Molecular Detection of Beta Lactamase Genes from *Salmonella Typhimurium* Isolated from Poultry Droppings in Nyanya, Abuja

Kanu N.V., Orji S.L., Okafor H.A., Adama R. I., Mustapha, F.A., Oluwafemi M. P., Offiong A. B., Tsebam B.C., Donli, S.C and Ekwe E.O

National Biotechnology and Research Development Agency, Lugbe Abuja, Nigeria

DOI: https://doi.org/10.51584/IJRIAS.2024.908020

Received: 28 June 2024; Revised: 07 July 2024; Accepted: 12 July 2024; Published: 02 September 2024

ABSTRACT

Salmonella infections are one of the major global public health problems. During the last decade, antibiotic resistance and multiresistance of *S. typhimurium.* This study aimed at molecular detection of Beta Lactamase genes from *S. typhimurium* Isolated from poultry droppings in Nyanya, Abuja. Fecal droppings samples were collected from two different Poultry farms using sterile screw tubes. *S. Typhimurium* was isolated and identified using standard microbiology methods. Antimicrobial susceptibility was carried out using Clinical and Laboratory Standards Institute method. Out of 180 samples collected the overall occurrence of *S. typhimurium* was 20 (11.1%). *S. typhimurium* isolated were higher in Farm A 9(10.0%) than in farm B 11(12.2%). The *S. typhimurium* isolates from Farm A were highly resistant to Ampicillin with 77.7% but less resistant to gentamicin with 33.3%, ofloxacin, ciprofloxacin and Sulphamethoxazole/trimethoprim with 44.4%. Isolates from Farm B were highly resistant to Ampicillin and Sulphamethoxazole/trimethoprim 81.8%, but less resistant to Cefuroxime 36.3%. the most common phenotypic resistant observed were C-CIP-CAZ-CRO-AUG- OFL -SXT-CEF (22.2%) from Farm A. CIP-CRO-CAZ- AMP -AUG- OFL -SXT- CEF (18.1%) from Farm B, The commonest MAR index observed in this study was 0.8(33.3%) in farm A and (36.3%) in farm B. The order of occurrences of the 4 Beta Lactamase genes detected was: *blaCTX*-M-9 and *blaSHV* (75.0%) > *bla CMY2* and *Bla CTX*-M (50.0%). The resistance observed in this study indicates it will be difficult to use commonly use antibiotics to treat infection caused by *S. typhimurium* isolated in the study location

Keywords; Antibiotic, Resistance, *S. typhimurium,* Fecal droppings, Beta Lactamase

INTRODUCTION

Poultry and poultry meat products are considered as some of the main carriers of *Salmonella* Species and represent a significant share of the attributed sources of *salmonellosis* in Humans. The widespread occurrence of *Salmonella* in natural environment and the Intensive husbandry practice used in the meat, fish and shellfish industries have been a significant problem in public health [1].

Beta-lactamases are ancient bacterial enzymes that attacks the beta-lactam ring. The class B beta-lactamases (the metallo- beta-lactamases) are divided into three subclasses: B1, B2 and B3. Subclasses B1 and B2 are theorized to have evolved about one billion years ago and subclass B3s is theorized to have evolved before the divergence of the Gram-positive and Gram-negative eubacteria about two billion years ago [2].

Penicillinase was the first β-lactamase to be identified. It was first isolated by Abraham and Chain in 1940, Among Gram-negative bacteria, the emergence of resistance to expanded-spectrum cephalosporins has been a major concern. *Salmonella* infections are one of the major global public health problems [3].

During the last decade, antibiotic resistance and multi-resistance of *Salmonella* spp. have increased a great deal, especially in developing countries with an increased and indiscriminate use of antibiotics in the treatment of humans and animals. Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently worldwide and the rates of multidrug-resistance have increased considerably in recent years [4,5]. Even worse, some variants of *Salmonella* have developed *Salmonella* metabolism and its effect on host colonization of food-

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

producing animals by *Salmonella* generally results in asymptomatic colonization of the intestines. Recently, they have begun to recognize that nutritional requirements of *Salmonella* play an important role in colonization. *Salmonella typhimurium* possesses three mannose-family sugar phosphotransferase (PTS) [6] permeases which are absent in most closely related bacteria and whose expression is controlled by the transcription factor, Consistent with the hypothesis that *Salmonella*'s nutritional requirements play a role in colonization, and are important for *Salmonella* survival within the chicken gastrointestinal tract [7, 8]. This study focus on molecular detection of Beta Lactamase genes from *Salmonella Typhimurium* Isolated from poultry droppings in Nyanya, Abuja

METHODS

Study Area

The study was carried out in the satellite town in Abuja called Nyanya which is under Abuja Municipal `Area Council, it is bordered on the north and northeast by a range of hills, to the east and southeast by Maraba district of Nasarawa state to the south by Federal housing karu site and to the west by Abuja city

