained in Falcon tubes and incubated for 18–24 hours at 37°C for enrichment. After incubation, positive bacterial growth in EE broth was indicated by a color change to yellow. Subsequently, 100 µL of the enriched bacterial suspension was plated onto TBX agar medium using the spread plate method. The plates were incubated for 24 hours at 37°C, and the presence of *E.coli* was confirmed by the appearance of blue-green colonies on the TBX medium. Among the 92 samples tested, 70 (76%) were confirmed positive for *E.coli*[Table 23] based on this characteristic colony morphology. Further confirmation was carried out by culturing the isolates on UTI and MacConkey agar media, which also yielded positive results.

AREA	E Coli Positive Count	Total Area Count	Percentage Count
UTTARA	4	8	50
BADDA	3	6	50
GULSHAN	1	8	13
FARMGATE	5	5	100
INDIRA ROAD	3	3	100
TEJGAON	2	3	67
MIRPUR	8	10	80
BASHUNDHARA	6	6	100
NADDA	1	1	100
RAMPURA	4	4	100
AREA	**E Coli Positive Count**	**Total Area Count**	**Percentage Count**
AFTABNAGAR	2	3	67
BANANI	5	5	100
MOHAKHALI	5	5	100
BASHABO	6	5	120
MOHAMMADPUR	6	10	60
MALIBAG	3	3	100
KHILGAON	6	7	86
Total Count	70	92	76%

Table 23: Percentage of Areas Contaminated with *E.coli*

The analysis shows severe *E.coli* contamination, with 100% positive results in Farmgate, Indira Road, Bashundhara, Nadda, Rampura, Banani, Mohakhali, and Malibagh [Figure 11] High contamination was also found in Mirpur (80%) and Khilgaon (86%), while Gulshan (13%) had the lowest levels.

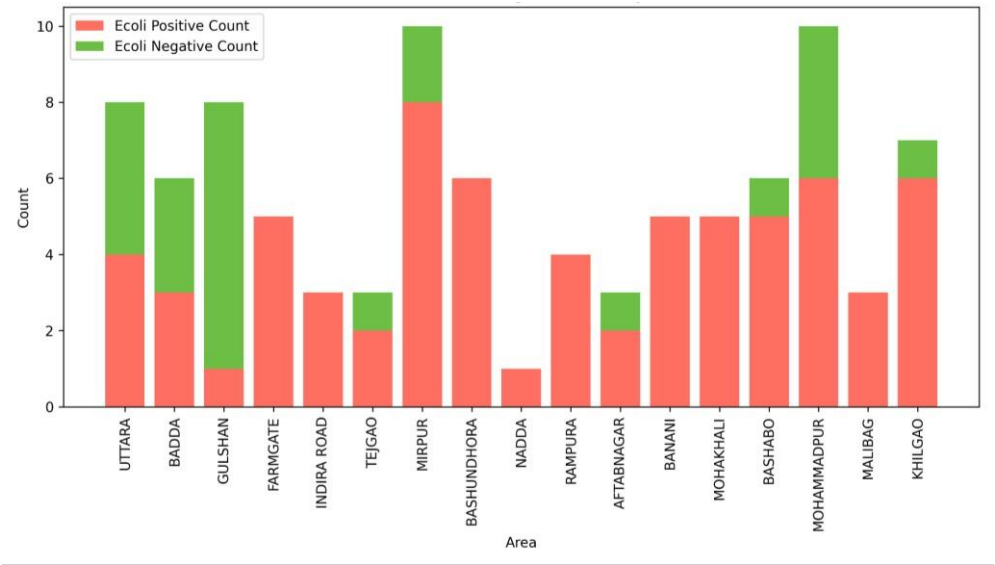

Figure 11: Prevalence of positive *E.coli* isolates in areas of Dhaka North

4.3: Molecular Detection of *E.coli*

The isolates derived from 70 samples, initially presumptive for *E.coli*, were cultured on TBX, UTI, and MacConkey media. All of these isolates, which tested positive on the respective media, were confirmed as *E.coli* through PCR analysis, yielding a 100% confirmation rate. PCR was conducted on the suspected *E.coli* isolates, yielding the desired bands. These bands were then visualized and confirmed using agarose gel electrophoresis, which determined the actual band size to be 585 bp. [Figure 12] Among all the presumptive 70 *E.coli* isolates showed positive results for molecular detection. This indicates that 70 of the presumptive *E.coli* isolates were conclusively identified as *E.coli* based on PCR results. [Figure 13]

Figure 12: Agarose gel electrophoresis of PCR assay of *E.coli* isolates. Here, Lane M is 100 bp DNA marker, and Lane (1-11) are some positive samples at 585 bp.

