Isolation of Multi-drug Resistant *Escherichia coli* from Hospital Wastewater and Adjacent Community Water Samples in Dhaka City, Bangladesh.

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirement for the Degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

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June, 2023

DECLARATION

It is hereby declared that

1. The thesis submitted is my/our own original work while completing the 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. I/We have acknowledged all main sources of help.

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APPROVAL

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ABSTRACT

Background: The emergence and spread of multi-drug resistant *Escherichia coli* (MDR *E. coli*) is a growing public health concern worldwide, including developing countries such as Bangladesh. Hospital wastewater and community water sources are potential reservoirs for MDR *E. coli* due to the discharge of antibiotics and other pollutants from healthcare facilities and urban areas. Therefore, there is an urgent need for effective surveillance systems, wastewater treatment, and infection control measures to prevent the spread of MDR *E. coli* in hospital wastewater and adjacent community water sources in Dhaka City. The aim of this study is to identify MDR *E. coli* in hospital wastewater and adjacent community water sources in Dhaka City.

Materials and method: From November 2022 to January 2023 a total of 18 water samples were collected (4 Hospital wastewater and 14 adjacent community water). To isolate *E. coli* those samples were cultured on a differential Hi-chrome chromogenic KPC agar medium and were selected based on colony morphology. For further identification conventional polymerase chain reaction (PCR) were performed using Eco-16s rRNA primer.

Findings: From 18 samples, 4 samples were positive with *E. coli*. Total 11 *E. coli* isolates (9 isolates from hospital wastewater and 2 isolates from community water) were selected after performing PCR. Furthermore, all confirmed isolates were characterized by Antimicrobial susceptibility test (AST). Hospital wastewater isolates showed 88.8% resistance to Ampicillin, 66.6% resistance to Tetracycline, 55.5% resistance to Cefixime and Ceftriaxone. On the other hand, community water isolates showed 100% resistance to Ampicillin, 50% resistance to Piperacillin, Imipenem , Aztreonam, Tetracycline and Amoxyclav. From these 11 isolates, 9 were MDR.

Conclusion: This study implies that it is crucial to monitor antibiotic resistance of *Escherichia coli*. The number or percentage will assist in implementing better control of the spread of antibiotic-resistant bacteria in hospital and community water sources.

Keywords: *Escherichia coli,* antibiotic resistance, multidrug resistance

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ABBREVIATIONS

Abbreviation	Elaboration
Bp	Base pair
CLSI	Clinical & Laboratory Standards Institute
dH2O	Distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
CRE	Carbapenem resistant Enterobacteriaceae
et. Al	and others
EtBr	Ethidium bromide
MDR	Multidrug resistant
Mm	Millimeter
TE	Tris-EDTA
TBE	Tris-borate-EDTA
rRNA	Ribosomal ribonucleic acid

pH	Power of hydrogen
MCT	Micro centrifuge tube
PCR	Polymerase chain reaction
rpm	Revolutions per minute

Chapter 1

Introduction:

The World Health Organization (WHO) has listed antimicrobial resistance (AMR) as one of the top ten public health threats facing humanity. (Word Health Organization, 2019). Several studies have investigated the antibiotic resistance of *Escherichia coli* in wastewater from different locations of different countries. Such as South Africa, Vietnam, South India, Bangladesh etc (Hossain et al., 2022; Kinge, Ateba, & Kawadza, 2010; Lien et al., 2017; Reddy et al., 2020). One study of South Africa tested 230 *E. coli* isolates from local wastewater and water-treatment plants, Modimola Dam and homes in the area. It found marked resistance to erythromycin (over 70%), tetracycline (over 70%), ampicillin (over 70%), chloramphenicol (over 70%), and norfloxacin (over 70%). (Kinge, Ateba, & Kawadza, 2010). In another study conducted in Dhaka City's wastewater treatment plant, 37 *E. coli* strains were tested against 15 antibiotic agents from 8 different antibiotic classes. Results showed that the highest resistance was to erythromycin (99%), followed by cefotaxime (91.9%) and amoxicillin (91.9%), streptomycin (89.2%), ampicillin (75.7%), cefuroxime (67.6%), ceftriaxone (64.9%), cefixime (62.2%), kanamycin (59.5%), ciprofloxacin (54.1%), nalidixic acid (51.4%), tetracycline (51.4%), trimethoprim/sulfamethoxazole (40.5%), gentamicin (40.5%), and chloramphenicol (21.6%). (Hossain et al., 2022). Similarly, a study conducted on *E. coli* isolated from a tertiary hospital treatment plant in Vietnam showed complete resistance to tetracycline (100%), sulphonamide (100%), ertapenem (100%), cefpodoxime (100%) and cefotaxime (100%). Additionally, 75% of the isolates were resistant to ciprofloxacin, ceftazidime and amoxicillin-clavulanate. (Lien et al., 2017). Moreover, another study conducted in South India tested 221 *E. coli* isolates from four sewage treatment plants receiving hospital and domestic wastewater in different proportions. Among the antimicrobials tested, ampicillin (20-90%) and cefazolin (20-90%) showed resistance, while nalidixic acid (15-75%) and ciprofloxacin (15-75%) showed moderate resistance. Chloramphenicol (2-20%) showed the least resistance. (Reddy et al., 2020).

These studies collectively highlight the widespread occurrence of MDR *E. coli* in wastewater samples from different locations. The high prevalence of resistance to commonly used antibiotics

in *E. coli* from wastewater samples is a serious public health concern, as it could lead to the emergence of antibiotic-resistant infections in humans and animals. The findings from these studies underscore the need for increased surveillance and monitoring of antibiotic resistance in environmental samples, as well as the development of novel strategies to control the spread of antibiotic resistance in the environment.

As filling the identified knowledge gaps is important, this study aimed to evaluate the occurrence of multidrug resistant *E. coli* bacteria in wastewater of three different hospitals in Dhaka city. Specifically, the prevalence of MDR *E. coli* bacteria present in the hospital wastewater.

Our findings are consistent with previous studies that have identified wastewater as a potential source of antibiotic-resistant bacteria in the environment (Kummerer, 2004; Pal et al., 2012). The high prevalence of MDR *E. coli* in hospital wastewater samples suggests that hospitals in Dhaka city may serve as reservoirs of antibiotic-resistant bacteria, which can pose a threat to public health (Tasnim et al., 2018).

Despite the growing concern over the emergence and spread of MDR *E.coli* in hospital and community settings, there are few significant studies regarding the percentage of MDR bacteria in Bangladesh, especially in Dhaka City. Specially limited studies have been conducted to investigate the percentage of resistant MDR *E.coli* in hospital wastewater and adjacent community water sources (Haque et al., 2019). This limitation presents a significant challenge in designing appropriate intervention strategies to mitigate the spread of MDR *E.coli*.

