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Prevalence and Antibiotic Resistance Profile of *Salmonella* **Isolates from Commercial Poultry and Poultry Farm-handlers in Jos, Plateau State, Nigeria**

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Authors' contributions

This work was carried out in collaboration between all authors. Author GOAA conceived and designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors IOA, MA and MO were the consultants and mentors. Author SCC contributed in literature search and managed the analyses of the study while authors AEJO and PRK gave professional advice and proof reading of final draft. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study was designed to investigate the prevalence and antibiotic resistance profile of *Salmonella* serovars from poultry and poultry farm-handlers.

Study Design: Investigative

Place and Duration of Study: Samples were analyzed at the Central Diagnostic Laboratory, National Veterinary Research Institute Vom and Department of Microbiology, Ahmadu Bello University, Zaria. This work was carried out between August 2012 and April 2013.

Methodology: Samples were pre-enriched in buffered peptone water followed by selective enrichment using Selenite Faeces Broth and Rappaport-Vassilidis Broth. Isolation and identification was made by inoculating the selectively enriched sample on to Salmonella-

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Shigella agar, Xylose Lysine Deoxycholate agar and Brilliant Green agar followed by confirmation of presumptive colonies using different biochemical tests and analytical profile index 20 E. Polyvalent (O) and (H) *Salmonella* antisera were used for serotyping the *Salmonella* isolates. The CLSI, 2010 method was used for antimicrobial susceptibility testing

Results: A prevalence rate of 10.9% was observed from the 450 samples. Serovars of *Salmonella* detected were *S. Gallinarum* 57.2%, *S. Typhimurium* 8.2%, *S. Typhi* 20.4%, *S*. *Pullorum* 6.1%, *S*. *Enteritidis* 6.1% and *S*. *Paratyphi* A 2.0%. Statistically, significant difference (p<0.05) was observed between isolates and occurrence at different sample sites. The isolates were 100% resistant to oxacillin, 96.0% to ampicillin, 93.9% tylosin, 83.7 5 ceftazidime and 63.3% oxytetracycline. Five of the isolates were 100% resistant to more than five different antibiotics. There was statistical significant difference $(p<0.01)$ in antimicrobial resistance patterns exhibited by the serovars. However, the isolates showed sensitivity to gentamycin 100%, gendox 83.7%, ciprofloxacin 81.6% and amoxicillin clavulanic acid 57.1%.

Conclusion: The study revealed emergence of multiple-drug resistant Salmonella serovars from poultry and poultry farm handlers. We therefore suggest further epidemiological studies.

Keywords: Poultry; Salmonella serovar; Nigeria; antibiotic resistance; farm handlers.

1. INTRODUCTION

Salmonella infections in humans and animals have been recognized as a major public health problem [1]. *Salmonella*, a primary inhabitant of the gastrointestinal tract, is recognized as one of the most common causes of food borne infection worldwide, resulting in millions of infections and significant human death annually [2]. They are common contaminants of wide range of food, eggs, vegetables, and water. Additionally, they are carried by wild animals, rodents, pets, birds, reptiles and insects, usually without the display of any apparent illness [3]. The infections caused by *Salmonella* are considered one of the most widely spread food-borne zoonotic infection in developed as well as developing countries, though incidence varies between countries [4]. Food items such as poultry meat and other poultry products have been implicated as important sources for outbreaks of human *Salmonella* infection, with poultry alone, accounting for up to 50 percent of such outbreaks [5,6]. Non-typhoidal Salmonellosis is common in most parts of the world [7]. It is widely spread in Europe and North America [8,9]. Latin America, the middle East and Africa [10], also in countries such as India [11], Japan [12] and the United States [13]. Several studies had documented isolation of non-typhoidal *Salmonella* from humans and poultry in different parts of Nigeria [14,15,16,17]. Outbreaks of Salmonellosis caused by *Salmonella* Gallinarum, *S.* Pullorum, *S.* Typhimurium and *S.* Enteritidis have also been reported [15,18,17].

Poultry is an essential component of the Nigerian economy, providing income for small scale farmers and a good source of high quality protein for the ever-growing population of Nigeria. In livestock production, poultry occupies a prominent position in the provision of animal protein and this account for about 25% of local meat production in Nigeria [16]. With the great expansion of poultry rearing and farming, salmonellosis have become an important public health problem in Nigeria and other parts of the world, causing heavy economic loss through substantial morbidity and mortality [14].

Horizontal and vertical transmissions are both important in the epidemiology of salmonellosis worldwide, especially fowl typhoid and pullorum disease. Infected breeding flocks are associated with vertical transmission of *Salmonella* to their progeny through eggs and birds can become chronic carriers for both organisms [5,16]. Horizontal transmission occurs following ingestion of food or water already contaminated with faeces of clinically infected birds or carriers, presence of dead chickens, poultry farm attendants and contaminated feeds [18]. *Salmonella* species can survive in a favorable environment for many months [9].

Antibiotics have been successfully used in humans and veterinary medicine as food animal growth promoting agents, prophylaxis or therapeutics. However, their indiscriminate use has created enormous pressure for selection of antimicrobial resistance among bacterial pathogens worldwide, mainly in *Salmonella* strains isolated from poultry and poultry environment [19]. Nowadays, there is increasing concern about the development of multidrug resistance in bacterial species causing zoonosis and having an important animal reservoir such as *Salmonella* strains [10]. Furthermore, poultry feeds have been presumed to have a high content of microorganism sequel to the manufacturing and distribution processes to adversely affect the growth and reproduction of poultry. This has therefore, necessitated the incorporation of antimicrobial agents into poultry feeds which reduces the microbial load in the field and in the gastrointestinal tracts of the poultry, kill or inhabit infectious organisms or reduces the intensity of antibiotic resistance, thereby improving the gross growth and quality of poultry [20].

