# Isolation of Antibiotic Resistant Salmonella spp. from Chicken samples Collected from Local Wet Markets of Dhaka City

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**A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment 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 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 have acknowledged all main sources of help.

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# Approval

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## Abstract

In Bangladesh poultry chicken is popular as a great protein source but it contains Zoonotic disease called Salmonellosis causing bacteria like *Salmonella spp.* Salmonellosis is a foodborne illness that is brought on by a number of non-typhoidal *Salmonella enterica* (NTS) serovars, primarily serovars *Enteritidis* and *Typhimurium*. The aim of this project was to Isolate the Zoonotic disease causing *Salmonella* and the rate of their antibiotic resistance. For this purpose 8 poultry Chicken samples were collected from different areas of Dhaka city. The highest amount of *Salmonella* isolates were found from flesh and that was 49.5%, then 22.7% came from cloacal swabs and 27.8% came from Liver. Antibiotic susceptibility test of these isolates was performed by using 10 antibiotics belonging to 7 groups which revealed that all the isolates (100%) were resistant to Tetracycline, Amoxicillin (93.8%), and Streptomycin (62.9%). However, commonly used antibiotics Meropenem, Imipenem and Ceftriaxone were reported to be very effective antibiotics against *Salmonella spp.* with sensitivity rates of 93.8%, 78.4% and 67%, respectively. This result indicates that there is a significant risk of cross-contamination and uncontrol use of different antibiotics to the poultry chicken. At the final stage PCR and Gel electrophoresis was performed for the Molecular confirmation of the *Salmonella spp.*



**Keywords:** *Salmonella spp.*;chicken samples; resistance; sensitivity; MDR, MAR index; PCR

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# CHAPTER 1

# INTRODUCTION

## 1.1 Study background

*Salmonella* species are zoonotic bacteria found in the intestinal tract of many animals, including cattle, pigs, horses, other mammals, reptiles, amphibians, and poultry (e.g. chickens, ducks, geese, and turkeys) (1) Nontyphoidal salmonellosis and (2) Typhoidal. *Salmonella* infection typically manifests as acute gastroenteritis that develops 12–72 hours after exposure. It is a rod shape, gram negative flagellated facultatively anaerobic bacilli that can cause salmonellosis (Giannella, 1996). *Salmonella* infection results can be different depending on the patient's age and immune system. As a result of this is diarrhoea, nausea, and vomiting, but it typically results in dehydration. Fever is another common occurrence. It can proceed to septicemia or localised infection in immunocompromised people. The costs associated with the condition are frequently astonishing, despite the fact that the sickness typically does not result in human fatality. It has the potential to kill weak persons, including young children, the elderly, and those with weakened immune systems (Castro-Vargas et al., 2020). Although *Salmonella* is commonly transmitted through food, recent outbreaks have highlighted direct or indirect contact with animals as a frequent route of transmission.

Both direct and indirect contact with infected animals can lead to human salmonellosis. Indirect transmission can occur through contact with anything in areas where animals live and roam or consumption of food/drink prepared in contaminated environments. Live poultry infected with *Salmonella* typically appear healthy, but can intermittently shed bacteria. *Salmonella* is widely known as a significant zoonotic pathogen, which has a negative impact on public health and causes significant economic losses.

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## 1.2 Transmission of *salmonella*

*Salmonella* is a foodborne pathogen that can be isolated by contaminated food, zoonotic animals and birds. It is a host restricted serovars as it is restricted to one specific host. For example *Salmonella* can be transmitted and colonised by invading, attaching and bypassing through the gastrointestinal path. It can be transmitted by a vertical transmission pathway such as a contaminated chicken can pass pathogenic *Salmonella* by laying eggs in part of moving from cloaca to reproduction organs. *Salmonella* also can spread through live and dead poultry, feeds, livestock and also the soil. For instance, through direct contact

salmonellosis outbreak occurs in live and dead chicken, farm chicken, feed, hatchery etc. which is the main cause of the epidemic situations (Hossain et al., 2021).

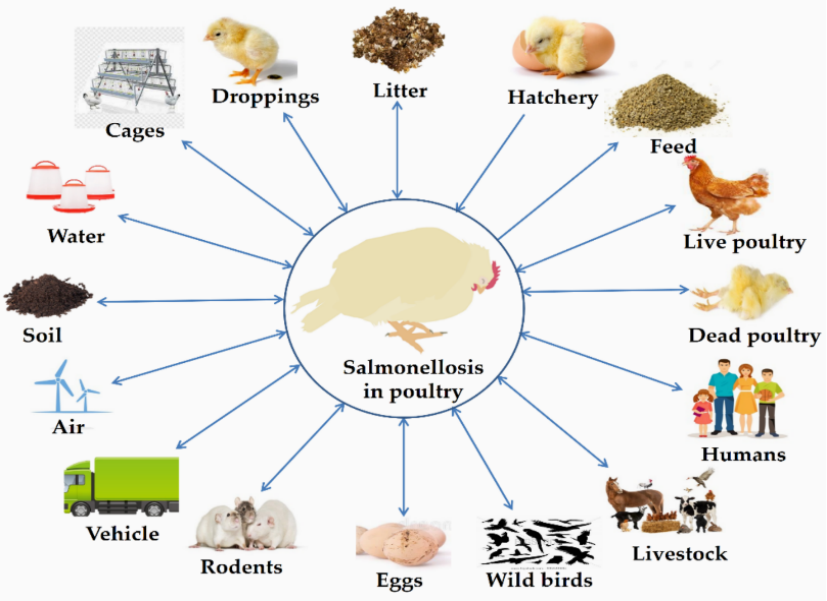



**Figure 1: Route of contamination of Salmonella spp from chicken poultries. The figure is adapted from (Hossain et al., 2021)**

## 1.3 Zoonotic Salmonellosis and its risk factors

*Salmonella spp.* can be transmitted from animal to human or conversely. ("Salmonella as a Zoonosis - SVA," 2020). Salmonella pathogen can be transmitted by food like meat, eggs etc, even from person to person known the disease as Salmonellosis. Everyone is susceptible to *Salmonella* pathogens. But aged people, infants and weak immune people are more sensitive to *Salmonella* pathogens. The incubation period is 1 to 3 days (Hossain et al., 2021)

If a person gets infected with *Salmonella* pathogen they can cause stomach pain, nausea, vomiting, diarrhoea, and fever ("Importance of Salmonellosis as a Zoonosis - Etiology and Symptomatology," n.d.). Infections frequently show very mild symptoms, but they might also go completely. Death is unusual. Reactive arthritis, an immune reaction that causes joint pain and fever, can occasionally develop as a subsequent consequence to the infection (Hossain et al., 2021).

