

INVESTIGATION ON ANTIMICROBIAL RESISTANCE
PATTERN OF *Escherichia coli* ISOLATED FROM
PATIENTS OF BANGLADESH

By

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A thesis submitted to the Department of School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

School of Pharmacy

BRAC University

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Declaration

It is hereby declared that.

1. The thesis submitted titled “**INVESTIGATION ON ANTIMICROBIAL RESISTANCE PATTERN OF *Escherichia coli* ISOLATED FROM PATIENTS OF BANGLADESH**” is my original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Investigation on antimicrobial Resistance Pattern of *Escherichia coli* isolated from patients of Bangladesh” submitted by Jarin Tasnim (21346063), of Spring, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 30 August, 2025

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Ethics Statement

This study does not involve any animal and human trial. This project work has been funded by Research Seed Grant Initiative (RSGI), BRAC University and approved by the Biomedical Research Review Board (IRB), BRAC University (Ref No. BRACUIRB125012).

Abstract

In Bangladesh, the rise of antibacterial resistance in *Escherichia coli* is concerning and has become a growing public health threat. We conducted a retrospective analysis of clinical records of culture sensitivity data from Khulna for the last three years (2023–2025). Among 600 infection-positive samples, *E. coli* identified as the most prevalent organism, accounting for 34.5% of female and 27.5% of male cases. A subset of 35 *E. coli* isolates was cultured for identification by PCR. By Kirby-Bauer disk diffusion, AST was estimated and explained with CLSI criteria. Among 35 *E. coli* isolates, 30 (85.7%) were PCR-positive. Multiple drug resistance was detected in 93% and only 7% classified as Non-MDR, showing high resistance levels to all antibiotic classes which is a major concern. Highest resistance level was exhibited against metronidazole (100%) followed by cephalexin (93.4%), amoxiclav (92.2%) and azithromycin (90.4%). The speed with which resistance can spread in the country confirms the worrying rise of public health problems and necessitates immediate attention to overcome AMR.

Keywords: *Escherichia coli*; antimicrobial resistance; multidrug resistance; CLSI; AST; PCR, Kirby–Bauer disk diffusion; resistance.

Dedication

This research is dedicated to my beloved parents, whose sacrifices and constant encouragement have been my greatest strength, and to myself, for the determination and hard work invested throughout the journey.

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All praise and gratitude are due to Almighty Allah for giving me enough strength, patience and determination to complete the research. I extend my heartfelt gratitude to my supervisor, Dr. Muhammad Asaduzzaman, Professor, School of Pharmacy, BRAC University, for his guidance, encouragement, and constant feedback to this research. Special thanks to Dr. Fahim Kabir Monjurul Haque, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, whose contribution was crucial in the success of this research. I appreciate and grateful to my research assistant Fahim Faisal Nakib for his cooperation and encouragement during my lab-work. Also thanks to Dr. Md. Zaber, Assistant Professor, Department of Microbiology, Gazi Medical College, Khulna for providing me enough information and samples for the research.

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Table of Contents

Declaration.....	ii
Approval	iii
Ethics Statement.....	iv
Abstract.....	v
Dedication	vi
Acknowledgement	vii
List of Tables	xi
List of Figures.....	xii
List of Acronyms	xiii
Chapter 1 Introduction.....	1
1.1 Background.....	1
1.2 The Growing Challenge of AMR in Bangladesh.....	1
1.3. AMR of <i>E. coli</i> and Virulence: a converging threat around the world.....	2
1.4 Major Types of Antibiotics.....	3
1.5 Emergence of Multiple drug and Extensively Drug-Resistant	5
1.6 Drug Resistance through Active Efflux.....	6
Aims and Objectives:	7
Chapter 2	8
Methodology	8
2.1 Research Framework.....	8

2.2 Type of samples	8
2.3 Retrospective study: Data collection	9
2.4 MAR Index Derivation	10
2.5 Statistical Investigation.....	10
2.6 Prospective study: Materials and Methods.....	10
2.7 Sample Collection.....	11
2.7.1 Clinical Sample Collection and Preparation	11
2.7.2 Bacterial Isolation & Identification.....	12
2.7.3 Bacterial Inoculation, Preservation.....	12
2.8 DNA Extraction and Purification	13
2.8.1 DNA Concentration and Purity	13
2.9 Protocol for DNA Amplification	13
2.9.1 Targeted Genes and Primers	14
2.10 Gel Electrophoretic Analysis.....	14
2.11 Antibiotic Efficacy Evaluation.....	15
2.11.1 Preparation of Inoculum and Interpretation	15
Chapter 3 Results and Discussions	15
3.1 Prevalence of <i>E. coli</i> Among the pathogens.....	15
3.2 Gender distribution of <i>E.coli</i> by specimen.....	16
3.3 Ratio of age range between males and females	17
3.4 Prevalence of <i>E. coli</i> in multiple Specimens Types	18

3.5 Distribution of MDR vs Non-MDR isolates	19
3.6 Different antibiotic sensitivity & resistant pattern of <i>E.coli</i>	20
3.7 Antibiotic resistance categorization (MDR, Non-MDR, XDR) & MAR Index of tested isolates of <i>E. coli</i>.	22
3.8 Assessment of Nucleic Acid Purity	23
3.9 PCR results:.....	23
3.10 Antibiotic Susceptibility Analysis of <i>E. coli</i>	24
Chapter 4	27
4.1 Limitations.....	27
4.2 Conclusion.....	27
References	29

List of Tables

Table 1: Major <i>E. coli</i> Pathotypes: Virulence Determinants and Syndromes.....	3
Table 2: A table containing the class and names of antibiotics, gram spectrum, action type, MOA	4
Table 3: Type of Sample for both Retrospective and Prospective study.....	8
Table 4: Primers and Targeted gene for this Study.....	14
Table 5: Comparison between Male & Female according to specimen.....	19
Table 6: Sensitivity and Resistance pattern.....	20
Table 7: DNA concentration and purity of tested samples.....	23
Table 8: AST Results comparison between Hospital & Laboratory for <i>E. coli</i>	24

List of Figures

Figure 1: <i>Escherichia coli</i>	2
Figure 2: Antimicrobial Resistance Mechanism of <i>E. coli</i>	3
Figure 3: Mechanism of Action of Antibiotics.....	5
Figure 4: Mechanism of multiple antibiotic resistance system.....	6
Figure 5: Increased Activation of Efflux Puump.....	7
Figure 6: Survey questionnaire.....	9
Figure 7: Protocol for Laboratory Analysis.....	11
Figure 8: A - Purple colony on Hi-Crome UTI agar and B - Growth of <i>E. coli</i> on nutrient agar (NA).....	12
Figure 9: Prevalence of Organism.....	16
Figure 10: Gender Distribution of <i>E. coli</i> by Specimen.....	17
Figure 11: Ratio of age range A – Female, B – Male.....	18
Figure 12: Distribution of MDR vs Non-MDR isolates. A. All bacteria, B. only <i>E. coli</i>	20
Figure 13: Sensitivity data for <i>E. coli</i>	21
Figure 14: Antibiotic Resistance Categorization of <i>E. coli</i>	22
Figure 15: MAR Index of test isolates: <i>E. coli</i>	22
Figure 16: PCR-amplified <i>E. coli</i> -targeted gene showing bands at 585 bp.....	24
Figure 17: Heatmap Representation of Antibiotic Susceptibility Profile of <i>E. coli</i>	25
Figure 18: AST plates showing resistance & sensitivity.....	25
Figure 19: Antibiotic Susceptibility Profile of Clinical Isolates of <i>E. coli</i>	26

List of Acronyms

GLASS	Global Antimicrobial Resistance and Use Surveillance System
AMR	Anti-Microbial Resistance
AST	Antimicrobial Susceptibility Testing
MAR	Multiple Antibiotic Resistance
MDR	Multidrug-Resistant
WHO	World Health Organization
MHA	Mueller-Hinton Agar
NA	Nutrient Agar
TE	Tris-EDTA Buffer
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ESBL	Extended-Spectrum Beta-Lactamase
<i>E. coli</i>	<i>Escherichia coli</i>
CLSI	Clinical and Laboratory Standards Institute

Chapter 1

Introduction

1.1 Background

Antimicrobial resistance (AMR) is one of the leading and major threat to public health which is very concerning. In 2019, it primarily causes 1.27 million deaths and approximately 4.95 million deaths around the world (Murray et al., 2022). The Global Antimicrobial Resistance Surveillance System (GLASS) is organized by the World Health Organization (WHO) to align and optimize the collection of AMR data across countries. GLASS particularly prioritizes key bacterial pathogen like *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, owing to their resistance to frontline antimicrobial agents and their profound implications for public health (WHO, 2023). Surveillance outputs provide essential evidence for policy making, antimicrobial stewardship and strengthening global strategies to combat AMR.

Among these pathogens, *E. coli* accounts for the largest share of AMR-related mortality, contributing to approximately 829,000 AMR-associated deaths and 219,000 attributable deaths in 2019 (Murray et al., 2022). *E. coli* exhibited high resistance patterns that lowers the efficacy of first-line therapeutic options as it is one of the major reason for critical urinary tract infections and frequent source of infections spreading throughout the body (Murray et al., 2022).

