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Evaluation of Prevalence and Antimicrobial Resistance of *Salmonella* spp Isolated from Chicken Eggs Sold in Ilorin, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOM and ADF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOA and AS managed the analyses of the study. Authors AOM and AS managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Salmonella is zoonotic bacteria which causes serious economic loss in poultry production and infections in human population. The use of antibiotics for prevention and treatment of diseases has become commonplace in intensive Poultry farming. However, there is a growing concern regarding the development of drug-resistant bacteria. This study was conducted to evaluate the prevalence and antimicrobial resistance of Salmonella spp isolated from eggs sold for human consumption in Ilorin, Nigeria.
Study Design: A randomized design was used to collect 480 eggs from 6 collection points (5 poultry farms and 1 market). Ten samples were collected per week from each point. Descriptive statistics was used to analyse the data
Place and Duration of Study: Poultry farms and a market in Ilorin, Nigeria were randomly

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sampled for eggs over 8 weeks between December 2015 and January 2016.
Methodology: Egg samples were collected to avoid cross contamination using sterile bags to transport the eggs from the collection points weekly to the Agricultural Laboratory, Kwara State University. The eggs were stored under sterile conditions at 4^0C until analysed. Standardized microbiological methods were used to isolate Salmonella spp. and identify the serotypes. Disc diffusion technique was used to carry out antimicrobial sensitivity test. Descriptive statistics was utilized in data analysis.
Results: An average Salmonella prevalence of 67% was discovered with a multi-drug resistance to all the antimicrobials used except gentamicin. There were 40%, 60%, 80% and 100% resistance to Ceftriaxone, Ofloxacin, Cloxacillin and Cefuroxime respectively. Also *Salmonella enteriditis* which is important in human health was the most prevalent serotype (47%) in chicken eggs.
Conclusion: The prevalence of Salmonella isolated from chicken eggs sold in Ilorin metropolis was very high and multi-drug resistance found along this important human food chain require urgent regulation on the use of antibiotics in poultry.

Keywords: Salmonella; chicken eggs; antibiotics; antimicrobial resistance; zoonosis.

1. INTRODUCTION

Multi - Drug Resistance (MDR) in bacteria has become a major health challenge both in human health care and livestock management. The development of resistance among bacterial populations exposed to antibiotics is a growing public health issue worldwide [1-2]. The imperativeness of research effort geared towards the gathering of relevant data to assess the level of contamination of multi drug resistant bacteria along the human food chain is very important to monitor prevalence of resistance to antibiotics not only in human populations but also in farm animals in order to detect transfer of resistant bacteria or resistant genes from animal origin to humans and vice versa [2-3].

Salmonella is a zoonotic bacterium that causes infection in both human and farm animals, especially chicken. *Salmonella pollunarum* and *Salmonella gallinarum* are host specific, non-motile and produce clinical disease (Fowl typhoid) with variable mortality in chickens [4]. They are prevalent in most medium scale commercial poultry farms in Nigeria and have been identified as one of the major sources of health challenges in egg production because of their persistence in poultry pens and difficulty in eradication as a result of multi-drug resistance acquired [5]. Consequently, this cause increased cost of production, low productivity, low feed conversion efficiency and high production mortality within the egg laying flocks despite huge amount spent on vaccination and medication [6].

Although *Salmonella pollunarum* and *Salmonella gallinarum* are only infectious in poultry, Chickens are reservoirs for other serovars of Salmonella that causes clinical disease in human. *Salmollena enteritidis* and *Salmollena typhi* are indicted as zoonotic strains that are transmitted from chicken to man causing typhoid fever. There are typically no clinical signs in birds infected with *Salmonella enteritidis* to suggest to farmers that the egg they produce might pose a public health threat [7]. Egg contamination associated with *Salmollena enteritidis* is believed to occur before the deposition of egg shell by vertical (internal) transmission to the content of the egg (yolk or albumen) via the reproductive tract [8]. The process of acquisition of resistance to antimicrobial agents in bacteria and mechanism of exchange of genetic materials between various strains of bacteria was reviewed by [2].

Chicken is an important food source to man. The meat provides relished dishes during festive periods while the egg is an important cheap protein source in the daily diet of average Nigerian families. The important role chicken eggs play in providing adequate dietary protein to low income segments of the economy in developing nations is been undermined by antimicrobial resistant bacteria contamination along the food supply chain.

Previous studies have shown that Salmonella serovars were isolated from chicken blood and feacal samples in Ibadan, South-West Nigeria [9]. Prevalence of *Salmonella enteritidis* in chicken meat in Maiduguri, North-East Nigeria was found to be 27% [10] indicating that chicken products in Nigeria are prone to Salmonellosis contamination. In Nigeria, there is paucity of information on the prevalence in *Salmonella*

serovars in chicken eggs causing human salmonellosis. Additionally the mode of transmission of food-borne pathogens from the food chain to humans has not been well elucidated [9]. The need to generate relevant data in Nigeria as it relates to prevalence of antimicrobial resistant Salmonella within the supply chain of poultry eggs that will provide basis for effective monitoring and regulation of poultry farms' management and use of antibiotics in commercial poultry egg production therefore provide relevance for this study.

