

Occurrence of *Salmonella* Species in Poultry Eggs and Their Susceptibilities to Antibiotics

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Abstract- The study on the occurrence of *Salmonella* species in poultry eggs and their susceptibilities to antibiotics was conducted. A total of thirty (30) poultry eggs samples were collected from three (3) poultry farms (A, B and C) in Gwagwalada. The antibiogram was carried out using commercially made sensitivity disc. The eggshell recorded the highest bacterial load of $1.8 \times 10^4 \pm 0.1$ CFU/ml while the egg content had bacterial load of $1.5 \times 10^2 \pm 0.2$ CFU/ml as its highest. Out fourteen (14) *Salmonella* spp isolated from the poultry eggs, six (6) were isolated from Farm B, five (5) from Farm A while three (3) were isolated from Farm C. However total number isolates in this study showed that *Salmonella enterica* serovars Typhimurium were 8 while *Salmonella enterica* serovars Enteritidis were six. Eleven (11) *Salmonella* spp were recorded in eggshell while only three (3) were recorded in the egg content. *Salmonella enterica* serovars Typhimurium was the most frequently isolated which represented 57.14% while *Salmonella enterica* serovars Enteritidis represented 42.86 % of the total isolates. The *Salmonella* species isolated from all the samples showed varying levels of susceptibility and resistance to the tested antibiotics. *Salmonella* Typhimurium was susceptible to cefoxitin, ceftazidime, ciprofloxacin and gentamicin but resistant to ampicillin, tarivid and nalidixic acid. Also, *Salmonella* Enteritidis was susceptible to chloramphenicol, cefoxitin, ampicillin, ceftazidime, ciprofloxacin and gentamicin but resistant to tarivid and nalidixic acid. This study revealed the presence of *Salmonella* serovars on eggs surfaces and egg content with potential antimicrobial-resistant traits. Therefore, it is recommended that preparation of eggs for domestic use should be handled with extreme care.

Index Terms- *Salmonella*, Antibiotics, Eggs

I. INTRODUCTION

Salmonella is one of the main factors contributing to the current global epidemics of food-borne illness [1]. Outbreaks due to *Salmonella* have been associated with a wide variety of foods such as poultry products (meat and egg) [2]. The ingestion of poultry products that are contaminated could potentially lead to infections. Contamination of these foods can occur during production, processing and even distribution [3]. During the process of egg development in the reproductive system of hens or through contact with faeces from the environment, bacteria have the potential to contaminate both the eggshells and the contents of the eggs. Several outbreaks of salmonellosis have been reported where eggs are the source or the intermediate of human infection [4].

Salmonella enterica is one of the most significant food-borne pathogens worldwide and remains the major cause of infectious gastroenteritis. Cases are often related to the consumption of food of animal origin, mainly poultry products, such as eggs and raw chicken [5]. Annually, approximately 93 million instances of gastroenteritis are attributed to non-typhoidal *Salmonella* worldwide, resulting in about 155,000 fatalities [6]. The disease manifestation depends on the serotype involved, virulence factors, infective dose, and host immunity. Immunocompromised patients, infants, and aged people tend to be more susceptible and suffer more fatal clinical symptoms, including sepsis [7].

Numerous studies have demonstrated that the ongoing use of antibiotics in animal husbandry and other agricultural practices, such as the breeding and production of poultry birds, as one of the major factors contributing to the emergence and spread of antimicrobial-resistant *Salmonella* [8]. The prevalence of *Salmonella* and other bacteria that are resistant to drugs is increasing significantly, primarily because antimicrobials are being improperly utilized as growth promoters and preventive measures in the rearing of food-

producing animals, including poultry birds, and livestock [9]. Continual resistance of bacteria to widely used antimicrobial agents remains a significant global public health concern, and this phenomenon restricts the options for antibiotic therapy in some bacterial-related infections or diseases. Thus, calls for the investigation into the occurrence of *Salmonella* species in poultry eggs and their susceptibilities to antibiotics.

II. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Gwagwalada, FCT. Along with Abaji, Kuje, Bwari, and Kwali, Gwagwalada is one of the five Local Government Area Councils of the Federal Capital Territory of Nigeria, which also includes the City of Abuja. Gwagwalada lies between latitude 8°-25" and longitudes 6°-45" and 7°-45" east of Greenwich, and an area of 1069.589 km². It is located in the semi-seasonal equatorial climate zone, which has contrasting rainy and dry seasons associated with it [10]. In the daytime, Gwagwalada Area Council temperatures may reach 28°C to 30°C and nighttime temperatures between 22°C to 23°C. April marks the commencement of the rainy season, which extends all the way through October. The daytime temperature can go as low as 12°C during the dry season. Rainy season is from March to November with mean annual rainfall of about 1400 mm [11].

