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Full Length research paper

Antimicrobial resistance profiles of *Salmonella gallinarum* isolates from free-range chickens in Nasarawa state, Nigeria

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The development of antimicrobial resistance in bacteria represents a global challenge to the control of bacterial diseases. The aim of this study was to investigate antimicrobial resistance in *S. gallinarum* isolated from free-range chickens in Nasarawa State, Nigeria. The isolates (n=49) were screened for antimicrobial resistance profiles against 13 antibiotics using the disc diffusion method. Overall, 81.7% resistance was recorded against ciprofloxacin, followed by gentamicin, (76.1%); ampicillin, (65.8%); chloramphenicol and cotrimoxazole, (66.2%); tetracycline, (58.1%), while neomycin, nalidixic acid, colistin, oxytetracycline, norfloxacin, kanamycin and amoxycillin had 43.9%, 42.2%, 35.5%, 33.4%, 30.1%, 24.4% and 12.9% resistances respectively. These resistances were found to be statistically significant ($p<0.05$), with the exception of nalidixic and tetracycline which were insignificant ($p>0.05$). Nasarawa West Senatorial Zone had an overall percentage drug resistance of 55.1%, followed by Nasarawa North, 44.2% and Nasarawa South, 40.6%. There was no significant difference ($p>0.05$) in antimicrobial resistance in the three senatorial zones. *S. gallinarum* resistance against the various tested antibiotics was generally low in Assakio and Andaha when compared with the drug resistance of the isolates in other study areas. Since free range chickens hardly receive any antibiotic medication, it is probable that the drug resistance observed may be a reflection of the consequence of drug use among human and animal populations in the study areas. This is a threat to the control of fowl typhoid in Nasarawa State.

Key words: Antimicrobial resistance, *S. gallinarum*, free range chickens, Nasarawa State, Nigeria.

INTRODUCTION

S. gallinarum is the causative agent of fowl typhoid (OIE, 2008), a severe systemic disease of chickens and other galliforme birds (Shivaprasad, 2000) that causes heavy economic losses in poultry through mortality and reduced egg production (Khan *et al.*, 1996).

Fowl typhoid was first reported in England by Klein as infectious enteritis in 1888 and was later named fowl typhoid in 1902 by Curtice in Rhode Island (Curtice, 1902).

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Although the disease has been eradicated from modern poultry in the developed countries of the world, it has increasesd in incidence in most developing countries of South America, Asia and Africa (Onunkwo, 1981; Bouzoubaa and Nagaraja, 1984; OIE, 2005). In Nigeria, for example, with the continuous expansion of poultry industry, fowl typhoid has also gained ground as a major disease of poultry (Agbaje *et al.*, 2010). Recent reports put the prevalence of fowl typhoid as 18.6% and 19.3% in local and exotic chickens respectively in Jos, Plateau State (Okwori *et al.*, 2007) and 18.4% in exotic chickens in Kaduna (Mbuko *et al.*, 2009), northern Nigeria.

Efforts including vaccination and antimicrobial therapy have been made for the control of fowl typhoid outbreak in Nigeria. Antimicobials such as amynoglycosides, B-lactam penicillins and fluoroquinolones have been widely used for treatment of the disease in animals and other bacterial diseases in humans However; the efficacy of these drugs has been threatened due to development of resistance. Antimicrobial resistance is the capacity of bacteria to survive exposure to a defined concentration of antimicrobial substance and antimicrobial use and especially misuse has been found to be the most important selecting force in bacterial antibiotic resistances (Okeke *et al.*, 1999; Moreno *et al.*, 2000; Okoli *et al.,* 2002a). The use of a particular antibiotic in a host in an environment may increase the risk of infection with resistant organisms in other hosts that have not receive that antibiotic but shares the same environment with hosts that have been treated with the antibiotic (Lipstich and Samore, 2002).

The free range chickens also called village or local chickens are the most important of the poultry species in terms of number and development (Oluyemi and Roberts, 1979). Acording to FLDPCS (1992), out of the 104.3 million poultry population in Nigeria, 72.4 million are chickens. The local chickens are managed under the extensive systems and are usually neither vaccinated nor receive any antibiotic medication. In Nigeria, the local chickens are kept by over 90% of rural households, especially women as asset and represent a significant part of rural economy in particular and national economy as a whole (Ajala *et al.,* 2007). They form the bedrock of poultry industry, contributing to over 80% of poultry products.

