Detection of Extended Spectrum β-lactamase (ESBL) And Carbapenemase Encoding *Escherichia coli* Isolates from Hospital Effluent Wastewater And Hospital Adjacent Community Tap Water in Dhaka Metropolitan City

By
Salman Habib Tishan
19136050
Md Najmul Haque
19136056
Arnab Hasan
19126058

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology and Bachelor of science in Microbiology.

Department of Mathematics and Natural Science
School of Data Science
BRAC University
August 2023

Declaration

It is hereby declared that,

1. The thesis submitted titled **"Detection of Extended Spectrum β-Lactamase (ESBL) and Carbapenemase encoding *Escherichia coli* isolates from Hospital Effluent Wastewater and Hospital Adjacent Community Tap Water in Dhaka Metropolitan City"** is our original work while completing our 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 that 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.

Student's Full Name and Signature:

Salman Habib Tishan
19136050
Department of Mathematics and Natural Science,
Biotechnology Program

Md. Najmul Haque
19136056
Department of Mathematics and Natural Science,

Biotechnology Program

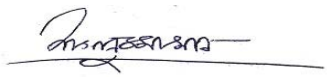
Arnab Hasan
19126058
Department of Mathematics and Natural Science,
Microbiology Program

Approval:

The thesis titled **"Detection of Extended Spectrum β-Lactamase (ESBL) and Carbapenemase encoding *E.coli* isolates from Hospital Effluent Wastewater and Hospital Adjacent Community Tap Water in Dhaka Metropolitan City "** submitted by 1. Salman Habib Tishan 19136050. 2. Md Najmul Haque- 19136056 3. Arnab Hasan-19126058. has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Microbiology in August 2023

Examining Committee:

Supervisor:



(Member) _____

Md. Hasanuzzaman
Senior lecturer,
Department of Mathematics and Natural Sciences
BRAC University

Program Director: _____

Dr. Munima Haque, PhD,
Associate Professor and Director of Biotechnology program
Department of Mathematics and Natural Sciences,
BRAC University

Program Coordinator: _____
(Member)

Dr. Nadia Sultana Deen
Associate Professor,
Department of Mathematics and Natural Sciences
BRAC University

Department Head:
(Chair)

_____

A.F.M Yusuf Haider
Professor and Chairperson,
Department of Mathematics and Natural Sciences
BRAC University

Ethics Statement

For the completion of this study, samples from selected venues were collected following all the necessary precautions. All the experiments were done at BRAC University Life Sciences Laboratory. It should also be noted that no animal or human models were used in this study.

Abstract

Escherichia coli is a gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. *E.coli* is an organism of bacterium that can be identified living in a variety of conditions, such as

warm blooded animals' and humans' gastrointestinal tracts, where it is a component of the gut microbiota and frequently released into the environment through wastewater effluent or diarrhea. The most common way to develop *E.coli is by* consuming contaminated food.

In this project we collected hospital sewage water and tap water from the community which are located 300 meters from the hospital. The main purpose of our project was to identify the *E.coli* ESBL and carbapenems encoding strain from hospital sewage water and 300 meter range of nearby community tap water. From November 2022 to June 2023 a total 125 isolates of confirmed *E.coli* were found and among these 125 isolates 84 isolates were found in hospital sewage water and 41 isolates were found in community tap water which was collected from 300 meters range from the hospitals. Out of 125 isolates, 26 *E.coli* isolates were found to be ESBL, while the remaining 6 isolates were found to be carbapenemase encoded genes. We identified 60 out of 125 isolates from the Dhaka Shishu Hospital and nearby community. Accordingly, 52 isolates were found from the National Cancer Research Institution and Hospital. The remaining 13 isolates found out from DNCC COVID-19 Dedicated Hospital and their nearby area.

After identify the 125 isolates by looking at their Antimicrobial Susceptibility Test (AST) pattern, 64 isolates were selected based on their phenotypic characteristic For identification of ESBL encoded *E.coli* from the 26 *E.coli* 14 isolates were found positive for CTX-M (53.8%) and 12 isolates found positive (46.2%) for bla-TEM. Moreover, 18.8% isolates tested positive for NDM but no positive result for SHV and KPC for identification of carbapenemase resistance genes. After observing the ratio, the vast majority of *E.coli* isolates found ESBL resistant and most of the isolates identified from hospital sewage.

Keywords: *Escherichia coli*, Enterobacteriaceae, ESBL, Carbapenem, Antimicrobial Susceptibility Test, Hospital, Wastewater, Community, Resistant.

Acknowledgement

We would like to start by thanking Almighty Allah for giving us the chance and the willpower to

finish this research. We are also appreciative of His blessings on our daily existence, excellent health, and sound minds. We acknowledge our esteemed **Professor A F M Yusuf Haider, Ph.D.,** Professor and Chairperson of the Department of Mathematics and Natural Sciences, BRAC University, for allowing and encouraging us to complete our undergraduate thesis. Also, our heartfelt gratitude and appreciation goes to **Dr. Munima Haque, PhD** Program Director of Biotechnology program and **Nadia Sultana Deen**, Program Coordinator of the Microbiology program, Department of Mathematics and Natural Sciences, BRAC University, for all the help, love, and direction she always gave us when we needed it.

Regards, gratitude, obligation of gratitude, and admiration to our venerable supervisor **Md Hasanuzzaman**, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his ongoing oversight, frank feedback, knowledgeable direction, passionate encouragement to seek novel concepts, and unending inspiration during the whole course of our study effort. Additionally, we would like to say thank you to **Akash Ahmed**, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his direction and help.

We sincerely thank you for everything to Laboratory Officers **Md. Mahmudul Hasan, Shamima Akhter Chowdhury**, and **Asma Binte Afzal** for their constant generosity and guidance. Additionally, we want to thank **Nishat Tasnim Ananna**, our research assistant, for her amazing generosity and help. We would like to express our genuine appreciation for helping us write the report and occasionally give advice on how to set up experiments, evaluate the data, and choose the next steps for the entire project. It was made feasible to submit our report with their gracious aid.

We also want to thank the lab assistants **Ashik-E Khuda, Tanzila Alam,** and office assistant **Nadira Yeasmin** for their help throughout the duration of our eight months in the lab. In

conclusion, we would like to thank all of the people that helped us in the lab and collaborated with us.

Table of Contents:

List of Graphs:

List of Figures:

List of Tables:

Chapter 1

Introduction

1.1. *Escherichia coli* (*E. coli*)

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, facultative anaerobic bacteria that is motile with flagella that are peritrichous or nonmotile. Theodor Escherich initially defined this

bacterium in 1885. The bacteria Escherichia coli (*E. coli*) tends to thrive in the lower intestine of organisms that are warm-blooded. The majority of *E. coli* strains are safe but a few can cause serious food-borne illnesses. Enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and diffuse adherent (DAEC) *E. coli* strains cause gastroenteritis in humans (Microbiology of Waterborne Diseases, n.d.). A complex cell wall surrounds Escherichia coli cells, consisting of two concentric lipid bilayers, the outer membrane and the cytoplasmic membrane, with a periplasmic distinction in between. This cell wall provides a variety of purposes including defense, transport, motility, sensing, detoxification, and energy production. The typically zoonotic bacterial pathogen has been responsible for outbreaks of infection in humans caused by contamination of drinking and water used for recreation in both developing and developed countries (Cho et al. 2018) *E. coli* is a feces borne coliform that is more specific in detecting contamination by feces than other fecal coliforms. As a result, the presence of *E. coli* in drinking water implies fecal contamination. Water contaminated with *E. coli* is an alarming signal of sewage or animal waste pollution. Many different forms of pathogenic organisms can be found in sewage and animal waste. Consumption can lead to serious health issues. Children under the age of five, people with weakened immune systems, and the elderly are most at risk (Microbiology of Waterborne (Diseases, n.d.).

In this project, *E.coli* isolates were isolated from hospital wastewater and surrounding community tap water in Dhaka Metropolitan City. A four-month study (December 2022 to March 2023) was done in three separate hospital sites and their surrounding communities, with isolates obtained mostly based on appearance in bacteriological media, followed by genus-specific PCR and Antimicrobial Susceptibility Test. That follows, ESBL and Carbapenem-resistant gene developments were concentrated on the samples.

People become ill from infectious diseases as a result of using and drinking this type of water. As a result, the purpose of this project is to discover and analyze the *E.coli* resistant pattern in hospital wastewater and community tap water. Thus, this initiative will also assist us in

determining the present state of the spread of prevalent infectious diseases caused by pathogenic *E.coli.* This project will help to safeguard Bangladesh's healthcare difficulties in the future. So, the target was set in anticipation of similar studies conducted in industrialized countries, the findings of which indicate that there are significant risks for the health sector in current times. Bangladesh, on the other hand, is a developing country with an overcrowded capital city and weak architectural infrastructure. Furthermore, this research will focus on analyzing the wastewater supply line and emphasizing the use of antibiotics in the treatment of infectious diseases.

The ability to cope with various antibiotics emerged as a result of the misuse of antibiotics on rather than pathogen-specific treatment, in-patients are treated subjectively by clinicians. The Patients' wastes are dumped off in the sewage system, where germs spread due to the hospital's inappropriate wastewater purification system. It is critical to treat wastewater according to the regulations provided so that microbes from wastewater that is not treated do not enter community tap waters in large numbers and cause severe infections such as nosocomial infections, putting public health at risk of harm. Over time, because of spreading of resistant *E.coli* in community water, the isolates can get the resistant genes through mutation and selection, as well as genetic exchange, resulting in a significant problem. So, this research project aims to emphasize the potential risk associated with untreated hospital wastewater, which can and will contaminate the tap water in the nearby community.

1.2 Literature review:

Escherichia coli (E. coli) is a gram-negative bacillus responsible for a variety of diarrheal diseases, such as dysentery and diarrhea. Although it is considered to be a normal part of the intestinal flora, it can also lead to extraintestinal and intestinal diseases in people. The most likely infection causing uncomplicated cystitis is *E. coli*. Both human and animal bacterial contamination of environmental water sources is a cause for concern. Wastewater release, sewage leaks, failed septic tanks, as well as municipal, household, medical, and industrial waste resources, are examples of

potential human sources. Animal inducing sources include raccoon and deer excrement, animal dung deposited on the ground, pet waste dumped in parks, and runoff from animal farms. Having a presence of pathogenic *E. coli* in the water might raise the risk of human illnesses as a result of coming into contact with these water sources because surface waters are frequently used for drinking and for recreational activities (Cho S et al., 2018). The gene contents of different strains of *E. coli* differ according to how they prefer certain environments and hosts. According to Bergthorsson and Ochman (1998), the *E. coli* wild isolates genome sizes range from 4.5 to 5.5 megabases. The lab strain K-12 MG1655 with 4,639,221 base pairs (bp) and two isolates of the enteric pathogen O157:H7 with 5,528,445 bp are the two strains of pathogenic *E. coli* that have been fully sequenced. Within the *E. coli* species, K-12 and O157:H7 are closely related.

1.2.1. Pathogenic Categories of *E.coli*:

E. coli pathogens fall into two categories as intestinal pathogens that cause diarrhea and extraintestinal *E. coli* (EPEC) pathogens that cause a number of illnesses in animals as well as humans, including urinary tract infections, meningitis, and septicemia (Johnson et al., 2003; Riley, 2014). While the EPEC major clone sequence type (ST) 131 has crossed over and spread around the world, other clones are equally prevalent.(i.e., ST10, ST38, ST69, ST73, and ST405) (Nicolas Chanoine et al., 2014; Manges et al., 2019).

1.2.2. Pathogenic Categories of *E.coli* based on Genomic Structure:

On the other hand, diarrheagenic *E. coli* have been divided into pathovars depending on their virulence gene arsenal. Humans contribute immensely to the spread of *E. coli* in the environment via wastewater. These wastewaters are processed in effluent treatment plants (WWTPs) in advanced nations. However, *E. coli* is present in WWTP outputs and is discharged into rivers.(Bréchet et al., 2014).With 2,000 genes present in 247 islands in one pathotype that are absent in K–12, the genomic structure of the *E. coli* pathotypes that have been sequenced so far displays a staggering mosaic pattern. Pathogenic *E. coli* can have up to 0.53 MB of DNA missing, which is also common in K–12.