1.2 The Growing Challenge of AMR in Bangladesh

Because of antimicrobial resistance, there are fewer treatment availability, increased period of illness and higher mortality. Current projections suggest a worsening trajectory, with AMR expected to cause as many as 39 million deaths globally by 2050 (Naghavi et al.,

2024). The situation in Bangladesh is worsened due to uncontrolled use of antibiotics, with up to 59.4% of non-prescribed antibiotics dispensed from WHO's "Access" group (Salam et al., 2023). The country's weak state is shown in its Global Health Security Index score of 35 and ranking of 113 out of 195 countries, underscoring systemic vulnerabilities in detecting and responding to the situation (Hoque et al., 2020b). Unfortunately, *E. coli* has developed strong resistance to third-generation cephalosporins and fluoroquinolones, whereas urinary isolates retain moderate nitrofurantoin susceptibility ($\approx 70\text{--}80\%$), and carbapenems remain the most reliably active agents (often $\geq 90\%$), underscoring the need to conserve last-line drugs through robust antimicrobial stewardship (WHO GLASS; IEDCR, 2016–2023; Habib et al., 2021; Hossain et al., 2020). In Bangladesh, national surveillance similarly reports high resistance among clinical isolates, underscoring the need for stewardship and infection-prevention measures.

1.3. AMR of *E. coli* and Virulence: a converging threat around the world

Belonging to the Enterobacterales group, *E. coli* is a Gram-negative, rod-like bacterium. Various pathotype such as enterotoxigenic (ETEC), enterohemorrhagic/Shiga toxin-producing (EHEC/STEC), enteropathogenic (EPEC), and uropathogenic (UPEC) strains are major etiological agents of diarrheal disease, urinary tract infections, and systemic infections such as sepsis.



Figure 1: *Escherichia coli* (Ross, 2019)

Pathotype	Virulence determining gene	Usual clinical syndrome(s)
ETEC	(<i>eltAB</i>), (<i>estA</i>), (CF/CS pili)	Traveler's diarrhea, pediatric watery diarrhea
EPEC	LEE island encodes T3SS; BFP (<i>tEPEC</i> only)	Infantile watery diarrhea
EHEC/STEC	Shiga toxins <i>Stx1/Stx2</i> (phage-encoded); enterohemolysin <i>ehxA</i>	Hemorrhagic colitis, risk of HUS
EIEC	Large invasion plasmid pINV (T3SS)	Dysentery-like diarrhea with fever
UPEC	Type 1 fimbriae (<i>FimH</i>), P fimbriae (<i>pap</i>), α -hemolysin (<i>hlyA</i>)	Cystitis, pyelonephritis

Table 1: Major *E. coli* Pathotypes: Virulence Determinants and Syndromes (Croxen and Finlay, 2010)

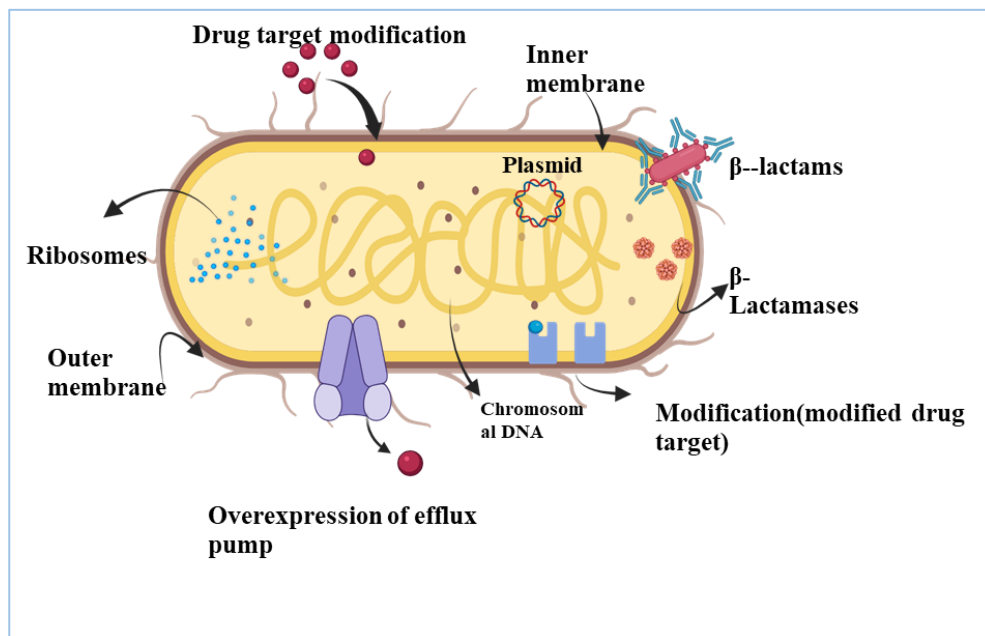


Figure 2: Antimicrobial Resistance Mechanism of *E. coli*

1.4 Major Types of Antibiotics

The fundamental role of antibiotics is to either destroy bacteria (bactericidal effect) or suppress their growth (bacteriostatic effect), thus supporting the host immune system in eradicating infections.

Class of Antibiotics	Names of the Antibiotics	Gram spectrum	Action type	Mechanism of action
Cephalosporins	Cefepime, Cefuroxime, Ceftriaxone	Gram (+) and Gram (-)	Bactericidal	Inhibits cell wall synthesis by blocking PBPs
Tetracyclines	Doxycycline	Gram (+) and Gram (-)	Bacteriostatic	Inhibits protein synthesis
Fluoroquinolones	Ciprofloxacin, Levofloxacin	Gram(+) and Gram(-)	Bacteriostatic	Inhibits DNA Replication
Lacosamide	Clindamycin	Gram (+)	Bacteriostatic	Inhibits protein Synthesis
Aminoglycosides	Amikacin, , Tobramycin, Gentamicin	Gram (-)	Bactericidal	Inhibits protein synthesis
Sulfonamides	Sulfasalazine, Sulfamethoxazole	Gram(+) and Gram (-)	Bacteriostatic	Inhibits folic acid synthesis (essential for DNA or RNA)
Macrolides	Azithromycin, Erythromycin	Gram(+)	Bactericidal	Inhibits protein Synthesis
Carbapenems	Meropenem	Gram(+) and Gram (-)	Bactericidal	Inhibits cell synthesis
Penicillin	Amoxicillin	Gram(+) and Gram(-)	Bactericidal	Inhibits cell wall synthesis by blocking PBPs
Glycopeptides	Vancomycin	Gram (+)	Bactericidal	Inhibit cell synthesis

Table 2: A table containing the class and names of antibiotics, gram spectrum, action type, MOA

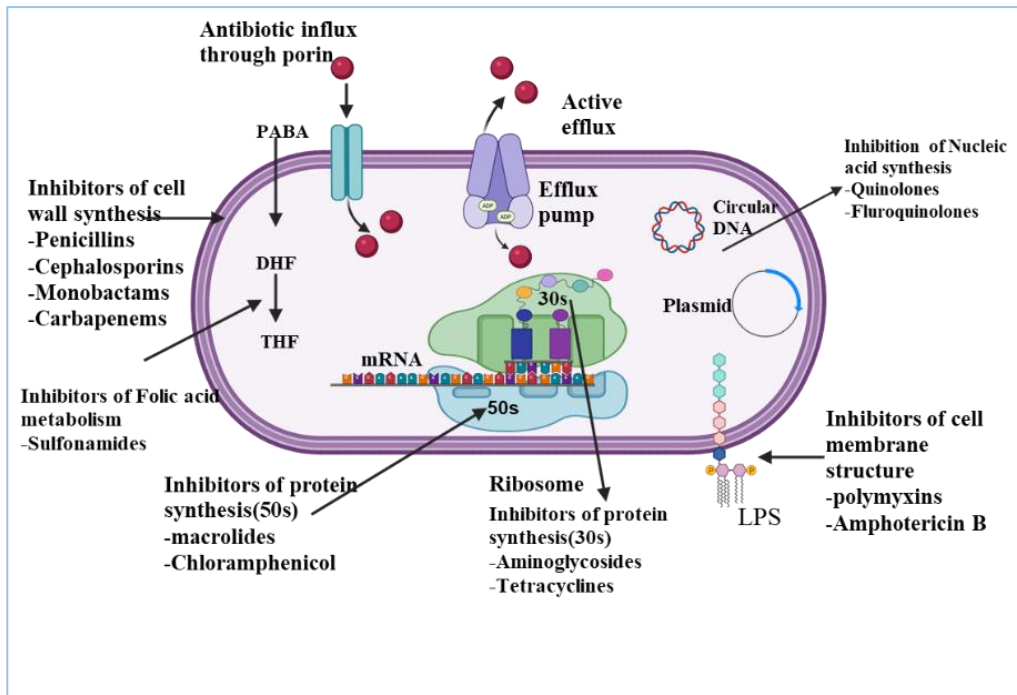


Figure 3: Mechanism of Action of Antibiotics

1.5 Emergence of Multiple drug and Extensively Drug-Resistant

Multiple drug-resistant (MDR) and extensively drug-resistant (XDR) bacteria have emerged as major growing public health threats. MDR organisms are defined as resistant to at least one agent in three or more antimicrobial classes, whereas XDR strains exhibit resistance to nearly maximum available antimicrobial agents, leaving only one or two therapeutic options for treatment (Magiorakos et al., 2012; WHO, 2021). The rise of MDR strains has been particularly associated with patients receiving long-term antibiotic therapy, where bacterial populations evolve complex survival strategies (Munita & Arias, 2016).