2.2 Preparation and Sterilization of Media and Materials

All the media used in this study were obtained in powdered form and constituted in distilled water according to the manufacturers' instructions. The various quantities and volumes of water depended on the particular medium. A weighed quantity of each medium was dissolved in specific volume of de-ionized water in a chemical flask, which was stoppered properly. It was sterilized by autoclaving at 121 °C and 15 pounds per square inch for 15 min and cooled to 45 - 50 °C before dispensing into pre-sterilized Petri dishes. These were left to gel on the workbenches. Glass materials used in this work were also sterilized by autoclaving at 121 °C for 15 min. They were then brought out and allowed to cool down properly before use.

2.3 Sample Collection

A total of thirty (30) poultry eggs samples were

collected from three (3) poultry farms in Gwagwalada. Ten (10) samples were gotten from each farm and transported to the Microbiology Laboratory, University of Abuja, for isolation of *Salmonella* spp and the sample were analyzed within 4 hours on the day of collection. At the point of collection, sterile cotton swabs previously moist with sterile normal saline water was used to swab the egg shell's entire surface area before putting it back into the container. The eggs used for collecting shell samples were also used for sampling the contents inside the eggs.

2.4 Isolation of *Salmonella* spp. from Egg

Isolation of *Salmonella* spp from eggs was done using the spread plate technique [12]. The egg's surfaces were sterilized using 70% ethanol and then cracked open with sterilized scalpel blade and forceps to carefully remove the eggshell from the contents. The egg whites and yolk were then separated from the shell and placed in a sterile stomacher bag, which was then homogenized for one minute. To facilitate pre-enrichment, 1 ml of the homogenized egg content was mixed well with 10 ml of buffered peptone water (BPW) from Oxoid (product code CM1049). Similarly, the swab sticks were aseptically dipped into 10 mL of buffered peptone water to form stock.

Tenfold serial dilutions of the pre-enriched broth were made in sterile water medium, 1.0 ml of the dilution sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile water. The contents were mixed thoroughly and ten-fold dilutions of solution were made up to 10⁻⁵ and 0.1 ml was inoculated on the Nutrient agar (10⁻⁵), MacConkey agar (10⁻³) and xylose lysine desoxycholate (XLD) agar (10⁻³) using the spread plate method.

The plates were incubated at 37 °C for 24 hours and the number of colonies on nutrient agar and MacConkey agar were counted using colony counter. The colonial density was calculated as the count multiplied by the dilution factor and the mean count obtained was recorded and expressed in colony forming unit per milliliter (cfu/ml) of the sample analyzed. The same technique was utilized for every individual sample [12].

Representatives of each colony type (that is discrete colonies) on Mac Conkey agar was aseptically

transferred to freshly prepared sterile Xylose Lysine Desoxycholate (XLD) agar to obtain pure cultures. The pure cultures of the bacteria were grown at 37 °C for 24 h and maintained on nutrient agar slants and stored at 4°C for further studies ^[12].

2.5 Identification of *Salmonella* spp

Bacterial isolates obtained were identified on the basis of microscopy. Following Gram staining, biochemical tests and morphological characteristics of the colonies as described by Cheesbrough ^[12]. Biochemical tests which were carried out include indole test, citrate utilization test, catalase test, methyl red test and Voges-proskauer test.

2.6 Molecular Characterization of Bacterial Isolates

2.6.1 DNA extraction

Genomic DNA extraction of isolated bacteria was carried out using the Bacterial DNA Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer ^[13].

Overnight cultures grown in nutrient broth (NB) were centrifuged for 10 min at 5000 x g, to harvest cells. The pellet was washed 3 times in Tris-EDTA buffer (TE buffer) (10mM Tris-HCl pH 8.0, 0.1 Mm EDTA) and the sample was incubated at 37°C for 30 minutes in an incubator, after which proteinase K and extraction buffer were added and mixed by vortexing. The mixture was then incubated at 56°C in a water-bath for 30 minutes. Ethanol (96-100% v/v) was added to precipitate the DNA, which was subsequently transferred into a DNeasy Mini spin column for DNA binding to the column. The column was washed twice with 500 µl washing buffers, and the DNA was eluted with 200 µl elution buffer. The resulting DNA was stored at -20 °C.