The extensive management system of keeping rural poultry exposes them to a number of infectious disease agents, which may also include drug resistant strains, since they often share common environments with human and other animals hosts that may habour drug resistant organisms. The aim of this study was to determine the antimicrobial resistance profiles of *S. gallinarum* isolated from free range chickens in Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study Area and Climatic Condition

Nasarawa State is located in the North Central Nigeria otherwise known as the Middle-Belt region, with Lafia as its capital. The state is divided into 13 Local Government Areas (LGA) for administrative purposes, which are further grouped into three senatorial zones. Nasarawa State is bounded in the north by Kaduna State, in the west by the Federal Capital Territory, in the south by Kogi and Benue States and in the east by Taraba and Plateau States.The state has agriculture as the mainstay of its economy and is located between latitude 8'35^{o}N and longitude 8'32^{o}E, with mean temperature of 32^{o}C (Hassan *et al.*, 2013). Nasarawa State has a total area of 27, 117 Km^{2} and population of 2, 040, 097 with density of 75/ km^{2} (190/sq mi), according to 2005 Nigerian National Cencus.

Selection of Study Areas

A preliminary field investigation was carried out in the state to identify the study sites. Three villages and a local government headquarter were randomly selected from each senatorial zone of the state. A total of 12 villages/towns were selected, which included Lafia, Keana, Agbashi and Asakkio in Nasarawa South Senatorial Zone; Akwanga, Andaha, Nasarawa Eggon and Wamba in Nasarawa North Senatorial Zone and Keffi, Garaku, Gadabuke and Gunduma in Nasarawa West Senatorial Zone. The selected villages/towns are located in 10 (76.9%) out of the 13 LGAs of Nasarawa State.

Sampling Procedure and Sample Collection

A total of 83 households were randomly selected from the selected villages and all chickens in a selected household were considered as one flock (Mdegela *et al.*, 2000). In each local government headquarters, samples were collected from chicken markets. Chickens of various types (pullets, cocrels and layers) and ages were sampled.

Cloacae swabs were collected from a minimum of 5 chickens randomely selected from a flock at each study location using sterile commercial swab sticks (Antec R). Swabs were placed in Bijou bottles containing 15ml Selenite F broth (Oxford, Basingtoke, Hampshire, England). In addition, samples from visceral organs (liver and spleen) were collected at the chicken markets into properly label polythene bags, using sterile forceps and scapel blades. Samples were transported to the laboratory in cold boxes. In the laboratory, tissues were homogenized in small volumes of nutrient broth and thereafter inoculated into Selenite F broth. Inoculated Selenite broths were incubated at 37^{o}C for 24 hours for selective enrichment.

Isolation and Identification of *S. gallinarum*

The procedures described by HPA (2011) and Fasure *et al,* (2012) were followed. Subcultures were made from Selenite F broth onto MacConkey agar (MCA, Oxoid Ltd, UK, without salt) and smooth colourless colonies (non-lactose fermenters) suggestive of *Salmonella*, observed

Table 1. Distribution of *S gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria in Relation to Study Location.

Location	No. of Samples Tested	No. of *S. gallinarum* Isolated	Isolation Rate (%)
Lafia	530	7	1.3
Keana	80	4	5.0
Agbashi	100	3	3.0
Assakio	100	4	4.0
Akwanga	230	2	0.9
Andaha	90	3	3.3
Wamba	80	4	5.0
N/Eggon	90	3	3.3
Keffi	380	9	2.4
Garaku	100	3	3.0
Gadabuke	100	3	3.0
Gunduma	90	4	4.4
Total	1970	49	2.5

