Identification and Antibiotic Susceptibility Profiling of *Escherichia Coli* Isolated from Chicken Cloacal Sample in Dhaka Wet Market, Bangladesh

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Ethics Statement

For completion of this study, samples from selected venues were collected following all the necessary precautions. All the experiments were done in BRAC University Laboratory. Our research complies with all applicable national animal welfare regulations and guidelines to ensure the ethical treatment of animals. We also take the permission from the sellers of wet markets under the supervision of microbiology department lab protocols. No animals were harmed in the research we conducted.

Abstract/ Executive Summary

Background:

The rise of antibiotic-resistant *Escherichia coli* (*E. coli*) in poultry is a major public health concern, as these bacteria can transfer to humans, posing risks of difficult-to-treat infections. Understanding *E. coli* prevalence and antibiotic resistance in poultry, especially in high-density commercial farming systems, is essential for developing strategies to manage this issue.

Method:

A total of 60 cloacal swab samples were collected from live chickens across twelve locations in Dhaka. Samples were spread on Macconkey agar and presumptive *E. coli* colony was taken based on Colony morphology which was round in shape and light pink in color. These colonies were picked and further sub-cultured on UTI media for screening purposes. On HI chrome UTI media purple color colonies were suspected as *E.coli*. From there four isolates were collected and streaked on Nutrient agar media for DNA extraction and other molecular identification processes. After that performing Polymerase Chain Reaction (PCR) and gel electrophoresis presumptive *E.coli* was identified from the chicken cloacal sample. Furthermore, antibiotic susceptibility testing was also done for these positive isolates by using Kirby-Bauer disk disc diffusion method followed by CLSI guideline

Results:

Our study found a high prevalence of *E. coli* in commercial broiler chicken and backyard poultry chicken (Shonali and Desi) with an overall prevalence rate of 78.33% across sampled locations. Broiler chicken showed higher *E.coli* contamination which is 66%. Comparatively, native breeds (Shonali and Desi chickens) showed lower *E. coli* prevalence, 21% and 15% respectively. A high resistance to tetracycline (89%), ciprofloxacin (59%), Amoxicillin (44%) was observed in antibiotic susceptibility tests and lower resistance was noted for meropenem and imipenem.

Conclusion:

The findings demonstrate that high-density commercial poultry farming is associated with increased *E. coli* prevalence and higher levels of antibiotic resistance, particularly for commonly used antibiotics like tetracycline. In contrast, indigenous breeds raised in less intensive conditions exhibit lower rates of *E. coli* contamination and resistance. This study highlights the need for stricter antibiotic regulations and improved farming practices to curb the spread of antibiotic-resistant *E. coli* in poultry.

Keywords: *Escherichia coli*, antimicrobial resistance, poultry farming, cloacal samples, antibiotic susceptibility, Dhaka, Bangladesh, intensive farming practices, indigenous breeds.

Dedication

“To our beloved family”

Acknowledgement

The people and organizations listed below deserve our sincere gratitude and appreciation for their important support and advice in helping me finish this thesis. We must first express my gratitude to Associate Professor Fahim Kabir Monjurul Haque, Ph.D., our supervisor, for his unflinching support, priceless insights, and constant encouragement. Additionally, his knowledge and direction were crucial in determining the focus and caliber of this research. Md Feroze H. Haque, Ph.D., Associate Professor and Chairperson of the Department of Mathematics and Natural Sciences at BRAC University, and Program Director and Associate Professor Nadia Sultana Deen, PhD are also praiseworthy of our sincere gratitude for their kind cooperation throughout this study as well as for their insightful comments, constructive criticism, and helpful suggestions that significantly improved the quality of this work overall. We are appreciative of Brac University's staff and faculty for creating a supportive learning environment and giving us access to the tools we needed to finish this project successfully. The Department of Mathematics and Natural Sciences' assistance has been helpful. We owe a great deal of gratitude to the study participants who voluntarily donated their time and wisdom. Their openness to sharing their insights and thoughts has greatly improved our research. We want to sincerely thank Golam Niaz Murshidi, our mentor and Research Assistant, for helping us with every aspect of my thesis. We remained focused and resolute because of their confidence in us and ongoing encouragement. Finally, we would want to thank all the researchers, academics, and writers whose work we have cited in this thesis. Their contributions have melded our perspective and given this study a strong foundation. Lastly, we are grateful for all the support, inspiration, and direction we have received and feel privileged to have been given the chance to work on this research project

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List of Acronyms

E. coli	*Escherichia coli*
MDR	Multi drug resistance
PCR	Polymerase chain reaction
XDR	Extensively drug-resistant
MIU	Motility Urease test
MHA	Mueller–Hinton agar
Bp	Base pair
DNA	Deoxyribonucleic acid
EPEC	Enteropathogenic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
Min	Minute
Sec	Second
TAE	Tris-acetate-EDTA

Glossary

MDR:	Multidrug resistant (MDR) is acquired resistance to at least one antimicrobial agent from three or more antimicrobial groups.
XDR:	Extensive drug-resistant, or XDR, is characterized as resistance to at least one antimicrobial agent in all but two or fewer antimicrobial categories.
PCR:	Polymerase chain reaction is a laboratory technique for amplifying millions to billions of copies of a given section of DNA in a short period.
Isolation:	Bacterial isolation is the process of isolating one species of bacteria from a mixed culture of bacteria using various plating methods such as pouring, spreading, streaking, and serial dilution.
Pathogenicity:	Pathogenicity is the characteristic or state of being pathogenic, or the propensity to cause illness, whereas virulence is the capacity of an organism to cause disease, or its degree of pathogenicity within a group or species

Chapter 1

Introduction

1.1 Background on *E.coli* and Its Importance in Food Safety

Escherichia coli (*E. coli*) is a Gram-negative bacterium that naturally inhabits the intestines of humans and animals, particularly warm-blooded species (World Health Organization 2023). Its presence in any environment outside of the gastrointestinal tract, such as food or water, typically indicates fecal contamination, making it a critical indicator organism in public health. Among food sources, poultry is especially significant due to its high consumption rates worldwide and the widespread use of antibiotics in its production. In countries like Bangladesh, where the poultry industry is a major source of affordable protein, controlling bacterial contamination is essential for protecting public health and comprehensive surveillance of *E. coli* in poultry not only protects animal welfare but also safeguards community health outcomes.