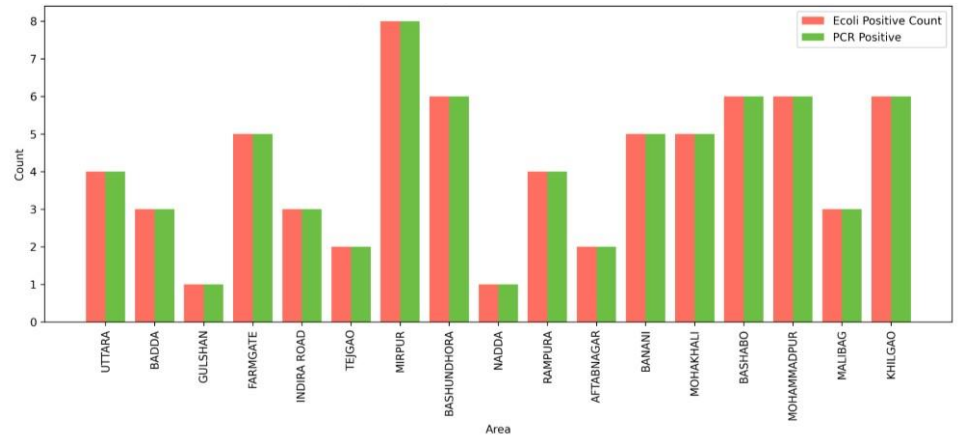

Figure 13: Comparison of Culturally Positive and Molecularly Detected *E.coli*

4.4 Confirmation of pathogenic isolates

Among 70 confirmed *E.coli* isolates tested for pathogenicity, molecular characterization identified varying levels of positivity for genes associated with pathogenic *E.coli* strains. Overall, 20% of the isolates were pathogenic. Notably, 53.33% of the pathogenic isolates were positive for the rbfE gene, a marker for *E.coli* O157:H7, while none tested positive for the fliC H7 gene. Furthermore, 46.67% of the pathogenic isolates were identified as Shiga toxinproducing *E.coli* (STEC), with a higher prevalence of the stx2 gene compared to the stx1 gene.

Interestingly, all isolates were negative for the CVD432 gene, specific to enteroaggregative *E.coli* (EAEC), and no isolates tested positive for markers of Enteroinvasive *E.coli* (EIEC).

4.4.1 Confirmation of rbfE, flicH7 GENE (*E.coli* O157:H7)

E.coli isolates confirmed for the presence of the rbfE gene exhibited a distinct band at 497 bp under UV light following gel electrophoresis. However, no bands were observed at 625 bp, corresponding to the fliC H7 gene.

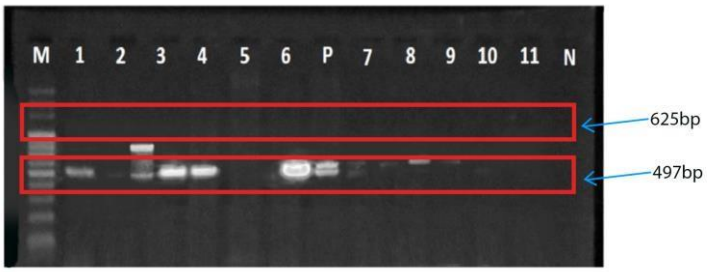

Figure 14: Agarose gel electrophoresis of PCR assay of rbfE gene and flicH7 gene. Here M lane is 100 bp DNA marker, and lane(1-11) are some positive samples band at 497bp no positive samples observed in 625bp.

4.4.2: Confirmation of STX1, STX2 gene (STEC):

E.coli isolates confirmed for the presence of the stx1 gene exhibited a distinct band at 180 bp and stx2 at 255 bp under UV light following gel electrophoresis.

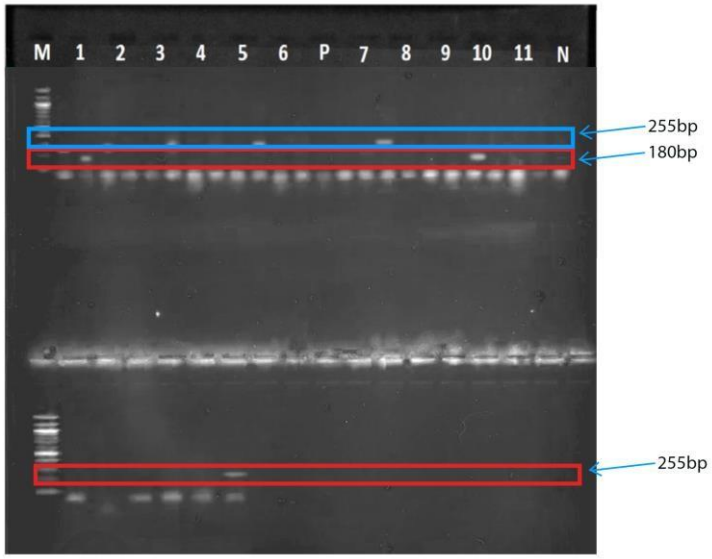

Figure 15: Agarose gel electrophoresis of PCR assay of STX1 gene and STX2 gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some positive samples band at 255bp and 180 bp.

4.4.3: Confirmation of IpaH GENE (EIEC)

No isolates showed a band at 423bp, which is the band size for Ipah gen, indicating negative result.

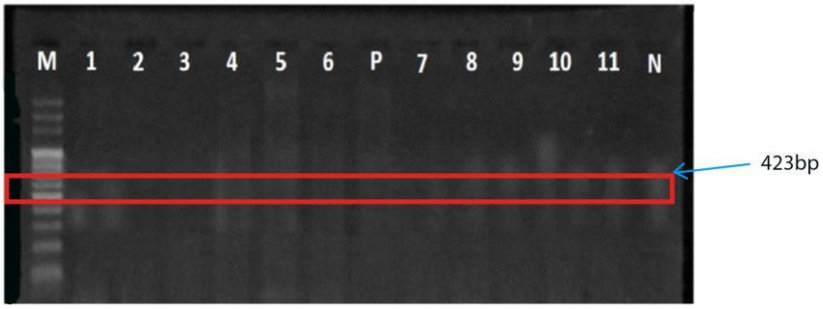

Figure 16: Agarose gel electrophoresis of PCR assay of IpaH gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some samples that did not show band at 423 bp.

