

## Article

# *Salmonella* characterization in poultry eggs sold in farms and markets in relation to handling and biosecurity practices in Ogun State, Nigeria

Michael Agbaje<sup>1</sup>, Patience Ayo-Ajayi<sup>2,3</sup>, Olugbenga Kehinde<sup>4</sup>, Ezekiel Omoshaba<sup>1</sup>, Morenike Dipeolu<sup>4</sup> and Folorunso O. Fasina<sup>5,6,\*</sup>

<sup>1</sup> Department of Veterinary Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria; mikeagbaje@gmail.com (MA); omoshabao@funaab.edu.ng (EO)

<sup>2</sup> Department of Veterinary and Pest Control Services, Federal Ministry of Agriculture and Rural Development; drtoyinayo@gmail.com

<sup>3</sup> Nigerian Field Epidemiology and Laboratory Training Programme, 50 Haille Selassie Str, Asokoro, Abuja

<sup>4</sup> Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria; gbengakehinde210@yahoo.com (OK); drmorenike@gmail.com (MD)

<sup>5</sup> Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Dar es Salaam, Tanzania; Folorunso.fasina@fao.org

<sup>6</sup> Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa; daydupe2003@yahoo.co.uk

\* Correspondence: daydupe2003@yahoo.co.uk; Tel.: (+255 686 132 852)

**Abstract:** *Salmonella* remains one of the notable food-borne bacterial pathogens. It is associated with poultry and poultry products including eggs. This study investigated *Salmonella* distribution in egg-shell and content, their antimicrobial resistance pattern, and the possible risk factors driving contamination in Ogun State, Nigeria. A total of 500 eggs (5 eggs pooled into one sample) were collected and culturally examined for the presence of *Salmonella* serovars. Isolates were further characterized biochemically using Microbact 20E (Oxoid) and Antimicrobial susceptibility determined by the Kirby-Bauer disk diffusion method. A total of 14 *Salmonella* isolates spread across 10 serovars were recovered from the 100 pooled egg samples; 10 (10%) from the market and 4 (4%) farms, 13(13%) eggshell, and 1(1%) egg content. All tested serovars were susceptible to ampicillin, chloramphenicol, florfenicol, and kanamycin. Resistance was mostly observed in sulfamethoxazole 8 (80%), followed by ciprofloxacin 5 (50%) and tetracycline 3 (30%). Sales of eggs in the market appears to be a strong factor encouraging contamination in addition to poor biosecurity and unhygienic handling of eggs on the farm.

**Keywords:** antimicrobial resistance; biosecurity; egg; Nigeria; poultry; *Salmonella*.

## 1. Introduction

Poultry eggs provide significant amount of animal protein in Nigeria and other developing nations Sub-Saharan since they are cheap, available and have little or no limitation in acceptance across socio-cultural and religious divide (1,2). With a poultry population of approximately 180 million, Nigeria produces an average of 3.8 million eggs annually (3). However, safety and wholesomeness of eggs can pose serious public health challenge since when contaminated. Eggs are the major sources of human non-typhoidal (NT) salmonellosis (4, 5).

Salmonellosis is an important public health burden in most developing countries and constitute a major food borne pathogen in the developed world (6). It is largely self-limiting but serious consequences may result where infected individuals are immune-compromised or co-infected with malaria or human immunodeficiency virus (HIV) (7,8). In the

United States, about 1.4 million people are infected annually, with approximately 15,000 hospitalizations and 4000 deaths (9).

Consumption of raw and improperly cooked eggs and egg products have been identified as the major route for the transmission of NT *Salmonella* (10) but cross contamination in the kitchen has also been reported in the outbreaks of *Salmonella* in humans (11,12). *Salmonella* contamination of egg impose a public health burden on human population as well as economic losses in the poultry sector.

In Nigeria, NT *Salmonella* have been mostly characterized in the poultry sector from faeces, dust, environment and poultry meat (13,14,15,16). However, information on the drivers of egg contamination and transmission is scanty due to lack of coordinated national surveillance program.

Also, increasing reports of multi-drug resistance to antimicrobial agent from *Salmonella* strains isolated from egg shells and contents (17,18) signals a need for similar investigations in the poultry sector in Nigeria. Consequent on the above, this study seeks to establish: a) a baseline survey of *Salmonella* occurrence in poultry eggs, ii) determine the circulating *Salmonella* serovars and their antimicrobial resistance profile and iii) determine possible risk factors that may be driving *Salmonella* contamination of eggs.

## 2. Results

Prevalence and diversity of *Salmonella* isolates on shells and in contents of eggs

Of the 100 samples pooled from 500 eggs, 14 (14%) were positive for *Salmonella* spread across shell (13/14, 92.9%) and content (1/14; 7.1%) (Table 1). *Salmonella* in egg shell was significantly higher ( $p < 0.05$ ) than that of egg content.