Media

Bacteriological media that was used, Methyl Red Voges Proskauer Medium, xylose-lysine deoxycholate (XLD) agar, Bismute susphate agar (BSA) and *Salmonella-Shigella* agar (SSA), Nutrient Agar, Simmon's Citrate Agar, Urea broth, and Peptone Water, Mueller-Hinton Agar (MHA)

Primers

The primers used in this study and their characteristics are as given in table 3.1**.**

Table 1: Primers sequence, target genes and their amplicon sizes

S/N	Target Genes	Sequence	Amplicon Size	References
1	blaTEM	5'-TCGGGGAAATGTGCGCG-3'	972	Matthew et al., [9]
		5'-TGCTTAATCAGTGAGGCACC-3'		
2	blaSHV	5'-GGGTTATTCTTATTTGTCGC-3'	615	Matthew et al., [9]
		5'-TTAGCGTTGCCAGTGCTC-3		
3	blaCTX-M	5'-ACGCTGTTGTTAGGAAGTG-3'	857	Matthew et al., [9]
		5'-TTGAGGCTGGGTGAAGT-3		

Sample Size Determination

The sample size was calculated manually using the formula below as earlier described by [10]

$N=Z^2P\sum/d^2$

Where: N= desired sample size (when the population >10,000); Z= standard normal deviate, usually set at 1.96, which usually correspond to 95% confidence level; P= proportion in the target population, set at 50% (0.5) d= tolerated margin of error.

The proportion was estimated as p< (0.5) and q< (0.5) for non-infection confidence estimated used was 95% (≥1.5) confidence interval with degree of accuracy of d (0.05). The designed effect of 1 was used. The sample

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

size was obtained

N= (1.16)2× 0.5× 0.5÷ (0.05)2

=0.9404÷0.0025 = **180**

Sample collection

A total of one hundred and eight (180) poultry fecal droppings samples were collected using sterile screw tubes. The samples were then transported to microbiology laboratory department of Nasarawa State University Keffi for the analysis.

Isolation of Salmonella species

A loopful of the poultry fecal droppings sample was transferred into 5ml enrichment nutrient agar broth and incubated at 37^0C for 24 h. After incubation, all the tubes showing turbidity after incubation were bacteriologically cultured on xylose-lysine deoxycholate (XLD) agar and Bismute susphate agar (BSA) (Oxoid, UK) for the selective isolation of *Salmonella* species. The plates were then incubated at 37^oC for 24 hours for bacteria. After incubation, the plates were observed for growth and subsequently sub-cultured unto freshly prepared media to obtain pure isolates while other conditions for incubation remain the same according to the methods describe by Langkabel et al. [11].

Identification of the Isolates

Representative colonies were chosen from each of the cultured plate on the bases of their colonial and morphological characteristics. Pure bacterial colonies were identify using Gram staining reaction and biochemical tests according to the methods of Cheesbrough [12]

Gram Staining Examination

The Gram staining technique was carried out as described by Cheesbrough [12] with some modifications; A small portion of cultural organism was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film is obtained, then the wire loop was ` re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through the flame. The slide was then flooded with crystal violet for 1 minute, and then rinsed with distilled water. The smear was again flooded with Lugol's iodine for 1 minute, and rinsed with distilled water and then decolorized, rapidly with acetone alcohol until no more colour appeared to flow from the preparation and rinsed appropriately with distilled water. The smear was then counter stained with neutral red for 1 minute and rinsed with distilled water and allowed to air dry and viewed microscopically using x100 oil immersion objective. Gram positive organism retains the dark blue colour inferred by the iodine/crystal violet complex, while Gram negative organisms appear red, maintaining the colour of the secondary dye. *Salmonella* species is a gram negative cocco-rod.

Biochemical tests

The conventional and commercial (Microgen Bioproducts) biochemical identification tests were used. Conventional biochemical tests were carried out according to the methods of Cheesbrough [12] and observed for reactions typical of *Salmonella* such as Triple Sugar Iron Agar (TSI); Indole Test; Lysine Decarboxylase Test: Methyl Red Test; Citrate Utilization test; Urease test; Motility Test

***Salmonella* serotyping**

Salmonella serotyping was carried out by standard slide agglutination test (CEN ISO/TR 6579-3:2014) with polyvalent and monovalent somatic (O) and flagella (H) antisera (Statens Serum Institute Denmark and Sifin, Berlin, Germany). Firstly, suspected colonies were picked up and tested with somatic O polyvalent and O polyvalent group antisera. In the case of a positive reaction, testing according to the Kaufman White scheme would be applied. If the suspect colony did not show any reaction with O polyvalent antisera, *Salmonella* species

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

and subspecies was identified by biochemical properties. Serotyping results was evaluated according to the Kaufmann White *Salmonella* serotyping scheme as described by Ibrahim *et al*. [13]

Determination of Antibiotic Susceptibility of the S. Typhi isolates

Antimicrobial Susceptibility Testing of the isolates were carried out using Kirby-Bauer disc diffusion method as described by the National Committee for Laboratory Standards (NCCLS) now the Clinical and Laboratory Standards Institute (CLSI) [14].

