Identification and Antibiotic Susceptibility of *Escherichia coli* Isolated from Chicken Samples Collected from Wet Markets in Dhaka city

By

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of
B.Sc. in Microbiology.

Department of Mathematics and Natural Science
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Declaration

It is hereby declared that

1. The thesis submitted is our own 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. We have acknowledged all main sources of help.

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Ethics Statement

This study was approved by the Department of Mathematics and Natural Science. The pursuit of scientific knowledge and advancements is very necessary to be guided through the ethical principles for the well-being of all living beings. In this research project, while isolating *Escherichia coli* from the chickens, we made sure to follow the ethical principles adequately in accordance with the Western Governors University guidelines (WGU, 2022):

I. Animal welfare: We acknowledge that chickens are living beings that are capable of experiencing any sort of pain, sufferings and comfort. Therefore, we made our first priority to minimize any sort of pain or distress caused to the chicken during our research.

II. Transparency and responsibility: We made sure to stay as transparent and responsible during our research as possible. We provided detailed information regarding the aim, methods, and possible benefits of the study to the population. We also maintained clear and open communication with the public in order to ensure transparency in our research practices. We also addressed all concerns or questions regarding our research to remain transparent.

III. Respect for animals: We respect the value of each living beings, and that's why, we tried to handle the chickens with utmost care. We also ensured minimum pain and distress during the handling or experimental procedures.

By following these ethical principles, we are aiming to make sure that our research on isolating *Escherichia coli* from chickens will not only contribute to the scientific knowledge, but will also contribute to the welfare and respect towards the animals.

Abstract

Pathogenic *Escherichia coli* can be found in the intestine and feces of the chickens. Even though most *E. coli* strains are not harmful, there are some deadly *E. coli* strains, which cause food-borne diseases in humans. Moreover, the recent increase in antibiotic resistant *E. coli* is becoming a huge threat to the public health. The aim of this project is to identify and determine the pathogenicity of *E. coli* and determine antimicrobial susceptibility of *E. coli*.

From the 8 chickens collected from 8 randomly selected wet markets in Dhaka city, a total of 32 samples (24 meat samples and 8 cloacal swab samples) were taken. Then the isolation of *E. coli* was done on MacConkey agar, Sorbitol MacConkey agar, and Nutrient agar medium. Finally, *E. coli* was detected through Polymerase Chain Reaction.

Furthermore, the antimicrobial susceptibility test showed these isolates were highly resistant to Amoxicillin (95.93%), followed by Ciprofloxacin (83.73%), and least resistant to Meropenem (0.81%).

This extensive research is crucial to understand the epidemiology of the disease outbreaks and emergence of antibiotic resistance.

Keywords: *Escherichia coli*; Chickens; Antibiotic Resistance; Pathogenicity; Antibiotic Susceptibility; Contamination.

Dedication

I dedicate my thesis to my parents for their unending love, support and guidance throughout my education. I hope I will be able to make them proud through this accomplishment.

Acknowledgement

Firstly, we are extremely grateful to Almighty Allah for giving us the required strength to fulfill our thesis project in the allocated time.

We would like to extend our heartfelt thanks towards Dr. Fahim Kabir Monjurul Haque (Assistant Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University), for his invaluable support and insights which lead to the writing of this research paper. Without his guidance, we would not have been able to accomplish our project so effortlessly.

We also would like to show gratitude towards each other, as we as a team worked continuously together with dedication, patience and faith towards each other.

Lastly, we wholeheartedly thank our parents for always supporting and encouraging us in our academic journey.

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List of Acronyms

E.coli	*Escherichia coli*
bp	Base pair
PCR	Polymerase Chain Reaction
AMX	Amoxicillin
CIP	Ciprofloxacin
AZM	Azithromycin
TE	Tetracycline
CFM	Cefixime
MRP	Meropenem
DO	Doxycycline
E	Erythromycin
CTR	Ceftriaxone
S	Streptomycin
IPM	Imipenem
PIT	Piperacillin
HUS	Hemolytic Uremic Syndrome
MDR	Multidrug resistant
DNA	Deoxyribonucleic acid

rRNA	Ribosomal Ribonucleic acid
TE buffer	Tris-EDTA buffer
TAE	Tris-acetate EDTA
EtBr	Ethidium Bromide
µL	Microliter
µg	Microgram
UV	Ultraviolet
CLSI	Clinical and Laboratory Standards Institute
dH20	Distilled water
LB	Lysogeny Broth
SMAC	Sorbitol MacConkey Agar
rpm	Revolutions per minute
TSB	Tryptic Soy Broth

Chapter 1

Introduction

1.1 Poultry farms and *E. coli*

The poultry industry in Bangladesh is a huge industry, which has been significantly growing over the past few decades. Chicken is one of the most popular and nutritious meat all around the world, including Bangladesh. It is a great source of protein. So, it can be clearly understood that the poultry industry plays a huge role in providing nutrition value to the people as well as a significant contributor to the economy of the country.

According to One Health Poultry Hub, currently 1 million entrepreneurs and almost 8 million people are able to produce 10.22 billion eggs and 1.46 million tons of poultry meat in the poultry farms of Bangladesh per year. There are a total of 16 grandparent farms, 206 breeder farms, and almost 70,000 commercial farms. These commercial farms are also increasing at 15% rate per year (One Health Poultry). This shows the high demand of chicken all around the country.

Even though chickens are delicious and have a lot of nutrition value, it can often be linked to being the source of many diseases, as it can lead to bloody diarrhea, food poisoning, *Escherichia coli* (*E. coli*) infections, *Salmonellosis*, Enterohemorrhagic *E. coli* infections and many more (Cleveland Clinic, 2020). This is because chickens can be easily associated with foodborne diseases, as it can get contaminated with bacteria, like *E. coli*, *Salmonella*, *Campylobacter*, *Clostridium perfringes* bacteria etc. According to CDC, about 1 million people from the USA become ill from consuming contaminated chickens per year (CDC, 2022).

