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Antibiotic Susceptibilities of *Salmonella* Serotypes Isolated from Visceral Organs of Post-mortem Chickens during Outbreaks in South-western Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author FMM designed the study, participated in the postmortem examination, wrote the protocol and the first draft of the manuscript. Author NDGI participated in postmortem diagnosis, supervised the work and reviewed the article. Author SNAS participated in supervising the work, managed the analyses of the study and reviewed the article. Author AAA participated in sample collection and isolation of the Salmonella isolates. Author AKFK managed the literature searches and performed the statistical analysis. Author PAA participated in the isolation and identification of the Salmonella isolates. Author MA participated in performing the antibiotic sensitivity test. Author CNK participated in supervising the work and reviewed the article. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Antimicrobial susceptibility profile of some motile *Salmonella* serotypes isolated from outbreaks of salmonellosis in commercial and backyard poultry farms were investigated in this study, to determine the therapeutic effectiveness of some common antimicrobial drugs used in Veterinary and Medical practices.

Place and Duration of the Study: Samples were collected from Lagos, Ogun and Oyo States, Nigeria. Bacterial culture and isolation; and antimicrobial susceptibility testing were carried out in the Department of Veterinary Microbiology, Federal University of Agriculture, Abeokuta. Confirmation of *Salmonella* isolates using Polymerase chain reaction (PCR) was done at the Biotechnology Laboratory, National Veterinary Research Institute, Vom. Serotyping was carried out at the World Organization for Animal Health/OIE, Italian Reference Laboratory for *Salmonella*, Istituto Zooprofilattico Sperimentale delle Venezie, Podava, Italy. The work was carried out over a period of 1 year between January and December, 2013.

Methodology: Tissue samples of the lungs, heart, liver, spleen, kidneys, proventriculus, small intestine, caecum and bile samples caecum from chickens submitted for postmortem examinations during outbreaks of salmonellosis were collected for bacterial isolation and identification. Confirmed *Salmonella* isolates were serotyped using the White-Kauffmann-Le Minor Scheme. The susceptibility profiles of the isolates were determined by the disc-diffusion method.

Results: The *Salmonella* serotypes were *Salmonella* Zega, *S*. Kentucky, *S*. Herston, *S*. Nima, *S*. Telelkebir, *S*. Colindale and *S*. Tshiongwe. All the seven *Salmonella* serotypes were 100% sensitive to Gentamycin, Ciprofloxacin, Enrolfloxacin, Ofloxacin, and Pefloxacin, but were 100% resistant to Erythromycin, Co-trimoxazol, Penicillin, and Ampicillin. They showed intermediate sensitivity to Cephalexin, Amoxycillin, Augumentin, Chloramphenicol, Ceftriaxone, Ceftazidime, Nolidixic acid, Oxacillin, Anicillin and Nitrofurontoin.

Conclusion: *Salmonella* serotypes identified in this study showed sensitivity to some antibiotics but were multidrug resistant (MDR) to various types used in both Veterinary and Medical practices, posing a serious therapeutic and public health challenges. All 7 *Salmonella* serotypes were resistant to 4 antibiotics. Also, all were MDR. We recommend for Polymerase chain reaction as a fast and accurate method for the detection of *Salmonella* species, and antibiotics testing before treatment in cases of outbreaks of avian salmonellosis.

Keywords: Salmonellosis; Salmonella; antimicrobials; susceptibilit; chicken; postmortem.

1. INTRODUCTION

Poultry production has contributed to the economy of many farmers and countries world-wide [1]. However, efficient poultry production requires healthy day-old chicks and good management practices [2]. The expansion of the poultry industry in Nigeria has been faced with various disease challenges in which salmonellosis takes high places, causing serious economic losses and public health problems [3].