1.2.3. Phylogroups of *E.coli*:

Agriculture additionally contributes to *E. coli* spread through engagements like spreading manure or sewage sludge.(Cabral, 2010; Niu and Phanikumar, 2015; Hocquet et al., 2016).Cattle feces additionally help significantly to the propagation of *E. coli* in the environment. (Cabral, 2010). *E. coli* affects practically all ecosystems, with both human-associated and non-human-associated *E. coli* found in rivers, lakes, groundwater, plants, and soils. (Brookes et al., 2004; John and Rose, 2005; Park et al., 2016).The phylogroups A, B1, B2, C, D, E, F, and G of *E. coli* bundle together. (Clermont et al., 2013, 2019). Strains from phylogroups A and B1 are widespread and have adjusted to humans or vertebrate animals. Phylogroup A strains are more prevalent in humans, while Phylogroup B1 strains prevail in animals.(Berthe et al., 2013). The exceptional phylogroup C has associations to phylogroup B1.(Moissenet et al., 2010). The most frequently encountered phylogroups detected in human EPEC infections are strains from phylogroups B2, D, and F.(Clermont et al., 2013).

1.2.4. Extended Spectrum Beta-lactamase (ESBL):

The beta-lactamase enzyme contains ESBL, or extended-spectrum beta-lactamases. ESBLs are plasmid-derived enzymes that can break down oxyimino-cephalosporin and monobactams but not carbapenems such as meropenem and imipenem. ESBLs are initially generated from a narrow spectrum parent ESBL enzyme and may neutralize penicillin, azithromycin, and broad-spectrum cephalosporins while eliminating carbapenems. This procedure functions through hydrolytic impact and the suppression of beta-lactamase inhibitors known as clavulanic acid. The oldest ESBLs are TEM-1, TEM-2, and SHV variants. *E.coli's* multidrug resistance (MDR) has been implicated in the establishment of fatal diseases.

ESBL is classified into three categories:

- ESBLA (Class A) enzymes - the most common ESBLs, CTX-M, SHV, and TEM enzymes. The enzymes can be transmitted horizontally and destroyed by clavulanic acid.

- ESBLM (miscellaneous ESBL) - divided into two sections: a) ESBLM-C (plasmid mediated AmpC, class C) and ESBLM-D (class D)
- ESBLCARBA (ESBLs that degrade carbapenems) - this category is divided into three sections: ESBLCARBA-A, ESBLCARBA-B, and ESBLCARBA-D are the three variants.

1.2.5. Virulence Factors of Pathogenic *E.coli*:

E. coli virulence factors might affect a variety of eukaryotic cellular functions, including cell signaling, ion secretion, protein synthesis, mitosis, cytoskeletal function, and mitochondrial function. These pathogenic *E. coli* components are usually encoded on genetic elements such as plasmids, bacteriophage, transposons, and pathogenicity islands, which can be deployed into various strains in order to generate novel virulence factor arrangements.

1.2.6. Resistance *E.coli*:

Extended-spectrum -lactamase (ESBL) is a very widespread cause of cephalosporin resistance in *E. coli.* ESBL-producing *E. coli* have expanded globally in recent decades, constituting a severe public health danger.(Pitout and Laupland, 2008). These strains with resistance were initially obtained from humans, animals, and food sources. (Mughini-Gras et al., 2019; Pärnänen et al., 2019). *E. coli* isolated from liquid hospital waste water was resistant to more than three antibiotics. Recent research examined the links between *E. coli* from humans, meat, cattle, and WWTPs in order to better comprehend the role of humans in the spread of *E. coli.* (Day et al., 2019; Ludden et al., 2019).

1.2.7. Epidemiology of *E.coli*:

Women had a greater overall incidence rate of *E.coli* bacterial infections than men. This link proved to be confined to young and middle-aged individuals; women and men had similar incidence rates in adults older than 60 years. In the small number of studies that reported incidence rates by setting of acquisition, rates for hospital-acquired *E.coli* bacteremia were 0.1 and 0.17 per 1000 patient-days [20, 21] (3650 and 6205 per 100 000 patient-years), respectively, though rates for community-onset, healthcare-associated *E.coli* bacteremia were 25.8 and 32 per 100 000 person-years**.** According to a review of databases and research from ten of the fourteen World Health Organization divisions, the global incidence of *E. coli* is 2.8 million cases per year. In children, *E. coli* O157:H7-induced HUS may trigger systemic morbidities which include acute renal failure.

Chapter 2

Methodology

2.1 Site of Sample collection:

For sample collection, three hospitals were chosen which were in three different locations where hospital samples were collected from hospital sewage water and the community sample was

collected from the neighboring community tap water between 300-meter radius. The three sample collection sites were **Dhaka Shishu Hospital (DSH)**, **National Cancer Hospital (NCH)**, **DNCC Dedicated Covid-19 Hospital (DNCC)**. From the DSH,

One hospital sample was collected and in the 300-meter radius two community tap water samples were collected as only two communities meet the requirement for our study. But from NCH and DNCH one hospital sample was collected and four community tap water samples were collected which were located near these hospitals. Every week, the samples were collected from these different sites.

2.2 Map of sites:

© from Google Maps

These black landmarks indicate the Hospitals location, from where we had collected our samples.

2.3 Sample transportation:

An ice box was used for collecting the water sample to maintain the temperature and also prevents the growth and proliferation of the sample. For the collection of hospital samples, an autoclaved falcon tube was used and for the community sample collection an autoclaved bottle was used. Before the sample collection, lab equipment such as membrane filtration apparatus, falcon tube, collection bottle was autoclaved because all the equipment must be in sterile condition. Also, necessary precautions were maintained such as using gloves and masks because various types of unwanted microorganisms can be infected during collecting the samples.

Figure 1: Ice Box

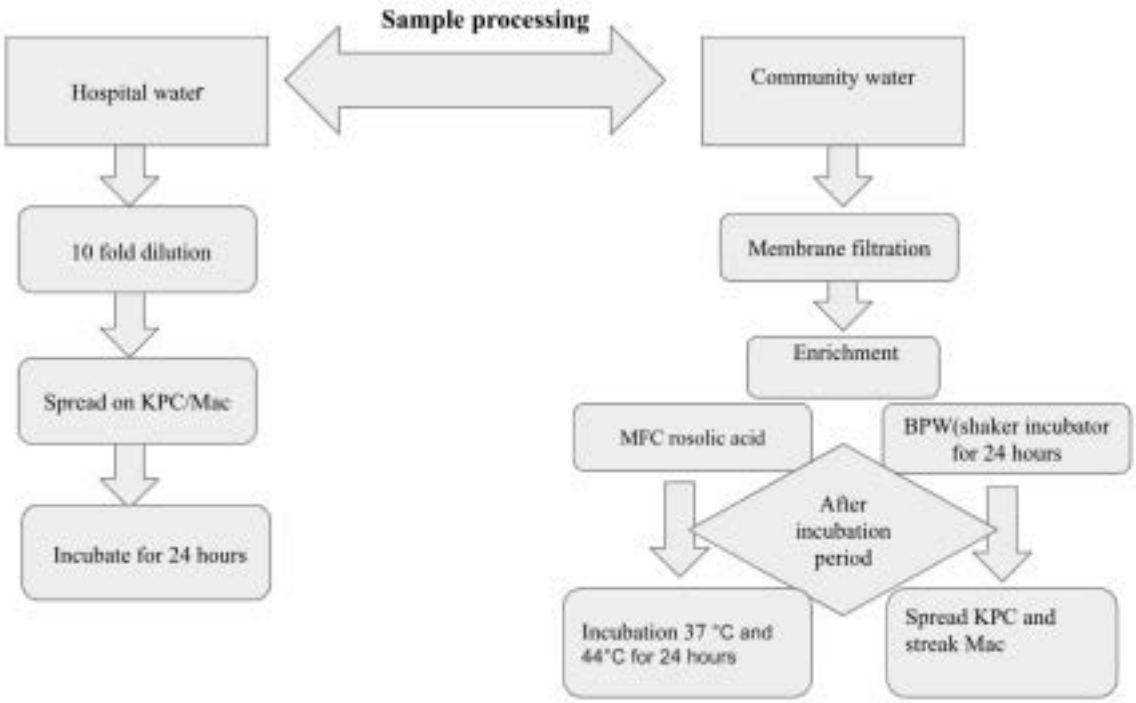
This icebox was used for sample collection and transportation.

2.4 Sample Processing in Laboratory:

For further processing the sample. The hospital samples were diluted 10-fold and spread in KPC

or MacConkey agar medium. On the other hand, membrane filtration was done to the community sample. For community samples, the filter paper was enriched on MFC agar media and in BPW.

2.5 Flow chart of Sample processing:



Here is the flowchart of our sample processing

2.6 Screening for Suspected *E.coli*:

After the sample processing, the culture plates were observed after 24 hours of incubation. *E.coli* was collected from the culture media by observing their morphology. After analyzing the morphology of *E.coli*, the M-FC culture plate with Rosolic Acid, *E.coli* shows pure blue color

colonies. On the other hand the *E.coli* shows purple color on KPC. And after that *E.coli* shows Dark pink color on MacConkey plates. Lastly on the EMB agar media *E.co*li shows a green shing color .

2.7 Membrane filtration:

Membrane filtration is a separation technique that may be utilized in order to eliminate dangerous compounds from liquids. Membrane filtration is commonly used for water purification where it is used to treat drinking water or wastewater by removing various types of contaminants like bacteria, viruses and other microorganisms.

After the sample collection, the community tap water sample was filtered through a membrane filtration process, 100 ml of community tap water sample was used during this procedure. After membrane filtration, the filter paper was enriched on MFC agar medium and BPW. The MFC agar medium was incubated for 24 hours at 37 degrees and 44 degrees Celsius.

2.8 Dilution:

A rich culture of microorganisms is diluted in a series of steps called "serial dilutions" to reduce the concentration of microorganisms. It is basically used to reduce the concentration of a substance in a solution progressively.

In our lab, a 10-fold serial dilution process was followed. Which means the concentration of a solution reduces by a factor of ten that is to 1/10 of the original concentrations. For the hospital sample, 9 ml of saline water was used to dilute 1 ml of our sample, 1000 µl.micropipette was used. By this procedure, hospital samples were diluted from 10^1 to 10^7 and also, after 24 hours of shaker incubation of BPW samples, the enriched samples were diluted.

2.9 Spread plate method:

The spread plate method is a method for plating a liquid sample containing bacteria so that it is simple to count and separate the germs. A spread plate allows the microbiologist to estimate a

viable microorganisms number of isolated bacterial colonies dispersed throughout it. After serial dilution, 100 µl of direct,10^2,10^3,10^4 dilution of hospital and BPW (Buffer peptone Water) enriched community water samples were spread on a selective KPC media and MacConkey media. After that, the sample was spread across the surface of the plate using a sterile spreading tool called a spreader by gently moving the spreader. Lastly, the plate was left to dry for 1-2 minutes to allow any excess liquid to be absorbed.

2.10 CFU Unit:

Colony Forming Units are known as CFUs. In a serving of a probiotic dietary supplement, this refers to the quantity of living, active bacteria. CFUs per gram or CFUs per milliliter are the usual units used to quantify this.

After the 24 hours of incubation, the plates were observed and the colony was counted from the KPC, MacConkey and MFC culture media.

2.11 Streak plate method:

In this method, a loop of culture is placed over an agar plate to space out individual cells sufficiently. The inoculum is gradually diluted by the streaking technique so that the bacteria may be counted as colony forming units (CFUs).

During our study, after the CFU count the isolates were selected by observing their colony morphology on selective media, for example, dark navy-blue colonies were collected from MFC culture plates, purple from KPC and mate pink colonies from MacConkey culture plates. The selected colonies were streaked on EMB agar media, which shows purple or green metallic sheen after 24 hours of incubation.

2.12 Spreading:

As previously mentioned, hospital samples were diluted, community samples were filtered, and

BPW enrichment was also used. A few dilutions were chosen, and spread plates were done. *E.coli* has various traits on MacConkey, EMB agar, KPC and MFC agar with Rosolic acid.

2.13 MacConkey Agar:

MacConkey agar is used for the isolation of gram-negative enteric bacteria. In MacConkey agar, the colony morphology of *E.coli* is red or pink and non-mucoid.

2.14. EMB Agar:

EMB agar is both a selective and a differential medium which is selective for gram negative bacteria. In EMB agar, the colony morphology of *E.coli* is purple or green metallic sheen.