E. coli is a type of Gram-negative, rod-shaped, coliform bacterium, which can be found in the guts and lower intestine of the humans, chickens and other animals (WHO, 2018). It is a facultative anaerobe, which means that *E. coli* can survive in both presence and absence of oxygen. Most of the strains of *E. coli* do not cause any harm to human being, chickens and other animals; as most of the strains of *E.coli* can be usually found inside the intestines of the living beings, but some strains can be extremely deadly (WHO, 2018). Some of the strains of *E. coli* like Shiga toxin-producing *E. coli* (STEC) can be extremely pathogenic and can lead to several illnesses. Also, some strains like *E. coli* 0157:H7 are also responsible for vomiting, nausea, bloody diarrhea, abdominal cramps and many more (Mayo Clinic, 2022). These deadly and pathogenic strains of *E. coli* can be passed through feces to the external surface of the chicken as well as to the environment, which can lead to the contamination of the chicken (Stromberg et al., 2017). Poor hygiene of the poultry farm, exposure to the chemicals and chemical industry, and contaminated environment can also cause contamination of the chicken (Gelli et al., 2019).

67``According to CDC, in July 1996, the largest *E. coli* 0157:H7 outbreak took place in Japan, which lead to approximately 10,000 people getting infected. The people who were infected were mostly school going children. It was later determined that the contaminated radish sprout of the cafeteria was the main reason behind the outbreak of *E. coli* (Watanabe et al., 1999). Moreover, another huge *E. coli* 0104:H4 outbreak took place in Germany in the year of 2011, which lead to 3500 people getting *E. coli* infection. It was found out that the spread of this infection occurred due to the consumption of contaminated sprouts (CDC, 2012).

Nevertheless, many studies were done on different parts of chicken, and the result showed that *E. coli* was present in all of the parts, and most of the antibiotics showed high resistance to *E. coli*. So, this is a serious threat to the public health, because of its developing antibiotic

resistance, which means chicken is at a possible risk for causing another *E.coli* outbreak. For this reason, in order to prevent the *E.coli* outbreaks through the consumption of chickens, proper handling of the chicken and regulatory measurements of the poultry farm is extremely crucial (FSIS, 2019).

1.2 Symptoms of *E. coli* and risk factors

E. coli can spread from the chickens to the humans through consuming the contaminated chicken meats. Due to this, *E. coli* infections can occur, which can lead to many mild and severe symptoms within the human body. Some of the symptoms caused by the *E. coli* are food poisoning, nausea, vomiting, diarrhea, high fever, abdominal cramps, pelvic pain, urinary tract infection (UTI), bloody diarrhea, dehydration and many more (CDC, 2022).

In most cases, people recovers from these types of symptoms within a week or two, however, in some of the extreme cases and in case of some young children and elderly people, people might get hospitalized due to complications, such as, hemolytic uremic syndrome (HUS), which can eventually lead to kidney failure. This hemolytic uremic syndrome usually happens as a result of the diarrheal infection, which is normally caused due to the *E. coli* 0157:H7 infection (Mayo Clinic, 2021). It can also cause the immune system of the elderly people and children to get weakened. Moreover, according to WHO, about 10% patients who is infected with STEC infection might develop into the HUS. This has the fatality rate from 3% to 5%. Moreover, the HUS is the most common reason behind acute renal failure among the young children. This can cause many complications like seizure, coma, stroke etc (WHO, 2018).

1.3 Cause of *E. coli*

One of the most common bacteria which is responsible for the outbreaks of foodborne diseases through the consumption of chicken is *E. coli* (Rachael, 2022). The chicken can

easily get contaminated with *E. coli*, due to the chicken coming in contact with feces. As *E.coli* is a bacterium that normally lives in the intestines and guts of chickens, it can easily spread through excrement and droppings of the chickens.

The contaminated water, contaminated soil, contaminated feed, rodents, diseased poultry, or even if there is some deceases animals within the poultry farm can lead to the spread of *E. coli* to the chicken, which then spreads to the human. The eggs of the chicken can also get contaminated with *E. coli*, which might also lead to the spread of *E. coli* to the humans (Islam et al., 2023). Also, if the chicken fecal matter is improperly handled during the processing or preparation of the poultry products, it can also lead to contaminations of the surface of the chicken as well as utensils or other foods (Ewers et al., 2009). This also increases the risk of the spread of *E. coli*.

1.4 Antibiotic resistance of *E. coli*

The antibiotic resistance is a huge problem in the modern world, as it makes the microorganisms develop a resistance to the antibiotics, which results in the survival of the microorganism and defeat of the antibiotics (CDC, 2022). According to the Government of Canada, one of the most common causes of antibiotic resistance is the overuse and misuse of antibiotics in order to treat an illness. Overuse of antibiotics take place when patients take antibiotics more than the required amount that was prescribed by the healthcare professionals. Misuse of antibiotics happen when patients self-medicate and shares antibiotics. It can also occur when antibiotics are given unnecessarily, and when patients take antibiotic for an illness which is not caused by a bacteria (Public Health Agency of Canada, 2021).

E. coli is a serious threat to the public health, because it has the ability to develop antibiotic resistance (Pormohammad et al., 2019). Due to the overuse and misuse of the antibiotics, the *E. coli* is getting stronger and is being able to resist many antibiotics. As most of the

antibiotics are currently failing to inhibit the growth of *E. coli*, this bacterium is also being called one of the multi-drug resistant (MDR) bacteria (Poirel et al., 2018). This can cause serious threat to the society, as antibiotic resistance will reduce the treatment options, increase medical expenses, extend the stays in the hospital, and increase the mortality rate (WHO, 2020).

Chapter 2

Materials and methods

2.1 Collection of samples

In order to collect the chicken samples, from October 2022 to March 2023, 8 wet markets randomly from different locations in Dhaka city were selected. The randomly selected wet markets were from Mohakhali small kachabazar, Mohakhali Haque kachabazar, Mohakhali kachabazar, Saat tola kachabazar, Korail BTCL bazaar, DCC market, Karwan bazaar kitchen market, and Mohammadpur krishi market. One chicken was purchased from each of these wet markets. But before slaughtering the 8 fresh chickens, the cloacal swabs of these chickens were collected aseptically with the help of sterile cotton swabs. These cotton swabs were then kept in separate sterile tubes containing 5 ml saline water.

Figure 1: Collection of cloacal swabs

After that, the chickens were slaughtered, and the breast muscle, thigh muscle and liver pieces were cut on top of sterilized aluminum foils to reduce the chances of environmental factors affecting the chicken meat. Then, the chicken breast, chicken thigh and chicken liver were wrapped up in separate sterilized aluminum foil and kept separately in zip lock bag. A total number of 32 samples were collected for the research purpose, where 24 samples were meat samples (chicken breasts, thighs, and livers) and 8 samples were cloacal swabs. These collected samples were then directly transferred to the microbiology lab in the icebox at 4° C, and processed within 24 hours (Sengupta et al., 2011).