2.6.2 Amplification of the 16S rRNA Genes by PCR

The genomic DNA was used to amplify the 16S rRNA gene via Polymerase Chain Reaction (PCR), utilizing bacteria universal primers (27F and 1492R). The PCR reaction was performed in a 50 µl mixture containing 25 µl of 2X PCR Master Mix, 1.5 µl of template DNA, 1 µl of both forward and reverse primers, and 21.5 µl of nuclease-free water. The experiment took place in a Techne TC-412 Thermal Cycler. It began with an initial denaturation step at 94 °C for 2 minutes, followed by 30 cycles. Each cycle consisted of three steps: 94 °C for 30 seconds, 52 °C for 30 seconds, and 72 °C for 2 minutes. The last stage of extension was carried out for a duration of 5 minutes at a temperature of 72 °C.

2.6.3 Assessment of Extracted DNA by Agarose Gel Electrophoresis

PCR products (amplicons) were separated by electrophoresis on a 1 % agarose with Tris-acetate-EDTA buffer (TAE) containing ethidium bromide. Where 4ul of the PCR product was mixed with 4 ul loading dye and ran at a voltage of 120V for 45min. This was later viewed under UV light to confirm the presence and quality of the bacteria DNA.

2.6.4 DNA Sequencing and Analysis

The PCR products obtained from the genomic DNA were subjected to sequencing using 518F and 800R primers through the ABI PRISM Big Dye Terminator cycle sequencer. The resulting gene sequences were aligned with sequences available in GenBank by using the Basic Local Alignment Search Tool (BLAST) search program from the National Center for Biotechnology Information (NCBI) for comparison.

2.7 Determination of frequencies of occurrence

The frequency of occurrence of isolated *Salmonella* spp. was determined using descriptive statistics. The sum of all the numbers of CfU/ml of the organisms in each sample and the percentage were calculated thus:

$$\frac{\text{Number of each Isolates}}{\text{Total number of Isolates}} \times 100 \quad i$$

2.8 Antibiotic susceptibility test

The antimicrobial susceptibility test was conducted using the disk diffusion method, in accordance with the guidelines provided by The Clinical and Laboratory Standards Institute ^[14]. Initially, a suspension of every *Salmonella* isolate colony was created using sterile saline, ensuring that it achieved a turbidity equivalent to the 0.5 McFarland standard. Next, a sterile cotton-tipped swab was dipped into the cell suspension and streaked onto a Mueller-Hinton agar plate (Himedia). Finally, sterile discs containing antibiotics (Oxoid, Hampshire, England) were placed aseptically on the surface of the agar.

The following antimicrobials were tested: amoxicillin (10 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), Tarivid (10 µg), gentamicin (31 µg), nalidixic acid (30 µg), tetracycline (30 µg). Following an 18-hour incubation period at 37 °C, the measurement of growth inhibition zones was conducted. The inhibitory zone diameters were assessed for growth inhibition zones using the

inhibitory zone diameter breakpoints established by CLSI [14].

2.9 Statistical analysis

Data obtained in this study was analyzed statistically using Analysis of Variance (ANOVA) from Ms Excel Statistics (Window 10 version) and the test applied was F-test statistic at $P < 0.05$.

III. RESULTS

3.1 Bacterial Loads

The bacterial loads from poultry eggs in Gwagwalada, Federal Capital Territory, Abuja is

shown in Table 4.1. For eggshell samples, the bacterial loads ranged from $1.4 \times 10^3 \pm 0.3$ to $1.8 \times 10^4 \pm 0.1$ CfU/ml for farm A in Gwagwalada, while the bacterial load for Farm B ranged from $1.5 \times 10^3 \pm 0.3$ to $1.8 \times 10^4 \pm 0.1$ CfU/ml and the bacterial loads from poultry Farm C ranged from $1.4 \times 10^3 \pm 0.3$ to $1.7 \times 10^4 \pm 0.1$ CfU/ml. For egg content samples, the total bacterial loads obtained from the Farm A ranged from $1.3 \times 10^3 \pm 0.2$ to $1.5 \times 10^3 \pm 0.1$ CfU/ml while the bacterial load for Farm B ranged from $1.3 \times 10^3 \pm 0.3$ to $1.5 \times 10^3 \pm 0.2$ CfU/ml and the total bacterial loads from Farm C were within the range of $1.2 \times 10^3 \pm 0.2$ to $1.5 \times 10^3 \pm 0.2$ CfU/ml (Table 4.1).