In countries like Bangladesh, where the poultry industry is a major source of affordable protein, controlling bacterial contamination is essential for protecting public health and comprehensive surveillance of *E. coli* in poultry not only protects animal welfare but also safeguards community health outcomes.

1.2 *E. coli* as an Indicator of Fecal Contamination

E. coli is part of the intestinal flora of healthy broilers; however, some strains of this bacterium, designated as avian pathogenic *E. coli* (APEC), are common causes of serious diseases such as systemic fatal colibacillosis and are considered a major cause of economic losses in many poultry farms worldwide (Pourhossein et al., 2020)

E.coli is a member of the fecal coliform group and a more specific indicator of fecal contamination than other fecal coliform species, its presence indicate possible presence of harmful bacteria which will cause diseases and it also suggests the extent as well as the nature of the contaminants (Preprints.org, n.d.) *E. coli* is not only a common gut bacterium but also a crucial indicator for assessing fecal contamination in food, water, and the environment. *E.coli* .bacteria is present in the intestine of man and animals which is released into the environment as a fecal material (Preprints.org, n.d.)Moreover, fecal bacteria affect rivers, sea beaches, lakes, surface water, recreational water which is used as an indicator of contamination. India has caused 10,738 deaths over the last 5 years since 2017.Uttar Pradesh has recorded the highest deaths due to diarrhea followed by Assam, West Bengal, Delhi and Madhya Pradesh (National Health Profile 2018). Its presence in poultry products suggests direct or indirect fecal contamination highlighting potential pathways for zoonotic transmission to humans often during farming, processing, or handling. While most strains of *E. coli* are harmless, certain types can cause severe illness in humans. But a few strains, such as *E. coli* O157:H7, can cause severe stomach cramps, bloody diarrhea and vomiting (Mayo Foundation for Medical Education and Research, 2022). The genetic diversity among *E. coli* strains, some of which possess virulent properties, complicates the risk assessment associated with poultry consumption and handling. It can enter the food supply chain, posing a risk of spreading *E.coli* to consumers leading to foodborne illnesses and potential outbreaks. Notably, pathogenic strains can lead to severe gastrointestinal infections in humans, including diarrhea, urinary tract infections, and even systemic infections. It can also affect other animals too by entering our food chain. The poultry industry can inadvertently become a vector for such pathogenic bacteria, facilitating the transmission of resistant *E. coli* strains to humans through direct handling, cross-contamination during food preparation, and consumption of undercooked poultry.

1.3 Antimicrobial Resistance in *E. coli* and Its Public Health Implications

Antimicrobial resistance (AMR) in bacteria like *E. coli* is a growing global health challenge. *E. coli* acquires antimicrobial resistance to isolates more easily than other common bacteria (Chuppava et al., 2018). Commensal and pathogenic *E. coli* strains therefore contain various antimicrobials resistance genes and entail a high risk of transmission of drug resistance to human microflora and pathogenic bacteria (Chuppava et al., 2018; von Wintersdorff et al., 2016; Badger et al., 2018). The development of resistance often stems from the misuse or overuse of antibiotics in animal husbandry. In many low- and middle-income countries, including Bangladesh, antibiotics are commonly used in poultry farming not only for treating infections but also for disease prevention and growth promotion. This practice creates selective pressure that encourages the proliferation of resistant strains. Resistant *E. coli* can then spread from animals to humans through the food supply chain, posing treatment challenges for infections caused by these resistant strains.

The World Health Organization (WHO) has identified AMR as a top public health threat, with projections suggesting that resistant infections could cause up to 10 million deaths annually by 2050. The persistence of resistant *E. coli* strains in poultry products is particularly concerning because it could lead to the transfer of resistance genes to other pathogenic bacteria in humans. This scenario complicates treatment protocols, as infections that were once easily manageable may become difficult or impossible to treat with commonly used antibiotics.

1.4 *E.coli* in Poultry Farming: Practices and Contamination Sources

E.coli is a part of the intestinal flora of healthy broilers; however, some strains of this bacterium, designated as avian pathogenic *E.coli* (APEC), are common causes of serious

diseases such as systemic fatal colibacillosis and are considered a major cause of economic losses in many poultry farms worldwide. In Bangladesh, poultry farming practices vary widely, with some farms operating at industrial scales and others functioning as small, backyard operations. Both types of farms face challenges related to hygiene and disease control. Fecal contamination can occur at multiple stages of poultry farming and processing: through feed, water, soil, or inadequate hygiene during slaughter. Antibiotics are frequently administered in feed and water to prevent diseases, but this can lead to antibiotic residues in chicken products and increased levels of resistant bacteria in the chicken gut, which may ultimately enter the food chain.

For our research purpose, we took 60 cloacal samples from three different types of chicken, they are-poultry chickens (broiler, sonali chicken and desi chicken). Cloacal samples from chickens serve as an excellent source for isolating *E. coli* for resistance testing because the cloacal is a common exit point for fecal matter. Assessing the resistance profiles of *E. coli* from these samples offers insight into the prevalence of AMR in local poultry farms and markets, providing critical data for guiding policy and practices to reduce AMR risks. Our research identified a notably higher prevalence of *E. coli* in broiler chickens compared to sonali and desi chickens. Broiler chickens, typically raised in high-density environments, are more susceptible to bacterial transmission due to overcrowding and frequent antibiotic use, which may contribute to the selection of antibiotic-resistant *E. coli* strains (Aarestrup et al., 2008 and Allen et al., 2013);Similar trends are observed in studies from India and Pakistan, where intensive farming and routine antibiotic use in commercial poultry operations have been linked to increased *E. coli* contamination and resistance (Chatterjee et al., 2018) and (Jamil et al., 2019);Conversely, sonali and desi chickens, raised in lower-density or free-range conditions, exhibited a reduced prevalence of *E. coli*. This aligns with findings from Kilonzo-Nthenge et al. (2008); and Rothrock et al. (2019); who reported lower pathogen loads in less confined

poultry environments. Additionally, indigenous breeds like the desi chicken in India and the aseel in Pakistan, often raised in backyard systems, show greater resilience to pathogens due to genetic adaptability and natural habitats (Kumar et al., 2021; and (Khan et al., 2017);