Antimicrobial agents have been used in the treatment and control of diseases and as food animal feed additives for growth promotion and prophylaxis in Veterinary medicine and are used as therapeutic agents in Medical practice [4]. Large varieties of antimicrobial drugs have also been used for prophylaxis and growth promotion in animal husbandry [5]. The indiscriminate use of antimicrobial drugs in both Veterinary and Human medicines has led to the emergence of antimicrobial resistant species of bacteria including *Salmonella* organisms [4,6]. The consequences of the emergence of Multidrug resistant (MDR) bacteria include an increase in chick mortality and persistence of disease carrier chickens due to ineffective therapeutic treatment [2]. The poultry husbandry has been reported to be a major reservoir of MDR bacteria species including *Salmonella* organisms [7,8]. To prevent the spread of antimicrobial resistant bacteria, it was suggested that a systematic and routine registration and analysis of patterns of resistance of pathogenic and non-pathogenic faecal bacterial flora be carried out [9]. Moreso, routine susceptibility testing and surveillance programs as essential measures to assess the prevalence of many of the drug resistant strains of bacteria were recommended [10,11]. Treatment of bacterial poultry diseases should also be based on susceptibility testing of bacteria in order to counter the resistant properties of the infecting bacteria [2,12,13].

This present study investigated the antimicrobial resistance and susceptibility profiles of some motile *Salmonella* serotypes isolated from tissue samples of chicken submitted for postmortem examination during outbreaks of salmonellosis in commercial and backyard poultry farms in Lagos, Ogun and Oyo States, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Lagos, Ogun and Oyo States within Southwestern Nigeria; which lies between longitude 30° and 7°E and latitude 4° and 9°N in the West of the lower Nigeria and South of the Niger Trough (Fig. 1) [14].

2.2 Postmortem Examination

This study was conducted between January to December, 2003 across backyard and commercial poultry farms. Flock sizes of the farm ranged from 5,000 to 160,000 birds. 8-13 dead birds from every outbreak of disease were examined at postmortem in the Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta (FUNAAB). The total number of outbreaks, chickens examined, their ages, breed and type of chickens were recorded according to the type of farm, month of examination and the State from which they were submitted. In each suspected case of salmonellosis presented, samples of the lungs, heart, liver, spleen, kidneys, proventriculus, small intestine, caecum and bile were collected from one representative bird. In total, 270 samples were collected from 30 commercial poultry farms, while 54 samples were obtained from 6 backyard farms. Samples were aseptically collected in pre-labelled sterile sample bottles, for bacterial culture and identification.

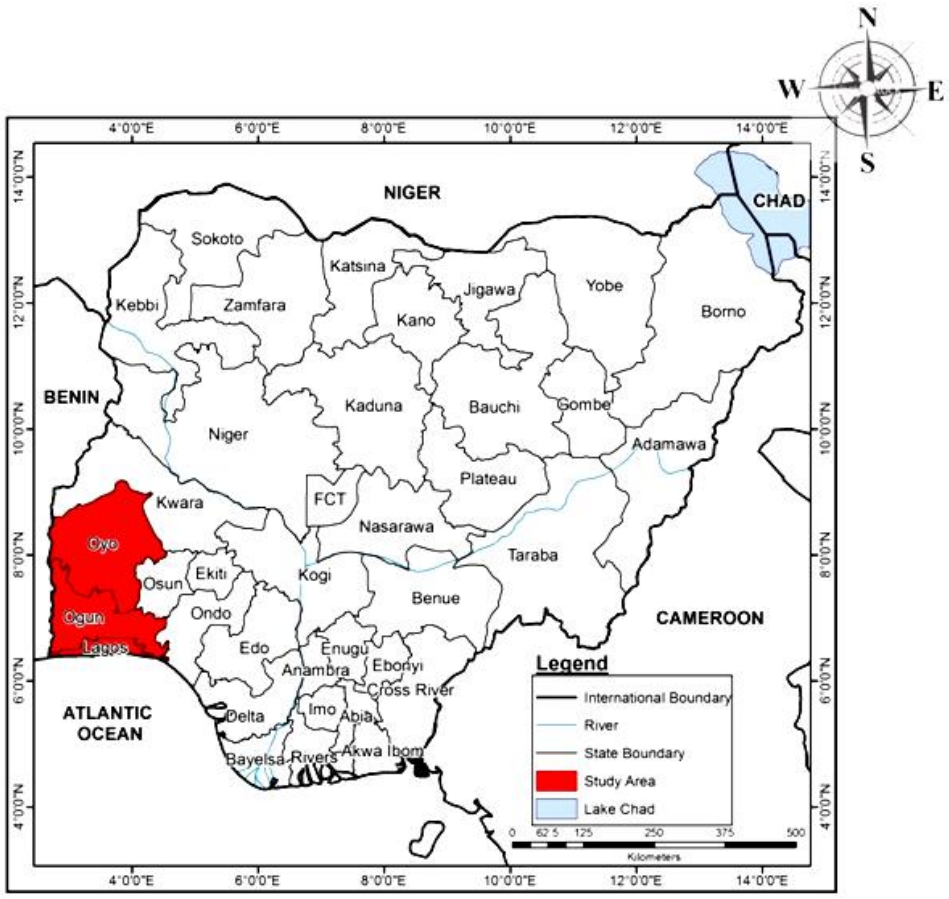

Fig. 1. Map of Nigeria showing the States (Lagos, Ogun and Oyo) under study (Source: Modified from the Administrative Map of Nigeria)