4.4.4: Confirmation of CVD 432 GENE (EAEC)

No isolates showed a band at 630 bp, which is the band size for CVD 432 gene indicating negative result.

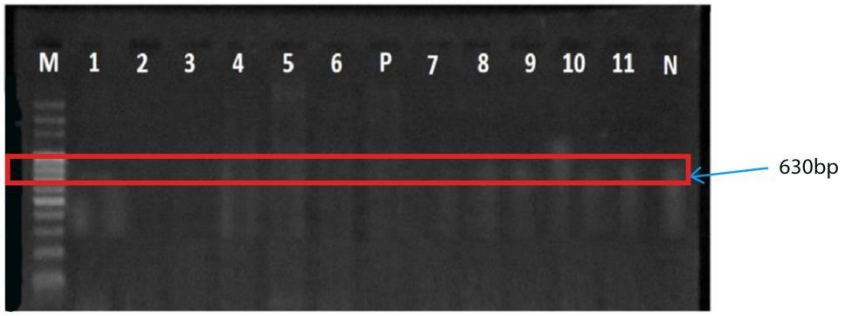

Figure 17: Agarose gel electrophoresis of PCR assay of CVD 432 gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some samples that did not show band at 630 bp.

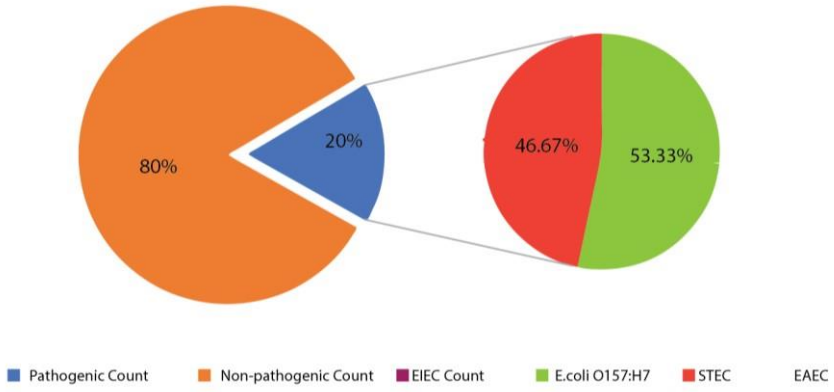

Figure 18: Distribution of diarrheagenic pathogens and different *E.coli* pathotype

4.5 Antibiotic susceptibility pattern of isolates

The analysis of antibiotic resistance data highlights significant patterns. Erythromycin and Ampicillin show complete resistance (100%), indicating their ineffectiveness against the isolates. Colistin exhibits alarmingly high resistance at 87%, despite being a last-resort antibiotic. Tetracycline also shows substantial resistance at 68%, reducing its therapeutic value.

Moderate resistance was observed for Ciprofloxacin (58%) and TrimethoprimSulfamethoxazole (52%). Resistance among 3rd and 4th generation cephalosporins remains low, with Ceftriaxone and Cefepime showing only 3% resistance. Similarly, Meropenem, a carbapenem, shows just 3% resistance, emphasizing its continued efficacy. [Table 24], [Figure 19]

Type of antibiotic	R Count	S Count	I Count	S%	I%	R%
AMPICILLIN	31	0	0	0	0	100
Ceftriaxone	1	25	5	81	16	3
Ciprofloxacin	18	0	13	0	42	58
Chloramphenicol	11	18	2	58	6	35
Trimethoprim-Sulfamethoxazole	16	13	2	42	6	52
Gentamicin	4	17	10	55	32	13
Meropenem	1	28	2	90	6	3
Nalidixic Acid	15	9	7	29	23	48
Tetracycline	21	7	3	23	10	68
Norfloxacin	11	19	1	61	3	35
Imipenem	5	18	8	58	26	16
Kanamycin	7	8	16	26	52	23
Erythromycin	31	0	0	0	0	100
Cefixime	5	17	9	55	29	16
Aztreonam	7	19	5	61	16	23
Ceftazidime	2	26	3	84	10	6
Piperacillin-Tazobactam	0	27	4	87	13	0
AMC Amoxicillin-Clavulanic Acid	11	14	6	45	19	35
Cefepime	1	28	2	90	6	3
Colistin	27	0	4	0	13	87