1.5 Public Health Concerns Related to Antimicrobial resistance (AMR) in Poultry in Bangladesh

AMR is a critical public health concern in Bangladesh. Research indicates that Bangladesh's densely populated areas, such as Dhaka, have increased demand for poultry due to its affordability and role as a primary protein source. This has led to intensive farming practices reliant on antibiotics to boost production and prevent diseases (Azad, M. A. K., et al. (2021). Studies have highlighted those antibiotics are often accessible without prescriptions in Bangladesh, with low awareness among farmers and consumers about the risks of antimicrobial resistance. This unregulated use significantly contributes to AMR (Hasan, B., et al. (2022). Bangladesh has laws, such as the *Bangladesh Fish Feed and Animal Feed Act 2010*, but their enforcement is weak, leading to inconsistent farm biosecurity practices. This results in the persistence and spread of resistant bacteria (Nhung, N. T., et al. (2017). Experts have emphasized the necessity of effective monitoring systems and public health interventions to mitigate AMR in poultry and ensure food safety. These measures include educating stakeholders and implementing stricter regulations (Azad, M. A. K., et al. (2021).

1.6 Significance of the Study

This research addresses the critical need for updated data on AMR in food sources in Bangladesh, particularly in poultry, which is consumed widely across all demographics. Understanding the extent of *E. coli* contamination and resistance profiles provides essential

information for public health authorities, policymakers, and the agricultural sector. The results of this study can support the development of guidelines for safer poultry production and handling practices. Moreover, it underscores the need for responsible antibiotic use in agriculture to mitigate the spread of AMR. Furthermore, by identifying the prevalence and resistance profiles of *E. coli* isolates from 12 different regions within Dhaka, this study can offer localized insights, guiding efforts to address AMR hotspots and improve food safety practices in specific areas. Such data also contribute to global efforts to monitor AMR trends and mitigate the threat posed by resistant bacteria in the food supply chain.

Escherichia coli (*E. coli*) is a feces-borne coliform that is particularly effective in indicating fecal contamination. Unlike other coliform bacteria, *E. coli* is closely associated with the intestines of warm-blooded animals, making it a highly specific indicator of recent fecal pollution. Thus, the detection of *E. coli* in any environment implies potential contamination with fecal material, highlighting a risk for pathogenic bacteria transmission. This is especially relevant in food production and animal husbandry, where contamination can easily lead to foodborne illness and further health risks for consumers. This study focuses on isolating fecal *Escherichia coli* from cloacal samples of chickens, a common and economical protein source in Dhaka, Bangladesh. By targeting cloacal samples, which contain fecal matter directly from poultry, this research aims to determine the prevalence of *E. coli* contamination in poultry farms and markets. Additionally, assessing the antibiotic resistance profiles of the *E. coli* isolates provides essential data on antimicrobial susceptibility. This analysis is crucial in understanding the extent of antimicrobial resistance (AMR) in local poultry, as chickens are often treated with antibiotics, which can create selective pressure for resistant bacteria. The study aims to provide a detailed analysis of fecal contamination levels and antibiotic resistance patterns in poultry within the Dhaka region, offering insights into food safety practices and potential public health risks associated with AMR. This investigation is timely and essential

for informing better management practices, policies, and awareness surrounding AMR and hygiene practices in poultry production.

1.7 Objectives of the Study

1. **Detection of *E. coli* in Cloacal Samples**: To identify the presence and prevalence of *E. coli* in cloacal samples collected from chickens across various markets in Dhaka.
2. **Antimicrobial Susceptibility Profiling**: To analyze the resistance patterns of isolated *E. coli* strains against commonly used antibiotics in the poultry industry in Bangladesh.

Chapter 2

Method and Materials

2.1 Collection of Samples:

Figure 1: specimen collection and processing

2.1.1 Area of Sample Collection:

For this research project chicken cloacal Samples were collected from the poultry sample from the three types of chicken from 12 different wet markets of Dhaka city.

The 12 areas are given in (Table 1):

1. Banasree	2. Rampura	3. Mohakhali	4. Gulshan 1
5. Gulshan 2	6. Badda	7. Banani	8. Rajarbag
9. Tejgaon	10. Mohammadpur	11. Farmgate	12. Khilgaon

Table 1: Area of sample

2.1.2 Names and Numbers of Selected Chicken Sample:

For this study our target was collecting samples from three types of chicken. Three types of chickens are Broiler chicken, Sonali and Desi chicken. We have collected 60 chicken samples based on these three types.

2.1.3 Preparation:

The test tubes and falcon were washed using disinfectant. After washing the test Tubes, both cotton swab and test tubes and falcon were sterilized using autoclave. Saline solution was also sterilized by autoclaving.

