

Prevalence of Carbapenem-Resistant *Escherichia coli* in the Drinking Water System in Dhaka

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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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Ethics Statement

There are no conflicts of interest in the entire research project. There was no use of a human or animal model in this experiment.

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Abstract

Water pollution could cause serious health hazards and Dhaka, Bangladesh is one of those heavily populated cities that are considered harmful effects related to health. This research evaluated the microbial quality of water across 11 zones of Dhaka with specific focus on detection of *Escherichia coli* and its antibiotic resistance capabilities including that of carbapenems, antibiotics that are now a last option in treating severe infections associated with multidrug-resistant species of Enterobacteriaceae. The emerging incidence of carbapenem resistant isolates has critically reduced the options of treatment, which has now become a major public health challenge.

A total of 200 water samples; tap, boiled and filtered; have been collected over the period October 2023-April 2024. Of the 410 bacterial isolates obtained, 103 were identified as *E. coli*. April had the highest average *E. coli* count in tap water at 50%. In contrast, January recorded the lowest average count for tap water at 3.3%. Overall, tap water consistently showed higher contamination levels than boiled and filtered water across the months. For example, in April, tap water had an average of 50%, while boiled water only had 13% and filter water was 19%. This trend indicates that tap water is generally more contaminated than the other types. Moreover, since all media contained meropenem with ZnSO₄ and *E. coli* still exhibited growth, the season-wise resistance pattern was found to be 91% in summer, 75% in fall, and 41% in spring. This indicates that the isolates show the highest resistance pattern to meropenem during the summer season. Antibiotic susceptibility testing revealed that all *E. coli* were found 100% resistant to Ampicillin and Amoxiclav, while 91.43% were resistant to Meropenem. In contrast, the isolates showed notable sensitivity to Ceftazidime (94.29%) and Tetracycline (82.86%).

PCR analysis of carbapenem genes, *bla*NDM-1 and *bla*OXA-2, was done in 35 *E. coli* isolates. The results provide critical evidence of multidrug-resistant *E. coli* presence in the water system of Dhaka which highlights a need for increased water treatment systems, strict regulations on antibiotic campaigns, as well as a public health program to control the spread of antimicrobial resistance.

Keywords: Antibiotic Resistance, Multidrug-Resistant *E. coli* (MDR), *Escherichia coli*, Carbapenem Resistance, Water Contamination, MAR Index, High-Risk Locations, Environmental Factors, Water Treatment Practices, Public Health Risk

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List of Acronyms:

E. coli	<i>Escherichia coli</i>
TSB	Tryptone Soya Broth
XLD	Xylose Lysine Deoxycholate
NA	Nutrient Agar
MHA	Mueller Hinton Agar
AST	Antibiotic Sensitivity Test
PCR	Polymerase Chain Reaction
NTC	No Template Control
bp	Base Pairs
CFU/ml	Colony Forming Units per milliliter
MAR	Multiple Antibiotic Resistance
MIC	Minimum Inhibitory Concentration
CRE	Carbapenem-Resistant Enterobacteriaceae
CPE	Carbapenemase-Producing Enterobacteriaceae
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
KPC	Klebsiella pneumoniae Carbapenemase
IMP	Imipenemase
ESBLs	Extended-Spectrum Beta-Lactamases
PBPs	Penicillin-Binding Proteins

Chapter 1:

Introduction

1.1 Water Contamination and Health Risks in Bangladesh:

Water as life and most of the vital resources required to sustain all living beings and perform regular day-to-day activities. Approximately, Bangladesh is a country with nearly 160 million inhabitants, out of which 13% are without safe drinking water access and 39% do not have adequate sanitation facilities. Inaccessible clean water and sanitation facilities have adverse effects that contribute to a high burden of waterborne diseases as pathogens, including those that are antibiotic resistant, are disseminated [1]. Fecal contamination usually harbors diverse waterborne pathogens, including *Escherichia coli* (*E. coli*). The organism is generally an endogenous organism of the human gastrointestinal tract and thus it is regularly used as an indicator organism while testing water quality. Specifically, its occurrence in drinking water usually indicates fecal contamination, which can cause many diseases that are subsequently transmitted through water. Water samples often contain *E. coli* that are accepted as confirming the presence of other pathogenic microorganisms in them. However, the most disturbing aspect of such infections is the role of antibiotic resistance. Several strains of *E. coli* developed resistance to most commonly used antibiotics and even to those which are considered the last option with lethal infections. It is a growing problem for concern across the globe and renders all the more difficult the management of waterborne diseases, particularly where access to healthcare and sanitation is poor. Water sanitation systems for risk areas of contamination should help manage waterborne diseases, including those involving antibiotic-resistant infections. Increased individual benefits in improvements to wastewater treatment, public health education, and monitoring systems will cumulatively add to lessening human exposure to acquiring harmful pathogens at the water sources including *E. coli*. The following table shows different levels of fecal contamination [2].

Count per 100 ml	Category and color code	Remarks
0	A(blue)	In conformity with WHO
1-10	B(green)	Low risk
10-100	C(Yellow)	Intermediate risk
100-1000	D(Orange)	High risk
> 1000	E(Red)	Very high risk

Table 1: Illustration of category and color-code scheme for thermo-tolerant (fecal) coliforms or *E. coli* in water supplies [3]

The table classifies the degree of fecal contamination of drinking water by the count of *E. coli* per 100 ml by color codes that reflect the health risk involved. Category A (Blue), 0 *E. coli* per 100 ml: complies fully with WHO guidelines and therefore is safe to drink. Category B (Green), 1-10 *E. coli*, are a low-risk category but must continue to be regularly monitored. Category C (Yellow) has 10-100 *E. coli* and is an intermediate category, which is risky and

may create health hazards to people using it, particularly with vulnerable groups. It should be treated prior to use. Category D (Orange) indicates high risk with 100-1000 *E. coli*. The water in this category is potentially capable of causing gastrointestinal ailments; therefore, treatment action is necessary right away, along with seeking out alternative sources of water. The last category is Category E-Red with over 1000 *E. coli*, indicating very high risk where the water is so contaminated and must not be contacted because of the high possibility of serious health consequences; urgent intervention will be needed to make it safe. These are the categories that are in use for assessment and management of safety of water, therefore helping guidance of decisions on public health.

1.2 Possibilities of infections via drinking water contamination:

There are several varieties of *E. coli* in water supplies that eventually can become major health disorders, such as diarrhea, renal failure, and meningitis. In addition, two other variants of Shiga toxins comprise Stx1 and Stx2 in the infection by several pathogens like *Shigella dysenteriae* and Enterohaemorrhagic *E. coli*-endemic variety, EHEC [4]. About 45,000 children in Bangladesh die of waterborne diseases every year due to the lousy water quality that in general terms originates from sewage or animal waste [5]. Some of the most important waterborne pathogens are listed in the following table.

Bacteria	Possible health effects
<i>Aeromonas hydrophilia</i>	Variety of diseases like septicemia, gastroenteritis in young children and elderly
<i>Enterobacter aerogenes</i>	Infections in urinary and respiratory tracts
<i>Enterococcus species</i>	Infections in urinary tract
<i>Escherichia coli</i>	Food poisoning, gastroenteritis and UTI
<i>Klebsiella species</i>	Cause nosocomial contaminations in the urinary tract, respiratory tract, lung, wound destinations.
<i>Listeria species</i>	Meningitis, endocarditis with high mortality rate
<i>Pseudomonas aeruginosa</i>	Diseases in newborns and elderly patients
<i>Salmonella species</i>	Typhoid
<i>Shigella species</i>	Abdominal pain, tenesmus, watery diarrhea
<i>Staphylococcus species</i>	Superficial skin lesions, food poisoning

Table 2: List of bacteria reported in drinking water in Bangladesh and their possible health effects: [6]

1.3 Water borne disease in Dhaka:

In the past few decades, Bangladesh made great strides in the improvement of the situation with regards to the access to safe water, however only 41 percent of the population, approximately 1.8 million individuals out of 165 million [7], had access to adequate safe drinking water in 2023. This consensus level is not sufficient as the country is currently addressing a high demand in Dhaka, its capital city, where the rapid increase of the population creates an acute shortage of safe drinking water. Having made significant investments into the development of the water supply system, safe and sanitary water sources like shallow tube wells and piped water have been integrated into most households with improved and safe sources alternative options like rivers, canals, dug wells, water collection, and vendors being utilized to meet the households' daily water requirements [8],[9]. These sources, in turn, are all facing growing risks of contamination due to poor sanitation, industrial waste, and agricultural runoff. This has increased the amount of fecal coliform and other injurious bacteria [10] with various adverse health impacts. [11], [12], [13], [14]. Use of the contaminated water from both safe and unsafe sources will result in different types of waterborne diseases like diarrhea, cholera, jaundice, typhoid, hepatitis, etc [15]. It is responsible for approximately 3.5 million annual fatalities worldwide, making it one of the leading causes of mortality across the world [16].

Several strains of *E. coli* in drinking water are the causes of diseases that threaten life, including diarrhea, renal failure, and meningitis. In a cohort study that was carried out among 884 children aged less than 5 years in Dhaka, Bangladesh found that children with feces found on the household compound had significantly higher odds for diarrhea, with an odds ratio of 1.71 (95% confidence interval: 1.23–2.38). Specifically, children whose household source drinking water had *E. coli* counts over 100 colony forming units/100 mL were at significantly increased odds of diarrhea, OR: 1.43; 95% CI: 1.06–1.92. Feces on the compound and source drinking water with over 100 colony forming units/100 mL *E. coli* were leading risk factors for diarrheal illness among the < 5-year-old children in slum areas of Dhaka, Bangladesh. These findings suggest an urgent need for comprehensive interventions to reduce fecal contamination on the compound in such households to protect vulnerable pediatric populations [17].

1.4 Antibiotic resistance of bacteria found in drinking water:

The development of antibiotic resistance emerged since the discovery of antibiotics, fully because bacteria have an exceptional ability to adapt to selective pressure. Bacteria can easily mutate, acquire genes by horizontal transfer, and gather mechanisms that enable them to survive when exposed to harmful conditions. Over time, resistance has been developed against all antibiotics; recently, carbapenems have fallen within this category. In the spreading of carbapenem resistance, human-related factors will be predominant.

These factors include:

a) The excessive prescription of antibiotics and the selling of these drugs to the public without any regulation in countries with weak regulatory mechanisms;

b) Poor infection control in health care when resistance to carbapenem class of antibiotics takes place; and,

c) Use of low-quality and inappropriate dosing of antibiotics for promoting growth in agriculture.

In addition, most areas of Bangladesh lack proper sanitation and clean water supply mechanisms with appropriate infrastructure, which predisposes toward contamination and propagation of resistant bacteria in water bodies.

Dhaka city, which is home to over 22.4 million citizens, also faces the dual challenge of providing adequate sanitation facilities to its citizens and increasing water pollution in municipal wastewater. This is partly because of rapid urbanization, poor sewage treatment, and inappropriate disposal practices, all contributing to the contamination of water bodies and thus offering ideal conditions for the multiplication of the pathogenic bacteria. Complications set in when these bacteria emerge resistant to multiple antibiotics, raising many questions concerning conventional treatment methods and public health implications. Furthermore, antibiotic resistance in municipal wastewater is more important as it would be directly linked to human households, representing the main contributor to contamination. With the rise in population and strategic use of antibiotics, resistant bacteria are fed into wastewater, thereby developing an antibiotic-resistant strain reservoir [\[18\]](#).

In the August 2023 study, two samples from each of the ten neighborhoods in Dhaka represent the municipal wastewater system scattered throughout Dhaka following the International Organization for Standardization (ISO) 2023 guidelines for all the species. All samples, 10 out of 10, revealed positivity for *Vibrio* spp., fecal coliform, *E. coli*, and *Salmonella* spp. The highest concentration of TVC, total *vibrio* spp., and total fecal coliform, total *E. coli* count, and total *Salmonella* spp. were present in Uttara 9×10^4 CFU/ml), (1.Bangshal (1.8×10^2 CFU/ml), and Lalbag (2.1×10^3 CFU/ml), Mirpur (3.70×10^2 CFU/ml), and Lalbag (6×10^2 CFU/ml) respectively [\[18\]](#).

Antimicrobial resistance of the selected *E. coli* isolates was studied against various antibiotics in this study, as represented in the figure below. The organisms demonstrated high sensitivity for Meropenem (MEM10) and Cefotaxime (CTX 30) with an 80% and 60% susceptibility rate, respectively, while these organisms demonstrated significant resistance against the drugs like Cefuroxime (CXM-30) and Ampicillin (AM10) as 50% and 50%, respectively. Certain antibiotics, such as Cefotaxime (CTX30) and Cotrimoxazole (COT-25), demonstrated an intermediate resistance that showed susceptibility at a moderate level. Isolates showed highly marked sensitivity to Erythromycin (E15) and Azithromycin (AZM15), where the rate of resistance was only 10% in each antibiotic.

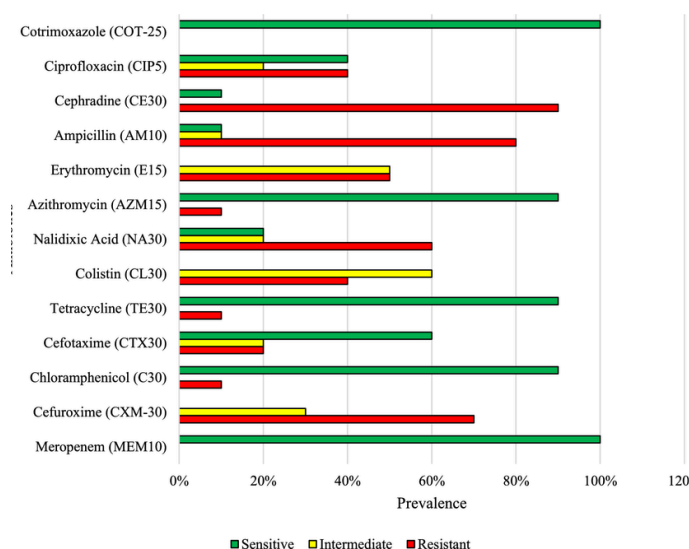


Figure 1: Antibiotic resistance to the selected isolates (*E. coli*) [18].

