ISOLATION AND ANTIBIOGRAM OF *SALMONELLA* SPECIES IN LOCAL CHICKENS (*GALLUS DOMESTICUS*) SOLD AT LIVE BIRD MARKETS IN JOS, PLATEAU STATE

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Abstract

The study was carried out to isolate and determine the antibiogram of *Salmonella* species in local chickens in Jos using colony morphology, biochemical test and disc diffusion method of the 150 cloacae samples collected and examined, 75 were positive for *Salmonella* giving an overall prevalence of 50.0%. Based on the location, the prevalence was highest in Jos East Local Government Area (72.0%) followed by Jos North (56.0%) and Jos South (22.0%) (P=0.000; Odds ratio=27.4; 95% CI=0.449-0.564). The antibiotics susceptibility profile of *Salmonella* isolates from the indigenous chickens characterized displayed resistance to one or more antibiotics. The antibiogram profile of the isolates showed varying degree of sensitivity. The result showed that 80.0% of the isolates were sensitive to streptomycin and only 5.3% to Tarivid. More so, 32.0%, 21.3%, 3.9%, 35.2%, 40.5% and 22.6% of the isolates were moderately sensitive to nalidixic acid, peflacine, gentamycin, ciproflox, ampicilin, and ceporex respectively. The result also showed that greater proportion (92.0 %) of the isolates were resistant to Tarivid. Only 4.0% showed resistance to streptomycin. This study showed that indigenous chickens sold at live bird markets in the study area harbor multi-drug resistant *Salmonella* species.

KEYWORDS: Salmonella species, Chicken, Market, Jos.

Introduction

Indigenous chickens are an essential resource benefitting the livelihood of poor people in developing countries (Msoffe *et. al.*, 2010). Over 80% of the chicken population in Africa is reared under the free range system (Gueye, 1998). They serve as an important source of food and limited income generation for rural poor (Msoffe *et. al.*, 2010). Many house hold keep indigenous free range chickens because they only require minimal resources and investment to maintain since they are scavengers almost taking care of themselves. Their management and feeding habit exposes them to getting in contact with pathogenic bacteria as well as anti-microbial resistance strains in the environment. (Hamisi *et. al.*, 2014) indicated that in developing countries, chickens are among the domestic animal threatened by being exposed to bacteria carrying resistance genes in the environment where they feed. These pathogens can thus be transmitted to other animal hosts and humans where they produce disease.

The most common source of human Salmonellosis is food of poultry origin since

poultry are the most crucial reservoirs of *Salmonella*. Poultry and poultry products are often involved in sporadic cases and in outbreaks of human Salmonellosis (Humphrey, 2000). Apparently healthy free range chickens brought from different places and sold at live bird market could serve as reservoir of multidrug resistant *Salmonella*. They could serve as source of transmission to other birds and as a source of transmission to humans. The spreading of *Salmonella* with antimicrobial resistance genes therefore has some global public health impact. This is because the spread to other countries and or regions by travelers or by trade is impossible to prevent (Collard *et. al*., 2007). There is dearth of information on the antibiotic susceptibility patterns of *Salmonella* in local chickens sold at live bird markets in Jos and environs. This present study on isolation and antibiotic susceptibility patterns of *Salmonella* in local chickens (*Gallus domesticus*) sold at live bird markets will provide useful information for determining control strategies.

Study area

The study was carried out in Jos and environs. Jos is the capital of Plateau State in Nigeria. Jos is a city in the middle belt region of Nigeria and the city is divided into three separate local, government areas: Jos south, Jos North, Jos East (Plateau State (n.d). Retrieved November, 29 2017 from https://en.wikipedia.org/wikis/Plateau _state). The city has a population density of 1.03 person per square mile (391 person per kilometer square) it is located at 9^{o} .56N, 8^{o}53E high on the Jos Plateau State (NPC, 2006). The city has an altitude of 4.062 feet above sea level and so enjoys a more temporal climate than most of the rest Nigerian region. Poultry has been ranked to be the most populous livestock in Plateau State followed by sheep and goats, dogs, pigs, cattle and cats being the least (Ndahi *et. al*., 2012).

Sampling technique

A cross-sectional study design was carried out. Live birds markets were identified in the three Local Government Areas (Jos South, Jos North and Jos East). Fifty birds from each LGA were sampled. Sterile swab sticks were inserted in the cloacae of each chicken and placed in sterile vial. Samples were transported on ice to the microbiology init of the Federal College of Animal Health and Production Technology, Vom.