2.2 Preparation of samples

At first, each of the samples i.e. chicken breasts, thigh muscle and liver pieces had to be sliced and minced thoroughly. It was easier to measure the minced meats to get the most accurate weight. These minced meat samples were placed on top of a sterilized aluminium foil to aseptically be measured in the weighing balance, and 10 grams of each sample were weighed for the next steps. Each of the 10 grams of meat samples were then added to small beakers which consisted of 40 ml of sterilized distilled water. Then, each sample was homogenized with the help of a homogenizer at 3500-4000 rpm for 1-2 minutes until the mixture is emulsified. These homogenized breast, thigh and liver samples were each taken in 1 ml and added to three different test tubes containing 9 ml of Tryptic Soy Broth (TSB), and they are incubated in the incubator at 37° C for 2-4 hours.

2.3 Isolation of *Escherichia coli*

After incubating the three test tubes containing Tryptic Soy Broth (TSB) and samples, from each of the three test tubes, 1 ml is taken and serial dilution is performed. Also, 1 ml from the cloacal tube was taken for the serial dilution. After the serial dilution, an amount of 80 micro liter of the culture suspension was taken from each tubes and spreading method was applied onto the MacConkey Agar plates. These plates were then incubated at 37° C for overnight.

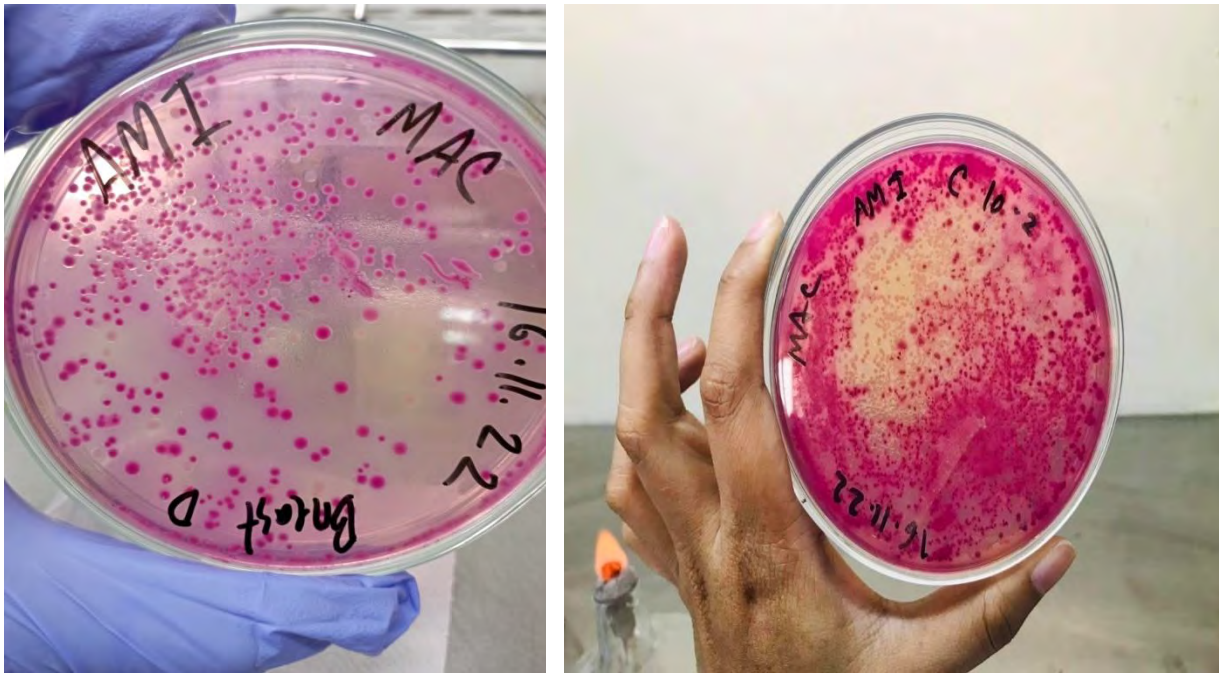

Figure 2: Growth of *Escherichia coli* in MacConkey Agar media (left one is of meat sample and right one is of cloacal sample)

The next day, the dark pink & round shaped colonies were considered as the *E. coli* (Rasool et al., 2016), and they are sub-cultured onto the Sorbitol MacConkey (SMAC) agar media. The SMAC agar media is a selective and differential media used in order to identify the *E.coli* 0157:H7 and other non- *E. coli* 0157:H7 strains. This media allows the growth of *E.coli* 0157:H7, whereas, it inhibits the growth of non- *E. coli* 0157:H7 strains (Universe84a, 2021). So, after sub-culturing onto the Sorbitol MacConkey (SMAC) agar media plates, these are also incubated at 37° C for overnight.

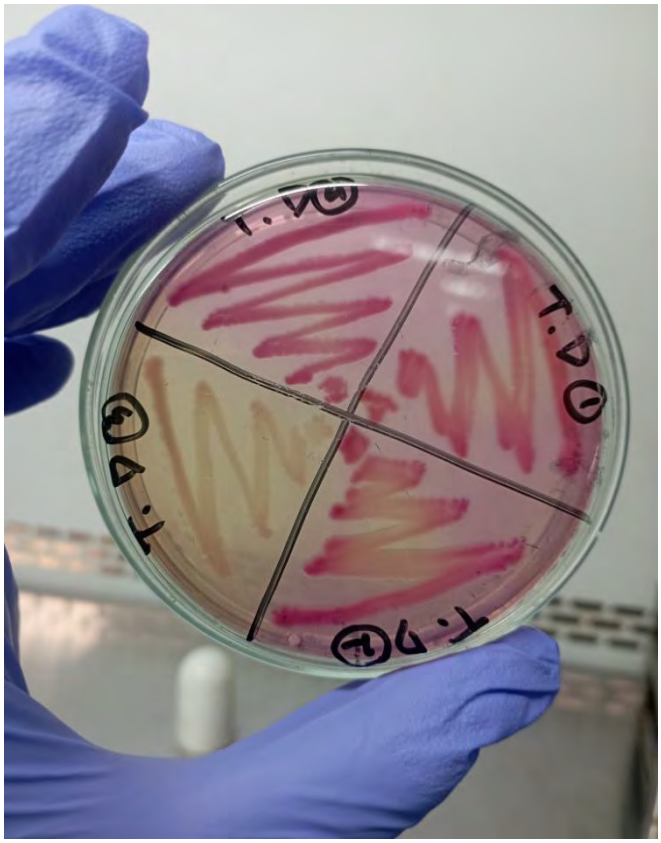

Figure 3: Growth of *Escherichia coli* in Sorbitol MacConkey Agar media

After that day, two types of colonies appeared on the SMAC media plates. According to March and Ratnam (1986), the colorless colonies can be presumed as the *E. coli* 0157:H7 and the pink colonies can be presumed as the non- *E. coli* 0157:H7. The *E. coli* 0157:H7 can be presumed as the colorless colonies because it is unable to ferment the sorbitol, on the other hand, non- *E.coli* 0157:H7 can be presumed as the pink colonies because it is able to ferment sorbitol (March & Ratnam, 1986).