1.5 Mechanism of antibiotic resistance in bacteria:

Antibiotics are expected to provide service to prevent and/or treat infections from bacteria. Resistance to these drugs occurs when bacteria adapt or change in response to the drugs. Resistance to antibiotics can be naturally acquired or through the acquisition of resistance, also referred to as horizontal transfer [19]. It is acquired mainly in four ways:

1. Impaired Uptake or Absorption of Antibiotics: Generally, antibiotics kill the bacteria by attacking certain specific sites. Here, the bacteria hinder the entry and reach of an antibiotic to its target site.

2. Inactivation of Antibiotics through Bacterial Enzymes: Antibiotics may be chemically modified or degraded by bacterial enzymes-for example, β -lactamase. Resistance genes on the bacterial plasmid encode enzymes such as β -lactamase that cleave the β -lactam ring structure of an antibiotic, such as penicillin, rendering it ineffective.

The β -lactam ring, while intact, is active and effective. But, when in contact with the enzyme β -lactamase, the ring is broken and, therefore, penicillin is not active. The enzyme breaks the C1-N bond of the β -lactam ring and thus deactivates the antibiotic properties of the medicine. Therefore, penicillin is not effective against bacteria. For example, *E. coli* produces the enzyme β -lactamase.

3. Target Site Modification:

If the bacteria cannot inactivate the antibiotic, then the target site is altered to no longer bind the antibiotic and the bacteria survive. For example, the antibiotic penicillin is a β -lactam. Penicillin exerts its action by binding to penicillin binding proteins (PBPs) within the peptidoglycan layer of bacterial cell walls. If the bacteria have genes that encode for altered PBPs, then penicillin cannot bind to the altered proteins and its action is unsuccessful. An

example of this is MRSA, which, through such modifications, has acquired resistance to an antibiotic known as methicillin.

4. Bacterial Efflux pump and poor permeability:

Resistance of bacteria to antibiotics is facilitated by active efflux mechanisms, involving efflux pumps, and by cell permeability reduction. The efflux pumps use energy to actively pump out the antibiotic-like Tetracycline outside the cell. The decreased cell permeability is a result of mutations in porins either by changing or reducing the porin channels, hence restricting the entry of antibiotics. These mechanisms decrease intracellular concentration of the antibiotic.

1.6 Carbapenem:

Carbapenems are beta-lactam antibiotics with a broad spectrum of activity against many Gram-positive and Gram-negative aerobic and anaerobic organisms used in the treatment of serious life-threatening infections not responding to standard antibiotic therapy. Carbapenems bind PBPs disrupting the integrity of the bacterial cell wall thus causing cell death. Its mechanism of action is similar to other beta-lactam antibiotics, which include cephalosporins, but carbapenems have a fused beta-lactam ring structure that renders them resistant to most of the beta-lactamases elaborated by bacteria to inactivate penicillin-like antibiotics. Carbapenems have excellent coverage and are very effective in treating infections caused by *streptococci*, *enterococci*, *staphylococci*, *Listeria*, Enterobacteriaceae, and many *Pseudomonas*, *Bacteroides*, and *Acinetobacter species*. MRSA are resistant to carbapenems. Carbapenems exhibit high potency against cephalosporin-resistant bacteria, which are producers of ESBLs. [\[20\]](#)

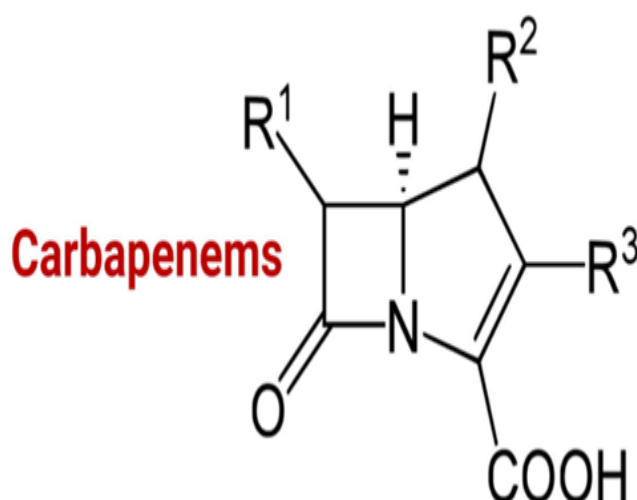


Figure 2: Chemical structure of carbapenems. [\[20\]](#)

Imipenem was the first carbapenem that was approved for clinical use. However, this drug needs to be used in combination with cilastatin because imipenem gets hydrolyzed in the human kidney via an enzyme called dehydropeptidase into a nephrotoxic metabolite. However, being a dehydropeptidase inhibitor, cilastatin prevents such a nephrotoxic metabolite from forming. However, meropenem is stable to human dehydropeptidase and does not require co-formulation with cilastatin. Other carbapenems include doripenem and ertapenem which are also given singly. Due to poor absorption from the gastrointestinal tract, all carbapenems are administered intramuscularly or intravenously. Similar to other beta-lactam antibiotics, the most serious adverse reaction is drug hypersensitivity. Liver enzymes may also mildly rise following carbapenem treatment.



Figure 3: Mechanism of action of carbapenemases [\[20\]](#)

1.6.1 Mechanisms of action of carbapenems:

Carbapenems work by inhibiting bacterial cell wall synthesis through their binding to most high-molecular-weight penicillin-binding proteins (PBPs). They enter gram-negative bacteria by passing through specific outer membrane proteins (OMPs) to reach the periplasmic space while the binding affinities differ between various carbapenems, they generally target PBPs 1a, 1b, 2, and 4, but exhibit lower affinity for PBP3. This weak interaction with PBP3 is thought to cause the formation of spherical cells without the development of long filaments during bacterial lysis. The broad-spectrum activity of carbapenems is due to their ability to bind to several PBPs across a range of bacteria. Like other β -lactam antibiotics, carbapenems act on the bacterial cell wall inhibiting its synthesis and subsequently causing lysis of the bacterial cell. Carbapenems are effective against both Gram-positive and Gram-negative bacteria and bactericidal. Carbapenems inhibit DD-transpeptidases, also called penicillin-binding proteins (PBPs), by binding to them. Transpeptidation - the final step of biosynthesis of the bacterial cell wall-peptidoglycan elongation and crosslinking - is impaired

due to this enzyme inhibition. As a result, bacteria cannot synthesize an adequate cell wall and eventually die. Moreover, inhibition of transpeptidation and crosslinking results in accumulation of incomplete peptidoglycan chains that induce production of autolytic hydrolases, which break the existing layer of peptidoglycan and enhance the bactericidal effect [21].

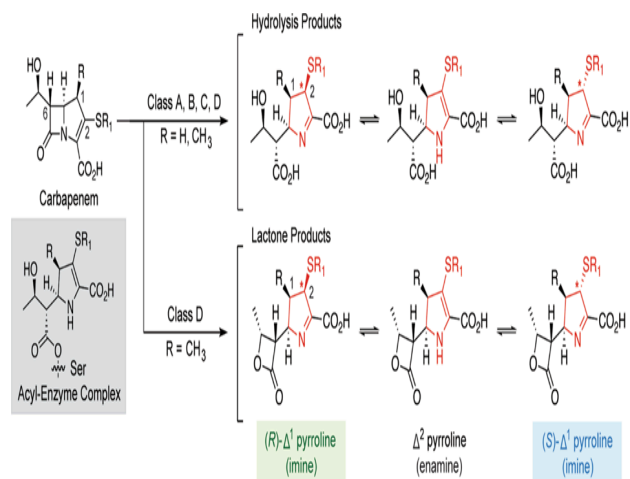


Figure 4: Mechanistic Insights into β -Lactamase-Catalysed Carbapenem Degradation Through Product Characterization [22]

1.7 Resistance of carbapenems:

Resistance to Carbapenem can be mediated through any one or a combination of the following mechanisms:

1. Hydrolyzing by β -lactamase enzymes
2. Reduced permeability due to loss or malfunction of specific outer membrane proteins in bacteria
3. Active efflux of the drug across the membrane
4. Production of altered or low-affinity targets, which is more common among Gram-positive bacteria.

The usual pattern of resistance in Gram-negative bacteria involves a combination of β -lactamase production and the modification of permeability or efflux or both because poor efficacy exists with one single mechanism in conferring substantial resistance [23].

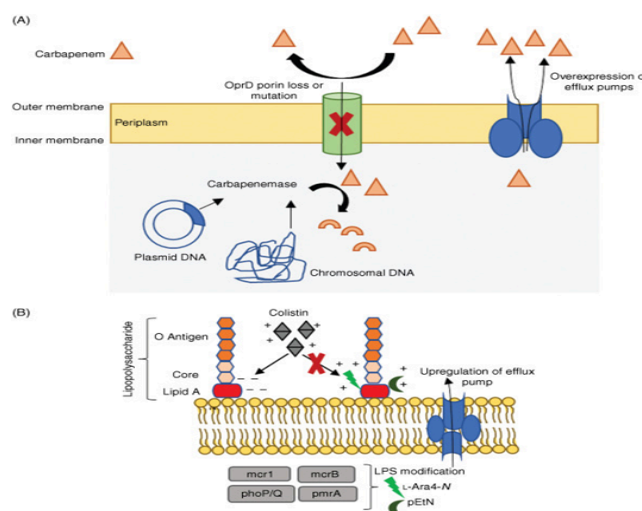


Figure 5: Carbapenem Resistance Mechanisms in Gram-negative Bacteria [\[23\]](#)

Resistance of Gram-positive bacteria to carbapenems is mediated through the production of low-affinity penicillin-binding proteins, including PBP2a in oxacillin-resistant *Staphylococcus* species and PBP5 in *Enterococcus faecium*. Thus, an estimated 60% of *S. aureus* strains are resistant to oxacillin and, by extension, carbapenems, and ~90% of *E. faecium* strains are resistant to ampicillin and carbapenems owing to the presence of low-affinity PBPs. Carbapenems are susceptible to easy hydrolysis by Ambler class B β -lactamases, which are zinc-dependent metalloenzymes. Certain non lactose-fermenting species such as *Stenotrophomonas maltophilia* and *Elizabethkingia meningoseptica* are intrinsically resistant to carbapenems through the production of class B β -lactamases. Furthermore, acquired class B β -lactamases, of which VIM and NDM are examples, are responsible for carbapenem resistance in a range of Gram-negative bacteria. Other class A β -lactamases, especially KPC-type carbapenemases, also possess carbapenem hydrolysis activity and have become major resistance determinants in *K. pneumoniae* and other Gram-negative pathogens worldwide.

The class D β -lactamases-OXAs that also possess carbapenem hydrolysis activity are widespread in *Acinetobacter baumannii* and are beginning to be reported in *K. pneumoniae* in some areas. In *Pseudomonas aeruginosa*, resistance to carbapenem is due to downregulation or loss of the porin protein OprD, especially when this is combined with production of AmpC β -lactamase. Of the carbapenems, imipenem is most heavily affected by the loss of OprD, but meropenem and doripenem are less so. The reduced expression of porins like OmpK35 and OmpK36 in *K. pneumoniae*. In *Enterobacter* species, carbapenem resistance has also been associated with the loss of OmpC and OmpF. Meropenem and doripenem, but not imipenem, are substrates of the MexA-MexB-OprM efflux system in *P. aeruginosa*, and upregulation of this system increases resistance to these drugs. Carbapenem resistance in Gram-positive bacteria may also be mediated by the production of low-affinity PBPs, including PBP2a in oxacillin-resistant *Staphylococcus aureus* and PBP5 in *Enterococcus faecium*. These

low-affinity PBPs render approximately 60% of *S. aureus* strains resistant to oxacillin and, by extension, carbapenems, and similarly, 90% of *E. faecium* strains resistant to both ampicillin and carbapenems [23].

1.7.1 Other contributing factors for carbapenem resistance:

Some AmpC β -lactamases, like CMY, and certain ESBLs confer resistance to carbapenems despite being classified as non-carbapenemases. This resistance is increased when combined with other mechanisms such as the loss of porins or modifications in efflux pumps. In addition, modifications in porins reduce the ability of the antibiotic to diffuse through the outer membrane of Gram-negative bacteria. This would give ample time for the remaining antibiotic to be degraded by ESBL and AmpC enzymes, hence leading to complete resistance. Changes in efflux pumps can increase the removal of the antibiotic from the cell before it can bind to penicillin-binding proteins, preventing its action. In addition, there are changes in the PBPs themselves, hindering the effective binding of β -lactams, a mode through which drugs become ineffective [24].

1.8 Beta lactamase:

β -lactam antibiotics remain one of the most clinically valuable classes of drugs for the therapy of bacterial infections. Resistance to β -lactams among Gram-negative bacteria is mediated primarily by the production of β -lactamase enzymes, which cleave the amide bond of the β -lactam ring. These enzymes are often disseminated by mobile genetic elements and play a major role in resistance among opportunistic pathogens like the Enterobacteriaceae, such as *Escherichia coli*, and the non-fermenting bacteria, such as *Pseudomonas aeruginosa*. β -Lactamases have been divided into four classes: the serine-based β -lactamases include classes A, C, and D, while zinc-dependent metallo- β -lactamases are grouped into class B. The carbapenem-hydrolyzing enzymes including *KPC* (class A), *NDM* (class B), and *OXA-48* (class D) are spreading all over the world.

