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

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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
Bsc. in Microbiology

Department of Mathematics and Natural Sciences
Brac University
September 2025

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing a 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. We 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 in order to assess microbial contamination and antibiotic resistance. Ethical approval for the study has been received from the relevant Institutional Review Board of Brac University. The study did not include human participants, personal data or direct contact with individuals. Water samples were accumulated from publicly accessible taps or with proper and informed consent of the household owners or authorities. The aim and purpose of the study was clearly elaborated to the contributors and none of their identifiable information was documented. All procedures were conducted in accordance with ethical guidelines and relevant regulations to ensure integrity, safety, and respect of the providers involved.

The findings of this research are solely with the intention of usage in public health betterment, with the conclusive goal to promote safe practices related to drinking water and spreading policy recommendations.

Abstract/ Executive Summary

Antimicrobial resistance (AMR) is an alarming increasing trend at present leading to a range of health complications. This study focused on the analysis of municipal household water in the south and north of Dhaka, Bangladesh, with conclusive results of 81.16% fecal coliform contamination in the samples and confirmed 53.33% of *Escherichia coli* presence. According to the molecular evaluation, the most pervasive ESBL gene was found to be *SHV* at 32.35% and in terms of carbapenem resistance, both *NDM* and *KPC* had similar trends at roughly 3%. Upon screening the isolates, 49% of them were revealed to be multidrug resistant and 21% portrayed extensive drug resistance, leading to complexity of therapeutic choices. The results suggest the urgency of improvements for the water treatment system in Dhaka as well as standard monitoring regularly in order to mitigate the prevalence and consequences of antibiotic resistance rooting from mainstream water supply in the urban areas.

Keywords: antimicrobial resistance; *Escherichia coli*; fecal contamination; extended spectrum beta-lactamase; carbapenem resistance; public health

Acknowledgement

We extend our gratitude to our supervisor, Dr. Fahim Kabir Mojurul Haque for his invaluable guidance, insightful feedback and consistent support throughout the research. From the beginning, he has not only been our academic parent but has always reminded us to take life lightly and enjoy the process of living it, even while pursuing a degree. We would also like to mention Sumaiya Sadia Momtaz, our respected senior who has taught us every single piece of hands-on knowledge we have learnt throughout the journey and without whom, we would still remain lost. We are also immensely thankful to our Research Assistant, to whom we could always run to in every minor inconvenience in the lab and the one who tolerated all of our silly mistakes with great patience. Moreover, we would like to acknowledge the AMR lab of BRAC University for providing the resources and necessary surroundings required for the study. Lastly, special thanks to my best friend, Shahazad Ruhul Rafid for walking every step of the way alongside me, from the first class to every lab experiment and to Sadia Chaity, our peer in this work who never failed to show up when needed.

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List of Acronyms

AMR	Antimicrobial Resistance
ESBL	Extended-Spectrum Beta-Lactamase
ETEC	Enterotoxigenic *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
CDC	Center for Disease Control and Prevention
GLASS	Global Antimicrobial Resistance and Use Surveillance System
UTI	Urinary Tract Infection
MNEC	Meningitis-associated *Escherichia coli*
ExPEC	Extraintestinal Pathogenic *Escherichia coli*
WHO	World Health Organization
PCR	Polymerase Chain Reaction
CFU	Colony Forming Unit
MDR	Multidrug Resistance
XDR	Extended-drug Resistance

Chapter 1

Introduction

1.1 Background

Antimicrobial resistance (AMR) has emerged as a defining health crisis of the 21st century, with *Escherichia coli* and other bacterial pathogens standing at the forefront of this global battle. 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 most recent Global Antimicrobial Resistance and Use Surveillance System (GLASS) reporting via World Health Statistics 2024, approximately 42 % of *E. coli* bloodstream isolates exhibit resistance to third-generation cephalosporins, underlining the persistent high resistance rates among common infections (same source n.d.). More than 50 % of individuals with UTIs in certain localities are no longer responsive to treatment due to *E. coli* resistance to fluoroquinolone antibiotics (Antimicrobial Resistance, n.d.). According to CDC findings, about 2.8 million antimicrobial-resistant infections occur each year in the United States, resulting in over 35,000 deaths (CDC, 2024). Projections still estimate that AMR could cause up to 1.9 million direct deaths annually by 2050, with associated deaths reaching 8.22 million per year, and the potential for yearly GDP losses 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 overwhelmingly replaced previous ESBL types like TEM and SHV. CTX-M-15 remains the dominant variant in both clinical and environmental isolates globally, reflecting the ongoing pan-regional pattern (Ribeiro et al., 2024). The TEM and SHV families continue to contribute to resistance in environmental *E. coli*, albeit at much lower levels than CTX-M. For instance, bla_TEM genes are still detected in various environmental samples—especially in settings with intensive agricultural and aquaculture antibiotic use—but represent <10 % of total ESBL prevalence (Ribeiro et al., 2024). The prevalence of specific ESBL genes shows marked regional variation: for example, studies in South Asia—particularly Bangladesh and India—report CTX-M-15 and CTX-M-1 in ~90 % of ESBL-positive environmental and clinical isolates (Bagaya et al., 2023). Environmental reservoirs, including wastewater treatment plants, urban surface water bodies, and agricultural runoff, have been repeatedly identified as significant hotspots for the spread of ESBL-producing *E. coli* (Bagaya et al., 2023; Ribeiro et al., 2024). A comprehensive analysis indicated that the pooled prevalence of ESBL‑producing *E. coli* from environmental sources was around 28.3% (95% CI = 13.5 to 50.1%) in broiler‑farm-related environmental samples. This is lower than earlier reports of ~39%, but still indicates a considerable risk of transmission to people via contaminated water supplies or food products. *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 remains a growing concern as it is one of the most significant challenges to treating infectious diseases. In Bangladesh, more recent data suggest that enterotoxigenic *E. coli* (ETEC) is still a major contributor to diarrheal infections: in 2022, stool surveillance at icddr,b found that about 12% of diarrheal cases were ETEC, second to *Vibrio cholerae* O1 at ~20%. Several studies have continued to report the isolation of pathogenic as well as resistant *E. coli* from environmental samples, which can participate

in horizontal gene transfer of resistance genes via plasmids and lead to outbreaks. For example, a recent study in urban surface waters (Goranchatbari, Dhaka) found that out of 266 *E. coli* isolates, 62 (≈ 23.3%) were phenotypically positive for ESBL, with nearly all (93.6%) carrying at least one ESBL gene (CTX-M, TEM, SHV, or OXA). 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. 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. For example, in Dhaka, hospital wastewater studies show >90% of *Pseudomonas aeruginosa* isolates from hospital effluents are multidrug resistant and many also form strong biofilms, with integron-associated genes present. ([University of Dhaka, 2024]) Several studies also show that residues of antibiotics in effluents co-select for resistance. 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 *fliC_H7*, which encodes the H7 flagellar antigen, and *rfbE_O157*, 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 a recent study found *E. coli* O157:H7 in all sampled rivers, with counts up to 4.2×10^5 cfu/100 mL, and persistence lasting up to two months in surface waters at ambient temperatures (≈ 15-25 °C) (from river water surveillance). outbreaks related to 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 β-lactamase (ESBL)‑producing strains of *Escherichia coli* O157:H7 have been detected with high resistance rates: in that same river study, ~58.5% of *E. coli* O157:H7 isolates were phenotypically ESBL producers, many carrying the *bla_TEM* gene (≈58.5%), *bla_SHV*, and some *bla_CTX-M* (~4.6%) among others. 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 ESBL-producing *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 1.4 million people die each year due to unsafe water, sanitation, and hygiene services (WASH) (2019 estimates), making these diseases one of the most prevalent causes of illness and death worldwide (WHO, 2020). A recent study of water, soil, and fresh vegetables from urban and peri-urban garden ecosystems (including DNCC, DSCC, and Gazipur) showed *E. coli* in 58.62% of samples (water, soil, and vegetables). Among *E. coli* isolates (54 randomly selected), 100% were resistant to ampicillin, and there was measurable resistance to ciprofloxacin (~25.9%), tetracycline (~14.8%), cotrimoxazole (~14.8%), imipenem (~9.3%) and fosfomycin (~1%). Multidrug resistance (MDR) was found in ~14.81% of those isolates. The blaTEM gene was present in ~81.48%, and tetA in ~3.70%. Another study in Barishal (2025) looked at water sources including household stored tank water (RTW), well water (VW), pond water (PW) etc. It revealed substantial microbial contamination and presence of *E. coli* with resistance to beta-lactams (ampicillin, penicillin, amoxicillin). However, some environmental isolates remained susceptible to colistin and

levofloxacin. Also, colistin-resistant *E. coli* were detected, which is alarming as colistin is often a last resort. Studies of surface water sources, such as rivers and lakes in Khulna city, found high proportions of antibiotic resistance among bacterial isolates, including *Escherichia* spp. Resistance patterns were especially high for ciprofloxacin (75-87.5%) and ceftriaxone (65.6-78.1%), though resistance to ampicillin was lower (9.4-18.8%). Importantly, the prevalence of ARB (antibiotic resistant bacteria) was higher during the wet seasons compared to dry seasons. A study of *E. coli* from environmental sources across different environments in Dhaka showed that ~27.9% of water samples were contaminated, and among all confirmed *E. coli* isolates from various sources, ~36% were MDR. There are significant knowledge gaps regarding microbial communities and the dissemination of resistance genes in the environment as a result of this lack of thorough monitoring. In order to fill in these knowledge gaps, this study will carry out a thorough evaluation of Dhaka City's residential water quality, focusing on the frequency of pathogenic E. coli strains and their virulence traits. Using molecular and microbiological methods, this study will provide crucial information on the distribution and frequency of various E. coli pathotypes in municipal water sources. Additionally, by creating a system for regular monitoring and assessment of home water quality, this study will help guarantee that households have access to safe and clean drinking water. These kinds of activities are essential for comprehending public health policies and interventions meant to safeguard the health of the community. In order to develop more efficient water management techniques that lower health risks, this study intends to locate the sources of contamination and get a deeper understanding of the behavior of pathogenic E. coli in urban water systems. In conclusion, resolving the problem of harmful E. coli in municipal water is essential to enhancing Bangladesh's public health. The results will not only fill in current knowledge gaps but also offer crucial information to health professionals and legislators who are working to improve urban water safety.

1.2 Research Objective

1.2.1 General Objective

The purpose of this study is to detect, isolate and enumerate fecal coliforms as well as verify the presence of *Escherichia coli* to assess the hygiene and safety limits of municipal water supply across various areas of Dhaka. The study focuses on assessing the existence of *E. coli* in water supply beyond the limits indicating health risks with the help of morphological analysis as well as molecular techniques like PCR. The study not only evaluates presence but also investigates the antimicrobial resistance capacity of the organism detected from the samples. The susceptibility of the targeted pathogen is analysed on the basis of the production of extended-spectrum betalactamase (ESBL) gene as well as carbapenem resistance genes. Furthermore, the investigation aims to reach conclusions regarding the comprehensive condition of water supply in Dhaka city in terms of safety and public health.