## 1.4 Antimicrobial resistance

Antimicrobial resistance is a significant threat to both human and animal health. Antibiotic resistance in *Salmonella* is an ongoing danger to public health. Disease causing *Salmonella* in people and animals is a big problem in both industrialised and developing nations, including Bangladesh, according to several surveys. *Salmonella* is often rarely spread from person to person in affluent nations, however there have been notable institutional outbreaks (Threlfall, 2002) Once more, it is seen that emerging nations experience a large number of invasions with a high mortality rate (Threlfall, 2002).

In order to target them for more antibiotic resistance testing, it is now increasingly concentrated on the supply of animal foods. *Salmonella* is challenging to eradicate from its reservoir hosts, and the disease is frequently found in food animals. The majority of infections, hospitalizations, and fatalities related to foodborne illness are caused by non-typhoidal *Salmonella*. (Threlfall, 2002)

Its risks are mostly related to the inability to effectively treat patients who are afflicted with diseases resistant to antibiotics and to the significant risk of transmission of such germs. The misuse of antibiotics, particularly their overuse in therapeutic treatments and usage as growth promoters in animal production systems, is a factor in the emergence of this resistance. This is a major problem because a lot of the antibiotic-resistant *Salmonella* have been found in contaminated foods of animal origin, putting human health at risk and driving up healthcare costs (Google Scholar, n.d.). By the year 2050, 10 million deaths will be brought on by diseases that are resistant to antibiotics, according to some academics (Castro-Vargas et al., 2020b).

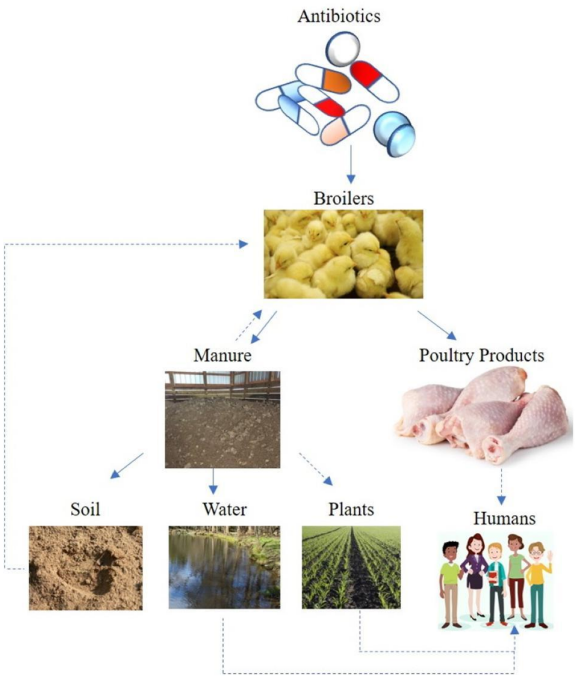


Figure 2 : Transmission route of potential AMR from broiler chicken. The figure is adapted from (Yang et al., 2019)

## 1.5 Cross contamination and prevention

Any disease that is dangerous and directly related to food processing is caused by cross contamination, which is a major problem. For instance, defeathering, scalding, eviscerating, and chilling are some processes that are acknowledged as the cause of salmonella pathogen cross contamination.("Cross-contamination and Recontamination by Salmonella in Foods: A Review," 2011) Pathogens might be transferred there when using hands and sharp tools to defeather. Once more, when water is scalded, pathogens can release to scald skin, feathers, and involuntary defecations. Hard scalding in hot temperatures, however, may not have much of an impact on microbes that are attached to skin, depending on the temperature. Cross contamination from the environments and even from the other animals might happen during the slaughtering process.("Cross-contamination and Recontamination by Salmonella in Foods: A Review," 2011)

So, prevention must be there. To prevent this, some steps must be followed.

1. Hands should be clean while handling raw materials and cooking.
2. Kitchen surfaces, and utensil also the equipments should be cleaned
3. Storing the chicken in the refrigerator is an another prevention method to avoid the cross contamination
4. To avoid cross contamination perishable food like chicken is refrigerated within 2 hours (*Salmonella and Food*, 2023).
5. Poultry chicken must be cooked within 165℉ or above (*Salmonella and Food*, 2023).

## 1.6 Aims and objective

Since the zoonotic infections are those that spread from one species of animal to another or conversely, chicken is a major source of this transmission around the world. As a result, *Salmonella spp.* can be quite hazardous to people. Therefore, the aim of this research is to determine their pathogenicity and raise awareness of Salmonellosis.

The objective of this study are given below:

1. Isolating *Salmonella spp.* from chicken samples around different areas in Dhaka city.
2. Checking the percentages of zoonotic Salmonellosis depending on the selected areas.
3. Comparing the percentage zoonotic disease causing *Salmonella* from different parts of chicken like flesh, liver and cloaca.
4. Differentiating the antimicrobial susceptibility result.
5. Focusing on the multi-drug resistance pattern of the *Salmonella spp.* by following the MAR index.

# CHAPTER 2

# MATERIALS AND METHOD:

## 2.1: Sample collection area

From November 15, 2022, to March 6, 2023, chicken samples were collected in the Mohakhali, Kawran bazar, Gulshan, and Mohammadpur regions. Due to their food choices and environment, broiler chicken was mostly the emphasis. We primarily concentrated on isolating *Salmonella spp.* for this experiment. For this, 8 chickens were collected from different wet markets around Dhaka city over a period of 5 months, and before processing the liver and meat, cloacal swabs were also collected while buying the chickens. In essence, the research was conducted to determine whether people may contract a foodborne illness from chicken.

Figure 3: Sample collection from chicken.

## 2.2 Sample processing

The chickens were bought from different wet markets targeted around Dhaka city. While buying the chicken, the cloacal swabs were also taken. The liver and flesh part from chickens were selected as samples. After taking the samples, they were taken to the BRAC University lab and flesh and liver were isolated. For processing, a very sterile sharp knife and scissors were used to cut the liver and flesh samples into small pieces. At first, the upper and lower surface of the flesh and liver were cut to avoid any contamination. Then, 10g flesh and liver were cut and separated & then transferred to room temperature distilled water that was autoclaved before. This mixture was homogenised and then filtered by using wattman filter paper. The solidifying portion was discarded and liquid was taken. Then 1ml mixture was transferred to 5 ml LB broth and waited for 4 hours for its growth. Cloacal swabs were not transferred to LB broth because of overgrowth. But they were taken only from the market by using a cotton swab and they were kept into falcon tubes where autoclaved saline was poured as transfer media.