2.3 Bacterial Culture, Isolation and Identification

Bacterial culture and identification was carried out in the Department of Veterinary Microbiology and Parasitology of the Federal University of Agriculture, Abeokuta. Swabs from aseptically sectioned tissue samples of the organs and bile collected at postmortem were taken and separately applied into Nutrient Broth (Oxoid, CM0001, Basingstoke, UK) and Buffered Peptone Water (Oxoid, CM0009, Basingstoke, UK) for pre-enrichment, and incubated at 37^{o}C for 24 h. Pre-enriched media (2 ml) were inoculated into 50ml of Rappaport-Vassiliades Broth (Oxoid, CM0669, Basingstoke, UK) and onto Tetrathionate Glucose Broth (Oxoid, CM0029, Basingstoke, UK) for selective enrichment and then incubated at 37°C for 24 h. Using a sterile wire loop, a loopful of incubated broth culture was inoculated onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid, CM0469, Basingstoke, UK) and incubated at 37°C for 24 h. The plates were examined for typical colonies of *Salmonella.* The colonies from these plates were further subcultured on XLD agar and incubated at 37°C for 24 h. The plates from the subculture were also observed for typical colonies of *Salmonella* as described by Doughlas et al. [15]. Suspected *Salmonella* colonies were inoculated onto MacConkey agar (Oxoid, CM0007, Basingstoke, UK) for purification.

Case fatality of salmonellosis was calculated as percentage of isolation of *Salmonella* serotype from each organ.

2.4 Polymerase Chain Reaction Procedure

One suspected *Salmonella* isolate from each of the 30 outbreaks in commercial poultry farms and 3 isolates from each of the 6 outbreaks in the backyard farms making 48 representative samples were put on nutrient slant, freeze-dried and sent to the National Veterinary Research Institute (NVRI), Vom, Plateau State for confirmation using conventional polymerase chain reaction (PCR).

Deoxyribonucleic acid (DNA) was extracted using a commercial kit ZR Fungal/bacterial DNA Miniprep^{TM} (Zymo Research Corp, USA). Extraction of DNA was done according to the manufacturer's instructions.

To confirm the identity of suspected isolates, a set of primer pair 139-141 targeting the *invA* gene of *Salmonella* species were used [16]. The primer sequences were as follows: invAf 5-3^{1-} GTGAAATTATCGCCACGTTCGGGCAA (sequence length of 26 bases) and invAr 5-3^{1}-TCATCGCACCGTCAAAGGAACC (sequence length of 22 bases) with amplification product of 284 base pair (bp).

DNA Amplification: PCR amplifications were performed in a final volume of 50 µl containing 10 µl of DNA template, 25 ul of 2X PCR master mix (10x buffer, 3 µl of 1.5 mM $MgCl_2$, 3 µl each of 2.50 mM deoxynucleoside triphosphate, 0.2 µl containing 1.25 unit Taq polymerase), 2 mM forward and reverse primers and make up the volume to nuclease free water [16]. Amplifications conditions were previously described using GeneAMP 9700 thermocycler (Thermocycler Applied Biosystem (AB) 9700, USA) [16]: Initial denaturation was at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 1sec, annealing at 55°C 1sec and final extention at 72°C for 7 mins. Amplification products were separated by electrophoresis on 1:2% agarose gel containing 5 µg/ml Ethidium Bromide with a 100-bp and 50-bp ladders (GibcoBRL) as molecular weight markers as described by Cha et al. [17].

2.5 *Salmonella* Species Serotyping

Thirty-seven *Salmonella* isolates, 1 from each of 35 outbreaks and 2 from 1, that were confirmed by PCR to be *Salmonella* species were inoculated into nutrient agar slope, freeze-dried and sent to the World Organization for Animal Health/OIE, Italian Reference Laboratory for *Salmonella*, Istituto Zooprofilattico Sperimentale delle Venezie, Podava, Italy for serotyping using the White-Kauffmann-Le Minor Scheme [18].