1.2.2 Specific Objective

- To identify and enumerate Fecal Coliforms from the household water supply.
- To distinguish the presence of *Escherichia coli* in the water supply.
- Cultural analysis with the basis of morphological characteristics using specific mediums such as TBX, UTI and MacConkey agar.
- Initial molecular verification of *E. coli* presence using Polymerase Chain Reaction on the basis of 16srRNA gene.
- Antibiotic susceptibility profiling of the detected organism.
- Molecular evaluation of antibiotic resistance for ESBL producers which carry CTX-M, SHV and TEM genes and carbapenem resistant gene carriers with NDM, KPC and VIM genes through primer specific PCR cycles.

Chapter 2 - Literature Review

2.1 Target 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*

Escherichia coli (*E. coli*) ranks among the most thoroughly researched microorganisms in the fields of microbiology, biotechnology, and medicine. In 1885, the German pediatrician Theodor Escherich was the first to discover it, isolating it from the stool of healthy infants. He originally designated it Bacterium coli commune to indicate its frequent existence in the colon. In 1919, Castellani and Chalmers paid tribute to Escherich by rebranding the bacterium as Escherichia coli (Kaper et al., 2004).

In the late 19th and early 20th centuries, *E. coli* was identified as a typical commensal bacterium in the human intestine, contributing significantly to digestion and the synthesis of vitamin K. Nonetheless, as time passed, it became associated with several pathogenic conditions, including diarrhea, urinary tract infections, and neonatal meningitis (Nataro & Kaper, 1998).

During the mid-20th century, *E. coli* emerged as a model organism in genetics and molecular biology. Its brief generation period, simplicity of cultivation, and comparatively straightforward genetics rendered it essential for research. During the 1940s and 1950s, E. coli strains (like K-12) played a crucial role in uncovering essential genetic processes, including bacterial conjugation (Lederberg & Tatum, 1946) and the operon model (Jacob & Monod, 1961).
In the 1970s, E. coli was pivotal in the advancement of recombinant DNA technology. E. coli plasmids and restriction enzymes were utilized to create the initial recombinant DNA molecules, setting the stage for contemporary genetic engineering and biotechnology (Cohen et al., 1973).

In more recent decades, pathogenic strains such as *E. coli* O157:H7 have been identified as significant causes of foodborne illness outbreaks, raising major public health concerns worldwide (Riley et al., 1983). Genomic studies have also revealed the extraordinary genetic diversity of *E. coli*, distinguishing between commensal, laboratory, and pathogenic strains (Tenaillon et al., 2010).

Today, *E. coli* remains both a beneficial model organism for research and biotechnology and a notorious pathogen responsible for outbreaks of diarrheal disease, hemolytic uremic syndrome (HUS), and urinary tract infections.

2.1.3 General Characteristics of *E. coli*

Escherichia coli (*E. coli*) is a rod-shaped, Gram-negative bacterium that is part of the Enterobacteriaceae family. First mentioned by Theodor Escherich in 1885, it is among the most extensively researched microorganisms in microbiology. The bacterium usually measures 1–2 µm in width and 2–6 µm in length, appearing as straight rods when viewed under a microscope. Its cell wall composition features a thin peptidoglycan layer and an outer membrane abundant in lipopolysaccharides, typical of Gram-negative bacteria (Madigan et al., 2018).

The majority of E. coli strains are capable of movement, featuring peritrichous flagella spread across the cell's surface. They possess fimbriae or pili, enabling them to stick to surfaces and contributing significantly to processes like colonization and conjugation. The bacterium is a facultative anaerobe, indicating it can thrive in both aerobic and anaerobic settings. It thrives at 37°C, which is the typical body temperature of humans, but it can withstand temperatures ranging from 15°C to 45°C.

E. coli proliferates easily on standard laboratory media. On nutrient agar, colonies look smooth, circular, moist, and grayish-white. On MacConkey agar, which has lactose, most strains ferment lactose, resulting in pink colonies because of acid production. The ability to ferment lactose is a significant diagnostic trait that aids in distinguishing E. coli from other enteric bacteria. Biochemically, the organism is positive for catalase and negative for oxidase, and it has the ability to ferment several sugars, resulting in acid and gas production.

Genetically, E. coli possesses a relatively compact genome of around 4.6 million base pairs, coding for roughly 4,000–5,500 genes varying by strain (Blattner et al., 1997). Laboratory

strains like E. coli K-12 are commonly utilized as model organisms in molecular biology and biotechnology because of their straightforward cultivation and thoroughly understood genetics.

From an ecological perspective, E. coli typically resides in the gastrointestinal tracts of humans and other warm-blooded creatures, aiding in digestion and the production of vitamins (including vitamin K). It is likewise utilized as a marker organism for fecal pollution in food and water. Nonetheless, some strains have gained virulence factors and developed into pathogens. These pathogenic categories consist of enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), uropathogenic (UPEC), and neonatal meningitis-associated (NMEC) strains (Kaper et al., 2004; Nataro & Kaper, 1998).

Consequently, *E. coli* serves as a helpful commensal bacterium and a notable pathogen, rendering it one of the most vital microorganisms in microbiology, medicine, and biotechnology.

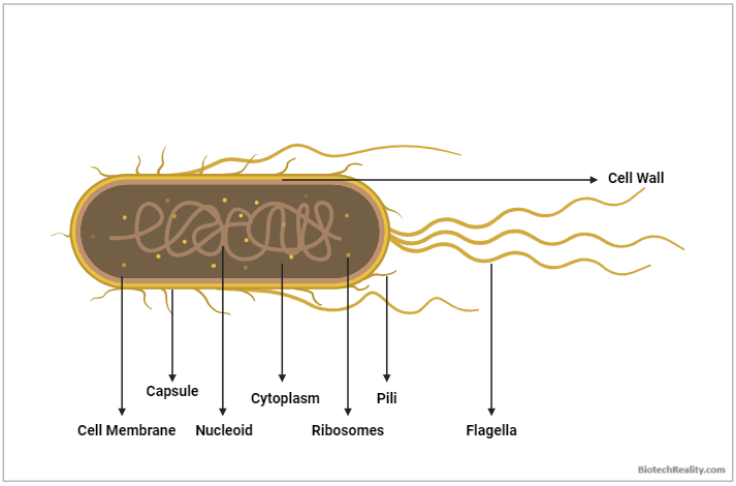

Figure 1: Basic structure of E. coli

2.1.4 Physiochemical properties of *E. coli*

Escherichia coli is a rod-shaped, Gram-negative bacterium measuring approximately 1–2 µm in width and 2–6 µm in length. The cell envelope consists of a cytoplasmic membrane, a

delicate peptidoglycan layer, and an outer membrane that includes lipopolysaccharides (LPS), resulting in a net negative charge on the cell surface and serving as a barrier to permeability. This arrangement also aids in resisting specific antibiotics and host defense systems (Madigan et al., 2018).The majority of E. coli strains exhibit motility because of peritrichous flagella, and fimbriae along with pili on their surface assist in adhesion and conjugation. The bacterium is a facultative anaerobe that can thrive in both oxygen-rich and oxygen-poor environments. It processes a broad variety of carbohydrates, such as lactose, which it ferments while producing acid and gas—a characteristic commonly employed in lab identification (Nataro & Kaper, 1998). E. coli thrives best at 37°C in cultural settings, but it can endure temperatures from 15 to 45°C. It favors a neutral pH of 6.0 to 7.5, yet it can live in slightly acidic or basic conditions. The bacterium shows moderate tolerance to osmotic stress, aiding its survival in various ecological habitats, such as food and water. 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

Escherichia coli is one of the most important members of the normal intestinal microbiota of humans and warm-blooded animals. It typically colonizes the gastrointestinal tract of infants within the first few hours to days after birth, often acquired from the mother and surrounding environment (Tenaillon et al., 2010). In healthy individuals, non-pathogenic strains of *E. coli* coexist with other commensal bacteria and play several beneficial roles.

1. Nutritional Contribution

E. coli assists in digestion and nutrient absorption by breaking down undigested carbohydrates and proteins that reach the large intestine. It also synthesizes essential vitamins, such as vitamin K and some B-complex vitamins, which are important for host metabolism and blood clotting (LeBlanc et al., 2017).

2. Maintenance of Gut Environment

Commensal *E. coli* helps maintain a stable intestinal ecosystem. By producing short-chain fatty acids (SCFAs) during fermentation, it contributes to lowering intestinal pH, which inhibits colonization by harmful bacteria (Kaper et al., 2004).

3. Colonization Resistance

As part of the gut flora, *E. coli* competes with pathogenic microbes for nutrients and attachment sites on the intestinal mucosa. This process, known as colonization resistance, prevents the overgrowth of harmful organisms, including pathogenic *E. coli* strains and other enteric pathogens like *Salmonella* and *Shigella* (Stecher & Hardt, 2011).

4. Immune System Modulation

Normal flora strains of *E. coli* play a role in stimulating and regulating the immune system. Their presence helps in the development of gut-associated lymphoid tissue (GALT), the production of secretory IgA, and the regulation of inflammatory responses. This immunomodulation ensures tolerance to commensals while priming the host to respond effectively against pathogens (Round & Mazmanian, 2009).

5. Indicator of Fecal Contamination

While not a direct benefit to the host, commensal *E. coli* serves as a valuable indicator organism for fecal contamination in water and food safety testing because of its consistent presence in the intestines of humans and animals (Madigan et al., 2018).

As a normal commensal, *Escherichia coli* contributes to nutrition, protection, and immune balance within the human host. Although certain strains can acquire virulence factors and

cause disease, the majority of *E. coli* strains function as beneficial members of the intestinal microbiota.

2.1.6 E. coli As A Model Organism

Escherichia coli, commonly known as *E. coli,* has been widely utilized as a model organism in research.research due to several advantageous characteristics that facilitate the exploration of essential biological mechanisms. Its straightforward nature as a single-celled organism allows scientists to alter and enhance it without the moral concerns associated with multicellular organisms, is a primary factor for its choice (Ogundare & Onifade, 2009). This species is frequently utilized in microbiological studies because of its easy availability, typically high virulence, and possibility for swift expansion. This organism flourishes readily on most laboratory media. at 37°C and develops extensive colonies after an overnight incubation. At present comprehension of DNA duplication, essential molecular mechanisms, and gene activation Control primarily comes from studies related to E. coli. Moreover, until these events were experimentally developed and examined in the bacterium, numerous biological processes such Genetic transformation processes that frequently happen in bacterial species have not been clearly understood. *E. coli* offers a superb basis for investigating gene functionality and regulation due to its well-known genomic organization and simplicity of genetic modification. The K-12 variant of Escherichia coli, for instance, has been crucial in key molecular biology. findings, like the comprehension of the genetic code and mechanisms of gene expression (FAQ, 2011). Additionally, *E. coli* acts as a host for recombinant DNA technology; scientists can place genes from different organisms into *E. coli* to generate proteins like Insulin, which has transformed biotechnology and medicine. *E. coli* serves as a versatile model for investigating metabolic functions and environmental relationships due to its ability to adjust to varied growth conditions and thrive in numerous environments (eLife, 2015). Its importance for examining human health and illness is also emphasized by the reality that it is a standard element of the plant life in the human intestine.