Table 24: Types of Antibiotic used in this study

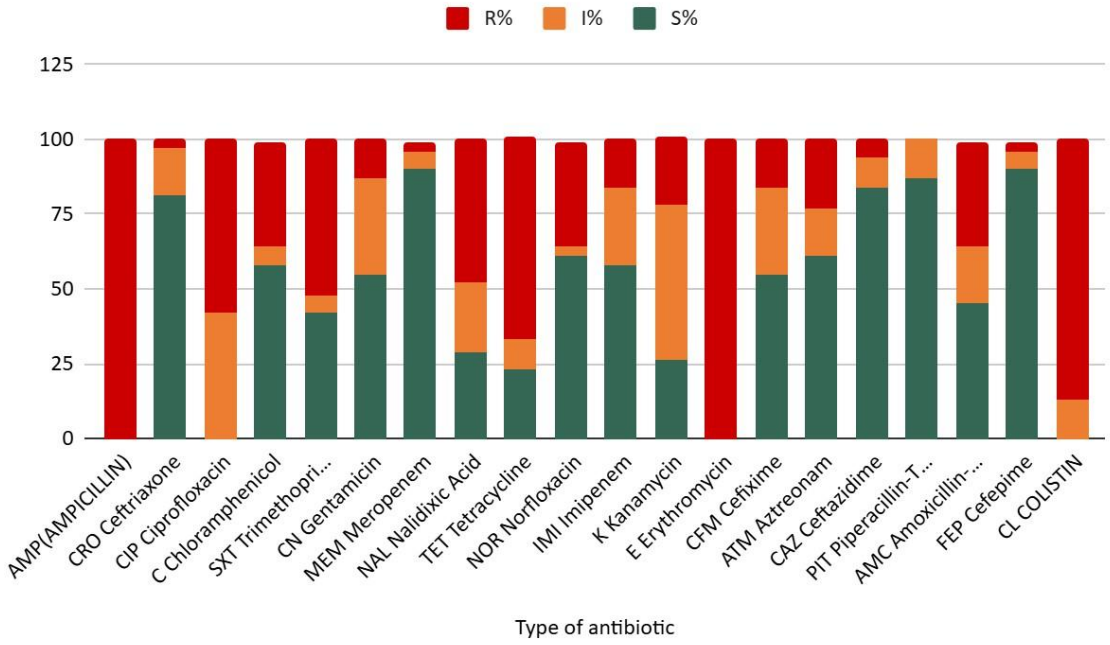

Figure 19: Antibiotic resistant patterns of *E.coli*.

The group-wise antibiotic resistance analysis reveals several important trends. In the Penicillin group, Ampicillin showed complete resistance at 100%, while Piperacillin-Tazobactam had no resistance (0%) and Amoxicillin-Clavulanic Acid exhibited moderate resistance at 35%. The 3rd Generation Cephalosporins demonstrated low resistance, with Ceftriaxone (3%), Ceftazidime (6%), and Cefixime (16%) remaining largely effective. 4th Generation Cephalosporins like Cefepime showed minimal resistance at 3%. In the Carbapenem group, Meropenem (3%) and Imipenem (16%) had low resistance, indicating their continued efficacy. The Aminoglycosides Kanamycin and Gentamicin showed moderate resistance, with values of 23% and 13%, respectively. In the Quinolone group, Norfloxacin (35%), Nalidixic Acid (48%), and Ciprofloxacin (58%) displayed varying levels of resistance, indicating a decline in their effectiveness. Overall, the data highlights significant resistance against some antibiotics, particularly Ampicillin and Ciprofloxacin, while others like Cefepime, Meropenem, and Piperacillin-Tazobactam remain largely effective. [Figure 20]

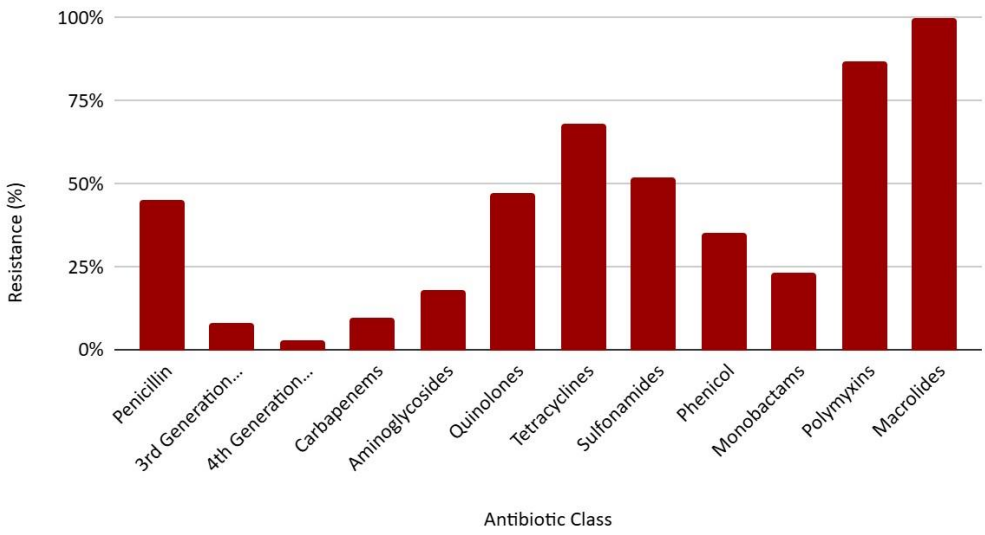

Figure 20: Percent of pathogenic *E.coli* isolates resistant to number of antibiotic classes.