Preparation and Standardization of Inoculum: At least three well isolated and identical colonies were selected and transferred aseptically with a wire loop into 5 ml sterile Mueller-Hinton broth. The tubes were incubated at 37ºC for 18h to match the turbidity of the 0.5 McFarland barium sulphate standard prepared by mixing 9.95 ml of 1% H_2SO_4 with 0.05 ml of 1.175% $BaCl_2.2H_2O$. Culture whose turbidity exceeded that of 0.5 McFarland was re-adjusted to equivalence using sterile broth. The turbidity of the standard and the test inocula was compared by holding both tubes in front of a stripped white background.

Inoculation of agar plates and application of antibiotics discs: Within 15 min of adjusting the turbidity of the inocula, sterile cotton swabs were immersed into the properly diluted inocula, then rotated firmly several times against the upper inside wall of the tubes to express excess fluid and used to inoculate (by streaking) the entire agar surface of the agar plates. Antimicrobial agent impregnated discs were aseptically placed on the inoculated agar using antimicrobial disc forceps. The discs were deposited such that the centers are 24 mm apart and left to stand for 1 h for pre-diffusion. The plates were subsequently incubated at 37ºC for 24 h.

Results and Interpretation: The diameters (in millimeters) of the various zones of inhibition of growth around each disc was measured with the aid of a ruler on the under surface of the plates without opening the lid. The results would be interpreted as susceptible, intermediate or resistant depending on the diameter of the zone of inhibition measured in millimeter as described in the CLSI [14] interpretation criteria.

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR Index was determined according to the method of Ibrahim et al. [13] as described by Ngwai *et al.* (2014). From the results of the antimicrobial susceptibility testing, MARI was calculated using the following formula:

MARI=a/b

Where a = number of antibiotics to which an isolate is resistant to

b = number of antibiotics against which the isolate was tested.

An isolate with MARI $\geq$ 0.2 is regarded to have originated from an environment where antibiotics are frequently used [13].

Phenotypic Detection of Extended Spectrum Beta-Lactamase

Extended Spectrum Beta-Lactamase production was detected by the conventional Double Disc Synergy Test (DDST) using ceftazidime (30 µg) and cefotaxime (30 µg) discs (placed 15 mm apart from the centre of the discs) with amoxicillin/clavulanic acid (20/10 µg) disc as recommended by the CLSI [14]. Positive result is indicated when the inhibition zones around any of the cephalosporin discs are augmented in the direction of the disc containing clavulanic acid. An increase of $\geq$ 5 mm in the inhibition zones of either cephalosporin in combination with clavulanic acid compared to the cephalosporin alone was interpreted as ESBL positive [14].

Molecular Detection of Extended-Spectrum Beta-Lactamase Resistance

1. *DNA Extraction*

The DNA was extracted from ESBL producing isolates using boiling method as described by Ibrahim et al. [13].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Briefly following, 1 pure colony of ESBL producing isolate, was inoculated into 2 ml of LB broth and incubated at 37ºC for 8 h and 200 µl of LB culture was transfer into Eppendorf tube and centrifuge in micro centrifuge as 3200 rpm for 2 min at room temperature and the supernatant was discard living the cells and the cells were wash twice with washing buffer. About 0.5ml of sterile phosphate buffer was added to the pellet and vortex for 5 sec after which it was heated at 90ºC for 10 min and rapid coiling was done by transferring the tubes into freeze for 10 min and thereafter it was centrifuge at 3200 rpm for 1 min to separate the DNA and the cell containing the DNA debris and 300 µl of supernatant, was transferred into 2 ml Eppendorf tube and stored at -10ºC until use.

2. DNA Amplification of Extended Spectrum β-Lactamase Genes

multiplex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes present in the isolates. The presence of blaCTX-M, blaSHV and blaTEM genes were tested for using previously published primer sets and conditions. The primer sequences and expected amplicon size for each gene are listed in Table 1. The reactions were carried out in 20 µl reaction volume made up of 10 µl of Mastermix (Qiagen), 0.32 µl of primers (0.16 µl each of forward and reverse primers), 3 µl of DNA and 6.68 µl of nuclease free water. The primer concentration stood at 0.2 M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine was closed. Conditions during the reactions were set as: 3 minutes of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 40 sec, initial extension at 72°C for 50 sec, final extension at 72°C for 3 min and a hold at 4°C infinitely

RESULTS

Isolation and Identification of Salmonella Typhimurium

The cultural, Morphological and Biochemical characterization of *Salmonella typhimurium* from poultry droppings in some selected poultry farm in Nyanya- Abuja as shown in Table 2. shows that *Salmonella typhimurium* is Gram negative staining, the morphology of the isolated bacterium was small, Gram negative rod shape bacteria, on xylose lysine Deoxycholate (XLD) plates, the presumptive *Salmonella typhimurium c*olonies appeared red with black centre appearing shows the presumptive and the biochemical characteristics of the *Salmonella* isolates.