Sub-culture was also done from the MacConkey agar media plates to the Nutrient agar plates, in order to get the pure and isolated colonies of the microorganism.

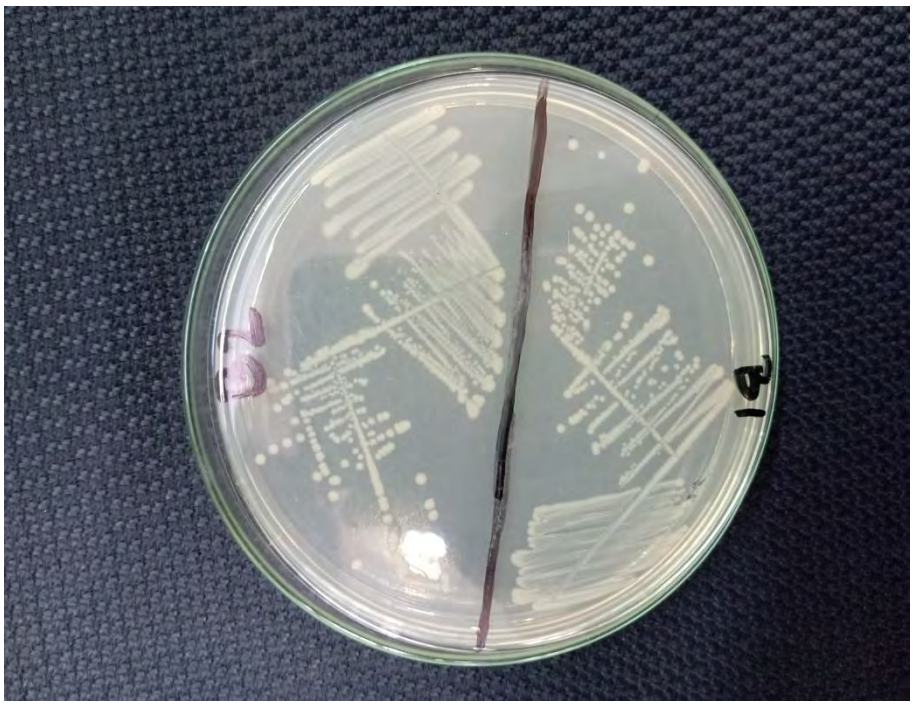

Figure 4: Isolation of *Escherichia coli* in nutrient agar media

After the sub-culture on nutrient agar, the pure and isolated colonies are then used in order to perform antimicrobial susceptibility tests, DNA extraction and purified stock DNA of bacteria.

2.4 DNA Extraction

The boiling method was used in order to extract the DNA from each of the isolates. It causes the lysis of the bacterial cells due to the intense amount of high temperature; this releases the DNA of the bacteria into the solution. At first, the pure culture of the *E. coli* bacteria were grown in the LB broth overnight, and then, 1 ml of the bacterial culture was collected to a sterile microcentrifuge tube in order to centrifuge the at 13500 rpm for 10 minutes. This will cause the pellet of bacterial cells. Afterwards, the supernatant was discarded carefully from

the top and the cell pellets were washed with 1 ml dH20. This was centrifuged once again at 14000 rpm for 5 minutes. Then the supernatant was discarded again, and 500 µl TE buffer was added in each of the tubes. The mixture is to be boiled at 100° C for 15 minutes in order to lyse the bacterial cells and release the DNA (Zhu et al., 2006). It is to be ensured that the parafilms of the tubes are sealed properly in order to prevent the loss of any sample. Then, after the boiling procedure, it has to be incubated in the ice bath for 10 minutes (Wu, et al., 2014). This incubation in the ice bath will ensure the DNA to reanneal and stabilize. After the incubation in the ice bath, it has to be centrifuged at 14000 rpm for 5 minutes, and the cell debris will precipitate at the bottom of each of the tubes. Lastly, it is required to collect the supernatant in a new tube, and it needs to be stored at -20° C.

2.5 Specific Detection and Confirmation of isolates as *E.coli* by PCR

Polymerase chain reaction (PCR) is one of the advantageous molecular techniques compared to traditional method, where determination of specific organism is way more sensitive and expeditious (Tonu et al., 2012). Explicit primer sequences (Bioneer, South Korea) for primary detection and confirmation of previously isolated *E. coli* targeted 16s rRNA gene (Messele et al., 2017). Isolated *E. coli* organisms were amplified by PCR, applying distinct primers which are: ECO-f and ECO-r, to target 16S rRNA where 585 bp amplicon was uncovered as well as narrated by Candrian et al. (1991) and also Wang et al. (1996) (Table 1).

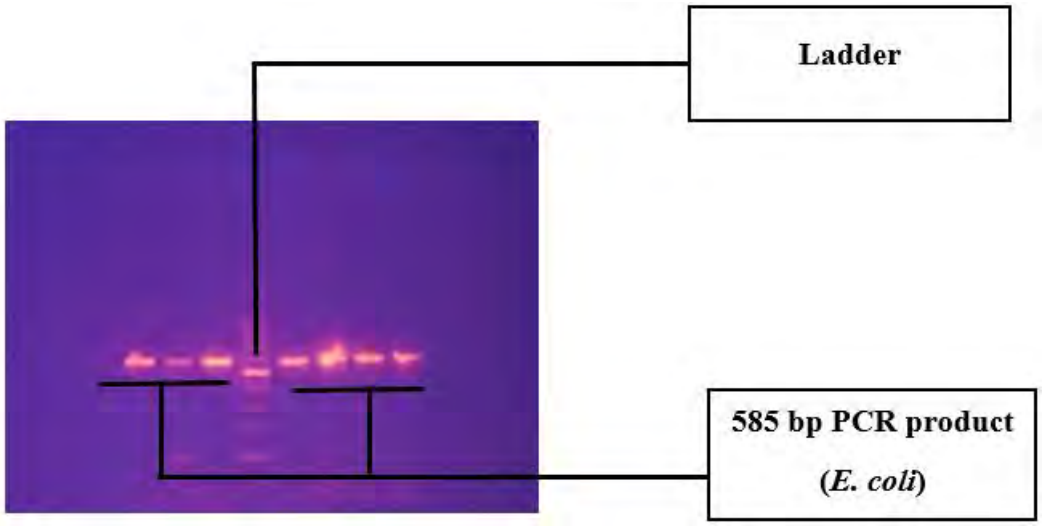

Figure 5:

The 585bp PCR product (depicted in the the illustration) after being amplified in field samples of locally isolate d *E. coli* is apparent on an agarose gel electrophoresis.