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

Escherichia coli is universally accepted as the most reliable biological indicator of fecal contamination in water, food, and the environment. This role comes from its close association with the intestinal tract of humans and warm-blooded animals, where it exists in very high numbers (up to 10^9 cells per gram of feces). Its detection in external environments, such as

drinking water or food products, almost always indicates that fecal material has entered the sample (Edberg et al., 2000).

The effectiveness of *E. coli* as an indicator organism is based on several important properties. Unlike other coliforms, which may be found in soil, plants, or water naturally, *E. coli* is specific to fecal sources and rarely occurs in clean environments (Ashbolt et al., 2001). It is present in much larger numbers than most intestinal pathogens, making it easy to detect even when pathogens are scarce. Moreover, it is relatively simple and inexpensive to isolate and identify using routine microbiological techniques. These features make it an excellent "sentinel organism" for fecal pollution. The presence of *E. coli* in water or food also serves as a warning sign of possible pathogen presence. Fecal contamination may carry enteric bacteria such as *Salmonella* and *Shigella*, viruses such as enteroviruses and noroviruses, and parasites like *Giardia* and *Cryptosporidium* (WHO, 2017). While *E. coli* itself is often harmless in its commensal form, its detection signals a breakdown in sanitary quality and indicates potential risk to human health. Regulatory bodies such as the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) recommend *E. coli* as the primary indicator of microbiological safety in drinking water. For example, WHO guidelines state that drinking water should contain zero detectable *E. coli* per 100 mL sample. Similarly, in food safety testing, high *E. coli* counts indicate fecal contamination during processing, handling, or storage (Jay et al., 2005). Recreational water quality is also monitored using *E. coli* counts, since exposure to polluted swimming water can result in gastrointestinal disease outbreaks (Ishii & Sadowsky, 2008). Despite its advantages, *E. coli* is not a perfect indicator. In tropical and subtropical environments, some strains have been found to survive or even multiply in soil and sediments, leading to occasional false positives (Ishii & Sadowsky, 2008). Nevertheless, due to its strong fecal association and practicality in monitoring, *E. coli* continues to be regarded as the gold standard organism for assessing fecal contamination and guiding public health protection.

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

Escherichia coli (*E. coli*) is a remarkably versatile bacterium that exists along a spectrum ranging from harmless commensals to highly pathogenic strains. In its commensal form, *E. coli* resides in the intestines of humans and other warm-blooded animals, where it contributes

positively to host physiology by aiding digestion, synthesizing essential vitamins such as vitamin K and some B vitamins, and maintaining the balance of the gut microbiota. These commensal strains are typically genetically stable, possessing mostly housekeeping genes necessary for survival and adaptation in the gut environment, and they rarely cause disease under normal circumstances. However, the genetic plasticity of *E. coli* allows certain strains to acquire additional genes through horizontal gene transfer, plasmids, bacteriophages, and genomic islands known as pathogenicity islands. These genes may encode virulence factors such as adhesins, invasins, toxins, capsules, and iron-acquisition systems, which enable these strains to adhere to, invade, and damage host tissues, thereby converting them into pathogenic forms (Kaper et al., 2004; Tenaillon et al., 2010). Pathogenic *E. coli* can be broadly categorized into intestinal (diarrheagenic) pathogens and extraintestinal pathogens (ExPEC). Intestinal pathogenic strains include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC, such as O157:H7), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC). These strains possess specific virulence mechanisms that allow them to colonize the intestinal mucosa, produce toxins, or invade epithelial cells, leading to a range of gastrointestinal illnesses from mild diarrhea to life-threatening conditions such as hemolytic-uremic syndrome. Extraintestinal pathogenic strains, including uropathogenic *E. coli* (UPEC) and neonatal meningitis-associated *E. coli* (NMEC), possess distinct virulence determinants that allow them to survive outside the gut, invade the urinary tract or central nervous system, and evade host immune defenses (Nataro & Kaper, 1998). The variability of *E. coli* is therefore shaped by a combination of genetic factors and environmental pressures, including the host immune response, gut microbiota composition, and exposure to antibiotics or other stressors. This genetic and phenotypic diversity allows *E. coli* to occupy multiple ecological niches, adapt rapidly to changing environments, and sometimes transition from a harmless commensal organism to a dangerous pathogen. Understanding this variability is critical for epidemiology, public health surveillance, outbreak investigation, and the development of preventive measures such as vaccines and targeted therapies (Tenaillon et al., 2010; Kaper et al., 2004). 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.

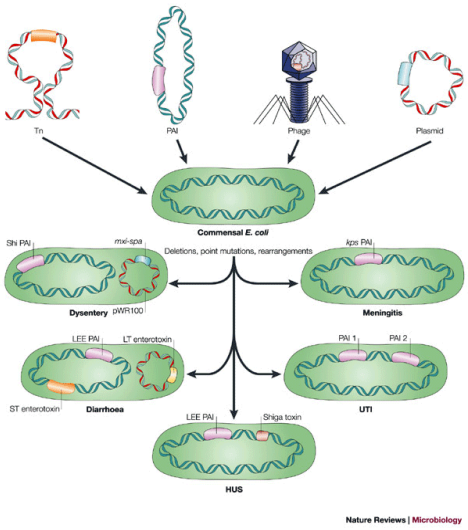

Figure 2: How commensal *E. coli* transform in pathogen[Toxin-linked mobile genetic elements in major enteric bacterial pathogens]

2.1.9 Clinical characteristics and epidemiology

2.1.10.1 Epidemiology

Escherichia coli (*E. coli*) is a highly diverse bacterium found worldwide, both as a commensal organism in the intestines of humans and animals and as a pathogen responsible for a range of infections. The epidemiology of *E. coli* varies depending on the strain, its virulence factors, and the mode of transmission. While most *E. coli* strains are harmless and contribute to gut health, pathogenic strains are a significant cause of diarrheal diseases, urinary tract infections, sepsis, and neonatal meningitis globally (Kaper et al., 2004). Pathogenic *E. coli* strains are primarily transmitted via the fecal-oral route, through contaminated food, water, or direct contact with infected individuals or animals. Foodborne outbreaks are often associated with undercooked meat, unpasteurized milk, raw vegetables, and contaminated water sources. Enterohemorrhagic *E. coli* (EHEC), such as the O157:H7 strain, is notorious for causing outbreaks of severe diarrhea and hemolytic-uremic syndrome (HUS), especially in children and the elderly. Epidemiological studies have documented outbreaks linked to contaminated meat, fresh produce, and unpasteurized juices in both developed and developing countries (Nataro & Kaper, 1998). Extraintestinal pathogenic *E. coli* (ExPEC), including uropathogenic *E. coli* (UPEC) and neonatal meningitis-associated *E. coli* (NMEC), often cause infections originating from the host's own intestinal flora. UPEC is

a leading cause of community-acquired and hospital-acquired urinary tract infections, while NMEC is a major cause of neonatal meningitis. ExPEC strains typically carry multiple virulence factors that enhance colonization, invasion, and immune evasion, contributing to their widespread prevalence in both community and healthcare settings (Russo & Johnson, 2000). Globally, *E. coli* infections impose a considerable public health burden. In low- and middle-income countries, diarrheagenic *E. coli* is a major contributor to infant and child morbidity and mortality due to poor sanitation, contaminated water, and limited access to healthcare. In high-income countries, foodborne EHEC outbreaks continue to pose significant risks, emphasizing the need for rigorous hygiene, food safety measures, and water quality monitoring. Ongoing surveillance, serotyping, and molecular epidemiology are crucial for tracking circulating strains, identifying outbreak sources, and implementing effective prevention strategies (Tenaillon et al., 201).

2.1.10.1.1 Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagic Escherichia coli (EHEC) is a type of *E. coli* bacterium that can cause serious intestinal disease in humans. It is a member of the Shiga toxin-producing *E. coli* (STEC) group. The most common strain is *E. coli* O157:H7, though other serotypes like O26, O103, O111, and O145 can also cause disease. EHEC is characterized by its ability to produce Shiga toxins (Stx1 and Stx2), which damage the cells lining the intestine. These toxins interfere with protein synthesis, leading to cell death, inflammation, and sometimes systemic effects. Among these, Stx2 is particularly associated with severe complications such as hemolytic uremic syndrome (HUS), a condition that can cause acute kidney failure, destruction of red blood cells, and low platelet count. The bacterium adheres to the intestinal lining using a surface protein called intimin, creating attaching-and-effacing (A/E) lesions. These lesions destroy microvilli, disrupt normal absorption, and trigger diarrhea. EHEC also produces hemolysins and uses a type III secretion system to inject effector proteins into host cells, which alters cell signaling and worsens intestinal injury. Clinically, EHEC infection usually begins with watery diarrhea and abdominal cramps, which may progress to bloody diarrhea. Severe cases can develop HUS, especially in children and the elderly. Fever is often mild or absent. Transmission occurs mainly through contaminated food or water, including undercooked beef, unpasteurized milk or juice, raw vegetables, and occasionally through direct contact with infected persons. The infectious dose is very low; ingestion of just a few bacteria can cause illness. Diagnosis relies on culture of stool samples, often using sorbitol

MacConkey agar to detect O157:H7, or molecular tests to identify Shiga toxin genes (stx1, stx2). Treatment is mainly supportive, focusing on hydration and monitoring for complications. Antibiotics are generally avoided, as they can increase toxin release and worsen outcomes. EHEC is a significant public health concern worldwide, causing foodborne outbreaks, hospitalizations, and occasionally fatalities. Proper food handling, hygiene, and cooking practices are key to preventing infection.

2.1.10.1.2 Enteropathogenic *E. coli* (EPEC)

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) is a pathogenic type of *E. coli* that primarily causes diarrheal illness, especially in infants and young children in developing countries, and in travelers to endemic regions (“traveler’s diarrhea”). Unlike some other pathogenic *E. coli* strains, ETEC generally does not cause bloody diarrhea or invade the intestinal lining. ETEC attaches to the small intestine using fimbrial adhesins, also called colonization factors, which help the bacteria colonize the mucosal surface. Once attached, the bacteria produce enterotoxins that disrupt the normal fluid balance in the intestines. These toxins include:

- Heat-labile toxin (LT): Functions like cholera toxin by increasing cAMP in intestinal cells, leading to secretion of water and electrolytes.

- Heat-stable toxin (ST): Increases cGMP, also causing fluid secretion.

The disease caused by ETEC typically manifests as watery diarrhea, sometimes accompanied by abdominal cramps, nausea, and mild or no fever. Severe dehydration may occur in vulnerable individuals, such as young children. Symptoms usually last a few days and are self-limiting in most healthy adults. ETEC is transmitted mainly via the fecal-oral route, through contaminated food or water. It is a major cause of childhood diarrhea in developing countries and the most common cause of traveler's diarrhea worldwide. Laboratory diagnosis requires detection of ETEC-specific toxins, as standard stool culture cannot reliably distinguish it from non-pathogenic *E. coli*. Treatment is primarily supportive, with rehydration therapy being crucial. Antibiotics are generally reserved for severe or persistent cases. Preventive measures include safe water, good hygiene, and careful food handling.