1.1 Objective

The objective of this study is to isolate *E.coli* from hospital wastewater and community water samples in Dhaka City to analyze the antibiotic resistance profiles of these two environments. The findings of this study will provide insight into the potential risks associated with the contamination of community water sources by multi-drug resistant *E. coli*.

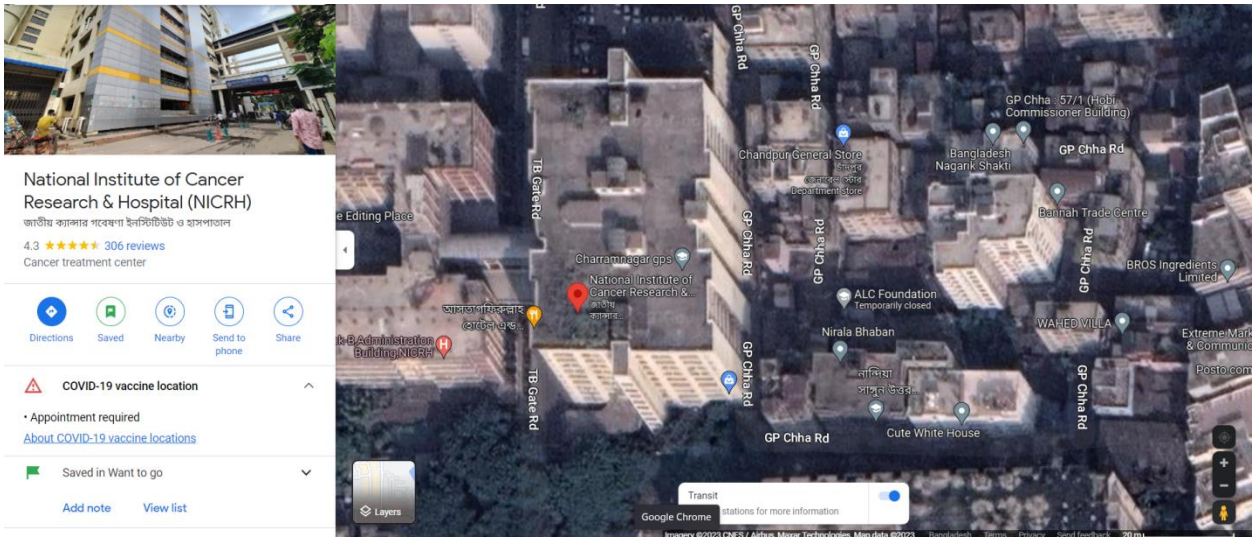
Chapter 2

Materials and Methods

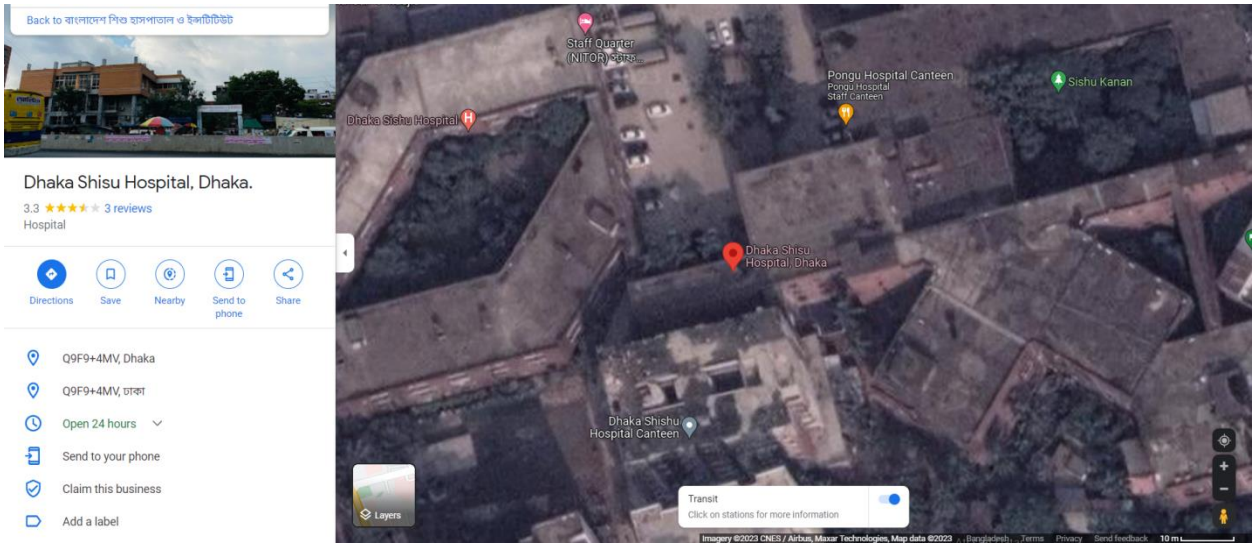
2.1 Sample collection:

2.1.1 Sample collection site of hospital waste water:

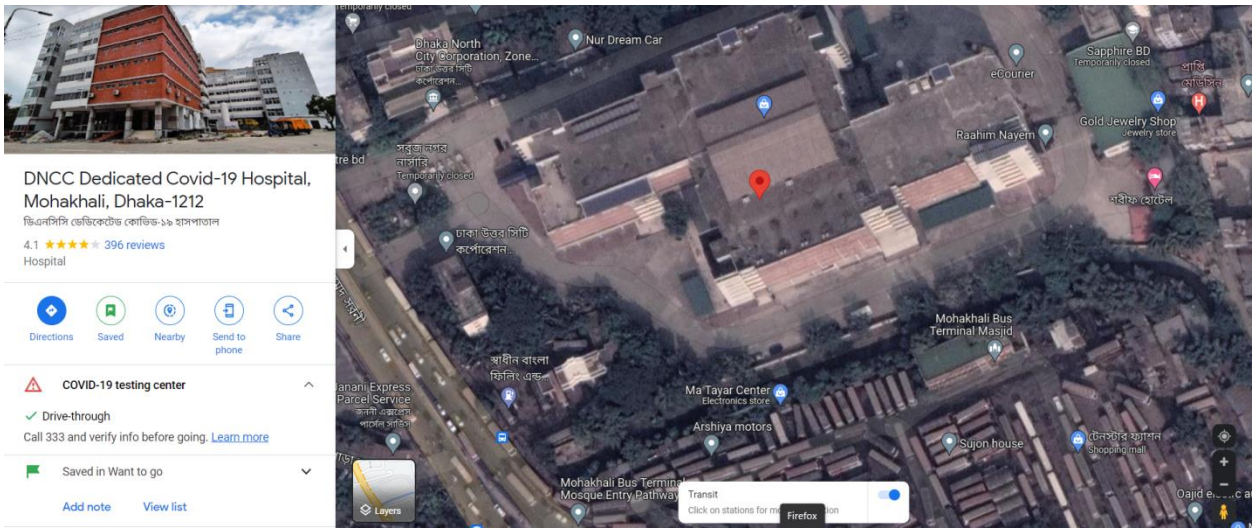
Hospital wastewater samples were collected from three well-known hospitals in Dhaka city. They are Dhaka Shishu Hospital, Dedicated Covid-19 Hospital (DNCC) and National Institute of Cancer Research and Hospital of Dhaka. Three different locations were set from where hospital waste was discharged to collect samples. Sample collection sites are shown in the pictures below.