## 2.3: *Salmonella* culture

Salmonella collection from the chicken parts were identified with different microbiological methods. The method that was applied for this research are given below,

### 2.3.1: Serial Dilution

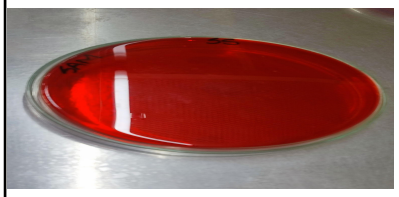
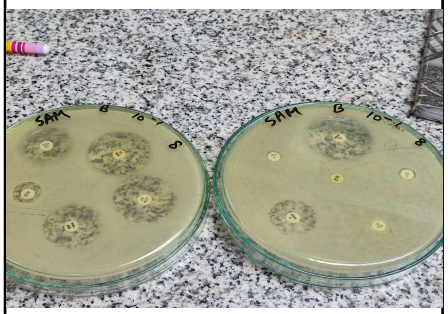
The collected samples were diluted into 10^1 fold for decreasing heavy growth of microbes and then mixed well by the vortex. For dilution we used 900 µl normal saline and 100 µl raw sample. The samples like liver and flesh, which was on incubation for enrichment were also diluted into 3 fold

### 2.3.2. Culture Media

In the lab we used different types of media for different purposes. For example, we used (1) Salmonella Shigella media (SS Media) for cultured purposes which is a selective media where only salmonella and shigella grows, (2) Nutrient Agar (NA media) as a subcultured media where we used it to culture the bacteria, (3) Mueller Hinton Agar (MHA media) which was used as antibiotic susceptible purpose, (4) Xylose lysine deoxycholate (XLD media) for confirming *Salmonella spp*. (4) Luria Bertani Broth (LB broth) for both as enrichment purpose & DNA extraction and lastly, (5) T1N1 media to stock the organism for further experiments.

### 2.3.3. Culture media procedure

Samples were collected from different areas and processed in the lab then the samples were spreaded on SS media to identify the salmonella. Salmonella gives black centre colonies on SS media that's why black centre isolated colonies were cultured on NA for storage, AST and DNA extraction. Then isolated colonies were picked from NA media and inoculated into saline then spreaded on the MHA media for AST. After performing the AST sensitivity, intermediate and resistance was measured. On the contrary, before DNA extraction , the isolated colonies were cultured on XLD to confirm and then single colonies were picked and inoculated into LB. Then DNA extraction was performed and separated DNA was used for PCR to get the confirmation. The media that were used for identification criteria for this whole research is shown in this **table 1**.

| Media | How it works | Figure |
|---|---|---|
| SS media | It was used for culture, incubated for 24 hours at 37˚ C. |  |
| NA media | This media was used for subculturing the organism for storing, DNA extraction & further workings. |  |
| MHA media | MHA media was used to know the efficiency of the antibiotics |  |

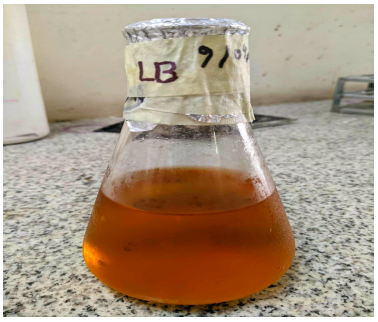
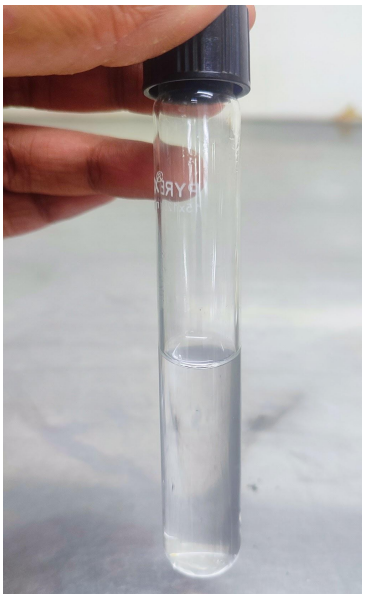
| | | |
|---|---|---|
| LB broth | This broth was used as an enrichment medium as it is rich in nutrients for the easy growth of any non-fastidious organisms, which is why this particular broth was used for enrichment and to check the growth if it was turbit or not, the Mcfarland formula was followed. The waiting period was 4 hours for the growth |   |
| T1N1 media | Salmonella spp. required a firmly bonded container to be stored in a cold, dry location away from harsh light, thus we employed T1N1 medium, which is often used to stock any isolate for subsequent investigations. Three years are allotted for storage. |  |
| Saline | Saline was used as a transport media that means saline was used to carry out the samples. Here the purpose of using saline was to take cloacal swabs from the local markets or wet markets. For the process, cotton swabs were used to take the samples and put it on the falcon tube where saline worked as a carrier to bring it to the lab. Furthermore, saline was used for serial dilution for the organisms cells viability and integrity. Saline did not have that property to react with biochemical properties and antimicrobial susceptibility tests. |  |

**Table 1: Names of media used for this research**

### 2.3.4: Sample plating:

Both 75 µl of raw and diluted samples were taken by using 100µl micropipette and we spreaded on an SS agar plate. For subculture isolated sure colonies were taken and strict on XLD plates than NA plates.

## 2.4 Antimicrobial susceptibility test.

Antimicrobial susceptibility tests were done following the Kirby-Bauer disk diffusion method . This was done for assessing an organism's sensitivity to or resistance to antibiotics, including *Salmonella*. Because of a higher diffusion rate, Special Mueller Hinton Agar (MHA) was used in tests to determine an antibiotic susceptibility. Toxins produced by bacteria so they did not interfere with medication, and it contained starch as an energy source that absorbs them. According to the Clinical and Laboratory Standards Institute, microorganisms were categorised as resistant (R), intermediate (I) and susceptible (S) based on their zone of diameter (mm) values (*Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*, n.d.).