Ten (10) *Salmonella* isolates were obtained from 40 samples (i.e  $40 \times 5 = 200$ ) eggs sold in the markets while the remaining four (4) were from 60 sample (i.e  $60 \times 5 = 300$ ) eggs obtained from the farm. The most isolates were from Egba ( $n=7$ ) followed by Yewa ( $n=4$ ), Ijebu ( $n=2$ ) and Remo ( $n=1$ ). All *Salmonella* isolates were obtained from egg shell except one (*S. Alachua*) from Remo zone was obtained from egg contents (Table1). In this study, 10 different *Salmonella* serovars were identified from 14 *Salmonella* isolates from eggs predominantly sold in the market and included: Agama, Durham, Bradford, Derby and Kentucky. Only serovars Kentucky was common in samples from the market and farms. Other serovars identified in the farm sourced eggs include Kingston, Colorado, Lattenkamp, Carno and Alachua (Table1).

**Table 1.** Zonal distribution of *Salmonella* serovars in eggs according to sources of samples and types.

| Zone        | Sources of Isolates |            |            |              | Identified Serovars     |               |                           |
|-------------|---------------------|------------|------------|--------------|-------------------------|---------------|---------------------------|
|             | Market (%)          | Farm (%)   | Shell (%)  | Contents (%) | Market(n)               | Farm (%)      |                           |
| Egba (n=25) | 5/10(50)            | 2/15(13.3) | 7/25(28)   | 0            | Agama(3)                | Kingston(1),  | Kentucky(1), Colorado(1), |
| Yewa(n=25)  | 4/10(20)            | 0/15(0)    | 4/25(8)    | 0            | Durham(2), Bradford(1), | Lattenkamp(1) |                           |
| Ijebu(n=25) | 1/10(20)            | 1/15(6.6)  | 2/25(8)    | 0            | Derby (1)               | -             |                           |
| Remo(n=25)  | 0/10(0)             | 1/15(6.6)  | 0          | 1/25(4)      | Kentucky(1)             | Carno(1)      |                           |
| Total       | 10/40(20)           | 4/60(6.7)  | 13/100(13) | 1/100(1)     | -                       | Alachua(1)    |                           |

*Salmonella* serovar resistance to antimicrobials and their resistance patterns

The antibiotic resistance profile of fourteen *Salmonella* serovars were subjected to 11 commonly used antimicrobial agents (Table 2). All tested serovars were susceptible to ampicillin, chloramphenicol, florfenicol and kanamycin. Resistance was most predominantly shown to sulfamethoxazole 8 (80%), followed by ciprofloxacin 5 (50%) and tetracycline 3 (30%). Gentamicin, nalidixic acid and streptomycin showed equal resistance 2 (20%) with the least resistance observed in trimethoprim 1 (10%) (Table2). The most resistant *Salmonella* serovar to antimicrobials was *S. Kentucky* (n=6), followed by *S. Carno* (n=4) and *S. Derby* (n=3). There was no resistance shown to any of the antimicrobials by *S. Colorado* (Table2).

**Table 2.** Frequency resistance of *Salmonella* isolates by serovars.

| Serotypes  | No of isolates tested | No (%) of resistant isolates | No (%) of resistant to antimicrobials |     |     |     |        |     |       |       |        |       |       | Antimicrob Type resist (n) |
|------------|-----------------------|------------------------------|---------------------------------------|-----|-----|-----|--------|-----|-------|-------|--------|-------|-------|----------------------------|
|            |                       |                              | Amp                                   | CHL | CIP | FFN | GEN    | KAN | NAL   | STR   | SMX    | TET   | TMP   |                            |
| Agama      | 3                     | 1 (33.30)                    | -                                     | -   | -   | -   | -      | -   | -     | -     | 1      | -     | -     | 1                          |
| Alachua    | 1                     | 1 (100)                      | -                                     | -   | 1   | -   | -      | -   | -     | -     | -      | -     | -     | 1                          |
| Bradford   | 1                     | 1 (100)                      | -                                     | -   | 1   | -   | -      | -   | -     | -     | 1      | -     | -     | 2                          |
| Carno      | 1                     | 1 (100)                      | -                                     | -   | -   | -   | 1      | -   | -     | 1     | 1      | -     | 1     | 4                          |
| Colorado   | 1                     | 0 (0)                        | -                                     | -   | -   | -   | -      | -   | -     | -     | -      | -     | -     |                            |
| Derby      | 1                     | 1 (100)                      | -                                     | -   | 1   | -   | -      | -   | -     | -     | 1      | 1     | -     | 3                          |
| Durham     | 2                     | 1 (50)                       | -                                     | -   | -   | -   | -      | -   | -     | -     | 1      | -     | -     | 1                          |
| Kentucky   | 2                     | 2 (100)                      | -                                     | -   | 2   | -   | 1      | -   | 2     | 1     | 1      | 1     | -     | 6                          |
| Kingston   | 1                     | 1 (100)                      | -                                     | -   | -   | -   | -      | -   | -     | -     | 1      | 1     | -     | 2                          |
| Lattenkamp | 1                     | 1 (100)                      | -                                     | -   | -   | -   | -      | -   | -     | -     | 1      | -     | -     | 1                          |
| Total      | 14                    | 10 (71.4)                    | 0                                     | 0   | 5   | 0   | 2 (20) | 0   | 2(20) | 2(20) | 8 (80) | 3(30) | 1(10) |                            |

AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; FFN: florfenicol; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; STR: streptomycin; SMX: sulfamethoxazole; TET: tetracycline; TMP: trimethoprim

Of the 14 positive *Salmonella* isolates spread across 10 serovars, five were resistant to two or more antimicrobials and included Kentucky, Bradford, Derby, Carno and Kingston while four including Agama, Lattenkamp, Durham and Alachua showed resistance to single antimicrobials (Table2). For the 7 *Salmonella* isolates tested from Egba zone, only one multi-resistance pattern (SMX-GEN-TET-STR-CIP-NAL) was observed in comparison to two patterns each from Yewa (SMX-CIP; SMX-TET-CIP) and Ijebu (CIP-NAL; SMX-GEN-STR-TMP) with four and two isolates respectively (Table 3).

**Table 3.** Antimicrobial resistance patterns of *Salmonella* isolates from eggs in Ogun State, Nigeria.

| Zones | No. (%) of isolates | <i>Salmonella</i> serovars (n)         | Resistance pattern         |
|-------|---------------------|--|----------------------------|
| Egba  | 7(50)               | Agama (2), Lattenkamp (1), Kingston(1) | SMX(4)                     |
|       |                     | Kentucky (1)                           | SMX-GEN-TET-STR-CIP-NAL(1) |
| Yewa  | 4 (28.6%)           | Durham (1)                             | SMX(1)                     |
|       |                     | Bradford (1)                           | SMX-CIP(1)                 |

|       |          |                           |                                  |
|-------|----------|---------------------------|----------------------------------|
|       |          | Derby (1)                 | SMX-TET-CIP (1)                  |
| Ijebu | 2 (14.3) | Kentucky (1)<br>Carno (1) | CIP-NAL(1)<br>SMX-GEN-STR-TMP(1) |
| Remo  | 1 (7.1)  | Alachua (1)               | CIP(1)                           |

Note: All antimicrobials tested were fully sensitive to two *S. Agama* isolates in Egba and one *S. Durham* isolate in Yewa zones

AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; FFN: florfenicol; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; STR: streptomycin; SMX: sulfamethoxazole; TET: tetracycline; TMP: trimethoprim

#### Biosecurity practices in the production and handling of eggs

The questionnaire results indicated a predominant cage system (73.3%) operations, compared to the deep litter system (26.7%). Eighty percent (48/60) of the farms were less than 500m away from other farms and the tendency for farms to be visited by wild birds. Twenty percent of the farms included antibiotics in their poultry feeds routinely.

A wide biosecurity concerns exist across most farms with only about 21.6% (13/60) of farm operations involve personal protective equipment (PPEs) where necessary. About half of the responding farms share tools with other farms, thereby encouraging pathogen transfer. All respondents (100%) do not clean their eggs in any form before selling. (Table 4). A fifth (12/60) of the respondents include antibiotics in feed as growth promoters or prophylactics while 41.68.3 % (41/60) of the respondents allow in-farms sales of eggs (Table 4).

**Table 4.** Husbandry and biosecurity practices in study area.

| Items  | Response    | Frequency | Percentage |
|--|-------------|-----------|------------|
| Husbandry system                               | Cage        | 44        | 73.3       |
|  | Deep litter | 16        | 26.6       |
| Presence of other farm <500m away              | Yes         | 48        | 80.0       |
|  | No          | 12        | 20.0       |
| Presence of wild birds and rodents around farm | Yes         | 53        | 88.0       |
|  | No          | 7         | 12.0       |
| <b>Sanitation</b>                              |             |           |            |
| Wearing of protective clothing                 | Yes         | 13        | 21.6       |
|  | No          | 47        | 78.3       |
| Sharing of tools with other farms              | Yes         | 32        | 53.3       |
|  | No          | 28        | 46.6       |
| Cleaning of eggs before sale                   | Yes         | 0         | 0.0        |
|  | No          | 60        | 100.0      |
| Inclusion of antibiotics in feed               | Yes         | 12        | 20.0       |

|                       |                   |    |      |
|-----------------------|-------------------|----|------|
|                       | No                | 48 | 80.0 |
| Traffic control       | Farm premises     | 41 | 68.3 |
| Point of sale of eggs | Off farm premises | 19 | 31.6 |

3. Discussion

In this study, a prevalence of 14% non-typhoidal *Salmonella* was detected from pooled egg samples. To the best of our knowledge this study provides the first detailed comparison of *Salmonella* serovars profile sold on farm and in open market. Our results corroborate *Salmonella* presence in eggs in Nigeria as previously reported (19). A previous national study reported 24.5% prevalence of NT *Salmonella* in poultry environments in Ogun State (15). The differences in the prevalence of the two studies may be attributed to the sample types investigated. While the national study employed a matrix of five samples (dust, litter, faeces, feed and water) from poultry environments, the current study focused on pooled poultry egg samples.