Table 1: Total Bacterial Load of Bacteria Isolated from Poultry Egg

Samples	Bacterial Loads (CFU/ml)		
	Farm A	Farm B	Farm C
Egg Shell			
1	$1.5 \times 10^4 \pm 0.2^a$	$1.6 \times 10^4 \pm 0.2^a$	$1.7 \times 10^4 \pm 0.2^a$
2	$1.7 \times 10^4 \pm 0.1^a$	$1.4 \times 10^3 \pm 0.1^b$	$1.6 \times 10^4 \pm 0.1^a$
3	$1.8 \times 10^4 \pm 0.2^a$	$1.7 \times 10^4 \pm 0.2^a$	$1.4 \times 10^3 \pm 0.0^b$
4	$1.4 \times 10^4 \pm 0.2^a$	$1.8 \times 10^3 \pm 0.1^b$	$1.7 \times 10^4 \pm 0.2^a$
5	$1.4 \times 10^3 \pm 0.2^a$	$1.4 \times 10^3 \pm 0.0^a$	$1.5 \times 10^4 \pm 0.2^b$
6	$1.4 \times 10^4 \pm 0.3^a$	$1.8 \times 10^4 \pm 0.2^a$	$1.7 \times 10^4 \pm 0.1^a$
7	$1.8 \times 10^4 \pm 0.1^a$	$1.7 \times 10^4 \pm 0.2^a$	$1.8 \times 10^4 \pm 0.2^a$
8	$1.7 \times 10^4 \pm 0.2^a$	$1.7 \times 10^4 \pm 0.1^a$	$1.7 \times 10^4 \pm 0.1^a$
9	$1.8 \times 10^4 \pm 0.1^a$	$1.5 \times 10^3 \pm 0.0^b$	$1.5 \times 10^3 \pm 0.1^b$
10	$1.5 \times 10^3 \pm 0.0^a$	$1.8 \times 10^3 \pm 0.2^a$	$1.4 \times 10^3 \pm 0.2^a$
Egg Content			
1	$1.4 \times 10^3 \pm 0.1^a$	$1.5 \times 10^2 \pm 0.2^b$	$1.4 \times 10^3 \pm 0.2^a$
2	$1.3 \times 10^3 \pm 0.2^b$	$1.4 \times 10^2 \pm 0.1^a$	$1.5 \times 10^2 \pm 0.2^a$
3	$1.5 \times 10^3 \pm 0.1^a$	$1.4 \times 10^3 \pm 0.0^a$	$1.5 \times 10^2 \pm 0.1^b$
4	$1.4 \times 10^3 \pm 0.0^a$	$1.5 \times 10^2 \pm 0.2^b$	$1.4 \times 10^3 \pm 0.2^a$
5	$1.5 \times 10^3 \pm 0.2^a$	$1.5 \times 10^3 \pm 0.2^a$	$1.3 \times 10^3 \pm 0.2^a$
6	$1.4 \times 10^3 \pm 0.2^a$	$1.3 \times 10^3 \pm 0.1^a$	$1.5 \times 10^2 \pm 0.2^b$
7	$1.4 \times 10^3 \pm 0.2^a$	$1.4 \times 10^3 \pm 0.2^a$	$1.3 \times 10^3 \pm 0.1^a$
8	$1.3 \times 10^3 \pm 0.1^a$	$1.3 \times 10^3 \pm 0.2^a$	$1.4 \times 10^2 \pm 0.1^b$
9	$1.3 \times 10^3 \pm 0.1^a$	$1.4 \times 10^3 \pm 0.0^a$	$1.4 \times 10^3 \pm 0.1^a$
10	$1.4 \times 10^3 \pm 0.0^a$	$1.3 \times 10^3 \pm 0.2^a$	$1.2 \times 10^3 \pm 0.2^a$

Key: ^{a, b} = Superscript,

Each Value Represent Mean \pm standard deviation from three replicate values

Values with the same superscript across the same row are not significantly different ($P < 0.05$).

3.2 Identification of *Salmonella* spp

The morphological and biochemical characterization of *Salmonella* spp isolated from poultry eggs is presented in Table 4.2. Isolates obtained were identified on the basis of microscopy,

biochemical tests, and morphological characteristics through macroscopic features. Table 4.3 shows the molecular characterization results of the *Salmonella* spp isolated from poultry eggs.