2.1.4 Sample Collection:

Samples were collected with the help of sterilized cotton swab from the wet market. The cloacal swab from the chicken was taken on a cotton swab. After taking the sample it was dipped in a sterile falcon containing 5ml sterile saline water. Later, the samples are taken to BRAC University Research Laboratory in isolated boxes or airtight bags by following all the necessary protocols on the same day they were collected. After collecting the sample was serially diluted and plated on selective media

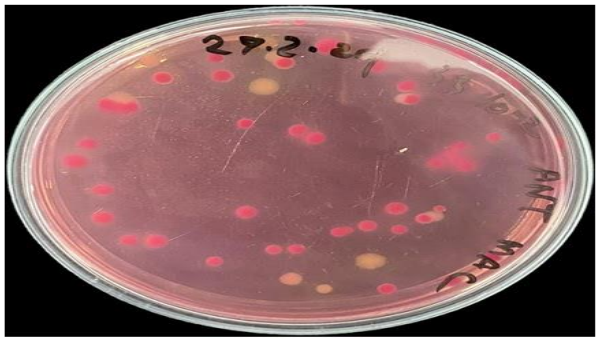
2.2 Isolation and Identification:

Spreading on MacConkey agar plate:

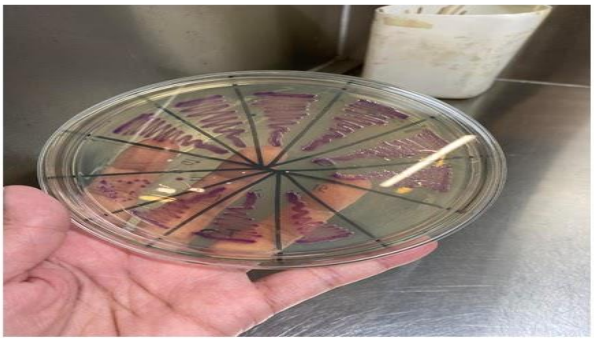
After completing the dilution, immediately spreading was performed on the Macconkey agar plate. The enriched samples were then processed following four-fold serial dilution and spread about 80ml on *a* MacConkey agar as quickly as possible with the help of a sterile glass spreader. To prevent contamination each step was carried out inside a laminar airflow and later

kept in an incubator for 24 hours at 37 degrees Celsius for further outcomes. Vortex the test tubes must every time before spreading.

Later after overnight incubation, the presumptive *E. coli* colony was taken based on colony morphology which was light pink in (Figure 2). These colonies were picked and further sub-cultured on HiChrome^{TM} UTI media plates for screening purposes. *E. coli* gives purple color on HiChromeTM UTI media as colony morphology in (Figure 1). A total of 4 isolates were taken as presumptive *E.coli* and then sub-cultured on NA .Furthermore, isolation of E. coli on Nutrient agar (NA) has been also used for further processes. NA is considered as a popular choice as it promotes the growth of various bacterial strains. It supplies necessary nutrients that are suitable for the subculture of a wide range of microorganisms. As the study needed pure culture of *E. coli* bacterial strains for various tests that is why, at least four putative single positive colonies with *E. coli* characteristic color (purple) were isolated on NA media and incubated, maintaining the same temperature for 24 hours.



a



b

Figure 2: *E. coli* colonies on MacConkey and HiChrome^{TM} UTI. a Pink Colored *E. coli* colony on MacConkey. b Purple Colored *E. coli* colonies on HiChrome^{TM} UTI.

2.3 Molecular Identification of *E.coli*

2.3.1 DNA Extraction:

The confirmed isolates of *E.coli* were inoculated into Nutrient Agar and incubated for 24 hours at 37°C. After 24 hours DNA was extracted by using the "Boiling method". This method was selected because of the efficiency, time and cost effectiveness. For that, 400 µl of TE buffer was taken in micro centrifuge tubes and a loopful of culture which was incubated for 24 hours at 37°C was suspended in the MCT (micro centrifuge tubes) tubes and vortexed for 15 seconds. After that the mixture was centrifuged at 13000 RPM for 10 minutes at 25°C. Then supernatant was discarded, and the pellet was again mixed with 400 µl of TE buffer and was heated at 95°C for 10 minutes using a dry heat block. After that the heated MCT tubes were centrifuged at 13,000 RPM for 10 minutes at 25°C. Then the supernatants were transferred into fresh MCT tubes and pellets were discarded. The collected supernatant was stored.

2.3.2 PCR Amplification:

The presumptive bacterial isolates were screened for confirmation by using primers dedicated for *E. coli* (ECO-1 & ECO-2 primers). PCR amplification was done with the following set of primers from **(Table 2)**:

Primers Name	Primer Sequence	Target Gene	Amplicon Size (bp)	Reference
ECO-1	5'-GACCTCGGTTTAGTTCACAGA-3'	16S rRNA gene	585 bp	(3)
ECO-2	5'-CACACGCTGACGCTGACCA-3'			

Table 2: Primers used for amplification of resistance genes by polymerase chain reaction (PCR).

2.3.3 PCR Condition:

The appropriate condition which was used for the primers described in [Table 3]

Steps	Temperature	Duration (Minutes)	Cycles
Initial Denaturation	95°C	7 minutes	1 Cycles
Denaturation	94°C	1 minute	35 Cycles
Annealing	55°C	1 minute	
Elongation	72°C	1 minute	
Final Elongation	72°C	7 minutes	1 Cycles

Table 3: Appropriate conditions for the ECO-1 & ECO-2 primers

2.3.4 PCR Mixture Preparation:

It is a laboratory technique for rapidly producing millions to billions of copies of a segment of segment DNA. This procedure quickly and easily makes numerous copies which helps in tests like molecular biology, forensic analysis, and medical diagnostics too (Britannica, T. Editors of Encyclopedia 2023). Within a few hours, rounds of replication using a PCR machine ((The Applied Bio systems 2720 Thermal Cycler). The extracted DNA was used as a template for PCR amplification during PCR mixture preparation. The total value of PCR mixture was 13 µl, where 2 µl was template and the rest 11 µl was master mixture, forward & a reverse primer and nuclease-free water. The calculation for a sample given below [Table 4]:

Reagent	Volume (µl)
Master Mixture	6
Forward Primer	1
Reverse Primer	1
Nuclease-Free Water	3
DNA Template	2
Total	13

Table 4: PCR preparation calculation for a sample.

2.3.5 Agarose Gel Electrophoresis:

It is considered that the agarose gel electrophoresis is one of the most effective methods for isolating DNA fragments ranging in size 100bp to 25 kp. The biomolecules are separated by size in the agarose gel matrix using an electric field to push charged molecules across the

material. The physical technique of agarose gel electrophoresis is one of many available for figuring out DNA size. In this technique, an electric current causes DNA to move through a strongly cross-linked agarose substrate. Since the DNA phosphates are negatively charged in solution, the molecule will move to the positive (red) pole. Three variables influence the speed at which DNA migrates through a gel which gives ideas on the size of the DNA as well as confirms the presence of it as particular DNA ladders were used. After the process of PCR was complete, the PCR products were analyzed by gel electrophoresis in 1.5% (w/v) agarose gel and the gel was stained with EtBr and was run at 110V for 55 minutes. The gel was made using 10X Tris-acetate EDTA (TAE) and TAE was used as the running buffer. After the run was completed UV trans illuminator to visualize the band size and 100bp ladder in (Figure 3).