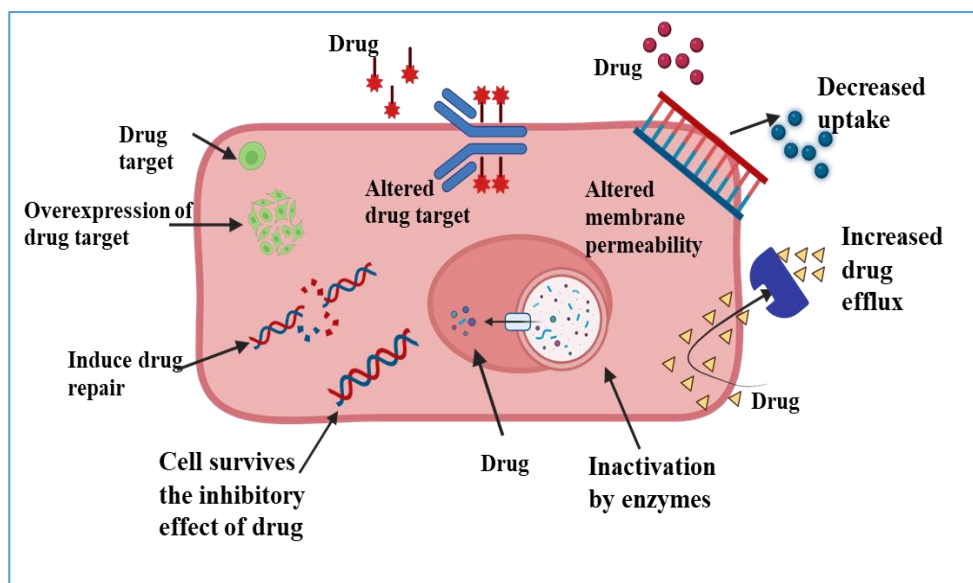


Figure 4: Mechanism of multiple antibiotic resistance system

1.6 Drug Resistance through Active Efflux

One of the major mechanisms facilitating MDR is the active efflux of antibiotics mediated by specialized membrane proteins. These carriers remove antimicrobial components from the inside or outside of the cell in Gram-negative pathogens which reduce effectiveness of the drug. Because of efflux pump, antibiotics such as macrolides, tetracyclines shown rapid activity against Gram-positive pathogens but unable to work against Gram-negative pathogens (Poole, 2005). Efflux pumps typically function as multidrug transporters capable of extruding diverse antibiotic classes, thereby conferring broad resistance. Resistance in quinolone occurs when the AcrAB-TolC efflux system in *E. coli* developed (Du et al., 2018). Furthermore, *E. coli* regulates porin expression (particularly OmpF and OmpC proteins) to modulate outer membrane permeability, thereby restricting antibiotic entry (Nikaido, 2003).

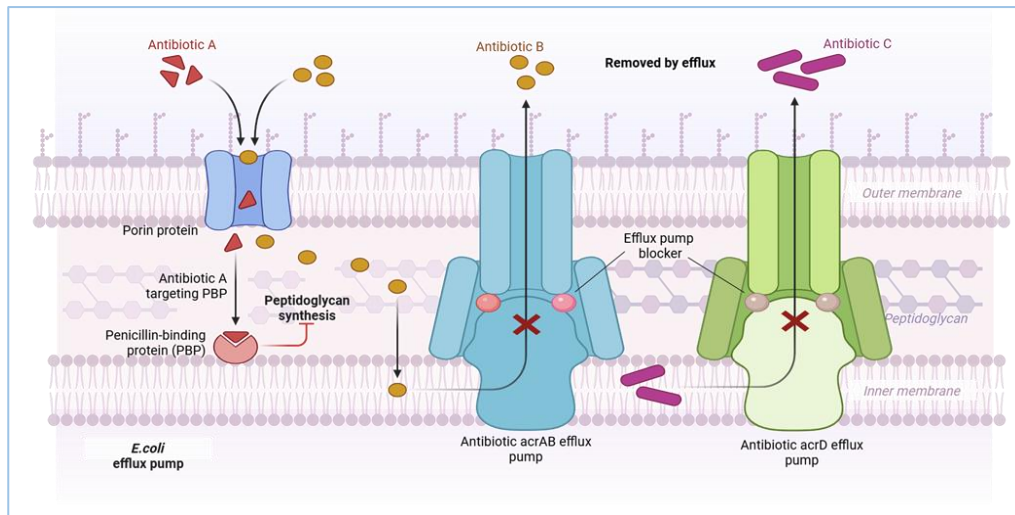


Figure 5: Increased Activation of Efflux Pump

Aims and Objectives:

- To analyze and evaluate the AMR trends of *E.coli* for last three years (2023–2025) in a selected hospital from Khulna division of Bangladesh with the goal of identifying resistance trends and supporting antimicrobial stewardship and infection control strategies.
- To collect, detect and confirm *E. coli* from patient-derived clinical specimens.
- To analyze the resistance and susceptibility patterns of bacterial samples using Antibiotic Susceptibility Testing (AST).
- To confirm the presence of *E.coli* based on PCR based detection targeted this gene.
- To analyze the prevalence of multiple antibiotic resistance and extensively antibiotic resistance from the *E. coli* isolates among the population.
- To determine resistance patterns of the clinical isolates against commonly prescribed antibiotics.

Chapter 2

Methodology

2.1 Research Framework

Between 2023-2025, a retrospective analysis was organized using 600 data from Khulna, Bangladesh & a prospective analysis was carried out using 35 *E. coli* isolates obtained from clinical samples across multiple centers located in Khulna, Dhaka, Brahmanbaria, and Feni.

2.2 Type of samples

Both retrospective and prospective samples (clinical isolates) were isolated from various biological specimens as outlined in table 3 below:

For Retrospective Study	For Prospective Study
Bile	Urine
Eye swab	Stool
Prostatic smear	Pus
Pus	High vaginal Swab
SPUTAM	
STOOL	
Synovial fluid	
Throat swab	
Umbilical Swab	
Urine	
Vaginal Swab	
Vulva	
Wound Swab	
Pleural fluid	

Table 3: Type of Sample for both Retrospective and Prospective study

2.3 Retrospective study: Data collection

A pre-designed questionnaire (Figure 6) was used to collect data, from the following data 1. Date, 2. Sample code, 3. Sample Type, 4. Organism name, 5. Patient information, 6. Antibigram result.

**Title: Investigation on Antimicrobial Resistance (AMR) pattern of
prevalent GLASS pathogens isolated from clinical samples of Bangladeshi
patients**

Data Collection Form

Date:

1. Sample Code:

2. Sample Type: Blood / Urine/ Stool/ Sputum/ Wound Swab/ Others
(.....)

3. Organism Name:

4. Patient Info:

a) Age:

b) Gender: M / F

5. Antibigram Result:

Name of the Antibiotics	R	S
Penicillin		
Amoxicillin		
Ampicillin		
Piperacillin		
Ceftriaxone		
Ceftazidime		
Cefotaxime		
Cefoxitin		
Cefuroxime		
Cefepime		

Figure 6: Survey questionnaire (All the antibiotics list tested is not shown here)

2.4 MAR Index Derivation

Multiple Antibiotic Resistance (MAR) index = A/B , where "A" refers the antibiotics number against where resistance was observed in the isolates, and "B" indicates the total antibiotics number evaluated in this research (Ayandele et al., 2020).

2.5 Statistical Investigation

With the help of Microsoft Excel, every correlative data between male and female participants were processed to statistical methods to identify significance.

2.6 Prospective study: Materials and Methods

Aim of this research is to examine the bacteriological profile and resistance trends of *E. coli* isolates from various clinical specimens (stool, urine, pus, high vaginal swab) have been collected from several centres in Bangladesh. Ethical clearance was granted to the research work by the concerned institution before initiating the research work, thereby fulfilling the requirement and protocol of ethics. Samples suspected of contamination during collection, handling, or processing were excluded. This strict criterion ensured the accuracy of the outcomes, preserving the research authenticity and validity.