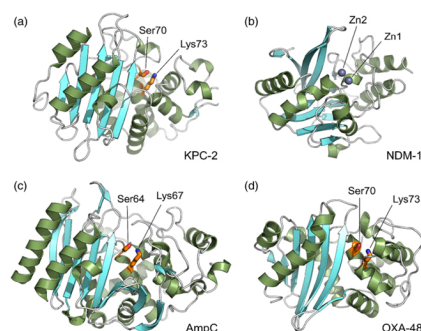


Figure 6: Crystal structures of β -lactamases from classes A, B, C and D. (a) Class A *KPC-2* (PDB 5ul8). (b) Class B *NDM-1* (PDB 5zgy). (c) Class C *AmpC* (PDB 1ke4). (d) Class D *OXA-48* (PDB 3hbr) [25].

1.9 Classification of carbapenemases:

Carbapenemases are enzymes that hydrolyze carbapenem antibiotics, hence rendering resistance to the bacteria. Based on their molecular structure, they could be divided into three main groups:

1.9.1 Class A Carbapenemases:

These are serine-based enzymes and hydrolyze a wide range of β -lactam antibiotics, including carbapenems. These have been found in bacteria such as *Klebsiella pneumoniae* and *E. coli*. Key members include the *KPC* (*Klebsiella pneumoniae* carbapenemase), which is the most clinically significant to date, and GES, SME, and IMI/NMC. *KPC* is plasmid-encoded and has spread globally [25].

1.9.2 Class B Carbapenemases (Metallo- β -lactamases):

- These enzymes require zinc for activity and can hydrolyze carbapenems but do not act on monobactams. They are resistant to the classical β -lactam inhibitors but are inhibited by metal chelators such as EDTA. Notable examples include *NDM* (New Delhi Metallo- β -lactamase), *IMP* (Imipenemase), and *VIM* (Verona integron-encoded Metallo- β -lactamase), which are widespread among pathogens such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Class D Carbapenemases: the *OXA*-type enzymes are serine-based and are much less inhibited by conventional β -lactam inhibitors. The most clinically important member, *OXA-48*, is usually found in species such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*. A total of over 489 variants of these enzymes have been reported; however, few are clinically significant.

These five-carbapenemases, *KPC*, *IMP*, *VIM*, *NDM*, and *OXA*-represent the carbapenemases of greatest clinical concern and have contributed most to the dissemination of carbapenem-resistant bacteria worldwide, challenging the treatment of infection [26].

1.10 The History of *NDM-1*:

In 2008, metallo-beta-lactamase 1 (*NDM-1*) was first identified in a strain of *Klebsiella pneumoniae* from a Swedish patient who had traveled to India. This enzyme's ability to make bacteria resistant to carbapenem antibiotics—often used as a last resort for treating severe infections—led to its classification [27]. By 2009, multiple cases of carbapenem-resistant Enterobacteriaceae were reported in Mumbai, primarily linked to *NDM-1*. The name "NDM" originates from New Delhi, India, where factors like inadequate sanitation and high antibiotic use played a significant role in its emergence and spread [28].

Action Plan:

NDM-1 is categorized as a metallo-beta-lactamase (MBL) class of enzyme. It breaks down beta-lactam medicines, including penicillins and carbapenems, reducing their effectiveness. The *NDM-1* gene is typically located on plasmids, which are genetic components that are moveable and can be exchanged by bacteria via horizontal gene transfer (HGT). This property makes it possible for *NDM-1* to spread quickly among different Enterobacteriaceae strains, including *Klebsiella pneumoniae* and *E. coli*, leading to broad antibiotic resistance [29].

The method of distribution of *NDM-1*:

Since its discovery, *NDM-1* has been detected in more than 70 countries worldwide. Despite the fact that the majority of cases originated in Pakistan and India, they have lately spread to other continents, including North America [28]. The transmission of *NDM-1* is often linked to individuals who have traveled to or received medical care in areas where the disease is prevalent. Notably, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have voiced worry that the resistance profile of *NDM-1* could result in catastrophic events for world health [30].

There are serious issues with the *NDM-1* transmission dynamics. It has been discovered in hospitals, particularly those without adequate infection control protocols, and in tainted water supplies. Notably, it has been demonstrated that *NDM-1*-producing bacteria can infect people who have never traveled, suggesting the possibility of local transmission [31].

1.11 History of *OXA-2*:

OXA-2 is a member of the *OXA*-type β -lactamase class, first identified in the late 1970s. These enzymes are referred to as *OXA* (oxacillinase) due to their ability to hydrolyze oxacillin, a penicillin derivative. Since its discovery in the early 1980s, *OXA-2* has primarily been associated with Gram-negative bacteria, particularly *Acinetobacter baumannii*. Its presence in various countries indicates a broad distribution and a significant role in antibiotic resistance [32].

Method of Action:

OXA-2, a β -lactamase enzyme, degrades β -lactam antibiotics, including penicillins and some cephalosporins.

Antibiotic Hydrolysis:

OXA-2 hydrolyzes the β -lactam ring of antibiotics, making them ineffective. This process disrupts the binding of penicillin-binding proteins (PBPs), which are essential for building bacterial cell walls. Without effective binding, the integrity of the bacterial cell wall is compromised, ultimately resulting in cell death [33].

Substrate Specificity:

OXA-2 primarily hydrolyzes oxacillin but also exhibits significant activity against certain cephalosporins and other β -lactam antibiotics like carbenicillin (Evans & Amyes, 2014b). However, its effectiveness can vary widely depending on the specific substrate. The enzyme's activity is influenced by its structural characteristics, including specific amino acid residues that affect substrate affinity and hydrolysis efficiency [34].

Spread of *OXA-2*:

There are multiple ways that *OXA-2* spreads among bacterial populations:

1. Horizontal Gene Transfer (HGT):

OXA-2 supports easy conjugation-based gene transfer between various bacterial species and is commonly located on plasmids or integrative conjugative elements (ICEs). This mobility allows for rapid dissemination in diverse environments, particularly in healthcare settings [35].

2. Environmental Reservoirs:

Soil and water, especially in unhygienic conditions, serve as environmental reservoirs for *OXA-2*-producing bacteria. This presence in the environment allows for transmission to humans through ingestion or direct contact [36].

3. Clonal Spread:

Clonal spread occurs when a resistant strain circulates among hospitalized patients. Hospitals have reported *OXA-2* epidemics caused by strains of *Acinetobacter baumannii*, often associated with insufficient infection control measures [37].

4. Combination with Other Resistance Mechanisms:

The effectiveness of *OXA-2* is often amplified by additional resistance mechanisms, such as decreased permeability of the bacterial outer membrane or overexpression of efflux pumps. This combination complicates the treatment of infections caused by *OXA-2*-producing organisms [38].

1.12 Carbapenem Resistant Enterobacteriaceae (CRE)

Carbapenem-resistant Enterobacteriaceae (CRE) represent a significant and growing global public health concern due to their resistance to carbapenem medications, which are commonly used as last resorts for bacterial infections [39]. The primary reason of CRE's emergence is carbapenemases, which are enzymes that deactivate carbapenems and other beta-lactam antibiotics. This resistance complicates treatment decisions and is associated with

notable mortality rates, which can vary from 18% to 48% depending on a variety of factors, including the severity of the ailment and the effectiveness of the chosen treatment [\[40\]](#).

Because CRE frequently carry several resistance genes, they are not only resistant to carbapenems but also to a wide range of other medications. Compared to infections produced by bacteria susceptible to carbapenem, infections induced by this multi-drug resistance often have higher costs, longer treatment durations, and more toxicity [\[40\]](#). Some of the most prevalent resistance mechanisms are as follows:

Enzymes that break down carbapenems are produced by carbapenemase. Efflux pumps are responsible for the removal of antibiotics from bacterial cells. Porin channel mutations are alterations that prevent antibiotics from penetrating the bacterial cell [\[40\]](#).

Options for Treatment

There are not many alternatives for treating CRE infections, although many different approaches are being used:

- Because of increased resistance, historically used polymyxins (such as colistin) are becoming less effective.
- Since tigecycline is not highly effective when taken alone, it is frequently utilized in combination therapy.
- One aminoglycoside that can occasionally be useful is amikacin; this is especially true when administered in combination regimens. For some infections, especially urinary tract infections, fosfomycin is a useful treatment choice [\[41\]](#).

New agents that expressly target CRE have emerged as a result of recent developments:

- Ceftazidime-avibactam works well against some bacteria that produce carbapenemase [\[39\]](#).
- Another innovative combination targeted at KPC producers is meropenem-vaborbactam.
- Cefiderocol and imipenem-relebactam are examples of novel medications that appear to be effective against resistant strains [\[42\]](#).

Combination Treatment

It is becoming more and more clear that combination therapy works better than monotherapy. According to studies, people with severe CRE infections might greatly increase their chances of survival by taking two or more active medications [\[41\]](#). For instance, when compared to single-agent regimens, regimens that combine meropenem with other active drugs have been linked to lower failure rates [\[40\]](#). High dosages and extended infusion times of carbapenems may potentially be advantageous for isolates with lower minimum inhibitory concentrations (MICs) [\[41\]](#).

Current Challenges and Opportunities

Despite the introduction of new antibiotics, resistance is still very likely to develop. Antimicrobial care and continuous observation of resistance patterns are crucial for efficiently limiting the spread of CRE. Ongoing research is necessary to identify novel therapy options and enhance existing treatments based on patient profiles and patterns of local resistance [\[43\]](#).

1.12.1 Detection of Carbapenem-Resistant Enterobacteriaceae (CRE)

Identification of Carbapenem-Resistant Enterobacteriaceae (CRE) Diagnosing carbapenem-resistant Enterobacteriaceae (CRE) is crucial because they significantly impact clinical outcomes and public health. Numerous methods have been developed to identify these resistant species; each has pros and cons [\[44\]](#).

Methods of Phenotyping

- **The Modified Carbapenem Inactivation Method (mCIM):** This evaluates bacteria's capacity to inactivate carbapenems, has a high sensitivity and specificity for identifying CRE, especially strains that produce KPC [\[45\]](#).
- **The Combined Disk Test (CDT) :** it is a technique used to assess how well carbapenems work in combination with other antibiotics. Although its accuracy is moderate, it might have trouble with specificity for some carbapenemases, such as metallo- β -lactamases [\[46\]](#).
- **Quick Colorimetric Measurements:** Although tests like Rapidec Carba NP yield results quickly, they may not be as sensitive for certain kinds of carbapenemase, such as *OXA-48* [\[47\]](#).

Molecular Methods

The polymerase chain reaction (PCR) and other molecular techniques are the gold standard for identifying specific carbapenemase genes. These methods can quickly identify resistance mechanisms directly from clinical data, even though they typically call for earlier culture stages [\[48\]](#).

According to research, mCIM usually outperforms other phenotypic approaches in detecting CRE, achieving high positive rates. For certain targets, molecular methods can sometimes yield low positive prediction values despite their high sensitivity. To ensure accurate CRE identification in clinical settings, a combination of molecular and phenotypic detection methods is recommended. This integrated strategy is essential for timely treatment and effective infection control strategies [\[49\]](#).

1.13 Carbapenemase-producing Enterobacteriaceae (CPE)

The production of carbapenemase (CPE) by *E. coli* is posing an increasing threat to global health. Particularly in those with compromised immune systems, it is associated with severe infections such as bloodstream infections, pneumonia, and urinary tract infections. Death rates from CPE infections can exceed 40%, depending on the patient's condition and the pathogen's resistance mechanisms. One of the main causes of CPE's spread is gastrointestinal colonization, which is usually asymptomatic and encourages transmission in medical environments while escalating antibiotic resistance [50].

One strategy to combat *E. coli* CPE is to improve diagnostic techniques like whole-genome sequencing and create global surveillance programs. Fighting these diseases requires interdisciplinary collaboration and stricter antimicrobial management to prevent the emergence and spread of resistance [50].

1.14 Carbapenem-resistance in *E. coli*:

E. coli is frequently isolated in clinical samples, and multidrug resistance (MDR) in this bacterium poses a significant challenge due to the limited availability of new antibiotics. Increasing resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides has led to carbapenems being the last-line treatment for severe MDR *E. coli* infections. However, rising carbapenem use has resulted in the emergence of carbapenem-resistant *E. coli*, raising global health concerns. The primary mechanisms of resistance include carbapenemase production, loss of porins, and efflux pump overexpression [51]. Notable globally prevalent carbapenemases include *KPC*, *NDM*, *VIM*, *IMP*, and *OXA*, often found on plasmids alongside other resistance genes, complicating treatment. Research indicates that *E. coli*'s outer membrane porins, specifically ompC andE ompF, play crucial roles in antibiotic resistance and membrane integrity [52].

1.15 Role of environment, treatment system, distribution system of water:

The spread of antimicrobial resistance through water systems is influenced by several factors, including environmental conditions, treatment strategies and distribution networks. Studies have shown that antibiotic-resistant bacteria and antibiotic resistance genes can persist in water environments, and treatment systems may not effectively remove all these microorganisms [53]. In addition, faulty distribution systems can result in the reintroduction of resistant bacteria and genes into the water supply [54].

Further studies have indicated that aquatic environments can act as reservoirs for antibiotic resistance due to contamination through agricultural runoff and wastewater effluents, which introduce antibiotic-resistant bacteria that can survive and multiply in water systems for extended periods of time [55]. Moreover, antibiotic-resistant bacteria have been detected in the effluents of drinking water treatment plants, proving that not all the antibiotic-resistant bacteria can be removed by conventional treatment processes [56].