2.6 Antimicrobial Susceptibility Test

Antimicrobial susceptibility of *Salmonella* serotypes to various drugs commonly used in Human and Veterinary Medicine was tested in-vitro, using the standard disk diffusion technique, according to the guidelines of NCCLS [19].

To test for susceptibility of the *Salmonella* serotypes to common antibiotics, isolates were emulsified in 5 ml of sterile normal saline and inocula were standardized using sensititre nephelometer (TREK Diagnostic Systems, UK)

after calibration [20]. The concentration of the bacteria was adjusted where necessary, with extra inoculums or diluents until 0.5 McFarland standards were obtained [19]. The dried surface of a 20 ml Mueller-Hinton agar plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking with the cotton swab over the entire sterile agar surface. The inoculated plates were air dried at 37°C before applying the antibiotic discs.

The antibiotic disks (Oxoid, Basingstoke,UK) were evenly dispensed onto the surface of the inoculated Mueller Hinton agar plate using a disc dispenser and gently pressed down the agar surface. The plates were incubated at 37°C for 24 hour. Twenty three antimicrobial drugs including Gentamycin (G) 10 µg, Ciprofloxacin (CP) 10 µg, Enrolfloxacin (EN) 5 µg, Cephalexin (CFX) 30 µg, Peflaxacin (PF) 10 µg, Erythromycin (ERY) 10 µg, Amoxicillin (AMX) 25 µg, Augumentin (AMC) 30 µg, Co-trimoxazol (SXT) 25 µg, Penicillin (P) 10 µg, Ampicillin (AMP) 10 µg, Chloramphenicol (CHL) 10 µg, Nitrofurantoin (NIT) 30 µg, Kanamycin (K) 10 µg, Norbaflaxacin (NOR) 10 µg, Ofloxacin (OFX) 10 µg, Oxytetracyclin (OXY) 30 µg, Nolidixic acid (NA) 30 µg, Ceftriaxone (CRO) 30 µg, Ceftazidime (CAZ) 30 µg, Oxacillin (OX) 5 µg, Streptomycin (S) 10 µg and Anicillin (AN) 10 µg were used for the test.

Following the application of antibiotic disks on the agar plates, they were incubated for 24 h in humidified incubator at 37°C. Determination and interpretation of susceptibility was carried out according to the guideline of NCCLS [19]. *Staphylococcus aureus* (ATCC 6538) was used as control [19,20,21].

3. RESULTS

3.1 Monthly Age Distribution of Chicken Examined at Postmortem

The overall number of disease outbreaks in commercial and isolated poultry farms in Lagos, Ogun and Oyo State that were examined at postmortem during the study period was 118. Of the 118 cases of disease outbreaks examined in this study, 16 (13.56%) were recorded in birds within the ages of 1-3 weeks. While 28 (23.73%) were seen in birds of ages 4-8 weeks and 74 (62.71%) were observed in 9 weeks and older birds (Table 1).

3.2 Monthly Distribution of Chicken Breed and Type Examined at Postmortem

Of the chicken breed examined, the highest outbreaks were recorded in Isa Brown (n=63; 53.39%), followed by Nera Black (n=35; 29.66). The least numbers of outbreaks occurred in the local breed (n=7; 5.93%) (Table 2). Also, layers were the highest chicken type recorded (n=66; 55.93%), followed by broilers (n=27; 11.02%), then pullets (n=13; 22.88%) and cockerels (n=11; 9.85%). The least outbreaks were observed in breeders (n=1; 0.85%).