after 24 hour incubation at 37^{o}C were purified using desoxycholate citrate agar (DCA, Oxoid Ltd, UK). The suspected *Salmonella* colonies were gram stained and morphologically studied (Merchant and Packer, 1967). Gram negative rods suggestive of *Salmonella* species were tested for motility. Non motile suspected *Salmonella* were identified using biochemical reactions as described by OIE (2008). Isolates were confirmed as *Salmonella gallinarum* by testing for agglutinability with *Salmonella* polyvalent 'O' (A-G) and H antisera using slide agglutination (OIE Manual 2008). Cultures that tested positive (agglutination) with *Salmonella* 'O' antiserum and negative with *Salmonella* 'H' antiserum were further tested in the same manner with group-specific sera for *S. pullorum* and *S. gallinarum* ('O' 9 antiserum). Positive cultures were serogrouped as *S. gallinarum* and *S. pullorum*. Glucose fermentation (with gas production) and dulcitol fermentation tests were used to differentiate the two biovars (Trabulsi and Edwards, 1962; OIE, 2008). Pure cultures of *S. gallinarum* were stored in nutrient agar slants at 4^{o}C for antimicrobial susceptibility test.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test to determine the resistance profiles of *S. gallinarum* isolates was carried out using the disc diffusion method according to the (NCCLS, 2002). The antimicrobial agents tested were chloramphericol (30µg), gentamicin (10µg), cotrimoxazole (30µg), nalidixic acid (30µg), ampicillin (30µg), tetracycline (25µg), amoxycillin (30µg), kanamycin (30µg), colistin (10µg), oxytetracycline (30µg), neomycin (30µg), norfloxacin (10µg) and ciprofloxacin (50µg) (Sensi Disc TM BBL, USA). Sterile wire loop was used to pick two to three colonies of *S. gallinarum* from DCA plate and emulsified in 3 to 4 ml of sterile normal saline. Standardization of the bacterial suspension was performed by diluting the suspension until the turbidity matched the 0.5 McFarland standards. 20 ml of Mueller-Hinton agar (Fluka Biochemika, Spain) was dispensed into 100 mm disposable petri dishes (Sterlin, UK) to a level mark and allowed to solidify at room temperature in sterile laminar flow hood (ESCO, USA). Agar plates were inoculated using sterile cotton swab dipped into the standardized suspension of the *S. gallinarum* and drained. The inoculated plates were air-dried and antimicrobial discs were applied with a dispenser within 15 minutes. Antibiotic discs were gently pressed down on the agar to ensure contact. A standard locally isolated *Escherichia coli* was used as control. Plates were incubated aerobically at 37^{o}C for 24 hours, afterwhich plates were examined. The diameters of the zone of inhibition were measured to the nearest millimeter using a meter rule (NCCLS, 2002) and compared with a zone interpretation chart (Bauer, 1966).

STATISTICAL ANALYSIS

The analysis of variance (ANOVA) and Chi-square statistical tests in Statistical Package for the Social Sciences (SPSS) software version 17.0 were used to compare the variations in antimicrobial resistance.

RESULTS

Overall, 49 (2.5%) *S. gallinarum* isolates (Table 1), comprising 18 (2.2%) from Nasarawa South Senatorial

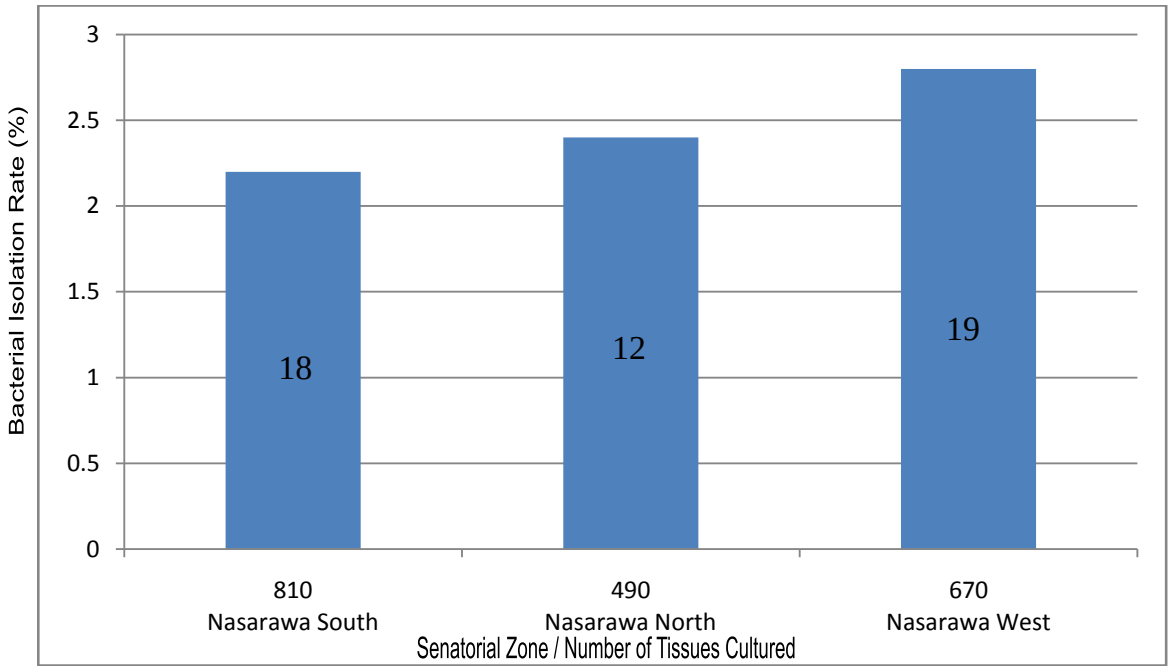

Figure 1. Distribution of *S gallinarum* Isolates from Free Range Chickens in in the Senatorial Zones of Nasarawa State, Nigeria.

Table 2. Antimicrobial Resistance of *S. gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria in Relation to Senatorial Zones.