Other studies have focused on the influence of distribution systems on the spread of antimicrobial resistance. Indeed, poor disinfection and maintenance of water distribution systems have been shown to recontaminate drinking water with antibiotic-resistant bacteria, thereby spreading these bacteria to consumers and contributing to the spread of antimicrobial resistance [56].

Some studies have investigated the human behaviours which are responsible for spreading the antimicrobial resistance via water systems. For example, discharging untreated or poor quality-treated wastewater into water systems will lead to the introduction of antibiotic-resistant bacteria in such an environment [57]. Moreover, poor handling and disposal of antibiotics and waste containing antibiotic-resistant bacteria means any waste product, medical waste, wastewater, or agricultural runoff that contains antibiotic-resistant bacteria or resistance genes, which may contribute to the spread of antimicrobial resistance in the environment [58].

1.16 Selected zones of this study:

Dhaka is among the fastest-growing megacities in the world. The city spans an area of 300 square kilometers and is home to 18.237 million people. It ranks as one of the most densely populated regions globally, with a density of 23,234 individuals per square kilometer. Dhaka is divided into several zones.

Samples were gathered and analyzed from areas such as Gulshan, Banani, Niketon, Baridhara, Badda, Khilgaon, Mohammadpur, Lalmatia, Uttara, Mirpur, Mugdapara, Tolarbagh, Moghbazar, Shanti Nagar, Jatrabari, Bangshal, Wari, Dhanmondi, Farmgate, Tejgaon, Mohakhali, Rampura, Bashundhara, and the Korail slum. Mirpur and Mohammadpur are the most populous neighborhoods in Dhaka. Mirpur covers a total area of 58.66 square kilometers, with a population of 1,074,232. In Mohammadpur, which has an area of 11.65 square kilometers, there are 355,843 residents. Gulshan and Uttara are regarded as the most prestigious areas in Dhaka, with population densities of 29,086 individuals per square kilometer and 25,701 individuals per square kilometer, respectively.

1.17 Research rationale:

Reviewing the previous research articles, the following research gaps were found:

- There is a lack of comprehensive studies in recent years specially focusing on the microbial quality of supplied water in urban areas of Dhaka. It could also be partly due to insufficient investigation into how rapid urbanization and poor waste management contribute to the prevalence of antibiotic-resistant *E. coli*.
- The need to explore other resistance mechanisms or genetic factors that could contribute to the carbapenem resistance in these isolate

1.18 Research hypothesis:

The number of carbapenem-resistant *E. coli* strains will rise with the increase in the number of collected samples. High numbers of samples will be having increased *E. coli* strains resistant to Carbapenems.

1.19 Objectives of the Study:

The purpose of this study is to investigate the microbial quality of supplied water across 11 different zones in Dhaka, with a particular emphasis on the presence and resistance patterns of *E. coli*. By analyzing water samples treated through a series of selective media, this research will find out the prevalence of *E. coli* and its resistance to carbapenem to add the most valuable insights into public health related to the quality and safety of water in urban settings. The recommendations from this study will be done to inform authorities about probable risks from water consumption and provide guidance on necessary interventions to promote standards of water safety.

Chapter 2:

Materials and

Methods

2.1 The work plan

Workflow

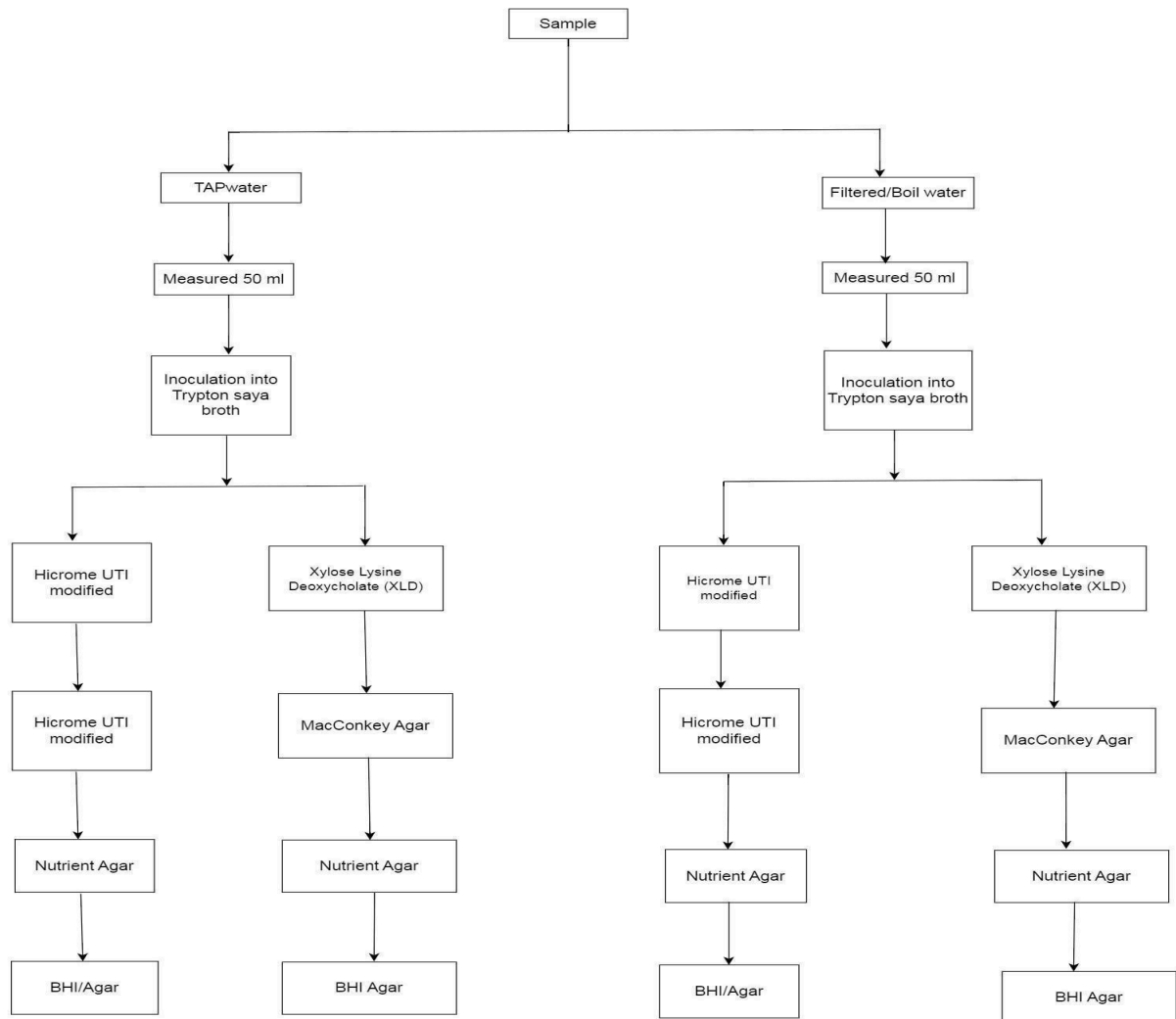


Figure 7: Workflow of sample processing to stocking of the isolated organisms

2.2 Sampling sites:

A total of 200 water samples were collected, resulting in the identification of 410 isolates. Out of these, 200 isolates originated from tap water samples, 100 from filtered water samples, and 110 from boiled water samples.

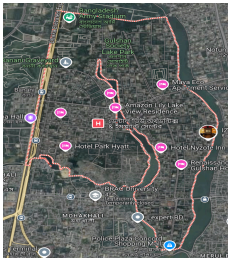
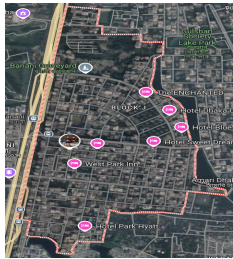
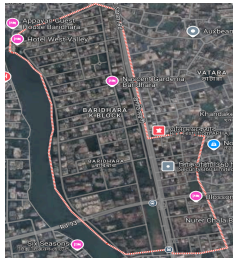
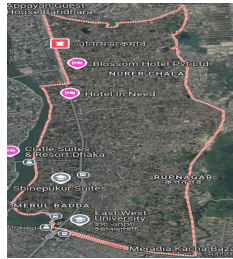
Area 1 (Gulshan, Banani, Niketon, Baridhara, Badda)			
Gulshan	Banani	Baridhara	Badda
			

Figure 8: Sampling Site of Gulshan, Banani, Niketon, Baridhara, Badda

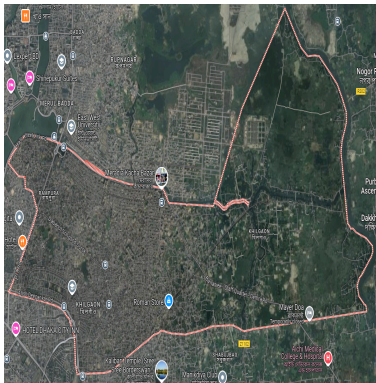
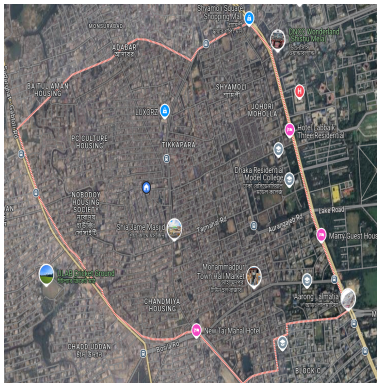
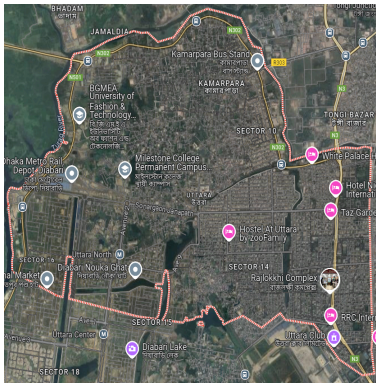
Area 2 (Khilgaon)	Area 3 (Mohammadpur)	Area 4 (Uttara)
		

Figure 9: Sampling Site of Khilgaon, Mohammadpur, Uttara

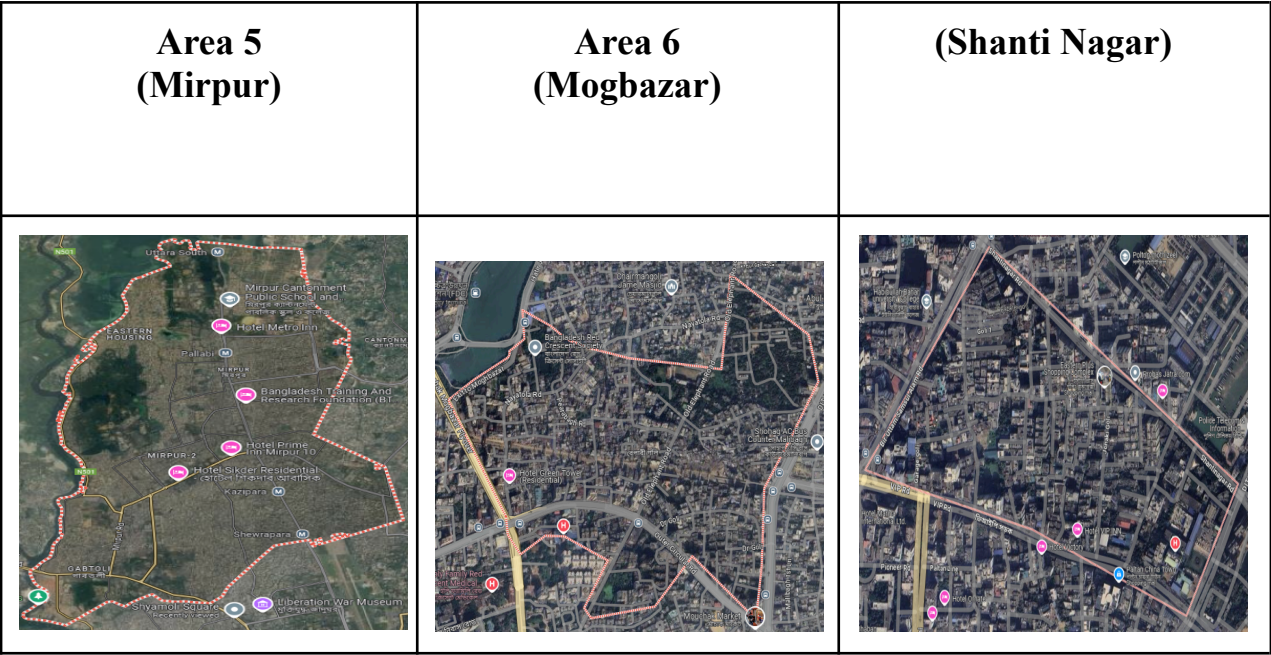


Figure 10: Sampling Site of Mirpur, Moghbazar, Shanti Nagar

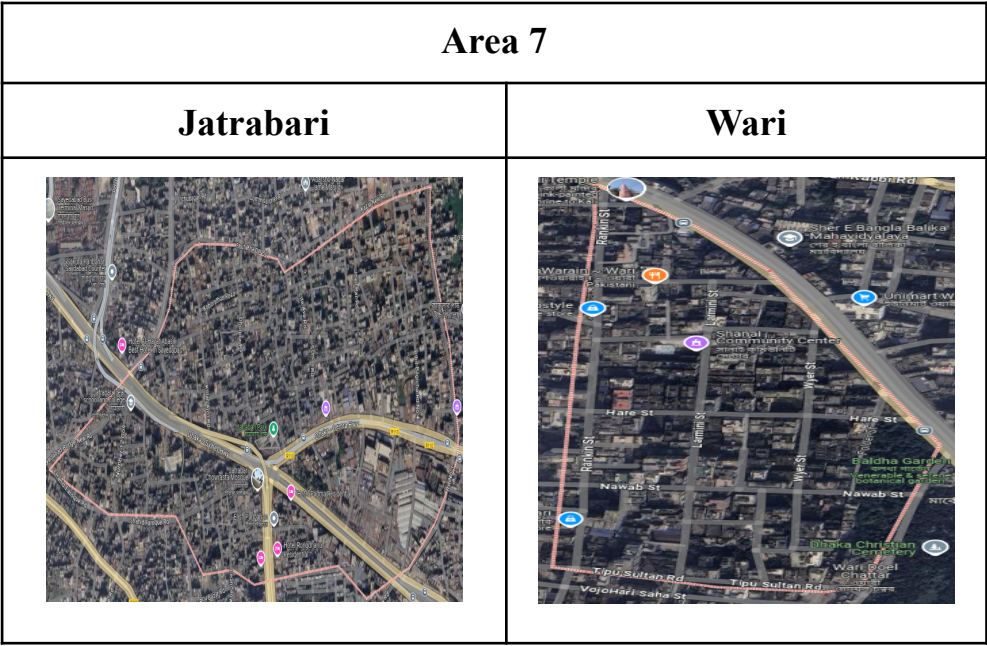


Figure 11: Sampling Site of Jatrabari, Wari

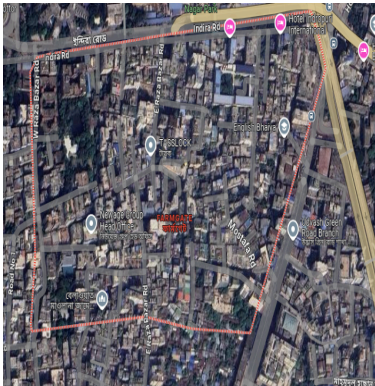
Area 8 (Dhanmondi, Farmgate, Tejgaon)		
Dhanmondi	Farmgate	Tejgaon
		

