

Sokoto Journal of Veterinary Sciences



(P-ISSN 1595-093X; E-ISSN 2315-6201)



<http://dx.doi.org/10.4314/sokjvs.v19i2.4>

Agbaje *et al.* /Sokoto Journal of Veterinary Sciences, 19(2): 98 - 105.

Diverse non-typhoidal *Salmonella* serovars with multi-drug resistance potentials isolated from chicken faeces in Ogun State, Nigeria

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Publication History:
Received: 02-10-2020
Revised: 02-03-2021
Accepted: 08-03-2021

Abstract

This study was carried out in selected poultry farms to determine the prevalence, distribution and antimicrobial resistance patterns in *Salmonella* serovars in Ogun State, South-western Nigeria. A total of 200 faecal samples were aseptically collected from the four geographical zones of Ogun State, Nigeria. Seventy-eight *Salmonella* isolates spread across 39 serovars and representing a prevalence of 39% was recovered. *Salmonella Urbana* (n=7), *Salmonella Kingston* (n=6) and *Salmonella Agama* (n=5) serovars were more commonly isolated. Resistance was most common to ciprofloxacin (29.5%; n=23/78). Multi-drug resistance (MDR) was observed in 15.4% (n=12/78) of the isolates spread across 7 serovars: *S. Kentucky*, *S. Telelkebir*, *S. Virchow*, *S. Blockley*, *S. Chomedey*, *S. Haifa*, and *S. Isangi*. The study showed the diversity of *Salmonella* serovars and the increasing trend of antimicrobial resistance in poultry farms in Ogun State, Southwestern Nigeria.

Keywords: Antimicrobial-resistance, Chicken, Multi-drug resistance, Nigeria, *Salmonella*

Introduction

Foodborne infections are serious public health issues all over the world (Djeffal *et al.*, 2018) and are mostly caused by zoonotic pathogens originating from apparently healthy food animals (Heredia &

Garcia, 2018). Annually, the World Health Organization (WHO) and the United States (US) Center for Disease Control and Prevention report an increasing number of human foodborne diseases

due to contaminated food consumption (CDC, 2019). One of such contaminating pathogens in the food chain is the zoonotic non-typhoidal *Salmonella enterica*, which has been associated with diverse food-producing animals and their products (Acha & Szyfres, 2001; Davies *et al.*, 2004).

Human non-typhoidal (NT) salmonellosis manifests clinically as self-limiting gastroenteritis in healthy individuals but may be severe in populations with compromised or low immunity (the young, the elderly and people with debilitating disease conditions) especially in developing nations (Motarjemi *et al.*, 1996; Hohmann, 2001). Transmission of NT *Salmonella* to humans is mostly linked to the consumption of contaminated poultry and poultry products (Braden, 2006; Heredia & Garcia, 2018).

While in the developed world, the incidence of *Salmonella* contamination along the food chain is treated seriously with proactive measures incorporated in the food chain to prevent outbreaks (Álvarez-Fernández *et al.*, 2012); the reverse is the case in the developing nations where food-borne infections are given less attention. The lack of focused surveillance systems and data collection on circulating *Salmonella* serovars in most developing countries make it difficult to define the severity of the problem. One major contributing factor may be due to the burden of other debilitating infections such as human immunodeficiency virus (HIV) that relegate foodborne infections like NT salmonellosis to the priority list in most developing countries (Oosterom, 1991).

Previous studies on NT salmonellosis in Nigeria have revealed diverse *Salmonella* serovars in both animals and man (Fashae *et al.*, 2010; Smith *et al.*, 2016; Agbaje *et al.*, 2019). However, the risk of infection with *Salmonella* has been worsened by the acquisition and spread of resistance traits to antimicrobials, a possible consequence of excessive and widespread use of antimicrobials in animal productions (Ojo *et al.*, 2012; Omoshaba *et al.*, 2017).

Multi-drug resistance has become the main attribute of non-typhoidal serovars of *Salmonella* involved in human salmonellosis especially in developing nations (Leegard *et al.*, 1996), like Nigeria, where routine surveillance and laboratory confirmation of these pathogens are seldom taken seriously.

To reduce the risk of multi-drug resistant NT *Salmonella* in the food chain, there is a need for continuous surveillance and relevant data collection to support risk management and policy decisions. An integrated approach may include up scaling existing

epidemiological surveillance and monitoring programs of apparently healthy poultry populations. Therefore, this study was conducted to determine the prevalence, anti-microbial susceptibility pattern and serovars distribution of NT *Salmonella* serovars in some selected poultry farms in Ogun state, Southwest Nigeria.