	No. (%) Resistance/Location			
Antimicrobial Agent	**Nasarawa South**	**Nassarawa North**	**Nassarawa West**	**Mean**
Chloramphenicol	12 (66.7)	7 (58.3)	14 (73.7)	66.2
Gentamicin	13 (72.2)	8 (66.7)	17 (89.5)	76.1
Cotrimoxazole	12 (66.7)	6 (50.0)	15 (78.9)	65.2
Nalidixic Acid	3 (16.7)	5 (41.7)	13 (68.4)	42.3
Ampicillin	8 (44.4)	7 (58.3)	18 (94.7)	65.8
Tetracycline	11 (61.1)	6 (50.0)	12 (63.2)	58.1
Amoxycillin	3 (16.7)	2 (16.7)	1 (5.3)	12.9
Kanamycin	3 (16.7)	3 (25.0)	6 (31.6)	24.4
Colistin	5 (27.8)	5 (41.7)	7 (36.8)	35.4
Oxytetracycline	5 (27.8)	3 (25.0)	9 (47.4)	33.4
Neomycin	3 (16.7)	2 (16.7)	2 (10.5)	14.6
Ciprofloxacin	13 (72.2)	10 (83.3)	17 (89.5)	81.7
Norfloxacin	4 (22.2)	5 (41.7)	5 (26.3)	30.1
Total Number of Isolates	18	12	19	
Overall Mean % Resistance	40.6	44.2	55.1(p. value 0.326)	

Zone, 12 (2.4%) from Nasarawa North and 19 (2.8%) from Nasarawa West Senatorial Zone were obtained (Figure 1). Bacterial isolation rate from individual study site was highest in Keana and Wamba (5.0%) and lowest in Akwanga (0.8%) (Table1). The overall mean percentage antimicrobial resistance of *S. gallinarum* isolates from the three senatorial zones against the various tested antimicrobial drugs is shown in table 2. Among the drugs tested, ciprofloxacin had the highest resistance of 81.7% followed by gentamicin 76.1% while amoxicillin had the least resistance of 12.9%. The order of increase in antimicrobial resistance is shown in figure 2. The percentage resistance variations were statistically significant ($p<0.05$). Further statistical treatment for resist-

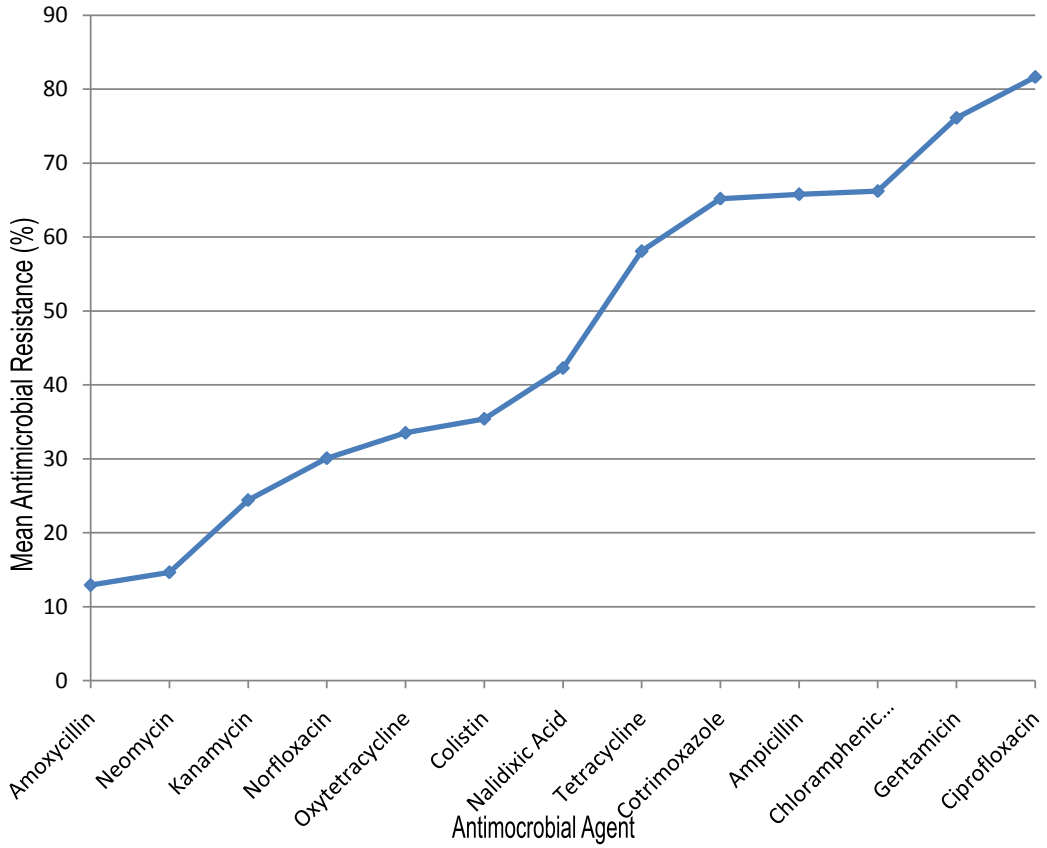

Figure 2. Progression in Antimicrobial Resistance of *S. gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria.