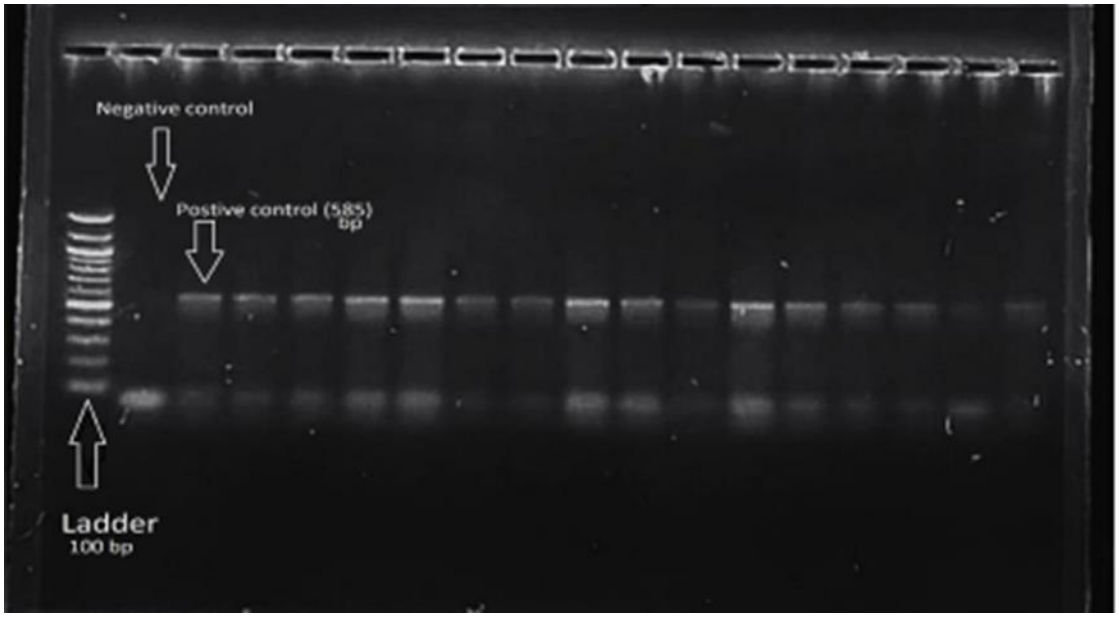


Figure 3: *E. coli* confirmatory PCR result. [Here the ladder was a 100 bp DNA marker. Lane 1 is a 100bp ladder. Lane 2 was negative control, lane 3 was positive control. From lane 4-16 different samples were loaded). Amplicon size 585 bp.]

2.4 Antibiotic Susceptibility Test (AST):

The disc diffusion method was followed to conduct the test and the range of susceptibility and resistance was evaluated by CLSI guideline. By using the guideline, if an isolate was resistant or susceptible was found out. MHA agar was used to perform the Antibiotic Susceptibility Testing (AST). To conduct the test, a cotton swab was used to collect isolated colonies of *E. coli* that were mixed in 5ml saline and vortexed to mix it properly which was then matched to the McFarland 0.5 standard. The saline solution was then evenly spread through the MHA agar plate using sterile cotton swabs. After that antibiotic disk were put into the plate and the plates were incubated at 37°C for 24 hours and the result was observed.

This specific type is particularly designed for antibiotic susceptibility tests used in disk diffusion methods i.e., Kirby-Bauer disk diffusion method. It has been recommended by the CLSI as the ideal medium for AST mostly because of its medium's nonselective and non-differential nature (Vasylevskyi S. et al., 2018). Due to the presence of starch, toxins caused by the bacteria are absorbed because it prevents them from interfering with antibiotics. Moreover, it shows good reproducibility from batch to batch. As it is a loose agar therefore, its facilities better diffusion of antibiotics. Hence, MHA has been used for the AST so that proper results come out from the study

As the broth dilution method is time consuming, Kirby-Bauer disk diffusion method was used to determine the antimicrobial specialized susceptibility profiles of the *E. coli* isolates for the cloacal sample. This test is mostly used to evaluate how susceptible or resistant aerobes or facultative anaerobes are to various antibiotic classes (Aryal S. et al., 2022). By determining how well antibiotics inhibit organism growth, this approach helps health officials in selecting various treatment alternatives.

Range of susceptibility and resistance and intermediate are in (Table 5):

Group Name	Antibiotic	Disc Code	Disc Potency(mcg)	Susceptible (mm) or more	Intermediate(mm)	Resistance (mm) or less
Aminoglycoside	Amikacin	AK 30	30	>=17	15-16	<=14
Carbapenem	Imipenem	IPM 10	10	>=23	20-22	<=19
	Meropenem	MRP 10	10	>=23	20-22	<=19
Penicillin & Beta-lactamase	Piperacillin Tazobactam	PIT 100/10	10/100	>=21	18-20	17
Polymyxin	Collistin	CL 10	10	18	17-11	<=11
Fluoroquinolone	Ciprofloxacin	CIP 5	5	>=20	16-20	<=15
Tetracycline	Tetracycline	PE 30	30	>=16	12-15	<=11
Macrolide	Azithromycin	AZM 15	15	>=13	-	<=12
Cephalosporins	Ceftriaxone	CRO 30	30	>=18	15-17	<=14
Beta-lactamase inhibitors	Amoxyclav	AMC 30	30	>=18	14-17	>=13
Sulfamethoxazole	Amoxicillin	AML 30	30	>=16	11-15	<=10