Materials and Methods

Study location and data collection

The cross-sectional study was carried out in selected poultry farms within the four zones in Ogun State, Nigeria. Ogun State consists of 20 Local Government areas divided into four zones namely Egba, Ijebu, Yewa and Remo. It lies between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E at an elevation of 169 feet with an area of 16,762 square kilometres and 4,054,272 populations (Adebowale *et al.*, 2016). A stratified probability random sampling design was adopted for this study such that poultry farms from the four zones of Ogun state were evenly represented in the final sample.

Salmonella Isolation and Characterization

Sample collection: For this study, fresh faecal samples from the floor of poultry farms were used for *Salmonella* detection. A total of 200 fresh faecal samples were collected based on convenience from layer flocks representing 50 samples from each zone. Samples were randomly collected from the four zones of Ogun state i.e. Abeokuta (Egba), Sagamu (Remo), Ijebu (Ijebu-mushin) and Ilaro (Yewa). A total of 20 farms were sampled representing 5 (five) per zone. A sample constituted aggregated faecal materials pooled together from at least two different points. Samples were collected using sterile nylon gloves and placed in pre-labelled sterile plastic bags and transported to the laboratory in iced packs. Samples were stored at 4°C till the time of analysis.

Isolation and presumptive identification of *Salmonella*: *Salmonella* isolation was performed according to the ISO 6579 standard, with minor modifications (ISO, 2002). In particular, each faecal sample (10g) was pre-enriched in 90 ml of sterile buffered peptone water (BPW, Oxoid CM0509, UK) and then incubated at 37°C for 18 to 24 h. From the BPW culture, 0.1 ml was transferred into 9.9 ml of modified semi-solid Rapport-Vassiliadis selective enrichment broth (MSRV, Oxoid CM0910, UK) supplemented with novobiocin (Oxoid, SR0161, UK) and then incubated at 42°C for 24 to 48 hours. Afterwards, the MSRV was observed for the typical migration pattern of *Salmonella enterica* (≥ 20 mm migration) every 24 h until 72 h. A loopful of

observable bacterial spread was taken from the periphery of the MSRVR medium and streaked simultaneously onto plates of Brilliant Green Agar (BGA, Oxoid CM0263, UK) supplemented with sulphamandelate (Oxoid SR0087, UK) and onto Xylose Lysine Desoxycholate agar (XLD, Oxoid, UK). The inoculated plates were incubated at 37 °C for 18 to 24 hours and examined for bacterial colonies. Typical *Salmonella* red colonies with the black centre were picked and subjected to biochemical and serological tests.

Gram staining, catalase and oxidase were employed in the initial identification of bacteria based on standard procedures while Microbact 24E (Oxoid, UK) biochemical kit was used to further identify *Salmonella* organisms (Cheesbrough, 2002).

Serotyping of *Salmonella* Isolates: Presumptive *Salmonella* isolates were shipped to the Office International des Epizooties (OIE) Reference Laboratory for *Salmonella*, IZSVe Legnaro (PD), Italy. All strains were serotyped by agglutination tests with specific O and H antisera and classified according to the Kauffman-White scheme as previously described (Grimont & Weil, 2007).

Phenotypic antimicrobial susceptibility testing: Susceptibility to 14 antimicrobials was carried out to determine the minimum inhibitory concentrations (MIC) using broth micro-dilution following the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (CLSI, 2009; EUCAST, 2012). Antimicrobial Susceptibility Testing (AST) was carried out with commercially prepared dehydrated broth microdilution panels (Sensititre CMV3AGNF, National Antimicrobial Monitoring System (NARMS); Trek Diagnostic Systems, Westlak, Ohio), following manufacturers' guidelines. The 14 antimicrobials tested were: ampicillin (AMP), cefotaxime (FOT), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), florfenicol (FFN), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulphamethoxazole (SMX), tetracycline (TET) and trimethoprim (TMP).

Owing to the unavailability of the resistance breakpoints of some antimicrobials on EUCAST, MIC results were interpreted following two sets of guidelines as published in 2009 by the CLSI and those published by EUCAST (2012).