The list of Antibiotics that were used are given in **Table 2:**

| S. No. | Antibiotic classes/group | Antibiotics | Sensitivity (mm) | Intermediate (mm) | Resistance (mm) |
|---|---|---|---|---|---|
| **1** | **Penicillins** | **Amoxicillin (AMX)** | **18** | **14-17** | **13** |
| **2** | **Quinolones** | **Ciprofloxacin (CIP)** | **31** | **21-30** | **20** |
| **3** | **Macrolide** | **Azithromycin (AZM)** | **13** | **-** | **-** |
| **4** | **Tetracycline** | **Tetracycline (TE)** | **15** | **12-14** | **11** |

| | | | | | |
|---|---|---|---|---|---|
| **5** | **Cephalosporin** | **Cefixime (CFM)** | **19** | **16-18** | **15** |
| **6** | **Aminoglycoside** | **Streptomycin (S)** | **15** | **12-14** | **11** |
| **7** | **Carbapenem** | **Imipenem (IPM)** | **23** | **20-22** | **19** |
| **8** | **Carbapenem** | **Meropenem (MRP)** | **23** | **20-22** | **19** |
| **9** | **Penicillin combination** | **Piperacillin (PIT)** | **25** | **21-24** | **20** |
| **10** | **Cephalosporin** | **Ceftriaxone (CTR)** | **23** | **20-22** | **19** |

**Table 2: The list of the Antibiotics**

## 2.5 DNA extraction:

DNA extraction was done to isolate the DNA so the polymerase chain reaction can be done. It is a molecular level test where DNA gets purified by using physical or chemical methods. The method that was used for this research was a hot water bath. Here, the overnight growth of salmonella colonies were put in the LB and waited for 24 hours. Then, several steps like, phosphate buffer saline or PBS was used for cleaning and TE buffer was used. Heat shock and cold shock was given and cold shock was given immediately as it gave the DNA protection.

## 2.6 PCR:

After the DNA extraction process was finished, PCR was conducted. A 35 cycle with primer annealing at 60°C for 30 seconds, DNA extension at 72°C for 60 seconds, and heat denaturation at 95°C for 30 seconds was required to obtain the desired results (*Rapid Molecular Approach for Simultaneous Detection of Salmonella Spp., Shigella Spp., and Vibrio Cholera*, 2016).

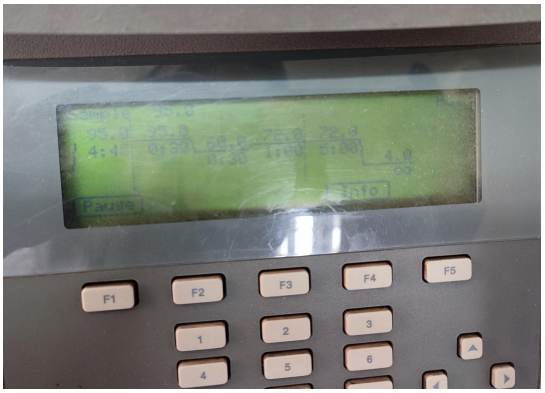



**Figure 4: PCR running**

## 2.7 Agarose Gel electrophoresis

Agarose Gel electrophoresis was done to determine whether the band size was accurate or not. DNA samples were inserted into wells at one end of a gel and pulled through the gel by an electric current after the fragmented DNA had been loaded on the wall and placed there.

# CHAPTER 3

# RESULT

## 3.1 *Salmonella spp*. colonies on culture media

After a 24-hour incubation period at 37°C, all 97 isolates produced colourless colonies with black centres on SS agar plate and XLD agar plate, which represented the desired growth appearance of *Salmonella spp.* on this specific growth media. Also NA plate was used to increase the growth of presumptive *Salmonella spp.*

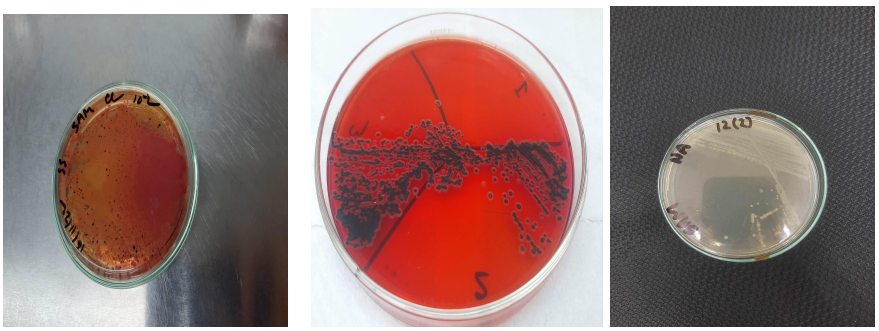


**Figure 5: *Salmonella* growth on SS, XLD & NA plates**

## 3.2 Polymerase Chain Reaction or PCR

PCR was done for the confirmatory test and 97 were confirmed by these molecular methods.

## 3.3 Gel electrophoresis

Gel electrophoresis was used for isolating *Salmonella spp.* According to the primer, along with its condition, it was noted that the primer size should be 403 bp.

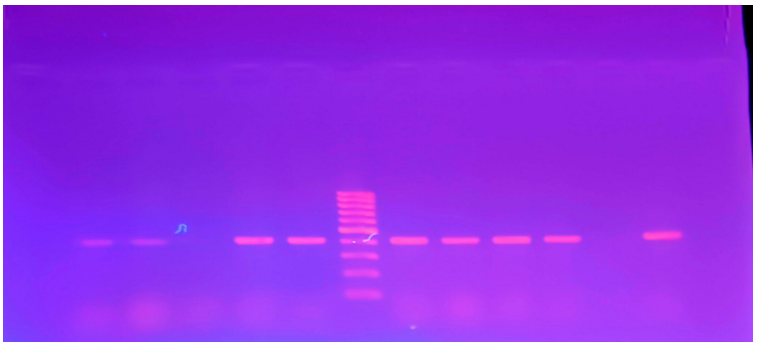


**Figure 10: Confirmed *Salmonella* at 403 bp**

## 3.4 *Salmonella spp.* in chicken samples

We had collected 8 chickens and from each chicken three sample parts (cloaca, liver and flesh) were selected and thus resulting as (3x8)=24 samples were tested further for isolating *Salmonella*.

From a total of 8 chicken, *Salmonella spp.* was detected from all the chickens among a total 8 cloacal swab, *salmonella spp.* were detected from 4 and both 8 livers and 8 fleshes, *Salmonella spp.* were detected by all of them. So from a total 24 chicken samples, we got *Salmonella spp.* in 20 samples.