Table 2: Morphological and Biochemical Characterization of Bacterial Isolates

Samples	Isolate Code	Gram Rxn	Cell Morphology	Catalase	Methyl Red	VogesProskauer	Citrate Utilization	Indole Test	Probable Identity
Egg Shell									
1	S1	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
2	S2	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
3	S3	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
4	S4	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
5	S5	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
6	S6	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
7	S7	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
8	S8	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
9	S9	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
10	S10	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
11	S11	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
Egg Contents									
12	S12	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
13	S13	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp
14	S14	-	Rod	+	-	-	+	-	<i>Salmonella</i> spp

Keys: + =Positive, - = Negative

Table 3 Molecular Identity of *Salmonella* spp from Poultry Eggs

S/N	Sample ID	ORGANISM MATCH	Primers used	Strain	SIMILARITY (%)	MATCHED ACCESSION
1	S1-S11	<i>Salmonella enterica</i> serovars Typhimurium	16s F&R	FDAARGOS_319	97.09	CP027412.1
	S12-	<i>Salmonella enterica</i> serovars Enteritidis	16s F&R	EQAS2016S1	99.07	CP017232.1
2	S14					

3.3 Integrity test for extracted nucleic acid

Plate 1 showed the integrity test for quality assessment of extracted genomic DNA by agarose gel electrophoresis. High molecular band size indicates the presence of DNA in each sample

loaded in the wells. Similarly, polymerase chain reaction (PCR) result viewed under UV light to confirm the presence of the amplified PCR products with 1500bp. Gel electrophoresis micrograph of the PCR products from the isolates is shown in Plate 2.

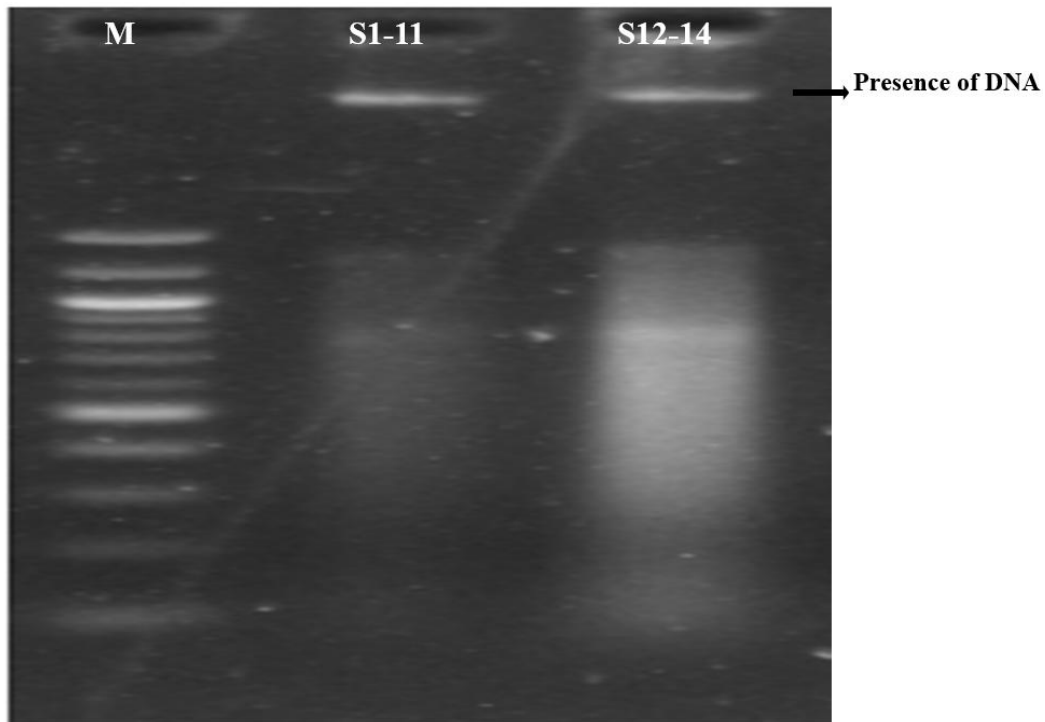


Plate 1: Agarose Gel Electrophoresis showing Presence of DNA

Key: From left to right, Legend: M = 1kb – Ladder, S1-11 = isolate S1-11, S12-14 = isolate S12-14.

