Isolation, Identification and Antimicrobial Susceptibility Profile of *Salmonella spp.* from Chicken Meat Samples Collected from Wet Markets of Dhaka City

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A Thesis Report submitted to the Programme of Microbiology in the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Undergraduate Programme.

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Declaration

It is hereby declared that,

1. The thesis report submitted is my own original work while completing undergraduate degree at BRAC University.

2. The report 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 report 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

Raw chicken commonly carries *Salmonella spp*., a major source of highly predominant foodborne disease. Improper handling or undercooking increases contamination risk. This study examined isolation, molecular detection and antibiotic resistance of *Salmonella spp*. from Chicken meat samples to understand its spread and enhance food safety practices. 50 raw meat samples collected from local markets of Badda, Gulshan, Dhanmondi, Uttara, and Segunbagicha of Dhaka city. The samples were homogenized and diluted to four folds (10-4) and were spreaded on Xylose Lysine Deoxycholate (XLD) agar and *Salmonella-Shigella* (SS) agar. Presumptive Salmonella colonies, characterized by red colonies with black centers, were selected. The Polymerase Chain Reaction (PCR) was used to confirm suspected *Salmonella* colonies sub-cultured on Nutrient Agar. The Kirby-Bauer disc diffusion method was used to test the antibiotic susceptibility of *Salmonella* isolates. A total of 15 positive *Salmonella* samples were found, which is 30% of the total samples. Among the positive ones, 40% was obtained from gizzard, 33% from liver, and 13% from meat. 15 positive isolates and 10 antibiotics were used for the Antimicrobial Susceptibility Test (AST), Ampicillin and Tetracycline were resistant to most of the strains of *Salmonella spp.*, which is in between 66.66% to 73.33%. Additionally, Sulfamethoxazole had the lowest moderate resistance whereas Tigecycline and Nalidixic Acid had the maximum moderate resistance which is 33.33%. In contrast, no isolates were able to develop resistance against Ciprofloxacin. Notably, only 46.77% isolates were sensitive to the antibiotics whereas 53.33% were multi-drug resistant. *Salmonella spp*. is a major cause of food-borne outbreaks. Most infections by *Salmonella* are attributed to consumption of contaminated food, especially those foods involving poultry and its products. This is a significant public health concern as it limits treatment options for *Salmonella* infections because of antibiotic resistance genes.

Keywords: *Salmonella spp*., Foodborne disease, Antimicrobial Susceptibility test, Multidrug resistance, Polymerase Chain Reaction, Dhaka city markets.

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List of Acronyms

AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
DNA	Deoxyribonucleic acid
MDR	Multidrug Resistance
MHA	Mueller Hinton Agar
NA	Nutrient Agar (NA)
PCR	Polymerase Chain Reaction
SB	Selenite Broth
SS	*Salmonella-Shigella* Agar
TE	Tris-EDTA
UV	Ultraviolet
XLD	Xylose Lysine Deoxycholate Agar
ZOI	Zones of Inhibition

Chapter 1

Introduction

1.1 Introduction

Salmonella, a significant foodborne pathogen, is often associated with disease outbreaks across the globe. Its significant role in public health underscores the urgent need for effective interventions. Most infections by *Salmonella* are attributed to consumption of contaminated food-especially those foods involving poultry and their products. *Salmonella spp*. are infamous for causing enteric fever due to *Salmonella Typhi* and the non-typhoidal salmonellosis caused by *Salmonella enterica serovars*, the latter being highly predominant foodborne illnesses in both the developed and developing countries (Andino & Hanning, 2015; Tauxe et al., 2019). *Salmonella* is a common foodborne disease associated with the intestinal system of food-producing animals. *Salmonella* species cause many gastrointestinal illnesses in people.The global scale of the problem is staggering, with an estimated 1.3 billion cases of salmonellosis and 155,500 deaths annually (Sun et al., 2021)**.** In 2018, *Salmonella* was the second most prevalent cause of foodborne illnesses within the European Union, with 91,856 reported cases (Lanier et al., 2018). Similarly, in China, 70–80% of foodborne illnesses have been linked to *Salmonella* infections (Sun et al., 2021). These statistics underscore the severity of the issue.Over 2,500 serovars of *Salmonella* have been identified globally (Antunes et al., 2016). *Salmonella* is a genus of gram-negative bacteria (family Enterobacteriaceae) containing common foodborne pathogens (Deguenon et al., 2019). *Salmonella* has the highest infection rate compared to other foodborne pathogens, causing many countries a more significant economic burden (Ramirez-Hernandez et al., 2018). Salmonellosis is primarily associated with consuming food items contaminated with

Salmonella (Ehuwa et al., 2022; Rotana et al., 2021). Animal-based foods, particularly chicken and other poultry products are the primary sources of *Salmonella* transmission to humans (Sun et al., 2021). After ingesting a significant amount of *Salmonella*, the pathogen colonises the intestinal tract, leading to various clinical outcomes, including gastroenteritis, bacteremia, and typhoid fever. Numerous outbreaks of salmonellosis associated with poultry consumption highlight the critical role these products play in spreading *Salmonella* (Antunes et al., 2016; Lee et al., 2015). The increasing prevalence of *Salmonella* infections presents considerable difficulties for developing nations due to the high expenses linked to treatment, preventive, and control strategies (Lee et al., 2015). The extensive variety of *Salmonella serovars* and the advent of novel serotypes, frequently associated with antibiotic resistance, have heightened apprehensions among researchers and the public (Antunes et al., 2016).

The poultry industry in Bangladesh has become a very vital and lucrative sector that contributes to the economy and employment opportunities within the country. With over 350 million poultry, as reported by the Department of Livestock Services (DLS), poultry provides an affordable source of protein and nutrition for people across all income levels (Islam et al., 2020). *Salmonella* contamination in raw chicken meat poses significant risks to Bangladesh, given the country's heavy dependence on poultry as a primary protein source and the expansion of its poultry sector. *Salmonella* infections, frequently resulting from the ingestion of undercooked or mishandled poultry, pose considerable public health issues, including epidemics of gastroenteritis, typhoid fever, and several other foodborne disorders. These infections impose a significant burden on the healthcare system, especially in rural regions where access to medical services is constrained. Additionally, the prevalence of multidrug-resistant (MDR) *Salmonella* strains in poultry exacerbates treatment challenges and elevates the risk of extended illness and increased healthcare expenses (Hoque et al., 2019). In Bangladesh, insufficient sanitary procedures in poultry processing, inadequate cold chain management, and a lack of consumer understanding regarding food safety intensify the dangers (Ehuwa et al., 2022). This condition jeopardises public health and endangers the economic viability of the poultry sector, a crucial component of Bangladesh's agricultural economy. This disease causes high mortality rates and decreased productivity in the poultry sector (Pal et al., 2015; Gast, 1997). Additionally, salmonellosis is responsible for conditions such as fowl typhoid and pullorum disease, which can lead to various infections, even affecting embryos (Gast, 1997).

The prevalence of *Salmonella* infections is quite devastating since it causes massive illness and deaths annually across the world. The economic repercussions stemming from the illness burden of these infections are substantial, manifesting as treatment expenses and preventive actions implemented by the healthcare and food sectors (Scallan et al., 2011).

1.2 Source and Transmission of *Salmonella spp.*

Raw or undercooked chicken meat is the most frequently contaminated with extended-spectrum β-lactamases (ESBL) producing *Salmonella* compared to other meat sources (Hoque et al., 2019). The extent of *Salmonella* contamination in chicken meat is primarily influenced by factors such as slaughtering procedures, sanitation during processing and packaging, maintenance of an adequate cold chain from processing to retail and consumer levels, and personal hygiene practices during handling at the retail stage (Hoque et al., 2019).

Poultry and poultry products have been considered major reservoirs for *Salmonella,* since chicken meat and organs are sources of infection after contamination by means of poor handling, processing, and improper storage. CDC (2020) states that *Salmonella* contamination in poultry production can occur during slaughter, processing, or through contact with contaminated surfaces.

Infection can be transmitted to individuals through the consumption of undercooked or raw poultry, necessitating contamination control measures in poultry to mitigate human infections, as stated by (Meakins et al., 2003). Wet markets, prevalent in underdeveloped nations such as Bangladesh, offer fresh, unpackaged food items and live animals, rendering them accessible and economical. In Dhaka, these marketplaces are essential for fresh poultry, seafood, and vegetables. Nevertheless, elevated foot traffic, inadequate refrigeration, and exposure to environmental conditions foster an environment conducive to bacterial proliferation, hence heightening the risk of infection, especially with *Salmonella.* Research indicates that poultry in these marketplaces is particularly vulnerable to contamination, heightening public health concerns in metropolitan regions such as Dhaka (Aung & Chang, 2021).