While SMX and KAN CLSI resistance breakpoints were ≥ 512 $\mu\text{g/ml}$ and ≥ 64 $\mu\text{g/ml}$ (CLSI, 2009), EUCAST epidemiological cut-off was applied for other antimicrobials including ceftazidime (>4 $\mu\text{g/ml}$), cefotaxime (>2 $\mu\text{g/ml}$) and ciprofloxacin (>1

$\mu\text{g/ml}$) respectively (EUCAST, 2012), with *Escherichia coli* (ATCC 25922) as the quality control strain.

Results

A total of 78/200 *Salmonella* isolates were recovered. This represents a prevalence of 39% (5% CI: 32.2 – 45.8%). The majority of the isolates were recovered from the Remo zone (40/78; 51.3%) followed by the Yewa zone (20/78; 25.6%), Egba zone (12/78; 15.4%) and Ijebu zone (6/78; 7.7%) of the state. The 78 *Salmonella* isolates were spread across 39 serovars, with *S. Urbana* (n=7), *S. Kingston* (n=6) and *S. Agama* (n=5) serovars more commonly isolated (Table 1). *Salmonella Urbana*, *S. Ituri* and *S. Durham* were geographically clustered in the Remo zone of the State while *S. Kingston* and *S. Agama* clustered in the Egba and Yewa zones respectively (Table 1).

All the isolates were susceptible to extended-spectrum cephalosporins, colistin and florfenicol (78/78, 100%), however, resistance was most common to quinolones especially ciprofloxacin (23/78, 29.5%), sulphamethoxazole (19/78, 24.4%), and tetracycline (12/78, 15.4%). Resistance to one antimicrobial was observed in 16 serovars (identified in 28/78; 35.9% isolates) while 23 serovars (identified in 50/78; 64.1% isolates) were pan-susceptible (Table 2). Twelve (12/78; 15%) of the resistant isolates were MDR (resistance to 3 or more antimicrobial classes). MDR isolates belong to 7 serovars (Table 2) including *S. Blockley* (1 isolate), *S. Chomedey* (1 isolate), *S. Haifa* (2 isolates), *S. Isangi* (1 isolate), *S. Kentucky* (3 isolates), *S. Telelkebir* (3 isolates), and *S. Virchow* (1 isolate).

Discussion

The prevalence of *Salmonella* spp. isolated in this study suggests that poultry is an important reservoir with possible adverse consequences on human health when contaminated poultry and poultry products are consumed. The prevalence observed in this study is similar to Orji *et al.* (2005) with a prevalence of 38.3%, however, Enabulele *et al.* (2010) reported a higher prevalence of 80% from poultry faeces but greater than Oyekunle *et al.* (2003) with a prevalence of 16.7% from poultry faeces. Thirty-nine *Salmonella* serovars were identified in this study, suggesting a high diversity of *Salmonella* serovars within the poultry farms in Ogun State, Southwestern Nigeria. Commonly reported non-typhoidal serovars associated with human foodborne infections such as serovars Enteritidis, Newport and Typhimurium were not

Table 1. Distribution of *Salmonella* serovars isolated from poultry faeces in Ogun State, Nigeria