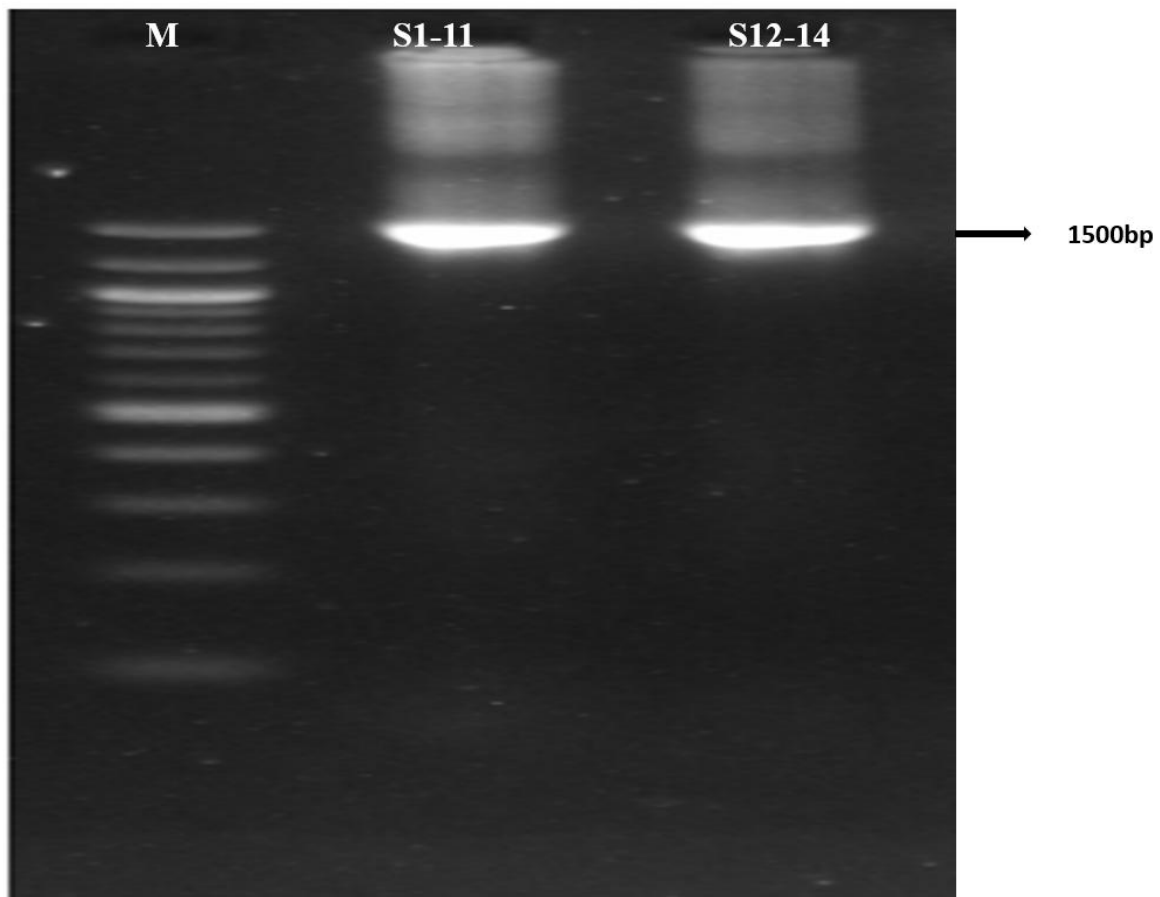


Plate 2: Agarose Gel Electrophoresis showing PCR Result with the Bond Point

Key: From left to right, Legend: M = 1kb – Ladder, S1-11 = isolate S1-11 (*Salmonella enterica* serovars *Typhimurium*), S12-14 = isolate S12-14 (*Salmonella enterica* serovars *Enteritidis*).

3.4 *Salmonella* spp Isolated from Poultry Eggs

Table 4 showed the frequency of occurrence of *Salmonella* spp isolated from Poultry eggs from different Farms in Gwagwalada. Out of a total of fourteen (14) *Salmonella* spp isolated from the poultry eggs, five (5) were isolated from Farm A, six (6) were from Farm B and while three (3) were isolated from Farm C. From the same Table 4.5, total number of isolates gotten from poultry eggs in this

study shows that *Salmonella enterica* serovars *Typhimurium* were 8 while *Salmonella enterica* serovars *Enteritidis* recorded were 6.

Meanwhile, eleven (11) *Salmonella* spp were recorded in eggshell while only three (3) *Salmonella* spp were recorded in the egg content as represented in Table 5.