2.1.10.1.4 Enter invasive *E. coli* (EIEC)

Enteroinvasive Escherichia coli (EIEC) is a pathogenic strain of *E. coli* that causes dysentery-like diarrhea in humans. Unlike other diarrheagenic *E. coli* such as ETEC, EIEC invades the cells of the large intestine (colon) rather than producing toxins. This invasion leads to cell destruction, inflammation, and ulceration of the intestinal lining. EIEC is closely related to *Shigella* and shares many virulence mechanisms. It uses a plasmid-encoded invasion system to enter colonic epithelial cells and multiply inside them. A type III secretion system delivers effector proteins that manipulate the host cell cytoskeleton, allowing the bacteria to spread to adjacent cells. EIEC does not produce enterotoxins and is usually non-motile, similar to *Shigella* species. The infection typically begins with watery diarrhea, which can progress to bloody stools containing mucus and pus, along with abdominal cramps, fever, and tenesmus (painful straining during defecation). Severe dehydration is uncommon but can occur in vulnerable individuals. EIEC is transmitted mainly through the fecal-oral route, via contaminated food or water, and person-to-person spread can occur in areas with poor sanitation. Diagnosis relies on stool culture and molecular detection of

invasion plasmid genes (ipa genes). Treatment is primarily supportive, with rehydration therapy, while antibiotics are reserved for severe cases.

2.2 Transmission of *E. coli*

Escherichia coli (E. coli) is a bacterium that naturally resides in the intestines of humans and animals. While most strains are harmless and part of the normal gut flora, certain pathogenic strains can cause gastrointestinal disease, urinary tract infections, and systemic illnesses. Transmission of *E. coli* occurs mainly through pathways that introduce the bacteria from fecal matter into a new host. The primary route of transmission is the fecal-oral route, where bacteria from the feces of infected humans or animals contaminate food, water, or surfaces that are then ingested. Contaminated drinking water, especially untreated or poorly treated water, is a major source of infection in regions with inadequate sanitation. Similarly, undercooked or raw foods—particularly beef and other meats contaminated with animal feces—can transmit pathogenic strains like EHEC. Fresh fruits and vegetables can also carry E. coli if irrigated or washed with contaminated water, and unpasteurized milk or juices are additional sources, especially for enterotoxigenic strains. Poor hand hygiene, lack of sanitation facilities, and improper food handling practices further increase the risk of infection. Certain *E. coli* strains, such as enteroinvasive (EIEC) and enteropathogenic (EPEC) types, can also spread directly from person to person. This occurs when contaminated hands, diapers, or surfaces come into contact with another person's mouth, which is particularly common in daycare centers, hospitals, or crowded households. Strains like EHEC and EIEC require only a small infectious dose, making outbreaks possible even with minimal exposure. Zoonotic transmission is also important for specific strains, particularly EHEC, which often reside in the intestines of cattle and other ruminants without causing illness. Humans can become infected by consuming undercooked meat contaminated with animal feces, by direct contact with animals or their fecal matter, or via environments contaminated by animal waste. *E. coli* can survive outside the host in water, soil, and on surfaces, allowing for indirect environmental transmission. Recreational waters such as lakes, rivers, or swimming pools contaminated with fecal matter can act as sources of infection. Contaminated produce, particularly when washed with unsafe water or fertilized with untreated manure, can also transmit the bacteria. In summary, *E. coli* transmission occurs through contaminated food and water, direct person-to-person contact, animal reservoirs, and contaminated environments. Hygiene, sanitation, proper food handling, safe water, and handwashing are critical measures

to prevent infection. The ease of transmission and low infectious dose of some pathogenic strains make *E. coli* a significant cause of outbreaks and gastrointestinal disease worldwide.

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

Escherichia coli (E. coli) is commonly used as an indicator of fecal contamination in water. While many strains are harmless, some are pathogenic and may carry antibiotic resistance genes. The presence of antibiotic-resistant *E. coli* in water is a significant public health concern, as it can serve as a reservoir for resistance genes and facilitate their spread to humans and other bacteria. Antibiotic-resistant *E. coli* can enter water systems through

discharge of untreated or inadequately treated sewage, agricultural runoff containing animal feces, or contamination from livestock operations. Wastewater from hospitals and pharmaceutical industries can also introduce resistant strains and residual antibiotics into aquatic environments. These bacteria can survive in rivers, lakes, groundwater, and even drinking water sources, posing a risk for community-acquired infections. Mechanisms of resistance in *E. coli* include the production of β-lactamases (e.g., ESBL—extended-spectrum β-lactamases), efflux pumps, mutations in target enzymes, and acquisition of resistance genes through plasmids, transposons, or integrons. Resistance to multiple antibiotics, including penicillins, cephalosporins, tetracyclines, and fluoroquinolones, has been reported in environmental *E. coli* isolates. The presence of antibiotic-resistant *E. coli* in water can lead to:

- Direct human exposure: Through drinking contaminated water, swimming, or recreational activities.

- Indirect transmission: Via contamination of crops irrigated with polluted water or through animals that drink contaminated water.

- Spread of resistance genes: Environmental *E. coli* can transfer resistance genes to other bacteria, amplifying the problem of antimicrobial resistance (AMR) in the community.

Escherichia coli (E. coli) is commonly found in the intestines of humans and animals, and it serves as an indicator of fecal contamination in water. Some strains of *E. coli* are pathogenic and can carry antibiotic resistance genes, making them a significant public health concern. Antibiotic-resistant *E. coli* in water can arise from untreated or poorly treated sewage, hospital effluents, agricultural runoff from livestock treated with antibiotics, and contaminated surface or groundwater. These bacteria may carry genes that provide resistance to multiple classes of antibiotics, including penicillins, cephalosporins, tetracyclines, sulfonamides, and fluoroquinolones. Resistance is often mediated through β-lactamases, efflux pumps, target modifications, or acquisition of resistance genes via plasmids and other mobile genetic elements. The presence of antibiotic-resistant *E. coli* in water poses several risks: humans can be exposed through drinking contaminated water, recreational activities, or

consumption of crops irrigated with polluted water. 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-drug-resistant pathogens. Additionally, these bacteria can transfer resistance genes to other bacteria, increasing the spread of antimicrobial resistance in the environment. Water thus acts both as a reservoir and a transmission pathway for resistant bacteria. Surveillance studies worldwide have detected multidrug-resistant *E. coli* in rivers, lakes, and urban wastewater, highlighting the role of water as a reservoir and transmission pathway for AMR. Controlling antibiotic-resistant *E. coli* in water requires proper wastewater treatment, reduction of antibiotic use in humans and animals, and environmental monitoring.

Chapter 3

Materials and Methods

This study was carried out at Brac University under the Mathematical and Natural Sciences Department. The entire study methodology is elaborated ahead.

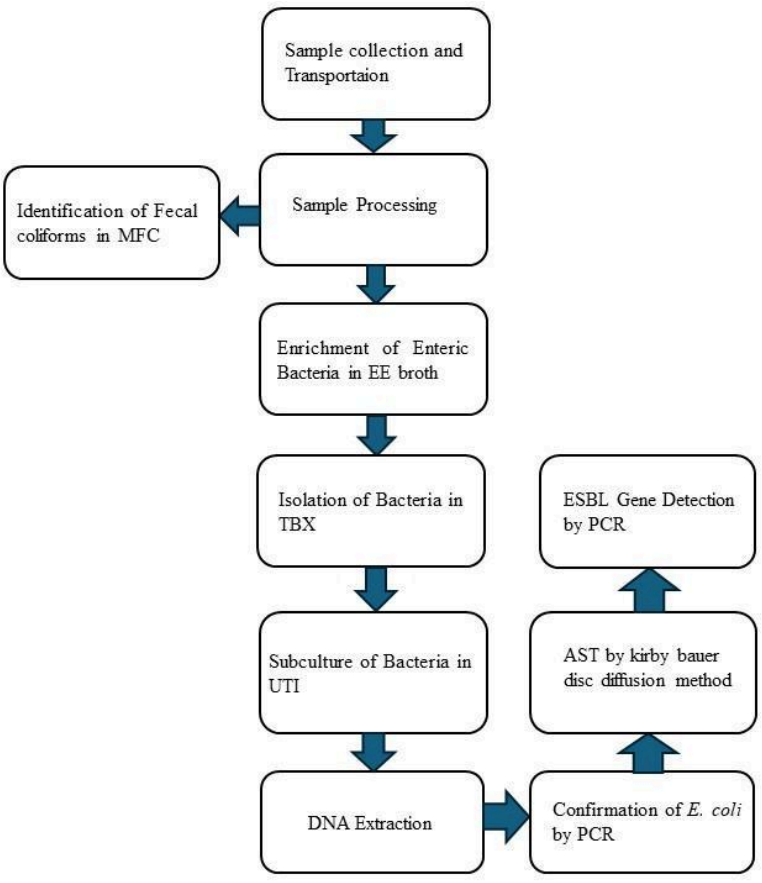

Figure 3: Work flow.

3.1 Sampling Site and Sample Collection

The objective of this study was to evaluate municipal water supply in household water across several areas of North and South Dhaka city. A total of 69 samples were collected from various locations which include Tejgaon, Indira Road, Hatirjheel, Gulshan, Niketon, Banani, Bazar Gate, Shatkhola, Badda, Aftabnagar, Rampura, Banasree, Mogbazar, Eskaton, Dhanmondi, Elephant Road, North Road, Central Road, Katabon, New Market, Shahbag, Gopibagh, Puran Dhaka, Wari, Agargaon, Mohammadpur and Bashundhara Residential Area (Figure 2). Sample collection was conducted from January 2025 to August 2025. For collection, sterile plastic bottles (NALGENE, USA) with the capacity of 500 ml were used in which roughly 500 milliliters of water was collected with required labelling. Post collection, during transportation, to maintain the cold chain, an insulated box carrying ice packs was used to achieve a temperature of 4°C to 10°C. All of the samples in this study were handled in terms with the standard procedures within 6 to 8 hours of collection.