<i>Salmonella</i> serovars	Geographical zones (n=78)				Total No (%)
	Egba	Ijebu	Remo	Yewa	
<i>S. Agama</i>	1	0	1	3	5 (6.41)
<i>S. Alachua</i>	0	0	0	1	1(1.28)
<i>S. Blockley</i>	0	0	0	1	1(1.28)
<i>S. Brancaster</i>	0	1	0	0	1(1.28)
<i>S. Bukavu</i>	0	0	0	1	1(1.28)
<i>S. Carno</i>	0	1	0	0	1(1.28)
<i>S. Chester</i>	0	0	0	1	1(1.28)
<i>S. Chomedey</i>	0	0	1	0	1(1.28)
<i>S. Colorado</i>	2	0	2	0	4(5.13)
<i>S. Cuckmere</i>	0	0	2	0	2(2.56)
<i>S. Dunkwa</i>	0	0	0	1	1(1.28)
<i>S. Durham</i>	0	0	4	0	4(5.13)
<i>S. Ekotedo</i>	0	0	1	0	1(1.28)
<i>S. Ent. Sub sp. Diarizonae</i>	1	0	0	0	1(1.28)
<i>S. Enterica sub.enterica gr.E</i>	0	0	0	1	1(1.28)
<i>S. Enterica sub.enterica gr.R</i>	0	0	0	1	1(1.28)
<i>S. Give</i>	0	2	0	1	3(3.85)
<i>S. Haifa</i>	0	0	1	1	2(2.56)
<i>S. Herston</i>	0	0	0	1	1(1.28)
<i>S. Hillingdon</i>	0	0	2	0	2(2.56)
<i>S. Idikan</i>	0	0	0	1	1(1.28)
<i>S. Isangi</i>	0	0	1	0	1(1.28)
<i>S. Ituri</i>	0	0	4	0	4(5.13)
<i>S. Kentucky</i>	2	0	1	0	3(3.85)
<i>S. Kingston</i>	4	1	0	1	6(7.69)
<i>S. Labadi</i>	0	0	2	0	2(2.56)
<i>S. Lattenkamp</i>	1	0	0	0	1(1.28)
<i>S. Limete</i>	0	0	1	0	1(1.28)
<i>S. Mbandaka</i>	0	0	2	0	2(2.56)
<i>S. Mississippi</i>	0	0	1	0	1(1.28)
<i>S. Nima</i>	0	0	0	1	1(1.28)
<i>S. Oakland</i>	0	0	0	3	3(3.85)
<i>S. Rubislaw</i>	0	0	0	1	1(1.28)
<i>S. Sanjuan</i>	1	0	0	0	1(1.28)
<i>S. Stanleyville</i>	0	0	2	0	2(2.56)
<i>S. Teitelkebir</i>	0	1	3	0	4(5.13)
<i>S. Urbana</i>	0	0	7	0	7(8.97)
<i>S. Virchow</i>	0	0	1	0	1(1.28)
<i>S. Wa</i>	0	0	1	0	1(1.28)
Total	12	6	40	20	100

Table 2. Antimicrobial resistance patterns in 28 *Salmonella* serovars isolated from the poultry faeces in Ogun State, Nigeria

Serovars	Antimicrobial resistance patterns												Frequency	
	A	B	C	D	E	F	G	H	I	J	K	L	Total	% (95% CI)
<i>S. Alachua</i>		1											1	3.57(0.0-10.44)
<i>S. Blockley</i>												1	1	3.57(0.0-10.44)
<i>S. Chomedey</i>										1			1	3.57(0.0-10.44)
<i>S. Ekotedo</i>			1										1	3.57(0.0-10.44)
<i>S. Enterica sub.enterica gr.R</i>			1										1	3.57(0.0-10.44)
<i>S. Give</i>	1	1	1										3	10.71(0.0-22.17)
<i>S. Haifa</i>								1			1		2	7.14 (0.0-16.68)
<i>S. Isangi</i>						1							1	3.57(0.0-10.44)
<i>S. Ituri</i>				1	2								3	10.71(0.0-22.17)
<i>S. Kentucky</i>							3						3	10.71(0.0-22.17)
<i>S. Kingston</i>			2										2	7.14(0.0-16.68)
<i>S. Labadi</i>	2												2	7.14(0.0-16.68)
<i>S. Mbandaka</i>	2												2	7.14(0.0-16.68)
<i>S. Mississippi</i>	1												1	3.57(0.0-10.44)
<i>S. Telelkebir</i>									3				3	10.71(0.0-22.17)
<i>S. Virchow</i>											1		1	3.57(0.0-10.44)
Total	6	2	5	1	2	1	3	1	3	1	2	1	28	

A- CIP

B- CIP-NAL

C- SMX

D- SMX-CIP

E- SMX-CIP-NAL

F- SMX-GEN-AMP-TET-STR-TMP-CHL-KAN-CIP (MDR pattern)

G- SMX-GEN-TET-STR-CIP-NAL (MDR pattern)

H- SMX-GEN-TET-TMP-CIP-NAL (MDR pattern)

I- SMX-TET-STR-CIP (MDR pattern)

J- SMX-TET-STR-CIP-NAL (MDR pattern)

K- SMX-TET-TMP-CIP-NAL (MDR pattern)

L- TET-STR-KAN-CIP-NAL (MDR pattern)

reported in this study. These three serovars may play minor epidemiological roles in the study location (Useh *et al.*, 2016; Fagbamila *et al.*, 2017). Of the serovars recovered in this study, serovars Urbana and Kingston appeared the most predominant. Geographical clustering of some serovars was observed in some zones: Remo (*S. Urbana*, *S. Ituri* and *S. Durham*), Egba and Yewa (*S. Kingston* and *S. Agama*).