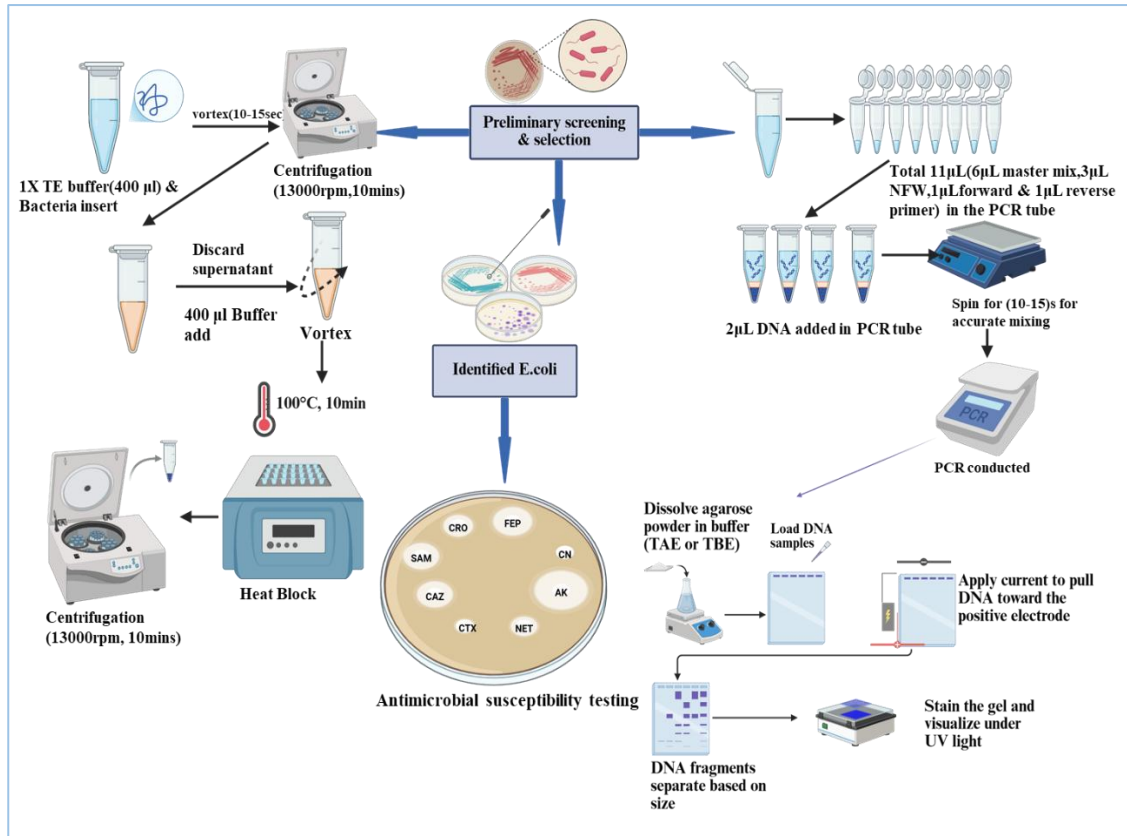


Figure 7: Protocol for Laboratory Analysis

2.7 Sample Collection

2.7.1 Clinical Sample Collection and Preparation

Clinical specimens were collected from patients using sterile vials, each labeled with unique patient identifiers. Samples were transported under strict cold chain conditions to ensure integrity and prevent contamination. Upon arrival at the microbiology laboratory, all procedures were carried out in compliance with biosafety protocols (CLSI, 2021). All experimental procedures were executed within a laminar flow biosafety cabinet under aseptic conditions. Subsequently, specimens were inoculated onto selective culture media for isolation ensuring accuracy and reliability of the outcomes.

2.7.2 Bacterial Isolation & Identification

To isolate and identify *E. coli*, portions of each clinical sample (urine, pus, high vaginal swab, and stool) were sub-cultured onto selective media to get the pure colonies. *E. coli* was isolated on Hi-Crome UTI agar, where chromogenic substrate reactions facilitated clear identification by producing purple coloured colonies, which indicates its chromogenic reaction. Then *E. coli* was sub-cultured onto nutrient agar (NA) for maintaining viable growth.

2.7.3 Bacterial Inoculation, Preservation

Following initial isolation on nutrient agar, bacterial colonies were transferred into nutrient broth (NB) to obtain liquid cultures with abundant biomass. For preservation over time, samples were stored as glycerol stocks at -20°C or -80°C , with glycerol acting as a cryoprotectant to prevent ice crystal formation and maintain bacterial viability (Green & Sambrook, 2012).

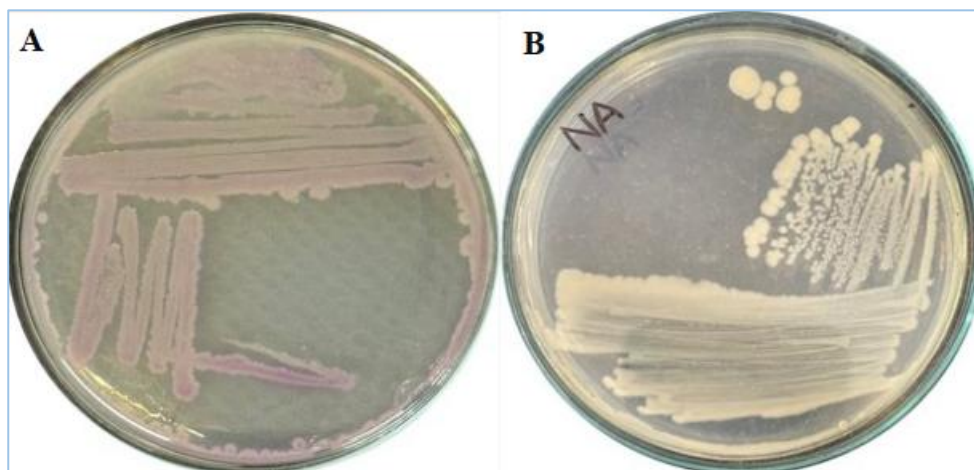


Figure 8: A - Purple colony on Hi-Crome UTI agar and B - Growth of *E. coli* on nutrient agar (NA)

2.8 DNA Extraction and Purification

By using boiling lysis method, genomic DNA was extracted with 1X Tris-EDTA (TE) buffer. Four hundred microliters of 1X TE buffer were added to an Eppendorf tube, and a loopful of bacterial culture was inoculated into the tube. The sample was vortexed to mix thoroughly and cells were pelleted by centrifugation at 13,000 rpm. After 10 minutes pellets were resuspended and placed in heat block at 100°C for 10 minutes, followed by cooling and debris removal through centrifugation. 200 µL supernatant was gently placed into a new Eppendorf tube. The resulting supernatant contained rich-quality DNA.

2.8.1 DNA Concentration and Purity

To check the DNA concentration as ng/µl, a Nanodrop[®] spectrophotometer is used and 1X TE buffer used as the blank. Purity of DNA was determined from A260/A280 ratios, where ~1.8 indicates pure DNA, with higher ratios reflecting possible RNA carryover and lower ratios considered protein or phenol contamination. Similarly, A260/A230 values of 2.0–2.2 are considered optimal, whereas reduced values may indicate residual salts, carbohydrates, or extraction reagents.

2.9 Protocol for DNA Amplification

Polymerase chain reaction (PCR) is a widely applied method which quickly amplifies certain DNA sequences, producing millions duplicates (Mullis & Faloona, 1987). By using Biosystems 2720[®] Thermal Cycler, the amplifications are completed. Each sample have (13 µL total volume) consisted of 2 µL of template DNA, 6 µL of EmeraldAmp[®] PCR master mix, 1 µL of forward primer, 1 µL of reverse primers, and 3 µL of nuclease-free water (NFW). The mixture vortexed (3–5 seconds) to ensure homogeneity before cycling under gene-specific thermal parameters. PCR products were subsequently preserved at –20 °C until further analysis (Sambrook & Russell, 2001).

2.9.1 Targeted Genes and Primers

The research focused on *E. coli* virulence-associated genes that causes wide range of infections. For example urinary tract infections (UTIs), gastroenteritis, hemolytic uremic syndrome (HUS), neonatal meningitis, pneumonia, and sepsis (Kaper et al., 2004; Croxen and Finlay, 2010). Samples analyzed included urine, stool, pus, and high vaginal swabs, which were screened for pathogenic strains harboring virulence markers. Identifying these genes was essential to understanding pathogenicity and guiding therapeutic strategies against resistant *E. coli* strains.

Name of Bacteria	Targeted Gene	Primer Sequences	Product size	Reference
<i>Escherichia coli</i>	16S rRNA	F: 5'- GACCTCGGTTT AGTTCACAGA- 3'	585 bp	(Lindsey et al., 2017)
		R: 5'- CACACGCTGA CGCTGACCA-3'		

Table 4: Primers and Targeted gene for this Study

2.10 Gel Electrophoretic Analysis

PCR products were analyzed by using gel electrophoresis (1.5% agarose) and transferred into TAE buffer. After that ethidium bromide (4 μ L) or MIDORI Green was incorporated as an intercalating dye for nucleic acid visualization (Brody & Kern, 2004). PCR products were loaded alongside a 50 bp DNA ladder as it is standard for evaluating product sizes by comparison. At 100V, the process was completed and bands were displayed under UV radiation (Sambrook & Russell, 2001).