(a) National Institute of Cancer Research and Hospital.



(b) Dhaka Shishu Hospital



(c) Dedicated Covid-19 Hospital, Mohakhali, Dhaka-1212. (DNCC).

(d) Hospital waste water discharge from where sample was collected.

Figure 1: Hospital waste collection site. (a) National Institute of Cancer Research And Hospital. (b) Dhaka Shishu Hospital. (c) Dedicated Covid-19 Hospital, Mohakhali, Dhaka-1212. (DNCC). (d) Hospital waste water discharge from where sample was collected.

2.1.2 Sample collection site of hospital adjacent community water:

Fourteen Community water samples (2 samples from adjacent sites of Dhaka Shishu Hospital, rest 12 samples from adjacent sites of Dedicated Covid - 19 Hospital(DNCC) and National Institute of Cancer Research And Hospital) were collected from houses within 300 meters surrounding the hospitals. Four particular locations (houses) were set previously. Some pictures of the sample collection sites are shown below.

Figure 2: Hospital adjacent community locations.

2.2 Sample collection from hospital wastewater and adjacent community water:

Forty ml hospital wastewater samples were collected in a sterile falcon tube and adjacent community water samples were collected in sterile plastic bottles. Both samples were collected maintaining an aseptic technique and transferred into the lab within two hours in a cold box till sample processing to maintain sample quality.

2.3 Sampling handling and preliminary preparation:

The surface of the falcon tubes and bottles were disinfected with 70% ethanol solution before starting sample processing.

2.4 Hospital wastewater sample processing:

Hospital wastewater samples were diluted up to 10^{-10} following serial dilution method. Then the direct sample and dilution factor $10^{-2}, 10^{-3}$ and 10^{-4} were selected and spread plated on HiCrome KPC media. *Escherichia coli* colonies show small pink to purple/magenta colored colonies on HiCrome KPC media, whereas Klebsiella, *Enterobacter*, and *Serratia* species show bluish-green colonies. Here, HiCrome KPC is used as a differential media. After spread plating the plates were incubated for 24 hours at 37°c.

2.5 Hospital adjacent community water sample processing:

Community water samples were filtered thrice using membrane filtration. Then two filter paper was placed on two MFC media supplemented with rosalic acid. Supplement Rosolic acid in MFC media has an important purpose. MFC media without rosalic acid helps to grow *E.Coli*. Whereas, MFC-RA will help to grow both fecal coliform and total coliform. Moving forward, the MFC plates were placed in two different incubators of two different temperatures (37°c and 44°c).

In addition, the third filter paper were inserted in falcon tubes containing Buffer Peptone Water (BPW) for enrichment and were placed in a shaking incubator for 24 hours at 37°c.

Community water samples have less amount of organisms than the required amount for detection through molecular analysis. For that purpose Buffered Peptone Water was used as an enrichment broth. This allows the recovery and growth of the organisms present in the community water sample. After 24 hours BPW was diluted up to 10^{-7} following the serial dilution method. Then dilution factors 10^{-2}, 10^{-3}, and 10^{-4} were selected and spread plated on Hi-Chrome KPC media. Then the plates were incubated for 24 hours at 37°c. [Table 1]

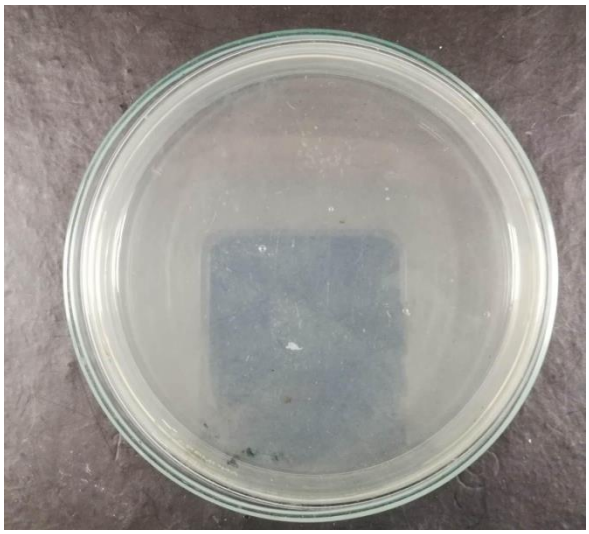
Selective and Differential Media	
Membrane Fecal Coliform Agar (selective) m-FC Agar is a selective membrane filtration medium used for the cultivation and enumeration of fecal coliforms.	
CHROMagar™ KPC (selective and differential media) CHROMagar™ KPC is a selective and differential chromogenic culture medium, intended for use in the qualitative direct detection of gastrointestinal colonization with carbapenem-resistant Enterobacteriaceae (CRE) to aid in the prevention and control of CRE in healthcare settings.	

Table 1: Name of the selective and differential media used for isolation of *E. coli*

2.6 Bacteria culture and identification:

To identify the presence of target microorganisms, 0.1ml of each dilution was spread on selective media and incubated for 24h at 37°C. After incubation, the primary identification was made based on colony morphology and color. [Table 2]

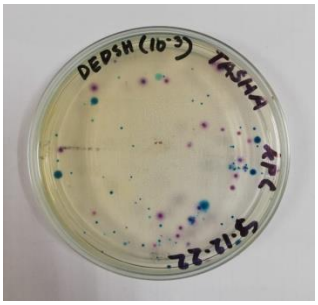
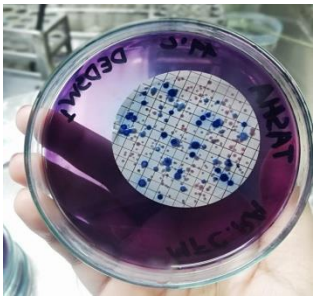
Sample	**Media**	**Organism**	**Colony morphology**	**Figures**
Hospital Wastewater sample	Hi-chrom KPC	*Escherichia coli*	Purple and smooth, round colonies	
Hospital adjacent community water sample	MFC		Blue colonies	

Table 2: Isolation with Hicrome KPC agar and MFC agar

2.7 Bacterial DNA extraction:

The isolates (*E. coli*) were inoculated into Nutrient Agar for subculture and were incubated at 37°C for 24 hours. The DNA of the selected isolates was extracted via the "Boiling method' due to its efficiency, simplicity, and cost-effectiveness (Dimitrakopoulou et al., 2020).