Similar observations were reported in Mushin Local Government Area of Lagos, Nigeria (Smith *et al.*, 2016) and the Northwestern part of Nigeria (including Sokoto, Kebbi and Zamfara States) (Jibril *et al.*, 2020). The clustered serovars may have established ecological niches in the respective zones, hence, their restrictions in the geographical areas (Galanis *et al.*, 2006). Interestingly, sixteen of the *Salmonella* serovars (*S. Labadi*, *S. Mbandaka*, *S. Mississippi*, *S. Give*, *S. Ekotedo*, *S. Ituri*, *S. Kingston*, *S.*

Enterica sub enterica gr. R, and *S. Alachua*, *S. Blockley*, *S. Chomedey*, *S. Haifa*, *S. Isangi*, *S. Kentucky*, *S. Telelkebir*, *S. Virchow*) were pan-susceptible to some antimicrobials. All the isolates were susceptible to extended-spectrum cephalosporins and multi-drug resistance was only observed in 7 serovars comprising of twelve isolates. Worthy of note in this study was the high frequency of resistance observed within ciprofloxacin (CIP), sulfamethoxazole (SMX) and Tetracycline (TET).

A similar resistance pattern against the three antimicrobials by *Salmonella* was previously reported in Poultry from Kaduna State, Nigeria (Agbaje *et al.*, 2019). We have previously shown ciprofloxacin to be one of the most commonly used antimicrobials in poultry farms in Ogun State (Oluwasile *et al.*, 2014). This emerging resistance pattern is further supported by a three-nation (Nigeria, Sudan and South Africa) African wide study on veterinary students' ranked perception of abuse

of antimicrobial agents. Sulphonamides and tetracycline were in the uppermost three ranked antimicrobials (Fasina *et al.*, 2020). It may be cautiously inferred that non-prescription abuse and misuse of SXT contribute to the emerging resistance in the study location.

Resistance to tetracycline as seen in this study was expected since it was extensively used as prophylaxis and additives in feed and water to boost performance in the poultry sector in Nigeria (Oluwasile *et al.*, 2014). Also, the observed resistance to CIP may be possibly due to its indiscriminate use in the poultry sector around the study area.

Quinolones as a large family of antimicrobials have been widely used due to their advantages which include bioavailability, broad-spectrum activity and a large volume of distribution. It is the drug of choice in the treatment of human salmonellosis, including infections caused by multi-resistant *Salmonella* serotypes (Reina *et al.*, 1993; Parvej *et al.*, 2016; Pribul *et al.*, 2017). Hence, increased resistance to ciprofloxacin raises public health concerns.

Furthermore, in this study, all the serovars of importance NT *Salmonella* including *S. Kentucky*, *S. Virchow*, *S. Blockley* and *S. Chomedey* were observed to be MDR and low in the frequency of occurrence in the study area (Table 2). Nevertheless, these findings are important from an epidemiological and public health standpoint. Similar findings have demonstrated *Salmonella* species as a potential transmission vehicle of MDR from poultry faeces to man (Helms *et al.*, 2004; Marshall & Levy, 2011). MDR *Salmonella* serovars can cause a severe foodborne outbreak that is refractory to treatment and with limited antimicrobial alternatives especially when most of the resistant isolates were resistant to fluoroquinolones (Gragg *et al.*, 2013; Madoroba *et al.*, 2016). The observed AMR pattern in this study calls for awareness of the prudent use of antimicrobials in poultry farms within Ogun State, Nigeria. The indiscriminate use of antimicrobials among the poultry farms, especially in developing countries like Nigeria where there is little to no restriction to clinically important antimicrobials is worrying.

In conclusion, *Salmonella* serovars identified in this study were diverse and susceptible to extended-spectrum cephalosporins but seven serovars from 12 isolates were multi-drug resistant (MDR) and included important NTS such as *S. Kentucky*, *S. Virchow*, *S. Blockley* and *S. Chomedey*. Most worrying in this study was the high resistance shown to ciprofloxacin (29.5%). The AMR pattern in Ogun

State poultry sector is an indication of indiscriminate use of antimicrobials among the poultry farms and suggests the need for restriction to clinically important antimicrobials commonly used in livestock and human populations.

Acknowledgements

We acknowledge the invaluable contribution of Istituto Zooprofilattico Sperimentale delle Venezie, National and OIE Reference Laboratory for Salmonellosis, Legnaro, Italy in this study.

Conflicts of Interest

The authors declare no conflict of interest.

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