2.11 Antibiotic Efficacy Evaluation

Antimicrobial susceptibility testing (AST) was estimated in Mueller-Hinton Agar plate by a method called Kirby-Bauer disk diffusion (Bauer et al., 1966). Commercial discs (HiMedia Laboratories Pvt. Ltd, India) were applied to evaluate the susceptibility of *E. coli* isolates. Then place it in the incubator and after that the zone diameters measured and explained by standardized guidelines. This method enabled determination of resistance phenotypes, providing clinically relevant insights for guiding effective therapy and informing public health surveillance strategies (CLSI, 2021).

2.11.1 Preparation of Inoculum and Interpretation

Microbial suspensions adjusted to mirror 0.5 McFarland turbidity standard ($\sim 1.5 \times 10^8$ CFU/mL) before doing inoculation (Andrews, 2001). Fresh colonies (3–5) were transferred into sterile saline, vortexed. By using a sterile swab, the suspension of bacteria distributed evenly in Mueller-Hinton agar plate. Antibiotic discs were aseptically placed with ethanol-flamed forceps. Plates were transferred to the incubator for 24 hours at 37°C. The Zone of inhibitions are recorded and explained by CLSI guideline, categorizing isolates as S, I, R (Bauer et al., 1966; CLSI, 2021).

Chapter 3

Results and Discussions

3.1 Prevalence of *E. coli* Among the pathogens

In this research, out of 600 positive samples indicating infection (Figure 9), caused by various pathogens, the most frequently isolated organism was *E. coli*, accounting for 34.5% of cases in females and 27.5% in males (Figure 9). *E. coli* found in both sexes and more frequent in females (53.2%) than males (26.6%) (Gu et al., 2021). A national tertiary-

hospital study likewise reported *E. coli* results in female (~55% overall) predominance below age 60 and a relative shift in older males (Jahan & Anwer, 2025). A recent hospital study from Iraq similarly found *E. coli* dominated (56.7% of positives), with marked female skew (41.7% of all positives in females vs 15% in males) (Mohammed et al., 2025).

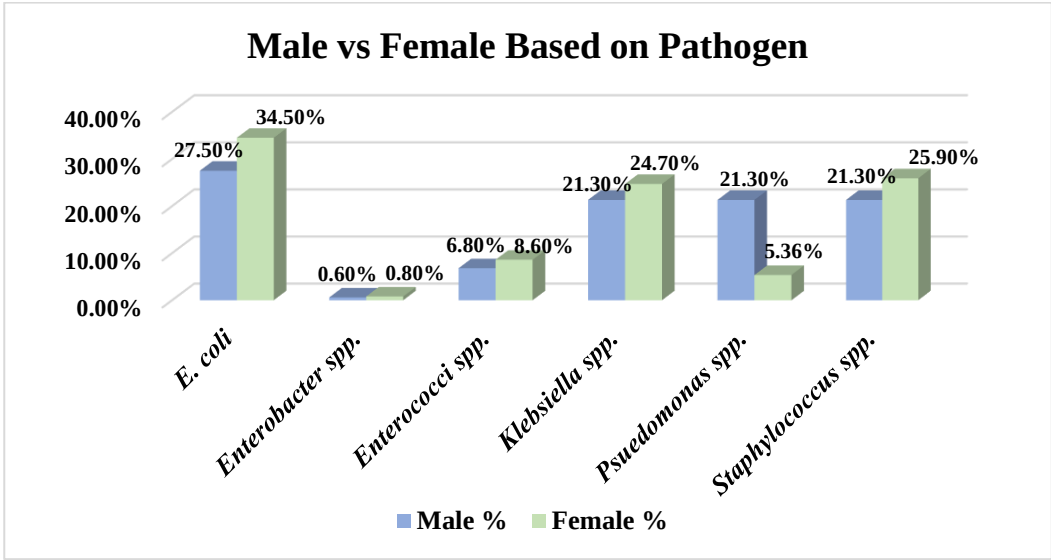


Figure 9: Prevalence of Organism

3.2 Gender distribution of *E.coli* by specimen

The majority of *E. coli* isolates were predominantly found from urine specimens, with the higher prevalence among female patients, while stool samples showed slightly greater recovery in males. Pus specimens yielded the fewest isolates, almost exclusively from male patients (Figure 10).

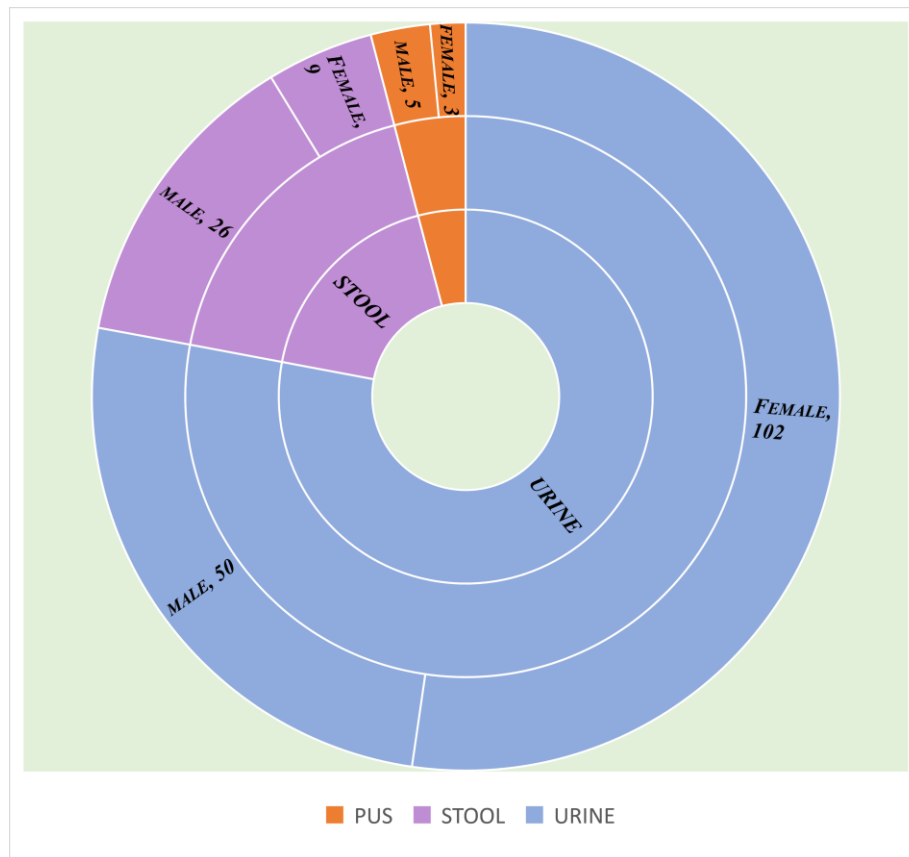


Figure 10: Gender Distribution of *E. coli* by Specimen

3.3 Ratio of age range between males and females

UPEC isolates in Dhaka peaked among 21-30-year-olds (38%) and were mostly female ($\approx 81\%$), while a larger Dhaka surveillance of community-acquired UTIs found adults more affected than adolescents/children and women composing roughly three-quarters of cases (Rahman et al., 2022; Islam et al., 2022). Similar result found in (Figure 11-A), the majority of *E. coli* infections occurred in the 21–30 year groups, in females almost equally represented. A population study from Pakistan observed the highest participation in the 26–35-year group with *E. coli* the leading uropathogen, consistent with concentration in reproductive ages that is similar with the results of this study (Khatoon et al., 2023). In Bangladesh, male children results 71% resistant to 3rd generation cephalosporins and 10% resistant to carbapenem (Islam et al., 2022), almost matching with the age group (1-10) from (Figure 11-B). Imam Khomeini Hospital from Iran reports significance resistance in

ciprofloxacin (~72%), nalidixic acid (~74%), cotrimoxazole (~71%) in male age range is 41 to 50 (Shirvani et al., 2023) mirrors with my findings (Figure11-B).