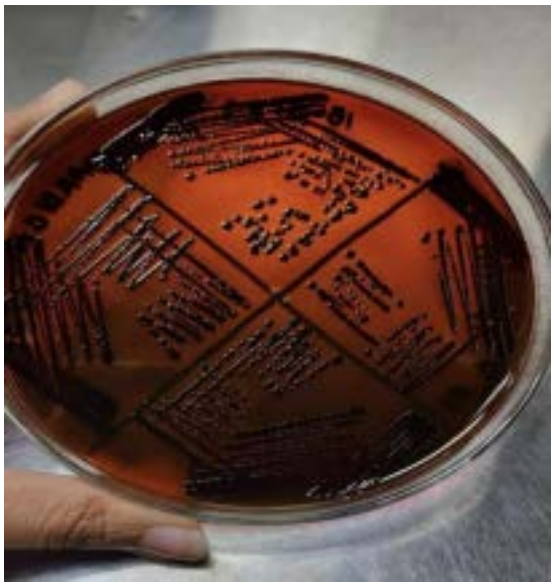


Figure 2: EMB agar media plate of sample

2.15. MFC Agar with Rosolic Acid:

In MFC agar with rosolic acid media, the colony morphology of *E.coli* is blue color on the membrane filter which is identified as fecal coliform, on the other hand, pink color colonies on the membrane filter is identified as total coliform.

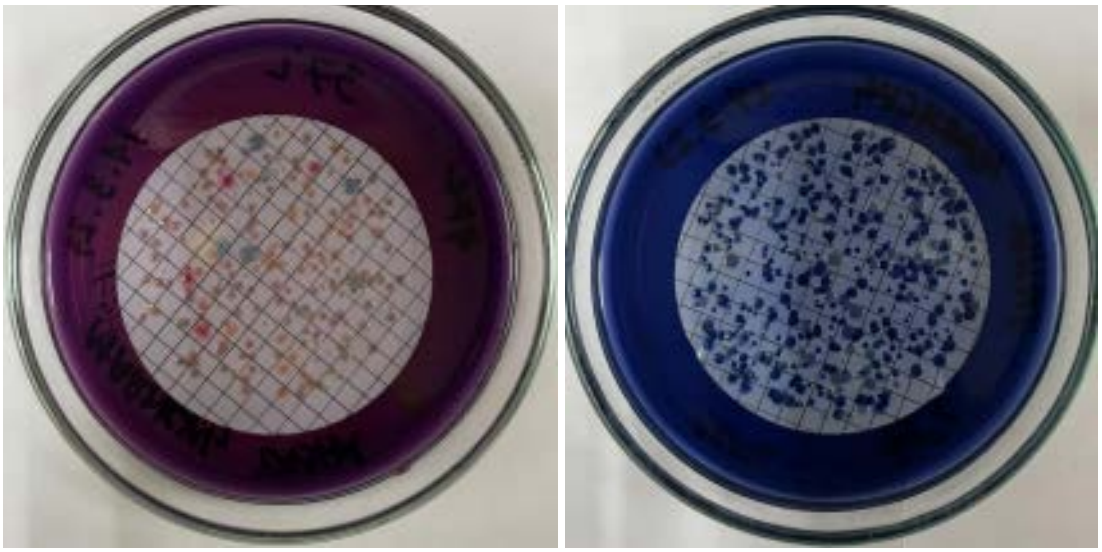


Figure 3: MFC plate of sample

2.16: DNA Extraction:

DNA extraction is the process of isolating DNA from the cells or tissue of an organism isolated from a sample. In our laboratory, after the streaking was done and the culture was incubated overnight, we started to do our next steps which is called DNA extraction. For that, 150 µl of TE Buffer was taken by a sterile pipette in a MCT tube. After that, a loop full of cultures which was incubated for 24 hours on the NA Plate was taken and carefully suspended in the TE Buffer. Then according to the boiling method, the MCT was boiled for 15 minutes at 100 degrees Celsius. After that, the boiled MCT tube was centrifuged at 13,000 rpm for 5 minutes and the cell debris was precipitated. After centrifugation, the supernatant was collected and transferred to another MCT

and the pellet or cell debris were discarded. The collected supernatant was stored at -20 degree Celsius.

2.17: PCR Amplification and Nucleotide Sequence:

The isolates which had been primarily identified as *E.coli* were confirmed by PCR using ECO primer. The PCR reaction was conducted in a final volume of 13µl, containing 2µl of DNA template, 2.5µl of nuclease-free water, 1µl of primers (o.5µl each), and 7.5µl of Taq Polymerase PCR master mix. A 30-cycle amplification procedure that used 95 °C for 30 seconds, 58 °C for 45 seconds, and 72 °C for 45 seconds, with a final extension step of 72 °C for 10 minutes, was used after an initial incubation at 95 °C for 15 minutes. After PCR reaction, Gel Electrophoresis was conducted at 110 volts for 50 minutes on 2% agarose gel. The gel was stained with ethidium bromide for 10 minutes after electrophoresis for the gel visualization.

Primer Name	Sequence	Product Size	Tm (°C)
ECO	F- 5’-GACCTCGGTTTAGTTCACAGA-3’ R- 5’-CACACGCTGACGCTGACCA-3’	585-bp	58°C
SHV	F- 5'-TACCATGAGCGATAACAGCG-3' R- 5'-GATTTGCTGATTTCGCTCGG-3'	450-bp	58°C
CTX-M	F- 5’-ACGCTGTTGTTAGGAAGTG-3’ R- 5’–TTGAGGCTGGGTGAAGT-3’	759-bp	58°C
NDM	F- 5’-GGTTTGGCGATCTGGTTTTC-3’	621-bp	58°C

	R- 5’-CGGAATGGCTCATCACGATC-3’		
bla-TEM	F- 5’-TACGATACGGGAGGGCTTAC-3’ R- 5’-TTCCTGTTTTTGCTCACCCA-3’	1080-bp	51°C

Table 1: Primer sequence

2.18 Antimicrobial Susceptibility Test (AST):

An antibiotic sensitivity test is utilized to determine the most effective method of treatment for bacterial illness. All antibiotics have a unique range of susceptibility that is evaluated by CLSI. By using the susceptibility range, we can recognize the infections that are resistant and carry out additional steps. The procedure required, spectrophotometer and Mc-Farland 0.5 standard and Kirby-Bauer method was used. The single colony which was a 24-hours incubated sub-cultured isolate was inoculated in 5ml saline. Optical density was measured, here 0.5 McFarland was used as standard which is OD= 0.1. After confirming the desired value, the Kirby-Bauer method was carried out. There had been a total of 11 antibiotics used in accordance with the CLSI Standard:

1. Ampicillin (AMP 25)
2. Amikacin (AK 30)
3. Aztreonam (ATM 30)
4. Cefixime (CFM 5)
5. Ceftazidime (CAZ 30)
6. Ceftriaxone (CTR30/CT30)
7. Chloramphenicol(C30)
8. Gentamicin (GEN10)
9. Imipenem (IMP 10)

10. Meropenem (MRP 10)
11. Tetracycline (TE 30)

Zone Size Interpretative Chart as per CLSI:

Antimicrobial agent	**Symbol**	**Disc content**	**Sensitive mm or more**	**Intermediate mm**	**Resistant mm or more**
Amikacin	AK	30 mcg	17	15-16	14
Ampicillin	AMP	25 mcg	17	14-16	13
Aztreonam	ATM	30 mcg	21	18-20	17
Cefixime	CFM	5 mcg	19	16-18	15
Ceftazidime	CAZ	30 mcg	21	18-20	17
Ceftriaxone	CTR	30 mcg	23	20-22	19
Chloramphenicol	C	30 mcg	18	13-17	12
Gentamicin	GEN	10 mcg	15	13-14	12
Imipenem	IMP	10 mcg	23	20-22	19
Meropenem	MRP	10 mcg	23	20-22	19
Tetracycline	TE	30 mcg	15	12-14	11

Table 2: Antimicrobial Susceptibility Testing - Zone Size Interpretative Chart

2.19 PCR Assay:

PCR (polymerase chain reaction) is quick and can accurately diagnose certain viral infection and genetic alteration. Using the polymerase chain reaction, a specific DNA segment can be quickly multiplied (amplified) into millions or billions of copies. PCR involves using short synthetic DNA fragments which are called primers to select a segment of the genome to be amplified. Multiple rounds of DNA synthesis to amplify that segment. PCR is also done to identify the specific pathogens, and ECO primer was used for the identification of *E.coli*. The band size of ECO primer is 585 bp and is specific only for *E.coli* identification. The sequence of ECO primer is:

30

ECO-F GACCTCGGTTTAGTTCACAGA
ECO-R CACACGCTGACGCTGACCA

PCR method was used for the quantification and identification of bacterial species which were collected from hospital wastewater and community water samples. PCR was conducted.

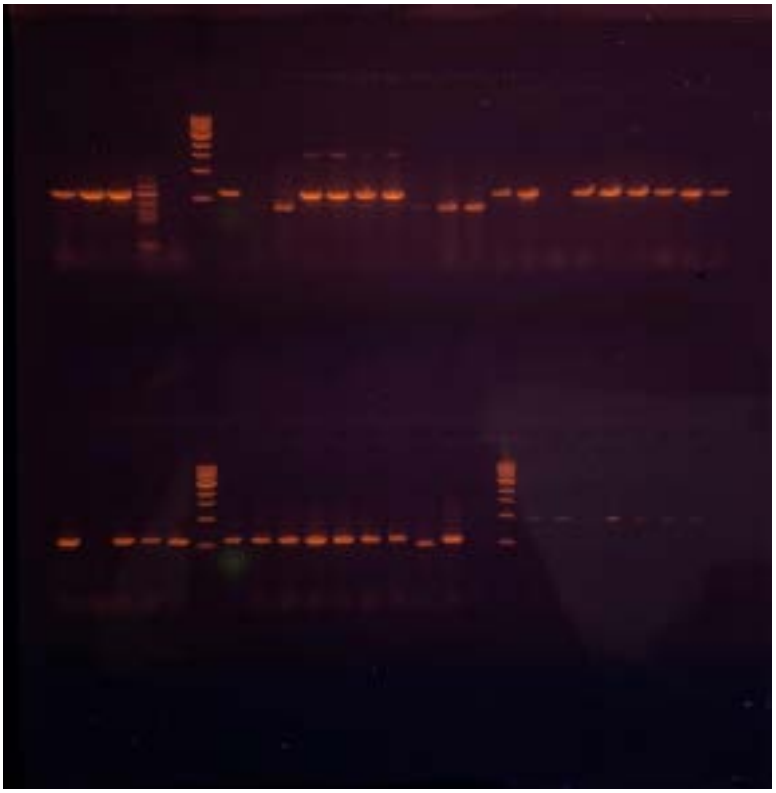


Figure 4: Gel of samples

Chapter 3

Result Interpretation and Observation

3.1 Isolation of *E.coli*:

During the study period from November 2022 to June 2023 a total of 78 samples from hospital sewage and community tap water had been collected. Eighteen of the 78 samples were collected from hospital wastewater and the remaining 60 samples were collected from tap water in the community around the hospital. From the 78 samples, 125 isolates were identified as *E.coli*, by observing the colony morphology on selective KPC, MacConkey and EMB agar media. Out of those 125 isolates, 84 isolates were found in hospital wastewater and the remaining 41 were found in tap water from the local community. PCR was frequently performed to determine whether or not these isolates were *E.coli*.