1.3 Antimicrobial Resistance in *Salmonella Species*

An upsurge in the rate of AMR within *Salmonella,* particularly serovars associated with poultry, is considered the most severe form of contamination by this bacterium. Inappropriate use and overuse of antibiotics in farming will continue to afford the best opportunity for development of antibiotic-resistant bacteria and more easily spread to humans via consumption of contaminated chicken. Antimicrobial-resistant strains of *Salmonella* provide a significant public health concern, as infections from these strains are difficult to treat, leading to prolonged illnesses, elevated healthcare expenses, and increased mortality rates. It is particularly vital for poor nations where controls on antibiotic usage in agriculture are low and public awareness of the problems associated with antimicrobial resistance is lacking, as exemplified by Bangladesh.

Antimicrobial resistance has emerged as a critical global concern, affecting all dimensions of health. By 2050, it is anticipated that antimicrobial resistance (AMR) would result in hundreds of millions of human fatalities, a significant economic downturn, substantial harm to livestock output and the impact of AMR will be especially severe in low- and middle-income countries, including Bangladesh (Rahman et al., 2020; Islam et al., 2021). Antimicrobial resistance jeopardises food security by resulting in production losses within the chicken industry, with antimicrobial-resistant *Salmonella* emerging as a significant worldwide public health issue. Human activities have contaminated poultry farm habitats with antibiotic residues and resistant bacteria (Orubu et al., 2021). The emergence of antimicrobial-resistant *Salmonella spp*. is frequently associated with the indiscriminate application of antimicrobials in chicken farming, resulting in treatment failures (Rahman et al., 2020). Antimicrobial resistance (AMR) has intensified the economic losses attributed to salmonellosis in Bangladesh, resulting in elevated mortality rates in poultry from antimicrobial-resistant *Salmonella* strains relative to non-resistant variants (Islam et al., 2021).

The extensive use of antimicrobials in poultry farming has become a widespread practice aimed at treating and preventing diseases, as well as enhancing growth performance in poultry. Nonetheless, this has played a crucial role in the rise of multidrug-resistant (MDR) *Salmonella*, which presents a serious public health issue worldwide (Page & Gautier, 2012). Over the past decades, the prevalence of MDR *Salmonella* has risen, with strains exhibiting resistance to multiple classes of antibiotics, complicating treatment options and increasing the risk of transmission through the food chain (Hindermann et al., 2017; White et al., 2001).

The significance of the current study is established here, reliance on poultry as staple food and wet markets as the primary source for fresh poultry has thereby raised *Salmonella* contamination to a public health concern. This study was designed to determine the actual prevalence and antimicrobial susceptibility of *Salmonella spp*. in poultry items available in the wet markets of Dhaka metropolis. This will clarify the common occurrence and resistance patterns that could indicate contamination in these marketplaces, emphasising the potential risks to public health. This information is essential for intervention and policy efforts focused on enhancing food safety standards, reducing the transmission of *Salmonella spp.*, and addressing the risks linked to antimicrobial resistance (AMR).

Salmonella contamination in raw chicken meat in Dhaka city's wet markets and supermarkets is poorly studied. Most research on foodborne pathogens have focused on other bacterial contaminants or general food safety issues without analysing *Salmonella* infection in consumer-sold chicken. Furthermore, there is a significant gap in understanding the antibiotic resistance profiles of *Salmonella* strains isolated from poultry in Bangladesh. Due to the poultry industry's broad and unregulated use of antibiotics, updated data on *Salmonella* strain resistance to common antibiotics is needed. This information is needed to estimate *Salmonella* and antibiotic-resistant illness risks. We also lack a comprehensive understanding of where contamination risks are highest and what variables contribute to them since current research does not thoroughly evaluate *Salmonella* prevalence and resistance trends across retail outlets. This study aims to fill these gaps by providing updated data on *Salmonella* prevalence in raw chicken, testing antibiotic resistance of isolated strains, and evaluating the differences in contamination across various market types in Dhaka City.

1.4 Objectives of the Study:

1. To detect the presence of *Salmonella spp. from* spp. from raw chicken samples collected from different wet markets.

2. To identify the resistance pattern of *Salmonella* by using antibiotic resistance test.

1.5 Rationale:

- *Salmonella* is a leading cause of foodborne illnesses, and chicken is a common source of infection. By determining how widespread *Salmonella* contamination is in broiler raw chicken sold in Dhaka City, this research will help identify the risk to consumers and where food safety improvements are most needed.
- With the growing threat of antibiotic-resistant bacteria, this study's focus on testing the *Salmonella* strains for resistance to common antibiotics is especially relevant. The findings will shed light on whether antibiotic-resistant *Salmonella* is present in the local food supply, which can make infections harder to treat and pose a serious health threat.
- This research will help raise awareness among consumers against *Salmonella* and also the importance of proper food preparation to avoid the infection. This awareness could minimise the risk of illness and could lead to healthier practice in homes.

Chapter 2

Materials and Methods

2.1 Sample Collection and Processing:

Fifty samples of chicken meat, liver and gizzard were collected from local wet markets of Badda, Gulshan, Dhanmondi, Uttara and Segun Bagicha of Dhaka, Bangladesh from September 2023 to October 2024. To elaborate, 15 samples each of meat and liver, and 20 samples of gizzard were collected. Each sample was immediately transported to the laboratory while being stored in sterile collection bags with ice packs (Sodagari et al., 2015) and then processed by homogenization, using a homogeniser.

Ten grams of each sample were handled aseptically using a sterile tweezer and pair of scissors, and then weighed using a mass balance. They were then homogenised in 90 ml of sterilised distilled water in a sterile beaker at 400 rpm for 15 minutes, and then filtered using a sterile strainer into a sterile test tube. Afterwards, the filtrates were enriched using Peptone and Selenite Broth (SB). Two ml of filtrate was enriched with 5 ml of Peptone and incubated for 4 hours in a shaker incubator at 37℃. Meanwhile, 20 ml of filtrate was enriched with 10 ml of SB for 24 hours at 37℃. After enrichment in Peptone, the filtrates were serially diluted (1:10) to 10^{-4}, plated on selective media via the spread plate method and incubated for 24 hours at 37℃. The filtrates enriched with SB were streaked on selective media and incubated for 24 hours at 37℃, as described in Gebeyehu et al. (2022) with some modifications.

Peptone was used for non-selective enrichment of the homogenised meat samples, to increase the load of *Salmonella spp.* This is because Peptone supports microbial growth, by acting as a source of nitrogen and providing long-chain amino acids. Peptone also does not contain any inhibitors, which allows sub-lethally damaged *Salmonella* cells to recover and remain viable (Aryal, 2022a).

Meanwhile, SB was used for selective enrichment of *Salmonella spp.* Here selenite serves as a selective agent against coliforms, streptococci and other Gram positive bacterial species, thereby promoting the growth of *Salmonella spp.* Simultaneously, the presence of sodium phosphate as a buffer and lactose as a carbohydrate source supports the recovery of *Salmonella* cells exposed to preservatives, marginal heat and food processing procedures such as homogenisation (*Selenite Broth*, 2008).

2.2 Isolation of *Salmonella spp.* by Culture:

From the selective media, isolated colonies were selected depending on colony morphology and phenotypic characteristics. The selected colonies were then streaked on Nutrient Agar for pure culture, and incubated for 24 hours at 37℃. The selective and non-selective media used in this experiment are given below (Table 2.1).

Selective Media	Non-selective media
1. Xylose Lysine Deoxycholate Agar (XLD)	1. Nutrient Agar (NA)
2. *Salmonella-Shigella* Agar (SS)	

Table 2.1: Selective and Non-selective media used in this experiment.

XLD and SS agar were used since they are both selective and differential for *Salmonella spp*. For XLD agar, the differential agent is the amino acid lysine. The medium also contains sugars including xylose, lactose, and sucrose as carbohydrate sources, but the fermentation of xylose by *Salmonella spp.* causes the decarboxylation of lysine. This leads to a decrease in the pH of the medium, and the production of hydrogen sulphide. For further differentiation, the medium contains sodium thiosulfate and ferric ammonium citrate, which serve as indicators of hydrogen sulphide production. The presence of hydrogen sulphide is finally observed as the characteristic black centres on the red colonies of *Salmonella spp.* on XLD agar (Aryal, 2022c).