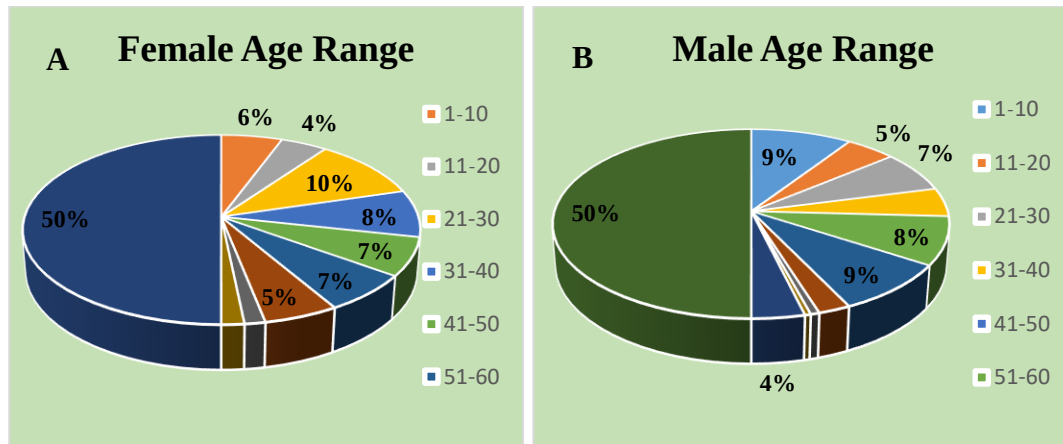


Figure 11: Ratio of age range A - Female, B - Male

3.4 Prevalence of *E. coli* in multiple Specimens Types

Among all the diverse clinical samples, urine samples yielded the highest number of pathogen for 72% of cases in females and 55.8% in males (Table 5). Throughout Bangladesh, *E. coli* consistently leading UTI organism ($\approx$ 50-60% of positive cultures), with a consistent female predominance a pattern that mirrors my report of higher *E. coli* isolation among females than males (Islam et al., 2022; Gu et al., 2021). A hospital in Dhaka analysis (2019–2020), similar result found in *E. coli*, $\sim$ 66% of positive urine cultures with most UTI patients being female (82%) and *E. coli* isolates far more frequent in females than males (139/160 vs 21/160) (Hossain et al., 2024).

Type	Male %	Female %
Bile	0	0.3
Eye swab	0	0.3
Pleural fluid	0	0.3
Prostatic smear	1.5	0.3
PUS	26.79	18
SPUTAM	0.75	0.3
STOOL	11.7	2.7
Synovial fluid	0	0.9
T/S	0	0.6
Throat swab	1.13	1.8
Umbilical Swab	0	0.3
urine	55.8	72
Vaginal Swab	0	1.5
Vulvul	0	0.6
Wound Swab	2.3	0.3
Total	100	100

Table 5: Comparison between Male & Female according to specimen

3.5 Distribution of MDR vs Non-MDR isolates

According to (Figure 12-A), the total MDR of all data shows 94% & Non-MDR of all data shows 6% where in (Figure 12-B), 93% of *E.coli* shows multidrug resistance (MDR), while only 7% shows non-MDR.

Similar study found in Bangladesh, recent hospital and community studies reports MDR *E. coli* $\approx 74\%$ in community-acquired UTIs and $\approx 71\%$ MDR among Gram-negative uropathogens, while another Dhaka tertiary-care study found MDR *E. coli* $\approx 73.9\%$ and overall MDR uropathogens $\approx 71.2\%$ (Islam et al., 2022; Chowdhury et al., 2024). A research with 100 *E. coli* isolates reported 98% MDR, very close to my study of MDR 93% (Jain et al., 2021).

This study results is closely echoed by international data, hospitals in Khartoum, Sudan reported 92.2% MDR *E. coli*, leaving $\sim 7.8\%$ non-MDR, which nearly matches my 93/7 split

(Ibrahim et al., 2012). In addition, a tertiary center in Abuja, Nigeria found 95.3% MDR *E. coli* and ~4.7% non-MDR (Medugu et al., 2022), almost matches with (Figure 12-B).

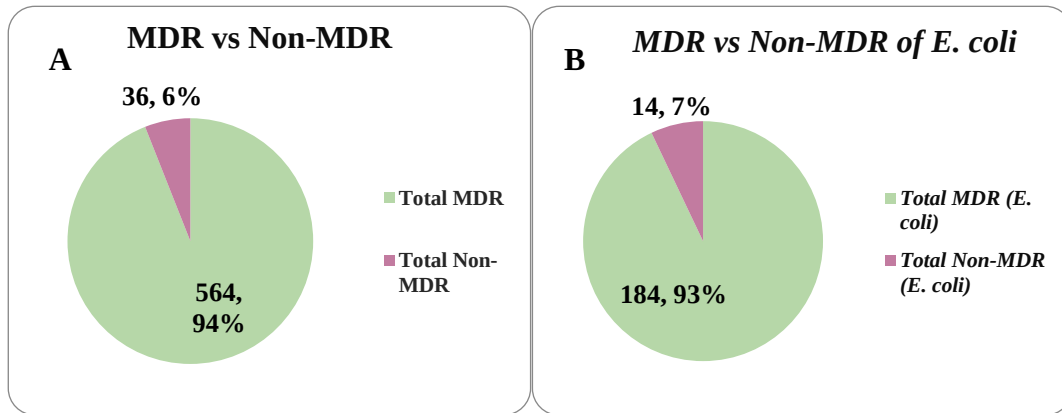


Figure 12: Distribution of MDR vs Non-MDR isolates. A. All bacteria, B. only *E. coli*

3.6 Different antibiotic sensitivity & resistant pattern of *E.coli*

High resistance ($R \geq 75\%$)	High sensitivity ($S \geq 80\%$)
Amoxiclav (92.2%)	Clarithromycin (100%)
Azithromycin (90.4%)	Colistin sulphate (97.0%)
Aztreonam (80.0%)	Meropenem (80.3%)
Ceftazidime (79.7%)	Vancomycin (95.7%)
Cefuroxime (79.8%)	Tigecycline (89.0%)
Cephalexin (93.4%)	
Cotrimoxazole (76.4%)	
Doxicycline (87.2%)	
Mecillinum (78.6%)	
Metronidazol (100%)	
Nalidixic acid (85.0%)	
Oxacillin (75.0%)	
Tetracycline (80.6%)	

Table 6: Sensitivity and Resistance pattern

In this research (Table 6) maximum resistance was exhibited against metronidazole (100%), cephalexin (93.4%), amoxiclav (92.2%), azithromycin (90.4%) and maximum sensitivity found in clarithromycin (100%), vancomycin (97%), colistin (97%), tigecycline (89%) and meropenem (80.3%). Bangladeshi and global evidence closely reflect the above pattern of this research: studies from Dhaka and national surveillance show *E. coli* with high resistance

to amoxiclav, cotrimoxazole, 1st or 3rd generation cephalosporins, nalidixic acid while carbapenems and colistin remain comparatively effective, mirroring with this study of high-R and high-S clusters (Hossain et al., 2020; IEDCR, 2016–2023). Similar resistance patterns have been reported in Bangladesh, where national AMR surveillance confirmed >60% resistance of *E. coli* to third-generation cephalosporins (IEDCR, 2025). International datasets report the same ESBL-driven profile, with high aztreonam/3rd-gen cephalosporin resistance alongside preserved meropenem susceptibility, for example in a Saudi hospital UTI cohort and in WHO’s GLASS explained *E. coli* resistance trends, which align with my findings (Almutawif & Eid, 2023; WHO GLASS, 2022). In Nepal, the resistance trends of *E. coli* is >50% to nalidixic acid, cotrimoxizole and 3rd generation cephalosporins (Baral et al., 2021).

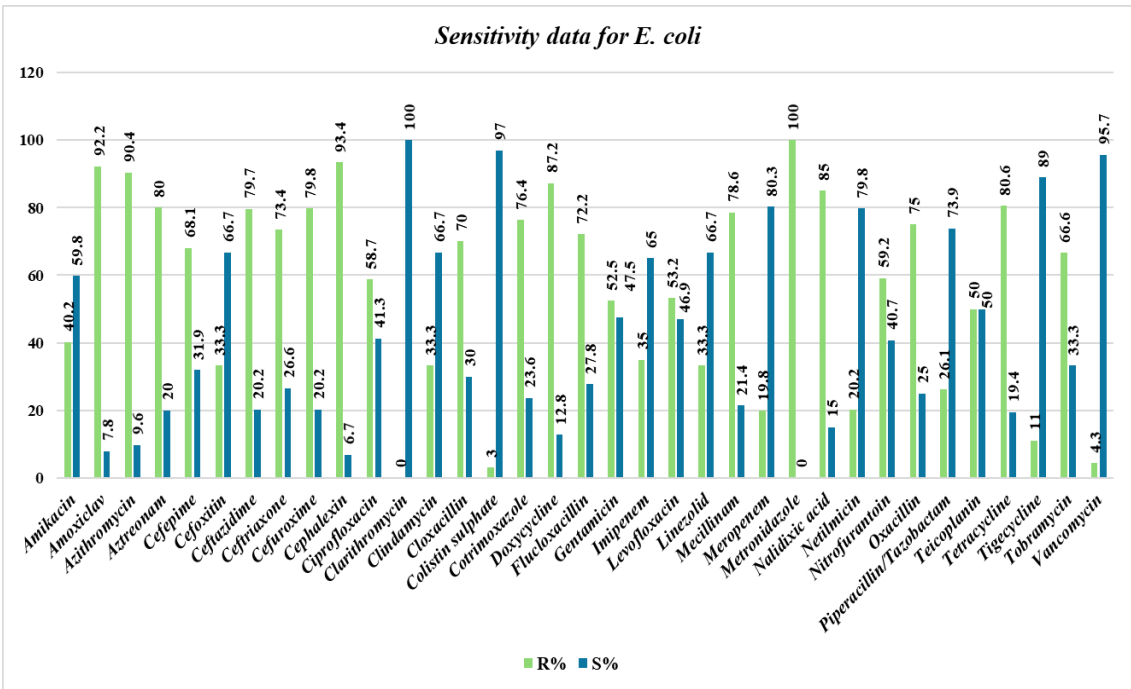


Figure 13: Sensitivity data for *E. coli*

3.7 Antibiotic resistance categorization (MDR, Non-MDR, XDR) & MAR

Index of tested isolates of *E. coli*.