Doxycycline	Cefixime	CFM 5	5	>=19	16-18	<=15

Table 5: Range of susceptibility and resistance and intermediate

Chapter 3

Result

3.1: Positive *E. coli* Results Based on Area:

A total of 12 areas around Dhaka city wet market have been selected from where a sum of 60 chicken cloacal samples were being tested in the lab. Out of these, 47 were the total sample count. A table (table 6) has been given below to give an accurate idea on how the live chicken cloacal sample were collected from these areas are contaminated with bacterial *E. coli.*

Area Name	**Number of collected chicken sample**	**Number of Positive chicken sample**	**Percentage of *E. coli* positive**
Banasree	5	4	80
Rampura	5	3	60
Mohakhali	5	4	80
Gulshan 1	5	5	100
Gulshan 2	5	5	100
Badda	5	2	40
Banani	5	5	100
Rajarbag	5	3	60
Tejgaon	5	4	80
Mohammadpur	5	4	80
Farmgate	5	3	60
Khilgaon	5	5	100
Total	60	47	78.33

Table 6: Number and percentage of Positive *E. coli* from chicken samples based on area.

3.2: Positive *E. coli* Sample Based on Chicken Type:

As mentioned above, out of a total 60 samples, 47 samples were detected with *E.coli* positive bacteria. We took 5 samples from each bazaar. Our initial goal was to take samples from only broiler live chicken. But we also intended to take swabs from both sonali and desi chicken. According to our study we found mostly pathogenicity in broiler type, less than deshi type. Moreover, there are positive samples of sonali as well as deshi type. A table (table 7) is given below:

Positive *E. coli* found in wet market						
Area	**Broiler**		**Sonali Type**		**Deshi**	
	Collected Sample Number	***E. coli* Positive**	**Collected Sample Number**	***E. coli* Positive**	**Collected Sample Number**	***E. coli* Positive**
Banasree	2	2	2	2	1	0
Rampura	2	2	1	1	1	0
Mohakhali	3	3	1	1	1	0
Gulshan 1	3	3	1	1	1	1
Gulshan 2	3	3	1	1	1	1
Badda	2	2	1	0	1	0
Rajarbagh	3	3	1	0	1	0
Tejgaon	3	3	1	0	1	1

Mohammadpur	3	3	1	1	1	0
Farmgate	2	1	2	1	1	1
Khilgaon	3	3	1	1	1	1
Banani	3	3	1	1	1	1
Total		31		10		6
percentage		66%		21%		15%

Table 7: Positive *E. coli* found in wet markets.

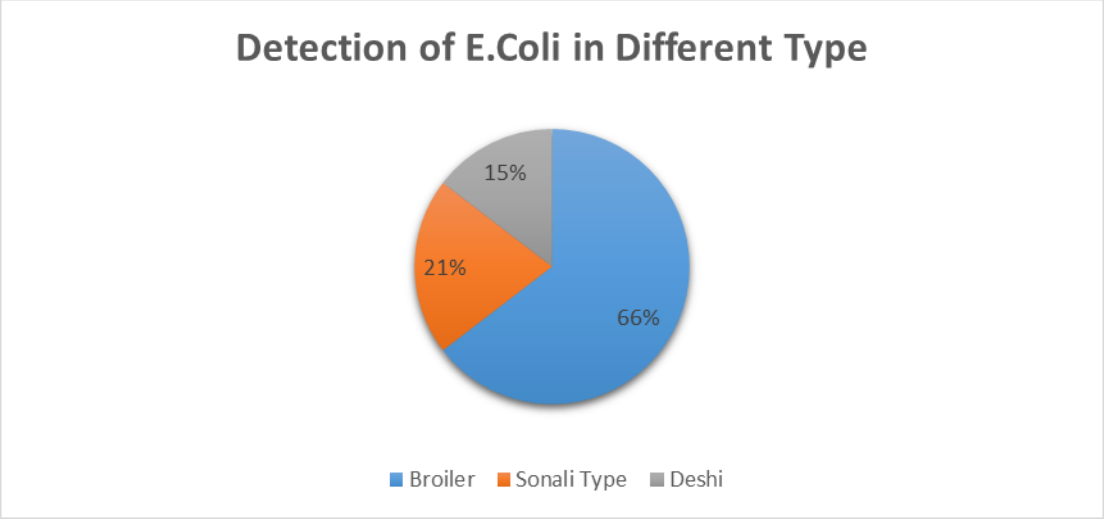


Figure 4: Pie chart of detected *E. coli* in different chicken types.

3.3: Antibiotic Susceptibility profiling:

Out of all the positive isolates, most of the strains were comparatively sensitive except Tetracycline, Ciprofloxacin, Azithromycin, Amoxicillin. A data table is given below to show how these antibiotics are working against them. In the table (table 8), it is seen that maximum bacterial strains have shown low intermediate where Amoxiclav is the highest i.e., 34% and Ceftriaxone having the lowest which is 6%. In contrast to it, Tetracycline has shown the highest

resistance towards the strains i.e., 89%, On the other hand, all the isolates have given minimum rate of intermediate which is between 5-34%. The patterns of antibiotic resistance demonstrated by other isolates were quite varied.

A data combining AST results from the positive isolates:

Antibiotics	**Susceptible**	**Susceptible%**	**Intermediate**	**Intermediate %**	**Resistance**	**Resistance %**
Piperacillin/ Tazobactam	81	86%	10	11%	3	3%
Ceftriaxone	82	87%	6	6%	6	6%
Azithromycin	49	52%	0	0%	45	48%
Amoxicillin	35	37%	18	19%	41	44%
Ciprofloxacin	22	23%	17	18%	55	59%
Amoxiclav	23	24%	32	34%	39	41%
Imipenem	84	89%	10	11%	0	0%
Meropenem	89	95%	5	5%	0	0%
Cefixime	54	57%	14	15%	26	28%
Amikacin	70	74%	16	17%	8	9%
Tetracycline	10	11%	0	0%	84	89%

Table 8: Percentage of Antibiotic Susceptibility profiles of 94 isolates.