Occurrence of Salmonella typhimurium

Out of the 180 samples collected twenty 20 (11.1 %) *S. typhimurium* were isolated. Farm A 9(10.0%) were isolated and from farm B 11(12.2%) were isolated as shown in Table 3.

Table 2: Cultural, Morphological and Biochemical Characteristics of *Salmonella typhimurium*

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics										Inference		
	Gram	Morphology OXD	MOT	UR	TDA	CUT	LYS	H_2S	ONPG	NIT	LAC	MAL	IN	MR	
Colonies that were colorless on MCA, black on SSA and black metallic sheen on BSA	-	Rod shape -	+	-	-	-	+	+	-	+	-	+	-	+	*Salmonella typhimurium*

OXD=Oxidase MOT= Motility test; UR: Urease test= TDA; LYS= Lysine utilization; H_2S= hyudrogen sulphide ; ONPG= Onpg-Galactosidase test; NIT= Nitrate test; LAC= Lactose fermentation test; MAL=Maltonate test; IN= Indole fermentation test; MR : Methyl red MCA: MacConkey agar; SSA: Salmonella-Shigella agar; BSA: Bismuth Sulfite agar

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Table 3: Percentage occurrence of *Salmonella typhimurium* in relation to Selected Poultry Farms

FARMS	Number of Samples	Number (%) of *S. typhimurium*
A	90	9(10.0)
B	90	11(12.2)
Total	180	20(11.1)

Antibiotics resistance of Salmonella typhimurium isolates

The *S. typhimurium* isolates from Farm A were highly resistant to Ampicillin with 77.7% followed by Ceftazidime and Chloramphenicol with 66.6%, Cefuroxime, Cefotaxime and Amoxicillin-Clavulanic acid with 55.5% but less resistant to gentamicin with 33.3%, ofloxacin, ciprofloxacin and Sulphamethoxazole/trimethoprim with 44.4% each. From Farm B the isolates were highly resistant to Ampicillin and Sulphamethoxazole/trimethoprim with 81.8%, Ceftazidime and Chloramphenicol with 72.7%, Cefotaxime with 63.6% but less resistant to Cefuroxime with 36.3%. as shown in Table 4.

Antibiotics Resistance Phenotypes

The antimicrobial resistance Phenotypes of isolates from Selected Poultry Farms is as shown in Table 5. The isolates were distributed into different resistant phenotypes and the commonest were C-CIP-CAZ-CRO-AUG-OFL -SXT- CEF (22.2%) from Farm A, CIP-CRO-CAZ- AMP -AUG- OFL -SXT- CEF (18.1%) from Farm B, C-CIP-CRO-AUG-AMP-SXT- CEF (12.5%) respectively.

Table 4. Antibiotics resistance of *Salmonella typhimurium* from the poultry Farms in part of Nyanya-Abuja.

Antibiotics	Disc Content (µg)	Resistance	
		Farm A N-9 (%)	Farm B N-11 (%)
Ofloxacin	30	4(44.4)	6(54.5)
Cefuroxime	30	5(55.5)	4(36.3)
Amoxicillin-Clavulanic acid	10	5(55.5)	6(54.5)
Ciprofloxacin	15	4(44.4)	5(45.4)
Ampicillin	30	7(77.7)	9(81.8)
Ceftazidime	30	6(66.6)	8(72.7)
Gentamicin	15	3(33.3)	5(45.4)
Cefotaxime	30	5(55.5)	7(63.6)
Chloramphenicol	30	6(55.5)	8(72.7)
Sulphamethoxazole/trimethoprim	30	4(44.4)	9(81.8)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Table 5. Antibiotics Resistance Pattern against *Salmonella typhimurium* Isolates

Antibiotics Resistance phenotype	**No (%) resistance (n=35)**	
	Farm A N-9	**Farm B N-11**
CIP-CRO-OFL- AMP –SXT	1 (11.1)	0(00.0)
C-AUG- OFL -CAZ-AMP	0 (00.0)	1(9.0)
CIP-AUG- OFL -CRO-AMP-CEF	0 (00.0)	1(9.0)
C-CIP-AUG- OFL -AMP-SXT	1 (11.1)	0(00.0)
C-CIP-CRO-AUG-AMP-SXT- CEF	0 (00.0)	0(00.0)
C-CIP-CAZ-AUG-AMP-SXT- CEF	1 (11.1)	0(00.0)
C-CIP-CAZ-AUG-AMP- OFL -SXT	0 (00.0)	2(18.1)
C-CN-CRO-CAZ-AMP-AUG- CEF- OFL	0 (00.0)	0(00.0)
C-CIP-CRO-CAZ-AUG- OFL -SXT- CEF	1 (11.1)	0(00.0)
C-CIP-CRO-CAZ-AUG- OFL -SXT- CEF	0 (00.0)	1(9.0)
C-CIP-CRO-AMP-AUG- OFL -SXT- CEF	0 (00.0)	0(00.0)
CIP-CRO-CAZ-AMP-AUG- OFL -SXT- CEF	0(11.1)	2(18.1)
C-CAZ-CN-CRO-AMP-AUG- OFL -SXT	1 (11.1)	0(00.0)
C-CIP-CAZ-CRO-AUG- OFL -SXT- CEF	2 (22.2)	1(9.0)
CN-CIP-CRO-CAZ-AMP-AUG-SXT- CEF	0 (00.0)	0(00.0)
C-CN-CIP-CRO-CAZ-AMP-AUG-SXT- CEF	0 (00.0)	2(18.1)
C-CIP-CRO-CAZ-AMP-AUG- OFL-SXT- CEF	1(11.1)	0(00.0)
C-CN-CIP-CRO-CAZ-AMP-AUG- OFL-SXT	0 (0.00)	1(9.0)
C-CN-CIP-CRO-CAZ-AMP- OFL-AUG- CEF –SXT	1 (11.1)	0(00.0)