Figure 12: Sampling Site of Dhanmondi, Farmgate, Tejgaon

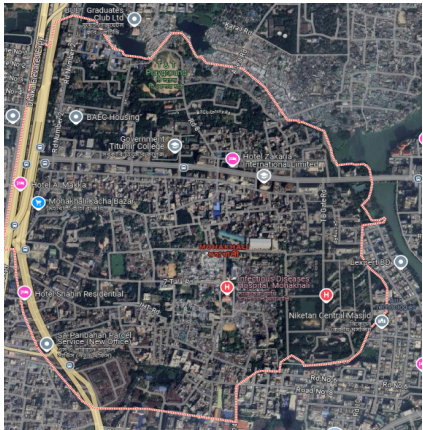
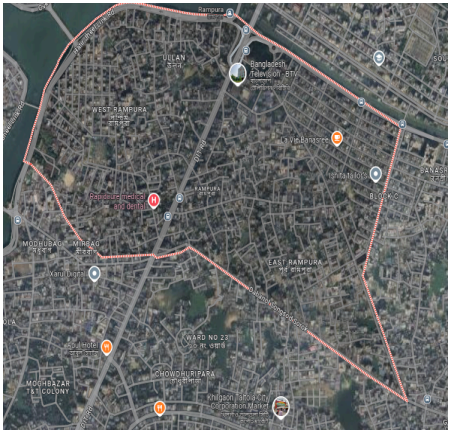
Area 9 (Mohakhali, Rampura)	
Mohakhali	Rampura
	

Figure 13: Sampling Site of Mohakhali, Rampura

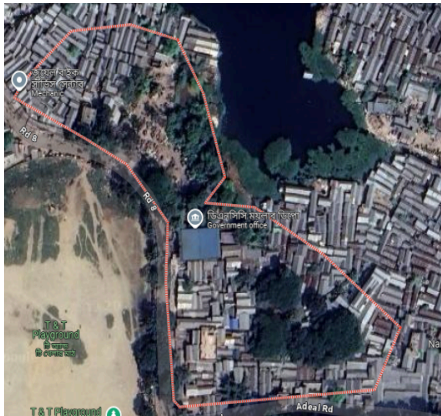
Area 10, 11 (Bashundhara, Korail slum)	
Area 10 (Bashundhara)	Area 11 (Korail slum)
	

Figure 14: Sampling Site of Bashundhara, Korail slum

2.3. Sample collection:

Water samples were collected twice a week from the designated areas in the morning between the time frame of 9 a.m. to 12 p.m from October 2023 to April 2024. The samples were collected in autoclaved sample collecting bottles. From each household, we collected TAP water and Filtered/Boiled water. While collecting the samples, one pair of gloves was used to avoid cross-contamination and to avoid leakage, the sample collecting bottles were sealed with caps properly. After collecting all the samples, the samples were transported to the lab within two hours then we started to process the samples.

2.4 Sample processing:

For sample processing autoclaved Tryptone Soya Broth (TSB) was used. To make the TSB, in a conical flask the TSB powder was measured for 225 mL but mixed into 200 mL of distilled water (dH₂O).

Then the conical flasks containing TSB were autoclaved. For both Tap water and filtered/boiled water individual conical flasks were taken and labeled. 50mL of water samples were measured and poured into the different conical flasks which contained TSB. After that the flasks were shaken properly to mix the samples and then sealed with foil paper and masking tape to avoid spilling. Then the flasks were kept into the shaker incubator for 24 hours at 37°C and 130 Rpm.

After 24 hours, 1 loopful of each sample was streaked into Hicrome UTI modified agar and Xylose lysine Deoxycholate (XLD) agar plates incorporated with meropenem and ZnSO₄. The streaked plates were incubated for 24 hours at 37°C. Again after 24 hours, the colonies that were found on Hicrome UTI modified agar plates were again transferred to fresh Hicrome UTI modified agar plates to get more pure colonies with specific colors. And from XLD agar plates the colonies were transferred to McConkey agar incorporated with meropenem and ZnSO₄. Then the streaked plates were incubated for 24 hours at 37°C. Then the specific colonies were picked and streaked into Nutrient agar (NA) and incubated for 24 hours at 37°C. The colonies from NA were stabbed into BHI agar stock media and incubated for 18 hours at 37°C. After incubation, paraffin oil was added to BHI media to keep the cell hydrated.

2.5 Antibiotic Sensitivity Test (AST):

Total 35 presumptive isolates were tested for antibiotic resistance using the following antibiotics: Meropenem(MEM10), Imipenem (IMI10), Kanamycin(K30), Gentamicin(CN10), Ceftazidime(CAZ30), Erythromycin(E15), Ampicillin(AMP10), Amoxycylav(AMC30), Streptomycin(S10), Tetracycline(TE30). Modified Kirby-Bauer disc diffusion test method was done for the Antibiotic Susceptibility Test (AST) of the isolates.

A small amount of pure fresh culture was taken by a loop from the Nutrient Agar (NA) plate and was inoculated in 0.9% Saline in a test tube to make a suspension. This inoculation created a turbidity of the suspension which was compared with the 0.5 McFarland turbidity standard. After that, a sterile cotton swab was dipped into the suspension and lawned on a fresh Mueller Hinton Agar (MHA) plate. The plate was kept aside to sit at room temperature. After a while, sterilized forceps were used to pick the antibiotic discs and place onto the agar plate, to make sure of complete contact with the agar surface the discs were slightly pressed down. Then all the agar plates were kept for incubation at 37°C overnight.

After incubation of 18-20 hours, the MHA plates were checked for a zone of inhibition. If a zone of inhibition was present, the diameter of the zone of inhibition was measured using a ruler to the nearest millimeter. According to the CLSI guidelines, the zone diameters of each antimicrobial agent were then translated into susceptible, intermediate, or resistant categories.

2.6 DNA Extraction protocol:

DNA extraction is a process used to isolate DNA from a sample by applying physical and/or chemical methods to separate it from cell membranes, proteins, and other cellular components. The goal of this technique is to achieve a high yield of DNA that is both pure and free from contaminants, ensuring good quality for further use [\[59\]](#).

Loopful of pure bacterial cultures that were grown on Nutrient Agar (NA) were inoculated in 5ml of Nutrient broth in a falcon tube. The samples were vortexed and then incubated for 6 hours. After 6 hours, 1ml of the samples were transferred to a microcentrifuge tube (mct) and

were centrifuged at 13000 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended and washed in 500µL of Nucleus free water (NFW). The bacteria suspension was centrifuged again at 13000 rpm for 10 minutes and a washed pellet was recovered. Then the pellet was lysed at 95°C for 10 minutes. The lysed cells were centrifuged at 13000 rpm for 10 minutes and the supernatant was transferred to a new microcentrifuge tube and the pellet was discarded. The supernatant contained the extracted DNA.

2.7 Raw DNA gel run:

The extracted DNA was checked by performing gel electrophoresis using loading dye and then stored at -20°C for further use.

2.8 Preparation of primer for PCR:

Primers that were used in this study were of 10x concentration. Stock solutions of both primers for *blaNDM-1* and *blaOXA-2* were present in the lab. Each time before performing PCR, working solutions were prepared from stock solutions.

To prepare the working solutions of *blaNDM-1* primer (10x concentration), 10 microliters of forward and reverse primers were taken in two different microcentrifuge tubes. 90 microliters of nuclease free water were then transferred in each tube. After mixing the tubes were then vortexed and short spun for 20 seconds. The same procedure was repeated in the case of *blaOXA-2* primers.

2.9 Sequence of primer:

Gene	Primer Sequence	Product size (bp)	Reference
<i>blaNDM-1</i>	Forward: 5'-ACCGCCTGGACCGATGACCA-3' Reverse: 5'-GCCAAAGTTGGGCGCGGTTG-3'	264	[60]
<i>blaOXA-2</i>	Forward: 5'-TTCAAGCCAAAGGCACGATAG-3' Reverse: 5'-TCCGAGTTGACTGCCGGGTTG-3'	702	[61]

Table 3: Sequences of primers used for amplification by PCR.

2.10 Preparation of controls:

Due to the absence of strains known to be positive for *bla*NDM-1 and *bla*OXA-2 in the laboratory, we had to prepare our positive control for these genes. To achieve this, we performed PCR to detect *bla*NDM-1 and *bla*OXA-2 in the selected isolates. Unfortunately, the results did not show amplification for either of the genes, and we could not obtain a positive outcome. For the negative control, we utilized a *Klebsiella pneumoniae* strain from the lab which is known to be negative for both genes, ensuring that any contamination or cross-reactivity would be minimized. Additionally, we included distilled water as the No Template Control (NTC) to rule out any possibility of contamination in the PCR reagents.

2.11 PCR:

The PCR was performed for Carbapenemase resistance genes *bla* NDM-1 and *bla* OXA-2 for 35 presumptive *E. coli* isolates.

The PCR assay was performed in tubes with a total volume of 13µl. The reaction mixtures commonly contained nuclease-free water 4µl, forward primer 0.5µl, reverse primer 0.5µl, and template 2µl. And master mix 6µl. Re-pipetting and spinning were carefully done for proper mixing and also to get rid of bubbles. After the initial preparation was taken, PCR for *bla* NDM-1 was performed under the following conditions: 35 cycles with initial denaturation at 94°C for 3 minutes, heat denaturation at 94°C for 45 seconds, primer annealing at 52°C for 45 seconds, and DNA extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes in microcentrifuge tubes gradient master cycler. And PCR for *bla* OXA-2 was performed under the following conditions: 35 cycles with initial denaturation at 94°C for 3 minutes, heat denaturation at 94°C for 45 seconds, primer annealing at 57°C for 45 seconds, and DNA extension at 72°C for 1 minute and final extension at 72°C for 5 minutes in microcentrifuge tubes gradient master cycler. For both the genes (*bla*NDM-1 and *bla* OXA-2) distilled water was used as a negative control to monitor the contamination by any external DNA in the PCR reagents in the PCR reaction.

2.12 Gel electrophoresis:

Conventional agarose gel electrophoresis was performed to confirm the presence of the amplified product. The amplicon was separated by 1.5% agarose gel electrophoresis and stained with 5 microliter ethidium bromide at 100 voltages for 40 minutes. We used 5 microliter pcr products for each well. The gel was then visualized by UV transilluminator. The 100bp DNA ladder was used to determine the size of the amplicon. PCR assay was performed at least two times to check the reproducibility.

Chapter 03:

Results

3.1 Growth, appearance, and isolation of strains on HiCrome UTI agar, Xylose Lysine Deoxycholate (XLD) agar, and MacConkey (MAC) agar.

3.1.1 Isolates on HiCrome 1

HiCrome UTI agar was used to isolate organisms from water samples. In this media, purple to magenta indicates *E. coli*, blue-green is *Enterococcus faecalis*, blue to purple is *Klebsiella pneumoniae*, colorless is *Pseudomonas aeruginosa*, and off-white or golden yellow is *Staphylococcus aureus*.

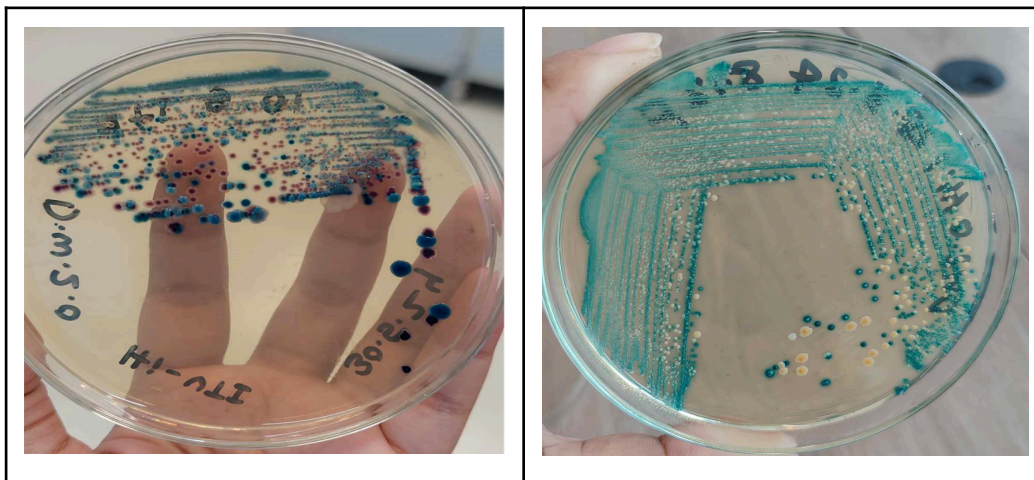


Figure 15: The appearance of different colored colonies on HiCrome 1

3.1.2 Isolates on HiCrome 2

The different-colored colonies from HiCrome 1 were transferred to HiCrome 2 to observe the consistency in colony morphology and obtain pure cultures.