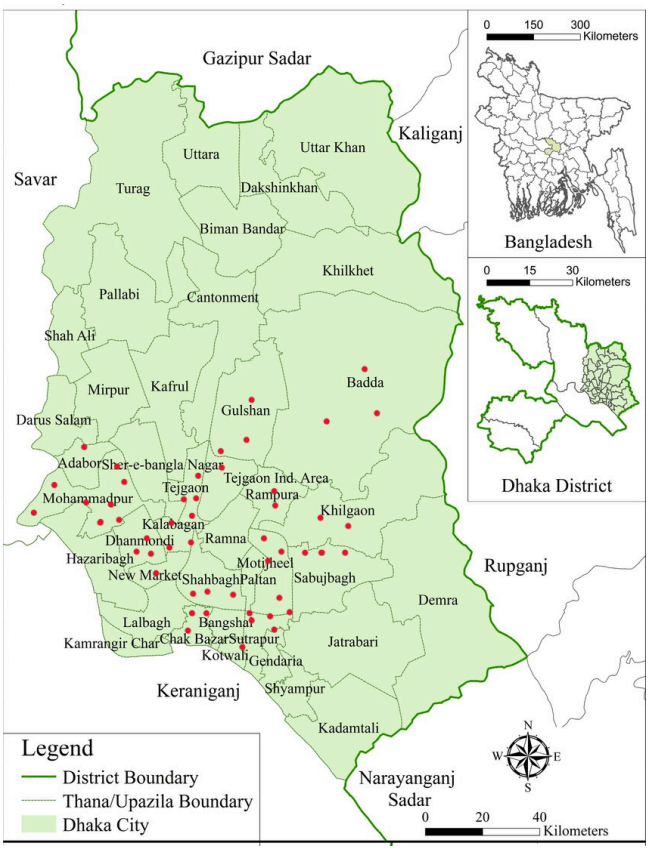

Figure 4: Mapping of sample collection

3.2 Sample Processing

The initial experimentation began with a membrane filtration method in which the samples are subjected to filtration using a 0.22 micrometre membrane filter (Sartious Stedim, Goettingen, Germany). 100 millilitres of each sample was taken for filtration and passed through a filtration unit containing the membrane filter. Further, the membrane filter was carefully shifted from the unit to an M-FC agar plate, facing up. Consecutively, another membrane filter following the same steps for the same samples were transferred to a falcon tube with proper labelling which contained EE broth. The incubation period for the M-FC agar plates was 44°C for 24 hours in order to isolate and enumerate fecal coliforms whereas the falcons with EE broth were incubated at 37°C for 24 hours, with lids loosely closed for the purpose of enrichment of *E. coli* present in the water samples.

3.2.1 Detection of Fecal Coliforms

Post incubation period of 24 hours at 44°C, the colonies that appeared blue in color were confirmed to be fecal coliforms. These positive colonies were enumerated in terms of colony forming units (CFU) and calculated as CFU/ml. (Figure 4)

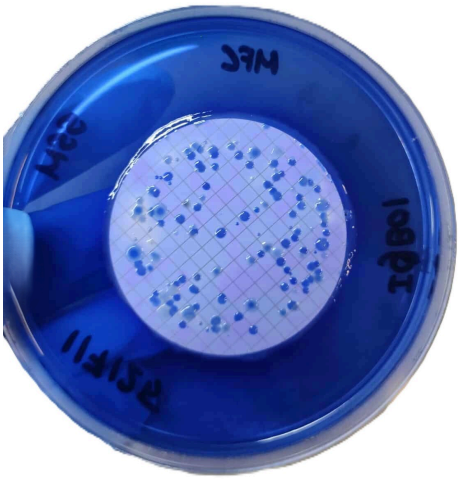

Figure 5 : Identification of Fecal Coliform on MFC

3.2.2 Isolation and Identification of *E. coli* on Selective Media

Post incubation period of 24 hours at 37°C, the enriched EE broth were subjected to serial dilution up to 10^{-6}. Aliquots were taken from each dilution and taken to TBX agar media (HiMedia) to carry out the spread plate method and then incubated at 37°C for 18 to 24 hours. Post incubation, the green-blue colonies were detected to be *E. coli* presumably. (Figure 5)

For further confirmation, single colonies blue-green in color were randomly selected and subjected to streaking on UTI and MacConkey agar plates and incubated at 37°C for 18 to 24 hours. The distinctive and identifiable morphological characteristics of the colonies suggested presence of *E. coli* such as colonies appearing purple in color on UTI medium and for MacConkey agar, they appeared as pink, dry and non-mucoid colonies. (Figure 6)

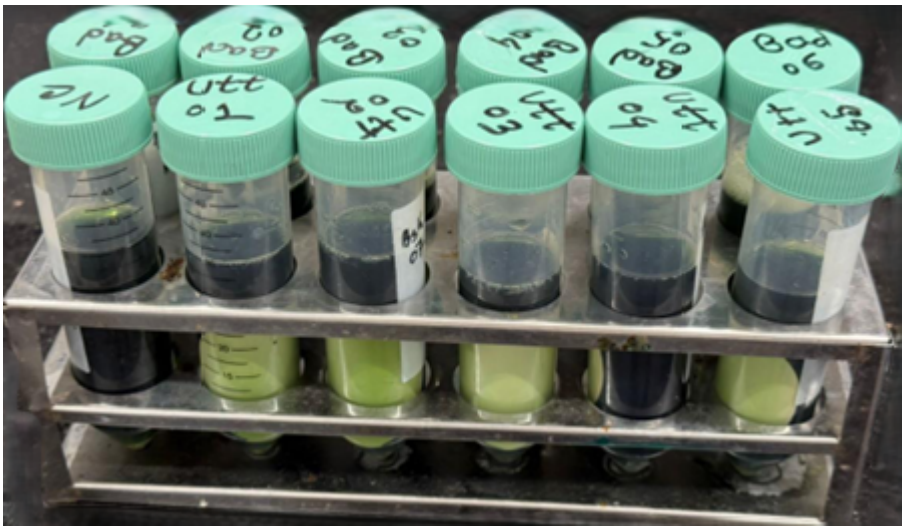

Figure 6: Enrichment of *E. coli* in EE broth

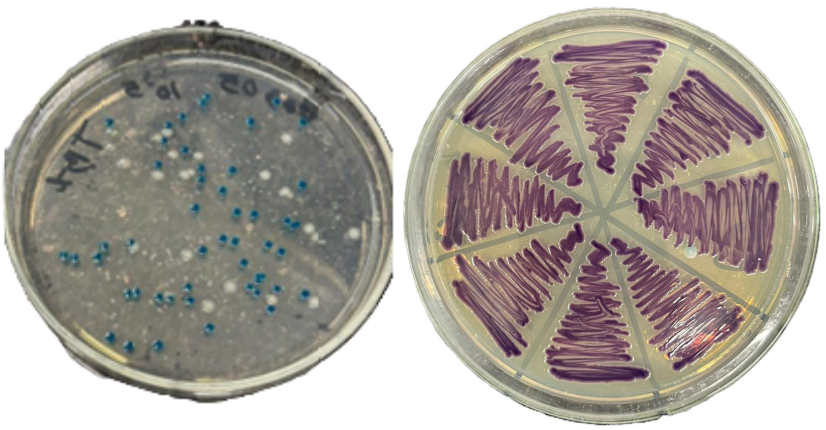

Figure 7: Cultural confirmation of *E. coli* on TBX, followed by UTI

3.2.3 Preparation of Stock Cultures for Further Analysis

For detailed experimentation further, stock cultures were prepared in two particular methods which were cryo-glycerol stock preserved at a temperature of -20°C as well as T1N1 stock stored at room temperature.

3.2.3.1 Cryo-preservation with Glycerol

After confirmed presence of *E. coli* through the said mediums, single colony was picked from UTI and MacConkey agar plates in order to culture them in LB broth of 7 millilitres taken in test tubes for further enrichment. Then, for incubation, the test tubes were kept in a shaking incubator at 37°C for 18 to 24 hours at 120 rpm. After that, to prepare a stock culture, 30% (v/v) glycerol was added as a supplement with the enriched culture (700 µL enriched culture + 300 µL glycerol) which was stored at -20°C.

3.2.3.2 T1N1 Stock

At first, following general procedures, T1N1 medium was made and autoclaved. Then, autoclaved vials were taken in which 3 mL of T1N1 medium was poured. From a subcultured Nutrient Agar plate in which a presumptive single colony of *E. coli* was grown taken from the UTI and MacConkey plates, an isolated single colony from this freshly grown overnight culture was taken using a sterile needle which was then stabbed into the T1N1 agar. The vials were then kept in an incubation period of 37°C for 24 to 48 hours. After this, 300 µL of autoclaved paraffin oil was poured into the vials and then capped to store at room temperature.

3.3 Molecular Identification of *E. coli* Isolates

Initially, following detection based on morphological characteristics of the colonies of the organism, PCR was performed species-specifically for the verification of *E. coli* presence at the molecular level. For PCR, DNA was obtained from fresh cultures using the boiling lysis or heat lysis method.

3.3.1 DNA Extraction

Extraction of DNA refers to separation of DNA from cell contents such as cell membranes, proteins and other biological elements either physically or chemically. In this study, after morphological confirmation of each colony of *E. coli* taken from TBX and streaked on UTI and MacConkey agar, a loop full of purple colonies were picked from the UTI plates using a sterile loop for the lysis method used. At first, microcentrifuge tubes (MCTs) were autoclaved and appropriately labelled with isolate numbers. The loop full of colonies taken were inoculated into 400 µL of TE buffer in the labelled tubes. Then, each of the tubes are vortexed to homogenise the contents. Afterwards, the tubes were subjected to centrifugation at 13000 rpm for 10 minutes and then the supernatants were discarded. In the residual pellets, another 400 µL TE buffer was added and vortexed again. Then, the tubes were loaded in a heat block machine at a temperature of 100°C for 10 minutes. Further, the samples were subjected to centrifugation again at 13000 rpm for 10 minutes. This time, the supernatants were carefully taken using a micropipette and transferred to new MCTs. Lastly, the extracted DNA was stored at -20°C for later use.

3.3.2 Polymerase Chain Reaction (PCR)

PCR is an extremely efficient method for the detection and confirmation of bacterial isolates in the molecular basis. It focuses on the amplification of distinct genes under specific conditions. After sample collection each week and post morphological analysis, DNA extracted from the samples were subjected to PCR routinely to confirm the presence of *E. coli,* targeting the amplification of 16srRNA genes, namely ECO-1 and ECO-2. (Figure 7)

The assay was accomplished in PCR tubes at a total volume of 13 µL. This contained 6 µL of 2X Emerald PCR Master Mix (Takara Bio), 3 µL of nuclease-free water, 1 µL of reverse and forward primer each and 2 µL of DNA template. Precise pipetting was maintained in each step to maintain volume accuracy and homogeneity and further the tubes were centrifuged to ensure uniform mixture while preventing bubble formation.

PCR cycles were implemented in the Applied Biosystems (Thermo Fisher) thermal cycler under given conditions. The data regarding the bacterial organism and its targeted genes, primer sequences, temperature of each step of the cycle and the amplicon sizes are provided in the Table.

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	

Figure 8: Primer sequence and PCR condition for 16srRNA gene (Band Size: 585bp) of *E. coli*

3.3.3 Electrophoretic Analysis of PCR-Amplified DNA

Agarose gel solution of 10 mL, 1.5% was formulated by measuring 1.5 grams of agarose in a conical flask. In this flask, 2 mL of 50x TAE buffer as well as 98 mL of distilled water were poured. The mixture was then heated in a microwave oven in order to dissolve the gel completely till the solution appears clear. Then, the solution needs to be cooled before the addition of 4 µL ethidium bromide (EtBr) with proper carcinogenic precautions. Afterwards, the gel prepped was poured into a gel tray and a comb was placed onto an end. Then, the gel was left uninterrupted for 10 minutes at room temperature for even solidification. Once the gel was solid, the comb was cautiously withdrawn and the tray was kept in an electrophoretic chamber in which the gel was completely submerged in a 1x TAE buffer. Here, 4 µL of PCR products were pipetted from each sample and loaded into the wells created by the comb in the gel along with a 100 base pair DNA ladder. A gel run of 100 V for 40 minutes segregated the PCR products into distinct band sizes in the gel. The gel was then visualised under UV light in order to observe the targeted gene.

