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DISTRIBUTION AND ANTIMICROBIAL RESISTANCE PATTERNS OF *Salmonella* SEROVARS IN POULTRY CARCASSES IN LAGOS STATE, SOUTHWEST, NIGERIA

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ABSTRACT

Salmonellosis is a major bacterial disease causing huge economic losses in the poultry industry globally. Most of the *Salmonella* infections in humans re-sults from the consumption of contaminated poultry and poultry products. This cross-sectional study determined the prevalence of non-typhoidal *Salmonella*, their serovars and the associated antibiotic resistance pattern in processed chicken carcasses in Lagos, Nigeria. A total of 100 dressed chicken carcasses spread across old layers (n=50) and broilers (n=50) were randomly collected in different live bird markets in Lagos, Nigeria. Culture and identification of *Salmonella* spp. were performed using standard bacteriological techniques followed by antimicrobial susceptibility test using the disk diffusion technique. Serotyping was performed at a *Salmonella* reference laboratory in Padova, Italy. A 16% *Salmonella* prevalence was obtained in this study spread across eight serovars (Bredeney, Wagadugu, Telelkebir, Corvallis, Chomedey, Kentucky, Nima and Hato). Five (31.25%) isolates were found in broilers while 11 (68.75%) isolates were from old layers. Of the 16 antimicrobial agents tested, seven of the positive *Salmonella* isolates were susceptible to all antimicrobial agents tested while out of the remaining 9 isolates, 6 were resistant to nalixidic acid (NAL), 5 showed resistance to each of spiramycin (SPR), sulphonamides (SSS) and tetracycline (TET). The presence of non-typhoidal *Salmonella* serovars and the antimicrobial resistant nature of some serovars impose public health challenges on the healthcare system and suggest poor hygiene practices and misuse/abuse of drugs in the poultry industry in the study location.

Key words: antimicrobial resistance, chicken, Nigeria, *Salmonella*, serovars.

INTRODUCTION

The livestock sector is vital to the socio-economic development of Nigeria and contributes about 9-10% of agricultural Gross Domestic Product (GDP)-FAO, 2006. The Poultry industry is one of the highest investments in Nigeria's agricultural sector with net worth of N250 billion (FAO, 2006).

Nigeria's chicken population is about 150 million, of which 25% are commercially farmed, 15% semi-commercially, and 60% in

backyards. As a result, livestock represents a major source of high-quality animal protein, providing about 36.5% of the total protein intake of Nigerians (UNDP, 2006).

However, while this industry contributes largely to the protein availability of the human population, it also serves as a means for disease transmission and the attendant antibiotic resistance associated with many of these disease-causing pathogens. One of the most important of these pathogens is the bacterium *Salmonella* (Ha and Pham, 2006), a genus divided into two species; *Salmonella bongori* and *Salmonella enterica* which is further subdivided into six subspecies namely, "*arizonae", "diarizonae", "enterica", "houtenae", "indica", and "salamae"* (Tindall *et al*., 2005). Of the six subspecies known, *S.* enterica subspecies enterica is further divided into over 2,500 serovars (Grimont and Weill, 2007), which are commonly found in the intestinal tract of warm-blooded animals including man (Grimont *et al*., 2000) and therefore often associated with foodborne infections.

Non-typhoidal salmonellae (NTS) are zoonotic agents with a wide host range including poultry birds (Hald *et al*., 2007). Poultry has been reported as one of the major reservoirs of non-typhoidal *Salmonella* zoonosis, with its accompanying economic, and health consequences (Brisabois, 2001).
Although antimicrobial agents have been used largely in animals and human treatment to contain the menace of salmonellosis, the continued development of resistance to some frequently used antibiotics still remain a concern. Antimicrobial abuse, especially in countries (developing or developed) with little or no restrictions on their usage has continued to encourage the emergence of multi-drug resistant *Salmonella* serovars (Brisabois, 2001). In developing countries like Nigeria, investigation and tracing of aetiology of this food borne pathogen are often not carried out. Hence, information on the sources of outbreaks is scarcely known (Kariuki *et al*., 2010).