Table 1. Monthly distribution of disease outbreaks in different ages of chickens at postmortem examination in 2013

Month	Number (%) of disease outbreaks according to age groups in weeks			
	1-3	4-8	9 and above	Overall
January	1 (7.14)	5 (35.70)	8 (57.14)	14 (11.86)
February	0 (0.00)	1 (35.70)	6 (86.71)	7 (5.93)
March	1 (9.09)	3 (27.27)	7 (63.63)	11 (9.32)
April	2 (15.38)	5 (38.46)	6 (46.15)	13 (11.02)
May	1 (8.33)	1 (8.33)	10 (83.33)	12 (10.17)
June	3 (25.00)	3 (25.00)	6 (50.00)	12 (10.17)
July	1 (10.00)	2 (20.00)	7 (70.00)	10 (8.47)
August	0 (0.00)	0 (0.00)	7 (100)	7 (5.93)
September	1 (16.67)	2 (33.33)	3 (50.00)	6 (5.08)
October	4 (44.44)	3 (33.33)	2 (22.22)	9 (7.63)
November	2 (22.22)	1 (11.11)	6 (66.67)	9 (7.63)
December	0 (0.00)	2 (25.00)	6 (75.00)	8 (6.78)
Total	16 (13.56)	28 (23.73)	74 (62.71)	118 (100)

Table 2. Monthly distribution of disease outbreaks in different breeds of chicken at postmortem examination in 2013

Month	Number (%) of disease outbreaks according to breed				
	Isa Brown	Nera Black	Local	Other	Overall
January	9 (64.29)	4 (28.57)	0 (0.00)	1 (7.14)	14 (11.86)
February	3 (42.86)	2 (28.57)	1 (14.29)	1 (14.39)	7 (5.93)
March	6 (54.55)	2 (28.57)	1 (9.09)	2 (18.18)	11 (9.32)
April	8 (61.54)	3 (23.08)	0 (0.00)	2 (15.38)	13 (11.02)
May	5 (41.67)	4 (33.33)	1 (8.33)	2 (16.67)	12 (10.17)
June	7 (58.33)	4 (33.33)	1 (8.33)	0 (0.00)	12 (10.17)
July	4 (40.00)	5 (50.00)	1 (10.00)	0 (0.00)	10 (8.47)
August	2 (28.57)	3 (42.86)	1 (14.29)	1 (14.29)	7 (5.93)
September	3 (50.00)	2 (33.33)	0 (0.00)	1 (16.67)	6 (5.08)
October	6 (66.67)	2 (22.22)	0 (0.00)	1 (11.11)	9 (7.63)
November	6 (66.67)	1 (11.11)	0 (0.00)	2 (22.22)	9 (7.63)
December	4 (50.00)	3 (37.50)	1 (12.50)	0 (0.00)	8 (6.78)
Total	63 (53.39)	35 (29.66)	7 (5.93)	13 (11.02)	118 (100)

3.3 Postmortem Findings

The cases of salmonellosis presented with acute (n=39; 70%) and chronic (n=16; 30%) pathological changes in visceral organs. In the acute form, the lungs were severely congested and oedematous; there were necrotic foci on the myocardium. The livers were markedly enlarged and had multifocal subcapsular necrosis in the visceral and parietal surfaces. The spleen and the kidneys were severely congested and enlarged. The proventriculus contained large amount of mucus and the mucous membrane were hyperemic. The intestines contained mucus admixed with diarrhoic faeces and the mucous membranes were necrotic and hyperemic. The mucous membranes of the caeca were necrotic and the lumen cantained dark red caecal cores. While in the chronic form of the disease, most of the changes observed in the acute form have resolved, but the carcasses were emaciated with pasted vents. There were foci of necrosis in the visceral organs.

3.4 Case Fatality of *Salmonella* Serotypes

Out of the 36 outbreaks, 21 were from Ogun State, nine from Lagos State and six from Oyo State. Of the 324 samples of the visceral organs collected, the lung showed the highest percentage of isolation (n=36; 86.1%) followed by the kidneys (n=36; 77.8%) and the bile (n=36; 77.8%). The proventriculus had the lowest percentage of isolation (n=36; 55.6%). The overall percentage of isolation was 86.1% (n=324) and *Salmonella* species were isolated in all the months of examination.

3.5 Polymerase Chain Reactions

In this study, 48 representative samples of suspected *Salmonella* isolates from the 36 natural cases of avian salmonellosis were tested using Polymerase Chain Reaction. Out of the 48 presumed *Salmonella* isolates, 41 were confirmed to be *Salmonella* species. Bands corresponding to 284 bp were observed in *Salmonella* positive samples and the positive control (*S*. Enteritidis) on the molecular marker ladder (Fig. 1).