Bacteria culture, isolation and identification

Bacteria culture, isolation and identification were carried out as described by Chesebrough, 2006. Briefly, within one hour of sampling, the swabs were directly inoculated into 9ml buffer peptone water for pre-enrichment, in screw capped bottles and incubated at 37^{0}C for 24hours. One ml of pre-enriched was streaked on to selective media of *Salmonella Shigella* agar and incubated at 37^{0}C for 24 – 48 hours. Plates were observed after 24 – 48 hours incubation for typical *Salmonella* colonies on *Salmonella Shigella* agar.

Determination of antibiotic susceptibility and resistant patterns of *Salmonella* isolates.

Antibiotic susceptibility tests were carried out by agar disc diffusion method. The standard disc diffusion method according to clinical laboratory standard institute, CLSI (2008) guidelines was applied. Briefly, the *Salmonella* isolates were enriched in buffer peptone water for 24hours at 37^{0}C before swabbing on to surface of dried nutrient agar plates. After 15mins of pre-diffusion time, antibiotic discs were placed on the Nutrient

agar surface, sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were then incubated at 37ºC for 24 hours. Diameter of inhibition zones were recorded and compared with the zone diameter interpretive chart for interpretation of results as resistant, intermediate and susceptible.

The antibiotics used and their concentrations are: Ciprofloxacin (10µg), Tarivid (10µg), Reflacine (10µg), Augmentin (30µg), Gentamycin (10 µg), Streptomycin (30µg), Ceporex (10µg), Nalidixic (30µg), Septrin (30 µg), Ampicilin (30 µg). The diameters of the zone of inhibition were measured in millimeters with a ruler and compared with a zone interpretation chart (Muragkar *et al*., 2004). McFarlland scale was used and the result was reported as sensitive, intermediate or resistant based on the size of zone of inhibition

RESULTS

Prevalence

The study was carried out to determine the antibiotics susceptibility pattern of *salmonella* in indigenous chicken in Jos. The result showed that of the 150 cloacal samples collected and examined, 43 were positive for *Salmonella* giving an overall prevalence of 28.7% (table 1). Table 2 showed that the prevalence was highest in Jos East Local Government Area (72.0%) followed by Jos North (56.0%) and Jos South (22.0%) (P=0.000; Odds ratio=27.4; 95% CI=0.449-0.564).

Antibiotic susceptibility

The antibiotics susceptibility profile of *Salmonella* isolates from the indigenous chickens characterized displayed resistance to one or more antibiotic (table 3). The antibiogram profile of the isolates showed varying degree of sensitivity. The result showed that 80.0% of the isolates were sensitive to streptomycin and only 5.3% to Tarivid. More so, 32.0%, 21.3%, 3.9%, 35.2%, 40.5% and 22.6% of the islolates were moderately sensitive to nalidixic acid, peflacine, gentamycin, ciproflox, ampicilin, and ceporex respectively. The result also showed that greater proportion (92.0 %) of the isolates were resistant to Tarivid. Only 4.0% showed resistance to streptomycin.

Table 1: Overall prevalence of *Salmonella* from cloacae swabs of indigenous chickens sold at live birds market in Jos.

Specimen	Number of sample	Number of positive	% prevalence
Cloacae swab	150	75	50.0

TABLE 2: Prevalence of *Salmonella* in indigenous chicken's based on location

Location	No. of samples examined	No. positive	% prevalence	P-value	Odds ratio	95% CI
Jos East	50	36	72.0			
Jos North	50	28	56.0	0.001	27.4	0.449-0.564
Jos South	50	11	22.0			

TABLE 3: Antibiotic susceptibility pattern of *Salmonella* isolated from indigenous chickens in Jos.

	Antibiotic susceptibility profile		
Antibiotics	**Susceptibility (%)**	**Intermediate (%)**	**Resistance (%)**
NA (30 µg)	10(13.3)	24(32.0)	41(56.0)
PEF(10 µg)	44(58.6)	16(21.3)	15(20.0)
CN(10 µg)	59(77.6)	3(3.9)	13(18.4)
AU(30 µg)	50(66.6)	0(0.0)	25(33.3)
CPX(10 µg)	18(24.7)	35(45.2)	22(30.1)
SXT(30 µg)	25(33.3)	0(0.0)	50(66.6)
S(30 µg)	60(80.0)	12(16.0)	3(4.0)
PN(30 µg)	19(24.0)	28(40.5)	28(35.4)
CEP(10 µg)	43(57.3)	17(22.6)	15(20.0)
OFX(10 µg)	4(5.3)	2(2.6)	69(92.0)

KEY: Nalidixic acid (NA), Reflacine (PEF), Gentamycin (CN), Augmentin (Au), Ciproflox (CPX), Septrine (SXT), Streptomycin (S), Ampicillin (PN), Ceporex (CEP), Tarivid (OFX).