Table 3. P-values from Chi-square Test on Antimicrobial Resistance *S. gallinarum*Isolates from Free Range Chickens in Nasarawa State, Nigeria

Antibiotics	Resistance	No Resistance	Total	P-value
Chloramphenicol	33	16	49	0.015^{S}
Gentamicin	38	11	49	0.000^{S}
Cotrimoxazole	33	16	49	0.015^{S}
Nalidixic acid	21	28	49	0.317^{NS}
Ampicillin	33	16	49	0.015^{S}
Tetracycline	29	20	49	0.199^{NS}
Amoxycillin	6	43	49	0.000^{S}
Kanamycin	12	37	49	$<0.001^{S}$
Colistin	17	32	49	0.032^{S}
Oxytetracycline	17	32	49	0.032^{S}
Neomycin	7	42	49	0.000^{S}
Cyprofloxacin	40	10	49	0.000^{S}
Norfloxacin	14	35	49	0.000^{S}

^{S}Significant (P < 0.05); ^{NS}Not significant (P>0.05)

ance against the antimicrobial drug revealed that, with the exception of nalidixic acid and tetracycline, resistance against the remaining antimicrobials were statistically significant (p<0.05) (Table 3). The overall mean resistances

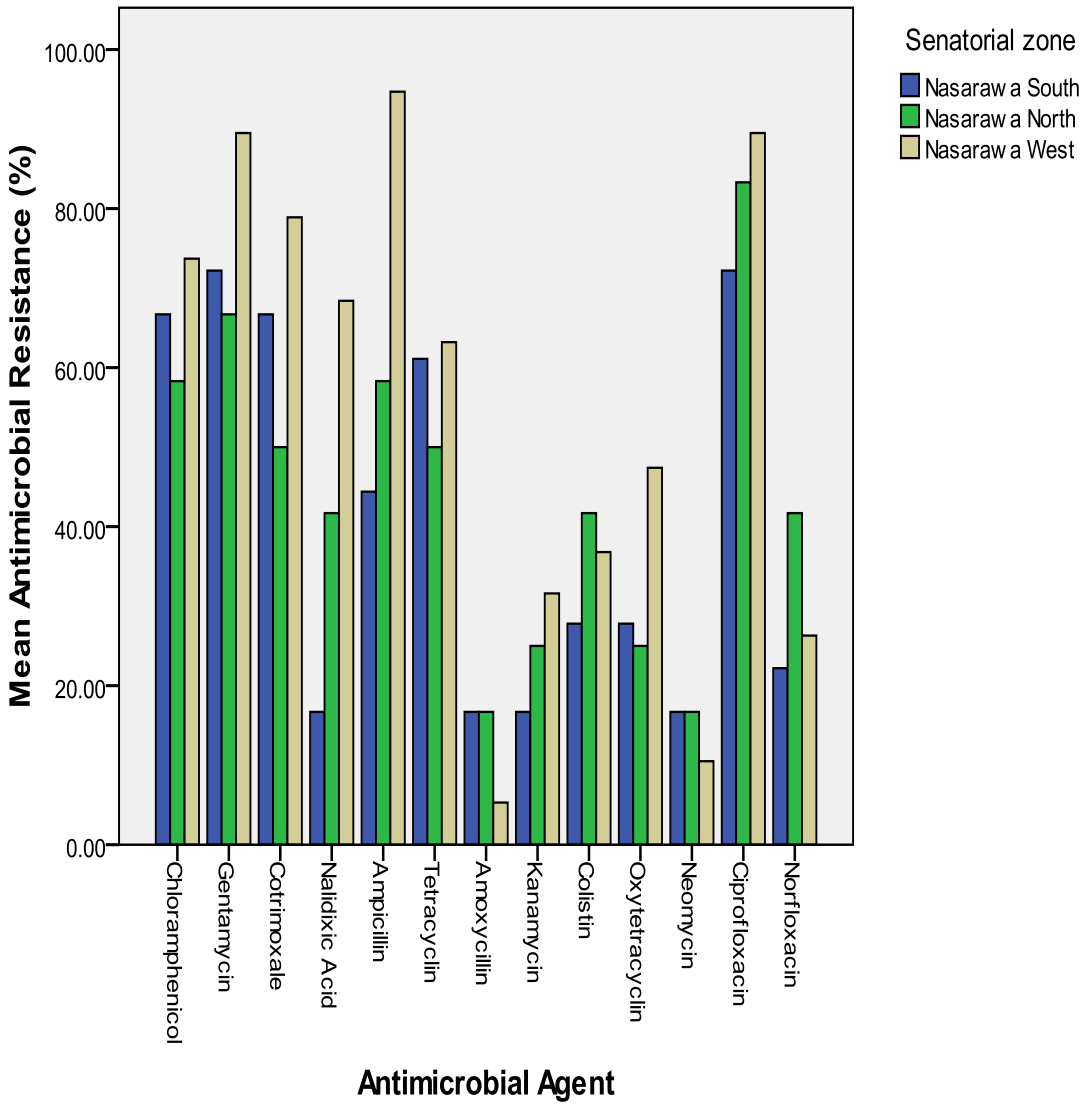

Figure 3. Comparison of Overall Antimicrobial Resistance of *S. gallinarum* Isolates from Free Range Chickens in Senatorial Zones of Nasarawa State, Nigeria.

of 55.1, 44.2 and 40.6% recorded in Nasarawa West, Nasarawa North and Nasarawa South Senatorial Zones respectively, were however found to be statistically indifferent ($p>0.05$).

Resistances of *S. gallinarum* isolates from the three senatorial zones against the tested antimicrobial drugs are compared in figure 3. It shows that the bacterial isolates from Nasarawa West Senatorial Zone were more highly resistant to most of the drugs tested when compared with those from Nasarawa South and Nasarawa North. For example, while 94.7% resistance was recorded against ampicillin in Nasarawa West, 58.3% and 44.4% resistances were observed against the same drug in Nasarawa North and Nasarawa South, respectively. Similarly, 89.5% resistance observed against ciprofloxacin and gentamicin, respectively in Nasarawa West was higher than the resistance observed against the same drug in the Nasarawa North and Nasarawa South. Other antibiotics with similar resistance