Alternatively, in the case of SS agar, lactose serves as a differential agent. Lactose fermenting microorganisms produce pink-red colonies, while lactose non-fermenting microorganisms such as *Salmonella spp.* produce colourless colonies. SS agar also contains the same hydrogen sulphide indicator system of sodium thiosulphate and ferric ammonium citrate found in XLD agar. As a result, colourless colonies with black centres are characteristic of *Salmonella spp.* in SS agar (Aryal, 2022b).

NA was used to obtain a pure culture of *Salmonella spp.* colonies selected from XLD and SS agar. This is because it is a non-selective medium that promotes the growth of a wide range of microorganisms. Due to its simple composition, NA is also ideal for extended preservation of cultures with a lower likelihood of contamination. Because of this, NA was used to preserve pure cultures of *Salmonella spp.* before performing DNA extraction (Sapkota, 2022).

2.3 Polymerase Chain Reaction to Confirm the Identity of *Salmonella spp.*:

2.3.1 DNA Extraction:

DNA extraction is the process by which Deoxyribonucleic acid (DNA) can be purified from a sample. During this process, physical or chemical methods are used to separate DNA from other cell components such as membranes and organelles. Briefly, in the process of DNA extraction, the cells are lysed to release the DNA, which is then purified and solubilised in a buffer (Gupta, 2019).

For this study, the genomic DNA of *Salmonella spp.* isolates were extracted using the Boiling Method, as described in Pavon et al. (2022), with some modifications. A loopful of colony was collected from pure culture using a sterile inoculating loop, and mixed with 400 µl of Tris-EDTA (TE) Buffer in a sterile microcentrifuge tube using a micropipette, then vortexed for 15 seconds. The mixture was centrifuged at 13000 rpm for 10 minutes at 25℃. After this, the supernatant was discarded and the pellet was mixed with 400 µl of TE Buffer, and vortexed again for 15 seconds. The mixture was then placed onto a heating block set at 100℃ for 10 minutes, and then allowed to cool for 10 minutes at room temperature. Afterwards, it was centrifuged at 13000 rpm for 10 minutes at 25℃, and 300 µl of the final supernatant containing the genomic DNA was transferred to a sterile microcentrifuge tube. Finally, the microcentrifuge tubes containing the extracted DNA were stored in the freezer at -20℃.

2.3.2 Polymerase Chain Reaction:

The Polymerase Chain Reaction (PCR) is used to amplify sequences of DNA. The steps of PCR include Denaturation, Annealing and Elongation. In the Denaturation step, the double stranded

DNA sequence is converted to single stranded DNA. Primers are then annealed to the single stranded sequences, leading to elongation of the target DNA. Primers are short, single stranded DNA sequences that are complementary to the ends of the target DNA sequence. They are required to begin the process of DNA synthesis, along with the thermostable enzyme DNA polymerase. The steps of PCR are repeated for a number of cycles, while the reaction takes place in a thermocycler (Gupta, 2019).

For this study, conventional PCR was used to confirm the identity of *Salmonella spp.* by targeting the gene *invA*, a chromosomal virulence gene specific for the genus *Salmonella* (Shanmugasamy et al., 2011; Yanestria et al., 2019). 13 µl of reaction mixture was prepared in a sterile PCR tube using 2 µl of extracted DNA, 6 µl of PCR master mix, 3 µl of nuclease free water, and 1 µl each of forward and reverse primer. The PCR master mix contained equal amounts of deoxynucleotide triphosphate (dNTP), magnesium chloride and Taq polymerase. The PCR was carried out under conditions suitable for each gene primer, outlined in Table 2.2.

The products were then analysed by Agarose Gel Electrophoresis. This is a technique used to separate DNA fragments based on their size, and push the fragments across the agarose gel matrix using an electric field. It is also used for the visualisation of PCR products using ethidium bromide, an intercalating agent used as a fluorescent tag, a DNA ladder to calibrate the distance moved by the DNA fragments, Tris acetate-EDTA (TAE) as a running buffer and ultraviolet (UV) light for visualisation. For this study, gel electrophoresis was performed using 1.5% agarose gel, 4 µl of ethidium bromide, 4 µl of 100 bp DNA ladder and 100 V of electricity. Gel electrophoresis was conducted as described in (Lee et al., 2012) with a few modifications, and the results were observed in a UV transilluminator.

Primer	**Primer sequence**	**PCR conditions**	**Number of cycles**	**Amplicon Size**	**Reference**
Salmonella	*Salmonella*-F: 5'-GTATTGTTGATTAATGACATCCG-3' *Salmonella*-R: 5'-ATATTACGCTACGGAAACACGTT-3'	95°C for 5 minutes 95°C for 30 seconds 60°C for 30 seconds 72°C for 1 minute 72°C for 10 minutes	35	403 bp	(Ranjbar et al., 2016)
invA	*invA*-F: 5'-GTGAAATTATCGCCACGTTCGGGCAA-3' *invA*-R: 5'-TCATCGCACCGTCAAAGGAACC-3'	95°C for 2 minutes 94°C for 30 seconds 53°C for 30 seconds 72°C for 1 minute 72°C for 7 minutes	30	284 bp	(Ghoddusi et al., 2019)

Table 2.2: PCR conditions for confirming the identity of *Salmonella spp*. by targeting the *invA* gene.

2.4 Antibiotic Susceptibility Testing:

Antibiotic Susceptibility Testing (AST) is a method that studies the ability of a microorganism to survive in the presence of an antibiotic. The purpose of the test is to observe the sensitivity of pathogenic aerobic and facultative anaerobic bacteria to a variety of antibiotics (Hudzicki, 2009). Though primarily used in clinical settings in order to formulate treatment plans for patients, AST provides valuable insights into the progress of antimicrobial resistance, and its results can be used to assess the strength of common antibiotics against a wide range of bacterial species.

For this study, the Kirby-Bauer disc diffusion method described in (Sharma, 2023) was used to study the antibiotic susceptibility of 10 antibiotics from 10 distinct classes. Before the test, the turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard. The purpose of this is to obtain an estimate of the number of bacteria present in the inoculum. The suspension was then inoculated onto Mueller Hinton Agar (MHA) plates using sterile cotton swabs to create a lawn of bacterial growth, followed by placing of antibiotic discs using a sterile tweezer and 24 hour incubation at 37°C. MHA is used for the Kirby-Bauer disc diffusion test for multiple reasons. To begin with, it is non-selective and non-differential, which allows it to support the growth of a wide range of bacterial species. Additionally, the presence of starch reduces the effect of any toxins released by the bacteria, and also helps to mediate the diffusion rate of the antibiotics throughout the medium (Aryal, 2023).

The antibiotics used for this test included Meropenem (MEM, 10 µg), Tetracycline (TE, 30 µg), Ciprofloxacin (CIP, 5 µg), Ampicillin (AMP, 10 µg), Nalidixic acid (NA, 30 µg), Gentamicin (CN, 10 µg), Ceftazidime (CAZ, 30 µg), Sulfamethoxazole (SXT, 25 µg), Tigecycline (TGC, 15 μg) and Chloramphenicol (C, 30 μg). After incubation, the Zones of Inhibition (ZOI) were observed, which were the clear zones surrounding an antibiotic disc where bacterial growth had been inhibited. Their mean diameters were measured, and compared to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). Based on these guidelines, the ZOI were classified as Susceptible, Intermediate or Resistant. The groups and ZOI ranges of the antibiotics used in this study are given below (Table 2.3).