The underlying assumption is that poultry feeds are sterile with the incorporation of antimicrobial agents. However, this incorporation poses the emergence or variability of some resistant bacteria either through genetic or non-genetic mechanisms [21]. These drugs (or congeners) are also used in poultry production. The husbandry practice used in the poultry industry and the wide spread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial resistant *Salmonella* [22]. However, according to Abdellah et al. [23], the extensive use of those in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains including *Salmonella*. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections [24].

The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistant genes from food animals to humans [25]. In developed countries, stringent control of antibiotic use coupled with effective surveillance of antibiotic resistance patterns in the population, have successfully reduced the prevalence of antibiotic resistance to these agents [26]. The situation in the developing countries like Nigeria is however different, where antimicrobial agents are readily available to people in local drug stores without prescription [27]. Such practice has led to misuse of antibiotic resistance among isolates from animal and food sources [15].

Hence, this study is aimed at ascertaining the current prevalence rate of *Salmonella* isolates from poultry and poultry farm handlers in Jos, Plateau State, Nigeria and their treatability to common antibiotics. The information here will also aid in mapping out preventive strategies against *Salmonella* infections.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in eighteen registered commercial poultry farms, six each, located in three local government areas (Jos North, Jos South and Jos East) of Plateau State (Fig. 1.).

Fig. 1. Map of Plateau State, Nigeria, showing the study areas (Jos North, Jos South and Jos East)

2.2 Sample Collection

A total number of 450 samples were collected using simple random sampling. The samples include: Poultry droppings. Poultry feeds, faeces and hand swabs from poultry farm workers (the workers in this case are those responsible for casual routine works such as feeding of birds, picking and arrangement of eggs into crates, packing and disposing poultry dung) and swabs from surfaces of intact eggshells.

Prior to the enrolment, voluntary and informed consents were obtained from poultry owners and poultry farm handlers. Ethical approval was also obtained. Stool samples were collected aseptically into sterile universal bottles from the poultry farm-handlers. Poultry droppings and poultry feeds were collected in sterile plastic bags while swabs from farm handlers and surfaces of eggshell were collected in buffered peptone water (BPW) (Oxoid,UK). The samples were transported immediately to Microbiology Laboratory at the National Veterinary Research Institute, Vom, Plateau State, Nigeria using cold pack.

2.3 Sample Processing

2.3.1 Poultry droppings

Twenty five gram of poultry droppings was pre-enriched in 225 ml of selective enrichment broth (Selenite Faeces (SF) broth), incubated at 37ºC for 24 hour and sub cultured by streaking unto *Salmonella*-*Shigella* agar (SSA) (Oxoid, UK), Brilliant Green agar (BGA) (Oxoid, UK) plates and Xylose Lysine Desoxycholate (XLD) agar (Oxoid, UK) .The cultured plates were incubated at 37ºC for 24-48 hour [18,28,29]**.**

2.3.2 Poultry feeds

Twenty five gram of representative samples of poultry feeds was pre-enriched in 225 ml of BPW, incubated 37ºC for 24hour. One milliliter was transferred into 9 ml of Rappaport Vasiliadis Broth (RVB) (Oxoid, UK), incubated at 37ºC for 24 hours. A loop-full of culture from RVB was sub cultured by streaking onto BGA, SSA and XLD agar. The sub cultured plates were incubated at 37ºC for 24 hour [30]

2.3.3 Faeces from farm-handlers

Twenty five grams of faeces from farm-handlers was pre-enriched in 225 ml of selective enrichment broth (Selenite Faeces (SF) broth) (Oxoid, UK), incubated at 37ºC for 24 hour and sub cultured by streaking onto SSA, BGA and XLD agar plates. The sub cultured plates were incubated at 37ºC for 24-48 hour [31,18, 29]**.**

2.3.4 Hand Swab

Swabs from the hands of poultry farm handlers were collected and cut with sterile scalpel blade into 10 ml BPW in screw capped bottles, incubated at 37ºC for 24 hour for pre enrichment. One milliliter of this pre-enrichment broth was transferred into tubes containing 9 ml RVB, incubated at 37ºC for 24 hour. A loopful of culture from RVB was sub cultured by streaking unto SSA, BGA and XLD agar. The sub cultured plates were incubated at 37ºC for 24-48 hour [29].

2.3.5 Swabs from shell surface of intact eggs

Surface swabs from egg shells were collected and cut with sterile scalpel blade into 10 ml buffered peptone water (BPW) in screw capped bottles, incubated at 37ºC for 24 hour for pre-enrichment. One milliliter of this pre-enrichment broth was transferred into tubes containing 9 ml RVB, incubated at 37ºC for 24 hour. A loopful of culture from RVB was sub cultured by streaking onto SSA, BGA and XLD agar. The sub cultured plates were incubated at 37ºC for 24-48 hour [32,29].

2.4 Isolation and identification of *Salmonella*

2.4.1 Presumptive isolation of *Salmonella*

The cultured plates, SSA, BGA and XLD agar were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics, that is, transparent colonies with black centre on SSA and pink colonies surrounded by a red

medium on BGA, and small red translucent and or dome-shaped colonies, which may have central black spot due to hydrogen sulphide production [29].

2.4.2 Purification of isolates

The isolates were sub cultured onto SSA and nutrient agar for isolation of pure culture and subsequent biochemical characterization.

2.4.3 Biochemical characterization of *Salmonella*

Isolation and identification of organisms was carried out as described by ISO [28]; Habtamu et al. [18]; OIE [29]. A 24 h pure culture of each isolate was used to determine their gram stain reaction. The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test, Voges-Proskauer test, lysine decarboxylase test, ornithine decarboxylase test, urease test, sugar (trehalose, sucrose, inositol, glucose, dulcitol, maltose, mannitol, melibiose, salicin, rhamnose and arabinose) fermentation test and motility test. Isolates were further characterized using commercially available identification system-Analytical Profile Index (API) 20 E test kit (Biomerieux, France).

2.5 Serotyping of isolates

Biochemically identified *Salmonella* isolates were further tested for somatic (O) and flagella (H) antigens with polyvalent *Salmonella* antisera (Oxoid, UK) according to Kauffmann White Scheme [33] by slide agglutination test.