profiles were chloramphenicol, cotrimoxaxole, nalidixic acid, tetracycline, kanamycin and oxytetracycline. Antimicrobial resistance of *S. gallinarum* isolates from the various study sites is shown in table 4. Generally, higher drug resistances (70%-100%) against the tested drugs were more observed in Agbashi, Lafia, Akwanga, Wamba, Keffi, Garaku, Gadabuke and Gunduma. In comparison, striking low resistances (0-49%) occurred more frequently in N/Eggon, Andaha and Assakio, while

Table 4. Antimicrobial Resistance Frequencies of *S. gallinarum* Isolates from Free Range Chickens in Relation to Study Locations in Nasarawa State, Nigeria.

		Antimicrobial Agent/Resistance (%)												
Site	**n**	**CH**	**GN**	**CO**	**NA**	**AM**	**TE**	**AX**	**KN**	**CL**	**OT**	**NM**	**CF**	**NF**
Lafia	7	7(100)	6(85.7)	7(100)	1(14.3)	6(85.7)	6(85.7)	0(0)	1(16.7)	1(14.3)	3(42.8)	(28.6)	7(100)	2(28.6)
Keana	4	2(50)	3(75)	3(75)	0(0)	1(25)	2(50)	1(25)	1(25)	1(25)	1(25)	2(50)	4(100)	1(25)
Agbashi	3	3(100)	3(100)	2(66.6)	2(66.6)	2(66.6)	3(100)	1(33.3)	1(33.3)	2(66.6)	1(33.3)	0(0)	2(66.6)	2(66.6)
Assakio	4	1(25)	2(50)	1(25)	0(0)	0(0)	1(25)	1(25)	0(0)	2(50)	1(25)	0(0)	1(25)	0(0)
Akwanga	2	2(100)	2(100)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)	2(100)
Andaha	3	0(0)	0(0)	1(33.3)	1(33.3)	0(0)	2(66.6)	0(0)	1(33.3)	1(33.3)	0(0)	1(33.3)	3(100)	0(0)
Wamba	4	4(100)	4(100)	4(100)	2(50)	4(100)	1(25)	2(50)	1(25)	0(0)	0(0)	0(0)	4(100)	1(25)
N/Eggon	3	0(0)	1(33.3)	0(0)	2(66.6)	1(33.3)	2(66.6)	0(0)	1(33.3)	1(33.3)	0(0)	0(0)	3(100)	1(33.3)
Keffi	9	8(88.9)	9(100)	7(77.8)	(66.7)	8(88.9)	5(55.6)	0 (0)	2(22.2)	4(44.4)	5(55.6)	2(22.2)	7(77.8)	1(11.1)
Garaku	3	3(100)	2(66.7)	3(100)	3(100)	3(100)	2(66.7)	0(0)	1(33.3)	2(66.7)	1(33.3)	0(0)	3(100)	1(33.3)
Gadabuke	3	3(100)	3(100)	1(33.3)	2(66.7)	3(100)	3(100)	0(0)	2(66.7)	1(33.3)	1(33.3	0(0)	3(100)	2(66.7)
Gunduma	4	0(0)	3(75)	4(100)	2(50)	4(100)	2(50)	1(25)	1(25)	0(0)	2(50)	0(0)	4(100)	1(25)
Total	49	33(67.3)	38(77.6)	33(67.3)	21(42.8)	33(67.3)	29(59.2)	6(12.2)	12(24.5)	17(34.7)	17(34.7)	7(14.3)	40(81.6)	14(28.6)