Data from this study further highlight the potentials continuous relevance of poultry eggs as important transmission reservoir of *Salmonella* in humans. Thirteen out of the 14 *Salmonella* isolates identified in this study were found on the egg shells and may suggest faecal, environmental or handling contamination (20). Only one *Salmonella* isolate (S. Alachua) was detected in egg content, the route of contamination was not investigated. S. Alachua was recently reported from faecal samples in the Northern part of Nigeria (21). Further study will be required to determine if S. Alachua was an accidental finding in the egg content, vertically transmissible or has the ability to penetrate egg shells into content.

The fourteen *Salmonella* isolates identified in this study were spread across 10 serovars, which depicts high serovar diversity. Studies across Nigeria have reported similar observations reported (15, 16, 21). Plausible reasons for this observation is the indiscriminate importation of poultry birds and eggs with no coordinated national screening and control programme in place for salmonellosis. While S. Agama (n=3) was the most occurring in this study, all three isolates were from the same market and zone. It is then possible that all three are clonally related, although clonal relatedness was not explored in this study. Two S. Kentucky serovars were identified, each from a different zone. Fagbamila et al (15) reported S. Kentucky in 11 states out of the 12 that were sampled in Nigeria. Other studies have similarly reported S. Kentucky across Nigeria, thereby suggesting this serovar as widely circulating in Nigeria (13,22,23,21). S. Kentucky has a world-wide distribution and was previously thought to be endemic in Africa with public health significance (24,25).

Notably in this study, *Salmonella* serovars commonly associated with foodborne infections (S. Enteritidis and S. Typhimurium) (26) were absent. It is possible that S. Gallinarum vaccine commonly used in Nigeria protects against other group D-strains such as S. Enteritidis (27,15). In addition, Fagbamila et al., (2017) and Useh et al., (2016) have suggested that these two serovars likely play minor roles in the Nigerian poultry sector. Put together, serovar diversity may be attributable to a number of reasons but not limited to poor sanitary and biosecurity conditions, indiscriminate importation of poultry chickens and eggs without adequate screening for *Salmonella* and lack of focused national *Salmonella* surveillance and control program.

The abuse and misuse of antimicrobial agents in the poultry sector has been linked to increased resistance to antimicrobials (28). In this study, antimicrobial resistance to *Salmonella* was highest in sulfamethoxazole (SMX), followed by Ciprofloxacin (CIP) and Tetracycline (TET) respectively. A previous study on NT *Salmonella* occurrence in freshly dressed poultry meat in northern Nigeria reported high *Salmonella* resistance pattern to

SMX, CIP and TET (16). This emerging resistance pattern is corroborated by studies on veterinary students' ranked perception of abused antimicrobials in Africa in which sulphonamides and tetracycline were in the uppermost three ranked antimicrobials (29, 30). However, in contrast to our study, earlier investigations in Zimbabwe by Makaya et al. (31) and Adesiyun et al. (32) in the Caribbean region reported no resistance to SXT. It is then possible that increased use and misuse of SXT in the Nigeria poultry sector may be a driving factor in the resistance observed in SXT. In addition, resistance to ciprofloxacin raises concern since this is the drug of choice in the treatment of human invasive salmonellosis. Resistance to tetracycline is not surprising considering its extensive prophylactic usage and as additives in feed and water to enhance performance in Nigeria (33). It may then be inferred that, the frequency of *Salmonella* spp resistance to these antimicrobials reflect their intense application in the poultry sector in Nigeria. The observed lack of stringent control on the availability and non-prescription use of antibiotics in poultry practice in the study area is concerning. In Nigeria, over the counter availability of most antimicrobials in local drug stores makes the control of antibiotic usage cumbersome (14).

Although not statistically significant, the occurrence of *Salmonella* in eggs from markets (20%) was higher than farms (6.7%). Contamination of eggs may occur during packing, grading, transporting and sales in the market, as multiple buyers visually inspect, touch and select eggs during sales in the study area (34). In the present study, unhygienic egg handling practices were common in all farms and markets involved. Also, all farms involved in the questionnaire survey have no egg sanitation programs in place.

In this study, data from the questionnaire indicated about 90% of the farms in the study area are accessed by wild birds and rodents. Similar to our results, a study involving three Caribbean countries also reported rodents in 90% of contaminated farms (35). Investigations in Australia have demonstrated the role of environmental vectors in the epidemiology of *Salmonella* in farm settlements (36,37). High and unchecked rodents population have been associated with increased *Salmonella* shedding in the environment (38) and are the most effective in the spread of *Salmonella* pathogen around farms (39). It is then imperative to initiate robust vector prevention programs in farm houses which may include secured access doors and windows, sealing of holes, repair of torn wire net and the use of baits to help control contamination of farms houses (40).