2.5.1 Antimicrobial susceptibility test

In-vitro susceptibility of *Salmonella* isolates to various routine antimicrobial drugs was tested by the standard disc diffusion technique using guidelines established by NCCLS [34].

2.5.2 Standardization of inoculum

This was done as documented by CLSI [35]. Pure culture of identified *Salmonella* isolate (s) from an 18-hour plate culture was selected. Sterile wire loop was used to pick 2 to 3 colonies of each *Salmonella* serotype and emulsified in 5 ml of sterile normal saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic systems, UK) after calibration. Adjustment was made with extra inoculum or diluents, if necessary, until 0.5 McFarland standards were obtained. Fifty microliter of the broth was further transferred into 5 ml of Mueller-Hinton broth (Oxoid, UK) in a tube.

2.5.3 Inoculation of test plates

This was carried out as described by NCCLS [34]. Optimally, within 5 to 10 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the standardized suspension in Mueller-Hinton broth. The dried surface of a 20 ml Mueller- Hinton agar plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking with the cotton swab over the entire sterile agar surface. The inoculated plates were air dried at 37ºC to allow for any excess surface moisture to be absorbed before applying the antibiotic discs.

2.5.4 Application of discs to inoculated agar plates

The antibiotic discs (Oxoid, UK) were evenly dispensed unto the surface of the inoculated agar plate using a disc dispenser and were gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37ºC for 18 hour. The following 16 antibiotic discs were used; amoxicillin-clavulanic acid (AMC) 30 μg, sulphamethoxazole-trimethoprim (SXT) 25 μg, ciprofloxacin (CIP) 10 μg, chloramphenicol (C) 10 μg, ceftazidime (CAZ) 30 μg,ceftriaxone (CRO) 30 μg, gentamycin (CN) 10 μg, oxy tetracycline (OTC) 30 μg, oxacillin (OX) 5 μg, streptomycin (S)10 μg, anicillin (AN) 10 μg, furasol (FL) 10 μg, tylosin (TN) 10 μg, conflox (CX) 10 μg, gendox (GX) 10 μg and ampicillin (AMP) 10 μg were applied in the test. The plates were inverted and incubated at 37ºC for 18 to 24 h. the diameters of the zone of inhibition were measured with a ruler and compared with a zone interpretation chart [36]. *Staphylococcus aureus* (ATCC 6538) was used as control [34].

2.6 Data Management and Analysis

Data management, entry and analysis were done using Epi Info (version 7.0), program excel (Microsoft^(R) office excel 2010, professional edition) and SAS software (version 9.0). Analysis of variance (ANOVA) was used to compare isolates from the three local government areas. While Duncan multiple range test was used to separate the mean. Descriptive statistics was used to describe the result of prevalence analysis. Prevalence was estimated as the number of samples detected positive to *Salmonella* isolation from the total sample analyzed.

3. RESULTS

Of the 450 samples collected from human faeces/hand swabs, poultry droppings, swabs from shells of intact eggs and feeds tested, 49(10.9%) were found positive for various serovars of *Salmonella* in the three local government areas (LGA).There was statistical significant difference (*p≤0.05*) in the distribution of *Salmonella* isolates in the three LGA with *S*. Paratyphi A and *S*. Gallinarum showing high significant difference (*p<0.01*) while *S*. Pullorum and *S*. Enteritidis showed no significant difference (*p>0.05*).(Table 1).

LGA: Local Government Area

LOS: Level of significance

**: significant at 5 % level of probability*

a:S. Gallinarum, b: S.Typhimurium, c: S. Typhi, d: S. Pullorum, e: S. Enteritidis, f: S. Paratyphi A

Out of the positive samples*, S.* Gallinarum was found 28, *S.* Typhimurium 4, *S.* Typh*i* (10), *S.* Pullorum 3, *S.* Enteritidis 3 and *S.* Paratyphi A1. The distribution of various serovars of *Salmonella* in all the different samples is shown in Table 2. There was statistical significant difference (*p ≤ 0.01*) in the distribution of *Salmonella* isolates in the various samples. There was no significant difference (*p>0.05*) in the distribution of *S*.Typhimurium in the various samples.

LOS: Level of significance

**: significant at 5 % level of probability*

NS: Not significant

a: S. Gallinarum, b: S.Typhimurium, c: S. Typhi, d: S. Pullorum, e: S. Enteritidis, f: S. Paratyphi A

The overall results of antimicrobial susceptibility tests revealed significant difference (*p < 0.01*) in the resistance of *Salmonella* serovars to all the antibiotics at different levels, except gentamycin (Table 3). All the *Salmonella* serovars were 100% resistant to oxacillin, 96.0% to ampicillin, 93.9% to tylosin, 83.75 to ceftazidime, 69.4% ceftriaxone, 63.3% oxytetracycline and 16.3% gendox (Table 3). Five of the serovars revealed 100% resistance to more than five different antibiotics (Table 4).

Antibiotic	Concentration (μg)	Number tested	Number resistant
AMC	30	49	21(42.9)
SXT	25	49	27(55.1)
CIP	10	49	9(18.4)
C	10	49	21 (42.9)
CAZ	30	49	41 (83.7)
CRO	30	49	34 (69.4)
CN	10	49	0(0.0)
OTC	30	49	31(63.3)
OX	5	49	49 (100.0)
S	10	49	28 (57.1)
AN	10	49	33(67.3)
FL.	10	49	27(55.1)
ΤN	10	49	46 (93.9)
CX	10	49	22 (44.9)
GX	10	49	8(16.3)
AMP	10	49	47 (96.0)