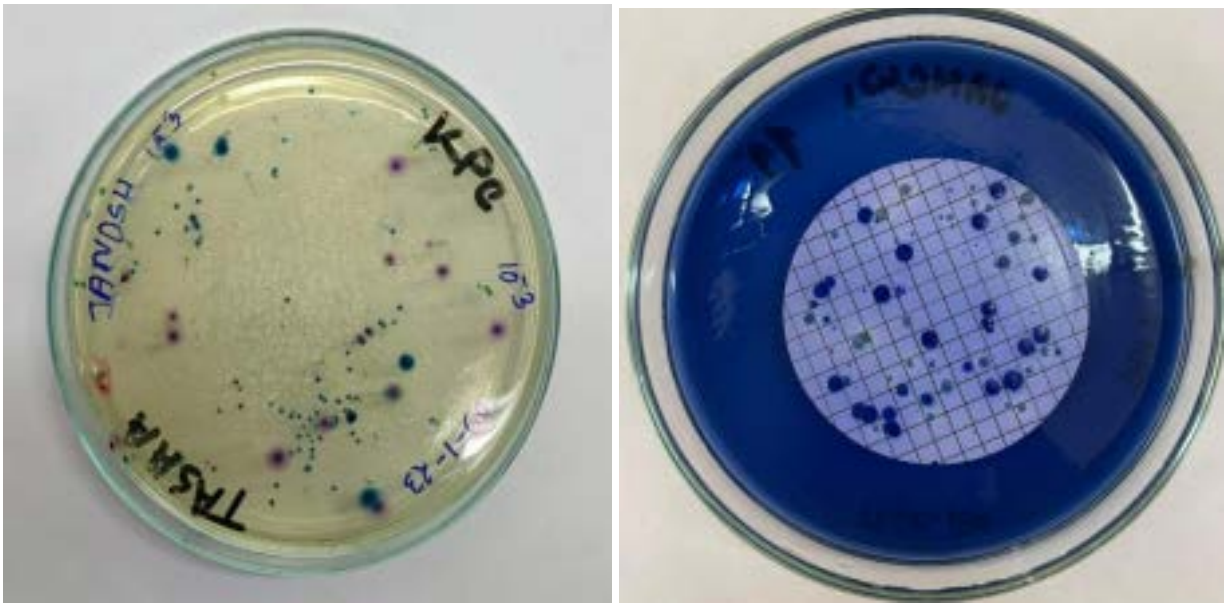
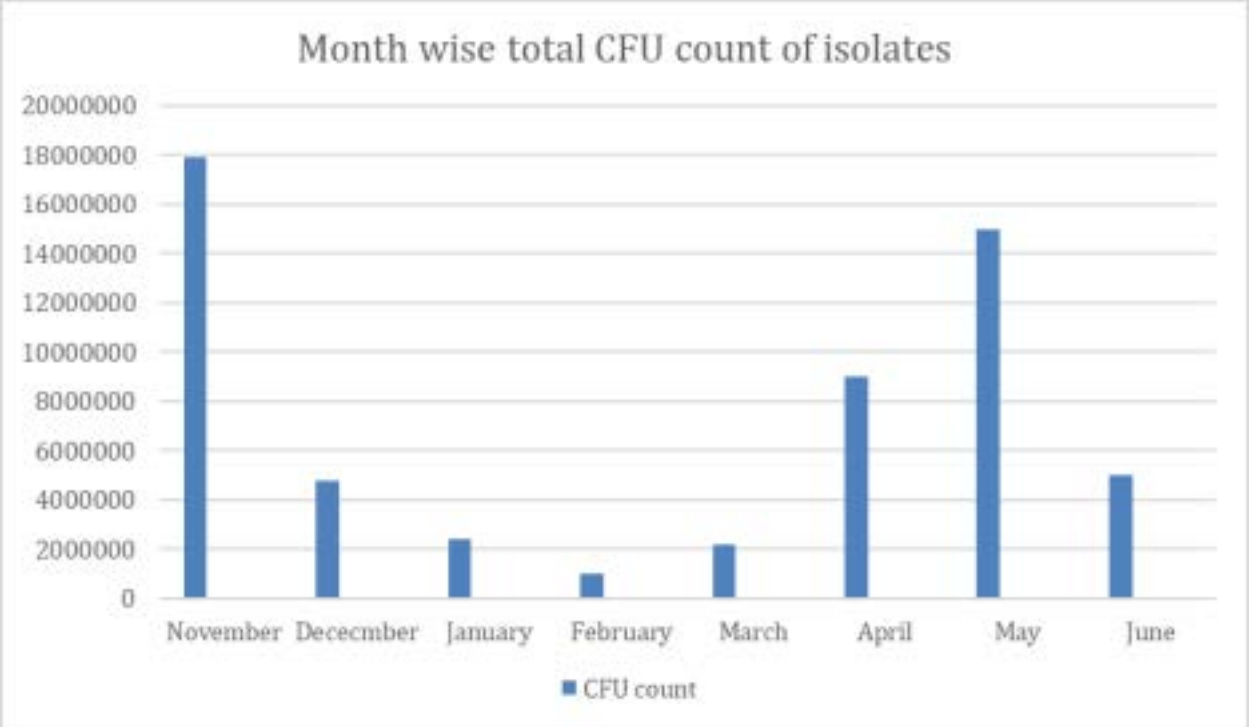


Figure 5: Purple color colonies on KPC agar media (Left) and Dark blue color colonies on MFC agar media.

The maximum number of colony formation was recorded in November 2022 at the National Cancer Research Institute and Hospital, by analyzing total CFU counts of hospital samples. However, total CFU counts at the DNCC COVID-19 Dedicated Hospital sharply declined in the same month. The greatest total CFU count was recorded in November 2022 and May 2023,

whereas the lowest total CFU count was recorded in February. According to month wise interpretation, the CFU rate declined from December 2022 to February 2023 and rapidly increased from March to May 2023. which indicates that, during the winter season the CFU count was the lowest and after the winter rapid increase of CFU was observed. The National Cancer Research Institute and Hospital provided the highest total CFU count, on the other hand, DNCC COVID-19 Dedicated Hospital provided lower total CFU counts.



Graph 1 : Total CFU count of isolates from November 2022 to June 2023

3.2 Identification of *E.coli* by PCR assay and Gel Electrophoresis:

By using ECO primer, which is species specific for *E.coli*, PCR and Gel electrophoresis was conducted and the gel was observed under UV illuminator. During the sample collection period

from November to June, a total 125 *E.coli* isolates were confirmed. The agarose gel which was observed under UV is given below:

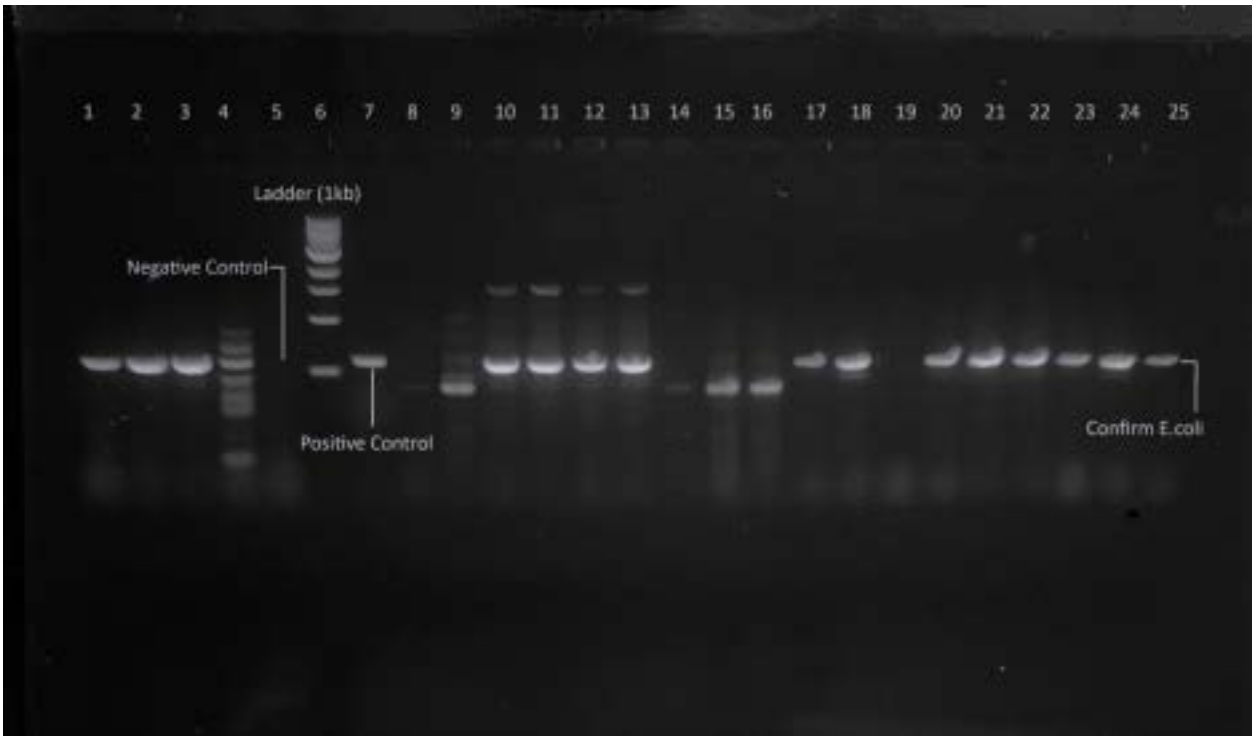
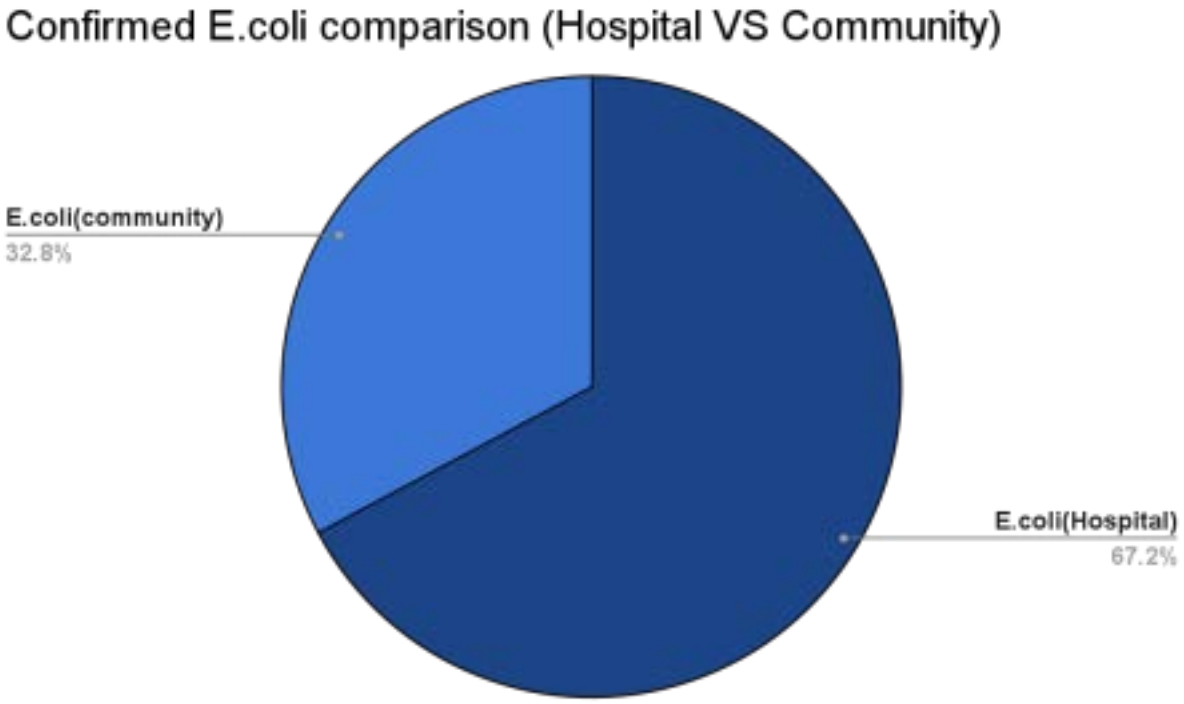