CH, Chloramphenicol; GN, Gentamicin; CO, Cotrimoxazole; NA, Nalidixic acid, AM, Ampicillin; TE, Tetracycline; AX, Amoxycillin; KN, Kanamycin; CL, Colistin; OT, Oxytetracycline; NM, Neomycin; CF, Ciprofloxacin; NF, Norfloxacin. n, number of isolates tested.

moderate resistances (50-69%) were more observed in remaining study areas. The table also shows that the drug resistance varies among study sites. For example, while there was 100% resistance against gentamicin and chloramphenicol in Agbashi, Akwanga, Wamba and Gadabuke, 0% resistance against the same drugs was observed in Andaha and 0% resistance against chloramphenicol in N/Eggon and Gunduma.

DISCUSSION

Antibimicrbial resistance in *Salmonella* has assumed alarming proportion worldwide (Aggarwal *et al.*, 1983; Dorn *et al.*, 1992). It is associated with improper use of antimicrobial agent and has been reported to occur mostly in hosts that receive the antimicrobial drug. For example, antibiotic resistance in *Salmonellae* has been reported in commercial chickens (Oh *et al.,* 2000; Hernandez *et al.,* 2002; Min *et al.,* 2010; Lee *et al.,* 2003; Ludovico *et al.,* 2005; Murugkar *et al.,* 2005; Fasure *et al.,* 2012). In the present study, *S. gallinarum* isolates from free range chickens that rarely receive any antibiotic treatment were observed to be resistant against the various tested antimicrobial drugs. Although there are no reports on antimicrobial resistance in *S. gallinarum* from free-range chickens in Nigeria, antibiotic resistance has been reported in an *E. coli* isolate from free-range chickens (Okoli, *et al.*, 2005; Okoli, 2006). This is not incidental but probably a reflection of resistance events in other hosts sharing the same environments with the local chickens (Okoli, *et al.*, 2005). The free range birds may maintain close contact with organisms originating from other hosts in their environments such as commercial poultry and other animal hosts that had been previously exposed to various antibiotics. For example, in Nigeria wastes from commercial poultry are not properly disposed and most rural farmers use these wastes as manure, which are often kept at the backyards before moving them to farms.

These poultry wastes may serve as source of enteric organisms that habour novel resistance factors for birds that feed on such wastes. It has been reported that the use of a particular antibiotic in a host for example humans, in an environment may increase the risk of colonization by or infection with resistant organisms in other hosts that have not received that set of antibiotics but are sharing a common environment with the treated ones (Okoli, 2006). This indirect effect of antimicrobial use experienced by members of a population has been described as the enhancement of risk for acquiring resistance by organisms, because of the use of antimicrobials in other hosts in the group or population (Lipsitch and Samore, 2002). The *Salmonella* may subsequently colonize the intestine of the infected chickens and constitute enormous reservoir for genes encoding resistance against these antibiotics (Stern, 1992; On *et al.*, 1998) and become the locus for spread of resistance factor.

In this study, there was generally high resistance against ciprofloxacin, gentamicine, chloramphenicol, cotrimoxazole, ampicillin and tetracycline by *S. gallinarum* isolates from free-range chickens. The observed high resistance against these antibiotics probably reflects the high usage of the drugs in the study sites. Moreover, these are inexpensive, first line broad-spectrum and readily available antibiotics (Okeke *et al.*, 2000; Chah *et al.*, 2000; Uwaezuoke *et al.*, 2000; Chah *et al.*, 2003). These drugs may have become seriously compromised and probably are currently ineffective for the treatment of fowl typhoid disease in the study areas. An increase in the incidence of antibiotic resistance in *Salmonella* isolated from animals related to exhaustive application of antibiotics has been documented worldwide (Chruchaga *et al.*, 2001; Okoli *et al.*, 2006). This is expected since antimicrobial use has been shown to be the most important selecting force in bacterial antibiotic resistance (Moreno *et al.*, 2000; Okoli *et al.*, 2002a). On the other hand the low resistance observed against the remaining antimicrobial drugs may be linked to infrequent use of the drugs. Such drugs may probably be effective for the chemotherapy and prophylaxis against fowl typhoid in the study areas.