3.6 *Salmonella* Serotypes

Thirty seven *Salmonella* isolates were serotyped in which seven serotypes were identified. They included *S.* Zega, *S.* Kentucky, *S.* Herston, *S.* Nima, *S.* Telelkebir, *S.* Colindale, and *S.* Tshiongwe. The predominant serotype was *S.* Zega (n=13; 35.14%) followed by *S.* Kentucky (n=9; 24.32%), then *S.* Herston (n=6; 16.22%), *S.* Nima (n=4; 10.81%), *S.* Telelkebir (n=3; 8.11%), *S.* Colindale (n=1; 2.70%), and *S.* Tshiongwe (n=1; 2.70%). Out of the 37 serotypes, 13.51% were isolated from Lagos State, 78.38% from Ogun State and 8.11% from Oyo State. All the *S.* Hertson, *S.* Nima, *S.* Telelkebir as well as the *S.* Tshiongwe and *S.* Colindale serotypes were isolated from Ogun State (Table 3).

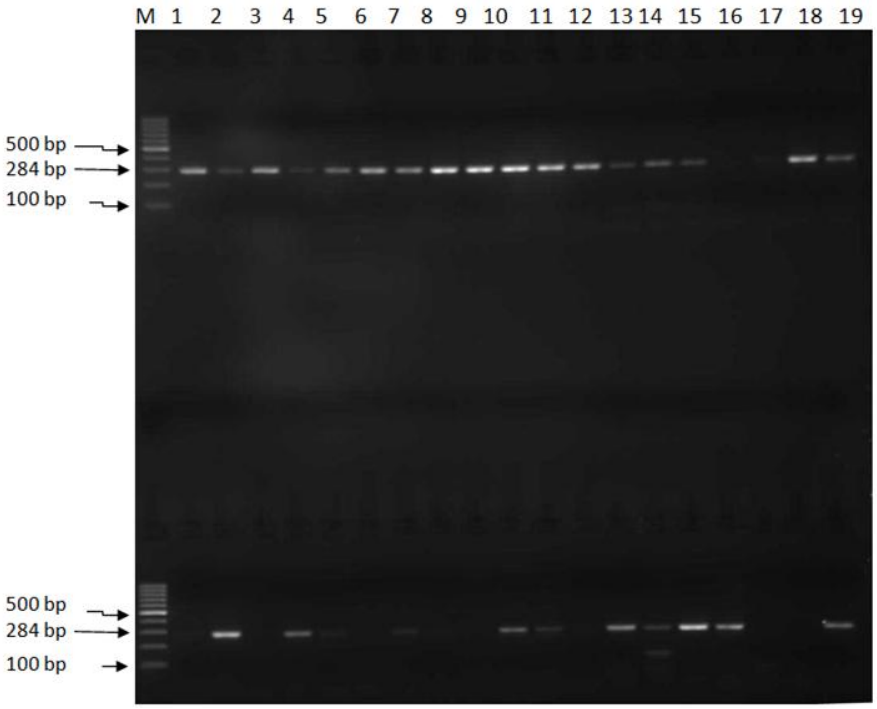

Fig. 1. PCR amplicons on 1.5 % gel for *Salmonella species* from Lagos, Ogun and Oyo States.
Lane M: Molelcular markers of 100 bp ladder each; Lane 1 to 19 (first row): Samples; Lane 1 to 17 (second row): samples; Lane 18 (second row): negative control, Proteus vulgaris; Lane 19 (second row): positive control, Salmonella Enteritidis. Samples of Salmonella species and the positive control, Salmonella Enteritidis correspond to the 284 bp.

Table 3. *Salmonella* serotypes isolated from chicken submitted from Lagos, Ogun and Oyo States, Nigeria for postmortem examination, in 2013

	***Salmonella* serotypes**	**States**		
	Lagos	**Ogun**	**Oyo**	**Total**
S. Herston	0 (0.00)	6 (20.69)	0 (0.00)	6 (16.22)
S. Zega	2 (40.00)	9 (31.03)	2 (66.67)	13 (35.14)
S. Kentucky	3 (60.00)	5 (17.24)	1 (33.33)	9 (24.32)
S. Nima	0 (0.00)	4 (13.79)	0 (0.00)	4 (10.81)
S. Tshiongwe	0 (0.00)	1 (3.45)	0 (0.00)	1 (2.70)
S. Telelkebir	0 (0.00)	3 (10.34)	0 (0.00)	3 (8.11)
S. Colindale	0 (0.00)	1 (0.00)	0 (0.00)	1 (2.70)
Overall	5 (13.51)	29 (78.38)	3 (8.11)	37 (100)