100% of strains are classified as MDR for macrolides, penicillins, aminoglycosides, quinolones, tetracyclines, sulfonamides, phenicol, monobactams, and polymyxins. In contrast, third-generation and fourth-generation cephalosporins and carbapenems show no MDR strains. Furthermore, 100% of strains are XDR for macrolides and penicillin. Total 34.2% isolates were identified as MDR and 5.8% isolates were classified as XDR. [Figure 21]

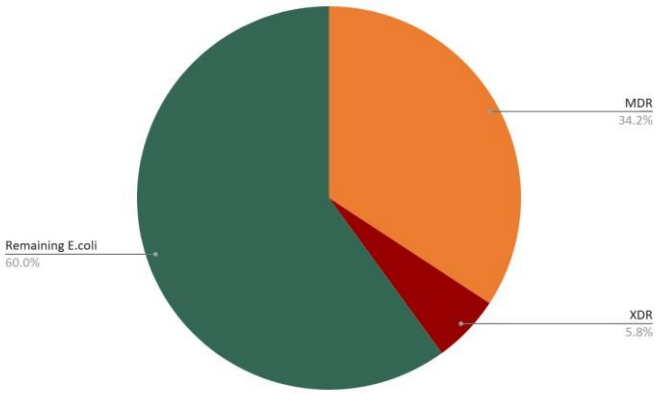

Figure 21: Percentage of XDR and MDR in contrast to total Isolates.

4.6 Confirmation of antibiotic resistant genes

4.6.1 ESBL

The results show that the blaCTX-M gene was present in the majority of isolates (54.29%), followed by blaTEM (30%) and blaSHV (28.57%). This indicates that the blaCTX-M gene is the most common among the isolates, suggesting a higher prevalence of CTX-M-type betalactamase-producing bacteria in the sample. [Figure 22]

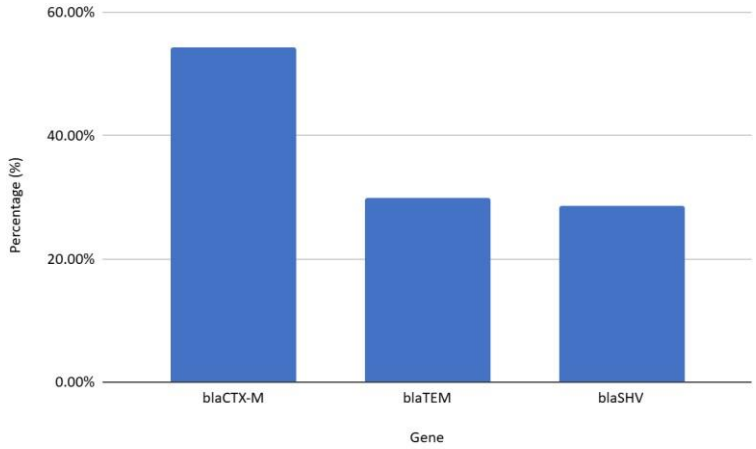

Figure 22: Distribution of ESBL genes in isolates

4.6.1.1 SHV

E.coli isolates confirmed for the presence of the blaSHV 450 bp gene exhibited a distinct band at bp under UV light following gel electrophoresis.

Figure 23: Agarose gel electrophoresis of PCR assay of blaSHV gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some positive samples band at 450 bp.

4.6.1.2 TEM

E.coli isolates confirmed for the presence of the blaTEM gene exhibited a distinct band at 1100 bp under UV light following gel electrophoresis.

Figure 24: Agarose gel electrophoresis of PCR assay of TEM gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some positive samples band at 1100 bp.

4.6.1.3 CTX-M

E.coli isolates confirmed for the presence of the blaCTX-M gene exhibited a distinct band at 759 bp under UV light following gel electrophoresis.

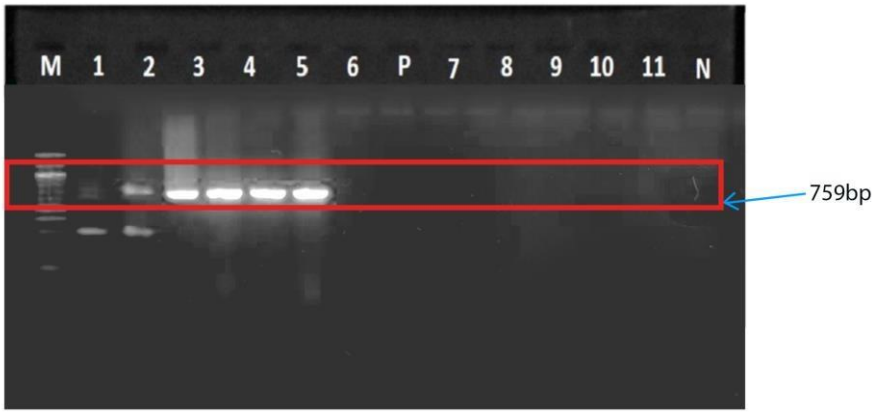

Figure 25: Agarose gel electrophoresis of PCR assay of CTX-M gene. Here M lane is 100 bp DNA marker, and lane (1-16) are some positive samples band at 759 bp.

4.6.2 Carbapenem resistant gene

The study of the genetic presence of KPC, VIM, and NDM genes in the isolates indicated no isolates contained the blaKPC gene, but 19.12% carried blaVIM. The blaNDM gene has been identified in 10.29% of the isolates. These data show that the VIM and NDM genes are more frequent among the isolates than KPC. [Figure 26]

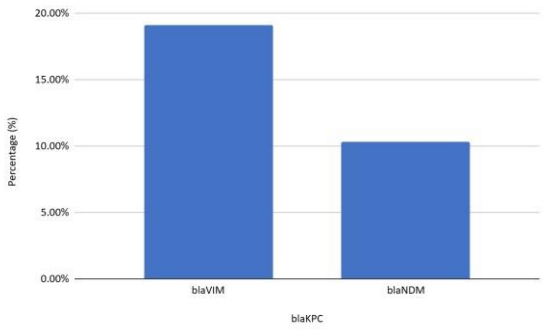

Figure 26: Distribution of carbapenem resistant genes in isolates

4.6.2.1 KPC

No isolates showed a band at 498 bp, which is the band size for blaKPC gene indicating negative result.