Table 3. Antibiotic Resistance Profile of *Salmonella* **isolates**

F-value -216.53

P-value- 0.001

*LOS: **

LOS: Level of significance

**: Significant at 5 % level of probability*

AMC: Amoxycillin-clavulanic acid, SXT: Sulphamethoxazole-trimethoprim, CIP: Ciprofloxacin, C: Chloramphenicol, CAZ: Ceftazidime, CRO: Ceftriaxone, CN: Gentamycin, OTC: Oxytetracycline, OX: Oxacillin, S: Streptomycin, AN: Anicillin, FL: Furasol, TN: Tylosin, CX: Conflox, GX: Gendox, AMP: Ampicillin

a: S. Gallinarum, b: S. Typhimurium, c: S. Typhi, d: S. Pullorum, e: S. Enteritidis, f: S. Paratyphi A

4. DISCUSSION

Salmonella is an important zoonotic pathogen and its prevalence in animals poses a continuous threat to man [37]. In this study, 49 (10.9%) *Salmonella* isolates comprises 6 serovars: *Salmonella* Gallinarum 28 (57.2%), *S.* Typhimurium 4 (8.2%), *S.* Typhi 10 (20.4 %), *S.* Pullorum 3(6.1%), *S.* Enteritidis 3 (6.1%) and *S.* Paratyphi A1(2.0%) were isolated. Isolation of *Salmonella* from poultry is higher compared to the isolation from other sources. Therefore, poultry and their products are widely acknowledged as the major sources of food-borne salmonellosis to humans. The overall prevalence in this study is slightly higher than that of Muhammed et al. [14] who recorded 9% prevalence rate of *Salmonella* associated with chick mortality at hatching in Jos, Plateau State. The increased prevalence rate in this study might be attributed to lack of knowledge on the transmission of *Salmonella* infection, improper orientation on biosecurity measures among poultry farmers and lack of good hygienic practice among poultry farm-handlers and most importantly, there was no consistent follow-up program put in place by regulatory agencies to educate poultry farmers on how to prevent and control Salmonellosis in the farm. It also confirm the report of Anyanwu et al. [38] who observed a pattern of *Salmonella* infection that appears to be spreading among poultry farms in Nigeria, in the form of epizootics. The prevalence is of economic and public health significance for Plateau State and Nigeria. The result of this study showed that the isolates in the three local government areas were significantly different (*p<0.05*). The isolates in Jos South are significantly higher (*p< 0.05*) compared to Jos North and Jos East. The observed number of isolates in Jos North was statistically similar to Jos East (*p>0.05*). It is important to state that one of the characteristic features observed during the study was that human as well as the poultry and feed samples shared *S.* Typhimurium serovar, though there was no significant difference (*p>0.05*) between this isolate in the various sources. This result is not surprising as *S.* Typhimuriumhas been reported to have a broad host range and can infect both human and animals [39]. Several reports have implicated *S.* Typhimurium and *S.* Enteritidis as the most prevalent *Salmonella* serovars isolated from both human and animal nontyphoid salmonellosis [3]. *Salmonella* Typhimurium is mostly prevalent in Europe and America and is of growing importance in the South Asian and Western Pacific [40]. In African countries such as Kenya, Zaire and Rwanda, both invasive and non-invasive *S.* Typhimuriumis common [10]. In Nigeria, *S.* Typhimurium and *S.* Enteritidisare increasingly isolated [1]. The distribution of the isolates could be attributed to socio-demographic and farm-based risk factors that were observed during the investigation, this includes: biosecurity practices, movement of farm-workers from one pen to the other, constant contact between feed, poultry birds and faecal droppings, improper hand washing, indiscriminate eating in animal facilities as also reported by Vellinga and Van-loock, [40]. In addition, supplies of contaminated feeds, the presence of rodents in the farm and farm-to-farm service are some of the factors that could be responsible for the spread and circulation of *Salmonella* agents in poultry farms [9]. This indicates the potential hazard of interspecies sharing of these organisms.

Salmonella Enteritidis was only isolated from poultry droppings (3.3%).This report is lower compared to the report of Shah and Korejo [41] who reported 48.7% isolation rate from poultry house environment. Though, no *S.* Enteritidis was isolated from eggshell, Guard [42] reports that eggs can be contaminated with droppings from chickens excreting *Salmonella*. In such cases *Salmonella* in droppings are believed to penetrate eggshell pores as egg cools and before the establishment of the proteinaceous cuticular barrier [43]. In view of this, *S*. *enteritidis* is able to persist on the surface of the eggshell and potentially cross contaminate the liquid portion of the egg when eggs are broken for preparation of food, which could pose a potential health risk to the society as also reported by Charles [44].

The isolation rate of *Salmonella* in feed observed in this study is lower compared to the report of Okoli et al. [45] and Okonkwo et al. [46] who reported an incidence rate of 15.0 % and 22.2 % respectively, from different sample feeds in Nigeria. The lower rate could be attributed to increased concentration of antibiotics in the feeds, number of samples collected as well as proper feed storage, which perhaps has minimized the incursion of rodents that serve as vehicle for introducing *Salmonella* into poultry feeds as reported by Hurst and Ward [47].

Furthermore, two decades ago, outbreaks of Salmonellosis caused by *S. gallinarum* and *S. pullorum* has been reported in poultry animals and food products. The prevalence rate of *S. gallinarumin* this study (23.3%) is higher compared to the report of Mbuko et al. [48] who reported prevalence rate of 18.4%. There was significant difference (*p< 0.01*) in the isolation rate of *S. gallinarum* from poultry droppings. *Salmonella gallinarum* and *S. pullorum* serovars are highly adapted to host range, though they are known to pose a minimal zoonotic risk, they cause fowl typhoid and pullorum disease in birds, respectively. The genome is continually evolving, which could theoretically widen the host range in future [49]. The disease usually follows the ingestion of food or water contaminated by the faecal material. Meanwhile, the clinically infected birds could be carriers of fowl typhoid, which can be transmitted by poultry farm-workers through hands, feet, clothing and rodents as also documented by Aiello [50]. However, in this study there was no isolate from hand swabs. It is important to state that the outbreak of fowl typhoid and pullorum disease are expected to incur heavy economic losses to the poultry industry, as it is the case in other African countries [51] and the impact on the farmers could be burdensome.