3.4 Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of *E. coli* isolates were analysed from total n positive samples out of n tested. From each of the positive samples, two isolates were tested for AST through the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. A total of 20 antibiotic discs depicting n antibiotic groups were screened to assess the susceptibility levels of the isolates. For the testing, commercially available antibiotic disks were employed (Thermo Scientific™ Oxoid™). The antibiotic disks in use were: Ampicillin (10µg), Ceftriaxone (30 µg),

Ciprofloxacin(5 µg), Chloramphenicol (30µg), 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(30µg),Ceftazidime(30µg),Piperacillin-tazobactam(110µg),Amoxcillin-Clavulanic acid (20 µg), Colistin (10 µg), Cefepime.

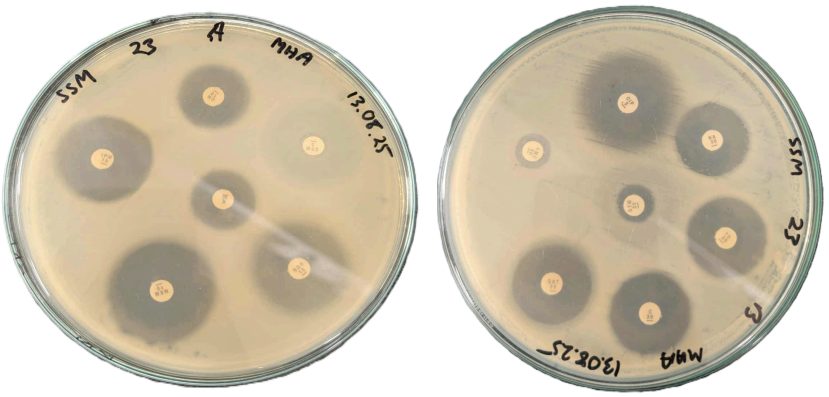

Figure 9: Antibiotic Susceptibility test by Kirby Bauer disk diffusion method

3.4.1 Procedure

The PCR confirmed positive isolates were subcultured in nutrient agar plates and incubated at 37°C overnight to gain freshly grown isolates. In test tubes containing 0.9% normal saline, a loop full of these pure bacterial colonies were suspended and vortexed. The turbidity levels of these tubes were compared to 0.5 McFarland standard and adjusted accordingly. Then, sterile cotton swabs were dipped into these suspensions as required and spread on Mueller Hilton agar (MHA) evenly to acquire bacterial lawns. Then, each of the disks are carefully placed onto the lawned MHA plates with the help of sterile forceps to allow proper diffusion. These plates were then labelled, stacked and incubated at 37°C for 18 to 24 hours. Afterwards, the plates were examined to measure the diameter of the inhibited clear zones found due to bacterial growth, recorded in millimetres. The antibiotics were observed at specific concentrations per disk and the critical values for each one are listed in figure 9. Multi-drug resistant (MDR) strains were interpreted as isolates portraying resistance to a minimum of one agent in three or more categories. (need a reference here). Extensively-drug resistant (XDR) strains were categorised as those resistant to all but two or lesser antibiotics (need a ref here). The detailed list of antibiotics, their suitable groups, potency and their interpretation

criteria are listed in table n. This research provides insights into the patterns of susceptibility of *Escherichia coli* isolates and emphasises the levels of resistance to clinically noteworthy antibiotics.

3.4.2 Interpretation

In accordance with the data of the diameters that define the inhibition zones for each of the antibiotics tested, the isolates were characterised as susceptible, intermediate or resistant as per CLSI guidelines.

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
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

Figure 10: List of the antibiotics that were used.

3.4.3 PCR Amplification for Detecting Antibiotic Resistant Genes

PCR evaluation was undertaken on PCR positive isolates to detect extended-spectrum beta lactamase (ESBL) genes as well as carbapenem resistance genes. The objective of this molecular analysis was to classify the isolates in groups of multidrug resistance (MDR) and extensive drug resistance (XDR). The ESBL genes examined included *blaTEM, blaCTX,* and *blaSHV.* The carbapenem resistant genes targeted were *blaNDM, blaVIM* and *blaKPC.*

Types of primers	Target gene	Primer name	Sequence	Product size	Initial denaturation (©), Time (min/sec)	Denaturation (©) Time (min/sec)	Annealing (©), Time (min/sec)	Extension (©) Time (min/sec)	Final Extension	Segment	Reference
ESBL primers	Bla*TEM*	TEM-F	F-AAAATTCTTGAAGACG	1080bp	95©, 3min	95©, 30s	51©,30s	78©, 7min	72©,10min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	(Sharma et al., 2010)
		TEM-R	R-TTACCAATGCTTAATCA								
	Bla*CTX-M*	CTX-M-F	F-ACGCTGTTGTTAGGAAGTG	759bp	94©,5min	94©,30s	58©,30s	72©,30s	72©,7min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	(Altayb et al., 2020)
		CTX-M-R	R-TTGAGGCTGGGTGAAGT								
	Bla*SHV*	SHV-F	F-TACCATGAGCGATAACAG	450bp	94©,5min	94©,30s	58©,30s	72©,30s	72©,7min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	(Doosti et al., 2015)
		SHV-R	R-GATTTGCTGATTTCGCTCGG								
Carbapenem resistant gene primers	Bla*KPC*	KPC-F	F-CATTCAAGGGCTTTCTTGC	498bp	95©,5min	95©,50s	57©,30s	72©,40s	72©,10min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	Mushi et al., 2014)
		KPC-R	R-ACGACGGCATAGTCATTT								
	Bla*NDM*	NDM- F	F-GGTTTGGCGATCTGGTTTT	624bp	94©,5min	94©,30s	58©,30s	72©,30s	72©,7min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	(Shoja et al., 2016a)
			R-CGTGTTTGGTCGCATATCG								
	Bla*VIM*	VIM-F	F-GGTGTTTGGTCGCATATCG	501bp	95©,5min	95©,45s	60©,45s	72©,1min	72©,10min	Segment 1=initial denaturation, Segment 2 (35 cycles) =denaturation, Segment 3 = final extension	(Shoja et al., 2016b)
		VIM-R	R-ATTCAGCCAGATCGGCAT								

Figure 11 : List of Primers used in ESBL gene detection

Chapter 4

Result

The study intended to detect the presence of fecal coliforms in household water samples and isolation of *Escherichia coli,* the bacterium accountable for a range of enteric and extraintestinal infections depending on its pathogenicity, starting from diarrhoea, urinary tract infections (UTIs) and meningitis in serious cases. A total of 69 samples were collected, 43 from the North of Dhaka such as Agargaon, various areas of Mohammadpur, Bashundhara Residential Area, Banani, Gulshan, Tejgaon, Badda, Banasree and 26 from the South of Dhaka such as Elephant Road, Central Road, Mogbazar, New Market, Shahbag, Puran Dhaka and Wari. Starting from morphological analysis through culture techniques to molecular experiments were used to confirm the presence, pathogenesis and antibiotic resistance of *E. coli.*

4.1 Fecal Coliform Count in CFU/mL

After proper collection and sampling, 100 ml of each sample were taken and filtered using a membrane filter of 0.22 µm pore size. This filter paper was placed on M-FC agar plates and incubated at 44°C for 18 to 24 hours for appropriate growth conditions. Post incubation, fecal coliforms were calculated in terms of colony forming units per millilitres (CFU/ml). Out of the 69 samples examined, 56 (81.16%) were confirmed to be fecal coliforms among which 32 (74.41%) were from North Dhaka and 24 (92.3%) were from South Dhaka. Among these areas, fecal coliform contamination was the most prominent at Iqbal Road, one distinct area of Mohammadpur at the North and in case of South, the same trends were observed at the New Market area. The analysis of FC count portrayed a remarkable distinction relatively between the North and South of Dhaka. In terms of North, areas that met the standards for safety limits of water according to WHO were Gulshan, Adabor and Niketon at 0 CFU/100 ml whereas some areas crossed the safe limits significantly such as Tejgaon (51 CFU/100 ml), Hatirjheel (40 CFU/100 ml) and Banani (32 CFU/ml). In the South side, areas that had no contamination were Dhanmondi, Mogbazar and Noyapara, Puran Dhaka at 0 CFU/100ml. The area that posed the highest occurrence of fecal coliforms was Central Road with colony counts of 29 CFU/100ml and 14 CFU/100ml. The locations that had the greatest health risks portraying TNTC (Too Numerous to Count) was New Market on the Southern and Iqbal Road on the Northern side. (Figure 11)

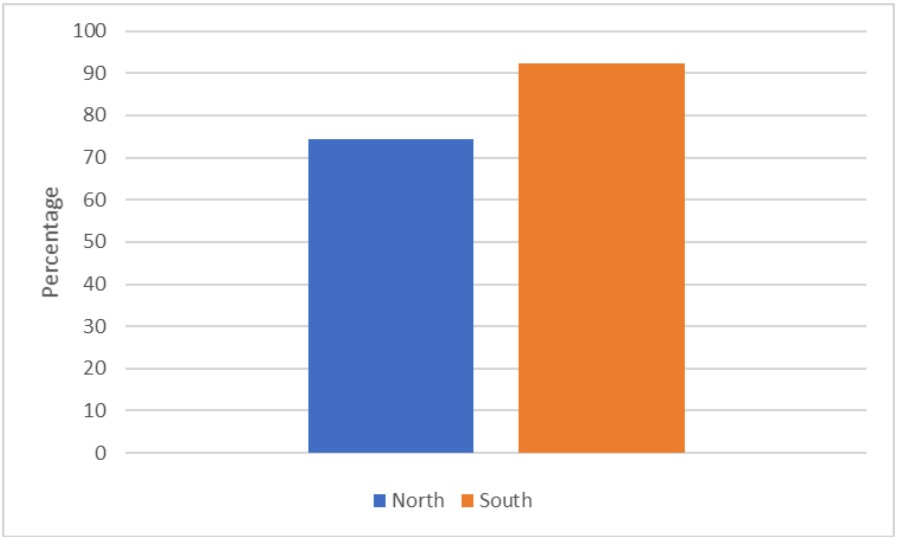

Figure 12 : Percentage of Fecal coliforms found in north and south

4.2 Results of the Spread Plating and Sub-Culture Method

Similar to M-FC plating, followed by sampling, another membrane filter of 0.22 µm pore size was used to filter each of the samples. This filter paper was transferred to falcons consisting of EE broth which were then incubated at 37°C for 18 to 24 hours to allow enrichment. Post incubating, the falcons with the color changed to yellow indicated positive *E. coli* growth. From these presumed positive falcons, 100 µL enriched bacterial suspension was pipetted and plated onto TBX agar medium following the spread plate technique using sterile glass spreader. These plates had an incubation period of 37°C for 24 hours which resulted in blue to blue-green positive colonies in case of *E. coli* presence. Among the 69 samples examined, 30 (43.47%) were culturally confirmed to be positive based on the morphology. Further justification of the presence was carried out on UTI and MacConkey agar medium which also led to positive outcomes. (Figure 6

4.3 Molecular Detection of ***E. coli***

The total of 30 samples with presumptive confirmation on TBX, UTI and MacConkey agar were further subjected to PCR which yielded a ratio of 53.33% (16) confirmed samples. The suspected isolates were run through PCR cycles at appropriate conditions and then observed under UV following the method of gel electrophoresis which yielded band sizes of 585 base pairs. This displayed that 16 out of 30 samples were conclusively decided to be *E. coli* based on PCR evaluation. (Figure 12)

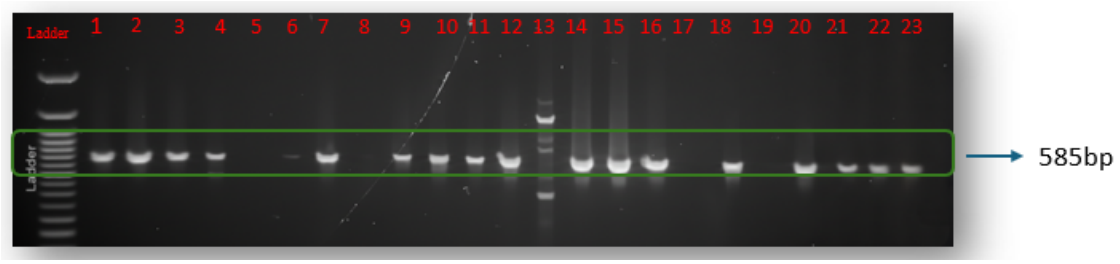

Figure 13 : Agarose gel electrophoresis of PCR assay of E. coli isolates. Here, the ladder is a 50 bp DNA marker, and the lanes with the clearer bands are some positive samples at 585 bp.