Furthermore, our results revealed certain practices which may encourage *Salmonella* occurrence and/or persistence in farms. Poor adherence to strict biosecurity measures on farms. The use of protective clothing as barrier to infectious agents was unpopular among majority of the farmers and may contribute to increase chances of contamination. Also, certain high-risk cross-contamination practices such as unhygienic picking of eggs with bare hands, sharing of tools with nearby farms (mostly <500m away) and sales of eggs on the farm were observed. These practices all increase the risk of contamination and transfer of pathogens (41,21,37).

The findings in this study are subject to at least two limitations. First the pooling of samples which ultimately reduced the sample size. While investigating individual egg sample will have provided a more detail data, pooled samples are considered more effective for the successful detection of *Salmonella* in the context of this study (42). Second, was our inability to match individual samples collected with corresponding husbandry and biosecurity questionnaires in the data analyses. Considering that the positivity rate of *Salmonella* was 14%, it was considered that analyzing data as per the region will be more informative than individual sample-farm analysis.

#### 4. Materials and Methods

##### *Study Location*

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 6.20N and 7.80N and longitude 3.00E and 5.00E at an elevation of 169 feet with an area of 16,762



square kilometers and 4,054,272 populations (43). A stratified probability random sampling design was adopted for this study such that poultry farms and markets from the four zones of Ogun state were evenly represented in the final sample.

### ***Sample Collection***

Egg samples from poultry farms and markets were used for *Salmonella* determination. A total of five hundred (500) eggs were collected representing 125 eggs per each of the four zones. From each zone, 75 eggs were obtained from 3 poultry farms and 50 eggs from 2 markets. Samples were analyzed in pools of 5 making a total of 25 samples units per zone (15 from farms and 10 from markets). Samples were collected into sterile bags using sterile nylon gloves and transported to the laboratory at 37°C.

### ***Isolation of Salmonella***

The egg surface was disinfected with 70% alcohol and alcohol residue removed by flaming. A sterile thumb forceps was used to aseptically separate the shell from the interior content. Five ml of homogenized egg was poured into 45 ml of buffered peptone water and incubated at 37 °C for 18 h.

The pre-enrichment broth culture was mixed and 1 ml was transferred with a sterile pipette into a tube containing sterile 9 ml of Mueller-Kauffmann Tetrathionate novobiocin (MKTn) broth. Moreover, 0.1 ml of the pre-enrichment broth culture was transferred in drops onto 9ml of sterile Modified semi solid Rappaport Vassiliadis agar (MSRV). Inoculated MKTn broth was incubated at 37°C for 24 h and MSRV was incubated at 41.5 °C for 24 h.

After incubation for 24 h, a loopful each of MRVS and MKTn cultures was streaked onto the surface of XLD agar (Xylose lysine desoxycholate agar) and Mac-Conkey agar (MAC). The plates were incubated at 37°C for 24 h. After incubation, the plates were checked for growth of typical *Salmonella* colonies. Typical colonies of *Salmonella* grow on XLD agar with a black centre and a lightly transparent zone of reddish colour due to the colour change of indicator, while typical colonies of *Salmonella* grows on MAC agar as pale/colourless translucent colonies and slightly convex (ISO, 2002).

### ***Biochemical Identification of Salmonella***

Suspected colonies were subjected to catalase and oxidase tests. For further identification different biochemical tests were carried out using MICROACT™ GNB 24E KIT (OXOID) for Gram negative bacteria and result was interpreted using the computer software package (Oxoid Microact® 2000 version 2.03) according to (Cheesbrough, 2006).

### ***Antibiotic Sensitivity Testing***

The isolates were subjected to antibiotic sensitivity test according to the Bauer-Kirby technique to evaluate the antimicrobial susceptibility pattern (CLSI, 2015). Nine (9) antibiotics commonly used in the study area were used namely; Penicillin (10µg), Enrofloxacin (5µg), Ciprofloxacin (5µg), Tetracycline (25µg), Gentamycin (10µg), Trimethoprim (5µg), Amoxicillin (25µg), Streptomycin (10µg), and Cloxacillin (10µg).

### ***Procedure:***

Overnight culture of test isolate in peptone water broth was adjusted to an equivalent of 0.5 McFarland concentration (approximately 1x10<sup>8</sup>cfu/mL). This was evenly spread on the surface of freshly prepared Mueller Hinton agar (MHA) plate. Antimicrobial discs were carefully and firmly placed at equidistance. This was incubated at 35±2°C for 18 h. The diameter of zone of inhibition around each disc was measured and result interpreted according to CLSI, 2015.

### ***Determination of biosecurity practices on poultry farms***

A test questionnaire was distributed among 15 farms not included in the final data collection to determine clarity of questionnaire. Based on the feedbacks from the respondents, adjustments were made in the final questionnaire.