3.7 Anbiotic Susceptibility of *Salmonella* Serotypes

All the seven *Salmonella* serotypes identified in this study were 100% sensitive to Gentamycin, Ciproflaxacin, Enrolfloxacin Ofloxacin, and Pefloxacin, but were also 100% resistant to Erythromycin, Co-trimoxazol, Penicillin, and Ampicillin (Table 4). While the serotypes exhibit variable sensitivity, majority showed intermediate sensitivity to Cephalexin, Amoxycillin, Augumentin, Chloramphenicol, Ceftriaxone, Ceftazidime, Nitrofurontoin, Nalidixic acid, Oxytetracyclin, Streptomycin, Kanamycin, Norbaflaxacin, Oxacillin and Anicillin (Table 4). The sensitivity of the *Salmonella* serotypes to the various antimicrobials varied slightly from 30.4% recorded with *S.* Zega to 47.8% in *S.* Hertston. However, all the *Salmonella* serotypes showed similar resistance profile to four antimicrobial drugs tested (Table 5).

Table 4. Percentage susceptibility of *Salmonella* serotypes to some antibiotics

Drugs	Susceptibility of *Salmonella* serotypes							
	***S.* Zega**	***S.* Kentucky**	***S.* Herston**	***S.* Nima**	***S.* Telelkebir**	***S.* Colindale**	***S.* Tshiongwe**	**Number (%) of serotypes susceptible**
G	+++	+++	+++	+++	+++	+++	+++	7 (100)
CP	+++	+++	+++	+++	+++	+++	+++	7 (100)
EN	+++	+++	+++	+++	+++	+++	+++	7 (100)
CFX	+++	++	+++	+++	++	+++	+++	5 (71.4)
PF	+++	+++	+++	+++	+++	+++	+++	7 (100)
ERY	-	-	-	-	-	-	-	0 (0.00)
AMX	++	++	+++	++	++	+++	++	2 (28.6)
AMC	++	++	++	++	++	++	++	0 (0.00)
SXT	-	-	-	-	-	-	-	0 (0.00)
P	-	-	-	-	-	-	-	0 (0.00)
AMP	-	-	-	-	-	-	-	0 (0.00)
CHL	++	++	++	++	+++	++	++	1 (14.3)
NIT	++	++	++	++	++	++	++	0 (0.00)
K	++	++	++	++	++	++	++	0 (0.00)
NOR	++	++	++	++	++	++	++	0 (0.00)
OFX	+++	+++	+++	+++	+++	+++	+++	7 (100)
OXY	++	++	+++	+++	++	+++	+++	4 (57.1)
NA	++	++	++	++	++	+++	++	1 (14.3)
CRO	++	++	++	++	++	++	++	0 (0.00)
CAZ	++	++	+++	++	++	+++	++	2 (28.6)
OX	++	++	+++	+++	+++	++	+++	4 (57.1)
S	+++	++	++	+++	++	++	++	2 (28.6)
AN	++	++	+++	++	+++	++	+++	3 (42.9)
Overall	7	5	11	9	8	10	9	59 (36.6)

+++: sensitive; ++: intermediate sensitivity; -: resistant

Table 5. Percentage (%) susceptibility of *Salmonella* serotypes to some tested drugs

Salmonella serotypes	Number of drugs tested	Number (%) of drugs with sensitive effect	Number (%) of drugs with intermediate sensitivity effect	Number (%) of drugs with resistant effect
S. Zega	23	7 (30.4)	12 (52.2)	4 (17.4)
S. Kentucky	23	5 (21.7)	14 (60.9)	4 (17.4)
S. Herston	23	11 (47.8)	8 (34.8)	4 (17.4)
S. Nima	23	9 (39.1)	10 (43.5)	4 (17.4)
S. Telelkebir	23	8 (34.8)	11 (47.8)	4 (17.4)
S. Colindale	23	10 (43.5)	9 (39.1)	4 (17.4)
S. Tshiongwe	23	9 (39.1)	10 (43.5)	4 (17.4)