Key: OFX = Ofloxacin; CXM = Cefuroxime, AMC = Amoxicillin-Clavulanic acid; CIP = Ciprofloxacin; TE = Tetracycline, NOR = Norfloxacin; CN = Gentamicin; CTX = Cefotaxime; C = Chloramphenicol; STX = Trimethroprim-sulfamethoxazole.

Multiple Antimicrobial Resistance (MAR) index

The MAR index of the isolates from Selected Poultry Farms is as shown in Table 6. The isolates were distributed into different MAR index and the commonest MAR index were 0.8(33.3%) from farm A and (36.3%) from farm B,

Occurrence of ESBL resistance genes

The occurrences of the ESBL resistance genes in the 4 cefuroxime, cefotaxime and amoxicillin-clavulanic acid resistant isolates are as given in Table 7. The order of occurrences of the 4 genes detected was: *blaCTX*-M-9 and *blaSHV* (75.0%) > *bla CMY2* and *Bla CTX*-M (50.0%).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Table 6. Multiple Antibiotic Resistance (MAR) Index

			No. (%) of isolates	
No. of antibiotics resistance to (a)	**No. of antibiotics tested (b)**	**MAR Index (a/b)**	**Farm A (N-9)**	**Farm B (N-11)**
10	10	1.0	1(11.1)	0(00.0)
9	10	0.9	1(11.1)	3(27.2)
8	10	0.8	3(33.3)	4(36.3)
7	10	0.7	1(11.1)	2(18.1)
6	10	0.6	1(11.1)	1(9.0)
5	10	0.5	1(11.1)	1(9.0)
4	10	0.4	0(00.0)	0(00.0)
3	10	0.3	0(00.0)	0(00.0)
2	10	0.2	0(00.0)	0(00.0)
1	10	0.1	0(00.0)	0(00.0)

Table 7 Occurrence of ESBL resistance genes

ESBL Resistance Genes	**No. (%) of *Salmonella typhimurium* (N=4)**
blaCTX-M-9	3(75.0)
bla CMY2	2(50.0)
Bla CTX-M	2(50.0)
blaSHV	3(75.0)

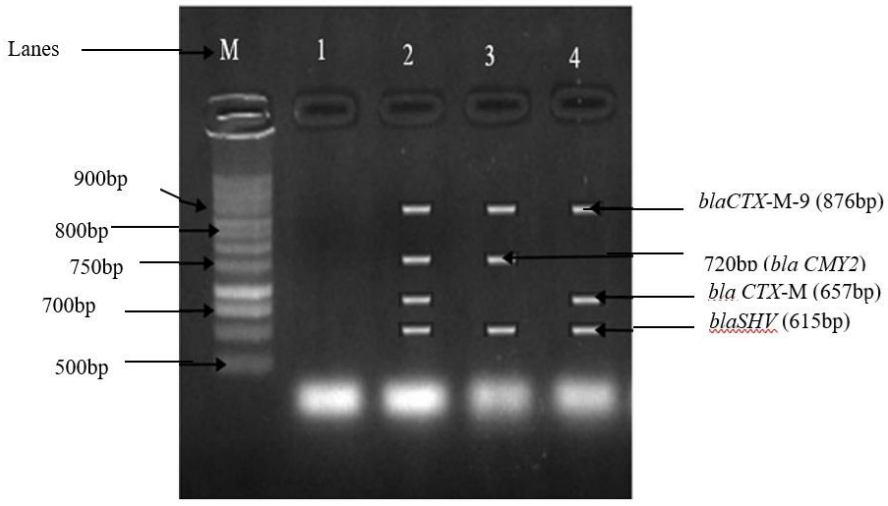

Plate 4.1: Agarose gel electrophoresis showing the amplified ESBL genes in antibiotic-resistant Salmonella typhimurium. Lane 1and 4 represent esbl gene; lane 2-4 represent blaCTX-M-9 (876bp) gene, lane 2 and 3 represent bla CMY2 (720bp) gene; lane 2 and 4 represent blaCTX-M (651bp) gene and lane 2,3 and represent blaSHV (615bp) gene while lane M represent 1000 bp molecular ladder

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

DISCUSSION OF FINDINGS

Salmonella Typhimurium were isolated from 180 faecal droppings across the two (2) Local poultries in Nyanya-Abuja. The isolation of *Salmonella* serotype such as *S. typimurim* in our study indicate the public health significance of these *Salmonella typhimurium* [15] as poultry product contaminated with *Salmonella* may pose serious health hazards [16,17]. *S. typimurium* has been implicated in bacteremia, septicemia and gastroenteritis.