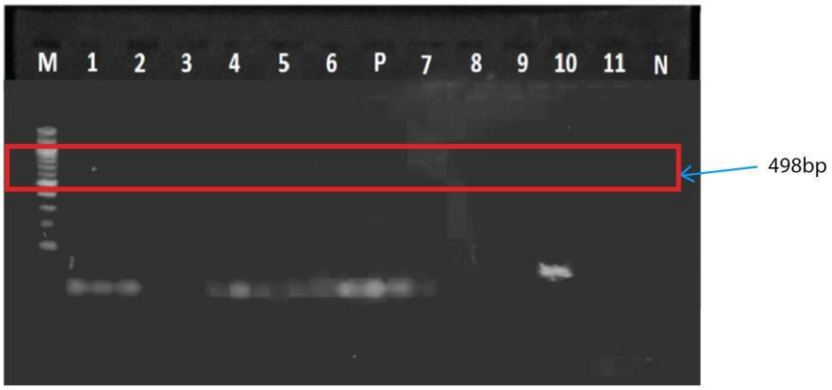

Figure 27: Agarose gel electrophoresis of PCR assay of blaKPC gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some samples that did not show band at 498 bp.

4.6.2.2 VIM

E.coli isolates confirmed for the presence of the blaVIM gene exhibited a distinct band at 501 bp under UV light following gel electrophoresis.

Figure 28: Agarose gel electrophoresis of PCR assay of blaVIM gene. Here M lane is 100 bp DNA marker, and lane (1-11) are some positive samples band at 501 bp.

4.6.2.3 NDM

E.coli isolates confirmed for the presence of the blaNDM gene exhibited a distinct band at 621 bp under UV light following gel electrophoresis.

Figure 29: Agarose gel electrophoresis of PCR assay of blaNDM gene.

Here M lane is 100 bp DNA marker, and lane (1-11) are some positive samples band at 621 bp.

Target gene	Antibiotic resistant gene	Percentage of resistance isolates in *E.coli*
ESBL	blaCTX-M	54.29%
	blaSHV	28.57%
	blaTEM	30%
Carbapenem	blaKPC	0
	blaVIM	19.12%
	blaNDM	10.29%

Table 25: Percentage of ESBL and Carbapenem resistant genes

Chapter 5

Discussion

Water is necessary for survival, yet many people lack access to safe drinking water, leading to bacterial diseases and death. Water is a good vehicle to transmit diseases, and most bacterial diseases like cholera, typhoid and bacterial dysentery spread through water. In developing countries like Bangladesh two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases(Fenwick, 2006).According to the World Health Organization (WHO), over 5 million people die annually from waterborne diseases. More than half of these deaths are due to microbial intestinal infections, with cholera being the leading cause. Furthermore, pathogenic *E.coli* strains namely enterotoxigenic (ETEC, namely O148), enterohemorrhagic (EHEC, namely O157) and enteroinvasive serotypes (EIEC, namely O124) can be transmitted through contaminated water(Olaolu et al., 2014).Furthermore, In recent years, there has been a reported increase in antimicrobial resistance among enteropathogens, including *E.coli*, which at times results in

situations where no effective antibiotic treatments are available(Pitout & Laupland, 2008a).This is particularly concerning as the spread of ESBL and carbapenemases (CARBase) is leading to resistance against vital β-lactam antibiotics. With the high levels of *E.coli* contamination in surface water, most *E.coli*-related diseases are waterborne. This study examined antibiotic resistance, pathogenic types, ESBL phenotypes, and the presence of key ESBL genes in samples collected from household water supplies in Dhaka city. The findings also revealed fecal contamination in household water, which serves as an indicator of water quality and potential health risks, suggesting the possible presence of harmful pathogens such as viruses, parasites, or other bacteria. In this study, it was observed that approximately 78.26% of the water samples showed high levels of fecal coliform contamination (>100 CFU/ml), and *E.coli* was detected in 76% of the samples. Similar results were also observed in another study held in Dhaka, Bangladesh, where they focused on the south part of Dhaka city with densely populated areas where low income people live(Talukdar et al., 2013a).However, another study relevant to this held in Mysensign, Sherpur and Gazipur , where the among 20 water sample only 2 sample (10%) was contaminated with fecal coliform indicating that the water quality of Dhaka City is worse than Mysensingn and adjunct areas(Hassan et al., 2018). In this study we also identified 76% of the samples are contaminated with *E.coli* indicating the presence of microorganisms in the water supply, which could pose a potential risk to human health, also signals fecal contamination. This becomes a significant concern when these *E.coli* strains display resistance to multiple antibiotics and possess pathogenic traits that can lead to enteric diseases in individuals who consume the contaminated water. Another study found that high levels of *E.coli* contamination in household drinking water are particularly prevalent in districts such as Dhaka, Bandarban, Netrokona, and Sunamganj, where more than 70% of household water samples exhibit high concentrations(Khan & Bakar, 2020).This finding aligns with the results of the present study. Another study held in Bangladesh on this topic, *E.coli* was detected in 100% of the samples tested from the entry points to users' homes in both areas, highlighting