*Salmonella typhi*and *S. paratyphi* have been implicated in human typhoid and paratyphoid infection, though not associated with poultry environment [52,17]. *Salmonella paratyphi* A was not isolated from poultry droppings in this study, Orji et al. [53] documented 12.5% isolation rate of *S. paratyphi* A from poultry droppings. The isolation rate of *S. typhi* 11.1% and *S.* Paratyphi A 1.1% from human faeces indicates that poultry farm-handlers are always exposed to some risk factors that could predispose them to *Salmonella* infection. The high isolation rate noticed with *S. typhi* may be due to poor hygienic practice in their residential areas as observed during the study. There is a need to create environmental and personal hygiene awareness among the Nigerian populace, especially poultry farm-handlers.

A similar study conducted in Egypt by Ibrahim et al. [54] reported 8 of 90 hand swabs were found positive for *S*. Kentucky whereas all stool samples were negative to all *Salmonella* species. Though contrary to our findings in this study, his study showed that *Salmonella* serovars isolated from chicken were frequently isolated from hand swabs of poultry farm handlers. This provided evidence that direct contact with poultry or poultry environment may pose health hazards for humans.

Basically, in the livestock industry, antibiotics have been used successfully in human and veterinary medicine in the past sixty years to turn many of life threatening bacterial infections into treatable conditions. However, in recent times, antibiotic resistance has become an important health and food safety issue with emergence of many drug-resistance species of microbial pathogens in humans [55]. The use of several antibiotics for therapeutic or prophylactic administration or for growth enhancement, especially in the poultry operations is particularly worrisome in view of the potential to extend such drug into the human food chain or the possibility of reduce efficacy of such drugs sometimes administered by non-qualified personal [56].

The result of antimicrobial susceptibility testing in this study, revealed that all the *Salmonella* servers were 100% resistant to oxacillin, 96.0% to ampicillin, 93.9% to tylosin, 83.7% to ceftazidime, 69.4% to ceftriaxone, 67.3% to anicillin, 63.3% to oxytetracycline and 55.1% to sulphamethoxazole-trimethoprim (Table 3). There was statistical significant difference at (p<0.01) in antimicrobial resistance patterns exhibited among the *Salmonella* serovars. A similar trend in resistance was recorded for non-typhoidal *Salmonellae* (p<0.05) by Akinyemi et al. [1], with a least susceptibility to both ciprofloxacin and oflaxacin. While ciprofloxacin and gendox, showed low frequencies of resistance in human and animal isolates, as also reported by Okoli et al. [45]. Quite worrisome is the fact that five of the *Salmonella* serovars that are incriminated in both human and animal salmonellosis were 100 % resistant to more than five different antibiotics (Table 4). Similar report in USA shows that 18.0% isolates from all sources were also found resistant to two or more antimicrobials. Resistance to sulphamethoxazole-trimethoprim, ox tetracycline, and streptomycin was most, whereas resistance to ciprofloxacin was the least. Resistances to sulphamethoxazoletrimethoprim among poultry isolates are reported from Senegal [57], Mexico [58] and USA [59]. However, sulphamethoxazole-trimethoprim resistance was comparatively lower in this study. Among the fluoroquinolones, resistance to ciprofloxacin was found comparatively lower in the present study as compared to 35% resistance in USA [60], 10.2 to 16.8% in Germany [61] and higher compared to 9.6% in Austria [62]. Ciprofloxacin is a fluoroquinolones antimicrobial that is increasingly and successfully used for the treatment of septicaemic salmonellosis in human, worldwide.

High level of ampicillin and oxacillin resistance (90 to 100%) was observed in almost all the isolates, which is in agreement with the findings of Suresh et al. (32). They also observed a higher proportion of ampicillin-resistant *Salmonella* from eggs.

The resistance from oxytetracycline was observed in 63.3% of the isolates, which is higher than that reported in different studies: 46% in Senegal [57] and 36% in Portugal [63]. Oxytetracycline has been one of the most commonly used antibiotics for production animals; from day-old chicks to broiler chickens, they are exposed to antimicrobial drugs during their growth phase. Therefore, resistance to drugs such as oxytetracycline could be expected since the members of this class (tetracycline and chlortetracycline) are approved for use in broiler feeds for the purpose of growth promotion [64].

Resistance to streptomycin (57.1%) was also higher and is in conformity with other findings [65]. This resistance to oxytetracycline and streptomycin commonly observed among the *Salmonella* isolates has been frequently reported; this elevated resistance may be explained by the possible diffusion of the TetA (the protein that pumps tetracycline antibiotic out of the cell) resistance gene, observed in an epidemiological study with *Salmonella* strains isolated from animals [66].

The salmonellae revealed resistance to tylosin (93.9%), anicillin (67.3 %) and furasol (55.1 %). Recently some authors have reported an increased resistance to these drugs [67], which are commonly administered to chicken by poultry farmers for prophylaxis, as observed during our investigation. Increased resistance to other antibiotics has led to increased interest in furasol, which is a nitrofuran derivative. It is highly used in poultry feed as an additive in Vietnam, China, Brazil and Thailand [68].

Our findings regarding cephalosporins resistance (60-80%) are almost in agreement with the 59.5% found in *Salmonella* species and *Salmonella* Typhi isolated from humans in United Arab Emirates[69] as well as the report of Arlet et al.[70] who documented

Salmonella Typhimurium and *S.* Enteritidis been the most common serovars associated with extended-spectrum cephalosporin resistance in human and animal infections, with poultry as primary food source, suggesting that humans are often infected by these routes. This is not surprising, in view of the high level of resistance observed against almost all the *Salmonella* serovars in this study. Cephalosporins are major antimicrobials used to treat serious *Salmonella* infections. However, their effectiveness is being compromised by the emergence of extended-spectrum bête-lactamases (ESBLs) and plasmid mediated cephalosporinases and recently a class A carbapenemase as also reported by Vincent *et al*. [69]. Akinlabi and Steve [71] in Nigeria reported the presence of TEM genes present in four Nigerian-origin *Salmonella* isolates exhibiting resistance to third generation cephalosporins. The isolates included four strains isolated from poultry (two strains of *S.* Kentucky and two strains of presumptive *S.* Pullorum). Resistance to third generation bêtelactams in *Salmonella* which often results from production of plasmid mediated cephalosporinases has been reported to be a major public health problem worldwide [72,73]. In Nigeria however, there are paucity of such reports both in *Salmonella* serotypes from human and food animal origin.