4.4 Antibiotic Susceptibility Patterns of Isolates

When we tested a range of antibiotics against these bacterial isolates, we got a crystal-clear picture of which ones are still effective and which ones have lost their punch. The results show a dramatic split: a few drugs are still good enough while many others, especially some of the older ones, are now consistently failing. The most glaring issue was the massive resistance seen in common antibiotics, highlighting the risk of just guessing which drug to

use.For instance, Ampicillin was almost completely ineffective, failing a staggering 91.9% of the time. Cefixime wasn't far behind, proving useless in about 87% of cases. On the flip side, we have some real heroes. Piperacillin-Tazobactam was the standout star, working beautifully with only a tiny 2.7% resistance rate. Ciprofloxacin also performed exceptionally well, succeeding over 94% of the time (Figure 13).

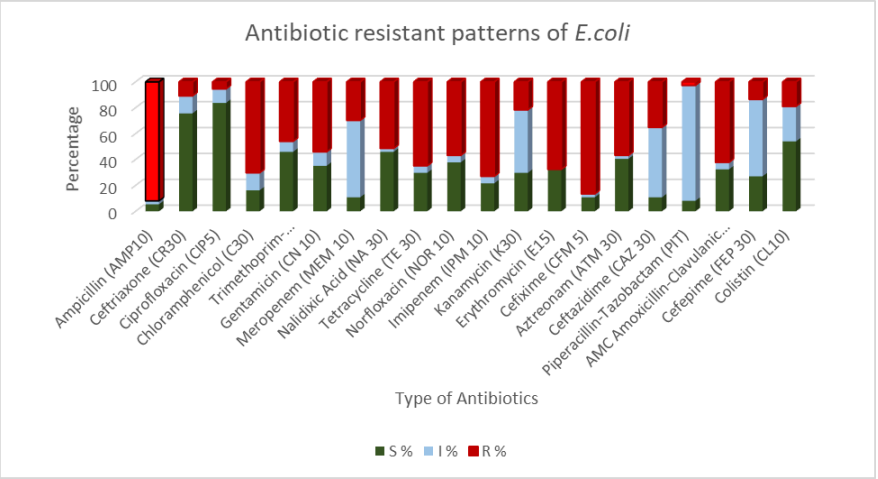

Figure 14 : Antibiotic resistant patterns of *E. coli*

The group-wise antibiotic resistance analysis reveals several important trends. In the Penicillin group, Ampicillin showed extremely high resistance at 91.9%, while Piperacillin-Tazobactam had almost no resistance (2.7%) and Amoxicillin-Clavulanic Acid exhibited high resistance at 62.2%. The 3rd Generation Cephalosporins demonstrated varied results, with Ceftriaxone having low resistance (10.8%) while Cefixime was largely ineffective (86.5%). The 4th Generation Cephalosporin, Cefepime, showed minimal resistance at 13.5%. In the Carbapenem group, Meropenem had moderate resistance (29.7%), but Imipenem showed very high resistance at 73.0%. The Aminoglycosides Kanamycin and Gentamicin showed moderate to high resistance, with values of 21.6% and 54.1%, respectively. In the Quinolone group, Ciprofloxacin was highly effective with only 5.4% resistance, while Norfloxacin (56.8%) and Nalidixic Acid (51.4%) displayed high levels of resistance, indicating a decline in their effectiveness. Overall, the data highlights significant resistance against some antibiotics, particularly Ampicillin and Imipenem, while others like Piperacillin-Tazobactam, Ciprofloxacin, and Cefepime remain largely effective. (Figure 13)

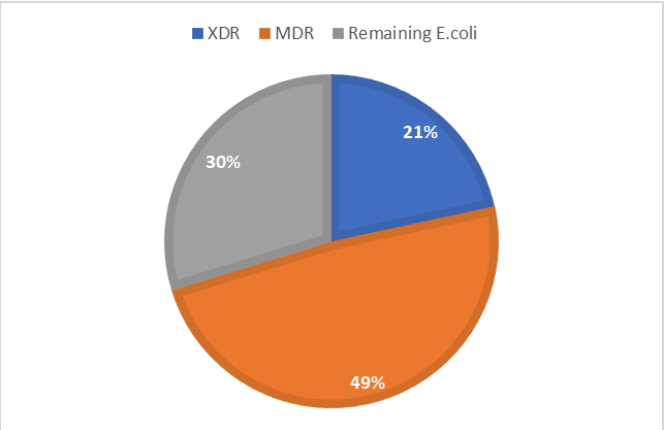

Figure 15 : Percentage of XDR and MDR in contrast to total Isolates.

For macrolides, penicillins, aminoglycosides, quinolones, tetracyclines, phenicol sulfonamides,polymyxins and monobactams 100% of strains are classified as MDR. Compared to third-generation and fourth-generation cephalosporins and carbapenems show no MDR strains. Additionally, 100% of strains are XDR for macrolides and penicillin. Total 49%. isolates were identified as MDR and 21% isolates were classified as XDR. (Figure 14)

4.5 Confirmation of Antibiotic Resistant Genes

4.5.1 ESBL

PCR analysis using specific primers targeting ESBL producer genes demonstrated that the majority of the isolates carry *blaCTX-M* at 29.4%, followed by *blaSHV* gene at 23.52% whereas *blaTEM* yielded no significant bands. The outcome suggested that CTX-M category of betalactamase producers were the most prevalent among the samples.

4.5.1.1 CTX-M

Under proper conditions of gel electrophoresis with UV, the isolates carrying *blaCTX-M* were observed as the band size of 759 base pairs. (Figure 15)

Figure 16: Agarose gel electrophoresis of PCR assay of CTX-M gene. Here the DNA ladder lane is 50 bp DNA marker, and the marked lane are some positive samples band at 759 bp.

4.5.1.2 SHV

The presence of *blaSHV* was ensured due to the band size exhibition of 450 base pairs through gel electrophoresis. (Figure 16)

Figure 17: Agarose gel electrophoresis of PCR assay of blaSHV gene. Here the DNA ladder is a 50 bp DNA marker, and the marked lanes with the clear bands are positive samples band at 450 bp.

4.5.1.3 TEM

The band size for *blaTEM* is expected at 1100 base pairs under UV which was not detected for the isolates in this study.

4.5.2 Carbapenem Resistant Gene

The genetic confirmation of carbapenem resistant genes were carried out through primer specific PCR which concluded the occurrence of *blaNDM* and *blaKPC* at 2.9% whereas *blaVIM* producers were at n%. This depicted a similar ratio of NDM and KPC genes in the isolates tested.

4.5.2.1 NDM

1 isolate was positive for NDM was obtained at a band size of 621 bp through gel electrophoresis. (Figure 17)

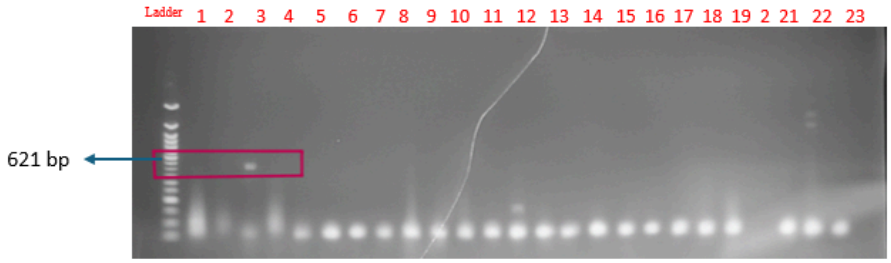

Figure 18: Agarose gel electrophoresis of PCR assay of blaNDM gene. Here the DNA ladder lane is 50 bp DNA marker, and the marked with the single band is a positive band at 621 bp.

4.5.2.2 KPC

Isolates were observed at the band size of 498 bp for the KPC gene under UV. (Figure 18)

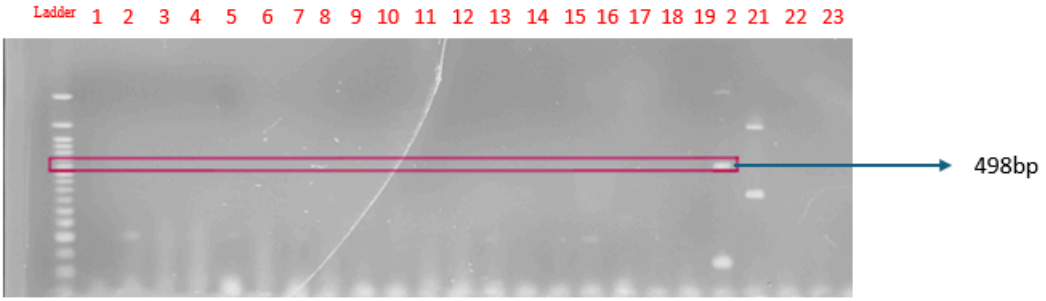

Figure 19: Agarose gel electrophoresis of PCR assay of blaKPC gene. Here the DNA ladder lane is 50 bp DNA marker, and the marked lane with the single band is a positive band at 498 bp.

4.5.2.3 VIM

No isolates were found to be positive for *E. coli* carrying *blaVIM* migrated to the band size of 501 base pairs in gel electrophoresis.