This work seeks to screen fresh poultry carcasses for the presence of *Salmonella* in live bird markets in Lagos, south-west Nigeria. This will give further information on the predominant serovars as a prelude to further understanding their epidemiology as well as their antimicrobial resistance pattern.

METHODOLOGY

Study area and Sample Collection

A total of 100 live birds (old layers=50; broilers=50) were randomly identified from two popular live bird markets in Lagos, southern Nigeria. Lagos was selected for this project because of its cosmopolitan nature and human population. 25g each of neck skin from freshly slaughtered and processed chickens were collected in sterile bags and transported in ice packs to the Veterinary Microbiology Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta for analysis. Neck skin is preferred in this study design owing to its higher yields than whole carcass rinse and other skin parts (Wu *et al*., 2014).

Isolation, identification and serotyping of Salmonella strains

Salmonella organisms were culturally isolated and biochemically identified as previously described (Agbaje *et al*., 2019). Presumptive *Salmonella* isolates were serotyped at the Office International des Epizooties (OIE) Reference Laboratory for salmonellosis, 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 by Grimont and Weil (2007).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed using the disk diffusion method. *Salmonella* colonies from nutrient agar plates were emulsified into tubes containing 5 mL of peptone water broth (Oxoid, UK). The broth culture was incubated at 37 °C for 4 h until it attained 0.5 McFarland turbidity concentration (approximately $1x10^8$cfu/ mL). A sterile cotton swab was used to create a uniform bacterial lawn over the surface of Muller Hinton agar plate, followed by a brief incubation at room temperature for 30 min to allow drying. Antimicrobial disks were carefully and firmly applied on MHA at equidistance. This was incubated at 35±2°C for 18 h. The diameter of zone of inhibition around each disk was measured and results interpreted according to the National Committee for Clinical Laboratory Standards Institute (CLSI, 2012) Sixteen (16) antibiotics commonly used in the study area were used, namely: Amoxycillin/ Clavulanic acid **(**AMC**,** 30mg), ampicillin (AMP, 10mg), Ceftazidime (CAZ, 10mg), Cefotaxime (CTX, 5mg), Cephalothin (CEF, 30mg) Chloramphenicol (CHL, 30mg), Ciprofloxacin (CIP, 5mg), Colistin (COL, 50mg), Enrofloxacin (ENR, 5mg), Gentamicin (GEN, 30mg), Kanamycin (KAN, 30mg), Nalidixic acid (NAL, 30mg), Spiramycin (SPR, 100mg), Sulfamethoxazole/ Trimethoprim (SXT, 30mg), Sulphonamides (SSS, 200 mg), Tetracycline (TET, 25mg).

Data analysis

Data collation and management was done using Microsoft Excel. Descriptive statistics was used to describe frequencies and prevalence.

RESULTS

Bacterial isolates from chickens

Out of the 100 chicken neck skin samples collected, 16 isolates (16%) were positive and spread across eight serovars (Bredeney, Wagadugu, Telelkebir, Corvallis, Chomedey, Kentucky, Nima and Hato). Five isolates (31.25%) were found in broilers while 11 (68.75%) isolates were from layers. *Salmonella* Corvallis (8/50%) was the most predominant serovar, followed by *S*. Telelkebir (2/12.5%). Each of *S*. Chomedey, *S*. Hato, *S*. Nima, *S*. Kentucky, *S*. Bredeney and *S*. Wagadugu occurred once i.e 1/6.25% (Table 1).

Antimicrobial susceptibility test

Sixteen *Salmonella* isolates were subjected to 16 antimicrobial agents. Seven of the isolates comprising *S*. Corvallis (4/16) and one each of *S*. Bredeney, *S*. Wagadugu and *S*. Nima were susceptible to all antimicrobial agents tested. Of the nine resistant isolates observed in this study, 6/9 were resistant to nalixidic acid, 5/9 showed resistance to each of spiramycin (S), sulphonamides (SSS) and tetracycline (TET). Also, three isolates showed resistance to ampicillin (AMP), two isolates were resistant to each of kanamycin (KAN) and sulfamethoxazole/ trimethoprim (SXT) while one isolate each was resistant to cephalothin (CEF), ciprofloxacin (CIP), enrofloxacin (ENR) and gentamicin (GEN) Table 1.