The Gene Bank tool BLAST was employed for the confirmation of applied primers being complimentary with the target species but not with other species where no similarities were found (Seidavi et al., 2010).

A modest adjustment was made to the PCR process outlined by Schippa et al. (2010).

Table-1: Primers used for the detection and confirmation of *E. coli*

Target Gene	Primer	Primer Sequence (5′-3′)	Amplification Product size (bp)	Reference
16srRNA	ECO-F ECO-R	5'GACCTCGGTTTAGTTCACAGA3' 5'CACACGCTGACGCTGACCA3'	585 bp	Schippa et al.,2010

13µl per PCR tube reaction containing 1X TAQ polymerase PCR master mix (6 µl), 1µl Forward primer, 1µl Reverse primer, 3µl Nuclease free water and 2µl sample. After a preliminary 3 min incubation stage at 95°C, a 30-cycle amplification regimen was carried out which incorporated of 45s at 94°C, 45s of annealing at 58°C, 60 s of extension at 72°C and 3 min of final extension at 72°C and hold for 4°C (Hassan et al., 2014) (Figure 6). Each isolate's double-stranded DNA was extracted, afterwards, the existence of PCR-compatible DNA was verified by performing a PCR analysis on the DNA (Seidavi et al., 2010).

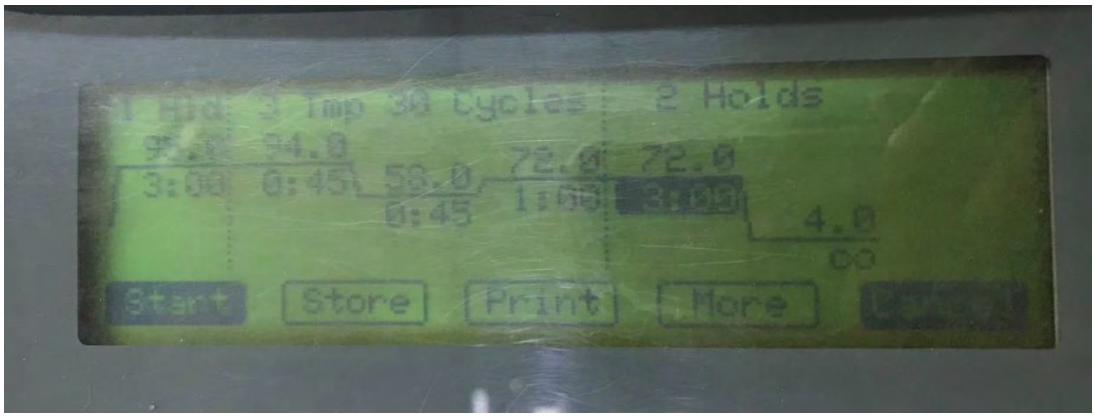

Figure 6: PCR conditions for this paper

2.6 Agarose Gel Electrophoresis

Electrophoresis was the method employed to separate amplified products on 1% agarose gel with ethidium bromide. The agarose gel was generated with 1g of agarose powder, 100ml of 1X TAE buffer and 5µl of ethidium bromide which was narrated from Fisher Biotech in New Jersey and Genei in Bangalore, India.In 1X TAE buffer, agarose gel electrophoresis was carried out at 110 V for 40 minutes. For electrophoresis, a 100 bp ladder (Ggibco BRL) (Bioneer, South Korea) was employed as a molecular weight marker and a loading dye. A transilluminator was used to observe bands while exposed to UV light.Agarose gel electrophoresis was carried out with a little modification to the method outlined by Tonu et al., 2012.

2.7 Antimicrobial Susceptibility Test

In order to perform the antimicrobial susceptibility testing of the isolated *Escherichia coli*, the Kirby-Bauer disc diffusion method was followed. The Mueller-Hinton agar media was selected to perform this disc diffusion method. In order to test the antimicrobial susceptibility of the isolated *Escherichia coli*, a total number of 13 antibiotics were used, where two of them were used as an alternative.

Figure 7.1: Antibiotic inhibiting the growth of *E.coli*

The 13 antibiotics and their concentrations were Amoxicillin (AMX/10µg), Ciprofloxacin (CIP/5µg), Azithromycin (AZM/15µg), Tetracycline (TE/30µg), Cefixime (CFM/30µg),

Streptomycin (S/10μg), Imipenem (IPM/10μg), Meropenem (MRP), Piperacillin (PIT), Ceftriaxone (CTR/30μg), Amoxiclav (AMC), Doxycycline (DO/30μg), and Erythromycin (E/15μg). During some samples, Doxycycline was used as an alternative for Tetracycline, and Erythromycin was used as an alternative for Azithromycin. In order to measure the zone of inhibition, the diameters of the clear circular area around the antibiotic discs are measured.