Chapter 5

Discussion

Water is a prerequisite for human health and survival fundamentally, however, a significant amount of the world's population are unable to access potable water as well as usage in the households due to lack of maintenance and quality assurance. The degradation of water quality due to negligence may lead to a range of infectious diseases imposing varied health risks. Water borne diseases are not uncommon in such areas due to easier access of pathogens to grow and thrive in the conditions provided. The World Health Organization gives an estimate of over 2 million people who are deprived of safe water and further related to water-borne pathogens oriented health dangers as well as a forecast of 5% of all deaths due to the same reasons in developing nations, emphasising effective monitoring and interventions (Iza and Ogwu, 2025). According to the official WHO website, for 2019 stated that 1.4 million deaths could be prevented through WASH services with a stark 69% of diarrheal deaths. This study focused on the detection and harmful ranges of Escherichia coli, an organism which is a frequent cause of enteric symptoms ranging from minimal to potent. For instance, virulent genes related to 52% pathogenic E. coli strains such as avian pathogenic E. coli and enteroaggregative E. coli were found due to rainfall driven contamination of water sources that deemed the water unfit for consumption (Rabiu et al, 2022). Moreover, multidrug resistance in E. coli is increasingly becoming a serious concern recorded not only in humans but also in veterinary medicine worldwide, the most problematic mechanism being the accumulation of genes that code for extended-spectrum betalactamases that confer resistance to broad-spectrum cephalosporins and carbapenemases resistant to carbapenems. (Poirel et al, 2018). Based on these water quality concerns associated with the effects of E. coli contamination, this study investigated presence of the target organism as well as the antibiotic resistance detailing ESBL phenotypes and the key ESBL genes in the samples

taken from the households. The study also looked into fecal contamination in the samples which act as a parameter of water safety, indicating the probable existence of detrimental pathogens ranging from virus, parasites or other bacterial classes. This study culminated 81.16% of the samples with fecal coliform presence (CFU/ml). Moreover, among all the suspected samples, 53.33% were confirmed to be Escherichia coli. Findings similar to this were found in a study conducted in Dhaka, Bangladesh to screen the virulence factor and genetic diversity of E. coli which yielded 63% positive outcome (Talukder et al.,2013). Conversely, a recent study portrayed only 14% of water E. coli contamination while investigating the microbiological safety of widely consumed water (Islam et al., 2022). However, this only enlightens how recent conditions may have worsened according to the finding this research concluded. In a broader perspective in Bangladesh, research carried out in the rural Matlab-sub district of Chandpur concluded 82% of household-level water samples to be contaminated with E. coli depicting unsafe water handling and storage practices (Rownak et al., 2022). Another study entails 92.5% household water supply contaminated with E. coli conducted in 24 different sub-districts (upazilas) in rural Bangladesh (Ali et al., 2016). Such a stark ratio suggests the water safety levels in need of dire attention and stricter monitoring in such areas outside of Dhaka as well. The proportion of fecal contamination as well as presence of E. coli exceeding regular limits signals towards direct risk to human health in terms of consumption. The dangers become more prominent when these strains illustrate resistance to a number of antibiotics which not only may cause serious enteric infections but also intervene in effective treatment plans which involve usage of these broad spectrum antimicrobial agents. At present, there are no established vaccination programs designed for enteric infections caused by E. coli or any specific treatment workflow targeting the common symptoms, meanwhile antibiotics are going in vain due to the increasing trend of multidrug resistant strains (MDR). Spread of these resistant strains through municipal water

is an alarming threat as this water is easily accessible to humans and is always in direct contact and usage. In this study, the isolates tested were resistant to ampicillin at a rate of 91% belonging from penicillin group, chloramphenicol at a rate of 70%, third generation cephalosporin cefixime at 86.5%, fourth generation cefepime at 13.5% and erythromycin from the macrolide group at 67%. However, Piperacillin-Tazobactum from the Penicillin group portrayed about 2.7% of resistance whereas such low proportion was also evident in case of ciprofloxacin related to the quinolone group at 5.5%. In terms of the carpenems, 73% of the isolates were resistant to imipenem and 29% were resistant to meropenem. Similar to this study, a study focusing on household well water in Zaria, Nigeria found 82.1% ampicillin resistance, 89.3% erythromycin resistance and a similar third generation cephalosporin called ceftriaxone at 21.4% among all their E. coli isolates (Dahiru et al.,2019). Furthermore, study from Gurugram, India sampling drinking water from household and public sources reported moderate resistance to carbapenem as observed in this study where E. coli isolates were equally resistant to imipenem and meropenem at 4.17% (Jha et al., 2024). Though this study portrays significantly higher rates of carbapenem resistance. Resistance towards third and fourth generation cephalosporin observed signal towards massive alarms as these broad spectrum antibiotics are vital to treat severe infections such as pneumonia and complicated UTIs and without these, the last dependable option becomes carbapenems, risking overuse. The fact that carbapenem resistance is also rising, although comparatively less, may leave no effective broad term solutions at this rate. Such statistics highlight the immediate need of improvements in overall water quality starting from supply to storage as well as extremely cautious usage of antibiotics in safer boundaries as municipal water supply is a major transmission route. Moreover, in this study, 49% of isolates were found to be multidrug resistant (MDR) and 21% were confirmed to be extensively drug resistant (XDR). Such multidrug resistance (MDR) was also found in 59.2% E. coli isolates collected from Dhaka in

terms of antibiotic profiling (Talukdar et al., 2013). In another research assessing various drinking water sources throughout Bangladesh, 91.3% E. coli isolates carried MDR whereas 18.7% carried XDR (Parvin et al., 2021). In this study, CTX-M gene was detected in 11.8% of the isolates, 32.35% carried SHV gene while all of them tested negative for TEM gene , all of which are illustrative of ESBL (Extended Spectrum Beta Lactamase) genes, SHV gene being the most pervasive. In case of carbapenem resistance genes, 2.94% isolates were confirmed to have the NDM and KPC gene whereas the VIM gene was not found. A previous study on 14 specific MDR E. coli strains concluded the presence of CTX-M at an overwhelming majority of 92.9% (13 out of 14 isolates) whereas SHV was found in only 7.1% (1 out of 14 isolates) (Tudorache et al., 2022). The data contrasts this study as in this case, SHV gene presence is proportionately higher than CTX-M. In another study judging the community wastewater of Dhaka, Bangladesh, the most frequently observed gene was blaSHV at 13.9%, followed by blaNDM-1 at 8.9% and lastly blaCTX-M at 7.6% (Hossain et al., 2024). This recent study aligns with the findings of this research which portrays the current trends of the E. coli strains in terms of illustrating resistance. In contrast, 64.7% of blaTEM carrying isolates were found from drinking water sources of Iran, indicating high resistance to beta-lactam antibiotics although dissimilarly TEM was not visualised as an indicator in this study whereas blaVIM represented carbapenem resistance at 11.7% which aligned (Momtaz et al., 2013). Besides, a study in Haryana, India tested drinking water samples negative for blaKPC (Jha et al., 2024). Meanwhile, a multi-drug resistant study in the Tanga coastal region of Tanzania laid out mediocre prevalence for the KPC gene in environmental water samples at 7.7% (Mbwambo et al.,2023). The trend of mediocre prevalence correlates with this study while the data on absence of KPC indicate that depending on the sample intake, region and the strains under assessment, the detection of genes vary yet give a complete profile of antibiotic resistance as per ESBL and

carbapenemase grouping. To summarise, this research accentuates public health concerns related to unsafe usage and consumption of water due to presence of antibiotic resistant Escherichia coli in Dhaka's supply. Remarkable presence of fecal coliforms indicating contamination of the water as well as identification of extensive resistant strains means higher likelihood of waterborne diseases, especially for those who are susceptible, living in vulnerable conditions. Such growth in resistance causes the treatment of common infections difficult and costly as the organisms carrying these genes cause therapeutic programs to be unsuccessful, prolonging illness and leading to greater mortality rate as well as the necessity of drugs with higher potency as the last resort that comes with side effects. This increasing challenge is called into attention in research to deliver that contaminated water can be a crucial medium of spread which in turn calls for an integrated and multidisciplinary system of environmental monitoring, betterment of water and sanitation infrastructure as well as collaboration of "One Health" attempts that brings animal, human and environmental health sectors in unison to effectively regulate the risks of antimicrobial resistance (Sharma et al., 2023).

Chapter 6

Limitations, Prospects and Conclusion

6.1 Limitation and Prospect

The research conducted comes with a range of limitations that require intervention in order to optimise the findings and inferences. To begin with, the low quantity and imbalance of sample intakes questions the impact of the findings. Increased sampling from each area in multiple takes might allow extensive apprehension of municipal water adulteration. While the

sampling did reach both the North and South of Dhaka, there is stark distinction of sample numbers comparatively and the unequal distribution restricts the study to make an all encompassing conclusion. Further expansion of geographical coverage as well as sample intake would represent validated data. The unequal allocation applies to seasonal variation as well, as different trends were observed when the results of winter and summer sampling were compared inferring how temperature, atmosphere and other environmental factors influence contamination levels. However, lack of sample size restricts conclusive analysis of these fluctuations. Besides, for indisputable statements, further study of such focus should not only explore districts outside of Dhaka but also consider seasonal variation as one of the key variable factors to understand the water quality parameters.

Additionally, the study could not cover all aspects of antibiotic susceptibility as only a few major ESBL and carbapenem resistant genes were examined. In order to recognize and dive into the entire genetic and resistant profiling of the contaminants, a broader spectrum of susceptibility testing is necessary.

Furthermore, due to time constraints, this study was unable to detect the specific pathogenic strains of *E. coli* which is a significant step in understanding the correlation of virulence and public health consequences. The basic molecular detection by PCR is an effective tool for confirmation but the study requires primer specific assays further to classify the pathogenic strains. The assessment of pathogenic strains requires an integrated work flow of PCR, culture based techniques, toxin assays to whole genome sequencing for true verification of the target organism which would assure none of the variants are overlooked, offering a clear view of the health implications.

Besides, the study did not use biochemical tests for confirmation and validated the findings initially with a three step culture based approach which may appear as a gap in the workflow.

It also did not concentrate on some of the key techniques of identification such as immunological tests. For fruitful and confident outcomes of research in the future as well as elaborate and precise portrayal of household water hygiene, safety levels and health concerns related to it, these limitations need to be conveyed and managed.

6.2 Conclusion

To conclude, this particular research accentuates crucial public health dangers of antibiotic resistant *Escherichia coli* in Dhaka city from municipal water supply. Excessive fecal coliform proportions of 81.16% in the collected samples suggest severe hazard of waterborne infections due to high risk of contact with microorganisms through water, specifically for exposed demography. The discovery of multidrug resistant strains (MDR) of *E. coli*, particularly those consisting of extended spectrum betalactamase (ESBL) and carbapenemase genes such as *blaCTX-M, blaVIM or blaKPC* refers to the fact that treatment options are getting constricted and the current therapeutic regimens are losing efficacy. The confirmed isolates were proportionately detected as n% of MDR and n% of XDR which implies an immediate need of an integrated system to upscale water sanitation and hygiene management alongside greater focus on infection control which should be a prerequisite to supplying potable water to the mass. The shortcomings of this research starting from lack of sample intake at an adequate quantity or deeper seasonal evaluation call for extensive investigation further for improved apprehension of not only Dhaka's water supply and quality but also the whole of the country. Intense, prioritised monitoring and interventions for the issue keeping public health agendas in conscience is vital to tackle the consequences of antibiotic resistance, halt the increasing trend of AMR oriented life risks and fundamentally, provide safe, hygienic water to the general public.

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