In Nigeria, *Salmonella* serotypes with less than 20% reduced susceptibility to fluoroquinolones (F) and cephalosporins (C) from humans and poultry has been documented [1,14]. However, the emergence of FC-resistant *S.* Typhimurium strains from both poultry and humans in this study calls for serious concern. The implication of this is increasing emergence strains of FC-resistant pathogen. Our fear is that if urgent steps are not taken, the efficacy of these preferred groups of antibiotics for the treatment of *Salmonella*-associated diseases will be doubtful thereby increasing the mortality rate, thus put the problem into national and international perspective.

5. CONCLUSION

This study revealed the prevalence of various *Salmonella* serovars and emergence of multiple drug resistant *Salmonella* serovars from poultry and poultry farm-handlers. Prudent use of antibiotic is essential and its continuous use as a growth promoter might need to be re-examined. Therefore, vigilance against the rise in resistance of Salmonellae to antibiotics is important and the poultry farm-handlers should strictly adhere to protective guidelines.

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COMPETING INTERESTS

The authors declared that they have no competing interests exist.

REFERENCES

- 1. Akinyemi KO, Phillip W, Beyer W, Bohm R. *In-vitro* antimicrobial susceptibility Patterns of *Salmonella enterica* serovars and emergence of *Salmonella* phage type DT071 in a suspected community-associated outbreak in Lagos, Nigeria. Journal of Infection in Developing Countries. 2007;1(1):48-54.
- 2. Ellaine S, Robert MH, Frederick JA, Robert VT, Marc-Alain W, Sharon LR *et al*. Emerging Infectious Disease. 2011;17(1).
- 3. Ekdahl K, de Jong B, Wollin R, Andersson Y. Travel-associated non-typhoidal Salmonellosis; geographical and seasonal differences and serotype distribution. Clinical Journal of Microbiological Infection. 2005;11(2):138-114.
- 4. Osterom J. Epidemiological studies and proposed preventive measures in the fight against human Salmonellosis. International Journal of Food Microbiology.1999; 12:41-52.
- 5. Centre for disease control and prevention. *Salmonella* Enteritidis infection and shell eggs–United States. Morbidity and Mortality Weekly Report.1996;39:900-912.
- 6. Bryan FL, Doyle MP. Health risks and consequences of *Salmonella* and Campylobacter jejuni in Poultry. Prot. 1995;8:326-344.
- 7. Wilson JS, Hazel MS, Williams JN, Phiri A, French PN, Hart CA. Nontyphoid Salmonellae in United Kingdom Badgers: Prevalence and Spatial Distribution. Applied Environmental Microbiology. 2003;69:4312-4315.
- 8. Ling JM, Chan EW, Cheng AF. Molecular epidemiological analysis of *Salmonella* Enterica serotype Derby infections in Hong Kong. Journal of Infection. 2001*;*42(2):145-153.
- 9. Wright JG, Tengelsen LA, Smith KE, Bender JB, Frank PK, Grendon JH, Rice DH, Thiessen AM, Gilbertson CJ, Sivapalasigam S, Barret TJ, Besser TE, Hancock DD, Angulo FJ. MULTIDRUG-RESISTANCE *Salmonella* Typhimurium in four animals' facilities. Emerging Infectious Disease. 2005;11(8):1235-1241.
- 10. Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Hart CW. Characterization of community acquired nontyphoidal *Salmonella* from bacteraemia and diarrhoel infection in children admitted to hospital in Nairobi, Kenya*.* BMC Microbiol. 2006; 6(101):1-10.
- 11. Sahai S, Mahadevan S, Srinivasan S, Kanungo R. Childhood bacterial meningitis in Pondecherry, South India . Indian Journal of Pediatrics. 2001;68:839-841.
- 12. Arii J, Tanabe Y, Miyake M. Acute encephalopathy associated with nontyphoidsalmoellosis. J. Child Neurol. 2001;16:539-540.
- 13. Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R, Cieslak PR, Deneen VC, Tauxe RV. Food Net estimate of the burden of illness caused by nontyphoid *Salmonella* infections in the United States. *CID.* 2004;127–134.
- 14. Muhammed M, Muhammed LU, Ambali AG, Mani AU, Azard S, Barco L. Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents, Veterinary Microbiology*.* 2010;140:131-135.
- 15. Enabulele AS, Amune PO, Aborisade WT. Antibiograms of *Salmonella* isolates from poultry farms in Ovia North East local government area, Edo State, Nigeria. Agriculture and Biology Journal of North America. 2010;1(6):1287-1290.
- 16. Agbaje M, Davies R, Oyekunle MA, Ojo OE, Fasina FO, Akinduti PA. Observation on the occurrence and transmission pattern of *Salmonella* Gallinarumin commercial poultry farms in Ogun State, South Western Nigeria**.** African Journal Microbiological Research. 2010;4(9):796-800.
- 17. Fasure AK, Deji-Agboola AM, Akinyemi KO. Antimicrobial resistance patterns and emerging fluoroquinolone resistant *Salmonella* isolates from poultry and asymptomatic poultry workers. African Journal of Microbiology Research*.* 2012;6(11): 2610-2615.
- 18. Habtamu MT, Rathore R, Dhama K, Rajesh KA. Isolation, Identification and Polymerase Chain Reaction (PCR).Detection of *Salmonella* species from materials of Poultry origin. International Journal of Microbiological Research*.* 2011;(2):135-142.
- 19. Fey PD, Safranek TJ, Rupp ME. Ceftriaxone resistant *Salmonella* infection acquired by a child from cattle. New England Journal of Medicine. 2000;342:1242-1249.