3.4 Types of Resistance Bacteria:

To conclude this, there were 94 isolates where 75% of them were Multidrug Resistant (MDR) Piperacillin/Tazobactam, Ceftriaxone, Azithromycin, Amoxicillin, Ciprofloxacin, Amoxiclav, Cefixime, Amikacin, Tetracycline) and 58.33% of them were Extensively Drug Resistant (XDR) (Amoxicillin, Amoxiclav, Tetracycline, Amikacin, Cefixime, Azithromycin, Ciprofloxacin) Among them 77.77% MDR. (Figure 5)

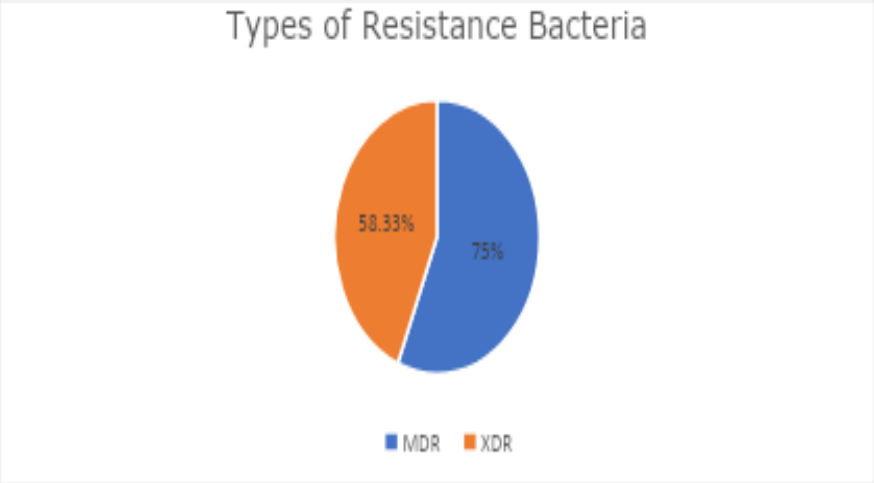


Figure 5: Multidrug resistant and extensively drug resistant percentage

Chapter 4

Discussion

The prevalence of *E. coli* in poultry farming is a major public health concern, particularly in lack of commercial poultry farming systems that results in bacterial contamination. Routinely used antibiotic in poultry for growth promotion and disease prevention creates antimicrobial resistance (AMR) in both animals and humans. Though most strains of *E. coli* are harmless and commonly found in the gut of humans and warm-blooded animals, some strains can cause severe foodborne illness in humans (Al Azad et el., 2019). In our research, among 60 cloacal swab samples of live chicken in Dhaka showed a positive detection rate of *E. coli* in 47 samples, resulting in an overall prevalence of 78.33%. The detection rate highlights significant contamination of chicken with *E. coli* in the areas, suggesting widespread exposure and potential health risks. The number of *E. coli* found in our research aligns with similar research in different regions. For instance, a study conducted in Nigeria reported *E. coli* prevalence in poultry as high as 80% (Oguttu et al., 2018), indicating similar contamination levels and public health implications. Similarly, a study in India detected *E. coli* in 72% of poultry samples, which correlates closely with our findings (Singh et al., 2020). These findings collectively suggest that high *E. col*i contamination in poultry is a common concern in many developing countries, potentially due to inadequate hygiene practices, lack of regulatory oversight, or intensive poultry farming.

Moreover, there is a noticeable fact we found in our experiment that a higher number of *E. coli* in poultry chickens where broiler chicken showed high contamination of *E.coli* which is 66% compared to sonali and deshi chickens. However, Jakaria et al., (2012) and Bashar et al., (2011) reported 82% and 100% prevalence of *E. coli* in broiler chickens, respectively from

cloacal swabs of chicken in Bangladesh. Whereas A study in Haryana, India, found a 100% prevalence rate of *E. coli* in broiler chickens from tested samples (Singh et al.,2020) which shows high contamination rate than our research. However, poultry chickens, typically raised in high-density environments, are more susceptible to bacterial transmission due to crowding and often routinely use of antibiotic, which can select for resistant *E. coli* strains (Aarestrup et al., 2008; Allen et al., 2013). Similar studies, such as those by Rasschaert et al., (2007) and Jones et al., (2019), reported higher *E. coli* presence in intensively raised poultry compared to free-range systems, highlighting the link between farming practices and bacterial contamination. Another cause of high contamination in poultry broiler chicken can be the excessive use of antibiotics to prevent infections. Studies from India and Pakistan have also highlighted the role of antibiotic use in poultry farming, which may contribute to higher bacterial contamination, especially antibiotic-resistant strains. For example, a study by Chatterjee et al., (2018) in India reported a high prevalence of *E. coli* in poultry farms where antibiotics were commonly used to prevent infections. Similarly, Jamil et al., (2019) found high rates of antibiotic-resistant *E. coli* in poultry flocks in Pakistan, which may increase the risk of infection and contamination in these commercial farming systems. In contrast, sonali and deshi chickens—often raised in lower-density or free-range conditions—showed lower *E. coli* prevalence which is 21% and 15% accordingly. This is consistent with research by Kilonzo et al., (2008) found reduced pathogen loads in chickens raised in less confined environments. Rothrock et al., (2019), he also found similar results. Indigenous breeds like the deshi chicken in India and in Pakistan are often more resilient to pathogens due to their natural habitat and genetic adaptability (Kumar et al., 2021). In Pakistan, a study by Khan et al., (2017) showed that indigenous poultry breeds raised in backyard systems had fewer *E. coli* colonies compared to broiler chickens raised in high-density conditions.