Table 4: Frequency of Occurrence of *Salmonella* spp isolated from Poultry Eggs from different Farms in Gwagwalada

Isolates	A	B	C	Total
<i>Salmonella enterica</i> serovars <i>Typhimurium</i>	2	4	2	8
<i>Salmonella enterica</i> serovars <i>Enteritidis</i>	3	2	1	6
Total	5	6	3	14

Table 5: Frequency of Occurrence of *Salmonella* spp in Egg shell and Egg Content

Isolates	Eggshell	Egg Content	Total
<i>Salmonella enterica</i> serovars <i>Typhimurium</i>	7	1	8
<i>Salmonella enterica</i> serovars <i>Enteritidis</i>	4	2	6
Total	11	3	14

3.5 Percentage Occurrence of *Salmonella* spp

Figure 4.1 shows the percentage occurrence of *Salmonella* spp isolated from poultry eggs. *Salmonella enterica* serovars *Typhimurium* was the

most frequently isolated which represented 57.14% while *Salmonella enterica* serovars *Enteritidis* represented 42.86 % of the total isolates.

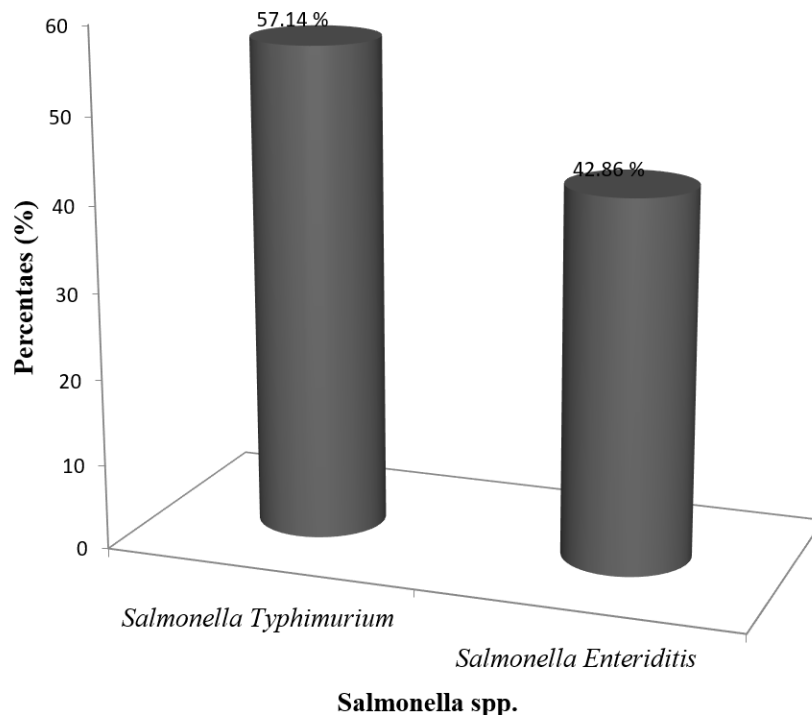


Figure 1: Percentage Occurrence of *Salmonella* Species Isolated from Poultry Egg

3.6 Antibiotics Susceptible pattern of *Salmonella* species isolated from poultry eggs

Table 4.6 showed the antibiogram of *Salmonella Typhimurium* and *Salmonella Enteritidis* isolated from poultry egg. *Salmonella Typhimurium* was susceptible to cefoxitin, ceftazidime, ciprofloxacin and gentamicin with intermediate activity to amoxicillin, chloramphenicol and tetracycline but showed resistance to ampicillin, tarivid and nalidixic acid. Also, *Salmonella Enteritidis* was susceptible to chloramphenicol, cefoxitin, ampicillin, ceftazidime, ciprofloxacin and gentamicin to intermediate

activity against amoxicillin, and tetracycline but showed resistance to tarivid and nalidixic acid.

However, Table 4.7 showed the antimicrobial susceptibility profile of the *Salmonella* species isolated from poultry eggs. The *Salmonella* species isolated from all the samples showed varying levels of susceptibility and resistance to the tested antibiotics. The inhibition zone diameter of the *Salmonella* species to the tested antibiotics less than or equal to 8 mm diameter is resistant while $\geq 9 \leq 18$ mm is intermediate while greater than or equal to 19 mm diameter is susceptible (CLSI, 2017).