Then, a single colony of each bacteria was added to the 150 µl TE buffer. The major purposes of using TE buffer are pH control, solubilization of DNA or RNA, and defense against enzymatic lysis of the nucleic acids. The components of the TE (Tris-EDTA) buffer are the pH buffer Tris and the metal chelating ion EDTA. It is used to lyse, wash, and dissolve DNA during DNA extraction procedures. These samples were then boiled or dry-heated at 100 degrees C for 15 minutes. After that, the samples were centrifuged for 5 min at 13rpm. The final DNA-rich supernatant was collected into new MCTs and stored at -20°C

2.8 PCR Amplification:

Each presumptive bacterial isolate was screened for confirmation by using ECO-1 and ECO-2 primers. PCR amplification was done with the following set of primers from Table-

Primer	Primer Sequence (5'-3')	Target gene	PCR condition	Annealing temperature	Amplicon size (bp)	Reference
ECO-1	F-GACCTCGGTTTAGTTCACAGA	malB-16SrRNA	95°c for 15 minutes 95°c for 30 seconds 58°c for 1 minute 72°c for 1 minute 72°c for 10 minutes 4°c 30 cycles	58°c	585	Candrian *et al*.(1991)
ECO-2	R-CACACGCTGACGCTGACCA					

Table 3: Primers used for amplification of resistance genes by polymerase chain reaction (PCR).

2.9 PCR master mix preparation:

The DNA extracts were used as a template for PCR amplification during this preparation. For each presumptive bacterial sample, 2µl template DNA, 7.5 µl Master Mix, 2.5µl of nuclease-free water,0.5µl forward primer, and 0.5µl reverse primer were adjusted to be a 13 µL of final solution

for PCR. The calculation given below is for 1 sample; for multiple samples, the amount will be multiplied with “n”. [Table 4]

Name of the Reagent	Total Volume= 13 µl
Master Mix (2x)	7.5 µl
F Primer (10 µM)	0.5 µl
R Primer (10 µM)	0.5 µl
Nuclease-Free Water	2.5 µl
DNA Template	2µl
Total	13µl

Table 4: PCR preparation calculation for 1 sample.

Primer annealing at various temperatures [Table 3] 95°C for 15 min, 30 cycles (95°C for 30 s, 58°C for 1 minute, 72°C for 1mnt), and final temperature at 72°C for 1 min 4°C.

2.10 Agarose Gel Electrophoresis:

Aliquots of PCR products were analyzed by gel electrophoresis in 2% (w/v) agarose gel and the gel was stained with EtBr and run at a constant voltage of 110V for 60 min. 2 % agarose gel using 50X TBE buffer, stained with ethidium bromide. The products were observed under a UV transilluminator to see the band size.

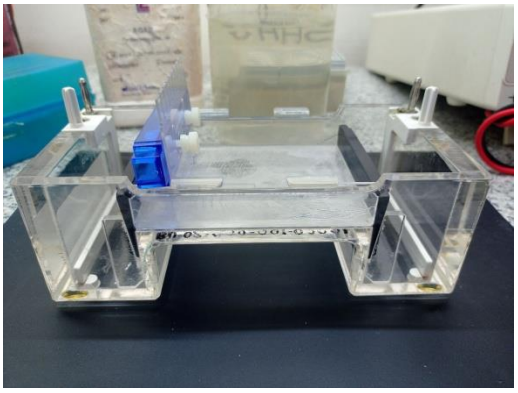
	
PCR tubes inside a Conventional PCR system.	Conditions setting parameters of PCR system.
	
DNA Ladder	Agarose Gel Electrophoresis system.

Table 5: Some of the tools used during PCR amplification and Agarose Gel Electrophoresis

2.11 Antibiotic Susceptibility Test:

The Kirby-Bauer disc diffusion protocol was followed for conducting the test, and the CLSI standards published in 2018 were used to interpret the zones of inhibition. The effectiveness, group, disc potency, and interpretive criteria of antibiotics used for the test are listed in Supplementary Table 6. Multidrug resistance (MDR) was defined as non-susceptibility to at least one antimicrobial agent in three or more categories as per the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) definition. MHA agar was used to perform the Antibiotic Susceptibility test (AST) for the targeted gram-negative organism *E.coli* in this study.