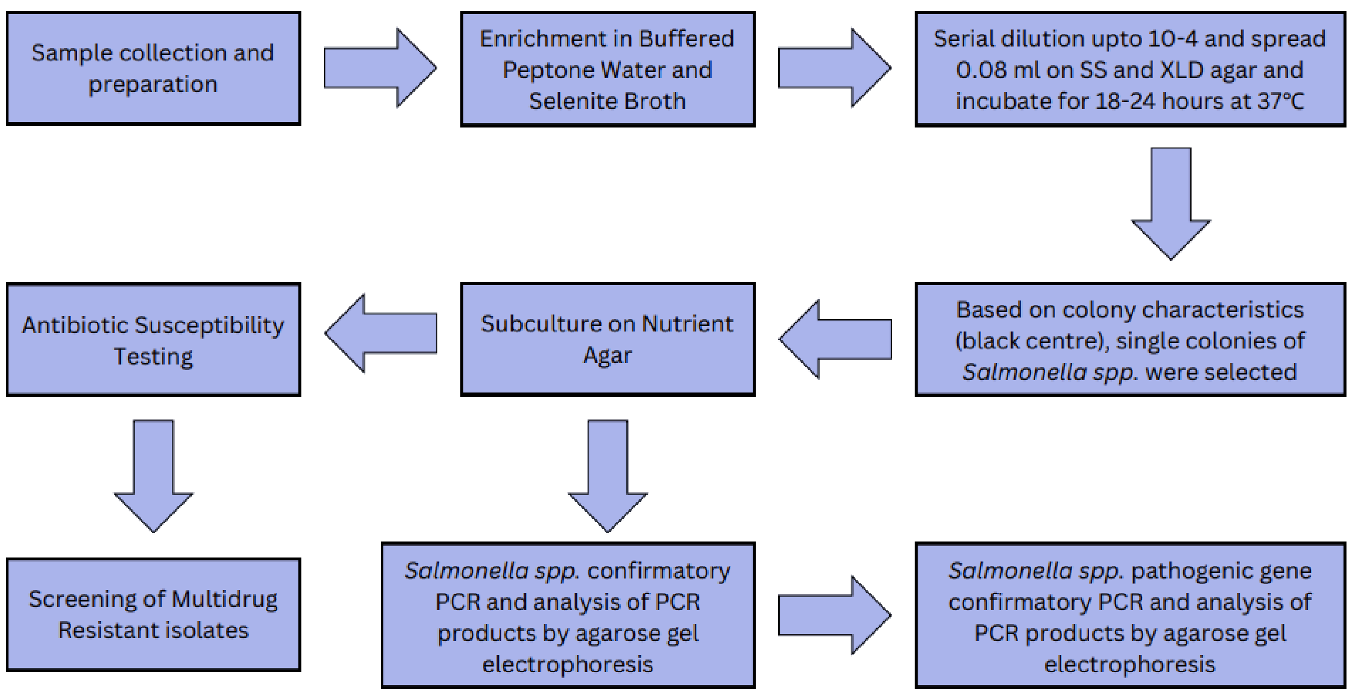
Antibiotic Group	**Antibiotic Name**	**Disc Code**	**Disc potency (μg)**	**Susceptible (mm)**	**Intermediate (mm)**	**Resistant (mm)**
Quinolone	Nalidixic Acid	NA	30	≥19	14–18	≤13
Beta-lactam	Ampicillin	AMP	10	≥17	14–16	≤13
Aminoglycosides	Gentamicin	CN	10	≥15	13–14	≤12
Tetracycline	Tetracycline	TE	30	≥19	15–18	≤14
Fluoroquinolone	Ciprofloxacin	CIP	5	≥21	16–20	≤15
Sulfonamides	Sulfamethoxazole	SXT	25	≥16	11–15	≤10
Cephalosporins	Ceftazidime	CAZ	30	≥18	15–17	≤14
Carbapenem	Meropenem	MEM	10	≥16	14–15	≤13
Glycylcycline	Tigecycline	TGC	15	≥17	13–16	≤12
Phenicols	Chloramphenicol	C	30	≥18	13–17	≤12

Table 2.3: Groups and ZOI ranges of the antibiotics used for the Antibiotic Susceptibility Test.

2.5 Screening of Multidrug Resistant Isolates:

The screening of Multidrug Resistant isolates was based on the results of the AST. Isolates which showed resistance against three or more classes of antibiotics were defined as Multidrug Resistant (Zhang et al., 2018).

2.6 Overview of the Followed Protocol:



Chapter 3

Results

3.1 Microorganisms Isolated from Chicken Meat:

Peptone was used to enrich the growth of *Salmonella spp.* (Fig A). After 4 hours of incubation, the sample was spreaded on the XLD and SS media again and sent for overnight incubation. The positive growth appeared as black-centred colonies (Fig C). On the other hand, after overnight incubating the sample in Selenite Broth (SB) for better enrichment of *Salmonella spp.*, the broth colour changes to orange indicating the presence of *Salmonella* (Fig B). Then, it was also streaked on XLD and SS agar and incubated for 24 hours. Following the isolation of probable *Salmonella spp.* colonies on XLD Agar and SS Agar, Nutrient Agar (NA) is used for subculturing (Fig D). Out of 50 samples, *Salmonella spp.* were found in 15 samples which is 30% of the samples. The positive samples exhibited distinctive black-centred colonies on Salmonella-Shigella Agar (SS Agar) and on Xylose Lysine Deoxycholate Agar (XLD Agar) *Salmonella* colonies are typically characterised by red colonies with black centres (Fig C).

A **B** **C** **D**

Fig 3.1: Growth of *Salmonella spp.* in Different Media.
(A: Enrichment in LB Broth; B: Enrichment in SB Broth; C: Black Centered Colonies on SS Agar; D: Growth on NA Media)

After handling 50 samples from Badda, Gulshan, Uttara, Segunbagicha and Dhanmondi of Dhaka City, we have achieved a total of 15 positive *Salmonella* samples, with a rate of 30%. From the following total samples, 20 samples of gizzard and 15 samples each of meat and liver were collected. Among these, 8 positive samples were obtained from gizzard, 5 positive samples from liver and 2 positive samples from meat. As a result, 40% of the positive samples were from gizzard, 33% from liver and only 13% from meat samples.

On the other hand, 10 samples were collected from each area. Therefore, 6 samples from Badda, 6 samples from Gulshan and Segunbagicha, 2 samples from Dhanmondi and 1 sample from Uttara area were positive.

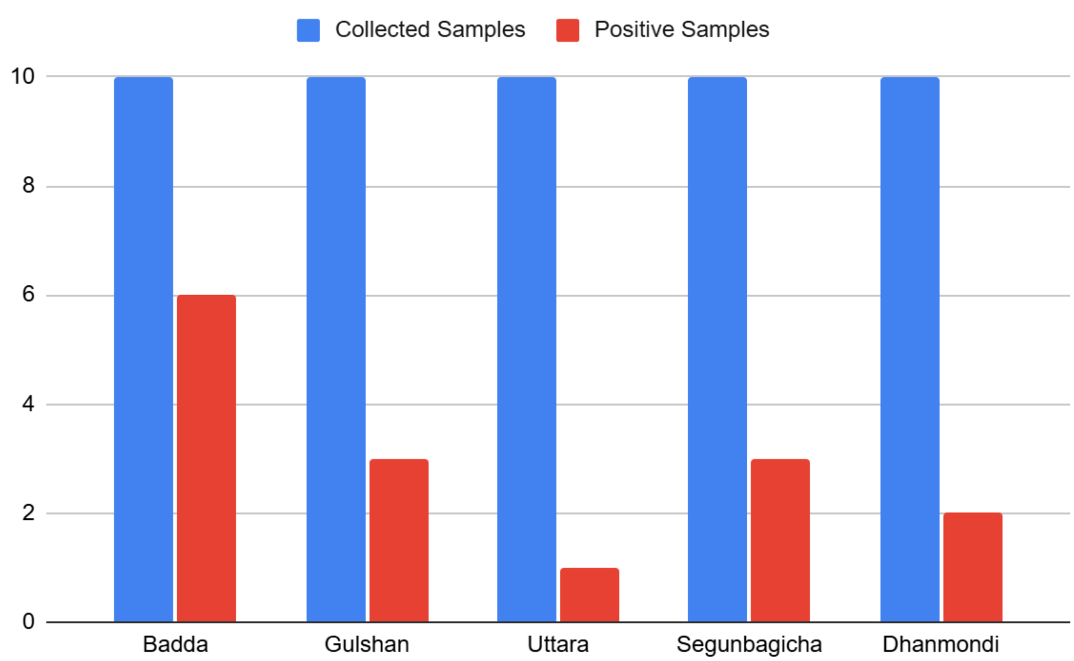


Fig 3.2: Graph of Positive Samples Based on Areas

From the above graph, we can determine that the highest positive samples were collected from the Badda area which is about 60%. Moreover, the rest of the areas have given us 10-30% positive isolated results from the samples.

3.2 Agarose Gel Electrophoresis Visualisation:

Following the protocol, after subculturing, DNA extraction was performed. After that, the isolates were mixed with PCR mix and sent to the PCR machine according to its condition. After the polymerase chain reaction is completed, we performed an agarose gel electrophoresis test to determine the base pair sizes of isolated genes. Transferring the solid agarose gel under the UV light, the band sizes were visualised. As we were identifying *Salmonella spp.,* 403 base pairs bands were visualised.