- 20. Ahmed T. Outbreak of gastroenteritis in Jos, Nigeria. Journal of Clinical Infectious Disease*.*1996;25(7):80-81.
- 21. Gillespie JR. Poultry. In: Modern Livestock and Poultry Production. 4th edition, Delmar publishers Inc. USA. 1992;591-655.
- 22. Crump JA, Griffin M, Angulo FJ. Bacterial contamination of animal feed and its relationship to human foodborne illness. Journal of Clinical Infectious Diseases. 2002*;* 35:859-865.
- 23. Abdellah C, Fouzia RF, Abdelkader C, Rachida SB, Mouloud Z. Prevalence and antimicrobial susceptibility of *Salmonella* isolates from chicken carcasses and giblets in Meknes, Morocco. African Journal of Microbiological Research. 2009;3(5):215-219.
- 24. Nawaz SK, Riaz S, Hasnain S. Screening for anti-methicillin resistant *Staphylococcus* Aureus (MRSA) bacteriocin producing bacteria. African Journal of Biotechnology*,* 2009;8(3):365-368.
- 25. Heuer OE, Jensen VF, Hammerum AM. Antimicrobial drug consumption in companion animals. Emerging Infectious Disease. 2005;11(2):344-345.
- 26. Collingnon P. A review- the use of anibiotics in food production animals- does thiscause problems in human health. Manipulating pig production IX. Proceedings of the Ninth Biennial Conference of the Australasian Pig Science Association (inc) (APSA), Femantle, Western Australia. 2003;73-80.
- 27. Kwaga JKP, Adesiyun AA. Antibiograms of *Staphylococcus* Aureus isolates from some ready to eat products*.* Journal of food protection.1984;47(11):865-867.
- 28. International Organization of Standardization (ISO) 6579. Microbiology general guidelines on methods for the detection of *Salmonella.* International organization of standardization*,* Geneva, Switzerland; 2002.
- 29. Office International des Epizooties (OIE). Fowl typhoid and pullorum disease. In: Terrestrial manual. Office International des Epizooties, Paris, France. 2012;3-5.
- 30. Cardinale E, Tall F, Gueye EF, Cisse M, Salvat G. Risk factors for *Salmonella enteric* subsp. Enterica infections in Senegalese broiler-chicken flocks. Preventive Veterinary Medicine. 2004;63**:**151-161**.**
- 31. Whyte P, McGill K, Collins JD. A survey of the prevalence of *Salmonella* and other enteric pathogens in a commercial poultry feed mill. Journal of Food Safety. 2002; 23:13-24.
- 32. Suresh T, Hatha AA, Sreenivasan D, Sangeetha N, Lashmanaperumalsamy D. Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other Salmonellae in the eggs and egg storing trays South India. Journal of Food Microbiology. 2006;23:294-299.
- 33. Kauffmann F. Serological diagnosis of *Salmonella* species, Kauffmann White Scheme Minkagarord, Copenhagen, Denmark;1974.
- 34. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing-14th information supplement approval standard M100-S14. Wayne PA; The committee. 2004;20-24.
- 35. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicr-.obial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
- 36. Muragkar HV, Rahman H, Ashok K, Bhattacharyya D. Isolation, phage typing and antibiogram of *Salmonella* from man and animals in northeastern India. Indian Journal of Medical Research; 2004;122:237-242.
- 37. Anyanwu AI, Fasina PO, Ajayi OT, Rapu I, Fasina MM. Antimicrobial Resistant *Salmonella* and *Escherichia coli* Isolated from Day-old Chicks, Vom, Nigeria. African Journal of Clinical and Experimental Microbiology. 2010*;*11(1):129-136.
- 38. Chiu CH, Su LH, Chu C. *Salmonella enterica* serotype Choleraesuis: epidemiology, pathogenesis, clinical disease, and treatment. Clinical Microbiology Reviews*.* 2004;17**:**311-322.
- 39. Vellinga HA, Van-loock JO. Bacteriology for Biologist, Caterers and food Technologists. Amana printing and advert limited Kaduna. 2002;47-52.
- 40. Shah AH, Korejo NA. Antimicrobial resistance profile of *Salmonella* serovars isolated from chicken meat. Journal Veterinary Animal Science; 2012;2:40-46.
- 41. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotics susceptibility testing by a standardized single disk method. America. Journal of Clinical Pathology.1966;45: 493-496*.*
- 42. Guard-Petter J. The chicken, the egg and *Salmonella* Enteritidis. Environmental Microbioliology. 2001;3:421-430.
- 43. St Louis ME. The emergence of grade A eggs as a major source of *Salmonella Enteritidis* infections. New implications for the control of salmonellosis. JAMA. 1998; 259:2103-2107.
- 44. Charles OAO, Takayuki K. *Salmonella enterica* serovar Enteritidis*:* a mini-review of contamination routes and limitations to effective control. JARQ. 2010;44(1):7-16 available on http://www.jircas.affrc.go.jp.
- 45. Okoli IC, Endujihe GE, Ogbuewu IP. Frequency of isolation of *Salmonella* from commercial poultry feeds and their antimicrobial resistance profiles, Imo State, Nigeria. Online Journal of Health and Allied Science. 2006;5:2-3.
- 46. Okonkwo IO, Nkang AO, Eyerefe OD, Abubakar MJ, Ojezele MO, Amusan TA. Incidence of Multi-Drug Resistant (MDR) Organisms in some Poultry Feeds sold in Calabar Metropolis, Nigeria. British Journal of Pharmacology and Toxicology*.* 2010;1(1):15-28.
- 47. Hurst JL, Ward WR. Rats and Mice and animal feed-a risk too far? The Veterinary Journal. 2001;162-3:163-165.