Discussion

In spite of the importance of poultry as the major element in human food chain, it has been frequently labeled as one of the most important or source of food poisoning due to *Salmonella* serovars causing the majority of food borne outbreaks worldwide (Nchawa and Bassey, 2015). Live bird markets can serve as potential hubs where disease agents are transmitted and maintained for prolonged periods of time. This study was therefore conducted to isolate and determine the antibiogram of *Salmonella* species in local chickens sold at live bird markets in Jos and environs. The prevalence of *Salmonella* species isolated from local chickens in this present study was 50.0%. This suggests a relative high prevalence compared to the 13.0% reported in free range indigenous chickens in Abeokuta, Nigeria by Ojo *et al*. 2012. Lower isolation rates from backyard chickens were also reported to be 6.0, 5.8, and 3.5% in Iran, Belgium, and Paraguay, respectively (Leota *et al*., 2010; Namata *et al*., 2009; Jafari *et al*., 2007). The high prevalence

in this study may suggest poor and unhygienic conditions of the environment or controlled used of antibiotics in local chickens in the area.

The prevalence of *Salmonella* in indigenous chickens sold at live bird market was highest in Jos East Local Government Area (72.0%) followed by Jos North (56.0%) and Jos South (22.0%). The difference in *Salmonella* isolation observed in the local chickens in the three Local Government Areas could be attributed to differences in the hygienic conditions in the poultry environment, and possibly, the incorporation of antimicrobials in poultry feeds.

In Nigeria, there is no policy guiding the use of antimicrobials in animals. Consequently, there is a high level of indiscriminate use of antimicrobials in animals (Alo and Ojo, 2007). The antibiogram profile of the isolates showed varying degree of sensitivity. Greater proportions of the isolates were sensitive to streptomycin and a greater proportion resistant to Tarivid. Some of the isolates were moderately sensitive to nalidixic acid, peflacine, gentamycin, ciproflox, ampicilin, and ceporex. The isolates were resistant to two or more antibiotics. This suggests that indigenous chicken in Jos harbored multi-drug resistant *Salmonella* and so may serve as a vehicle to human. Indiscriminate and improper use of sub-therapeutic doses of antibiotics may be the reason for the multidrug resistant isolates.

The high incidence of multi-drug resistant *Salmonella* in free-range chickens, as observed in the present study, may be due to the continuous exposure of these birds to resistant *Salmonella* in the environment. Factors such as levels of dependence on antimicrobial usage in animals, degrees of environmental pollution and prevailing climatic conditions from region to region may contribute to antimicrobial selection pressure and the emergence of resistant bacteria, the persistence and distribution of resistant bacteria, as well as the exposure of hosts to resistant bacteria. All these will influence the overall prevalence of antimicrobial resistant bacteria within an ecosystem. Although free-range chickens hardly receive any modern veterinary attention, they are exposed to potentially resistant bacteria harbored by other hosts (with previous exposure to antimicrobials) living in the same environment (Ojo *et al*., 2012). Free range chickens may acquire drug resistant *Salmonella* by contact with carriers or by ingestion of food and water that have been contaminated by fecal materials from other scavenging animals, which are more likely to receive veterinary care and treatment with antimicrobials. Poor sanitation and environmental pollution with human excreta due to inadequate toilet facilities, as observed in many rural communities in Nigeria, may expose free-range chickens to resistant bacteria of human origin. Poor management of effluent generated by abattoirs and commercial farms also contributes to environmental pollution and, hence, possible exposure of free-range chickens to resistant bacteria.

Conclusion

This study has revealed a relatively high prevalence (50.0%) of *Salmonella* in commercial indigenous chickens in the study area. The isolates were resistant to two or more antibiotics which suggests that indigenous chicken in the area harbor multi-drug resistant *Salmonella*. Commercial indigenous chicken may therefore serve as a vehicle for transmission of multidrug resistant *Salmonella* to humans. More so, live bird markets in the area with a poor level of biosecurity could play an important role in the dissemination of multidrug resistant *Salmonella* between animals and humans through their network

Recommendations

Further studies should be undertaken to identify the serotypes of *Salmonella* isolates in indigenous chickens in Jos, Plateau State. More so, there should be legislation on antibiotic use and usage. Farmers should raise chicken on improved conditions.

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