a critical issue of unsafe drinking water(Kormoker et al., 2017).These studies indicate high prevalence of *E.coli* in water supply with densely populated areas where low income people live(Talukdar et al., 2013b).This becomes a significant concern when these *E.coli* strains display resistance to multiple antibiotics and possess pathogenic traits that can lead to enteric diseases in individuals who consume the contaminated water. Another study found that high levels of *E.coli* contamination in household drinking water are particularly prevalent in districts such as Dhaka, Bandarban, Netrokona, and Sunamganj, where more than 70% of household water samples exhibit high concentrations(Khan & Bakar, 2020).This finding aligns with the results of the present study. In this study we have found 20% pathogenic isolates of *E.coli* by detecting pathogenic genes, where most of the pathogenic *E.coli* showed positive for having rbfE gene indicating *E.coli* O157:H7 bacteria, following STEC serotype. However, no isolates were found to be positive for EIEC and EAEC. Among the pathogenic isolates, 53% was probable *E.coli* O157:H7, and the remaining 46.67% was STEC.The findings of pathogenic isolates in this study are similar to those of another study on the municipal water supply of South Dhaka, where 7% of the isolates tested positive for pathogenic bacteria(Talukdar et al., 2013b).Another one relevant study mentioned that out of 357 isolates from stored drinking water, six (1.7%) were pathogenic, with enteropathogenic *E.coli* (EPEC) being the most prevalent at 1.1%. In contrast, no pathogenic *E.coli* was found in sanitary wastewater samples, despite the presence of EPEC in the drinking water consumed by the community. (Talukdar et al., 2013b) Which also validates this study that pathogenic strains of *E.coli* tend to be found in the water supply system of Bangladesh. Furthermore, a study conducted in Bangladesh also shows similar results, where 32% (34/107) of the samples tested positive for ETEC. Among these, 65% produced only ST, 9% produced only LT, and 26% produced both ST and LT(Begum et al., 2005). Also**,** this aligns with the study conducted in urban Bangladesh, which found that 31% (73 out of 233) of all samples, including rectal swabs and household samples, tested positive for one or more diarrheagenic *E.coli* virulence factors(Hossain et al., 2021).A

recent study found that ETEC forms biofilms in household drinking water, which can be present throughout the year, with higher levels during the summer and rainy season(Ahmed et al., 2013). Currently, there is no vaccine for *E.coli* diarrhea, and treatments, including antibiotics, are not very effective due to the rise of multidrug resistant (MDR) strains. Multiple ways of exposure likely contribute to the spread of MDR *E.coli* to humans, with household water supply being a major factor due to its high contamination and easy access for people. In this study, all *E.coli* isolates exhibited 100% resistance to ampicillin (from the penicillin group) and erythromycin (from the macrolide group). Additionally, 68% of the isolates were resistant to tetracycline, 58% to ciprofloxacin, and 52% to trimethoprim-sulfamethoxazole (SXT). Resistance was also observed in 3% of the isolates towards the 3rd generation cephalosporin ceftriaxone and the 4th generation cephalosporin cefepime. Among the carbapenems, 16% showed resistance to imipenem, and 3% were resistant to meropenem. The presence of resistance to 3rd and 4th generation cephalosporins and carbapenems further complicates treatment options, signaling the spread of multidrug-resistant bacteria. Since household water is a key transmission route, this highlights a serious public health risk, particularly for vulnerable populations, and calls for urgent measures to improve water quality and control antibiotic use. In this study, it is observed that 38.7% isolates were multidrug resistant (MDR), and 6.5% were extremely drug resistant (XDR). Similar results showed in similar type of study held in Dhaka south, where the percentage of MDR was 49%(Talukdar et al., 2013a).This study shows that the resistance frequencies among *E.coli* isolates are concerning, with approximately 58.1% exhibiting resistance to beta-lactams, 9.7% showing resistance to carbapenems, and 41.9% resistant to quinolones. Similarly to the findings of this study, other research has reported a higher prevalence of resistance to β-lactam, quinolone, and fluoroquinolone antibiotics. (Coleman et al., 2012)showed all multidrug-resistant ESBL-producing *E.coli* strains were resistant to ceftriaxone, cefotaxime, ceftazidime, ampicillin, and aztreonam, which aligns with this study as well as another research(Vaiyapuri et al., 2021).In this study, 54% of