4. DISCUSSION

Antibiotic susceptibility profile of non-typhoidal *Salmonella* serotypes isolated from chickens of all age groups and different breeds and types were examined in this study. Older chickens between 9 weeks of age and older were the most affected. Younger chickens within the ages of 1 to 3 weeks were less frequently encountered. Similar findings of high occurrence of diseases in older birds among commercial poultry farms have been reported in Canada [22]. The reason may be attributed to the long time exposure of older birds to infectious agents such as *Salmonella*; the persistent contamination in poultry houses for long period of time, especially in developing countries like Nigeria where resources for biosecurity is limited and consecutive generation of chickens that are being kept on the farm [22,23]. It is also possible that the low number of outbreaks of diseases recorded in young birds in this study was due to the strict preventive measures employed in the hatcheries where day-olds were procured. Isa Brown was the most affected breed probably because it was the most predominant breed kept on commercial poultry farms in the study area. While Layers were the most affect type because of the reason earlier given for older birds. Chickens are known to be carriers of food borne non-typhoid Salmonella that are often unassociated with mortality. However, the isolation of non-typhoid *Salmonella* serotypes from different tissue samples; and the pathological lesions in the visceral organs which were consistent with those reported in typhoidal serotypes, *S*. Gallinarum and *S*. Pullorum [24,25], suggest they were pathogenic strains and were responsible for heavy mortality in commercial and backyard poultry farms in the study area. The sensitivity of all the *Salmonella* serotypes to Gentamycin, Ciproflaxacin, Pefloxacin and Oflaxacin antimicrobial drugs but resistant to a considerable number including extended-spectrum cephalosporins such as Ceftriaxone that is being used in both Veterinary and Medical practices emphasize the need for antimicrobial sensitivity before treatment of avian salmonellosis. The results showed that the *Salmonella* serotypes identified in this study were also resistant to Ampicillin, Amoxycillin and Penicillin which belongs to same class of antibiotics. Multiple drug resistance of *Salmonella* isolates including *S*. Gallinarum, *S*. Pullorum, *S*. Enteritidis, *S*. Typhimurium, *S*. Heidelberg, *S*. Kentucky, *S*. Typhi and *S*. Paratyphi from poultry carcasses have been reported by various workers [26-30]. However, the resistance exhibited by the uncommon serotypes in the present study, such as the *S*. Zega, *S*. Hertston, *S*. Nima, *S*. Telelkebir, *S*. Colindale and *S*. Tshiongwe has not been reported. The findings on the antimicrobial susceptibility of these *Salmonella* serotypes suggest a common similarity in their susceptibility profile. The slight differences observed may be attributed to the difference in the frequency of their exposure to antimicrobials. From the results of the present study, *S*. Zega and *S*. Kentucky which were the most predominant serotypes appeared to be less sensitive to the antimicrobials tested. The resistance of the *Salmonella* isolates to commonly used drugs in the two fields of medicine pose serious therapeutic and public health problems [2,3,9,12,30,31,32]. Resistance to antimicrobial drug can result from repeated abuse [30] and therefore the high level of antimicrobial resistance of foodborne *Salmonella* isolates in the present study suggests an indiscriminate and continuous use of sub-therapeutic doses of such drugs in animals. There have been reports of gradual increase in the use of various antibiotics including Gentamycin, Neomycin, Tylosin and

Chloramphenicol by those persons that are not Veterinarians in different parts of the country [2,32], raising serious public health concern. The extensive use of antimicrobials in human and animals has led to an increase in bacterial MDR among *Salmonella* strains [33,34]. The various antimicrobials in extensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis or growth promotion [33,34]. However, the results of the present study suggest the need to discourage the use of antimicrobials for prophylaxis and growth promotion. It also suggests a possible significance of chicken meat as a source of multiple antimicrobial-resistant *Salmonella* for human infections and suggests the need for an indebt epidemiological study.

5. CONCLUSION

In conclusion, *Salmonella* serotypes isolated from postmortem tissue samples from chickens were susceptible to some antibiotics, but resistant to wide range of drugs that are commonly used in Veterinary and Medical practices. All 7 *Salmonella* serotypes were resistant to 4 among the antibiotics tested. Also, all were MDR. This is a serious therapeutic and public health challenge. We recommend that Polymerase chain reaction should be used as a fast and accurate method for the detection of *Salmonella* species. A wide range of antibiotics susceptibility testing should be carried out before treatment of bacterial diseases, including salmonellosis and the indiscriminate use of antibiotics by non qualified persons should be discouraged.

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ETHICAL DISCLAIMER

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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