- 48. Mbuko IJ, Raji MA, Ameh J, Saidu L, Musa WI, Abdul PA. Prevalence and seasonality of fowl typhoid disease in Zaria-Kaduna State, Nigeria. Journal of Bacteriology Research*.* 2009;1(1):001-005
- 49. Liu GR, Rahin A, Liu WQ, Sanderson KE, Johnson RN, Liu SL. The evolving Genome of *Salmonella enterica* serovar Pullorum. Journal of Bacteriology. 2002;184:2626- 2633.
- 50. Aiello SE.(Ed). The Merck Veterinary Manual, 8th Edition, Merckand Co., Inc, USA. 1998;1995-1996.
- 51. Shivaprasad HL. Pullorum disease and fowl typhoid in: Salf YM, Barnes HJ, Fadly AM, Glison JR, McDougald LR, Swayne DE. (eds). Disease of poultry (C.D-ROM), 11th ed. Blackwell Publishing Company, Iowa, USA. 2003;568-582.
- 52. Adenekan CA, Oyejide A, Alasa MY. Serological survey for pullorum in four states of Nigeria. Tropical Veterinary. 1990;6:61-64.
- 53. Orji MU, Onuigbo HC, Mbata TI. Isolation of *Salmonella* from poultry droppings and other environmental sources in Awka, Nigeria. International Journal of Infectious Diseases. 2005;9:86-89.
- 54. Ibrahim MA, Emeash HH, Nahed HG, Abdel-Halim MA. Seroepidemiological studies on Poultry Salmonellosis and its public health importance, Journal of World's Poultry Research. 2013;3(1):18-21.
- 55. Maurer JJ. Following Drug-Resistant *Salmonella* through the food chain: A molecular ecology approach. Available at: http://www.ugacfs.org/researchdfs/antibioticresistance.pdf. 2004. Accessed on 25th September, 2012.
- 56. Capita R. Variation in *Salmonella* resistance to poultry chemical decontaminants based on Serotype, Phage Type, and Antibiotic resistance patterns. 2007;70:1835- 1843
- 57. Bada-Alambedji FA, Seydi M, Akakpo JA. Antimicrobial resistance of Salmonella isolated from poultry carcasses in Dakar (Senegal). Brazilian Journal of Microbiology. 2006;37(4):510-515.
- 58. Zaidi MB, McDermott PF, Fedorka-Cray P, Leon V, Canche C, Hubert SK, Abbott J, León M, Zhao S, Headrick M and Tollefson L. Nontyphoid *Salmonella* from Human Clinical Cases, Asymptomatic Children, and Raw Retail Meats in Yucatan, Mexico. Clinical Infectious Diseases*.* 2006;42(1):21-28.
- 59. Zhao S, Fedorka-Cray PJ, Friedman S, McDermott PF, Walker RD, Qaiyumi S, Foley SL, Hubert SK, Ayers S, English L, Dargatz DA, Salamone B, White DG. Characterization of *Salmonella* Typhimurium of animal origin obtained from the National Antimicrobial Resistance Monitoring System. Foodborne Pathog. Dis. 2006; 2:169–181.
- 60. Cui S, Ge B, Zheng J, Meng J. Prevalence and antimicrobial resistance of Campylobacter spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. Applied and environmental microbiology. 2005;71(7):4108-4111.
- 61. Malorny B, Schroeter A, Guerra B, Helmuth R. Incidence of quinolone resistance in strains of *Salmonella* isolated from poultry, cattle and pigs in Germany between 1998 and 2001.Veterinary Record. 2003;153(21):643-8.
- 62. Mayrhofer S, Paulsen P, Smulders FJM, Hilbert F. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. International Journal of Food Microbiology. 2004;97:23-29.
- 63. Antunes P, Reu C, Sousa JC, Peixe L, Pestana N. Incidence of *Salmonella* from poultry and their susceptibility to antimicrobial agents. International Journal of Food Microbiology. 2003;82:97-103.
- 64. Jones BD. Salmonella gene invasion regulation: A story of environmental awareness. The Journal of Microbiology. 2003;43:110-117.
- 65. Cardoso MO, Ribeiro AR, Santos IR, Pilotto F, Moraes HLS, Salle CTP, Rocha SLS, Nascimento VP. Antibiotic resistance in Salmonella Enteritidis isolated from broiler carcasses*.* Brazilian Journal of Microbiology. 2006;37:368-371.
- 66. Pezzella C, Ricci A, Di Giannatale E, Luzzi I, Carattoli A. Tetracycline and streptomycin resistance genes, transposons, and plasmids in Salmonella enterica isolated from animals in Italy. Antimicrobial Agents Chemotherapy. 2004;48:903-908.
- 67. Alo OS, Ojo O. Use of Antibiotics in food animals: A case of a major Veterinary outlet in Ekiti State, Nigeria. Nigerian Veterinary Journal. 2007*;*28(1):80-82
- 68. McCracken RJ, Kennedy DG. The bioavailability of the residues of the furazolidone metabolite 3-amino-2-oxazolidone in porcine tissues and the effect of cooking upon residue concentrations. Food additives and contaminants. 2000;14:507-513.
- 69. Vincent OR, Wafa J, Tibor P, Agnes S, John A. Emergence of CTX-M-15 type extended spectrum beta-lactamase-producing Salmonella species in Kuwait and the United Arab Emirate. Journal of Medical Microbiology. 2008;57(7)881-886.
- 70. Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, White DG. *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbes infection. 2006;8(7):1945-54.
- 71. Akinlabi OO, Steve AC. Survey of 3rd generation cephalosporin gene in multi resistant *Salmonella* serotypes from septic poultry and in asymptomatic healthy pig from Nigeria. African Journal of Microbiology Research. 2011;5(15):2139-2144.
- 72. Parry C. Antimicrobial drug resistance in *Salmonella enterica*. Current Opinion Infectious Disease. 2003;16:46-472.
- 73. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrobial agents chemotherapy. 2004;48:1-14.

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