Phenotypic antimicrobial resistance of *Salmonella* isolates

Of the nine resistant isolates, five different resistant patterns (R-type) were identified with resistance to nalidixic acid being the most distributed (4/9; 44.4%) and found in *S*. Corvallis (n=3) and *S*. Hato (n=1). It was followed by AMP-KAN-SPR-SSS-SXT-TET

Table 1: Resistant Salmonella isolates tested against 16 antimicrobial agents as per CLSI 2012

Salmonella serovars	No of isolates	AMP	AMC	C	CAZ	CF	CIP	CL	CTX	ENO	GM	K	NA	SP	SSS	SXT	TE
S. Bredeney	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Wagadugu	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Telelkebir	2	2R	-	-	-	-	-	-	-	-	-	2R	-	2R	2R	2R	2R
S. Chomedey	1	-	-	-	-	-	-	-	-	-	-	-	-	R	R	-	R
S. Corvallis	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Corvallis	3	-	-	-	-	-	-	-	-	-	-	-	3R	-	-	-	-
S. Corvallis	1	-	-	-	-	-	-	-	-	-	-	-	R	R	R	-	R
S. Kentucky	1	R	-	-	-	R	R	-	-	R	R	-	R	R	R	-	R
S. Nima	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Hato	1	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-
No of isolates	16																
No of resistant isolates		3	0	0	0	1	1	0	0	1	1	2	6	5	5	2	5
Prevalence of resistance (%)		18.8	0	0	0	6.3	6.3	0	0	6.3	6.3	12.5	37.5	31.3	31.3	12.5	31.3

Key:
Amoxycillin/ clavulanic acid **(**AMC**,** 30mg), ampicillin (AMP, 10mg), ceftazidime (CAZ, 10mg), cefotaxime (CTX, 5mg), Cepahlothin (CF, 30mg) chloramphenicol (CHL, 30mg), ciprofloxacin (CIP, 5mg), Colistin (CL, 50mg), Enrofloxacin (ENO, 5mg), gentamicin (GM, 30mg), kanamycin (KAN, 30mg), nalidixic acid (NAL, 30mg), spiramycin (SP, 100mg), sulfamethoxazole/ trimethoprim (SXT, 30mg), Sulphonamides (SSS, 200 mg) tetracycline (TET, 25mg), R= resistant isolates.

Table 2. Phenotypic resistance patterns of *Salmonella* serovars isolated from chicken meat in Lagos, Nigeria

***Salmonella* serovars**	**Source of isolates**	**Phenotypic resistance pattern**
S. Telelkebir	Broiler	AM-K-SP-SSS-SXT-TE
S. Telelkebir	Broiler	AM-K-SP-SSS-SXT-TE
S. Chomedey	Layers	SP-SSS-TE
S. Corvallis	Layers	NA
S. Corvallis	Broilers	NA-SP-SSS-TE
S. Corvallis	Layers	NA-
S. Corvallis	Layers	NA
S. Kentucky	Layers	AM-CF-CIP-ENO-GM-NA-SP-SSS-TE
S. Hato	Layers	NA

pattern which occurred twice (22.2%) in S. Telelkebir (n=2) Table 2. Five out of the 9 resistant isolates were multi-drug resistant (MDR) to at least three antimicrobials with *S*. Kentucky being the most resistant of all the serovars with an R-type AMP-CEF-CIP -ENR-GEN-NAL-SPR-SSS-TET (Table 2).