**Table 3** is basically showing the sample isolated from chicken sample sites.

| Total chicken (8) | Sample carrying *salmonella* from the total samples (8x3=24) |
|---|---|
| Cloacal swabs (8) | 4 |
| Flesh (8) | 8 |
| Liver (8) | 8 |

**Table 3: Salmonella isolation from samples**

## 3.5 Salmonella spp in 20 samples

The total chickens were 8 and these were collected from different areas in Dhaka city. From a total of 24 samples 97 isolates were confirmed and collected for further results. Here is a **table 4(a)** of number of sample numbers and there specific isolated colonies from chicken sample sites

| **wet markets** | **Total 97 Isolated colonies** | **Liver** | **Flesh** | **Cloacal swab** |
|---|---|---|---|---|
| **Mohakhali small kacha bazar** | **15** | **6** | **5** | **4** |
| **Mohakhali hauque kacha bazar** | **19** | **8** | **7** | **4** |
| **Mohakhali kacha bazar** | **9** | **5** | **4** | **x** |

| | | | | |
|---|---|---|---|---|
| **Saat tola kacha bazar** | **5** | **x** | **5** | **x** |
| **Korail BTCL Bazar** | **20** | **4** | **5** | **11** |
| **DCC market** | **11** | **2** | **5** | **4** |
| **Karwan Bazar** | **8** | **x** | **8** | **x** |
| **Mohammadpur krishi market** | **10** | **x** | **10** | **x** |

**Table 4(a): Number of samples and their specific isolated colonies**

A total of 97 isolates out of 24 samples were collected. From this, it can be shown that 48 colonies were isolated from flesh and 27 were isolated from liver and 22 were isolated from cloacal swabs. That means, 49.5% where highest positive rate for *salmonella* came from flesh, 27.8% where highest positive rate for *salmonella* from liver, 22.7% where highest positive rate for *salmonella* from cloacal swabs.

Here is a **table 4(b)** of number of colonies from 24 sample-

| SAMPLE NAME | Total colonies & percentage |
|---|---|
| **CLOACAL swabs** | **22 (22.7%)** |
| **LIVER** | **27(27.8%)** |
| **FLESH** | **48(49.5%)** |

**Table 4(b): The list of sample names, total colonies & their percentage**

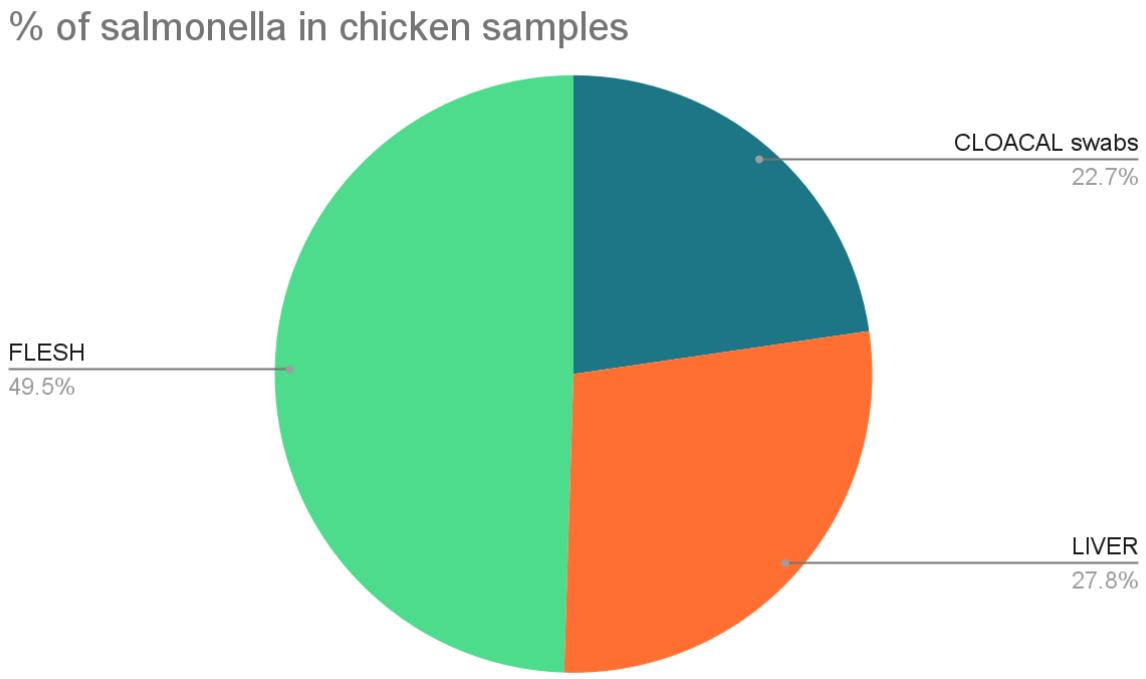



**Figure: Growth ratio of *Salmonella spp.* in chosen chickens' parts**

## 3.5 Percentage of *Salmonella spp.* positive chicken samples as area basis

According to this experiment, the positive *Salmonella spp.* were higher in Korail BTCL bazar which was 20.6%. After that, mohakhali haque kacha bazar, Mohakhali small kacha bazar , DCC market, mohammadpur krishi market, mohakhali kacha bazar, karwan bazar and last also the lowest percentage saat tola kacha bazar were respectively 19.6%, 15.5%, 11.3%, 10.3%,9.3%, 8.2% and lastly 5.2%

**Table 5** is showing the total percentages of Salmonella from each wet markets

| Wet market | Total colonies & percentage |
|---|---|
| **Mohakhali small kacha bazar** | **15(15.5%)** |

| | |
|---|---|
| **Mohakhali hauque kacha bazar** | **19(19.6%)** |
| **Mohakhali kacha bazar** | **9(9.3%)** |
| **Saat tola kacha bazar** | **5(5.2%)** |
| **Korail BTCL Bazar** | **20(20.6%)** |
| **DCC market** | **11(11.3%)** |
| **Karwan Bazar** | **8(8.2%)** |
| **Mohammadpur krishi market** | **10(10.3%)** |

**Table 5: Name of the wet markets, total colonies and total percentages**

## 3.4 Salmonella antimicrobial results:

Antimicrobial test was done to check the resistance of different antibiotics for *salmonella spp*. Here is a picture showing the no zone areas and zone areas.