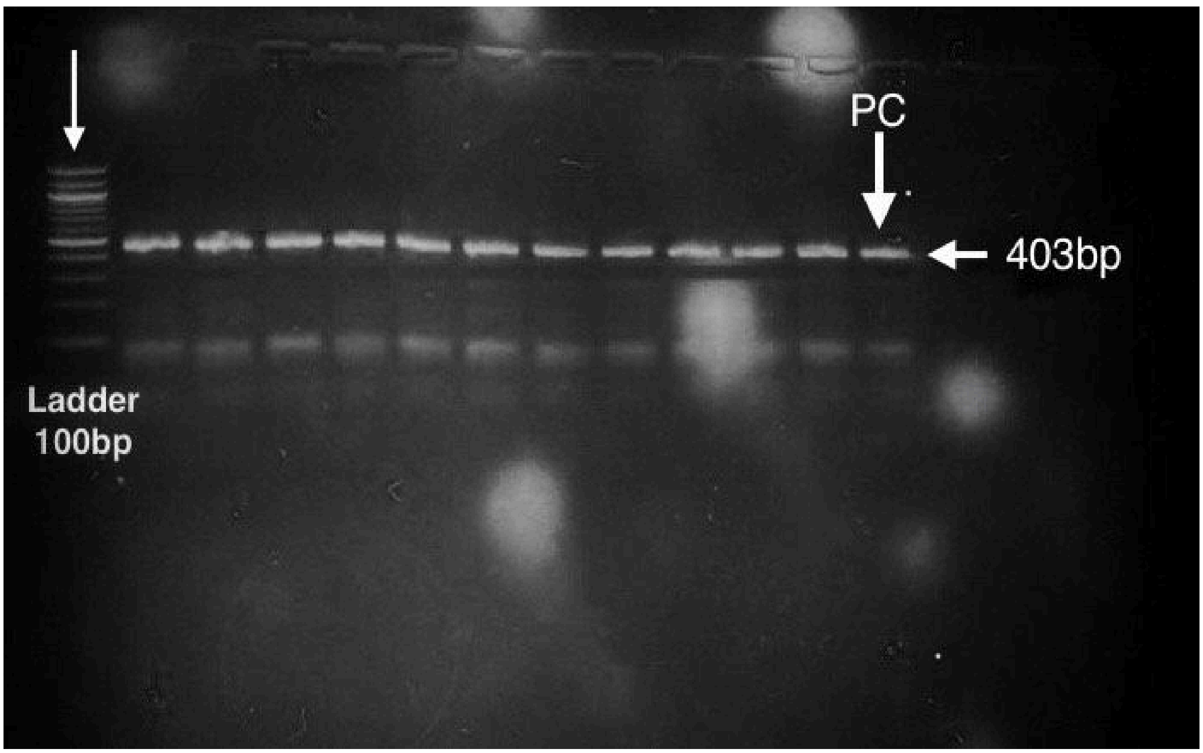


Fig 3.3: Agarose Gel Electrophoresis of PCR Products of *Salmonella spp.* isolated from Chicken Meat Targeting invA Gene (PC: Positive Control)

3.3 Antibiotic Susceptibility Test (AST) Analysis:

After performing AST with our 15 isolates and 10 different antibiotic groups, Zone of Inhibition (ZOI) was determined. This ZOI determines effectiveness of the antibiotics against *Salmonella* whether it is sensitive, intermediate or resistant.

Fig 3.4: Antibiotic Susceptibility Test on MHA Agar

Group Names	Antibiotic Name	Sensitive Bacteria Quantity	Sensitive (%)	Intermediate Bacteria Quantity	Intermediate (%)	Resistant Bacteria Quantity	Resistant (%)
Aminoglycosides	Gentamicin	11	73.33%	3	20.00%	1	6.66%
Carbapenem	Meropenem	14	93.33%	0	0.00%	1	6.66%
Cephalosporins	Ceftazidime	13	86.66%	0	0.00%	2	13.33%
Beta-lactam	Ampicillin	5	33.33%	0	0.00%	10	66.66%
Fluoroquinolone	Ciprofloxacin	13	86.66%	2	13.33%	0	0.00%
Phenicols	Chloramphenicol	9	60.00%	2	13.33%	4	26.66%
Tetracycline	Tetracycline	4	26.66%	0	0.00%	11	73.33%
Glycylcyclines	Tigecycline	5	33.33%	5	33.33%	5	33.33%
Quinolones	Nalidixic Acid	8	53.33%	5	33.33%	2	13.33%
Sulfonamides	Sulfamethoxazole	11	73.33%	1	6.66%	3	20.00%

Table 3.1: AST Profile of *Salmonella* Isolates

All of the *Salmonella* isolate strains were sensitive against the antibiotics. Between 11-14 strains were comparatively more sensitive to Gentamicin, Meropenem, Ceftazidime, Ciprofloxacin and Sulfamethoxazole resulting at 73.33% to 93.33%. Moreover, Ampicillin, Chloramphenicol, Tetracycline, Tigecycline, Nalidixic Acid sensitive strains range from 4 to 9. From the table, it is indicated that only between 1-5 strains has an intermediate profile against the antibiotics. Among these, Tigecycline and Nalidixic Acid had the highest intermediate profile which is 33.33% and Sulfamethoxazole having the lowest at only 6.66%. On the other hand, Ampicillin and Tetracycline were resistant to most of the strains of *Salmonella* which is in between 66.66% to 73.33%. Rest of the antibiotics were partially resistant to our isolates but Ciprofloxacin could not make any resistance against any isolates. The below graph (Fig 3.5) is to give a visualisation of the *Salmonella* isolates being resistant, sensitive and intermediate against 10 different antibiotic groups. To conclude, only 46.77% isolates were sensitive to the antibiotics whereas 53.33% were multi-drug resistant (Fig 3.6).

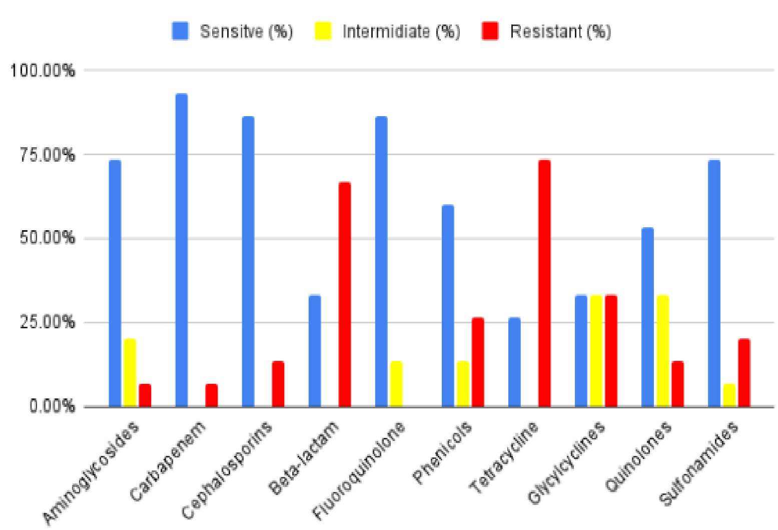


Fig 3.5: Graph on AST Profile

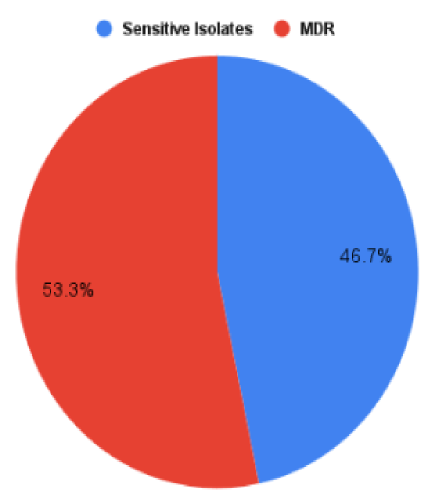


Fig 3.6: Percentage of Multi-drug Resistant and Sensitive Isolates

Chapter 4

Discussions

The main aim of this research was to study or evaluate the presence and antibiotic resistance of *Salmonella spp.* in poultry meat, liver & gizzard, a known foodborne pathogen that presents significant health hazards. The research found a total of 15 isolates whereas 12% from the chicken meat, 8% from liver & 10% from the gizzard from 50 chicken samples were contaminated with *Salmonella spp.*, the prevalence rates of *Salmonella* have been found to be 23.33% in poultry slaughterhouses, 23.53% in poultry supply chains, and 37.9% in poultry producing areas, according to previous research (Momtaz et al., 2018; Mumun et al., 2017; Mahmud et al., 2011). The occurrence in Dhaka's wet marketplaces, where fresh poultry is typically procured, is affected by insufficient sanitation and restricted refrigeration, fostering conditions conducive to bacterial contamination, particularly *Salmonella spp*.