The overall prevalence of *Salmonella typhimurium* isolated from faecal droppings in the poultry farms in Nyanya-Abuja was 11.1 %. The high prevalence of *Salmonella typhimurium* recorded in this study may be attributed to the improved hygienic condition in the poultry farms and the administration of antibiotics into the feeds and water in the farm [18]. This may account for the higher prevalence obtained in our study than Salihu *et al*., [19] in Nassarawa State, Nigeria, reported a prevalence of 2.5% which was lower than 11.1% reported in this study. In addition, Obi and Ike [4] reported a prevalence of 1.3% in Intensively Reared and backyard chickens in Nsukka Area, Nigeria. However, relatively lower but similar prevalence of 0.85 % was reported by Aiyelu [20] who isolated *Salmonella* Arizonae from chicken in Zaria, Kaduna State. The difference in prevalence observed may be due to the fact that the presence and distribution of *Salmonella* serotypes vary from region to region as reported by Uyttendaele *et al*.[21], Dominquez *et al*.[22], Agada *et al*., [23]. Similarly, the isolation rates might depend on the sampling plan as well as the detection limit of the methods of isolation adopted (15, 22].

The result of the antibiotic resistance in is present study showed that the four (4) *Salmonella* isolates were resistant to the various antibiotics used in the study. These organisms harbour multiple resistance genes. These findings agree with that of Kristiansen *et al*. [24] who reported multiple resistance characteristics to chloramphenicol, amoxicillin, sulfonamides and ampicillin. This indicates that fewer drugs would be available to treat infectious diseases caused by *Salmonella typhimurium*. Resistance to sulphamethoxazole – Trimethoprim and ampicillin among *Salmonella* isolates from poultry has been previously reported in Jos, Nigeria [23], in Ibadan, [25]. The observed resistance to sulphamethoxazole – Trimethoprim is likely due to its high usage in animal production [26].

The *Salmonella* showed resistance (66.6%) to the cephalosporins, this finding agrees with the 59.5% found in *Salmonella* species from poultry reported by Agada *et al.* [23] in Jos, Nigeria. Also, Arlet *et al*. [27] documented that *Salmonella* species is the most common serovars associated with extended spectrum cephalosporins resistance in poultry. The resistance to cefotaxime (100%) to *Salmonella* Arizonae observed in this finding is higher than 94.73% observed by Sivakumar *et al*. [28]. Results of multi drug resistant *Salmonella* recorded in this study was similar to the findings of other researchers (Davis *et al*., 2005; Akinyemi *et al*., 2007; Mohammed *et al.*, 2010). In many of these studies, non-typhoidal *Salmonella* species were found to be resistant to Amoxicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, sulphamethoxazole–Trimethroprim, Tetracycline, Cefotaxime. Similarly, Okeke *et al*. (2005) also reported that multiple antimicrobial resistances are common in *Salmonella* species in developing countries.

Multidrug resistant *Salmonella* reported in this study is in agrees with the findings of Hemen *et al*. [29] in Benue State Nigeria who reported the isolation of multi drug resistant *Salmonella* in poultry environment that was resistant to chloramphenicol, Augmentin and ampicillin. Fasure *et al*. [30] also revealed the emergence of multi - drug resistant *Salmonella* serotypes in Ogun state, Nigeria. In his study, resistant to ampicillin (100%), Tetracycline (90.6%), resistant strains were observed. Similarly, Mohammed *et al*. [31], also reported that *Salmonella* isolate from a poultry farm in Jos was resistant to Gentamicin and Ciprofloxacin. It was observed that 100% of the *Salmonella* species were resistant to the antibiotics in the study. The routine use of antibiotics for agricultural purposes, and disease prevention could be responsible for the emergence of multi-drug antibiotic resistance [30].

Polymerase Chain Reaction detection of antibiotic resistance genes analysis revealed that 75.0% and 50.0% of the isolates had *bla-CMY, blaCTX*-M-9, *Bla CTX*-M and *blaSHV* genes of the AmpC family. *Salmonella* resistance to cefuroxime, cefotaxime and amoxicillin-clavulanic acid could be due to the secretion of beta-lactamases into the periplasmic fluid. This enzyme hydrolyses the beta-lactam ring in beta-lactams into beta – amino acids which have no anti-microbial activity. The genes encoding for beta lactamases are typically carried

on plasmids [32]. The presence of beta-lactamases in this study is in line with the findings of Abdel-Maksoud *et al.* [33, 34] who reported the presence of these genes in *Salmonella* isolates from poultry samples. The detection of these genes in this study may be due to the widespread use of antibiotics in animals for disease prevention and animal production. Thus, the spread of resistance genes in poultry flock [35] that can infect human directly or transfer antibiotic resistance genes to other human pathogen [36].