Table 6: Antibiogram of *Salmonella Typhimurium* and *Salmonella Enteritidis* Isolated from Poultry Egg

Isolates	Zone Diameter of Inhibition (mm)	
	<i>S. Typhimurium</i>	<i>S. Enteritidis</i>
CHX	17	27
CEF	23	27
PN	06	21
AM	15	19
CPX	27	28
CFT	25	26
OFX	08	08
CN	21	26
NA	07	08
TET	14	16

Table 7: Antibiotics Susceptibility of Pattern of *Salmonella* species

Isolates	<i>S. Typhimurium</i>	<i>S. Enteritidis</i>
CHX	I	S
CEF	S	S
PN	R	S
AM	I	I
CPX	S	S
CFT	S	S
OFX	R	R
CN	S	S
NA	R	S
TET	I	I

Keys: AM = Amoxicillin, PN = Ampicillin, CEF= cefoxitin, CFT= ceftazidime, Chx= chloramphenicol, CPX= ciprofloxacin, OFX= Tarivid, CN= Gentamicin, NA= Nalidixic acid, TET= Tetracycline. Isolates were categorized as resistant (R) if ≤ 8 mm, intermediate (I) if $\geq 9 \leq 18$ mm or sensitive (S) if ≥ 19 mm to each antimicrobial using the inhibitory zone diameter breakpoints recommended by the CLSI 2 (2017).

IV. DISCUSSION

Salmonella is a type of bacteria that is commonly responsible for causing foodborne illnesses in humans, such as salmonellosis and gastroenteritis. These bacteria can be transmitted through food, especially those of poultry origin, when proper

hygiene and infection control practices are not followed. The study analyzed a total of 14 *Salmonella* isolates from poultry egg samples and found that a higher number of isolates were recovered from the eggshell compared to the egg content. This is consistent with a previous study of Detha and Datta ^[15] which suggested that faecal

contamination the eggshell can lead to penetration of pathogenic bacteria such as *Salmonella*. Meanwhile in this study, the occurrence of *Salmonella* spp. in eggs shell was significantly higher than the egg contents. Eggs can become contaminated during various stages including packing, grading, faecal contact, and transportation. Contamination could potentially arise within the study location due to the visual inspection, physical contact, and individual selection of eggs by numerous buyers during sales. All the farms participating in the current research lacked egg sanitation programs, leading to the prevalence of unsanitary egg handling practices. The findings of this study underscore the ongoing significance of poultry eggs as a significant source of *Salmonella* transmission to humans.

Among the 14 *Salmonella* isolates detected in this study, it was observed that 11 of them were present on the surfaces of eggshells, implying the possibility of contamination originating from fecal matter, the environment, or improper handling. The presence of only three *Salmonella* serovars *Enteritidis* in egg content suggests contamination, but the exact route of contamination was not examined. This study also revealed serovar diversity as the 14 *Salmonella* isolates were distributed across 2 serovars. The finding of serovar diversity among the *Salmonella* isolates in this study is consistent with the results reported by Kimura *et al.* [16]. This may be attributed to unregulated importation of poultry birds and eggs without an effective national screening and control program for salmonellosis.

In this study, the *Salmonella Enteritidis* isolate showed susceptibility to ampicillin, cefoxitin, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin and intermediately to amoxicillin and tetracycline but completely resistant to nalidixic acid and Tarivid while *Salmonella Typhimurium* isolates were resistant to ampicillin and, Nalidixic acid tarivid and intermediately to tetracycline, chloramphenicol, amoxicillin and tetracycline.

Additionally, *S. Typhimurium* isolates were classified as multiple drug resistant (MDR) with two different resistance patterns found with ampicillin and, Nalidixic acid, tarivid. The findings of this study regarding the antimicrobial resistance phenotypes observed are consistent with those reported by previous researchers, including Fernández Márquez *et al.* [17]. Isolates exhibiting

resistance to a minimum of three antibiotics that are structurally unrelated were categorized as multidrug resistant (MDR), as defined by Magiorakos *et al.* [18]. The level of antimicrobial resistance observed in the *Salmonella* species isolated in this study is similar to the findings of Okoli *et al.* [19], who demonstrated that *Salmonella* species from poultry sources often exhibit resistance to commonly available antibiotics. Additionally, the resistance of the *Salmonella* species in this study to some commonly available antibiotics supports previous research suggesting that *Salmonella* from poultry sources is often multidrug resistant, as reported by Devasia *et al.* [8] and Khan *et al.* [20].

The emergence of antimicrobial resistance genes in bacteria from the community, such as those found in poultry farms, is often linked to the use of antibiotics, which creates selective pressure and allows organisms to develop resistance. The consumption of poultry products has been linked to foodborne infections, including salmonellosis, caused by *Salmonella* species in humans. If left unaddressed, salmonellosis resulting from the consumption of contaminated or infected poultry products could pose a significant public health risk.

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