**Figure 8: Zone and no zone area in MHA plate.**

We performed an antimicrobial Susceptibility Test (AST) for *Salmonella spp.* Here 7 groups of antibiotics were used to do AST, those are Beta-Lactam, Cephalosporin, Carbapenem, Aminoglycoside, Tetracycline, Macrolide, Quinolones. From these groups we used 11 different antibiotics and among them Tetracycline was 100% resistant against the *Salmonella spp.* and Meropenem was 93.8% sensitive against the *Salmonella spp.* Among the 200 presumptive *Salmonella spp.* confirmed 97 *Salmonella* were taken for antibiotic susceptibility test.

Table 6 is showing the percentages of antibiotic resistance, intermediate and sensitivity.

| **Antibiotic classes** | **Antibiotics** | **Total percentage of resistance** | **Total percentage of intermediate** | **Total percentage or sensitive** |
|---|---|---|---|---|
| **Penicillins** | **Amoxicillin (AMX)** | **93.8** | **1** | **5.2** |
| **Penicillin combination** | **Piperacillin (PIT)** | **2.1** | **8.2** | **89.7** |

| | | | | |
|---|---|---|---|---|
| **Cephalosporin** | **Cefixime (CFM)** | **37.1** | **9.3** | **53.6** |
| | **Ceftriaxone (CTR)** | **25.8** | **7.2** | **67** |
| **Carbapenem** | **Meropenem (MRP)** | **1** | **5.2** | **93.8** |
| | **Imipenem (IPM)** | **13.4** | **8.2** | **78.4** |
| **Aminoglycoside** | **Streptomycin (S)** | **62.9** | **8.2** | **28.9** |
| **Tetracycline** | **Tetracycline (TE)** | **100** | **X** | **X** |
| **Macrolide** | **Azithromycin (AZM)** | **X** | **45.3** | **54.7** |
| **Quinolones** | **Ciprofloxacin (CIP)** | **90.7** | **9.3** | **X** |

**Table 6: Name of the antibiotics, their classes and results**

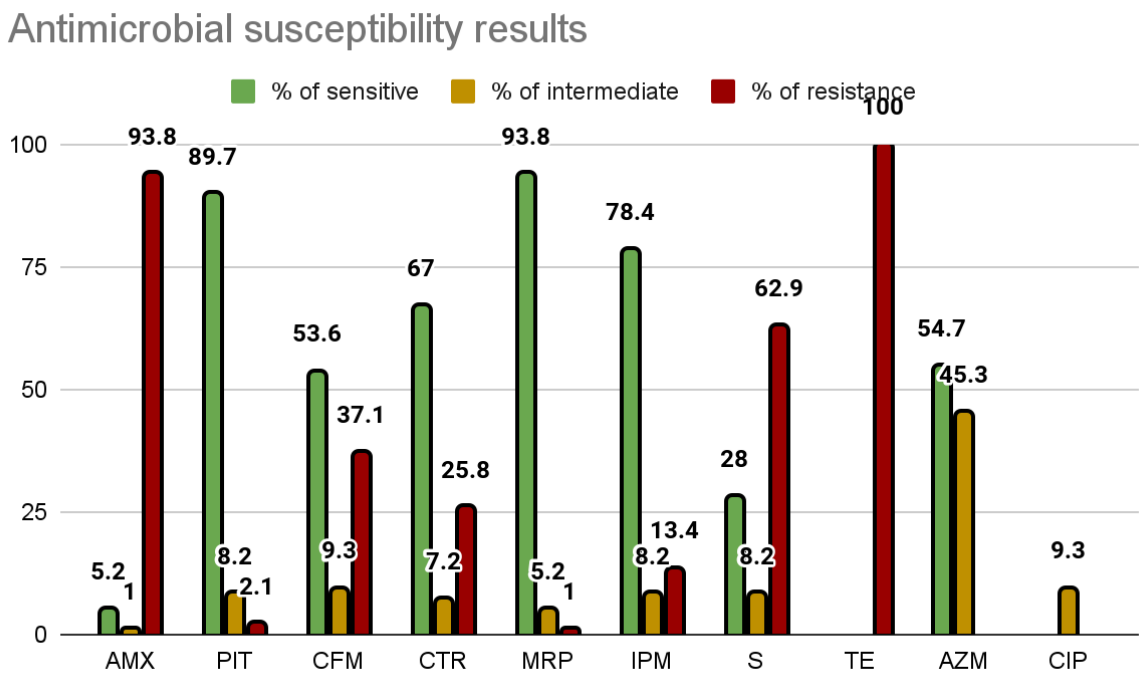



**Figure 9: Percentages of resistance rate, sensitive rate and intermediate rate of different antibiotics**

## 3.5 Measuring of Multiple Drug resistance (MDR) of the isolates

Isolates of Multiple Drug resistance (MDR) reveals the resistance at least in 3 types of antibiotic groups. In this research, there were at least 3 resistant antibiotic groups present in 97 isolated colonies .

## 3.6 Measuring of Multiple Antibiotic Resistance (MAR) Index of the isolates

The isolates of Multiple Antibiotic Resistance (MAR) Index revealed that all of them were resistant to at least one antibiotic of more than two different groups and had MAR Index of 0.28 and higher.

| Serial number | Samples of salmonella spp. | Resistance antibiotic (a) | Total tested antibiotic (b) | MAR (a/b) | Level of resistance |
|---|---|---|---|---|---|
| 1 | S1L10-1 | 4 | 10 | 0.4 | HIGH |
| 2 | S1L10-1 | 4 | 10 | 0.4 | HIGH |
| 3 | S1CS10-1 | 3 | 10 | 0.3 | HIGH |
| 4 | S1L10-1 | 3 | 10 | 0.3 | HIGH |
| 5 | S1CS10-2 | 4 | 10 | 0.4 | HIGH |
| 6 | S1B10-1 | 4 | 10 | 0.4 | HIGH |
| 7 | S1B10-1 | 5 | 10 | 0.5 | HIGH |
| 8 | S1L10-1 | 4 | 10 | 0.4 | HIGH |
| 9 | S1B10-1 | 4 | 10 | 0.4 | HIGH |
| 10 | S1L10-3 | 4 | 10 | 0.4 | HIGH |
| 11 | S1B10-1 | 4 | 10 | 0.4 | HIGH |
| 12 | S1CS10-1 | 4 | 10 | 0.4 | HIGH |