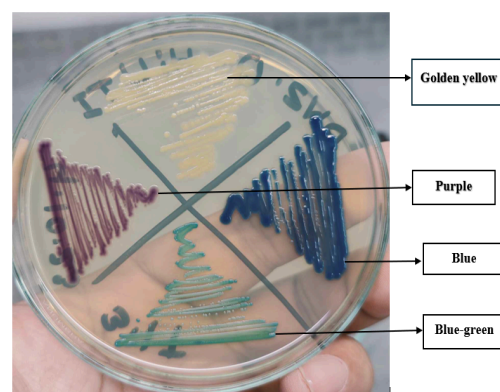


Figure 16: The appearance of different colored colonies on HiCrome 2

3.1.3 Isolates on Xylose Lysine Deoxycholate (XLD)

XLD agar was used as a confirmatory medium to further characterize the organism accurately. XLD agar was used to isolate organisms from water sample. In this media, *Salmonella* produces red colonies with a black center. *Shigella* and non-H₂S-producing *Salmonella* produce red colonies without black centers. Coliforms produce yellow colonies.

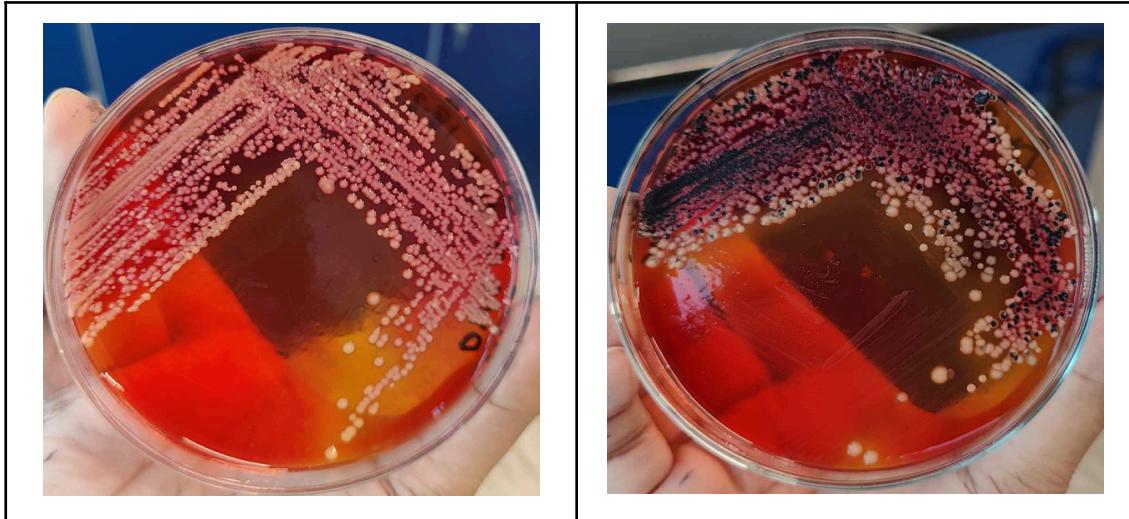


Figure 17: The appearance of different colored colonies on XLD

3.1.4 Isolates on MacConkey (MAC)

Colonies from XLD agar transferred to MAC. In this media, pink colonies indicate *E. coli* or *Klebsiella pneumoniae*, and colorless colonies indicate the presence of *Salmonella*, *Shigella*.



Figure 18: The appearance of different colored colonies on MAC

3.2 The number of strains isolated throughout the study

Throughout the study, a total of 410 isolates were obtained. 200 were from Tap water samples, 110 were from Boil water and 100 were from Filter water samples.

Sample Type	Number of Isolates	Percentage
Tap Water	200	48.80%
Boil Water	110	26.80%
Filter Water	100	24.40%
Total	410	100%

Table 4: The number of strains isolated throughout the study

3.2.1 The abundance of isolates obtained throughout the study:

Among the 410 isolates, 103 were *E. coli*, 76 were *Enterococcus faecalis*, 56 were *Klebsiella pneumoniae*, 28 were *Staphylococcus aureus*, 55 were *Pseudomonas aeruginosa*, 38 were *Salmonella* and 54 were *Shigella*.

E. coli was the most found organism followed by *Enterococcus faecalis* and after that *Klebsiella pneumoniae*. The least found organism was *Staphylococcus aureus*.

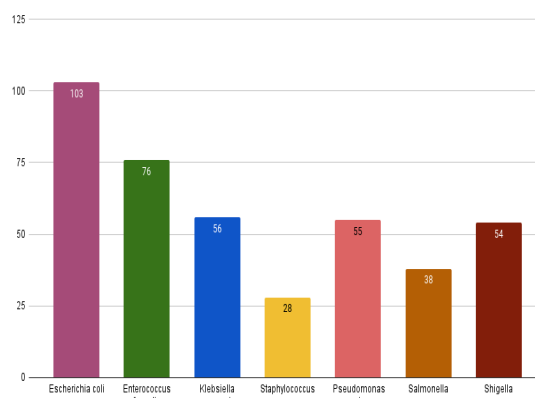


Figure 19: The abundance of isolated strains

3.3 Area-wise distribution of isolates

200 isolates were obtained from tap water samples, 110 from boil water and 100 from filter water. Among all the samples, the highest number of isolates were obtained from area- 1,

which is 100 isolates. Those samples were collected from Gulshan, Banani, Niketon, Baridhara and Badda. The least number of isolates were obtained from area 7, which is 11 isolates, and the area was Jatrabari and Wari.

Sample	Names of isolates (presumptive)	Number of isolates
Area 1	<i>E. coli</i> (16), <i>E. faecalis</i> (21), <i>P. aeruginosa</i> (17), <i>S. aureus</i> (4), <i>K. pneumoniae</i> (17), <i>Salmonella</i> (11), <i>Shigella</i> (14)	100
Area 2	<i>E. coli</i> (11), <i>E. faecalis</i> (11), <i>P. aeruginosa</i> (6) <i>S. aureus</i> (6) , <i>K. pneumoniae</i> (8), <i>Salmonella</i> (1), <i>Shigella</i> (7)	50
Area 3	<i>E. coli</i> (14), <i>E. faecalis</i> (8), <i>p. aeruginosa</i> (9), <i>S. aureus</i> (3) <i>K. pneumoniae</i> (6), <i>Salmonella</i> (3), <i>Shigella</i> (8)	51
Area 4	<i>E. coli</i> (2), <i>E. faecalis</i> (3), <i>P. aeruginosa</i> (4), <i>S. aureus</i> (1), <i>K. pneumoniae</i> (2), <i>Salmonella</i> (3), <i>Shigella</i> (4)	19
Area 5	<i>E. coli</i> (10) , <i>E. faecalis</i> (5), <i>P. aeruginosa</i> (4), <i>S. aureus</i> (2), <i>K. pneumoniae</i> (7), <i>Salmonella</i> (0), <i>Shigella</i> (8)	36
Area 6	<i>E. coli</i> (12), <i>Salmonella</i> (1)	13
Area 7	<i>E. coli</i> (11)	11
Area 8	<i>E. coli</i> (9), <i>E. faecalis</i> (10), <i>P. aeruginosa</i> (5), <i>S. aureus</i> (5), <i>K. pneumoniae</i> (4), <i>Salmonella</i> (1), <i>Shigella</i> (6)	40
Area 9	<i>E. coli</i> (7), <i>E. faecalis</i> (6), <i>P. aeruginosa</i> (5), <i>S. aureus</i> (2), <i>K. pneumoniae</i> (4), <i>Salmonella</i> (4), <i>Shigella</i> (5)	33
Area 10	<i>E. coli</i> (4), <i>E. faecalis</i> (2), <i>P. aeruginosa</i> (2), <i>S. aureus</i> (0), <i>K. pneumoniae</i> (3), <i>Salmonella</i> (2), <i>Shigella</i> (2)	15
Area 11	<i>E. coli</i> (7), <i>E. faecalis</i> (10), <i>P. aeruginosa</i> (3), <i>S. aureus</i> (5), <i>K. pneumoniae</i> (5), <i>Salmonella</i> (12), <i>Shigella</i> (0)	42
Total		410

Table 5: Area wise distribution of isolates

3.4 *Escherichia coli*

Out of the 410 isolates obtained in the study, 103 were presumptive *E. coli*. These were considered for the rest of the analysis throughout the study.

3.4.1 Area-wise distribution of presumptive *E. coli* isolates

Among the 103 presumptive *E. coli* isolates, 60 were obtained from tap water samples, 24 from boil water and 19 were obtained from filter water samples. Among the areas, the highest number of *E. coli* isolates was obtained from area 1 with a total of 16 and the least number came from area 4 and the total number was 2.

Sample	Number of <i>E. coli</i> isolates
Area 1	16
Area 2	11
Area 3	14
Area 4	02
Area 5	10
Area 6	12
Area 7	11
Area 8	09
Area 9	07
Area 10	04
Area 11	07
Total number of <i>E. coli</i> isolates	103

Table 6: The total number of presumptive *E. coli* isolates obtained from each area

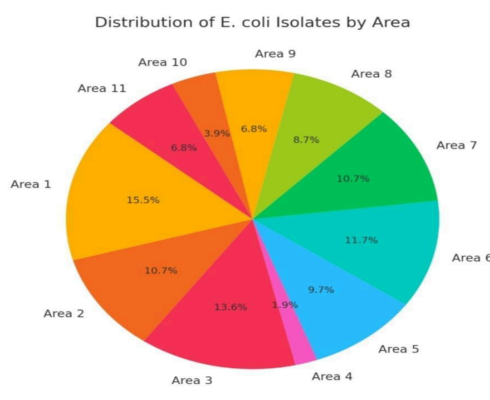


Figure 20: Location-wise distribution of isolates

3.5 Antibigram

After PCR 35 *E. coli* isolates were identified. Those isolates were considered for antibiograms.

3.5.1 Antibiotic Susceptibility Test on MHA media- Modified Kirby-Bauer disk diffusion method:

The antibiotic susceptibility test was done using the modified Kirby-Bauer disk diffusion method. Some isolates were found either resistant, intermediate, or sensitive to some of the antibiotics after incubation in MHA plates.

A few isolates fully developed resistance to all 10 tested antibiotics, as shown in Fig. 21. The specific antibiotic-resistant isolates had no zone of inhibition. On the other hand, sensitive ones or those that were resistant at an intermediate level had their zones of inhibition measurable.

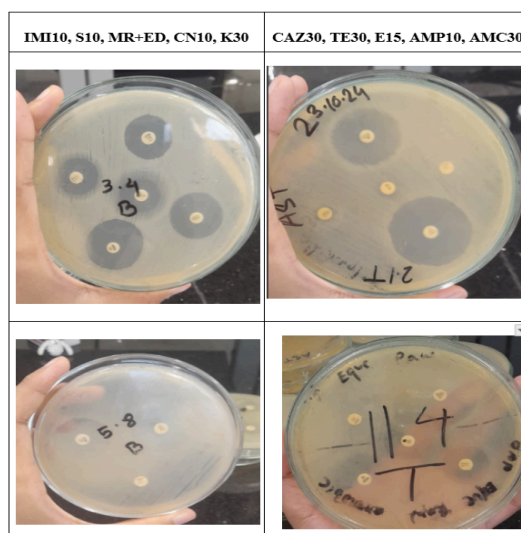


Figure 21: Resistant and sensitive to some of the antibiotics

3.5.2 Antibiotic sensitivity pattern of *E. coli* isolates:

All 35 isolates exhibited high resistance rates of Meropenem, Erythromycin, and Gentamicin (91.43%, 97.14%, and 88.57%, respectively), also showing a notable resistance rate of 77.14% to Imipenem, while in terms of Ampicillin and Amoxycylav, they showed complete resistance (100%). In contrast, the isolates demonstrated significant sensitivity to Ceftazidime and Tetracycline (94.29% and 82.86%, respectively). In the case of Kanamycin, they displayed a mixed profile, with 14.29% resistance, 20% intermediate, and 65.71% sensitivity, while they mostly remained resistant to Streptomycin (77.14%), with a smaller proportion being intermediate (14.29%). Overall, the data highlights the high levels of resistance of the 35 isolates of *E. coli* to several antibiotics, especially Ampicillin and Amoxycylav, while some, like Ceftazidime and Tetracycline, retain greater effectiveness.