The extensiveness of *Salmonella* in poultry around the world shows significant variation, shaped by elements like farming practices, biosecurity protocols, and local regulations. Countries with advanced economies frequently indicate reduced prevalence rates due to rigorous food safety measures. A review highlighted that the prevalence of *Salmonella spp.* in chicken and poultry products is lower in developed countries, such as the United States and the European Union compared to developing countries like Bangladesh and India (Bintsis, 2021). In Bangladesh, investigations have indicated alarmingly high prevalence rates of *Salmonella* in poultry. In research completed in Dhaka, it was established that a significant prevalence was carried by 52% of apparently healthy chickens (Rahman et al., 2020). Further, a prevalence of 23.33% was recorded in poultry slaughterhouse specimens (Haque et al., 2022). This highlights the urgent need for antimicrobial stewardship in addition to the activities directed at combating AMR. *Salmonella* infections and the excessive degree of resistance to antimicrobials in Bangladeshi hen are extreme public fitness problems. Effective measures have to be taken to deal with each of those threats via modifications in local agricultural and biosecurity practices and promotion of responsible use of antimicrobials offshore.This approach can assist reduce the occurrence of *Salmonella* infections and fight the escalating threat of antimicrobial resistance. The excessive prices may be related to inadequate biosecurity protocols, a lack of regulatory supervision, and problems executing robust meals protection measures. The growing global fear is the antibiotic-resistant stranger microbes associated with *Salmonella* traces gathered from poultry. A meta-evaluation concluded that the overall volume of AMR, the potential to face up to the results of antimicrobial tablets, in South Asia became 70% and confirmed temporal increment from 53% to 77% inside a span of ten years (Bhatt et al, 2023). It has been reported in Bangladesh that strains of reproducing *Salmonella* strains obtained from poultry have demonstrated multidrug resistant abilities. A certain investigation recorded such resisters to be all of the isolates. Bangladeshi poultry has hours comparable with *Salmonella* at a rate that is more significant than those of many developed countries (Bintsis, 2021; Haque et.al, 2022). There is a global rather worrying trend characterised by factors associated with AMR especially among *Salmonella* isolates in Bangladesh and other nations as well.

As it is, the level of resistance as reported from Bangladesh is in fact worrying because it poses a high level of MDR strains of the pathogens (Islam et al., 2021). This suggests to prevent the problems caused by the improper use of antibiotics as well as absence of active AMR surveillance. The context regarding public health in Bangladesh is alarming as there is presence of *Salmonella* in poultry. To meet such hurdles, it is necessary to develop comprehensive strategies that not only improve local farming and biosecurity standards, but also promote responsible antimicrobial use. This kind of strategy will help in curtailing the frequencies of salmonella infections as well as tackling the ever growing threat of antimicrobial resistance.

In addition, the AST results reveal the antibiotic resistance profile of the isolates. Using 10 different antibiotics gave us a comprehensive profile of the isolates. Gentamicin (73.33%), Meropenem (93.33%), Ceftazidime (86.66%), Ciprofloxacin (86.66%), and Sulfamethoxazole (73.33%) has the highest sensitivity against the isolates. The high percentage of sensitivity of these pathogens indicates the efficacy of these antibiotics as a *Salmonella* infection treatment. Additionally, Tigecycline and Nalidixic Acid have the maximum intermediate profile which is 33.33%. This indicates partial efficacy of the antibiotic against *Salmonella*. Tigecycline is well recognized for its usage against the multidrug-resistant isolates. Having intermediate resistant efficacy indicates the changing mechanisms of the pathogens. Also, Nalidixic Acid becoming intermediate resistant to the pathogens describes the under-developed resistance mechanisms of the pathogen which is related to the continuous gene mutations of the organism. At last, Ampicillin and Tetracycline have the highest resistance against the pathogen which is respectively 66.66% and 73.33%. These commonly used antibiotics being highly resistant to the pathogen are concerning for human health. Moreover, isolates are multi-drug resistant (MDR) to the antibiotics which is 53.33% and only 46.77% isolates are sensitive to the antibiotics. Most isolates being MDR is concerning for the human health in case of preventing infections or diseases caused by the *Salmonella spp.* This resistance takes us to the inappropriate and excessive usage of these antibiotics. As a result, the organisms could mutate its genes and generally become resistant.

The detection of *Salmonella spp.* in chicken meat, liver & gizzard which is as regular food for most of the people in Bangladesh and it becoming resistant to antibiotics poses a public health risk in Dhaka city and all over the country. Infections by *Salmonella* might lead to several severe diseases in the human body. Also, developing strains having antibiotic resistant genes will complicate the treatment process of the diseases. For a highly populated place like Badda, these findings need more surveillance, precaution measurements and more strict regulations in the poultry industries. By increasing the importance of hygiene protocols, giving poultry farmers proper knowledge on the impacts and prevention of these diseases could be a beneficial way. To completely eliminate *Salmonella* from chicken flesh, liver & gizzards farmers could use probiotics with the poultry food. *Salmonella* has the ability to horizontal gene transfer which could make other pathogens resistant. *Salmonella*, particularly those strains associated with poultry, are increasingly showing AMR and MDR. This is a significant public health concern as it limits treatment options for infections caused by these bacteria. Many infections may have fewer treatment options as a result, making management more challenging. However, healthcare systems may be severely impacted by MDR *Salmonella* infections, which can result in more hospital stays, longer treatment periods, and more medical expenses. However, improperly disposing of contaminated chicken waste can help MDR *Salmonella* spread throughout the environment, perhaps polluting soil and water supplies. To reduce these problems, enhancing sanitation, hygiene procedures and prudent use of antibiotics in both human and animal are two ways to stop the development of MDR *Salmonella*. The average rate of *Salmonella* may be influenced by variables such as geographic location, hygiene habits, and market size. However, the accuracy of results in a laboratory might be affected by the sensitivity and specificity of the isolation and detection techniques used. Additionally, mentioning about the Hygiene Procedures, *Salmonella* contamination can result from poor hygienic practices, such as handling, storing, and preparing poultry inappropriately. Moreover, analysing *Salmonella* in both human and animal populations can help determine possible routes of transmission and guide control measures.

Finally, according to future viewpoints, implementing a routine surveillance strategy to track the amount of *Salmonella* in chicken samples from wet markets can assist in recognising patterns and outbreaks. The efficiency of surveillance can be increased and resource allocation optimised by concentrating on high-risk regions and susceptible groups. Nonetheless, *Salmonella* isolates can be characterised by whole-genome sequencing, which can reveal important information about their genetic diversity, mechanisms of antibiotic resistance, and possible contamination sources. This study marks the connection between human and animal health. Proper coordination among human health development and veterinary sectors could bring this challenge down effectively. Lastly, limiting the sample size within 50 which is a small-scale study could lack the generalizability of our finding in a large-scale population. Broader study will definitely be providing more and unknown comprehensive views on *Salmonella spp.* in chicken meat, liver & gizzard.

In conclusion, this study concerns the incidence of *Salmonella spp.* found in chicken bodies in different populated Dhaka city areas, especially having a notable resistance to the commonly and regularly used antibiotics by humans. The findings of this case highlights the importance and need of strict observations of the authorities, increment of food safety efficacy and limited usage of antibiotics in a proper way to mitigate the infections caused by *Salmonella spp.*. Continuous and proper monetization and future research on this matter is crucial to ensure the food safety measurements to prioritise public health issues.