Sixty (60) well-structured questionnaire were administered to farmers and farms spread across the four zones of Ogun State to evaluate the husbandry practices and adherence to biosecurity measures on farms (Appendix I). Fifteen (15) questionnaires were distributed per zone and included farms from which egg samples were previously taken for *Salmonella* detection. Farmers were informed of their rights to discontinue participation at any stage of the project and anonymity and confidentiality of data was stressed.

### ***Data Management and Statistical Analysis***

Data collation and management was computed using Microsoft Excel. The responses to questionnaire were presented in percentages; descriptive statistics were used to describe the prevalence analysis. The Pearson's chi square value was determined for the data generated using Statistical Package for Social Sciences (SPSS) software, version 20.

## **5. Conclusions**

This study demonstrated the presence of diverse NT *Salmonella* serovars in eggs with potential antimicrobial resistant traits. Sales of eggs in the market seems to promote the risk of *Salmonella* contamination as well as other unhygienic biosecurity practices in the farm.

### **Author Contributions:**

Conceptualization, MD, MA, TA and OK.; methodology, MA.; optimization of methods, MA., TA. and EO.; formal analysis, EO.; investigation, TA.; resources, MD,MA,TA.; data curation, MA, FF.; writing—original draft preparation, MA, EO and TA.; writing—review and editing, FF, MD and OK; visualization, FF.; supervision, MD, MA and OK.; project administration, MD. All authors have read and agreed to the published version of the manuscript.

### **Funding:**

No funding was received for conducting this study

### **Institutional Review Board Statement:**

Not applicable

### **Data Availability Statement:**

Data set used during study are available from corresponding author on reasonable request.

**Acknowledgments:** This study was supported through serotyping of isolates by Istituto Zooprofilattico Sperimentale delle Venezie, National and OIE Reference Laboratory for Salmonellosis, Legnaro, Italy.

**Conflicts of Interest:** The authors declare no conflict of interest.

## **References**

1. Fagbamila, I.; Kabir, J.; Abdu, P.; Omeiza, G.; Ankeli, P.; Ngulukun, S., et al. Antimicrobial screening of commercial eggs and determination of Tetracycline residue using two microbiological methods. *Int. J Poultry Science* **2010**, *9*, 959-962.
2. Bettridge, J.M.; Lunch, S.E.; Brena, M.C.; Melese, K.; Dessie, T.; Terfa, Z.G., et al. Infection-interactions in Ethiopian village chickens. *Prev Vet Med.* **2014**, *117*, 358-366.
3. Food and Agriculture Organization. Livestock and livelihoods spotlight NIGERIA: Cattle and Poultry Sectors. 2018; Available from: <http://www.fao.org/3/CA2149EN/ca2149en.pdf>.