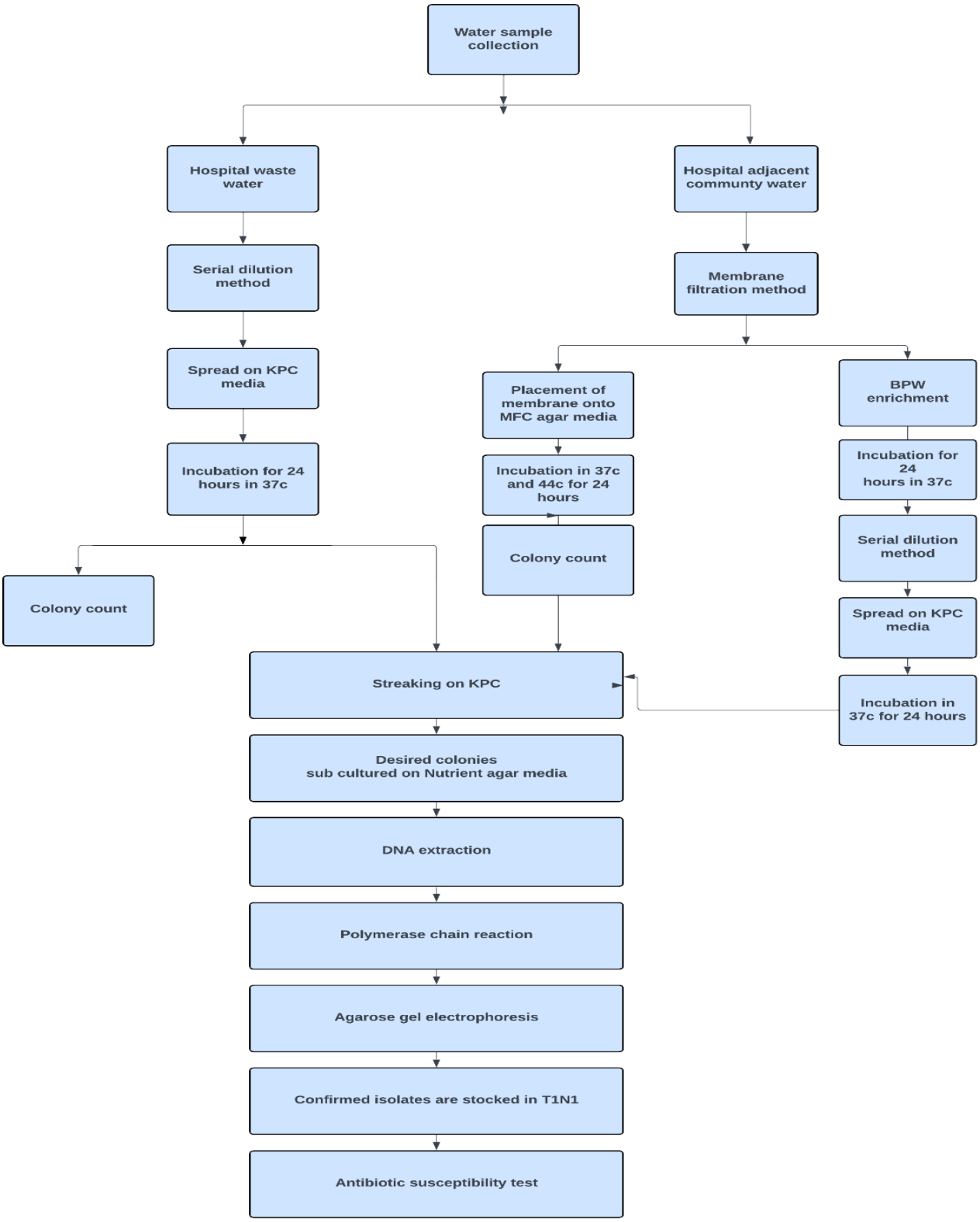
To conduct the test, a cotton swab was used to collect isolated colonies of *E.coli*. The organism was then mixed in 6 ml saline and vortexed to achieve a high concentration, which was then matched to the Mcfarland 0.5 standard. The saline containing the concentrated *E.coli* was evenly spread onto MHA plates with the help of a sterile cotton swab. Known concentrations of different antibiotics were added to the agar surface, maintaining a proper distance from each other, by placing filter paper disks. These plates were then incubated at 37°C for 18-24 hours. Following the incubation period, the plates were observed for results.

Serial no	**Antibiotics**	**Group**	**Effective against**	**Abbreviation of antibiotics**	**Disc potency (µg)**	**Resistance level (mm)**
1	Gentamicin	Aminoglycoside	Gram positive and gram negative	GEN	10	S: >=15, I: 13-14, R: <= 12
2	Ampicillin	Beta lactamase	Gram positive and gram negative	AMP	25	S:>= 17, I: 14-16, R:<=13
3	Meropenem	Carbapenem	Gram positive and gram negative	MRP	10	S:>=23, I: 20-22, R:<=19
4	Imipenem			IMP	10	S:>=23, I:20-22,

						R:<=19
5	Cefixime	Cephalosporin	Gram positive and gram negative	CFM	5	S:>=19, I: 16-18, R:<=15
6	Ceftriaxone			CTR	30	S: >= 23, I: 20-22, R: 20-22
7	Ceftazidime		Gram positive	CAZ	30	S: >=21, I: 18-20, R: <=17
8	Piperacillin tazobactam	Penicillin and beta- lactamase inhibitor	Gram positive and gram negative	PIT	10/100	S: >=21, I: 18-20,R:<=17
9	Amoxiclav		Gram negative	AMC	30	S: >=18, I: 14-17, R: <=13
10	Azithromycin	Macrolide	Gram positive and gram negative	AZM	15	S<=18; I:14-17, R=>13
11	Tetracycline	Protein synthesis inhibitor	Gram positive and gram negative	TE	30	S:>=15, I:12-14, R:<=11
12	Norfloxacine	Fluoroquinolone	Gram positive	NX	10	S:>= 17, I: 13-16, R: <=12
13	Aztreonam	Monobactam	Gram negative	ATM	30	S:>= 21, I: 18-20, R:<= 17
14	Trimethoprim-sulfamethoxazole	Sulphonamides	Most gram positive, some gram negative	COT	25	S:>= 16, I:11-15, R:<=10

Table 6: List of antibiotics used in the experiment.

2.12 Flowchart of the method of the experiment:



Chapter 3

Results

3.1 Isolation of *E. coli*:

3.1.1 Isolated *E. coli* from hospital wastewater sample:

From 4 hospital wastewater samples, *E. coli* was found in 2 samples. [Table 7]

3.1.2 Isolated *E. coli* from hospital adjacent community water sample:

From 14 Community water samples, *E. coli* was found in 2 samples. [Table 7]

Hospital waste water sample			**Hospital adjacent community water sample**		
Sample ID	Sampling area	*E. coli*	Sample ID	Sampling area	*E. coli*
NONCH	National Institute of Cancer Research And Hospital	2	NONCW1	Adjacent area of National Institute of Cancer Research And Hospital	0
			NONCW2		0
			NONCW3		0
			NONCW4		0
DEDSH	Dhaka Shishu Hospital	0	DEDSW1	Adjacent area of Dhaka Shishu Hospital	0
			DEDSW2		1

DENCH	National Institute of Cancer Research And Hospital	7	DENCW1	Adjacent area of National Institute of Cancer Research And Hospital	0
			DENCW2		1
			DENCW3		0
			DENCW4		0
JANDNCH	Dedicated Covid-19 Hospital	0	JANDNCW1	Adjacent area of Dedicated Covid-19 Hospital	0
			JANDNCW2		0
			JANDNCW3		0
			JANDNCW4		0
		Total=9			Total=2

Table 7: Total isolates with sample ID, confirmed *E. coli* count.

3.2 Identification:

3.2.1 Identification of *E. coli* by PCR:

After undergoing isolation and identification procedures based on their colony morphology, a total of 11 isolates were selected to be *E. coli* after performing PCR. 9 isolates were from hospital wastewater and 2 were from community water. [Table 7]

3.2.2 Agarose Gel Electrophoresis:

All the 11 *E. coli* isolates gave a band at 585bp. [Figure 3] shows the result of PCR amplification.