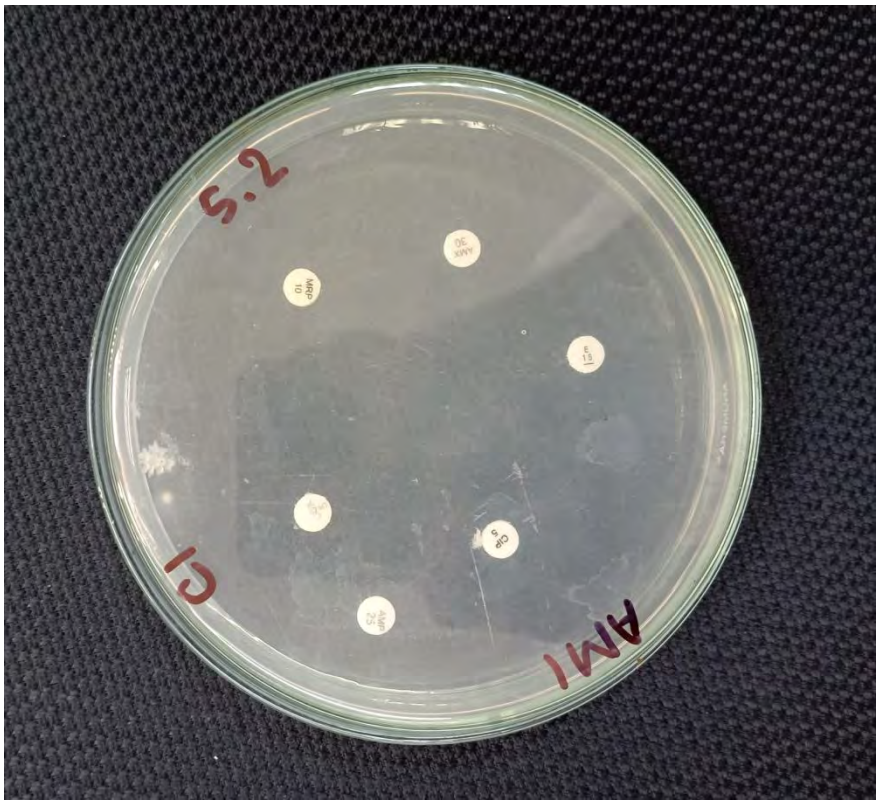

Figure 7.2: Antibiotic Resistance

Table-2: Antibiotics detail based on CLSI standard

Serial no	Antibiotic Name	Group	Disc code	Disc potency (µg)	Interpretative Criteria		
					Sensitive mm or more	Intermediate mm	Resistant mm or less
1	Amoxicillin	Penicillin	AMX	10	17	14-16	13
2	Ciprofloxacin	Fluroquinolones	CIP	5	26	22-25	21
3	Meropenem	Carbapenem	MRP	10	18	15-17	14
4	Imipenem	Carbapenem	IPM	10	23	20-22	19
5	Piperacillin	Penicillin Combination	PIT	100/10	21	18–20	17
6	Amoxiclav	Penicillin Combination	AMC	10	21	18-20	17
7	Azithromycin	Macrolide	AZM	15	18	14–17	13
8	Erythromycin	Macrolide	E	15	18	14–17	13
9	Tetracycline	Tetracycline	TE	30	15	12-14	11
10	Doxycycline	Tetracycline	DO	30	14	11-13	10
11	Cefixime	Cephalosporin	CFM	5	19	16-18	15
12	Ceftriaxone	Cephalosporin	CTR	30	23	20-22	19
13	Streptomycin	Aminoglycoside	S	10	15	12-14	11

From the used antibiotics, Amoxicillin (2^{nd} generation) falls under the penicillin type antibiotic (Diaz, 2019), Ciprofloxacin (2^{nd} generation) falls under Fluroquinolones (Sharma et al., 2017), Azithromycin (4^{th} generation) and Erythromycin (1^{st} generation) falls under Macrolides (Research Gate, 2020), Tetracycline (1^{st} generation) and Doxycycline (2^{nd}

generation) falls under Tetracycline type antibiotics, Cefixime (3rd generation) and Ceftriaxone (3rd generation) falls under Cephalosporin (NCI, 2022), Streptomycin (1st generation) falls under Aminoglycosides, Imipenem (2nd generation) and Meropenem (3rd generation) falls under Carbapenem (Zemelman et al., 2004), and Piperacillin (4th generation antibiotic) & Amoxiclav falls under Penicillin combination type antibiotic (Diaz, 2019).

Chapter 3

Results

There were total 32 chicken samples where cloacal samples were 8 and meat samples were 24 (liver 8, thigh 8, breast 8) and total isolates that we randomly picked were 123 in the current study where 30 of these isolates were from cloacal, 31 of these were liver, 31 were thigh and another 31 were from breast (Table-3).

Table-3: Overall overview of prevalence of *E.coli* from raw chicken sample

Sample Name	**Number of Sample Collected**	**Number of Isolates Randomly Selected**	**Number of *E. coli* presence in samples**
Cloacal	8	30	8
Liver	8	31	8
Thigh	8	31	8
Breast	8	31	8
	Total= 32	**Total= 123**	**Total= 32**

3.1 PCR Confirmation:

Following 1% agarose gel electrophoresis, 123 of *E. coli* isolates from each field sample were randomly chosen which had been locally isolated as well as demonstrated 585-bp products (Figure:5). Using ECO-f and ECO-r primers, which are distinct to *E. coli*, PCR has been carried out on the randomly chosen isolated *E. coli*. By performing a PCR analysis, all of the chosen isolates were discovered to be positive (Figure 5). In this research, 123 isolates were randomly chosen for the PCR. All the chosen isolates were depicted to be positive.

3.2 Antimicrobial resistance profiling:

Out of 123 *E. coli* isolates from raw chicken meat, 95.93% were resistant to Amoxicillin, 83.73% Ciprofloxacin, 0.81% Meropenem, 2.43% Imipenem, 3.25% Piperacillin, 61.78% Amoxiclav, 19.51% Azithromycin, 66.66% Erythromycin, 56.91% Tetracycline, 11.38% Doxycycline, 56.91% Cefixime, 8.94% Ceftriaxone and 39.83% Streptomycin. The majority of the *E. coli* isolates found in raw chicken meat were resistant to amoxicillin which is 95.93%, then 83.73% were Ciprofloxacin the lowest amount of resistant found in Meropenem which is 0.81%.