CONCLUSION

In this study *Salmonella typhimurium* were isolated from two poultry farms sampled. The occurrence of the isolates was high from farm A. the isolates were highly resistance to most of commonly used antibiotics to treat infection caused by *Salmonella* species. Most of the isolates were multidrug resistant and beta-lactam producing isolates. The beta-lactam resistant genes detected were *blaCTX*-M-9 and *blaSHV, bla CMY2* and *Bla CTX*-M.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

Authors declare no conflict of interest

REFERENCE

1. Brenner, F. W, Villar, R.G., Angulo, F. J., Tauxe, R.V. and Swaminathan, B. (2010) Salmonella nomenclature. Journal of Clinical Microbiolology,38: 2465-2467
2. Andrews, W. H., Flowers, R. S., Silliker, J and Bailey. J. S. (2011). Salmonella. in Compendium of Methods for the Microbiological Examination of Foods. Downes, F. P., and K. Ito, 4th Ed. American Public Health Association, Washington, D.C. Pp. 357–358.
3. Okoli, I.C., Endujihe G .E and Ogbuewu I .P. (2015). Frequency of isolation of Salmonella from commercial poultry feeds and their antimicrobial resistance profiles, Imo State, Nigeria. Online Journal of Health and Allied Science. 5:2-3
4. Obi, O. J. and Ike, A. C. (2015).Prevalence and Antibiogram Profile of Salmonellae in Intensively Reared and Backyard Chickens in Nsukka Area, Nigeria. Nigerian Journal of Biotechnology, 30: 18 – 25
5. Molbak, K., Olsen, J. and Wegener, H. (2016).Salmonella Infections, In H. Reimann, D. Cliver (eds.), Foodborne Infections and Intoxications, .Academic Press. Pp. 55-115
6. Muktar, Y., Abera, G., and Kemal, J., (2015).Antibiotic Resistance in SalmonellaSpecies, a Serious Public Health Problem: A Review. (2015).Global Veterinaria, 15 (5): 469- 479.
7. Garber, L., Smeltzer, M., Fedorka-Cray, P., Ladely, S. and Ferris, K. (2003).Salmonella enterica serotype Enteritidis in table egg layer house environments and in mice in US layer houses and associated risk factors. Avian Diseases, 47: 134-142
8. Doublet, B., Butaye, P., Imberechts, H., Boyd, D., Mulvey, M. R., Chaslus - Dancla, E. and Cloeckaert, A. (2004).Salmonella genomic island 1 multidrug resistance gene clusters in Salmonella enterica serovar Agona isolated in Belgium in 1992 to 2002. Antimicrobial Agents Chemotherapy,48: 2510-2517.
9. Matthew, E E., Nsikan S. U., Emmanuel, M. I., Kingsley H.E., Josiah A. L. and Kimboline, D. E. (2012). Antibiogram studies and extended spectrum beta-lactamase activity profile of Salmonella-like species isolated from poultry soil of the University of Uyo, Nigeria. Malaysian Journal of Microbiology,8 (4): 280-284
10. Niang L., Winn T. and Rusli B.N (2006). Practical isssues in calculating the sample sizes for prevalence studies. Archeological orofactory science. 1:9-14
11. Langkabel , N., Klose , 1 A., Irsigler , H., Jaeger , D., L. Bräutigam , L., Hafez , H. M., and Fries, R. (2014).Comparison of methods for the detection of Salmonella in poultry. Journal of Applied Poultry Research,23:403–408
12. Cheesbrough (2016).District Laboratory Practice in Tropical Countries Part 2 Second Edition. Cambridge university press
13. Ibrahim T., Ngwai Y.B., Ishaleku D., Tsaku P.A., Nkene I.H., Abimiku R.H (2022). Detection of extended spectrum beta-lactamase production in Salmonella Typhimurium isolated from poultry birds in Nasarawa State, Nigeria. Scientific African 16; e01243