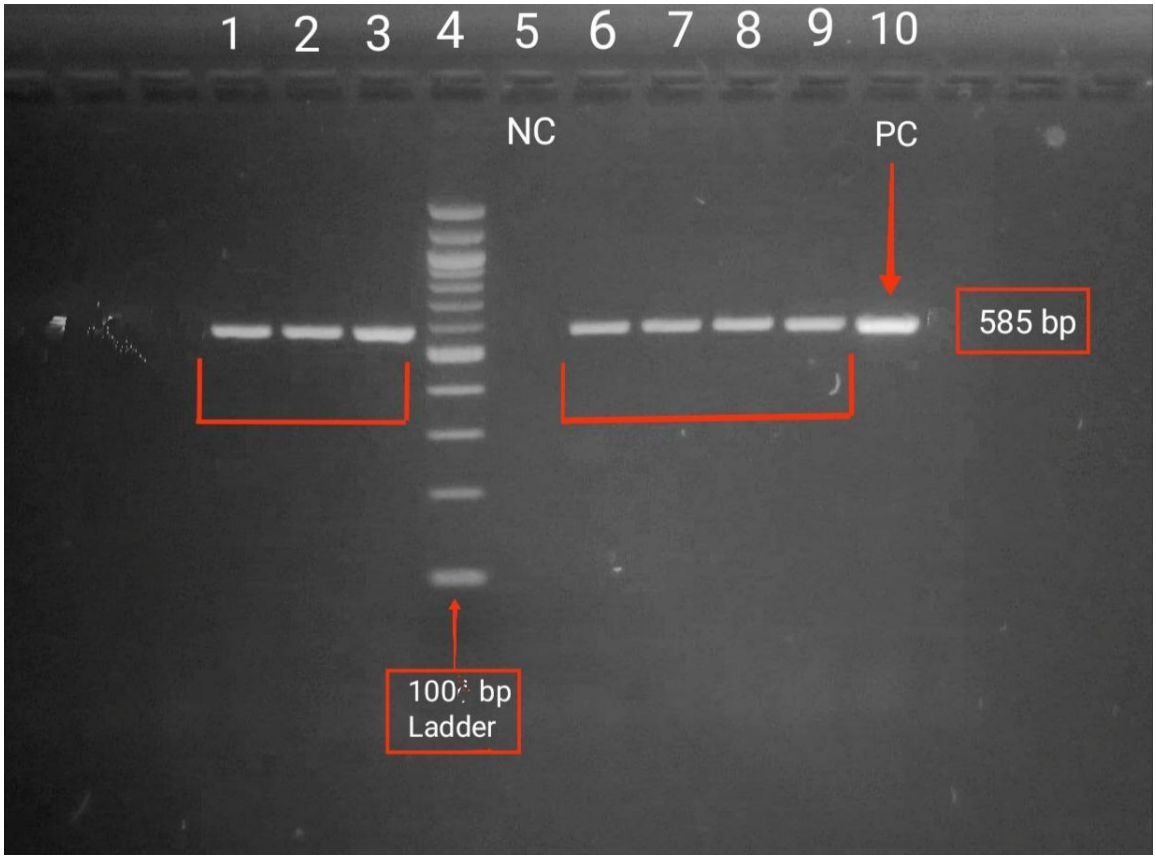


Figure 3: Agarose Gel electrophoresis of Escherichia coli, Lane number 1-3 and 6-9 are the sample, lane number 10 is positive control (PC), lane number 5 is negative control (NC) and lane number 4 is 100bp Ladder.

3.3 Antibiotic susceptibility pattern:

By using Kirby - Bauer disk diffusion method antibiotic susceptibility test for all the 11 confirmed *E. coli* was done. According to the CLSI guidelines, resistant, intermediate, or sensitive results were interpreted. [Table 8 and Table 9]

Antibiotic susceptibility pattern of 9 confirmed isolates of hospital wastewater samples			
Antibiotics	**Resistant n(%)**	**Intermediate (%)**	**Sensitive (%)**
Ceftriaxone	5(55.5%)	0	4(44.4%)
Piperacillin	2(22.2%)	5(55.5%)	2(22.2%)
Gentamicin	1(11.1%)	0	8(88.8%)
Ceftazidime	3(33.3%)	2(22.2%)	4(36.36%)
Norfloxacin	3(33.3%)	0	6(66.6%)
Trimethoprim-sulfamethoxazole/Co-trimoxazole Sulfa	3(33.3%)	0	6(66.6%)
Ampicillin	8(88.8%)	1(11.1%)	0
Imipenem	4(44.4%)	3(33.3%)	2(22.2%)
Cefixime	5(55.5%)	2(22.2%)	2(22.2%)
Aztreonam	4(44.4%)	1(11.1%)	4(44.4%)
Tetracycline	6(66.6%)	0	3(33.3%)
Meropenem	2(22.2%)	0	7(77.7%)
Azithromycin/Erythromycin	3(33.3%)	0	6(66.6%)
Amoxiclav	4(44.4%)	0	5(55.5%)

Table 8: Percentage of Antibiotic Susceptibility Test of 9 hospital wastewater sample isolates