4. Zhang, S.; Yin, Y.; Jones, M.B.; Zhang, Z.; Kaiser, B.L.D.; Dinsmore, B.A., et al. *Salmonella* serotype determination utilizing high-throughput genome sequencing data. *J Clin Microbiol.* 2015, 53(5), 1685–92. <https://doi.org/10.1128/JCM.00323-15> PMID: 25762776.
5. Ibrahim, G.M.; Morin, P.M. *Salmonella* serotyping using whole genome sequencing. *Front Microbiol.* 2018, 9, 1–8.
6. Herikstad, H.; Motarjemi, Y.; Tauxe, R.V. (2002). *Salmonella* surveillance: a global survey of public health serotyping. *Epidemiol Infect.* 2002, 129(01), 1–8.
7. Orji, M.C.; Onuigbo, H.C.; Mbata, T.I. Serological survey of mycoplasmosis and pullorum disease in Plateau State of Nigeria. *Int J Infect Dis.* 2005, 9(2), 86–89 doi: 10.1016/j.ijid.2004.04.016 PMID: 15708323.
8. Akinyemi, K.O.; Bamiro, B.S.; Coker, A.O. Salmonellosis in Lagos, Nigeria: incidence of Plasmodium falciparum-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. *J. Health Popul. Nutr.* 2007, 25(3), 351–358.
9. Voetsch, A.C.; Van Gilder, T.J.; Angulo, F.J.; Farley, M.M.; Shallow, S.; Marcus, R., et al. FoodNet Estimate of the Burden of Illness Caused by Nontyphoidal *Salmonella* Infections in the United States. *Clin Infect Dis.* 2004, 38, S127–S134. <https://doi.org/10.1086/381578>
10. Foley, S.L.; Nayak, R.; Hanning, I.B.; Johnson, T.J.; Han, J.; Ricke, S.C. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Appl Environ Microbiol.* 2011, 77 (13), 4273–4279.
11. Gorman, R.; Bloomfield, S.; Adley, C.C. A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *Int J Food Microbiol.* 2002, 76(1–2), 143–150. doi:10.1016/s0168-1605(02)00028-4
12. Whiley, H.; Ross, K. (2015). *Salmonella* and eggs: from production to plate. *Int J Environ Res public health.* 12(3), 2543–2556. <https://doi.org/10.3390/ijerph120302543>.
13. Muhammad, M.; Muhammad, L.U.; Mani, A.U.; Ambali, A.G. A survey of chick mortality at hatching in three selected hatcheries in Jos, Central Nigeria. *Int J Poult Sci.* 2009, 8(7), 656–659.
14. Fashae, K.; Ogunola, F.; Aarestrup, F.M.; Henriksen, R.S. 2010. Antimicrobial susceptibility and serovar of *Salmonella* from chickens and humans in Ibadan, Nigeria. *J Infect Dev Ctries.* 2010, 4 (8), 484–494.
15. Fagbamila, I.O.; Barco, L.; Mancin, M.; Kwaga, J.; Ngulukun, S.S.; Zavagnin P, et al. *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS One.* 2017, 12(3), 1–15.
16. Agbaje, M.; Lettini, A.A.; Olufemi, O.E.; Longo, A.; Marafin, E.; Antonello, K. et al. Antimicrobial Resistance Profiles of *Salmonella* serovars Isolated from Dressed Chicken Meat at Slaughter in Kaduna, Nigeria. *Rev. Elev. Med. Vet. Pays Trop.* 2019, 72. <https://doi.org/10.19182/remvt.31484>.
17. Dias de Oliveira, S.; Siqueira-Flores, F.; Dos Santos, L.R.; Brandelli, A. Antimicrobial resistance in *Salmonella* Enteritidis strains isolated from broiler carcasses, food, human and poultry related samples. *Int. J. Food Microbiol.* 2005, 97, 297–305.
18. Adesiyun, A.; Webb, L.; Musai, L.; Louison, B.; Joseph, G.; Stewart-Johnson, A.; Samlal, S.; Rodrigo, S. 2014b. Resistance to Antimicrobial Agents among *Salmonella* Isolates Recovered from Layer Farms and Eggs in the Caribbean Region. *J Food Prot.* 2014b, 77 (12), 2153–2160 doi:10.4315/0362-028X.JFP-14-162.
19. Obi, C.N.; Igbokwe, A.J. Microbiological analyses of freshly laid and stored domestic poultry eggs in selected poultry farms in Umuahia, Abia State, Nigeria. *Res J Biol Sci.* 2007, 2(2), 161–166
20. Ferreira, V.; Cardoso, M.J.; Magalhães, R.; Maia, R.; Neagu, C.; Dumitraşcu, L.; Teixeira, P.. Occurrence of *Salmonella* spp. in eggs from backyard chicken flocks in Portugal and Romania - Results of a preliminary study. *Food Control*, 2020, 113, 107180. doi:10.1016/j.foodcont.2020.107180.
21. Jibril, A.H.; Okeke, I.N.; Dalsgaard, A.; Kudirkiene, E.; Akinlabi, O.C.; Bello, M.B., et al. Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. *PLoS ONE*, 2020, 15(9), e0238190. <https://doi.org/10.1371/journal.pone.0238190>.
22. Ogunleye, A.O.; Steve, A.; Carlson, S.A. Emergence of an SGI1-bearing *Salmonella enterica* serotype Kentucky isolated from septic poultry in Nigeria. *J Infect Dev Ctries.* 2012, 6(6), 483–488. PMID: 22706190.
23. Useh, N.M.; Ngbede, E.O.; Akange, N.; Thomas, M.; Foley, A.; Keena, M.C. et al. Draft genome sequence of 37 *Salmonella enterica* strains isolated from poultry sources in Nigeria. *Genome Announc.* 2016; 4(3): e00315–e00316. PMID: 27151793
24. Le Hello, S.; Hendriksen, R.S.; Doublet, B.; Fisher, I.; Nielsen, E.M.; Whichard, J.M. et al. International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. *J Infect Dis.* 2011, 204, 675–684. doi: 10.1093/infdis/jir409 PMID: 21813512.
25. Le Hello, S.; Harrois, D.; Bouchrif, B.; Sontag, L.; Elhani, D.; Guibert, V. et al. Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *Lancet Infect Dis.* 2013, 13, 672–679. doi: 10.1016/S1473-3099(13)70124-5 PMID: 23721756.
26. Freitas, N.O.C.; Penha, F.R.A.C.; Barrow, P.; Berchieri, J. A. Sources of human non-typhoid Salmonellosis: a review. *Braz J Poultry Sci.* 2010, 12, 1–11.
27. Gast, R.K. Serotype-Specific and Serotype-Independent Strategies for Pre-harvest Control of Food-Borne *Salmonella* in Poultry. *Avian Dis.* 2007.
28. Sasanya, J.J.; Okeng, J.W.; Ejobi, F.; Muganwa, M. Use of sulfonamides in layers in Kampala district, Uganda and sulphonamide residues in commercial eggs. *Afr. Health Sci.* 2005, 5, 33–39.
29. Smith, W.P.; Agbaje, M.; LeRoux-Pullen, L.; van Dyk, D.; Debusho, K.L.; Shittu, A.; Sirdar, M.M.; Fasanmi, O.G.; Adebawale, O.; Fasina, O.F. (2019). Implication of the knowledge and perceptions of veterinary students of antimicrobial resistance for