According to (Figure 14), only 11% of *E. coli* shows multidrug resistance (MDR) & 1% of *E. coli* shows XDR, while 24% are Non-MDR. The MAR index analysis revealed significant variation in multidrug resistance patterns across bacterial isolates, with the highest resistance observed in BM21 (MAR index = 0.91), followed by KG2 and BM26 (MAR index = 0.80 each) (Figure 15). Notably, 16 out of 35 isolates (45.69%) demonstrated MAR index values ≥ 0.5 , indicating widespread multidrug resistance, while isolates BB3, BB18, BM20, BM27, BM28 and F6 showed relatively lower resistance profiles with MAR index values below 0.1 (Figure 15).

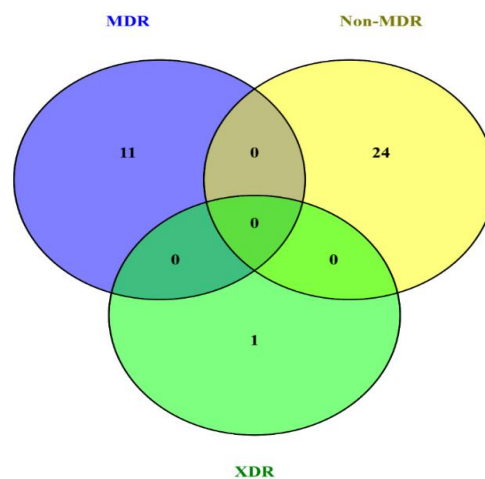


Figure 14: Antibiotic Resistance Categorization of *E. coli*

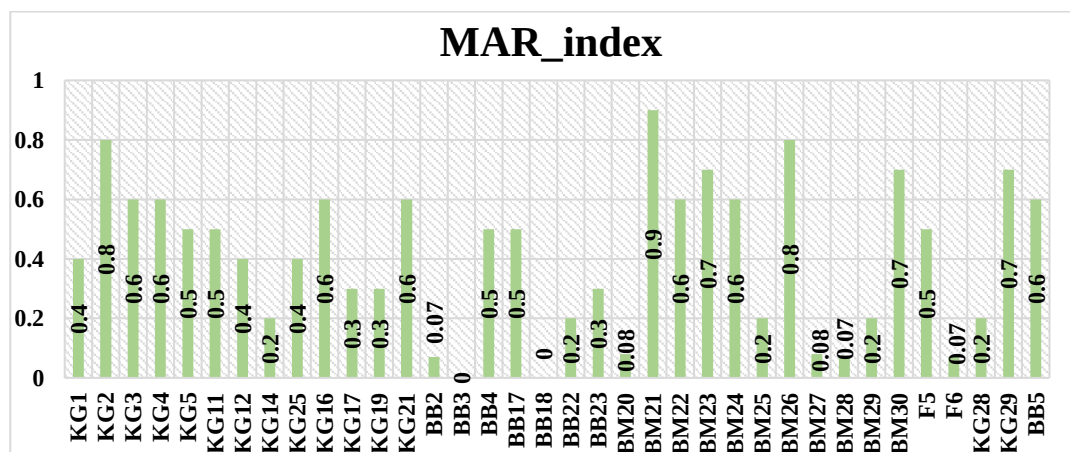


Figure 15: MAR Index of test isolates: *E. coli*

3.8 Assessment of Nucleic Acid Purity

Sample ID	Concentration (ng/ μ L)	A260/A280	A260/A230
BM29	84.14	2.24	1.18
BB3	216.03	2.22	1.40
BM26	120.22	2.18	1.26
KG1	145.1	2.21	1.31
BB18	178.45	2.27	1.45
KG4	70.4	2.27	1.32
KG17	121.1	2.25	1.34
BM20	102.7	2.22	1.33
BB5	119.8	2.20	1.32
KG28	84.89	2.14	1.21
BB17	147.31	2.18	1.28
KG11	82.9	2.12	1.17
BM30	147.31	2.22	1.32
BB23	82.9	2.16	1.19
KG21	139.52	2.29	1.29
KG25	150.2	2.29	1.22
KG29	55.14	2.22	1.34
BM24	81.81	2.47	1.45
BB4	137.7	2.53	1.27
F5	71.98	2.79	1.54
BB22	30.58	2.19	1.29
KG12	258.02	2.22	1.39
BM22	94.24	2.26	1.34
BB5.1	14.08	2.44	1.14
KG16	54.71	2.282	1.26
KG14	72.17	2.29	1.31
KG5	59.16	2.11	1.02
KG3	72.06	2.13	1.05
BM21	95.38	2.19	1.20
KG2	167.89	2.19	1.34
BM27	12.42	2.58	1.05
BM25	52.08	2.23	1.27
BM23	105.01	2.20	1.29
F6	94.128	2.20	1.30
BB2	125.15	2.27	1.41

Table 7: DNA concentration and purity of tested samples

3.9 PCR results:

PCR amplification produced distinct bands at approximately 585 bp in the positive control and the majority of test samples, confirming the target gene. Positive amplification in lanes 3–13, 15–17, 19–23, 25–35, and 37–38, confirming positive gene (Figure 16). The remaining lanes showed weak or absent amplification, indicating either the absence of the target sequence or suboptimal PCR efficiency. Overall, the findings demonstrate that most isolates tested were positive for the expected 585 bp amplicon.

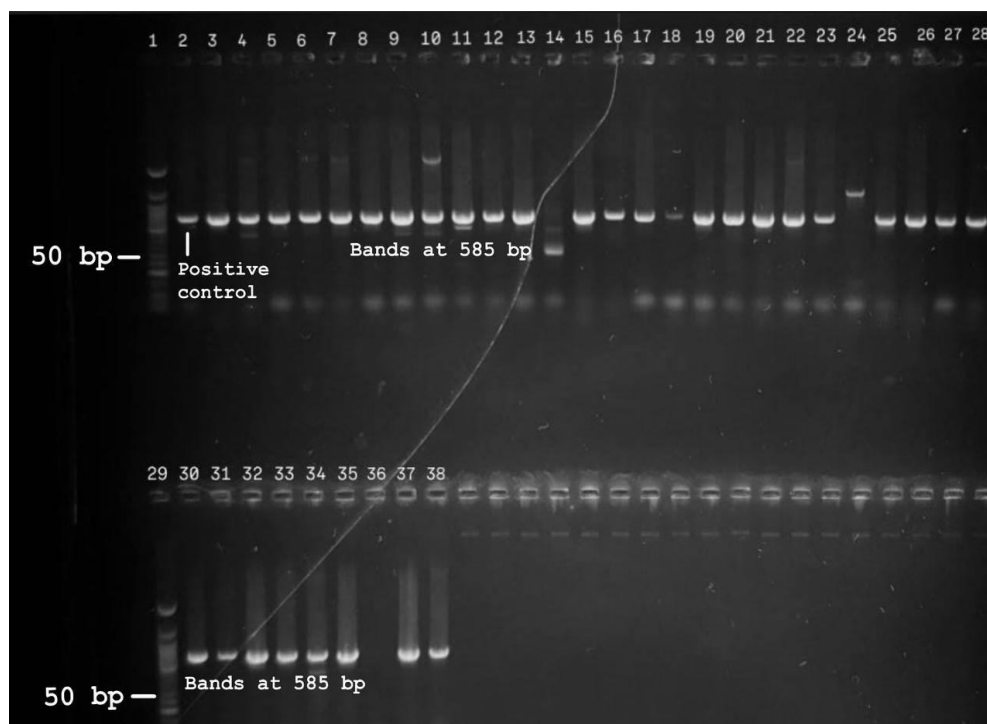


Figure 16: PCR-amplified *E. coli*-targeted gene showing bands at 585 bp

3.10 Antibiotic Susceptibility Analysis of *E. coli*

Antibiotic	Disc Content (µg)	Disc Potency (µg)	ZOI (mm) for <i>E. coli</i>			Laboratory AST Results		Hospital AST Results	
			≥ S	I	≤ R	KG 14	KG17	KG 14	KG17
Azithromycin	AZM 15	15	≥13	12	≤ 11	R(0)	S(16)	I	R
Chloramphenicol	C 30	30	≥18	13-17	≤ 12	R(0)	S(20)		
Piperacillin-Tazobactam	TPZ 110	100/10	≥21	18 - 20	≤ 17	S(21)	I(20) R	S	I
Cefepime	FEP 30	30	≥25	19 - 24	≤ 18	R(15)	I(22) R	I	I
Levofloxacin	LEV 5	5	≥17	14-16	≤ 13	R(0)	R(0)	S	S
Imipenem	IMP 10	10	≥23	20-22	≤ 19	R(0)	R(15)		
Meropenem	MEM 10	10	≥23	20 to 22	≤ 19	S(23)	I(22) R	S	S
Ciprofloxacin	CIP 5	5	≥21	16 to 20	≤ 15	S(21)	R(0)	S	R
Gentamicin	GEN 10	10	≥15	13-14	≤ 12	S(20)	S(20)	S	S
Amikacin	AK 30	30	≥17	15 to 16	≤ 14	S(17)	S(18)	I	S
Ceftazidime	CAZ 30	30	≥21	18 to 20	≤ 17	R(17)	I(18) R	R	I
Clindamycin	CL 30	2	-	-	-	-	-		
Cotrimoxazole	COT 30	1.25/23.75	≥16	11-15	≤ 10	R(0)	R(0)	R	R