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Meropenem	91.43% (32/35)	5.71% (2/35)	2.86% (1/35)
Erythromycin (E15)	97.14% (34/35)	0% (0/35)	2.86% (1/35)
Kanamycin (K30)	14.29% (5/35)	20% (7/35)	65.71% (23/35)
Ampicillin (AMP10)	100% (35/35)	0% (0/35)	0% (0/35)
Ceftazidime (CAZ30)	5.71% (2/35)	0% (0/35)	94.29% (33/35)
Tetracycline (TE30)	17.14% (6/35)	0% (0/35)	82.86% (29/35)
Streptomycin (S10)	77.14% (27/35)	14.29% (5/35)	8.57% (3/35)
Imipenem (IMI10)	77.14% (27/35)	14.29% (5/35)	8.57% (3/35)
Gentamicin (CN10)	88.57% (31/35)	11.43% (4/35)	0% (0/35)
Amoxycylav (AMC30)	100% (35/35)	0% (0/35)	0% (0/35)

Table 7: Showing resistance pattern of *E. coli* isolates

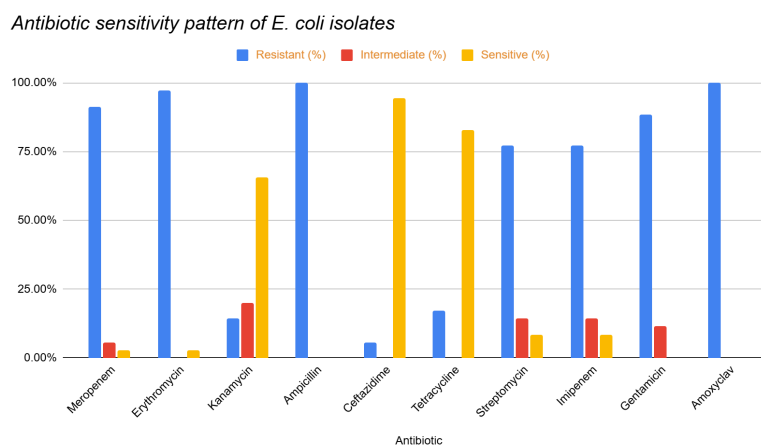


Figure 22: Resistance profile of *E. coli* isolates

3.5.3 Multiple Antibiotic Resistance (MAR) Index:

The MAR index is calculated by dividing the number of antibiotics to which a bacterial strain is resistant by the total number of antibiotics tested. A MAR index greater than 0.2 indicates that the contamination likely comes from a high-risk source where antibiotics are commonly used [94].

In this study, the MAR index was divided into four categories: 0.4, 0.5, 0.6, and 1, which were further classified into four risk levels: Low, High, Higher, and Highest. A total of 10 antibiotics were tested.

- The **low-risk group** included 5 isolates that were resistant to 4 antibiotics (MAR index 0.4). These isolates came from Baridhara/Badda (2 isolates), Gulshan (2 isolates), and Banani/Niketon (1 isolate).
- The **high-risk group** had 4 isolates resistant to 5 antibiotics (MAR index 0.5), with 3 from Khilgaon, Dhanmondi, Farmgate, and Tejgaon, and 2 from Mohammadpur/Lalmatia.
- In the **higher-risk group**, 15 isolates were resistant to 6 to 8 antibiotics (MAR index 0.6-0.8). Of these, 10 were from Mugdapara/Tolarbagh, and 5 were from Mirpur.
- The **highest-risk group** contained 11 isolates resistant to all 10 antibiotics tested (MAR index = 1). These were from Mohakhali (3 isolates), Rampura (2 isolates), Bashundhara (1 isolate), and the remaining isolates from Korail slum.

Most isolates were found in the high-risk group, with MAR values between 0.4 and 0.8.

Risk	MAR index	Number of isolates	Location
Low	0.4	5	Gulshan, Banani, Niketon, Baridhara, Badda
High	0.5	4	Khilgaon Dhanmondi, Farmgate, Tejgaon, Mohammadpur, Lalmatia
Higher	0.6- 0.8	15	Mirpur, Mugdapara, Tolarbag
Highest	1	11	Mohakhali, Rampura, Bashundhara, Korail slum

Table 8: Multiple Antibiotic Resistance (MAR) index

3.5.4 Monthly Variation and Resistance Pattern

In October, the average count for *E. coli* in tap water was 33%, in boil water 21% and in filter water 28%. In November, the average count for *E. coli* in tap water was 36%, in boil water 3% and in filter water was 10%. In December, no sampling was performed because of the campus shifting; the lab was off/shut down. In January, the average count for *E. coli* in tap

water was 3.3% and in filter water was 21%, but boiling water didn't show any growth for *E. coli*. In February, the average count in tap water was 26%, in boil water was 6% and in filter water was 13%. In March and April the average of tap water count showed 41% and 50%. Boil water count was 6% and 13%. Lastly, filter water showed the average count for *E. coli* 20% and 19%. After the discussion, it shows April is the month with the highest average count of *E. coli* in tap water, with an average of 50%. Looking at the overall average counts across all water types, again, April shows the highest average for tap water, and for filter water, the highest average count in March is 20%. The month with the lowest average count for *E. coli* in tap water is January, with an average of 3.3%. For boil water, November has the lowest average count at 3%, indicating minimal contamination. Analyzing the averages, tap water generally shows higher contamination levels compared to boil water and filter water throughout the months. For instance, in April, the tap water had 50%, while boil water had only 13%. This trend is replicated in other months, with tap water showing higher counts than the other types. In short, the highest growth of *E. coli* was observed in April, while the lowest was recorded in January. Tap water is always showing more contamination compared to boil and filter water, which may be due to differences in treatment and sources. Also after AST, the isolates showed variation in their resistance pattern based on the seasons. In summer the isolates resistance pattern was 91%, in fall it showed 75%, and again the isolates from spring showed the average resistance pattern of 41%.

Month	Tap Water	Boil Water	Filter Water
October	33%	21%	28%
November	36%	3%	10%
December	0	0	0
January	3.3%	0	21%
February	26%	6%	13%
March	41%	6%	20%
April	50%	13%	19%

Table 9: Monthly variation of *E. coli* isolates in tap, boil and filter water

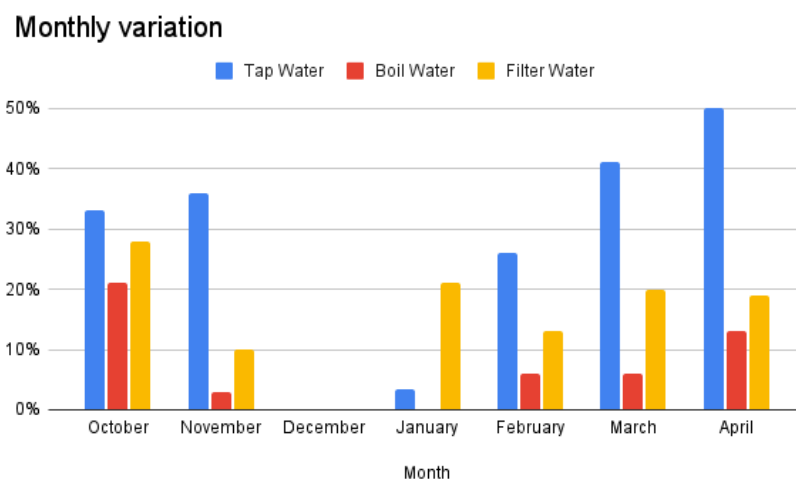


Figure 23: Monthly variation of organisms from different samples

Seasons	Average Resistant Pattern
Fall	75%
Spring	41%
Summer	91%

Table 10: Seasonal resistance pattern of *E. coli* isolates

Average Resistant Patterns

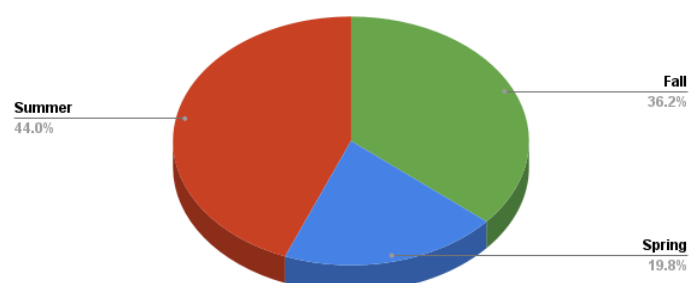


Figure 24: Resistance pattern in different seasons

3.6 PCR results :

PCR was done by using two primer pairs named; ECO-1 and ECO-2 to amplify the 16s rRNA gene (product size: 585 bp).

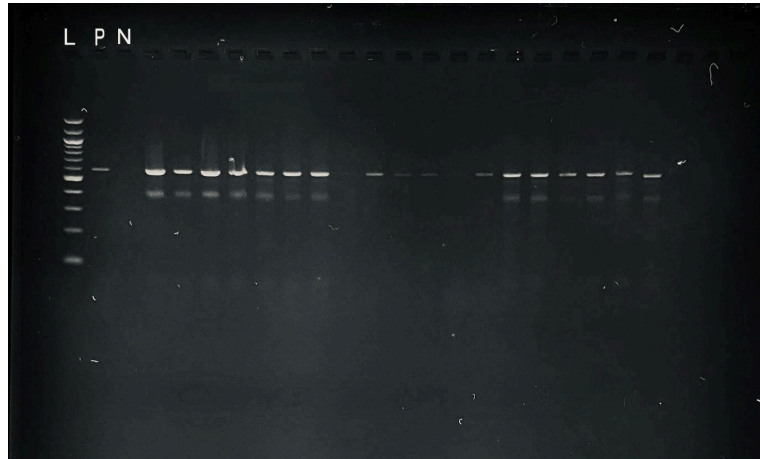


Figure 25: PCR results for *E. coli* isolates. Here, L represents the 100bp DNA Ladder, P represents the Positive Control, and N represents the Negative control (*K. pneumoniae* strain). positive isolates are in lanes. The faint bands signify a low concentration of DNA.

Again PCR was done by using two primer pairs named; *Bla-NDM1* and *BlaOXA2* to amplify the *blaNDM-1* gene (product size: 264 bp) and *blaOXA-2* gene (product size: 702bp) respectively.

PCR results for *blaNDM-1* gene:



Figure 26: *blaNDM-1* PCR results for *E. coli* isolates. Here, L represents the 100bp DNA Ladder, P represents the Positive Control, and N represents the Negative control (*K. pneumoniae* strain negative for the *blaNDM-1* gene).

PCR results for *blaOXA-2* gene:

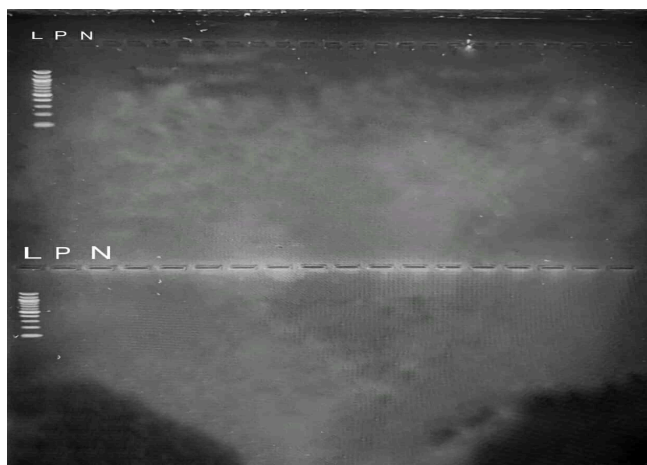


Figure 27: *blaOXA-2* PCR results for *E. coli* isolates. L represents 100bp DNA Ladder, P represents Positive Control, and N represents Negative control (*K. pneumoniae* strain negative for the *blaOXA-2* gene).

The PCR for detecting *BLA-NDM-1* and *BLA-OXA-2* genes could not be successfully completed.

Chapter 4:

Discussion

4.1 Discussion:

Carbapenem-resistant Enterobacteriaceae (CRE) are extremely dangerous, as carbapenems are often drugs of last resort that are used to treat multidrug-resistant infections [62].

E. coli is a gram-negative bacterium that is commonly found in the gut of humans and warm-blooded animals [63]. Most strains of *E. coli* are harmless and even help keep the digestive tract healthy. Some pathogenic variants, such as Enterohemorrhagic *E. coli* (EHEC), can cause severe gastrointestinal illness. Which can lead to diarrhea if one eats contaminated food or drinks polluted water [64]. Contamination of drinking water with *E. coli* typically occurs through fecal contamination from human or animal sources, particularly in regions with inadequate sanitation or compromised water infrastructure. Such contamination not only indicates potential health risks but also suggests the presence of other harmful Pathogens [65]. Carbapenem resistance among *E. coli* strains is a growing public health concern. It often carries resistance genes, which allow them to evade one of the last-resort classes of antibiotics used to treat severe bacterial infections. These resistant strains can survive in drinking water systems, especially in environments with insufficient disinfection or improper waste management, increasing the risk of widespread transmission. Identifying and addressing this resistance is crucial for ensuring safe drinking water and effective public health interventions [66]. The present research was done to isolate and assess the prevalence of carbapenem-resistant *E. coli* in raw and domestically treated water samples in Dhaka city.

Detection methods often require enrichment steps to amplify viable *E. coli* populations to detectable levels, overcoming low initial concentrations and recovering stressed cells caused by food processing. Most rapid genetic assays and traditional methods for detecting specific foodborne pathogens require an enrichment step, which extends the analysis time. However, this step offers significant advantages, such as increasing the target cell count to detectable levels, diluting inhibitory substances, distinguishing viable from non-viable cells, and recovering stressed or injured cells caused by food processing [67]. Enrichment is a critical step in the detection of *E. coli* in food samples. In this study, the enrichment cultures successfully promoted the growth of target bacterial strains, yielding colonies that could be further isolated and purified for identification and analysis. A similar study demonstrated that enrichment facilitated the detection of *E. coli* O157:H7 in contaminated beef samples, achieving reliable identification by allowing the bacteria to multiply to detectable levels [68]. Another study emphasized that the use of a pre-enrichment medium like Tryptone Soy Broth (TSB) before applying selective media is an effective method for recovering and isolating specific bacterial species from complex samples [69]. In this study, by this enrichment method, 103 *E. Coli* were found.