Figure 6: Identification of *E.coli* by using ECO primer

3.3. Hospital and Community samples isolate comparison:

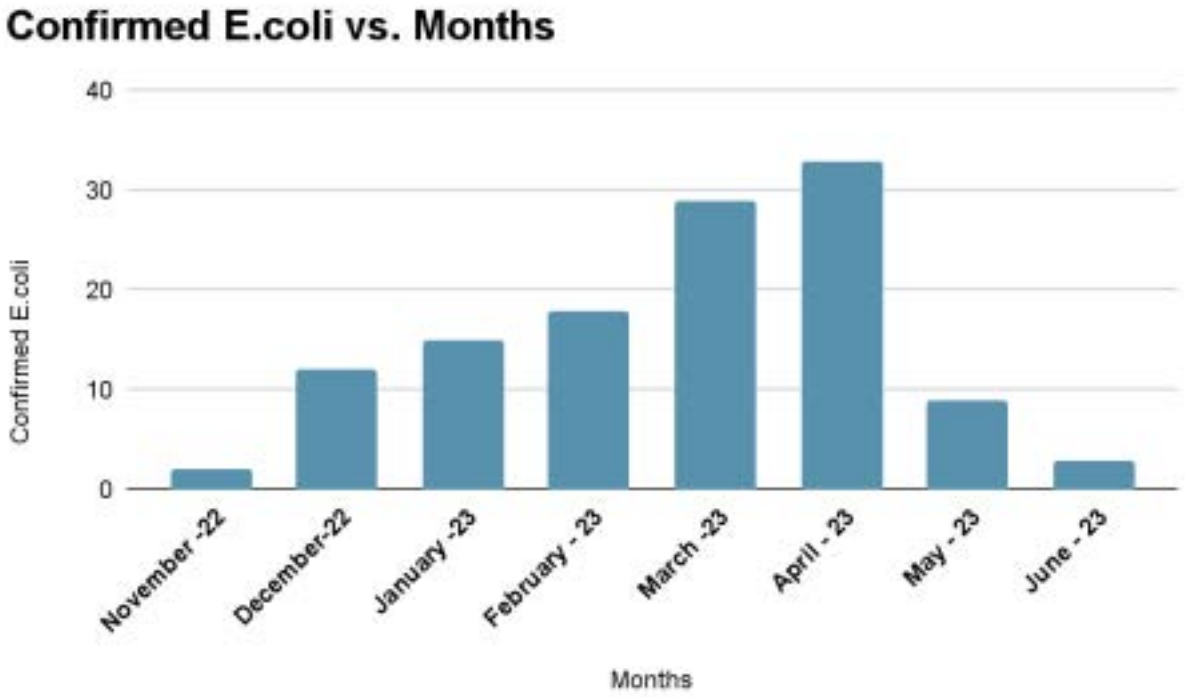
During the study period from November 2022 to June 2023 a total of 78 samples from hospital sewage and community tap water had been collected. A total 125 isolates were identified as *E.coli* by observing the colony morphology. By analyzing the result, it was identified that 67.2% (84 out of 125) isolates were from hospital wastewater samples. And the rest 32.8% (41 out of 125) isolates were from community tap water samples. Here is the graphical presentation:



Graph 2: Confirm *E.coli* comparison between Hospital and Community isolates

3.4 Month wise identification of confirm *E.coli*:

The study period to isolate *E.coli* from hospitals and their nearby community tap water in Dhaka city was from November 2022 to June 2023. During this study period, a total of 125 confirmed *E.coli* isolates were found from both the hospital and community samples. Following a breakdown of the confirmed isolates by month, it can be seen that there were 2 confirmed isolates in November 2022, 12 in December 2022, 15 in January 2023, 18 in February 2023, 29 in March 2023, 33 in April 2023, 9 in May 2023 and 3 in June 2023. Therefore, it is obvious that the maximum number of verified isolates occurred in March and April and the lowest number occurred in November 2022.

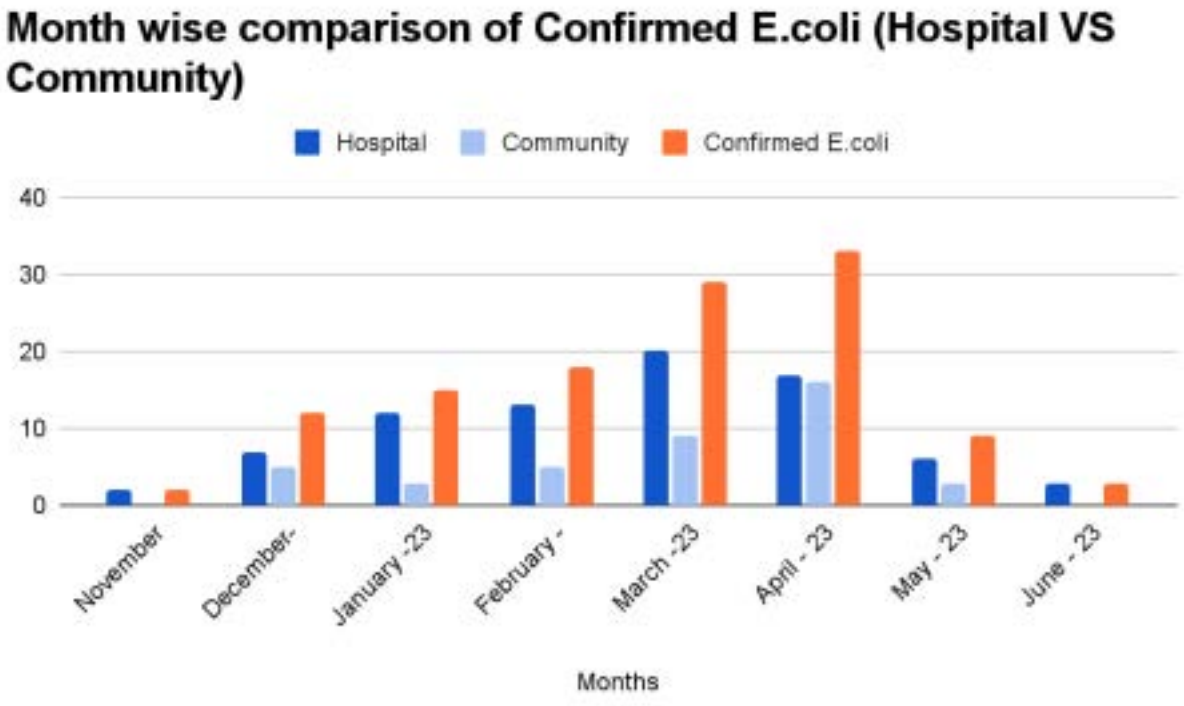


Graph 3: Month wise identification of confirmed *E.coli*

3.5 Month wise confirmed *E.coli* isolates comparison (Hospital VS Community):

During the study period, the graph shows that, in April there were the highest confirmed isolates of *E.coli* which was 33 isolates. From the 33 isolates, 17 isolates were identified from Hospital sewage water and 16 isolates were identified from Community tap water. From November to April, a decent increase of confirmed isolates was observed. In November 2022, only 2 confirmed *E.coli* isolates were observed which were isolated from Hospital sewage water. In December 2022, 12 isolates were identified as *E.coli*, where 7 isolates were from Hospital sewage water and 5 were from Community tap water. In January 2023, 15 isolates were identified as *E.coli*, where 12 isolates were from Hospital sewage water and the rest 3 isolates were from community tap water. In February 2023, 18 isolates were identified, where 13 isolates were from Hospital sewage water

and the rest 5 isolates were from community tap water. A greater number of isolates were identified in the March 2023 sample, where 20 isolates were from Hospital sewage water which is the highest confirmed *E.coli* in Hospital sewage water and the rest 9 isolates were from community tap water. But in May and June 2023, the rate of confirmed isolates decreased, which was 9 and 3 confirmed isolates.



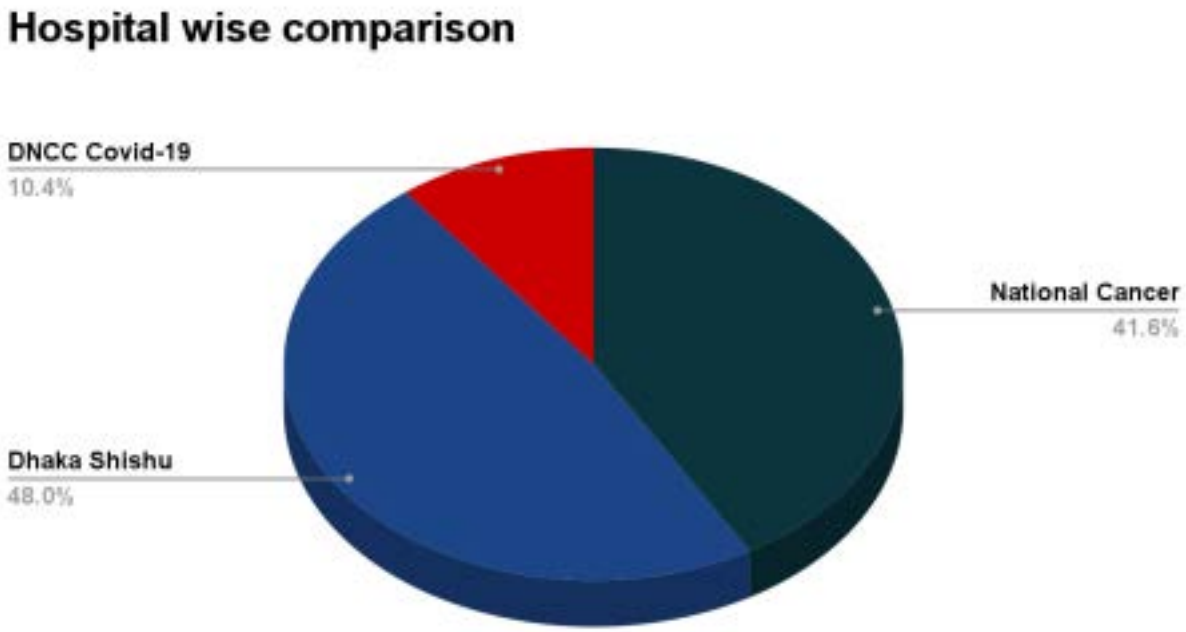
Graph 4: Month wise comparison of Confirmed *E.coli* (Hospital VS Community)

3.6 Hospital wise identification of Confirmed *E.coli*:

The pie chart compares the confirmed isolates of *E.coli* among the three Hospitals, as the three hospitals that were selected were located in densely populated areas. In both hospitals and the nearby neighborhood areas, there were excessive crowds of people. After reviewing the identification rate data by hospitals, it was discovered that the Dhaka Shishu Hospital had the highest percentage of confirmed isolates at 48%, which was 60 out of 125 isolates. The second

highest number of confirmed isolates were found at National Cancer Research Institute and

Hospital, where the rate was 41.6%, which was 52 out of 125 isolates. The lowest percentage of confirmed isolates, which was 10.4% (13 out of 125), was found in the DNCC Covid-19 Dedicated Hospital.



Graph 5: Hospital wise comparison of confirmed *E.coli*s

3.7 Interpretation of Antibiotic Susceptibility Test:

After species specific identification, antibiotic susceptibility test (AST) was performed on the confirmed *E.coli* isolates. About 11 listed antibiotics were utilized for the AST disk diffusion technique, which were used to identify resistance to ESBL and Carbapenamase isolates of *E.coli.* The MHA plates were observed after the 18-24 hours of incubation to measure the Zone of inhibition to determine whether the isolates were sensitive, resistant or intermediate to the antibiotics.

Here is the AST plates figure:

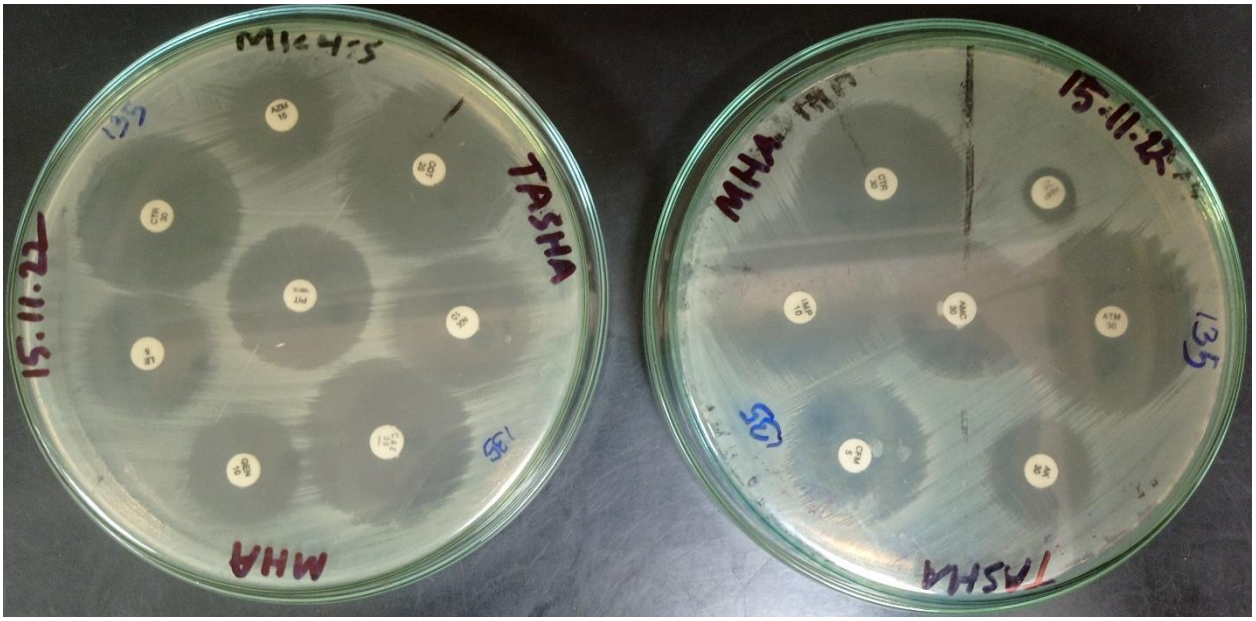
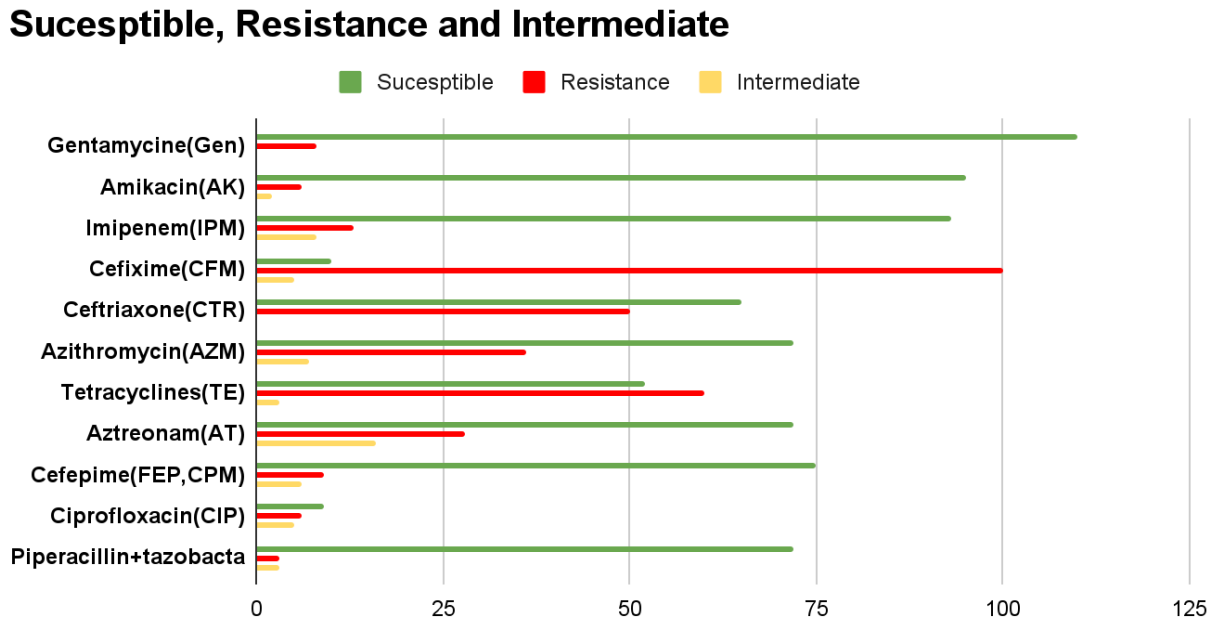


Figure 7: AST plate

For the Antimicrobial Susceptibility Test (AST) we used 11 antibiotics for disk diffusion technique. After observing the below Column chart, the sensitivity level was highest for Gentamicin (GEN) a total 118 disks were placed for and a highest number of isolates (110) were found out as sensitive and 8 isolates were found as resistant but no intermediate observed. The second highest for sensitive levels was Amikacin (AK) and the number was 95. Accordingly, Imipenem (IPM) susceptible has 93 isolates of Susceptible, Ceftriaxone (CTR) has 65 isolates, Azithromycin (AZM) and Piperacillin + Tazobactam (PIT) also Aztreonam (AT) has 72

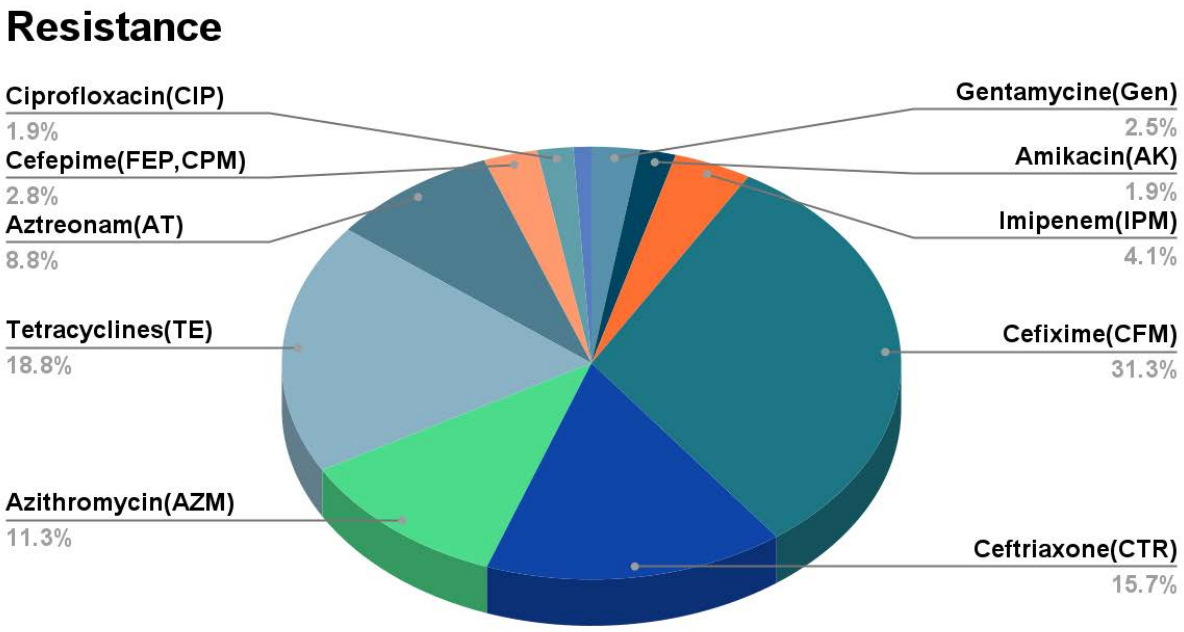
susceptible isolates, Cefepime (FEP, CPM) has 75 isolates, Tetracyclines (TE) has 52 susceptible isolates, Cefixime (CFM) and ciprofloxacin has a few susceptible isolates of 10 and 9.



Graph 6: Antimicrobial Resistance of confirmed *E.coli* isolated found from hospital wastewater and community tap water

After analyzing the pie chart of resistance isolates for hospital and community confirmed isolates of *E.coli* it shows that Cefixime shows the highest number of resistance rate of 31.3% (100 isolates resistance from 118 isolates). Following this, Tetracyclines show the second highest resistance rate of 18.8% (60 out of 118). Also, Ceftriaxone (CTR) has a 15.7% resistance rate. Also, the resistance isolates for the antibiotics and rates were: Azithromycin (AZM) 11.3%, Aztreonam (AT) 8.8%, Imipenem (IPM) of 4.1%, Cefepime (FEP, CPM) resistance rate was

2.8%, Gentamicin (GEN) rate was 2.5%, Ciprofloxacin (CIP) about 1.9%. Lastly the lowest resistance isolates antibiotic was Piperacillin + Tazobactam (PIT) and the rate is below 1%.



Graph 7: Comparison of Resistance *E.coli*

3.8 Multi-drug Resistant Gene Identification PCR assay and Gel electrophoresis:

After analyzing the phenotypic result for the antibiotics, genotypic analysis was carried away. For

the genotypic analysis of the 64 selected isolates which were resistant to most of the antibiotics were selected and identification was done for the presence of 6 different Multidrug Resistance specific genes which were basically ESBL and Carbapenems encoded. In order to check the resistance gene pattern Multidrug resistance gene (CTX-M, SHV, bla-TEM, bla-KPC and NDM

etc.) PCR was conducted. After analyzing the result, it was found that from the selected 64 isolates, 32 isolates give positive results for the selected multi-drug resistance Genes.

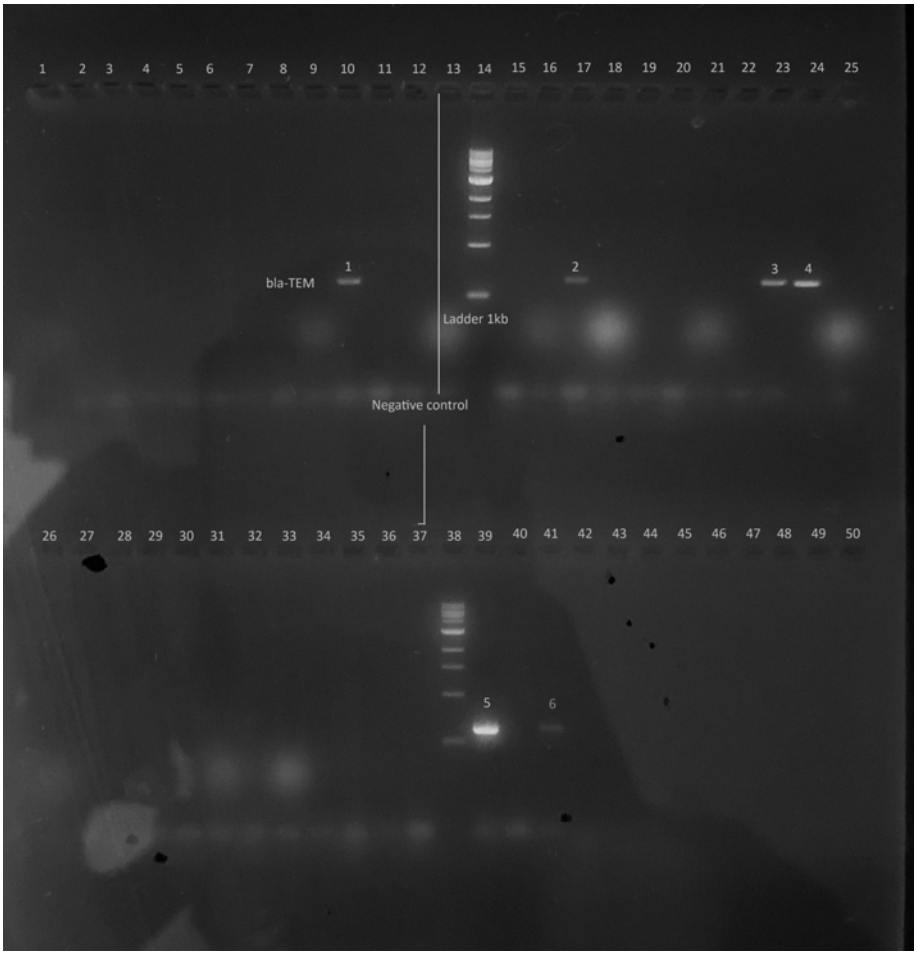


Figure 8: Multidrug resistance gene specific PCR (bla-TEM 1080bp)