The antibiotic resistance observed in local chicken isolates in some of the study areas may be a consequence of feeding on commercial poultry feeds, to which antibiotics are usually added as supplements and growth promoters (Murugkar *et al.*, 2005). For example, in most households visited for sample collection, it was observed that some owners of local chickens also owned small numbers of commercial (exotic) chickens, which were not separated from the local breeds. In these environments, the local chickens were not restricted access to commercial feeds; rather they were left to share such feeds and drinking water with the commercial chickens. The obvious lack of restriction of access for local chickens to commercial feeds may indirectly expose them to the antibiotics incorporated in such feeds, which may be a probable cause for the antibiotic resistance (Dorn *et al.*, 1992).

It was observed that drug resistance varied among the geographical study locations. For example, whereas isolates from Keffi, Garaku, Gadabuke, Wamba, Akwanga, Lafia and Agbashi were maximally resistant to chloramphenicol, isolates from Gunduma, Nasarawa Eggon and Andaha showed 0% resistance against the drug, while Assakio and Keana isolates showed moderate resistance against the same drug. This may be attributed to differences in exposure rates of the organism to such antimicrobial drugs in the study locations.

The high resistances against the fluoroquinolones observed in this study is a problem for control of bacterial diseases of poultry in general in the study areas considering the fact that fluoroquinolones are group of antimicrobials that are commonly used for the chemotherapy and chemoprophylaxis of fowl typhoid and other bacterial diseases of poultry. Fluoroquinolones are a class of synthetic antimicrobial agents that have been widely used in veterinary medicine since their introduction in the late 1980's and early 1990's. This class of antibiotics offers the advantage of oral administration, high potency against Gram negative organisms and low toxicity (Lee *et al.*, 2004). In the present investigation, two fluoroquinolone antibiotics (Ciprofloxacin and Norfloxacin) were used and results showed that out of the thirteen antibiotics tested, ciprofloxacin had the highest total percentage resistance of 81.6% while norfloxacin had 30.1%. The proportion of resistance of isolates from individual study area against the fluroquinolones showed that isolates from 8 (62.5%) locations were 100% resistant against ciprofloxacin and 1 (7.7%) against norfloxacin. It is probable that even though ciprofloxacin and norfloxacin belong to the first generation fluoroquinolones, the former may be the most frequently used because it is less expensive, more readily available and widely used. This may have consequently resulted to the high resistance observed. The high fluoroquinolone resistance may also be linked to the fact that fluoroquinolone antibiotics have found their way into feeds as a result of increasing smuggling of animal feeds into Nigeria (Fasure *et al.*, 2012). This has threatened the efficacy of fluoroquinolones, the preferred antibiotics for treatment of salmonella-associated diseases.

All the *S. gallinarum* isolates were resistant to more than one antimicrobial drug. This is indicative of multiple drug resistance in the isolates and confirms the presence of multiple drug resistant (MDR) strains of *S. gallinarum* in free-range chickens in the study areas. Although there is scarcity of published reports on occurrence of multiple drug resistance in *Salmonellae* in free range chickens in Nigeria, it has been reported in *S. gallinarum* isolates from

from commercial chickens (Fasure *et al.*, 2012) and *E. coli* isolates from free-range chickens (Okoli *et al.*, 2005; Okoli *et al.*, 2006). Multiple drug resistance in *S. gallinarum* have been reported in other parts of the world (Lee *et al.*, 2003a; Ludovico *et al.*, 2005; Min *et al.*, 2010).

Important finding in the current study is the occurrence of ciprofloxacin resistance in *S. gallinarum* isolates since fluoroquinolones (ciprofloxacin and norfloxacin) has implications for both veterinary and human therapy. The abuse of such drugs in poultry could result in the emergence of resistant zoonotic organisms. It has been reported that the excessive or improper use of antibiotics in the rearing of farm animals represents a major factor in the emergence, persistence and spread of resistant *Salmonella* even in the humans who are the *cul-de-sac* of food chain (Chruchaga *et al.*, 2001; Threlfall *et al.*, 1986).

These results confirms the importance of emphasizing on the proper use of antimicrobials in the study environment to reduce the selection and spread of resistant strains of *Salmonellae* to sustain the usefulness of the antibiotics in controlling salmonellosis on long-term basis. This underlines the need for integerated surveillance systems of antibiotic resistance in poultry, human and other animal hosts in Nigeria.

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