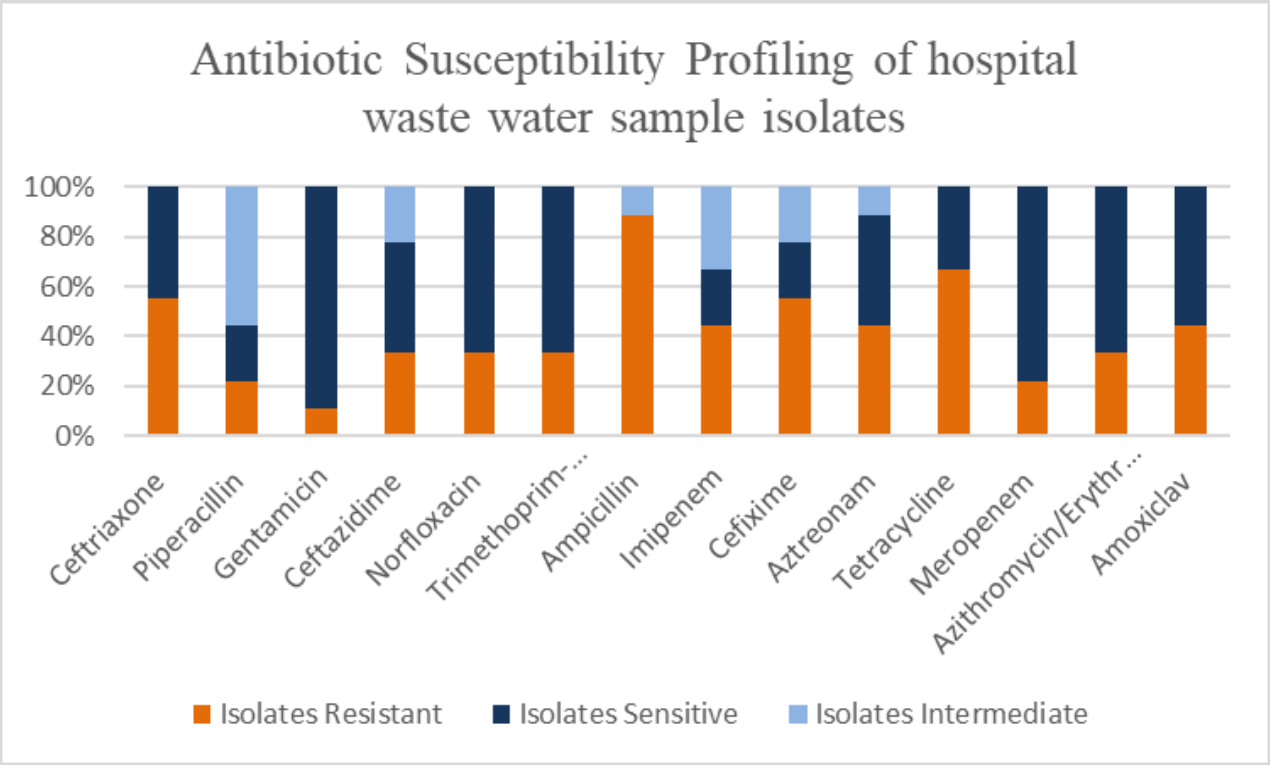


Figure 4: Percentage of Antibiotic Susceptibility Test of the 9 isolates derived from hospital waste water

Antibiotic susceptibility pattern of 2 confirmed isolates of community water samples			
Antibiotics	**Resistant (%)**	**Intermediate (%)**	**Sensitive (%)**
Ceftriaxone	0	0	2 (100%)
Piperacillin	1(50%)	0	1(50%)
Gentamicin	0	0	2(100%)
Ceftazidime	0	0	2 (100%)
Norfloxacin	0	0	2 (100%)
Trimethoprim-sulfamethoxazole/Co-trimoxazole Sulfa	0	0	2 (100%)
Ampicillin	2 (100%)	0	0
Imipenem	1 (50%)	0	1 (50%)
Cefixime	0	0	2 (100%)
Aztreonam	1 (50%)	0	1 (50%)
Tetracycline	1 (50%)	0	1 (50%)
Meropenem	0	1 (50%)	1 (50%)
Azithromycin/Erythromycin	0	0	2 (100%)
Amoxyclav	1(50%)	0	1 (50%)

Table 9: Percentage of Antibiotic Susceptibility Test of 2 community water sample isolates

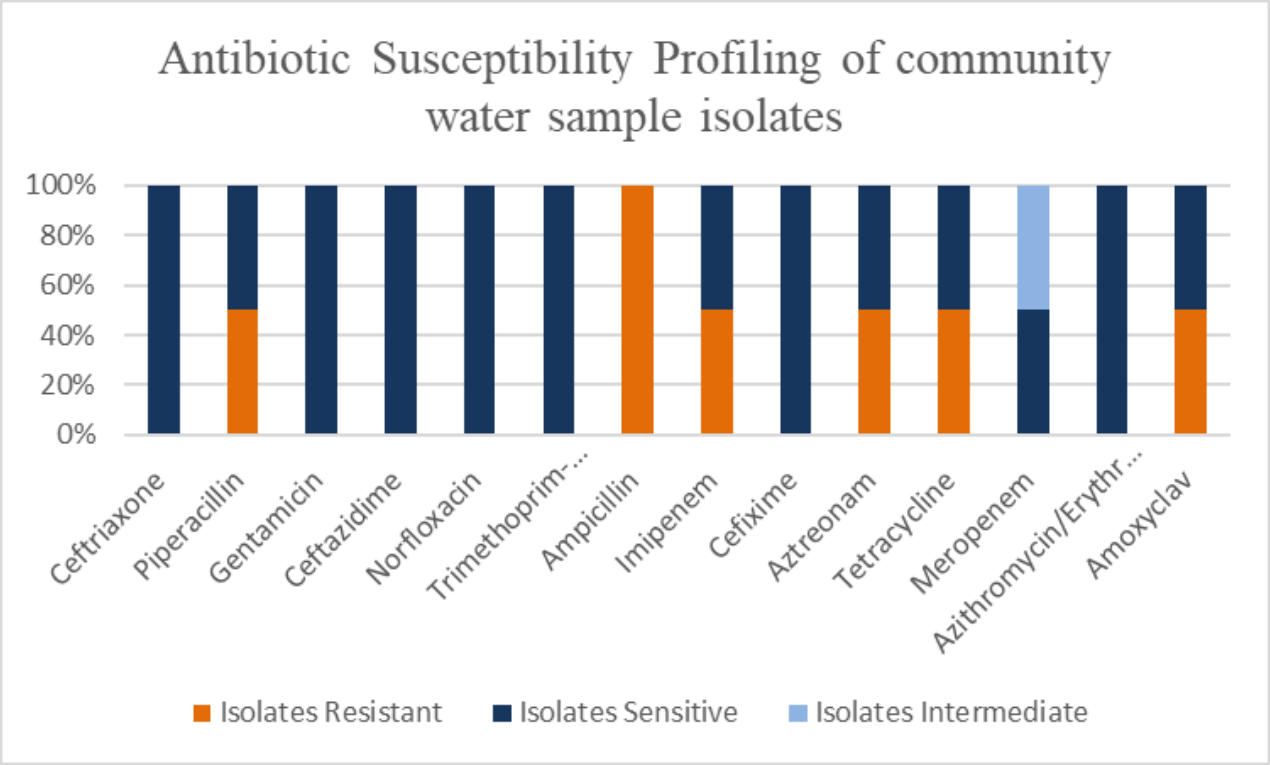


Figure 5: Percentage of Antibiotic Susceptibility Test of 2 isolates derived from community water

3.4 Multidrug Resistance of the isolates:

The ECDC criteria define MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Our study has found 9 MDR from 11 *E. coli*. 7 were from hospital waste water and 2 were from community water.