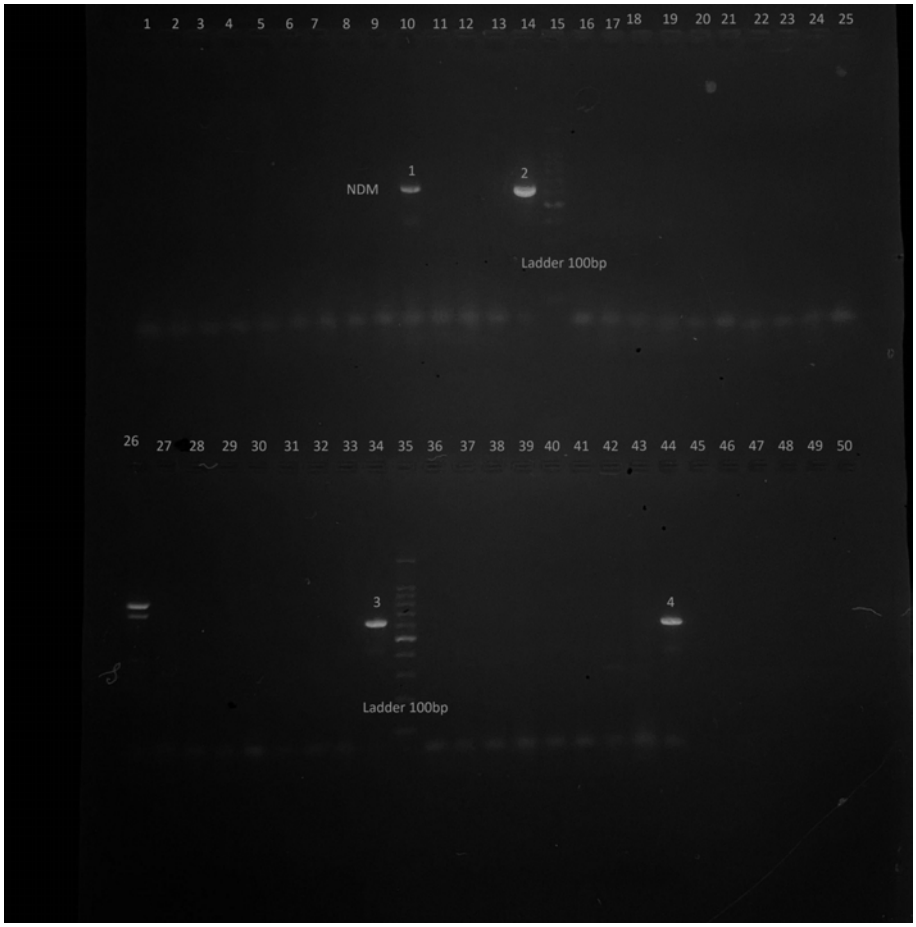
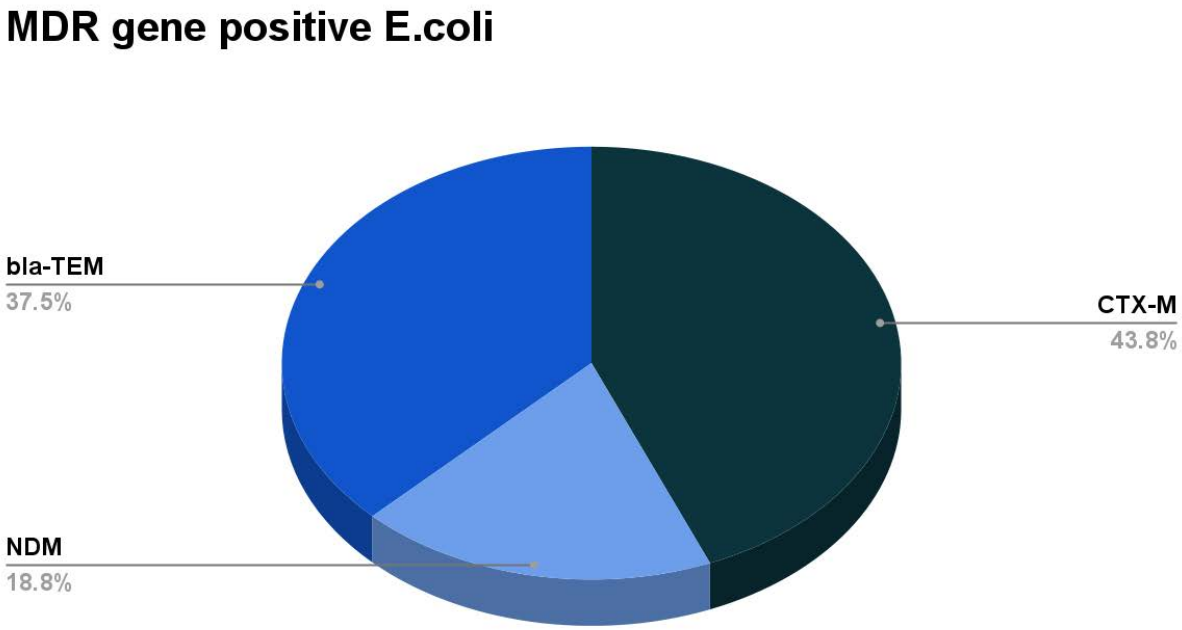


Figure 9: Multidrug resistance gene specific PCR (NDM 621bp)

After reviewing the specific data, it was found that the detection rate for CTX-M was high, which

was 43.8%, or 14 isolates out of 64, which was isolated from hospital sewage water but no isolates were detected from community tap water. The identification rate for bla-TEM was 37.5%, or 12 out of 64 isolates, and two of the isolates were from community tap water but the rest 10 isolates were from hospital sewage water. The identification rates for NDM-1 were 18.8% or 6 out of 64 isolates and one of the isolates were from the community tap water and the other 5 isolates were from hospital sewage water. But no *E.coli* confirmed isolates were observed in KPC and SHV specific PCR runs.

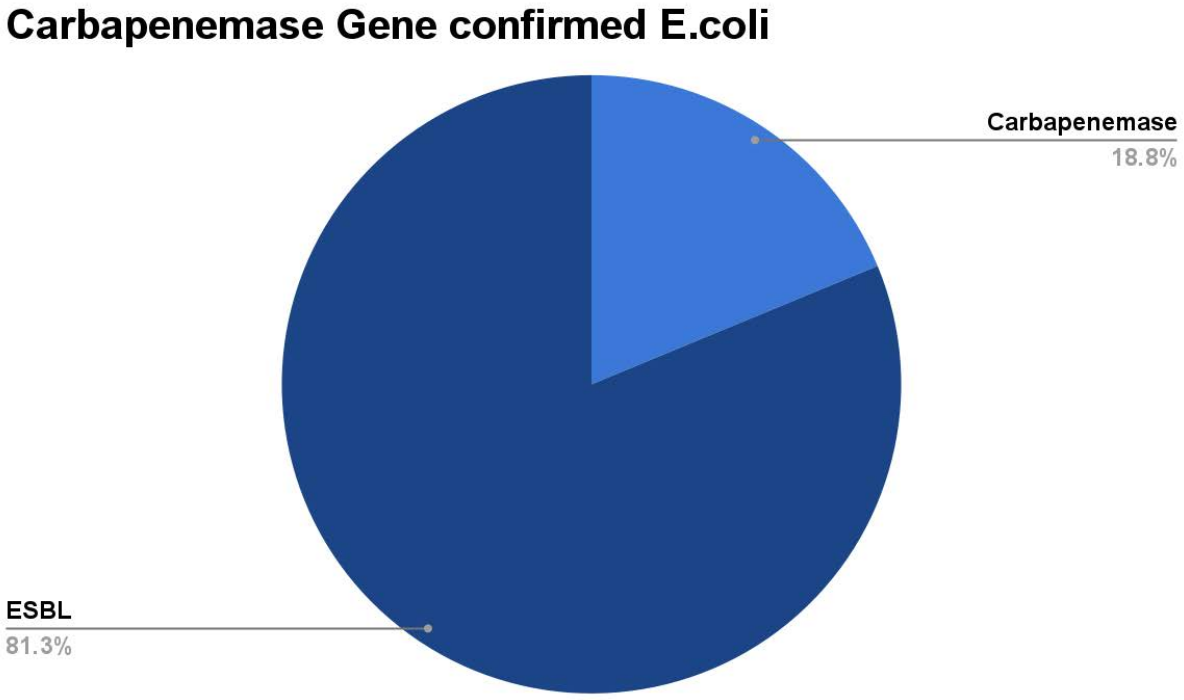


Graph 8: MDR gene positive *E.coli.*

3.9 Carbapenemase Resistance Gene confirmed *E.coli*:

Following the study, it was identified that some isolates included genes for carbapenemase

resistance. The carbapenemase resistance gene was located using the three gene-specific primers SHV, NDM, and KPC in the study. These genes were present after gene specific PCR process, and it was observed that 18.8% of the isolates tested positive for NDM. But no positive test result for SHV and KPC was observed.

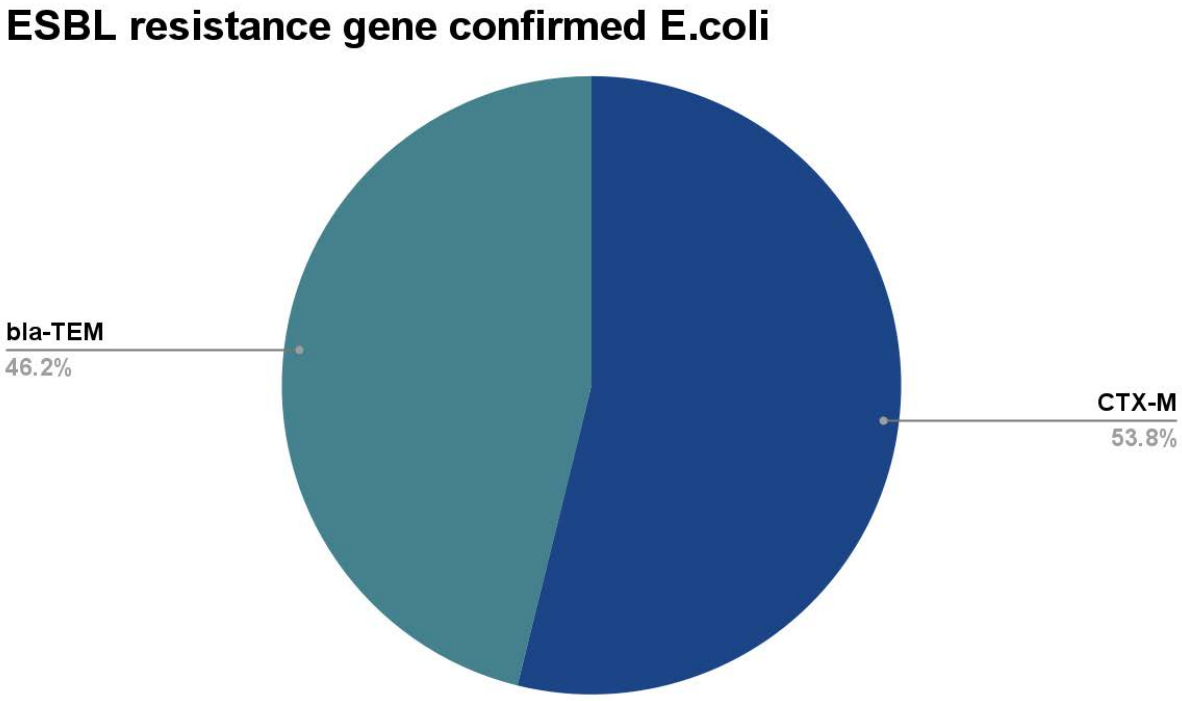


Graph 9: Carbapenemase Gene confirmed *E.coli*

3.10 ESBL resistance gene confirmed *E.coli*:

Following the investigation, it was shown that a number of isolates carried resistance genes for ESBLs. In this study, the ESBL-encoding resistance gene was located using the gene-specific

primers CTX-M and bla-TEM. When we looked at the rate at which these genes were present, we discovered that CTX-M was positive in 53.8% (14 out of 26) of the isolates. 46.2% (12 out of 26) of the isolates for bla-TEM showed positive results. Additionally, it was noted that there were two isolates which showed positive results for bla-TEM from community tap water.



Graph 10: ESBL confirmed *E.coli*

Chapter 4

Discussion on the Result

4.1 Discussion:

The bacteria *Escherichia coli* was first identified by Theodor Escherich, a German pediatrician in 1885 after isolating it from infant feces. *E. coli* is a gram-negative, non-sporulating, rod-shaped, facultatively anaerobic, and coliform bacteria that often lives in the environment, food, and the lower stomach of warm-blooded animals. Although the majority of *E. coli* strains are harmless, some serotypes can cause diarrhea when taken through contaminated food or drink, while others may result in urinary tract infections (UTIs), anemia, respiratory or kidney infections. To gain virulence factors, some strains of *E.coli* have transformed into harmful ones by employing plasmids, transposons, bacteriophages, or pathogenicity islands. Antibiotic resistance genes have been generated in many gram-negative bacteria and *E.coli* is not an exception. These bacteria have developed different mechanisms that grant them antibiotic resistance. *E.coli* is capable of producing extended-spectrum beta-lactamase (ESBL) which makes the bacteria resistant to beta lactams. On the other hand, *E.coli* that produce carbapenemase, have genes that provide carbapenem resistance. The purpose of the current study was to identify the *E.coli* ESBL and Carbapenems encoding strains from hospital sewage water and the nearby community tap water.