Antibiotic resistance in gram-negative bacteria, including carbapenem-resistant *E. coli*, is a growing global threat. These bacteria often carry resistance to multiple antibiotics, leaving few treatment options. Carbapenems, once highly effective, are now increasingly unreliable, highlighting the urgent need for alternative therapies and careful monitoring, especially in

environmental sources like drinking water [70]. In this study, the Antibiotic Susceptibility Test (AST) was done by using commercially available discs. The results of the test showed 0.00% sensitivity for *E. coli* isolates to ampicillin and amoxycylav. However, gentamycin showed 88.57% resistance. In a recent study conducted in Bangladesh by Sher-e-Bangla Agricultural University in Dhaka found *E. coli* species to be resistant to ampicillin (97.5%) and gentamycin (93.8%) in hospital wastewater [71]. That's a 2.5% increase in ampicillin resistance to *E. coli* in this study which suggests a rise in ampicillin resistance in Bangladesh. The high resistance of *E. coli* to ampicillin in Bangladesh reflects the widespread misuse of antibiotics due to easy over-the-counter availability and lack of regulation [71].

Furthermore, in this study, *E. coli* isolates found from drinking water samples were 91.43% resistant to Meropenem. In the case of other antibiotics not working, meropenem from the class of carbapenem is a last-resort antibiotic that is used to treat serious life-threatening infections. But the percentage of resistance of this last resort antibiotic for the *E. coli* isolates in this study is alarming which can lead to limited treatments resulting in higher morbidity and mortality. Imipenem in this study showed 77.14% resistance to *E. coli* which was higher than the 45% resistance against imipenem & 47% resistance against meropenem shown in a study by Primeasia University of urinary tract infected patients from different hospitals in Dhaka, Bangladesh [72]. The rise in resistance suggests that *E. coli* isolates in the drinking water are evolving resistance at a faster rate compared to clinical settings. This trend can severely limit the effectiveness of these last-resort antibiotics. The increasing resistance in community sources, like drinking water, implies a broader environmental contamination, further complicating the treatment of infections, leading to prolonged illness, higher healthcare costs, and an increased risk of morbidity and mortality. This underscores the urgent need for more robust infection control and antibiotic stewardship measures in both healthcare and environmental sectors. Also in this study, *E. coli* isolates were 97.14% resistant to erythromycin. A similar percentage of resistance to erythromycin (98.7%) was observed in Malaysia from foodborne *E. coli* isolates [73].

Antimicrobial resistance (AMR) patterns of this study suggest multidrug resistance (MDR) for *E. coli* isolates in drinking water. Although there are no antibiotics that showed 100% sensitivity for *E. coli* isolates, ceftazidime was 94.29% susceptible. Tetracycline was 82.85% susceptible which is higher than the 34.9% observed in Malaysia by waterborne *E. coli* isolates [74], [75]. Thus it is safe to say that these antibiotics can still be used as a treatment for carbapenem-resistant *E. coli*.

E. coli has been found to be the reservoir of antimicrobial resistance genes, which upholds the dissemination of resistance in bacterial populations. Since a great deal of research studies MDR in *E. coli* from animals, very little attention is given to non-antibiotic environments. A recent study investigated the microbiological quality and antibiotic resistance of bacterial isolates, particularly *E. coli*, from contaminated water sources [76]. A total of 49.48% prevalence of multidrug-resistant *E. coli* was observed, out of which 63% of the strains showed a MAR index >0.2, indicating frequent exposure to different classes of antibiotics, which is alarming. According to Mishra et al. (2013) and Thenmozhi et al. (2014) [77], [78],

MAR indices ≥ 0.2 indicate high-risk contamination from sources with more frequent use of antibiotics, while values ≤ 0.2 indicate less exposure to antibiotics. A MAR index ≥ 0.4 would specifically indicate contamination from human fecal sources. High MAR indices require urgent monitoring and interventional activities [79]. The study also shows that the bacterial isolates were highly resistant to penicillin (32.99%), cefuroxime (28.87%), and erythromycin (23.71%), while being highly susceptible to nitrofurantoin (93.8%), cefotaxime (91.75%), and amikacin (91.75%) whereas in our study, all the *E. coli* isolates exhibited high resistance to Meropenem + EDTA at 91.43%, Erythromycin at 97.14%, Gentamicin at 88.57%, and 100% resistance to Ampicillin and Amoxycylav. They showed higher sensitivity to Ceftazidime at 94.29% and Tetracycline at 82.86%, with a moderately resistant response to Imipenem at 77.14%. Kanamycin resistance was mixed, while the resistance to Streptomycin was high, at 77.14% and in terms of the MAR index, the low-risk group had 5 isolates resistant to 4 antibiotics (MAR 0.4), the high-risk group had 4 isolates resistant to 5 antibiotics (MAR 0.5), the higher-risk group had 15 isolates resistant to 6-8 antibiotics (MAR 0.6-0.8), and the highest-risk group had 11 isolates resistant to all 10 antibiotics (MAR 1). Most isolates were in the high-risk group (MAR 0.4-0.8) which shows the poor water quality in households in Bangladesh [76]. These carbapenem-resistant Enterobacteriaceae (CRE) are a global health threat because they represent critical antibiotic resistance, usually by the production of enzymes, efflux pumps, and porin mutations. Among carbapenemase enzymes responsible for resistance, KPC, MBLs, such as blaNDM-1, and OXA-48-like enzymes are the most important. The geographical predominance includes KPC in the United States and parts of Europe, blaNDM-1 in countries like India, Pakistan, and Sri Lanka, and OXA-48 in the Middle East and North Africa. These enzymes are plasmid-mediated, allowing rapid horizontal gene transfer and the wide diffusion of resistance. Addressing this threat, treatment strategies must include repurposing of existing antibiotics, dual therapies, and developing new β -lactamase inhibitors.

In this study, 410 isolates were collected, of which 103 were identified as *E. coli*. These isolates were derived from different water sources: 60 from tap water, 24 from boiled water, and 19 from filtered water. The distribution of *E. coli* isolates varied by area. Area 1 had the highest number, with 16 isolates, while Area 4 had the fewest, with just 2. The number of *E. coli* isolates in the other areas were as follows: Area 2 had 11, Area 3 had 14, Area 5 had 10, Area 6 had 12, Area 7 had 11, Area 8 had 9, Area 9 had 7, and Area 10 had 4. The variation in *E. coli* levels across different areas is influenced by factors such as water treatment, sanitation, and local infrastructure [84]. Areas with poor sanitation, inadequate water treatment, or contamination from human and animal waste tend to have higher *E. coli* levels. For example, untreated water systems or improper waste disposal increase the risk of contamination. Agricultural practices, especially manure use and livestock near water sources, also contribute to higher *E. coli* presence. Densely populated areas face more contamination due to higher waste production and strained sewage systems. Additionally, natural sources like contaminated soil or wildlife can also spread *E. coli* in rural areas.

Environmental factors like temperature, rainfall, and humidity significantly affect *E. coli* survival and growth [85]. Warmer months tend to see bacteria multiplying faster, while areas with higher humidity or frequent rainfall may experience more runoff, leading to higher concentrations of *E. coli* in water sources. A report states that warm temperatures and high relative humidity promote the emergence and survival of antimicrobial-resistant bacteria in the environment [86]. A total of 410 samples were collected over three seasons: summer, spring, and fall. The average temperature and humidity of the summer season in the sample collection areas of our study were 30°C to 40°C (86°F to 104°F), with high humidity levels, often reaching 80% or more. The average temperature and humidity of the spring season in the sample collection areas of our study were 25°C to 35°C (77°F to 95°F) and 65%. In fall usually, from September to November, Dhaka experiences relatively more pleasant weather. The average temperature and humidity were 25°C to 30°C (77°F to 86°F) and 75%. High temperature and humidity create an environment where *E. coli* can multiply quickly. This is why we observed higher levels of *E. coli* in the summer and fall compared to spring. Moreover, through this study it is quite clear from the data that the highest resistance patterns are seen in summer, followed by fall, and are low in spring. During the summer, the higher resistance pattern of 0.91 could mean that the bacteria are more exposed to antibiotics because of increased infections or use of antibiotics, leading to a high selection pressure for resistant strains. The fall resistance pattern is 0.75, still considerable, but somewhat lower than in summer, maybe due to a decrease in antibiotic usage or different bacterial strains circulating. This is much higher compared with the average resistance pattern of spring, which was 0.41. By and large, this analysis supports the fact that seasonal variations influence antibiotic resistance patterns to a great extent.

According to the MAR index of the meropenem resistance *E. coli* isolates in the present research, the locations for highest risk for carbapenem resistance are Mohakhali, Rampura, Bashundhara, Korail slum which indicates *E. coli* isolates from those areas are resistant to multiple antibiotics, also a significant level of antibiotic resistance among the *E. coli* populations in the supplied water. In these areas, water quality is usually compromised by a myriad of factors such as urbanization and a lack of adequate sanitation; poor practices in managing wastewater can lead to the contamination of water supplies. Rapid urbanization has been identified in a study in "water research" as usually leading to high levels of inadequacy in water supply and sanitation infrastructures, which increases the risks of contamination. Urban runoff and sewer overflows contribute to the presence of pathogens and antibiotic-resistant bacteria in water bodies[87]. A review in "Environmental Health Perspectives" underlines the agricultural practices that contribute to environmental spread of antibiotic resistance due to overuse of antibiotics leading to runoff that contaminates water[88]. According to other research published in "Science of The Total Environment, inadequately treated wastewater or untreated wastewater harbors resistant bacteria that can introduce it to water sources[89]. The areas with lower socioeconomic status have higher levels of antibiotic resistance due to the limited access to healthcare and sanitation. A study in "BMC Public Health" emphasizes the link between socioeconomic factors and the prevalence

of antibiotic-resistant infections in urban slums[90]. Overall, a combination of these problems contributes to the high levels of antibiotic-resistant *E. coli* in the water supply in these areas.

Among the three samples of water, in this study, the most resistant *E. coli* isolates were from tap water, then from the filter water sample, and lastly from the boiled water sample. This is because tap water is more prone to contamination from various sources including sewage, industrial discharge, and runoff. Residual antibiotics from pharmaceutical waste and agricultural runoff also easily mix with tap water, and the treatment process for tap water is not sufficient to eliminate all resistant bacteria. One review in "Nature Reviews Microbiology" discusses how environmental exposure to antibiotics can drive the evolution of resistance in bacterial populations since bacteria in tap water have to adapt to survive in the presence of antibiotics [91]. This in turn, does not provide equal efficiency of various methods of filtration in the complete removal of antibiotic-resistant bacteria, as they may survive in nutrient environments or biofilms that form on filter media. According to research in "Environmental Microbiology," biofilms may provide bacteria with protection against environmental stresses and agents of antimicrobial action, allowing the growth of resistant varieties [92]. However, in "Applied and Environmental Microbiology", it was pointed out that boiling successfully denatures proteins and causes metabolic impairment at an elevated temperature and thereby was regarded as effective against a variety of pathogens, including those showing resistance to antibiotic therapy; this treatment can therefore be recommended as efficient for reducing the microbial load, including *E. coli* [93].

4.2 Conclusion and future directions:

In conclusion, this study underscores the alarming prevalence of carbapenem-resistant *Escherichia coli* (CRE) in the drinking water systems of Dhaka, particularly from tap water sources, which poses a significant public health risk. The results reveal that these *E. coli* isolates exhibit high resistance to critical antibiotics, especially meropenem, a last-resort treatment for severe bacterial infections. The widespread resistance observed, including multidrug-resistant (MDR) strains, suggests the growing impact of antibiotic misuse and inadequate sanitation on the environment. Tap water, more prone to contamination from sewage, industrial discharge, agricultural runoff, and pharmaceutical waste, harbored the highest levels of resistant strains. This continued contamination with the ongoing risk of resistance may one day make antibiotics ineffective against various infections.

Interestingly, while filtration methods also demonstrated some level of resistance, boiling water was more effective in reducing the microbial load, including *E. coli*, due to the high temperatures achieved. This highlights the importance of proper water treatment practices in mitigating the spread of antimicrobial resistance (AMR).

A critical obstacle in fighting AMR is the health system. In poor settings where access to health services is limited, the normal response to such infections includes self-medication and

other forms of misuse, which further exacerbates resistance. There is a need to increase awareness about the risks associated with inappropriate use of antibiotics and stringently regulate antibiotic prescriptions.

Moreover, the study emphasizes the critical need for better regulation of water quality, especially in areas with rapid urbanization and low-income populations. These regions are more likely to experience higher contamination levels, thus compounding the challenges of AMR. High-risk zones identified in this study, such as Mohakhali, Rampura, and Korail slums, indicate that the issue of waterborne *E. coli* is closely linked to socioeconomic factors, with limited access to healthcare and sanitation services exacerbating the problem.

The findings also suggest that antibiotic resistance patterns in *E. coli* are dynamic and influenced by environmental conditions such as temperature and humidity. These conditions, particularly in the warmer months, support the rapid growth and multiplication of *E. coli*, contributing to the increased resistance levels seen in this study.

This research serves as a wake-up call for the urgent need to strengthen water infrastructure, implement more effective waste management practices, and enforce stringent regulations on antibiotic use, particularly in environments where they are overused, such as agriculture and healthcare. It also highlights the necessity for continuous monitoring of antimicrobial resistance in environmental sources to prevent the further spread of resistant pathogens and safeguard public health. Furthermore, basic research into alternative therapies, including phage therapy and probiotics, as well as technological advances in water purification systems, such as UV radiation, is critical for addressing the spread of resistance. Since AMR knows no borders, addressing it requires a coordinated response across all sectors. Without appropriate action, public health and modern medicine itself are at risk.

Chapter 5:

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