14. CLSI (Clinical and Laboratory Standards Institute (2012).Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement.M100-S22. Vol 32(3)
15. Manoj, J., Singh, M.K., and Singh, Y.P. (2015).The role of poultry in food borne salmonellosis and its public health importance. Advances in Animal Veterinary Science, 3(9):485-490
16. Butaye, P., Michael, G., Schwartz, S., Barett, T., Brisabois, A., and White, D. (2013). The clonal spread of multidrug-resistant non-typhi Salmonella 7 serotypes. Journal of Microbes and Infection,8:1891–1897
17. Graziani, C., Busani, L., and Luzzi I. (2008) Antimicrobial resistance in Salmonella enterica serovar Typhimurium from human and animal sources in Italy. Journal of Veterinary Microbiology 414–418
18. Carrique-Mas, J.J and Davies, R.H (2008). Sampling and bacteriological detection of Salmonella in poultry and poultry premises: a review. Revised Science Technology Office of International Epizootic, 27(3):665-677
19. Salihu, A. E., Onwuliri F. C., and Mawak J. (2012) Antimicrobial resistance profiles of Salmonella gallinarum isolates from free-range chickens in Nasarawa state, Nigeria. International Journal of Science and Technology, 2(8):2224-3577
20. Aiyelu, D.O. (2015). Prevalence, characterization and antibiotic susceptibility of Salmonella isolates from chicken in Zaria, Kaduna State, Nigeria. (Unpublished master's thesis).Ahmadu Bello University, Zaria, Kaduna State, Nigeria
21. Uyttendaele, M.R., Debevere, J.M., Lips, R.M., and Neyts, K.D. (1998).Prevalence of Salmonella in poultry carcass and their products in Belgium. International Journal of Food Microbiology, 40: 1-8
22. Dominguez, C., Gomenz, I., and Zumalacarregui, J. (2002).Prevalence of Salmonella and Campylobacter in retail chicken meat in Spain. International Journal of Food Microbiology, 72: 165-168
23. Agada, G. O. A., Abdullahi, I. O., Aminu, M., Odugbo, M., Chollom, S. C., Kumbish, P. R., and Okwori, A. E. J. (2014).Prevalence and Antibiotic Resistance Profile of Salmonella Isolates from Commercial Poultry and Poultry Farm-handlers in Jos, Plateau State, Nigeria.British Microbiology Research Journal, 4(4):462-479
24. Kristiansen, M., Sandvang, D., and Rasmussen, T. (2003). In vivo development of quinolone resistance in Salmonella enterica serotype typhimurium DT104. Journal of Clinical Microbiology, 41 (9), 4462-4464
25. Fashae, K., Ogunsola, F., Aarestrup, F.M., and Hendriksen, R.S (2010). Antimicrobial susceptibility and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. Journal of Infectious Disease in Developing Countries, 4(8):484-494
26. Threlfall, E.J., Ward, L.R., Frost, J.A., and Willshaw, G.A. (2012).The emergence and spread of antibiotic resistance in food- borne bacteria. International Journal of Food Microbiology, 62, 1-5
27. Arlet, G., Barrett, T.J., Butaye, P., Cloeckaert, A., Mulvey, M.R., and White, D.G. (2006).Salmonella resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbial infection, 8(7):1945-54
28. Sivakumar, T., Avinash, S. N., Prabhu, D., Shankar, T., and Vijayabaskar, P. (2012). Characterization of multidrug resistant patterns of salmonella sp. World Journal of Medical Sciences,7(2), 64-67
29. Hemen, J.T., Johnson, J.T., Ambo, E.E., Ekam, V.S., Odey, M.O., and Fila, W.A. (2012). Multi-Antibiotic resistance of some gram negative bacterial isolates from poultry litters of selected farm in Benue state. International Journal of Science and Technology, 2 (8), 543-547
30. Fasure, A.K., Deji-Agboola, A.M., and Akin yemi, K.O (2012). Antimicrobial resistance patterns and emerging fluoroquinolone resistance Salmonella isolates from poultry and asymptomatic poultry workers. African Journal of Microbiology Research, 6 (11): 2610-2615
31. Muhammed, M.A., Muhammed, L.U., Ambali, A.G., Mani, A.U., Azard, S., and Barco, L. (2010). Prevalence of Salmonella associated with chick mortality at hatching and their susceptibility to antimicrobial agents, Veterinary Microbiology,140: 131-135
32. Mascaretti, O. A. (2003). Bacteria versus Antimicrobial Agents: An Integrated Approach. ASM, Washington,D.C
33. Abdel-Maksoud, M., Abdel-Khalek, R., El-Gendy, A., Gamal, R.F., Abdelhady, H.M., and House, B.L. (2015). Genetic characterization of multidrug-resistant Salmonella enterica serotypes isolated from poultry in Cairo, Egypt. African Journal Laboratory Medical, 4(1):158, 7 pages.Retrieved from http://dx.doi. org/10.4102/ajlm.v4i1.158
34. Miko, A., Pries, K., and Schroeter, A. (2005). Molecular mechanisms of resistance in multidrug-resistant

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

serovars of Salmonella enterica isolated from foods in Germany. Journal of Antimicrobial Chemotherapy, 56(6):1025–1033

35. Angulo, F. J., Johnson, K. R., Tauxe, R. V., and Cohen, M.L. (2000). Origins and consequences of antimicrobial resistant nontyphoidal Salmonella: Implications for use of flouroquinolones in food animals. Microbiology Drug Resistance.6:77–78
36. Sturenburg, E, and Mack, D. (2003). Extended spectrum β-lactamases: Implications for the clinical microbiology laboratory, therapy, and infection control. Journal of Infectious Disease, 47(4): 273–295