During the study period, a total 125 isolates were identified as confirmed *E.coli* from hospital sewage water and community tap water. However, out of 125 isolates, 84 were found in hospital sewage water and remaining 41 were found in community tap water which is 300 meters in range from hospitals. By analyzing the CFU count, the microbial count variation was observed, because one is from hospital sewage water and the other is from community tap water which is used by locals in their daily lives. In a study which was conducted in the Czech Republic from August 2020 to April 2021, from the 408 confirmed *E.coli* isolates, researchers identified 389 ESBL genes and 7 confirmed Carbapenemase encoding genes (Davidova-Gerzova et al., 2023). During our study period from November 2022 to June 2023 a total 125 isolates were confirmed as *E.coli* both from hospital sewage water and community tap water. Among 125 isolates 26 *E.coli* isolates identified as ESBL and the rest 6 isolates were identified as carbapenemase encoded genes. According to the hospital, after the distribution of confirmed *E.coli* isolates, it was identified that 60 out of 125 confirmed isolates came from the Dhaka Shishu Hospital in Shyamoli, Dhaka. The number of confirmed isolates from National Cancer Research Institute and Hospital, located in

Mohakhali, Dhaka, was 52 out of 125, which was slightly fewer than those from Dhaka Shishu Hospital. Though being located in the same region, the National Cancer Research Institute and DNCC COVID-19 Dedicated Hospital (13 out of 125 isolates) have different numbers of isolates. This could be due to different numbers of patients being admitted to the hospitals.

The objective of our research was to identify *E.coli* that was ESBL-encoding and carbapenem resistant in both hospital wastewater and the nearby community tap waters. We confirmed 125 isolates during the research period of November 2022 to June 2023, and some of them either ESBL-encoding or Carbapenem resistance. And, Disk Diffusion Antibiotic Susceptibility testing was carried out to determine the antibiotic resistance pattern phenotypically. A total 11 antibiotic were used which were Ampicillin, Amikacin, Aztreonam, Cefixime, Ceftazidime, Ceftriaxone, Chloramphenicol, Gentamicin, Imipenem, Meropenem, and Tetracycline. After the phenotypic analysis of the 125 confirmed *E.coli* by looking at their AST pattern in order to identify the gene encoding for ESBL and carbapenem resistance *E.coli*, 64 samples were selected from hospital sewage water and nearby community tap water. In order to identify the ESBL encoding *E.coli*, CTX-M and bla-TEM genes were identified by using CTX-M and bla-TEM specific primers. And for the identification of Carbapenem resistant *E.coli*, NDM, SHV and KPC genes were identified by using their specific primers in PCR method.

The majority of the *E.coli* isolates identified in our research were ESBL-resistant, and the majority of them were isolated from hospital sewage water. Our goal was to identify identical resistant *E.coli* isolates in both community tap water and hospital wastewater. According to our study, MDR gene was identified both in hospital sewage water and nearby community tap water, but presence of the same kind of resistance gene varies between them.

This study demonstrates that hospital isolates that were found to be MDR were spread to the environment by their staff members or by their used medical supplies. If these transmissions continue for a long period, this poses a serious threat to the public health of our nation. The main objective of our research was to determine the connection between hospital wastewater isolates and the nearby community water. Perhaps for some reason, we were unable to discover our conclusive findings aligned with our goals. However, the major goal was to make professionals

aware of how overusing antibiotics could negatively impact patients and the environment in the near future.

There are a few limitations on this study. We looked into samples from Bangladesh's Dhaka North City Corporation, which might not accurately reflect the country as a whole. To track changes in resistance patterns over time, a more thorough investigation with a larger sample size and all the broader areas or divisions is highly desirable. From an epidemiological standpoint, determining the ESBL phenotypes could have been a useful addition to this study because antibiotic-susceptible isolates with unexpressed ESBL genes may facilitate horizontal gene transfer through these strains. Additionally, the unavailability of different antibiotics from different classes makes it impossible to determine AST results for other antibiotics. There must be several antibiotic classes for which the isolates might have a resistance pattern.

Chapter 5

Conclusion

We identified the isolates that have been confirmed to be multidrug-resistant pathogens throughout the entire procedure. Antibiotic resistance is a significant issue that is becoming worse every day. The treatment of infections with appropriate antibiotic doses, which will

ultimately fail due to resistance pattern changes, has an impact on every part of the world. If circumstances stay like that, it will be difficult to provide the right treatments to patients. Additionally, the presence of beta-lactamase and carbapenemase is rising, which is an important issue in health services. According to the samples that have been collected, the presence of carbapenemase and beta lactamase, which contribute to the pathogen's resistance pattern to popular antibiotics, appears to contrast with the spread of pathogens to the population from the hospital. Multidrug resistance *Escherichia coli* has grown into an alarming issue because it is inherently susceptible to almost every clinically important antimicrobial drug.

Additionally, the causative genes NDM, CTX-M, bla-TEM have been identified to coexist in the isolates collected, which is a more concerning outcome for the future considering that some of them are communicable. It is up to us to ensure that the risk of the transmission spreading across the community is as low as possible. Our research was executed in a small area comprising three hospital settings and over a short period of time. It is essential to perform additional research and studies regarding how to incorporate different antibiotic spectrums to reduce the probable impacts of becoming affected with multidrug resistant *E.coli*.

Chapter

6

Reference

s

1. Chaudhary, U. & Aggarwal, R. (2004). EXTENDED SPECTRUM β-LACTAMASES (ESBL) – AN EMERGING THREAT TO CLINICAL THERAPEUTICS. ScienceDirect. Retrieved from https://www.sciencedirect.com/science/article/pii/S025508572102884X

2. Novais, C. & Coque, T., C. (2005). Environmental Contamination with Vancomycin Resistant Enterococci from Hospital Sewage in Portugal. American Society for Microbiology. Retrieved from https://journals.asm.org/doi/full/10.1128/AEM.71.6.3364-3368.2005

3. Korzeniewska, E. & Harnisz, M. (2013). Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment. National Library of Medicine. Retrieved from https://pubmed.ncbi.nlm.nih.gov/23886578/

4. Moreira, I., V. & Nunes, O., C. (2014). Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. SciHub. Retrieved from https://pubmed.ncbi.nlm.nih.gov/23886578/

5. Qamar, M., U., Hassan, B. (2021). The present danger of New Delhi metallo-β-lactamase: a threat to public health. Future Medicine. Retrieved from https://www.futuremedicine.com/doi/full/10.2217/fmb-2020-0069

6. Dhillon, R., H., P. & Clark, J. (2011). ESBLs: A Clear and Present Danger? Hindawi. Retrieved from https://www.hindawi.com/journals/ccrp/2012/625170/

7. Kumar, M. & Dutta, R. (2015). Risk Factor Analysis in Clinical Isolates of ESBL and MBL (Including NDM-1) Producing Escherichia coli and Klebsiella Species in a Tertiary Care Hospital. National Library of medicine. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4668407/

8. Moellering, R., C. & Jr., M.D. (2010). NDM-1 — A Cause for Worldwide Concern. The New England Journal of medicine. Retrieved from https://www.nejm.org/doi/full/10.1056/NEJMp1011715

9. Bush, L. & Bradford, P., A. (2020). Epidemiology of β-Lactamase-Producing Pathogens. National Library of Medicine. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7048014/

10. Laraki, N. & Franceschini, N. (1999). Biochemical characterization of the Pseudomonas aeruginosa 101/1477 metallo-beta-lactamase IMP-1 produced by Escherichia coli. National Library of medicine. Retrieved from https://pubmed.ncbi.nlm.nih.gov/10103197/

12. Senda, K. & Arakawa, Y. (1996). PCR detection of metallo-beta-lactamase gene (blaIMP)

in gram-negative rods resistant to broad-spectrum beta-lactams. National Library of medicine. Retrieved from https://pubmed.ncbi.nlm.nih.gov/8940421/

13. Brolund, A. (2012). Overview of ESBL-producing Enterobacteriaceae from a Nordic perspective. Taylor & Francis Online. Retrieved from https://www.tandfonline.com/doi/full/10.3402/iee.v4.24555

14. s: L.M. Lima, B.N. Monteiro da Silva, G. Barbosa, E.J. Barreiro, β -Lactam antibiotics: an overview from a medicinal chemistry perspective, European Journal of Medicinal Chemistry, https://doi.org/10.1016/j.ejmech.2020.112829

15. Bush,K. (2018). Past and Present Perspectives on β-Lactamases. National Library of medicine. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6153792/ 16. Pfeifer,y., Cullik,A., (2010). Resistance to cephalosporins and carbapenems in Gram negative bacterial pathogens. Science direct. Retrieved from: https://www.sciencedirect.com/science/article/abs/pii/S1438422110000305 17. Kurokawa,H., Yagi,T. (1999) Worldwide proliferation of carbapenem-resistant gram negative bacteria. The lancet. retrieved from: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(05)75707-X/fulltext 18. Coquel, T., M. & Baquerol, F. (2008). Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Eurosurveillance. Retrieved from https://www.eurosurveillance.org/content/10.2807/ese.13.47.19044-en

19. Rupp, M., E. & Fey, P., D. (2003). Extended Spectrum β-Lactamase (ESBL)-Producing Enterobacteriaceae. SpringerLink. Retrieved from https://link.springer.com/article/10.2165/00003495-200363040-00002

20. Bonten & Johnson. (2020) *Epidemiology of escherichia coli bacteremia*: A systematic literature review. https://www.researchgate.net/publication/341408961_Epidemiology_of_Escherichia_coli_Bacteremia_A_Systematic_Literature_Review

21. Martak, D., Henriot, C. P., Broussier, M., Couchoud, C., Valot, B., Richard, M., Couchot, J., Bornette, G., Hocquet, D., & Bertrand, X. (2020, August 18). *High prevalence of human-associated escherichia coli in wetlands located in eastern France*. Frontiers. https://www.frontiersin.org/articles/10.3389/fmicb.2020.552566/full

22. Ameer, M., Wasey, A., & Salen, P., (2023, February 5). *Escherichia coli (e Coli 0157 H7)*. NCBI. https://www.ncbi.nlm.nih.gov/books/NBK507845/#:~:text=Review%20of%20database%20and%20studies,renal%20failure%2C%20primarily%20in%20children. 23. Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (n.d.). *Pathogenic escherichia coli*. Nature News. https://www.nature.com/articles/nrmicro818 retrieved from https://www.nature.com/articles/nrmicro818

24. Basavaraju, M., & Gunashree, B. (2023). *Escherichia coli*: An Overview of Main Characteristics. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.105508 25. Zhang, Y., Huang, F., Gan, L., Yu, X., Cai, D., Fang, J., Zhong, Z., Guo, H., Xie, Y., Yi, J., Wang, Z., & Zuo, Z. (2021). High prevalence of blaCTX-M and blaSHV among ESBL producing E. coli isolates from beef cattle in China's Sichuan-Chongqing Circle. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-93201-z

26. Shah, A. A., Hasan, F., Ahmed, S., & Hameed, A. (2004). Extended-Spectrum B Lactamases (ESBLs): Characterization, epidemiology and detection. *Critical Reviews in Microbiology*, *30*(1), 25–32. https://doi.org/10.1080/10408410490266429

1. 26.Cho, S., Hiott, L. M., Barrett, J. B., McMillan, E. A., House, S. L., Humayoun, S. B., Adams, E. S., Jackson, C. R., & Frye, J. G. (2018, May 8). *Prevalence and characterization of escherichia coli isolated from the upper Oconee watershed in Northeast Georgia*. PloS one. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5940194/

27. *Microbiology of waterborne diseases*. (n.d.). ScienceDirect. https://www.sciencedirect.com/book/9780124158467/microbiology-of-waterborne diseases

28. *Before you continue to Google Maps*. (n.d.). https://www.google.com/maps/dir/BRAC+University+UB01,+Mohakhali+Gulshan+Road,+Dacca/Dhaka+Shishu+Hospital+Canteen,+Dhaka/DNCC+Dedicated+Covid 19+Hospital,+Mohakhali,+Dhaka 1212,+Shaheed+Tajuddin+Ahmed+Avenue,+Dhaka/National+Institute+of+Cancer+Research+%26+Hospital+(NICRH),+TB+Gate+Road,+Dhaka/@23.7758773,90.3677856,14z/data=!3m1!4b1!4m26!4m25!1m5!1m1!1s0x3755c7715a0c8ffd:0x9ffd27f74a8d2db1!2m2!1d90.4071984!2d23.7801569!1m5!1m1!1s0x3755c1c179fa3fc9:0xf2cd38322fdaf0c0!

2

57 m2!1d90.3690948!2d23.7725786!1m5!1m1!1s0x3755c70167b1aff5:0xdc1b3c1531fea45f!2m2!1d90.400874!2d23.7742473!1m5!1m1!1s0x3755c776e63ab313:0x2d352dbacb50ba14!2m2!1d90.4094593!2d23.7782321!3e0?entry=ttu