- future prescription of antimicrobials in animal health, South Africa. *J S Afr Vet Assoc.* DOI: <https://doi.org/10.4102/jsava.v90i0.1765>.
30. Fasina, F.O.; LeRoux-Pullen, L.; Smith, P.; Debusho, L.K.; Shittu, A.; Jajere, S.M.; Adebawale, O.; Odetokun, I.; Agbaje, M.; Fasina, M.M.; Fasanmi, O.G.; van Dyk, D.; Abubakar, M.S.; Onakpa, M.M.; Ali, M.G.; Yousuf, H.S.; Elmgboul, W.E.; Sirdar, M.M. (2020). Knowledge, Attitudes, and Perceptions Associated With Antimicrobial Stewardship Among Veterinary Students: A Multi-Country Survey From Nigeria, South Africa, and Sudan. *Front. Public Health*, 2020, 8, 517964., doi: 10.3389/fpubh.2020.517964.
  31. Makaya, P. V.; Matope, G.; Pfukenyi, D. M. Distribution of *Salmonella* serovars and antimicrobial susceptibility of *Salmonella* Enteritidis from poultry in Zimbabwe. *Avian Pathol.* 2012, 41, 221– 226.
  32. Adesiyun, A., N.; Offiah, N.; Seepersadsingh, S.; Rodrigo, V.; Lashley, and L. Musai. Antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from table eggs. *Food Control* 2007, 18, 306–311.
  33. Oluwasile, B.B.; Agbaje, M.; Ojo, O.E.; Dipeolu, M.A. 2014. Antibiotic usage pattern in selected poultry farms in Ogun State. *Sokoto J. Vet. Sci.* 2014, 12 (1), 45-50, doi: 10.4314/sokjvs.v12i1.7.
  34. Rouger, A.; Tresse, O.; Zagorec, M. (2017). Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. *Microorganisms*, 2017, 5(3), 50. <https://doi.org/10.3390/microorganisms5030050>
  35. Adesiyun, A.; Webb, L.; Musai, L.; Louison, B.; Joseph, G.; Stewart-Johnson, A.; Samlal, S.; Rodrigo, S. 2014a. Survey of *Salmonella* contamination in chicken layer farms in three Caribbean countries. *J. Food Prot.* 2014, 77, 1471–1480.
  36. Chousalkar, K.; Gole, V.; Caraguel, C.; Rault, J.L. Chasing *Salmonella* Typhimurium in free-range egg production system. *Vet. Microbiol.* 2016, 192, 67–72.
  37. Sodagari, H.R.; Habib, I.; Whiddon, S.; Wang, P.; Mohammed, A.B.; Robertson, I.; Goodchild, S. Occurrence and Characterization of *Salmonella* Isolated from Table Egg Layer Farming Environments in Western Australia and Insights into Biosecurity and Egg Handling Practices. *Pathogens* 2020, 9, 56. doi: 10.3390/pathogens9010056.
  38. Lapuz, R.R.; Umali, D.V.; Suzuki, T.; Shiota, K.; Katoh, H. Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities. *Avian Dis.* 2012, 56, 29–34.
  39. Wales, A.; Breslin, M.; Carter, B.; Sayers, R.; Davies, R. A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. *Avian Pathol.* 2007, 36, 187–197.
  40. Trampel, D.W.; Holder, T.G.; Gast, R.K. Integrated farm management to prevent *Salmonella* Enteritidis contamination of eggs. *J. Appl. Poult. Res.* 2014, 23, 353–365.
  41. Namata, H.; Welby, S.; Aerts, M.; Faes, C.; Abrahantes, J.C.; Imberechts, H, et al. Identification of risk factors for the prevalence and persistence of *Salmonella* in Belgian broiler chicken flocks. *Prev Vet Med.* 2009, 90, 211–22. <https://doi.org/10.1016/j.prevet-med.2009.03.006> PMID: 19467722
  42. Carrique-Mas, J.J.; Breslin, M.; Sayers, A.R.; McLaren, I.; Arnold, M.; Davies, R. Comparison of environmental sampling methods for detecting *Salmonella* in commercial laying flocks in the UK. *Lett. Appl. Microbiol.* 2008, 47, 514–519.
  43. Adebawale, O.O.; Adeyemo, O.K.; Awoyomi, O.; Dada, R.; Adebawale, O. Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. *Rev. Elev. Med. Vet. Pays Trop.* 2016, 69, 41-45. doi: 10.19182/remvt.31170.