Chapter 5

Conclusion

To conclude, The purpose of this study was to separate and identify *Salmonella* species from chicken samples that were gathered from Dhaka City's wet markets. One of the main sources of *Salmonella* contamination is poultry, especially chicken. The high frequency of *Salmonella* infections in impoverished nations like Bangladesh is a result of inadequate food safety standards and poor hygiene practices. According to the study, out of 50 samples, pathogenic *Salmonella Spp.* were found in 15 samples. *Salmonella spp.,* plays a significant role in the onset of foodborne outbreaks. The likelihood of becoming infected with a sickness is likely to decrease as people's health consciousness grows. Therefore, this issue needs to be given more attention, and all protocols should be properly addressed. Since non-typhoidal *salmonellosis* is the most common cause of foodborne illness and *Salmonella spp.* are known to cause enteric fever, more research on *Salmonella spp.* is necessary to gain a comprehensive understanding of this pathogen's dangers to human health. In this study, Sample preparation and collection, enrichment, selective plating, subculturing, and molecular confirmation are some of the processes in the procedure. The accuracy of the procedure is further increased by the use of PCR and agarose gel electrophoresis for *Salmonella spp.,* confirmation and pathogenic gene detection & AST has been done to find out which antibiotic will be most effective in treating infection. Following AST testing on 15 isolates and 10 distinct antibiotic groups, we identified the zone of inhibitions that indicated whether the antibiotics were effective against sensitive, intermediate or resistant *Salmonella*. Screening for MDR resistant isolates was based on AST. Multiple antibiotic resistance in *salmonella* strains makes treatment more difficult and raises the risk of serious illness, difficulties, and even death. For this, strict hygiene procedures, such as washing and disinfection of surfaces and equipment, are essential for handling and producing chicken in order to reduce the risk of *Salmonella* infections. Need to make sure the poultry is cooked through to get rid of *Salmonella* infection. Additionally, we need to encourage proper personal hygiene, particularly we can greatly lessen the incidence of *Salmonella* infections and protect public health by addressing these important issues. Finally, The potential risk of infection can be decreased by offering market vendors training on good hygiene habits, such as handwashing, sanitation, and food handling. A more hygienic environment can be produced by investing in upgraded infrastructure, such as waste management and clean water supplies.

Last but not least, this thesis helps identify Salmonella contamination, such as It determined that chicken flesh sold in Dhaka's wet marketplaces frequently contains *Salmonella*. This information can be used to assess the risk of acquiring a foodborne illness from consuming chicken from these markets, although there can be some cross contamination. The study determined the isolates of Salmonella that demonstrated patterns of antibiotic resistance. The results can help shape public health laws and policies pertaining to wet market food safety and cleanliness. This could entail putting in place more stringent hygienic regulations, better sanitation procedures, and enhanced consumer education. On the other hand, The results can help shape public health guidelines and policies pertaining to wet market food safety and cleanliness. Future investigations into the presence of *salmonella* in food sources and the creation of more potent control strategies might use the study as a baseline.

References

Andino, A., & Hanning, I. (2015). *Salmonella* enterica: Survival, colonization, and virulence differences among serovars. FEMS Microbiology Reviews, 39(4), 341-361. https://doi.org/10.1093/femsre/fuv022

Islam, M.S.; Sabuj, A.A.; Haque, Z.F.; Pondit, A.; Hossain, M.G.; Saha, S. Seroprevalence and risk factors of avian reovirus in backyard chickens in different areas of Mymensingh district in Bangladesh. *J. Adv. Vet. Anim. Res.* 2020, *7*, 546–553.

Attia, Y.A.; Ellakany, H.F.; El-Hamid, A.A.; Bovera, F.; Ghazaly, S. Control of *Salmonella enteritidis* infection in male layer chickens by acetic acid and/or prebiotics, probiotics and antibiotics. *Arch. Geflügelk.* 2012, *76*, 239–245.

Pal, M.; Merera, O.; Abera, F.; Rahman, M.T.; Hazarika, R.A. Salmonellosis: A major foodborne disease of global significance. *Beverage Food World.* 2015, *12*, 21–24.

Gast, R.K. Detecting infections of chickens with recent *Salmonella pullorum* isolates using standard serological methods. *Poult. Sci.* 1997, *76*, 17–23.

Scallan, E., et al. (2011). Foodborne illness in the United States. *Emerging Infectious Diseases*, 17(1), 7–15. DOI: https://doi.org/10.3201/eid1701.P11101

Meakins, S., et al. (2003). *Salmonella* transmission and prevention. *Clinical Infectious Diseases*, 37(Suppl 2), S253–S259. DOI: https://doi.org/10.1086/376932

Aung, M. M., & Chang, Y. S. (2021). The effect of wet markets on food safety and public health. *Environmental Science and Pollution Research*, 28, 54550–54565. DOI: https://doi.org/10.1007/s11356-021-14335-8

Rahman, M.T.; Sobur, M.A.; Islam, M.S.; Ievy, S.; Hossain, M.J.; El Zowalaty, M.E.; Rahman, A.T.; Ashour, H.M. Zoonotic Diseases: Etiology, Impact, and Control. *Microorganisms* 2020, *8*, 1405.

Islam, M.S.; Sobur, M.A.; Rahman, S.; Ballah, F.M.; Ievy, S.; Siddique, M.P.; Rahman, M.; Kafi, M.A.; Rahman, M.T. Detection of *bla*TEM, *bla*CTX-M, *bla*CMY, and *bla*SHV Genes Among Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Migratory Birds Travelling to Bangladesh. *Microb. Ecol.* 2021, in press.

Islam, M.S.; Nayeem, M.M.H.; Sobur, M.A.; Ievy, S.; Islam, M.A.; Rahman, S.; Kafi, M.A.; Ashour, H.M.; Rahman, M.T. Virulence Determinants and Multidrug Resistance of *Escherichia coli* Isolated from Migratory Birds. *Antibiotics* 2021, *10*, 190.

Urmi, M.R.; Ansari, W.K.; Islam, M.S.; Sobur, M.A.; Rahman, M.; Rahman, M.T. Antibiotic resistance patterns of *Staphylococcus* spp. isolated from fast foods sold in different restaurants of Mymensingh, Bangladesh. *J. Adv. Vet. Anim. Res.* 2021, *8*, 274–281.

Orubu, E.S.F.; Samad, M.A.; Rahman, M.T.; Zaman, M.H.; Wirtz, V.J. Mapping the Antimicrobial Supply Chain in Bangladesh: A Scoping-Review-Based Ecological Assessment Approach. *Glob. Health Sci. Pract.* 2021, *28*, 5963–5970.

Davies, J.; Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 2020, *74*, 417–433.

Duong, V.T.; Tuyen, H.T.; Van Minh, P.; Campbell, J.I.; Phuc, H.L.; Nhu, T.D.; Tu, L.T.; Chau, T.T.; Nhi, L.T.; Hung, N.T.; et al. No clinical benefit of empirical antimicrobial therapy for paediatric diarrhoea in a high-usage, high-resistance setting. *Clin. Infect. Dis.* 2018, *66*, 504–511.

Iwamoto, M.; Reynolds, J.; Karp, B.E.; Tate, H.; Fedorka-Cray, P.J.; Plumblee, J.R.; Hoekstra, R.M.; Whichard, J.M.; Mahon, B.E. Ceftriaxone-resistant nontyphoidal *Salmonella* from humans, retail meats, and food animals in the United States, 1996–2013. *Foodborne Pathog. Dis.* 2017, *14*, 74–83.

Tyson, G.H.; Tate, H.P.; Zhao, S.; Li, C.; Dessai, U.; Simmons, M.; McDermott, P.F. Identification of plasmid-mediated quinolone resistance in *Salmonella* isolated from swine ceca and retail pork chops in the United States. *Antimicrob. Agents Chemother.* 2017, *61*, e01318-17.

Sun, T.; Liu, Y.; Qin, X.; Aspridou, Z.; Zheng, J.; Wang, X.; Li, Z.; Dong, Q. The Prevalence and Epidemiology of *Salmonella* in Retail Raw Poultry Meat in China: A Systematic Review and Meta-analysis. *Foods* 2021, *10*, 2757.

Antunes, P.; Mourao, J.; Campos, J.; Peixe, L. Salmonellosis: The Role of Poultry Meat. *Clin. Microbiol. Infect.* 2016, *22*, 110–121.

Lanier, W.A.; Hale, K.R.; Geissler, A.L.; Dewey-Mattia, D. Chicken Liver-Associated Outbreaks of Campylobacteriosis and Salmonellosis, United States, 2000-2016: Identifying Opportunities for Prevention. *Foodborne Pathog. Dis.* 2018, *15*, 726–733.

Deguenon, E., Dougnon, V., Lozes, E., Maman, N., Agbankpe, J., Abdel-Massih, R. M., Djegui, F., Baba-Moussa, L., & Dougnon, J. (2019). Resistance and virulence determinants of faecal Salmonella spp. isolated from slaughter animals in Benin. *BMC Research Notes*, *12*(1). https://doi.org/10.1186/s13104-019-4341-x

Kaur J, Jain SK, Malik RK. Salmonella pathogenesis: where do we stand and what do we aim to achieve? Reviews in Medical Microbiology. 2021;32(2):71–84.