The study was conducted to evaluate the prevalence of *Salmonella* in chicken eggs along the supply chain in Ilorin metropolis, identify the common serovars and determine their antibiotic resistance.

2. MATERIALS AND METHODS

Egg samples were collected from five medium scale commercial poultry farms randomly selected from 31 farms operating within Ilorin metropolis and a popular local market in Ilorin where local eggs were collected. A total of 480 eggs were collected comprising ten (10) egg samples from each of the six locations per week over an eight (8) week period. Each egg sample was placed separately in a sterile plastic bag and transported to the Agricultural Central Laboratory, Kwara State University, Malete at ambient temperature (34^0C). After arrival at the laboratory the egg sample was stored at 4^0C until examination.

Laboratory isolation of the bacteria was done from each egg sample which is a mix of yolk and albumin. A pre-enrichment of the egg sample was done by inoculating 20 ml of buffered peptone water (Oxoid, UK) with 2 ml of aliquot from the egg sample and incubated at 35^0C for 24-48 hours using GENLAB Incubator (GENLAB, Germany) as described by [11]. Biochemical characterization was done to identify each Salmonella Serovars following the procedures outlined by [11]. Briefly, each sample was inoculated into freshly prepared Nutrient broth (NB) and incubated at 37^0C for 24 hours aerobically in bacteriological incubator (GENLAB, Germany). The presence of characteristic colonies of Salmonellae was confirmed and Nailidixic acid (NA) medium was used to grow the organisms from the bacterial samples. The representative Salmonella colonies were characterized morphologically using Gram's staining method. For carbohydrate fermentation tests, triple sugar iron agar (TSIA) slant reaction, methyl red-Voges-Proskauer (MR-VP) and Indole reaction tests were carried out for identification of suspected Salmonella [12]. The carbohydrate fermentation test was performed by inoculating NB culture of the organisms into the tubes containing different sugar media. Acid production was indicated by the colour change reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube. A selective medium of triple sugar iron agar (TSIA) slant was used to detect the lactose, saccharose and dextrose fermenters. The medium also helped to determine the ability of the organisms to produce H_2S. Pinkish slant and yellow butt or black slant and yellow butt were recorded as the positive reaction for Salmonella. The MR test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. A red coloration was positive and indicated an acid pH of 4.5 or less resulting from the fermentation of glucose. A yellow coloration was considered as negative. In VP test, 2 millilitre of sterile glucose phosphate peptone water was inoculated with the 5 ml of test culture. Two millilitres of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours to conduct Indole test. Kovac's reagent (0.5 ml) was also added. A red colour in the reagent layer indicated indole. The motility test was performed to differentiate motile bacteria from the non-motile one. The motile and non-motile organisms were identified by observing motility in contrasting with Brownian movement of bacteria. Salmonella agglutinating antiserum poly 'O' and poly 'H' of S and E reagents was used to do the serotyping of the isolated Salmonella. It was noted that poly 'O' antiserum gives positive agglutination reaction with any serovars for preliminary screening of Salmonella and poly 'H' antiserum gives specific agglutination reaction for motile Salmonella [13,14]. Thereafter, the organisms isolated and identified as Salmonella from incubated tubes were streaked separately into the *Salmonella shegella* agar (Oxoid, UK). slants and incubated at 37ºC for 24 hours in bacteriological incubator.

Antibiotic/antimicrobial sensitivity testing was carried out by using the Kirby-Bauer disc diffusion method [15]. About 20 ml of Mueller Hinton agar was poured into sterile Petri dish aseptically. The surface of this Mueller Hinton agar was seeded with 0.2 ml of the bacteria isolate suspension. Thereafter the antibiotic sensitivity disc (Pfizer, New York) containing

ceftazidime (30 µg), cefuroxime (30 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Augmentin (30 µg), Gentamicin (10 µg), and Ofloxacin (5 µg) was laid on the seeded surface of the agar. Using sterile forceps to press it down to ensure contact with the plate and was incubated at 35°C for 24 to 48 hours. The antibiotic sensitivity result was interpreted by comparing the results with guideline of interpretive standard of Clinical Laboratory Standards Institute (CLSI). Characterization of the isolates was carried out at Kappa Biotechnology Laboratories Ibadan, Nigeria. The data collected from this study were analysed using descriptive statistics.

3. RESULTS

Out of the 480 eggs sampled for this study, 320 eggs were found positive for various *Serovars* of *Salmonella* which showed a prevalence of 67% of S*almonella* (Table 1). Characterization of *Salmonella spp*. showed that, *Salmonella enteritidis* had the highest incidence of 47%, while *Salmonella paratyphi* and *Salmonella typhi* recorded low incidence at 13%. The other Salmonella serovar that was identified was *Salmonella typhimurium* with 20% level of occurrence, while 7% of the serovars remain unidentified (Table 2). The incidence and distribution of different serovars (Table 3) showed *Salmonella enteritidis* as the most common serovar found in all the farms sampled, followed by *Salmonella typhimurium*, found in farms 1, 3 and 5. *Salmonella typhi* was found in farm 2 and local eggs and *Salmonella paratyphi* was present in only farm 1. However, a Salmonella serovar was unidentified in local eggs. The result of antimicrobial resistance test (Table 4) revealed that the Salmonella serovars had 100% resistance to Cefuroxime and Ceftazidime, 80% to Cloxacillin and Erythromycin, 60% to Ofloxacin and Augmentin, 40% to Ceftriaxone and 0% to Gentamicin.