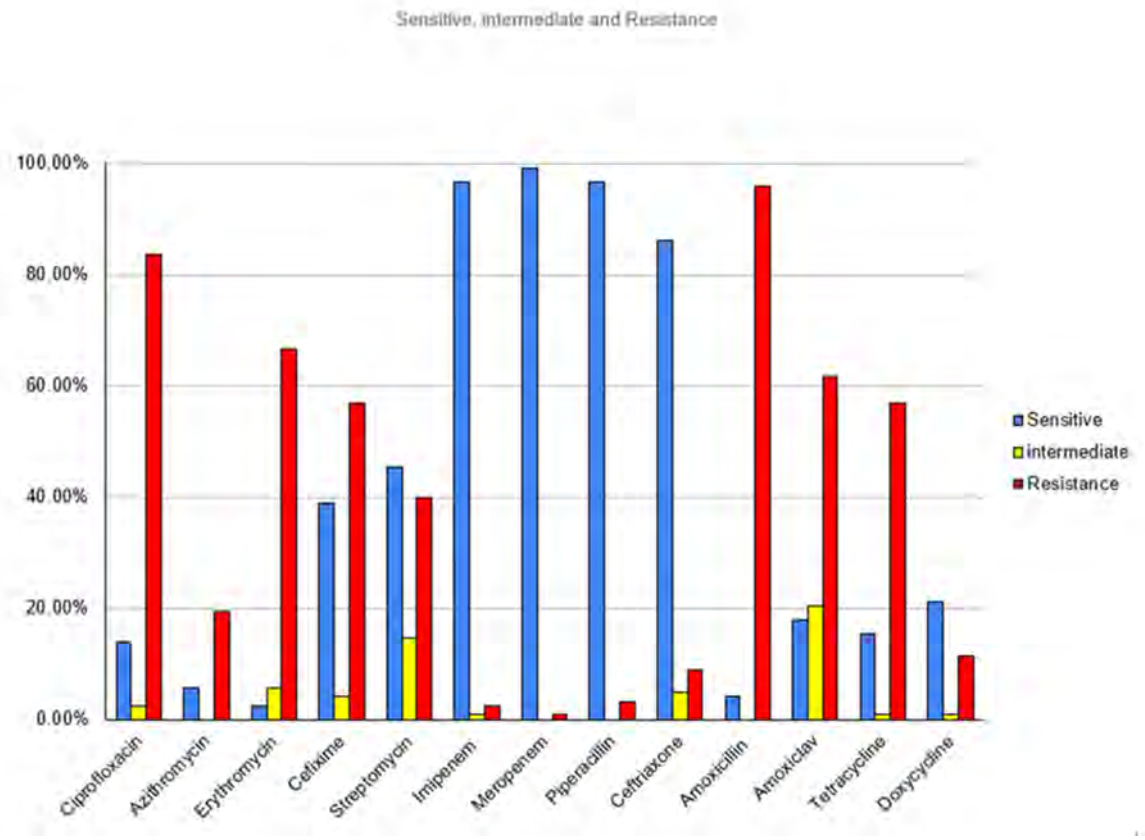

Figure 8.1: Antibiotic Susceptibility profile of *E. coli*

These 123 *E. coli* isolates from raw chicken flesh were investigated where 4.06% were sensitive to Amoxicillin, 13.82% Ciprofloxacin, 99.18% Meropenem, 96.74% Imipenem, 96.75% Piperacillin, 17.88% Amoxiclav, 5.69% Azithromycin, 2.40% Erythromycin, 15.44% Tetracycline, 21.13% Doxycycline, 39.02% Cefixime, 86.17% Ceftriaxone and 45.52% Streptomycin. According to this founding's, the highest amount of sensitivity was noticed in Meropenem and Piperacillin, Imipenem respectively, where the lowest amount noticed in Erythromycin.

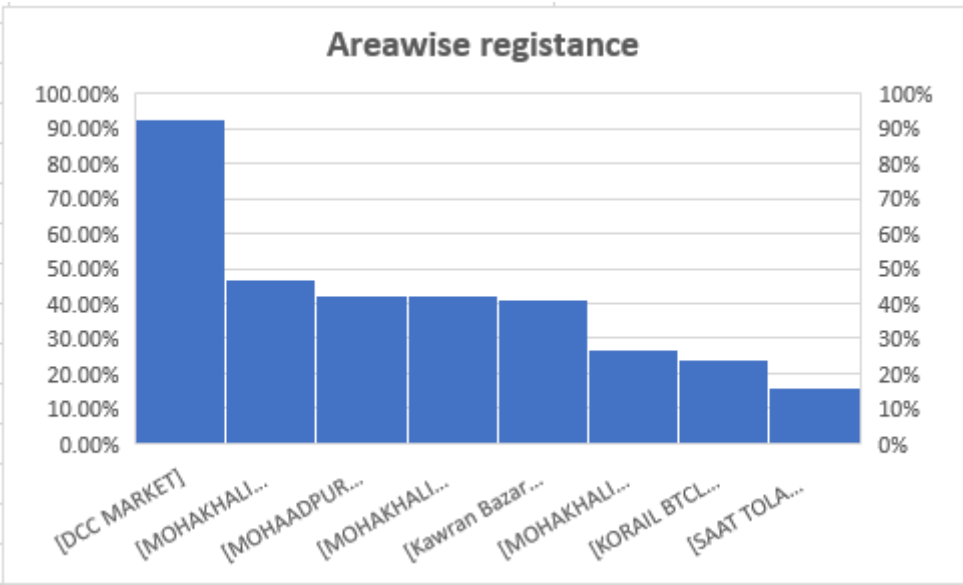

Figure 8.2: Area wise resistance percentage of *E. coli*

2.40 percent of 123 *E. coli* isolates from raw chicken flesh were found to be Ciprofloxacin intermediate, 0.81% Imipenem, 20.32% Amoxiclav, 5.69% Erythromycin, 0.81% Tetracycline, 0.81% Doxycycline, 4.06% Cefixime, 4.87% Ceftriaxone and 14.63% Streptomycin where Imipenem, Tetracycline and Doxycycline all of them showed similar intermediate parentage (Figure:8.1). All these data were verified using CLSI standard (Table 1). In this study, different area showed different resistant in chicken sample where the highest resistant was observed in DCC Market (Figure 8.2).

3.3 MDR:

Out of 123 isolates, 86.17% were multidrug (three or more medications) resistant. The total number of MDR in the isolates were 106, where 20 were cloacal, 31 were liver, 29 were thigh and 26 were found in breast isolates. In raw chicken meat, there were increased number of multidrug-resistant bacteria (Tonu et al., 2012) (Table:4).

Table 4: Total number of MDR detected in isolates

Sample Name	Number of MDR in Isolates
Cloacal	20
Liver	31
Thigh	29
Breast	26
	Total=106

Chapter 4

Discussion

Studies focused into the prevalence of *E. coli* and its susceptibility to antibiotics in raw chicken meat samples from several wet markets. In our study, raw meat has shown a very high *E. coli* prevalence where highest 95.93%, antibiotic resistance was found which is very alarming. Similar data can be seen from Messele et al., 2017. It signifies that raw chicken parts have an elevated amount of *E. coli* resistance, which has consequences for the risk to the general public's health. According to reports, unsanitary procedures are to blame for *E.coli* contamination in raw meat (Vahedi et al., 2011). The prevalence of *E. coli* in this investigation was similar to earlier studies conducted in various regions of Ethiopia by Haileselassie et al., 2012; Haimanot et al., 2010 and Bitew et al. 2010 who observed that the prevalence was 22.2, 26.6, and 20.3%, respectively. In the current research, chicken meat had drastically greater percentages of *E. coli* prevalence and resistance. This may be clarified

through small-holder farmers' preference for raising hens in their backyards to scavenge, together with their husbandry and production methods. Therefore, along with the food chain, *E. coli* might migrate from the normal intestinal flora of organisms to chicken (Fang et al., 2011). Overall, the disparities in reported prevalence and resistance may result from changes in animal breed, geographic origin, animal breed, and history of antimicrobial therapy.