| | | | | | |
|---|---|---|---|---|---|
| **13** | **S1B10-1** | **3** | **10** | **0.3** | **HIGH** |
| **14** | **S1L10-1** | **5** | **10** | **0.5** | **HIGH** |
| **15** | **S1CS10-3** | **4** | **10** | **0.4** | **HIGH** |
| **16** | **S2B10-2** | **4** | **10** | **0.4** | **HIGH** |
| **17** | **S2L10-1** | **4** | **10** | **0.4** | **HIGH** |
| **18** | **S2F10-1** | **4** | **10** | **0.4** | **HIGH** |
| **19** | **S2L10-1** | **3** | **10** | **0.3** | **HIGH** |
| **20** | **S2L10-1** | **5** | **10** | **0.5** | **HIGH** |
| **21** | **S2L10-1** | **4** | **10** | **0.4** | **HIGH** |
| **22** | **S2L10-1** | **6** | **10** | **0.6** | **HIGH** |
| **23** | **S2F10-1** | **4** | **10** | **0.4** | **HIGH** |
| **24** | **S2F10-1** | **5** | **10** | **0.5** | **HIGH** |
| **25** | **S2CS10-2** | **4** | **10** | **0.4** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **26** | **S2CS10-2** | **4** | **10** | **0.4** | **HIGH** |
| **27** | **S2CS10-2** | **5** | **10** | **0.5** | **HIGH** |
| **28** | **S2CS10-2** | **4** | **10** | **0.4** | **HIGH** |
| **29** | **S2B10-1** | **5** | **10** | **0.5** | **HIGH** |
| **30** | **S2B10-1** | **5** | **10** | **0.5** | **HIGH** |
| **31** | **S2B10-1** | **4** | **10** | **0.4** | **HIGH** |
| **32** | **S2L10-1** | **5** | **10** | **0.5** | **HIGH** |
| **33** | **S2L10-1** | **4** | **10** | **0.4** | **HIGH** |
| **34** | **S2L10-1** | **5** | **10** | **0.5** | **HIGH** |
| **35** | **S3TD** | **3** | **10** | **0.3** | **HIGH** |
| **36** | **S3LD** | **4** | **10** | **0.4** | **HIGH** |
| **37** | **S3TD** | **4** | **10** | **0.4** | **HIGH** |
| **38** | **S3TD** | **5** | **10** | **0.5** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **39** | **S3L10-2** | **4** | **10** | **0.4** | **HIGH** |
| **40** | **S3L10-1** | **4** | **10** | **0.4** | **HIGH** |
| **41** | **S3TD** | **4** | **10** | **0.4** | **HIGH** |
| **42** | **S3L10-1** | **4** | **10** | **0.4** | **HIGH** |
| **43** | **S3TD** | **4** | **10** | **0.4** | **HIGH** |
| **44** | **S4T10-2** | **6** | **10** | **0.6** | **HIGH** |
| **45** | **S4T10-3** | **7** | **10** | **0.7** | **HIGH** |
| **46** | **S4T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **47** | **S4TD** | **3** | **10** | **0.3** | **HIGH** |
| **48** | **S4TD** | **5** | **10** | **0.5** | **HIGH** |
| **49** | **S5T10-1** | **7** | **10** | **0.7** | **HIGH** |
| **50** | **S5CS10-2** | **5** | **10** | **0.5** | **HIGH** |
| **51** | **S5CS10-2** | **6** | **10** | **0.6** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **52** | **S5CS10-1** | **6** | **10** | **0.6** | **HIGH** |
| **53** | **S5TD** | **7** | **10** | **0.7** | **HIGH** |
| **54** | **S5LD** | **8** | **10** | **0.8** | **HIGH** |
| **55** | **S5LD** | **7** | **10** | **0.7** | **HIGH** |
| **56** | **S5TD** | **7** | **10** | **0.7** | **HIGH** |
| **57** | **S5LD** | **9** | **10** | **0.9** | **HIGH** |
| **58** | **S5LD** | **8** | **10** | **0.8** | **HIGH** |
| **59** | **S5TD** | **7** | **10** | **0.7** | **HIGH** |
| **60** | **S5CS10-1** | **7** | **10** | **0.7** | **HIGH** |
| **61** | **S5CS10-1** | **4** | **10** | **0.4** | **HIGH** |
| **62** | **S5CS10-1** | **4** | **10** | **0.4** | **HIGH** |
| **63** | **S4TD** | **7** | **10** | **0.7** | **HIGH** |
| **64** | **S5CS10-** | **8** | **10** | **0.8** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **65** | **S5CS10-2** | **4** | **10** | **0.4** | **HIGH** |
| **66** | **S5CS10-3** | **6** | **10** | **0.6** | **HIGH** |
| **67** | **S5CS10-3** | **4** | **10** | **0.4** | **HIGH** |
| **68** | **S5CS10-1** | **4** | **10** | **0.4** | **HIGH** |
| **69** | **S6LD** | **3** | **10** | **0.3** | **HIGH** |
| **70** | **S6LD** | **4** | **10** | **0.4** | **HIGH** |
| **71** | **S6CS10-2** | **5** | **10** | **0.5** | **HIGH** |
| **72** | **S6CS10-1** | **6** | **10** | **0.6** | **HIGH** |
| **73** | **S6CS10-2** | **4** | **10** | **0.4** | **HIGH** |
| **74** | **S6CS10-2** | **5** | **10** | **0.5** | **HIGH** |
| **75** | **S6TD** | **5** | **10** | **0.5** | **HIGH** |
| **76** | **S6TD** | **6** | **10** | **0.6** | **HIGH** |
| **77** | **S6TD** | **7** | **10** | **0.7** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **78** | **S6T10-1** | **4** | **10** | **0.4** | **HIGH** |
| **79** | **S6T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **80** | **S7T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **81** | **S7TD** | **5** | **10** | **0.5** | **HIGH** |
| **82** | **S7TD** | **7** | **10** | **0.7** | **HIGH** |
| **83** | **S7TD** | **7** | **10** | **0.7** | **HIGH** |
| **84** | **S7T10-2** | **6** | **10** | **0.6** | **HIGH** |
| **85** | **S7LD** | **6** | **10** | **0.6** | **HIGH** |
| **86** | **S7T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **87** | **S7T10-2** | **7** | **10** | **0.7** | **HIGH** |
| **88** | **S8T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **89** | **S8T10-1** | **7** | **10** | **0.7** | **HIGH** |
| **90** | **S8T10-1** | **7** | **10** | **0.7** | **HIGH** |

| | | | | | |
|---|---|---|---|---|---|
| **91** | **S8T10-1** | **5** | **10** | **0.5** | **HIGH** |
| **92** | **S8T10-2** | **4** | **10** | **0.4** | **HIGH** |
| **93** | **S8T10-1** | **6** | **10** | **0.6** | **HIGH** |
| **94** | **S8TD** | **5** | **10** | **0.5** | **HIGH** |
| **95** | **S8TD** | **5** | **10** | **0.5** | **HIGH** |
| **96** | **S8TD** | **6** | **10** | **0.6** | **HIGH** |
| **97** | **S8TD** | **5** | **10** | **0.5** | **HIGH** |