4. DISCUSSION

The study revealed a high prevalence of Salmonella in chicken eggs sampled in Ilorin metropolis. The average prevalence of 67% Salmonella serovars in chicken eggs and 100% local chicken eggs in the current study were higher than the 4.82% prevalence of Salmonella in Chicken eggs in North India [16], 2.7% prevalence in frozen chickens sampled in Brazil [17], 11% prevalence observed in poultry feacal samples in Ibadan, Nigeria [8] and 22.2% prevalence in poultry feeds sampled in Imo State, Nigeria [1]. The higher prevalence recorded in this study could be as a result of difference in management practice in poultry farms in the different localities. The higher prevalence observed in eggs in the current study could be due to the predominant route of transmission of salmonella in chicken through vertical transmission of Salmonella from the hen into the egg content before deposition of shell. In the local eggs, the 100% prevalence of Salmonella observed could be attributed to the lack of health management for the birds on extensive production system.

Predominance of *Salmonella enteritidis* in this study was in contrast to the report of [16] who reported a predominance of *Salmonella typhimurium* in chicken eggs in India. However, the predominance of *Salmonella enteritidis* reported in the present study was similar to the results of the study conducted on chicken carcasses in Brazil [17-19] and the United State of America between 2000 and 2005 [20] and Maiduguri, North-east Nigeria was [9]. The result from the current study agreed with previous report [21] that *Salmonella enteritidis* caused increase in the number of cases of human infections which was related to the consumption of chicken in the 1980s.

Table 1. Prevalence of *Salmonella*

Sample source	*Salmonella* positive		
	No. of eggs	No. of contaminated eggs	Contamination %
Farm 1	80	40	50
Farm 2	80	70	88
Farm 3	80	60	75
Farm 4	80	30	38
Farm 5	80	40	50
Local eggs	80	80	100
Total	480	320	67

Table 2. Prevalence of identified salmonella serovars

Salmonella serovar	Percent prevalence (%)
Salmonella enteriditis	47
Salmonella typhi	13
Salmonella typhimurium	20
Salmonella paratyphi	13
Salmonella spp	7
Total	100

Table 3. Identified *salmonella* strains

Source	Names of *Salmonella* species				
	Salmonella typhi	***Salmonella typhymurium***	***Salmonella paratyphi***	***Salmonella enteritidis***	***Salmonella spp***
Farm 1	-	+	+	+	-
Farm 2	+	-	-	+	-
Farm 3	-	+	-	+	-
Farm 4	-	-	-	+	-
Farm 5	-	+	-	+	-
Local eggs	+	-	-	-	+

Table 4. Antimicrobial resistance of salmonella to antibiotics tested

Antibiotics	Concentration (µg)	Number tested	Resistance (%)	Susceptibility (%)
CTR	30	5	40	60
ERY	5	5	80	20
OFI	5	5	60	40
CXC	5	5	80	20
CAZ	30	5	100	0
AUG	30	5	60	40
CRX	30	5	100	0
GEN	10	5	0	100

(CAZ) Ceftazidime (30 µg), (CRX) Cefuroxime (30µg), (CTR) Ceftriaxone (30 µg), (ERY) Erythromycin (5 µg), (CXC) Cloxacillin (5 µg), (AUG) Augmentin (30µg), (GEN) Gentamicin (10 µg) and (OFI) Ofloxacin (5 µg)

The varying degree of resistance to the 8 antimicrobial agents tested in this study except Gentamycin indicated the presence of multi-drug resistance in Salmonella serovars in chicken eggs. This multi-drug resistance to antimicrobial agents observed was similar to the findings of [16] and [8]. All serovars were resistant to one or more classes of antimicrobial agents. The resistance observed to ceftriaxone (40%), a third generation cephalosporin used in the treatment of human invasive salmonellosis, was similar to the observation of [17] in chicken meat. There was a high incidence of resistance against Augmentin (60%) Cloxacillin (80%) and Ofloxacin (60%). These are drugs of choice in the treatment of human and animal salmonellosis. The multi-drug resistance observed underlies its grave consequence in public health care provision against Salmonella infection in humans.

5. CONCLUSION AND RECOMMENDATIONS

This study revealed the prevalence of various Salmonella serovars that are important in human infections. It also confirms the emergence of multiple-drug resistant Salmonella serovars from poultry eggs especially from local chickens which represent the cheapest source of animal protein with its consumption cutting across all social strata of the economy. Prudent use of antibiotics in poultry farms is essential and its continuous use as a growth promoter need to be re-examined. It is strongly recommended that Food and Drug regulatory agencies in Nigeria

should monitor the use of antibiotics in both the production and value chain of poultry products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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