The overall outcomes of this study demonstrated an exceptionally high resistance rate to tetracycline (47.5%) and ampicillin (71.4%) where in case of our study, it was observed that 95.93% were resistant to Amoxicillin and 83.73% Ciprofloxacin, showed the high resistance rate. More significantly, compared to other meat origins, chicken meat involved a higher percentage of isolates of drug-resistant *E. coli* (Daniel et al., 2012). As anticipated, former antibiotics like ampicillin (Amoxicillin) (introduced in 1961) and Fluroquinolone (Ciprofloxacin) (introduced in 1978) were shown to have the strongest resistance (Daniel et al., 2012). In a similar manner Momtaz et al., 2012; revealed that the most prevalent findings were resistance to trimethoprim, chloramphenicol, sulfamethoxazole, and tetracycline, with prevalence rates of 91.2, 45.6, and 29.8%, respectively. Parallel research on diarrheal patients in Korem, Ethiopia revealed that tetracycline, chloramphenicol, and ampicillin had the lowest *E. coli* resistance levels. The determined *E. coli* is also exceedingly resistant to streptomycin, cephalosporin, tetracycline, ampicillin, and trimethoprim, as claimed by Hiko et al., 2008.The extensive and promiscuous use of antibiotics in animals for treatment and other preventive purposes may be the cause of the larger degree of antimicrobial resistance that has been documented (Messele et al., 2017). Amoxicillin is one of the most widely used antibiotics for the treatment of many diseases, including *E. coli*, despite having the second-highest rate of resistance in this study (Messele et al., 2017).

In this study, 123 isolates *E. coli* from chicken were examined, and 86.17% of those were MDR. Intestinal microbial population in poultry evolved into MDR, with 77.4% of the Saudi Arabian population responsible (Al-Ghamdi et al., 1999) and in Vietnam, 81.3% come from residences and small farms (Nguyen et al., 2015). *E. coli* was identified in 83.5% of chicken breast specimens assessed in a US research, with 38.9% of the isolates exhibiting MDR (Zhao et al., 2012). A substantial amount of *E. coli* in retail meats implies faecal contamination at the time of slaughter or during processing, whereas, this study showed 45.06% of MDR. A comparable finding was observed in South Africa, wherein 40.0% of chicken isolates were MDR (Fielding et al., 2012).

For the purpose of finding of pathogenic or non-pathogenic microorganisms such as *E. coli* in raw chicken meat, the PCR is particularly precise and specific approach (Cohen et al., 1993). Additionally, PCR is more dependable as well as quick than conventional culture methods (Carli et al., 2001). Similar findings have been identified in this particular study nonetheless. In this experiment, the isolated *E. coli* organisms from the collected raw chicken meats were cultured in nutrient broth, DNA was extracted and the *E. coli* 16S ribosomal DNA was amplified by PCR using primers ECO-f and ECO-r. After 1% agarose gel electrophoresis, a 585 bp amplicon was discovered. Other writers were found who had comparable results (Amith-Romach et al., 2004). This base pair is unique to *E. coli* and not to other bacteria (Amith-Romach et al., 2004).

The selection of standards is quite challenging when utilizing conventional techniques for developing *E. coli*. There is no widespread agreement regarding the most effective method for identifying this foodborne pathogen. As of now, culture techniques are the acknowledged

standard approach for identifying bacteria like *E. coli* in the gastrointestinal tract of broilers (Seidavi et al., 2010). According to theory, these approaches can identify just one viable cell in a sample after pre- and selective enrichment (Seidavi et al., 2010). However, greater sensitivity of PCR methods has been observed for the detection of *E. coli* in comparison to culture techniques (Gong et al., 2002). This might be explained by the fact that PCR can detect target sequences regardless of the growth capability of target cells (Seidavi et al., 2010). The approach described in this paper was created for the daily routine detection of multiple samples. As an outcome, a significant volume of the PCR mixture was made and aliquots were added to PCR tubes. These results indicate that an affordable PCR test may identify *E. coli* swiftly within a few hours(Seidavi et al., 2010). Future research could lead to the creation of a multiplex PCR test that would utilize the rapidly expanding pool of 16S RNA sequences to analyse a complex microflora in a single or a small number of reactions. The prevalence of *E. coli* was high in the chicken gut samples, but it was comparable to different nations reports by authors like Cheville and Arp (1978) and Sackey et al. (2001).

If we consider the increasing number of microbes in the environment, diet, water, litter, during slaughtering, the elevated rates of *E. coli* are not surprising. *E. coli* could have been found in live birds as a result of contaminated diet Sackey et al. (2001). Feeds for poultry can occasionally include dangerous bacteria, such as *E. coli*. Water that has been contaminated may also act as a transmission medium. Flying birds could carry enteropathogenic germs and used the same water source as the broilers. However, the results we obtained support and enhance the accuracy of *E. coli* detection. Our findings might have significant impact on chicken nutrition and health.

Chapter 5

Conclusion

To understand the epidemiology of disease outbreaks and the emergence of antibiotic resistance in *E. coli*, a prevalence investigation of commensal *E. coli* in raw chicken meat which appear to be in good condition is crucial. This study's findings offer some preliminary information about microorganisms which are present in raw chicken meat, that are resistant to antibiotics. In Bangladesh, there are several reports on the prevalence of pathogenic *E. coli*, but barely anything is known about commensal *E. coli.* Therefore, this study will include fundamental data on the occurrence of *E. coli* in raw chicken meat from various open markets in the study area. The location chosen and sample analyzed in this study, however, is quite small, and a study with a larger population size from another region of the country will disclose the true prevalence of *E. coli* in the raw chicken meat in that area. To have a better grasp of the exact situation across the country and to help reduce potential hazards, extensive research on this subject should be done longitudinally. On that account, it is crucial to implement training and awareness campaigns to prevent the unjustified use of antibiotics and, as a result, the spread of drug resistance in poultry and livestock.

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