**Table 7: List of the measures of Multiple Antibiotic Resistance (MAR) Index of the isolates & the level of resistance**

# CHAPTER 4

## Discussion:

The goal of this research was to isolate *Salmonella spp.* from chicken. Chicken is considered as a great source for Salmonellosis and many researches have shown that it might occur in children who are less than 6 years old and elderly people who are aged more than 65 and also people with weak immunity (*Salmonella and Food*, 2023). In Bangladesh, samples from poultry and their environments both had a similar amount of *Salmonella spp.* (25%) (Hassan et al., 2016). *Salmonella* has reportedly been shown to be a common microflora in raw animal feed, poultry feed, and other food sources. *Salmonella* contamination of fresh chicken poultries in many farms or warehouse are usually brought on by careless and unhygienic handling techniques, which then spread to poultry and poultry products, including eggs, plastic-wrapped poultry meat, and ready-to-eat foods, from food handlers with poor personal hygiene. Even it may be cross contaminated at the time of processing while buyers bought them and processed it.

The results of this research revealed that the largest concentration of proven *Salmonella spp.* was found mostly in the flesh, which was obtained from a chicken. The liver came next and finally the lowest rate from the cloaca was collected.

The research also investigated that among the areas the highest number of obtained *Salmonella spp.* was from the korail BTCL bazar which was 20.6%. After that, Mohakhali haque kacha bazar, Mohakhali small kacha bazar , DCC market, Mohammadpur krishi market, Mohakhali kacha bazar, Kawran bazar and last also the lowest percentage Saat tola kacha bazar were respectively 19.6%, 15.5%, 11.3%, 10.3%,9.3%, 8.2% and last 5.2%.

From the Antimicrobial Susceptibility Test (AST), we got a result showing the resistance, sensitivity and intermediate rate of various antibiotics. For the AST, 7 groups of antibiotics are used for the AST, these are Beta Lactam, Cephalosporin, Carbapenem, Aminoglycoside, Tetracycline, Macrolide, Quinolones. From these groups we used 11 antibiotics and among them Tetracycline was 100% resistant against the *Salmonella spp.* and Meropenem was 93.8% sensitive against the *Salmonella spp.* At the same time 90.7% of Ciprofloxacin and 93.8% of Amoxicillin was resistant against the *salmonella spp.* On the other hand, 89.7% of Piperacillin and 78.4% Imipenem was sensitive against *Salmonella spp.* Only 45.3% of Azithromycin was intermediate against the *Salmonella spp.*

The Multiple Antibiotic Resistance (MAR) index for *Salmonella spp.* is also determined using the overall ratio of the resistance antibiotics. It has long been understood that a high number of antibiotics should be indicated by a number greater than 0.2. The MAR index is a reliable, precise & affordable technique to determine where the antibiotic-resistant bacteria first appeared. The resistance number is quite high, as it can be seen in the result section. Since they will soon develop resistance, intermediate antibiotics are likewise considered to be the source of this resistance. Currently, it can be found that a sample named S5LD includes nine antibiotic resistant. The MAR index now places that higher. As a result of this, there is a large danger of cross-contamination with food products that have been contaminated with *Salmonella* and have a high level of rasistant antibiotics. All of the bacteria identified from chicken were reported to be multi-drug resistant.

Polymerase chain reaction was also done for the confirmation. This method is largely used as a molecular level confirmation where the DNA is amplified into multiple copies just to identify the *Salmonella spp.* In PCR, a section of the genome to be amplified is chosen using DNA fragments called primers. Multiple rounds of DNA synthesis are then used to amplify that segment (*Polymerase Chain Reaction (PCR)*, 2023), the principle is quite easy and effective. For this pure 16S gene should be accrued by DNA extraction and it was aligned against primer to identify the kind of bacteria (Barghouthi, 2011). Here a specific primer was used to detect *Salmonella spp.* To check the band size, later Gel electrophoresis was done. The band size was 403 bp. So by this, 97 samples were confirmed among 200.

# CHAPTER 5

## Conclusion

The research is based on how *Salmonella spp.* affects people from chicken. From the research people can understand its pathogenicity and how it can affect a large range of people. The research from zoonotic topics is not a new concept in Bangladesh as several researchers are working on the pathogenicity of zoonotic organisms. There is much research into zoonosis around the world as well as in our country. Chicken is a major sector that plays a great role in causing Salmonellosis. Chicken is consumed by people of almost every age. Even the poultry farms are also spreading Salmonellosis among humans that causes MDR *Salmonella*. According to many researches it can be seen that Salmonellosis is a very dangerous disease even if it may not be considered as life threatening by the general population. So some prevention must be there. Only proper knowledge, guidelines and hygiene maintenance can help reduce Salmonellosis. MDR *Salmonella* must be focused. Though the treatment of MDR bacteria is considered as expensive, it should be monitored and further work should be done.

Not only *Salmonella*, various pathogenic organisms can affect food if proper hygiene is not followed and many people like elders, children, even weak people are in the first line of this harmful disease. As poultry farms are the main source of chicken, which is spreading much more dangerous pathogens to humans that's why, poultry farms should be noted to the researchers and more and more research should be done on this topic. So, to conclude, the research should go further and alert people by giving information of its risk assessment on the microbiological quality of broiler chickens.

## Workflow:

**chicken cloacal swab**

collection of cloacal swab from healthy chicken

Transport samples to saline

Diluted into 3 folds and spreading onto SS agar

Marked the colony and subculture on XLD & NA Agar plate to get single colony

AST, DNA extraction and stock culture

PCR for final detection

**<u>chicken liver and flesh</u>**

collection of liver and flesh from healthy chicken

Transport samples to an icebox

Enriched on LB for 4 hours and diluted in 3 fold

Spread the samples onto SS agar

Marked the colony and subculture on XLD & NA Agar plate to get single colony

AST, DNA extraction and stock culture

PCR for final detection

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