Tamang MD, Gurung M, Nam HM, Moon DC, Jang GC, Jung SC. Antimicrobial susceptibility and virulence characteristics of *Salmonella enterica Typhimurium* isolates from healthy and diseased pigs in Korea. JFP. 2014;77(9):1481–6. https://www.sciencedirect.com/science/article/pii/S0362028X23064207?via%3Dihub

Litrup E, Torpdahl M, Malorny B, Huehn S, Christensen H, Nielsen EM. Association between phylogeny, virulence potential and serovars of *Salmonella enterica*. Infect Genet Evol. 2010;10(7):1132–9. https://www.sciencedirect.c

Ehuwa, O.; Jaiswal, A.K.; Jaiswal, S. *Salmonella*, Food Safety and Food Handling Practices. *Foods* 2022, *10*, 907.

Rortana, C.; Nguyen-Viet, H.; Tum, S.; Unger, F.; Boqvist, S.; Dang-Xuan, S.; Koam, S.; Grace, D.; Osbjer, K.; Heng, T.; et al. Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in Chicken Meat and Pork from Cambodian Markets. *Pathogens* 2021, *10*, 556.

Sun, T.; Liu, Y.; Qin, X.; Aspridou, Z.; Zheng, J.; Wang, X.; Li, Z.; Dong, Q. The Prevalence and Epidemiology of *Salmonella* in Retail Raw Poultry Meat in China: A Systematic Review and Meta-analysis. *Foods* 2021, *10*, 2757.

Antunes, P.; Mourao, J.; Campos, J.; Peixe, L. Salmonellosis: The Role of Poultry Meat. Clin. Microbiol. Infect. 2016, 22, 110–121.

https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(15)01030-7/fulltext

Hoque, M. N., Mohiuddin, R. B., Malik, M. M. H., Khan, A., Hannan, M. J., & Alam, A. (2019). Outbreak of *Salmonella* in poultry of Bangladesh and possible remedy. *Journal of Advanced Biotechnology & Experimental Therapeutics*, 2, 87-97. https://www.bibliomed.org/?mno=44217

Ehuwa, O., Jaiswal, A. K., & Jaiswal, S. (2022). *Salmonella, Food Safety and Food Handling Practices*. *Foods*, 10(907). https://link.springer.com/article/10.1186/s13104-019-4798-7

S., Page,, P. Gautier Use of antimicrobial agents in livestock. https://doc.woah.org/dyn/portal/index.xhtml?page=alo&aloId=31376

D., Hindermann,, G., Gopinath, H., Chase, F., Negrete, D., Althaus, K., Zurfluh, B.D., Tall, R., Stephan, M. Nüesch-Inderbinen *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010–2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2017.01322/full

D.G., White,, S., Zhao, R., Sudler, S., Ayers, S., Friedman, S., Chen, P.F., McDermott, S., McDermott, D.D., Wagner, J. Meng. The isolation of antibiotic-resistant *Salmonella* from retail ground meats https://www.nejm.org/doi/full/10.1056/NEJMoa010315

Zhao,, D.G., White, S.L., Friedman, A., Glenn, K., Blickenstaff, S.L., Ayers, J.W., Abbott, E., Hall-Robinson, P.F. McDermott Antimicrobial resistance in *Salmonella enterica s*erovar

Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. https://journals.asm.org/doi/full/10.1128/aem.01249-08

Aryal, S. (2022a, January 7). *Peptone Water- Composition, principle, preparation, results, uses*. Microbe Notes. https://microbenotes.com/peptone-water/

Aryal, S. (2022b, January 7). *Salmonella Shigella (SS) Agar- Composition, Principle, Preparation, Results, Uses*. Microbe Notes. https://microbenotes.com/salmonella-shigella-ss-agar/

Aryal, S. (2022c, January 7). *XLD Agar- Composition, Principle, Preparation, Results, Uses*. Microbe Notes. https://microbenotes.com/xylose-lysine-deoxycholate-xld-agar/

Aryal, S. (2023, January 13). *Mueller Hinton Agar (MHA)- Composition, Principle, Preparation, Results, Uses*. Microbe Notes. https://microbenotes.com/mueller-hinton-agar-mha/

Gebeyehu, A., Taye, M., & Abebe, R. (2022). Isolation, molecular detection and antimicrobial susceptibility profile of *Salmonella* from raw cow milk collected from dairy farms and households in southern Ethiopia. *BMC Microbiology*, *22*(1). https://doi.org/10.1186/s12866-022-02504-2

Ghoddusi, A., Fasaei, B. N., Salehi, T. Z., & Akbarein, H. (2019). Serotype distribution and antimicrobial resistance of *Salmonella* isolates in human, chicken, and cattle in Iran. *PubMed*, *74*(3), 259–266. https://doi.org/10.22092/ari.2018.120267.1190

Gupta, N. (2019). DNA extraction and polymerase chain reaction. *Journal of Cytology*, *36*(2), 116. https://doi.org/10.4103/joc.joc_110_18

Hudzicki, J. (2009, December 8). *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. American Society for Microbiology. Retrieved November 10, 2024, from https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

Lee, P. Y., Costumbrado, J., Hsu, C., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, *62*. https://doi.org/10.3791/3923

Pavon, R. D. N., Mendoza, P. D. G., Flores, C. a. R., Calayag, A. M. B., & Rivera, W. L. (2022). Genotypic virulence profiles and associations in *Salmonella* isolated from meat samples in wet markets and abattoirs of Metro Manila, Philippines. *BMC Microbiology*, *22*(1). https://doi.org/10.1186/s12866-022-02697-6

Ranjbar, R., Naghoni, A., Afshar, D., Nikkhahi, F., & Mohammadi, M. (2016). Rapid Molecular Approach for Simultaneous Detection of *Salmonella spp*., *Shigella spp*., and *Vibrio cholera*. *Osong Public Health and Research Perspectives*, *7*(6), 373–377. https://doi.org/10.1016/j.phrp.2016.10.002

Sapkota, A. (2022, May 12). *Nutrient Agar- Principle, Composition, preparation, Results, uses*. Microbe Notes. https://microbenotes.com/nutrient-agar-principle-composition-preparation-and-uses/

Selenite Broth. (2008, April 4). Acumedia.com. Retrieved November 10, 2024, from https://www.mibius.de/out/oxbaseshop/html/0/images/wysiwigpro/7155_PI.pdf

Sharma, R. (2023, May 17). *Kirby Bauer Disc diffusion method for antibiotic susceptibility testing*. Microbe Notes. https://microbenotes.com/kirby-bauer-disc-diffusion/

Sodagari, H. R., Mashak, Z., & Ghadimianazar, A. (2015). Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets in Iran.

The Journal of Infection in Developing Countries, *9*(05), 463–469. https://doi.org/10.3855/jidc.5945

Zhang, L., Fu, Y., Xiong, Z., Ma, Y., Wei, Y., Qu, X., Zhang, H., Zhang, J., & Liao, M. (2018). Highly prevalent Multidrug-Resistant *salmonella* from chicken and pork meat at retail markets in Guangdong, China. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02104

Momtaz S, Saha O, Usha MK, Sultana M, Hossain MA. Occurrence of pathogenic and multidrug resistant *Salmonella* spp. in poultry slaughter-house in Bangladesh. Bioresearch Communications-(BRC). 2018. Jul 1; 4(2):506–15. https://www.bioresearchcommunications.com/index.php/brc/article/view/80

Mamun. MA, Kabir, SL. Islam, MM. Lubna, M. Islam, SS. Akhter. AT, et al. Molecular identification and characterization of *Salmonella* species isolated from poultry value chains of Gazipur and Tangail districts of Bangladesh. Afr J Microbiol Res. 2017. Mar 21; 11(11):474–81. doi: 10.5897/AJMR2017-8431

Mahmud MS, Bari ML, Hossain MA. Prevalence of *Salmonella* serovars and antimicrobial resistance profiles in poultry of Savar area, Bangladesh. Foodborne Pathog 4. Dis. 2011. Oct 1; 8(10):1111–8. https://pubmed.ncbi.nlm.nih.gov/21689000/

Shanmugasamy, M., Velayutham, T., & Rajeswar, J. (2011). Inv A gene specific PCR for detection of *Salmonella* from broilers. *Veterinary World*, 562. https://doi.org/10.5455/vetworld.2011.562-564

Yanestria, S. M., Rahmaniar, R. P., Wibisono, F. J., & Effendi, M. H. (2019). Detection of invA gene of *Salmonella* from milkfish (Chanos chanos) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World*, *12*(1), 170–175. https://doi.org/10.14202/vetworld.2019.170-175