Table 8: AST Results comparison between Hospital & Laboratory for *E. coli*

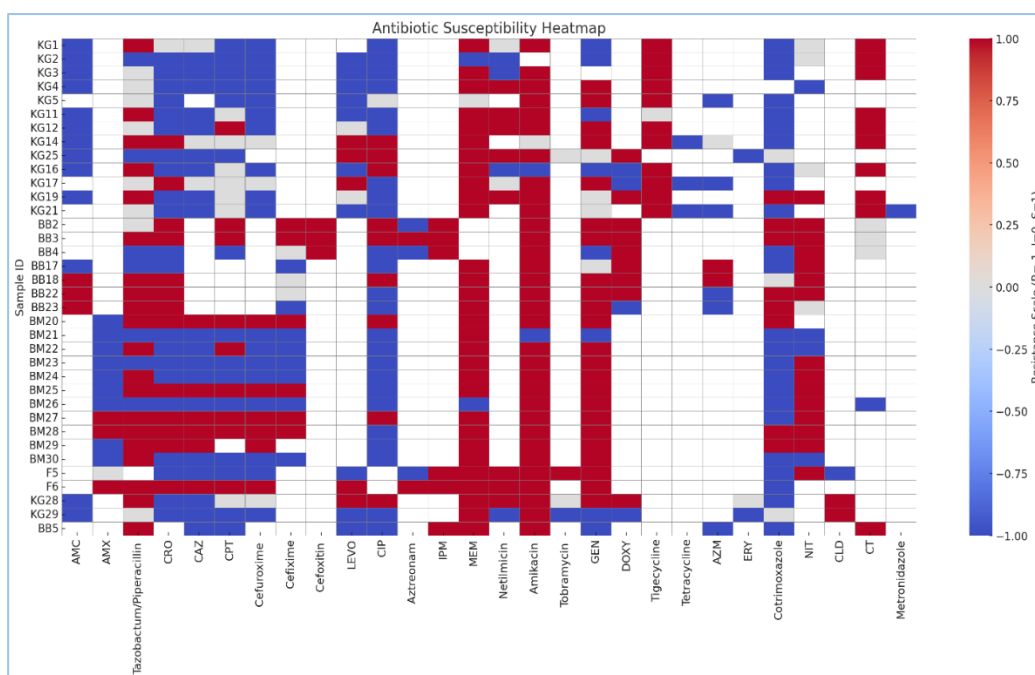


Figure 17: Heatmap Representation of Antibiotic Susceptibility Profile of *E. coli* where (Red - Sensitive, White - Intermediate, Blue -Resistance)



Figure 18: AST plates showing resistance & sensitivity

Total of 13 antibiotics are used to detect the sensitivity and resistant patterns of *E.coli* (Figure 18), where IMP, CI, GEN, AZM, LEV, FEP, TPZ are resistant while AK, MEM, C are sensitive.

Sample ID	BL			CEP							FQs	MON	CAR			AMG						TET		MAC			SUL	Nitrofuran	Others		NIM
	AMC	AMX	TZP	CRO	CAZ	CPT	CXM	CFM	FOX	LEVO	CIP	ATM	IPM	MEM	NET	AK	TOB	GEN	DOXY	TGC	TE	AZM	ERY	SXT	NIT	CLD	CL	MTZ			
KG1	R		S	I	I	R	R			R	R			S	I	S		R		S				R	I			S			
KG2	R		R	R	R	R	R			R	R			R	R			R		S				R	I			S			
KG3	R		I	R	R	R	R			R	R			S	R	S		R		S				R				S			
KG4	R		I	R	R	R	R			R	R			S	S	S		S		S					R						
KG5			I	R						R	I			I		S		S		S		R		R							
KG11	R		S	R	R	I	R			R	R			S	S	S		R		I				R				S			
KG12	R		I	R	R	S	R			I	R			S	S	S		S		S				R				S			
KG14	R		S	S	I	I	I			S	S			S		I		S		S	R	I		R				S			
KG25	R		R	R	R	R				S	S			S	S	S	I	I	S				R	I							
KG16	R		S	R	R	I	R			R	S			S	R	R		R	R	S				R		I		S			
KG17			I	S	I	I	I			S	R			S	I	S		S	R	S	R	R	R	R							
KG19	R		S	R	R	I	R			I	R			S	S	S		I	S	S				S		S		S			
KG21			I	R	R	I	R			R	R			S		S		I	S	S	R	R	R	R				S			
BB2			I	S		S		S	S	S	R	R	S		S		S	S	S					R		S		I			
BB3			S	S		S		S	S	S	S	S	S		S		S	S	S					R		S		I			
BB4			R	R		R		I	S		R	R	S		S		R	S						R		S		I			
BB17	R		R	R				R		R				S		S		I	S			S		R		S					
BB18	S		S	S				I		R				S		S		S	S				S		I		S				
BB22	S		S	S				I		R				S		S		S	S			R		R		S					
BB23	S		S	S				R		R				S		S		S	R			R		S		I					
BM20		R	S	S	S	S	S	S		S				S		S		S						S							
BM21		R	R	R	R	R	R	R		R				S		R		R						R		R					
BM22		R	S	R	R	S	R	R		R				S		S		S						R		R					
BM23		R	R	R	R	R	R	R		R				S		S		S						R		S					
BM24		R	S	R	R	R	R	R		R				S		S		S						R		S					
BM25		R	S	S	S	S	S	S		R				S		S		S						R		S					
BM26		R	R	R	R	R	R	R		R				R		S		S						R		S		R			
BM27		S	S	S	S	S	S	S		S				S		S		S						R		S					
BM28		S	S	S	S	S	S	S		R				S		S		S						R		S					
BM29		R	S	S	S		S			R				S		S		S						S		S					
BM30		R	S	R	R	R	R	R		R				S		S		S						R		R					
F5		I		R	R	R	R			R		R	S	S	S	S	S	S						R		S	R				
F6		S	S	S	S	S	S			S		S	S	S	S	S		S						R							
KG28	R		S	R	R	I	I			S	S			S	S	S	I	S	S				I	R			S				
KG29	R		I	R	R	R	R			R	R			S	R	S	R	R	R				R	I		S					
BB5			S		R	R				R	R			S	S		S		R			R		R				S			

Figure 19: Antibiotic Susceptibility Profile of Clinical Isolates of *E. coli* where (Red -Resistant, Yellow - Intermediate, Green - Sensitive)

Chapter 4

4.1 Limitations

Limited Sampling Scope: Samples were taken from only a few hospitals, so the results may not represent the whole of Bangladesh.

Molecular Detection Limitations: PCR was used for selected resistance genes only; other genes and detailed genomic information were not studied.

Limited resources:

1. Time shortage restricted the sample size and duration of the study, which may have limited the ability to observe seasonal or long-term resistance trends.
2. Due to limited laboratory resources, advanced methods such as Biofilm formation, Biochemical test, whole-genome sequencing could not be performed.

4.2 Conclusion

The research analyzed current AMR trends of *E. coli* isolated from patients in Khulna, Bangladesh. While multicenter Bangladeshi data are increasingly available, sub-national patterns vary by facility, patient population and frequency in antibiotic use practices. The local evidence gap is important because empirical therapy and stewardship protocols should be guided by recent, site-specific antibiograms rather than national averages.

This investigation of patient-derived *E. coli* demonstrates a concerning burden as it is mostly resistant to metronidazole, azithromycin, cephalexin and amoxiclav. Though several antibiotics are resistant, many antibiotics demonstrated significant level of sensitivity which includes clarithromycin, tigecycline, vancomycin and meropenem. Therefore, the above mentioned antibiotics may be recommended for treatment of resistant cases of *E. coli*

infection among the patients of Khulna. PCR-based confirmation of *E. coli* strengthened species identification, but ongoing surveillance is essential to detect shifts in resistance and to prevent erosion of last-line therapies. Future multi-center studies with larger sample sizes and molecular characterization of resistance determinants will help refine treatment guidelines and inform targeted interventions. The research and findings will provide insights that may guide clinicians in selecting appropriate antibiotics to reduce the overuse of drugs, to detect the resistance trends and help to understand which antibiotics have the potency against pathogen.

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