Antimicrobial resistance in chickens is a common problem in Bangladesh and other developing countries (Al Azad et el., 2019). We also analyzed the antimicrobial susceptibility profile of *E.coli* isolated from cloacal samples of live chickens . The findings reveal a mixed pattern of susceptibility, resistance, and intermediate responses across a range of commonly used antibiotics. These findings are consistent with trends observed in similar studies both within Bangladesh and internationally, highlighting significant concerns about antibiotic resistance in poultry-associated bacterial pathogens. According to our research, the isolates showed a high level of susceptibility to carbapenems, like Meropenem (95% susceptibility), Imipenem (89%), and Ceftriaxone (87%), show high effectiveness against *E. coli* isolates in our experiment, with very low resistance rates (0-6%). Similar results were observed in a study by Rahman et al., (2021), where poultry-associated *E. coli* isolates from Dhaka demonstrated above 90% susceptibility to carbapenems. The continued effectiveness of these antibiotics underscores the importance of limiting their use in veterinary settings to preserve their efficacy in human healthcare (Davies et el., 2010). These findings emphasize the importance of preserving carbapenem efficacy by avoiding its use in veterinary settings to mitigate the risk of resistance spillover to human pathogens (Willems et al., 2020). Our findings reinforce the importance of restricting their use in veterinary medicine to avoid cross-resistance with human infections. Antibiotics, such as Tetracycline (89% resistance), Amoxicillin (44%), and Ciprofloxacin (59%), demonstrate high levels of resistance, indicating limited effectiveness in treating *E. coli* infections. Studies from various regions, including Asia, have consistently reported high resistance rates in *E. coli* from poultry samples to common antibiotics like Tetracycline, Amoxicillin, and Ciprofloxacin. For example, research by Islam et al. (2022) on poultry in Bangladesh found similarly high resistance rates to Tetracycline (over 80%) and Amoxicillin, which aligns closely with our findings. A result was reported by Azad et al., (2017) who observed 100% resistance in *E.*

coli isolates to ampicillin, tetracycline isolated from broiler cloacal swab samples in Rajshahi area, Bangladesh. The reason behind such differences in detection of *E. coli* is unclear, several factors can contribute to such variations such as regional differences, sample collection techniques, season, and bacterial identification methods. Tetracycline and Amoxicillin are commonly used in poultry farming for growth promotion and disease prevention, leading to widespread resistance due to their frequent and often unregulated use (Islam et al., 2022; Marshall et al., 2011). The high resistance to these antibiotics in *E. coli* indicates that these drugs may no longer be effective for treating infections in poultry, potentially limiting treatment options in veterinary practice. The high resistance rates to Tetracycline, Ciprofloxacin, and Amoxicillin in this study underscore the need for a coordinated approach to antibiotic use in the poultry sector. Similarly, Ciprofloxacin showed high resistance (59%) and an intermediate sensitivity rate of 18%. This finding is in line with Rahman et al. (2023), who documented around 60% resistance to Ciprofloxacin in poultry-associated *E. coli* in Dhaka. A similar high resistance percentage to ciprofloxacin was reported in the previous studies conducted by Akond et al. (2009) (100%) and Bashar et al. (2011) (82%) from cloacal swab samples of broiler. The widespread use of fluoroquinolones like Ciprofloxacin in poultry for infection control has led to increased resistance, posing a challenge for treating both animal and zoonotic infections (Rahman et al., 2023). On the other hand, Amoxiclav and Azithromycin exhibited variable responses, with Amoxiclav showing 24% susceptibility, 34% intermediate, and 41% resistance, while Azithromycin showed 52% susceptibility and 48% resistance. Hasan et al. (2022) also reported mixed results with these antibiotics in *E. coli* isolates from poultry, indicating that their efficacy varies depending on the specific bacterial strain and regional factors. This variability may be due to inconsistent usage patterns or regional differences in resistance mechanisms, underscoring the need for region-specific antimicrobial guidelines (Hasan et al., 2022). The high resistance percentage

observed in the research, especially for Tetracycline, Amoxicillin, and Ciprofloxacin, indicate a critical need for improved antibiotic stewardship in the poultry industry. In Bangladesh and globally, excessive use of antibiotics in animal farming contributes to the spread of resistant bacteria, posing risks to both animal and human health. Effective antibiotic stewardship programs, as implemented in European Union countries, have shown success in reducing resistance levels by regulating the use of critical antibiotics in animal agriculture (European Medicines Agency, 2019). Implementing similar programs in Bangladesh could help curb resistance development, preserving the efficacy of essential antibiotics for both veterinary and human healthcare. Overuse of antibiotics not only reduces treatment options for veterinary infections but also increases the likelihood of transferring resistant bacteria to humans (Marshall et al.,2011). According to (McEwen et al., 2018; Singer et al., 2003), indiscriminate use of antibiotics without prescription contributes to the development and spread of antimicrobial resistance. One of the major concerns is the sale of antibiotics without prescription that promote irrational use, overuse, and misuse of antibiotics in the animal health as well as human health sectors in most of the developing countries including Bangladesh (Hassan et al., 2021; Kalam et al., 2021; Kumar et al., 2013; Masud et al., 2020). Policymakers should consider implementing strict regulations on antibiotic use in animal farming to limit the development and spread of antimicrobial resistance. Furthermore, promoting alternatives, such as vaccination and improved biosecurity, can reduce the reliance on antibiotics and help control resistance levels in poultry farming.

Chapter 5

Conclusion

The research highlights high percentage of *E. coli* and its antibiotic resistance patterns in poultry chickens where broiler chicken showed high number of *E.coli* contamination than shonali and deshi breed. The findings indicate that commercially raised poultry chickens, particularly those in high-density environments, higher levels of *E. coli* contamination and exhibit increased resistance to commonly used antibiotics, including tetracycline, ciprofloxacin, and amoxicillin. The frequent use of antibiotics in these commercial systems contributes to the emergence of resistant strains, posing a public health risk due to the potential transmission of these bacteria. In contrast, native sonali and deshi chickens, which are often raised in less intensive, lower-density, or free-range settings, demonstrated lower *E. coli* prevalence and resistance levels. This supports global research showing that traditional farming practices help reduce harmful bacteria and antibiotic resistance. There might be a solution if implementing improved biosecurity measures, reducing overcrowding, and promoting alternative, sustainable farming practices for poultry can be taken care of. Additionally, promoting native breeds and supporting lower-density farming systems may offer a viable approach to reducing public health risks.

Chapter 6

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