From hospital wastewater 7 MDR *E. coli* were found to be highly resistant because they showed 88.8% resistance to Ampicillin, 66.6% resistance to Tetracycline and 55.5% resistance to Cephalosporins (Cefixime and Ceftriaxone). In addition, from these 7 MDR *E. coli* 5 were Carbapenem resistant which are often used as last resort antibiotics to treat serious MDR infection and 2 were found to be resistant to most of the antibiotic categories. Both of these isolates were acquired from the National Cancer Hospital, Dhaka. Moreover, both the isolates of community

water were found to be MDR because they showed resistance to Penicillin , Amikacin and Imipenem.

Chapter 4

DISCUSSION:

Our study aimed to isolate multi-drug resistant *E. coli* from hospital wastewater and adjacent community water samples in Dhaka city, Bangladesh. However, there are currently relatively few data available in Bangladesh for the isolation and identification of MDR *E. coli* from hospital wastewater and community water.

In this study total 11 *E. coli* were selected from the samples, which were collected from four hospital wastewater (National Institute of Cancer Research And Hospital, Dhaka Shishu Hospital and Dedicated Covid-19 Hospital, Mohakhali, Dhaka-1212.) and fourteen hospital adjacent community water. From 11 *E. coli*, 9 were selected from hospital wastewater and 2 *E. coli* were selected from community water. Among the nine *E. coli* isolates found in hospital wastewater, the highest rate of resistance was observed against ampicillin, with (88.8%) of the isolates being resistant. Tetracycline resistance was found in (66.6%) of the isolates, while resistance to ceftriaxone and cefixime was observed in (55.5%) of the isolates. Additionally, (44.4%) of the isolates were resistant to a combination of three antibiotics: imipenem, aztreonam, and amoxiclav. Furthermore, (33.3%) of the isolates demonstrated resistance to four antibiotics: ceftazidime, norfloxacin, trimethoprim-sulfamethoxazole/co-trimoxazole sulfa, and azithromycin/erythromycin. Moreover, (22.2%) of the isolates were resistant to piperacillin, while only (11.1%) showed resistance to gentamicin. [Table 9]

In contrast, two *E. coli* isolates from community water exhibited complete resistance (100%) against ampicillin. Furthermore, (50.00%) of these isolates demonstrated resistance to five antibiotics: Piperacillin, Imipenem, Aztreonam, Tetracycline, and Amoxyclav. However, no resistance was observed against other antibiotics. [Table 10].

Furthermore, from these 9 isolates of hospital wastewater 7 were MDR as they showed resistance against 3 different types of antibiotic groups or more, where 2 isolates were found to be resistant to

most of the antibiotic categories. These two isolates were from the Dhaka National Cancer Hospital. Additionally, it was determined that both community water isolates were MDR since they displayed resistance to Penicillin, Amikacin, and Imipenem.

These findings suggest that there is a significant rate of MDR *E. coli* in hospital wastewater. The high levels of resistance to commonly used antibiotics such as Ampicillin and Tetracycline, as well as third-generation cephalosporins like Cefixime and Ceftriaxone, and also Carbapenem highlight the urgent need for more effective antimicrobial stewardship and infection control measures in both hospital and community settings. Further studies are required to better understand the epidemiology of MDR *E. coli* in water sources and to identify strategies for controlling their spread.

Moreover, this finding is consistent with previous studies conducted in Bangladesh and other countries, which have reported high levels of antimicrobial resistance in *E. coli* isolates from hospital settings and environmental sources (Tasnim et al., 2018; Islam et al., 2020). In one study of South African, 230 *E. coli* isolates were examined from nearby wastewater and water treatment facilities, the Modimola Dam, and adjacent residences. Though this study was not exactly about hospital waste water but it was about wastewater. It shows that resistance to erythromycin, tetracycline, ampicillin, and norfloxacin is quite prevalent, with resistance rates to each antibiotic exceeding 70%.(Kinge, Ateba, & Kawadza, 2010). In our study, from hospital waste water only ampicillin is with high resistance rate, which is 88.8% compared to this study of South Africa. The resistance rates for the other antibiotics are lower: 33.3% for erythromycin, 66.6% for tetracycline and 33.3% for norfloxacin. [Table 8]. However, in community water, both isolates were 50% resistant to erythromycin and tetracycline and 100% resistant to ampicillin, which is very high when compared to the South African study. Whereas norfloxacin was sensitive. [Table 9]. In a separate research conducted at a wastewater treatment plant in Dhaka City, a total of 37 strains of *E. coli* were examined for their resistance to 15 different antibiotics from eight distinct classes. The findings revealed that the highest level of resistance was observed against erythromycin, with a rate of 99%, followed by amoxicillin 91.9%, streptomycin 89.2%, ampicillin 75.7%, cefuroxime 67.6%, ceftriaxone 64.9%, cefixime 62.2%, ciprofloxacin 54.1%, nalidixic acid 51.4%, tetracycline 51.4%, trimethoprim/sulfamethoxazole 40.5%, gentamicin 40.5% and

chloramphenicol 21.6%. (Hossain et al., 2022). Comparing this study with our study on wastewater, only tetracycline and ampicillin is higher in rate, which is 66.6 % and 88.8% respectively. Other antibiotic rate is comparatively lower. Such as erythromycin 33.3%, ceftriaxone 55.5%, cefixime 55.5 %, gentamicin 11.1%. [Table 8]. On the other side if we compare our result of community water with this study then it is seen that only ampicillin is highly resistant, which is 100%. Also the resistance of tetracycline is quite close compared to this study, which is 50%. But in our study ceftriaxone and gentamicin is 100% sensitive [Table 9]. Where as in the study conducted at a wastewater treatment plant in Dhaka City ceftriaxone is 64.9% resistant and gentamicin is 40.5% resistant.

The high rate of MDR *E. coli* in community water sources is a cause for concern, as it indicates a potential risk of transmission to humans and animals. The presence of high rate of MDR *E. coli* in hospital wastewater may be attributed to the overuse and misuse of antibiotics in healthcare settings, which can promote the emergence and spread of antibiotic-resistant bacteria (Laxminarayan et al., 2013).

In conclusion, our study highlights the number or percentage of MDR *E. coli* in some hospital wastewater and adjacent community water sources in Dhaka city, Bangladesh. The presence of antibiotic resistance genes in the MDR *E. coli* isolates highlights the potential for the spread of ESBL-producing bacteria in the environment. Our findings underscore the need for effective infection control measures, improved sanitation and water treatment facilities, and increased surveillance of antibiotic resistance patterns to prevent the spread of antibiotic-resistant bacteria in the environment and protect public health.

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