the isolates were found to carry the CTX-M gene, 30% carried the TEM gene, and 28.57% carried the SHV gene, all of which are representative of ESBL (Extended Spectrum BetaLactamase) genes. Among these, the CTX-M gene was the most prevalent. For carbapenem resistance genes, 12% of the isolates tested positive for the VIM gene, 1.47% for the NDM gene, and none for the KPC gene. This finding perfectly correlates with another similar study showing CTX-M1 in most *E.coli* isolates(Talukdar et al., 2013b).The occurrence of CTX-M type β-lactamases in Enterobacteriaceae is on the rise, and in certain regions, they have become more common than TEM and SHV types(Falagas & Karageorgopoulos, 2009).In addition another study also showed a high amount of CTX-M-15 gene among ESBLs in *E.coli*(Falagas & Karageorgopoulos, 2009).A recent study on drinking water in New Delhi reported the presence of multiple classes of β-lactamases, including NDM-1. This finding is consistent with the results of this study, where NDM-1 was detected in 1.47% of the total *E.coli* isolates from household water samples(Walsh et al., 2011).Enterobacteriaceae containing NDM-1 gene were also found from the clinical samples in Bangladesh(Islam et al., 2012).A study conducted in Sudan, titled "Detection of Carbapenem-Resistant Genes in *Escherichia coli* Isolated from Drinking Water in Khartoum, Sudan," revealed that 2 out of 90 *E.coli* isolates (approximately 2.2%) were found to harbor the blaVIM gene. This finding aligns with the results of the current study. Additionally, in the study "Diversity of Carbapenemases in Clinical Isolates: The Emergence of blaVIM-5 in Bangladesh," it was reported that 11.42% of *E.coli* isolates were found to carry the blaVIM gene. Specifically, 4 out of 35 *E.coli* strains tested were found to harbor the blaVIM gene, indicating a notable presence of this resistance gene among the clinical isolates examined. These findings highlight concerns regarding the prevalence of metallo-beta-lactamase-producing bacteria in the region and emphasize the need for continuous monitoring and effective infection control measures(Rakhi et al., 2019).Interestingly unlike this study most of study held in Bangladesh, *E.coli* tend to have kpc

gene , one study titled "Detection of Extended Spectrum β-lactamase (ESBL) And Carbapenemase Encoding *Escherichia coli* Isolates from Hospital Effluent Wastewater and Hospital Adjacent Community Tap Water in Dhaka Metropolitan City," it was reported that 6.7% of the *E.coli* isolates were found to carry the blaKPC gene. Specifically, out of the total isolates examined, 3 out of 45 *E.coli* strains were positive for the KPC gene(Tishan et al., 2023). In summary, this study highlights the serious public health problem of antibiotic-resistant *Escherichia coli* in Bangladesh, especially in water sources. The high level of fecal contamination and detection of harmful strains pose a significant risk of waterborne diseases, particularly for vulnerable groups. With 76% of water samples contaminated and many strains showing multidrug resistance, including ESBL and carbapenemase genes like blaCTX-M, blaVIM, and blaKPC, there is an urgent need to improve water quality and strengthen infection control. The increasing resistance to antibiotics makes treating these infections harder, stressing the importance of better monitoring, sanitation, and collaborative efforts to reduce the dangers of contaminated water and antimicrobial resistance(Olsen et al., 2002)**,**(Pitout & Laupland, 2008b)**.**

Chapter 6

Limitations, prospects and conclusion:

6.1 Limitation and prospects:

This study has several limitations that should be addressed to enhance its findings and implications. Firstly, the relatively small sample size limits the generalizability of the results. Increasing the number of samples could provide more comprehensive insights into household water contamination. Additionally, the study did not cover the entire Dhaka City, particularly Dhaka South, which leaves gaps in understanding the full scope of contamination. Expanding the geographic coverage and sample size would yield more representative and accurate findings.

Another limitation is the absence of a seasonal variation analysis, which is critical when studying environmental samples like water quality. Seasonal fluctuations can significantly influence contamination levels, and analyzing these variations would offer valuable insights. Future research should extend sample collection to additional regions within Dhaka City and other districts across Bangladesh, incorporating seasonal variation assessments for a more holistic understanding of water quality issues.

The study also did not cover all pathogenic *E.coli* strains, and key methods like immunological assays and agglutination tests were not performed. While PCR for specific genes is a highly effective tool for detecting *E.coli* O157:H7 and assessing its pathogenic potential, it is not sufficient on its own. Comprehensive detection requires integrating PCR with culture-based methods, toxin assays, serotyping, or even wholegenome sequencing (WGS) for precise

identification of *E.coli* serotypes. This approach ensures no serotypes or pathogenic variants are overlooked, providing a clearer understanding of their health risks.

Furthermore, the study did not screen for all extended-spectrum beta-lactamase (ESBL) genes or carbapenem-resistant genes. Including a broader spectrum of antibiotic resistance gene screening would offer deeper insights into the genetic makeup of the contaminants and their resistance profiles. Addressing these limitations in future research will provide a more detailed and accurate picture of household water contamination and its associated health risks.

6.2 Conclusion

In conclusion, the research highlights the significant public health risk of antibiotic resistant *Escherichia coli* in Bangladesh, particularly in household water supplies. The high levels of faecal contamination, with 76% of water samples positive for *E.coli*, represent a major threat of waterborne infections, particularly among vulnerable populations. Notably, 20% of isolates were pathogenic, and a significant percentage tested positive for the rbfE gene, indicating the presence of potential *E.coli* O157:H7. The existence of multidrug-resistant (MDR) strains, especially those containing extended-spectrum beta-lactamase (ESBL) and carbapenemase genes like blaCTXM, blaVIM, and blaKPC, complicates treatment choices and casts doubt on the efficacy of existing treatments. With 34.2% of isolates classified as MDR and 5.8% as very drug-resistant (XDR), the findings highlight the urgent need for enhanced water quality management and infection control methods to minimize the dangers associated with contaminated drinking water. The study's shortcomings, such as its limited sample size and lack of seasonal fluctuation analysis, highlight the need for more research to better understand water quality concerns in Bangladesh. Addressing these issues via improved monitoring,

sanitation, and public health programs is critical to preventing antibiotic resistance and providing safe drinking water to all.

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