DISCUSSION

The 16% prevalence of *Salmonella* serovars isolated in this study suggests that poultry and poultry products still remain potent reservoir for the transmission of non-typhoidal salmonellosis with possible adverse consequences on human health when consumed. This result is lower compared to the 28% prevalence obtained from previous study on chicken neck skin in Kaduna, Northwest Nigeria (Agbaje *et al*., 2019), but higher than similar studies from the US with 4.6% and 2.3% prevalence respectively (USDA-FSIS, 2013; Wu *et al*., 2014). The variations in prevalence may be attributed to a number of factors including breed, age, and location (Uyttendaele *et al*., 1998). Also, unhygienic processing of products, lack of clearly separated dirty and clean areas during evisceration in the slaughterhouse as well as unhygienic processors and equipment may be contributory (Agbaje *et al*., 2019).

Sixteen of the *Salmonella* isolates identified in this study were distributed across eight serovars with six serovars obtained from layers and two serovars from broilers. Various studies in Nigeria have similarly reported diverse *Salmonella* serovar types in Nigeria (Fagbamila *et al*. 2017; Agbaje *et al*. 2019; Jibril *et al*. 2020). Major reasons for this development may be attributed to the unrestricted importation of poultry birds and eggs with little or no national screening and control programmes in place. Results from this study also highlight the role of layers in the possible transmission of non-typhoidal salmonellosis compared to broilers. Layers are known to be kept longer than broilers and may explain the reason for the increased yield of *Salmonella*.

Of the serovars identified in this study, *S*. Corvallis appeared the most commonly isolated followed by *S*. Telelkebir. This contrasts our previous study on freshly dressed chicken in Kaduna wherein *S*. Haifa was the most isolated (Agbaje *et al*., 2019). Also, this study varies with a study on retail meat (including chicken) in Lagos where *S*. Amoutive and *S*. Bargny were the predominant serovars (Smith *et al*., 2016). However, none of the *Salmonella* serovars described in the chicken meat study by Smith clustered with serovars identified from chicken in this study. The difference in serovar types in both studies, in spite of sampling from the same geographical location may be related to a host of factors including sample number and type as well as serotyping technique. In Smith's study (Smith *et al*., 2016), chicken meat was used as sample and sample size was small (n=30) compared to the present study where neck skin samples were used and sample size higher (n=100). Also, the Smith study employed a micro array based geno-serotyping compared to the Kauffman-White scheme agglutination technique in this study.

In this study, it was observed that the highest proportion of the isolates were resistant to nalidixic acid, with lower proportions to others. The lowest proportions were resistant to ciprofloxacin, ceftazidime and gentamicin. These findings represent progressive resistance to antibiotics of importance in human therapy. This worrying trend may be associated with unregulated access to pre-

scription antimicrobials (Beyene *et al*., 2015), often purchased and handled by unskilled individuals in the veterinary and human health sectors (Okeke *et al*., 2005). Compliance and monitoring for antimicrobials across levels in Nigeria is low to nonexistent. Additionally, sub-therapeutic or prophylactic dosages of antimicrobial agents in food animals potentiate the farm selection of antimicrobial resistance genes in *Salmonella* as well as other potential human and animal pathogens.

In general, 5 out of 16 isolates in this study exhibited a MDR phenotype to over 10 antimicrobials spread across four resistance phenotypes. MDR in *Salmonella* has progressively increased over the years. For example, in the US, 53.4% isolates from processed poultry have been reported to be MDR (Parveen *et al*., 2007) and 15–18% of the European isolates are MDR (Meakins *et al*., 2008). This intensive misuse/abuse of antimicrobial agents encourages selective pressures and may lead to the development of antimicrobial resistance. Also, the inclusion of antibiotics in the livestock diets as a prophylactics and/or a growth promoters has contributed to the development of antibiotic resistant strains of *Salmonella* (Oluwasile *et al*., 2014). Overall, abundance of the MDR *Salmonella* in poultry meat indicates potential public health concern.

CONCLUSION

Data from this study indicate the contamination by *Salmonella* spp. in the selected live bird markets and the possible risk of salmonellosis occurring in consumers of such contaminated products. The study also highlights the continued development of resistance by pathogens to drugs employed in the management of disease in human and animal health and hence, presents a need for improved safe processing of foods and increased monitoring of antibiotics handling, prescription and availability to the poultry industry.

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