# Incidence of Fluoroquinolone and Multidrug Resistant *Salmonella* species in Intensively Reared Pigs and Chickens in Nsukka, Nigeria

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**Abstract:** Fluoroquinolones are broad spectrum antimicrobial agents used to treat infections caused by microbial pathogens especially those resistant to other classes of antimicrobial drugs. The introduction of fluoroquinolones into veterinary therapy has been accompanied by an increase in resistance. Salmonella represents a challenge in modern animal production. This study determined the incidence of fluoroquinolone (and non fluoroquinolones) resistant Salmonella from intensively reared pigs and chickens in Nsukka, an area representative of the emerging trend in intensive animal production in Nigeria. Standard cultural techniques were used to randomly sample 120 animals (60 pigs and 60 chickens) from 12 medium to large scale farms, and from which were obtained a total of five (5) Salmonella isolates (4.17%). Two isolates showed 100% (5/5) resistance to 5 fluoroquinolones while 3 isolates showed 100% (5/5) susceptibility to fluoroquinolones. For non fluoroquinolones two isolates showed 11.1% (1/9) resistance, 22.2% (2/9) intermediate resistance and 66.7% (6/9) susceptibility; one isolate showed 100% (9/9) susceptibility; 1 isolate showed 33.3% (3/9) resistance and 66.7% (6/9) susceptibility. Four isolates were more susceptible to non fluoroquinolones than fluoroquinolones while 2 isolates were more susceptible to fluoroquinolones than non fluoroquinolones. The implications of this resistance patterns are discussed.
**Keywords** *Salmonella, Antibiotic resistance, Fluoroquinolones, Pigs, Poultry*

## INTRODUCTION

The introduction of antimicrobial agents in human and veterinary medicine was one of the most significant biomedical achievements of the 20th century. The first antimicrobial agents were introduced in the 1930's, followed quickly by a large number of new compounds. However, shortly after the introduction, resistance began to emerge. These days, antimicrobial resistant pathogenic agents poses a risk to human and animal health by reducing the efficacy of antimicrobials in the treatment of infectious diseases in both animal and human medicine (European Medicines Agency, 2006) and increasing the cost of antimicrobial chemotherapy and animal production.

*Salmonella* is a Gram negative, non-spore-forming member of the enterobacteriaceae and is widely distributed in nature. The primary habitat of *Salmonella* spp. is the intestinal tract of animals such as birds, reptiles, farm animals, humans and occasionally insects. The organisms are excreted in faeces from which they may be transmitted directly or by insects, other living creatures and fomites to a large number of places and media including water and wastewater (DANMAP, 2009). Due to their role as causative agents of food borne (particularly food of animal origin) diseases in humans, *Salmonella* represents a challenge in modern animal production and husbandry. Their widespread occurrence among food animals including poultry, pigs, cattle, etc., means they may frequently be encountered in raw foods from such sources. *Salmonella* food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers and morbidity and mortality associated with infection in Nigeria and worldwide, continues to increase. In agriculture, several studies have shown that *Salmonella* infection especially in poultry is of critical importance (Molla *et al.,* 2003; Okoli, 2006; Ogunleye *et al.,* 2008; Akoachere *et al.,* 2009; Agbaje *et al.,* 2010; Ojo *et al*., 2011; Felix *et al.,* 2012) and the sources of infections include contaminated feeds, feed ingredients, human wastes, rodents and animal droppings, among others (Hayashi and Yamazaki, 1996).

Fluoroquinolones represent a complex group of synthetic broad-spectrum antimicrobials widely used in the treatment of human and animal bacterial infections. Fluoroquinolones are derived from 1, 4-dihydro-4-oxoquinoline-3-carboxylic acid. They inhibit the activity of bacterial DNA gyrase thereby inhibiting DNA replication and transcription. Fluoroquinolones are the fourth generation of the quinolone antimicrobials and have a broader spectrum of activity than the three previous generations. They are highly bioavailable broad spectrum agents with activity against Gram negative and positive pathogens (Jurado *et al.,* 2008; Cheng *et al.,* 2012). The introduction of fluoroquinolones as effective broad spectrum antimicrobials into veterinary medicine soon resulted in their widespread use. Prominent indications for their use include respiratory and enteric infections in farm animals. They have been used not only as therapeutic agents but also for diseases prevention prophylaxis and as growth promoters (Anderson *et al*., 2003).

The widespread use of antibiotics (including fluoroquinolones) in poultry and livestock industry to treat and prevent infectious bacterial disease and as growth promoters at sub-therapeutic levels in feeds has led to increased bacterial resistance to antibiotics (Apata, 2009). A major problem with the use of antibiotics in food animal production is that many of such antibiotics come from antibiotic classes that are also in use in human medicine thus leading to cross resistance to such antibiotics. Even more alarming is the possibility that the use of one antibiotic could select for multiple antibiotic resistance to functionally unrelated antibiotic classes because antimicrobial resistance genes could be associated with mobile DNA element - transferable plasmids and transposons (Marshall and Levy, 2011).

Recently, with improving agricultural policies and credit schemes, there has been a dramatic increase in intensive animal production in Nigeria. With this has come also increasing use of various antibiotics in treatment, prophylaxis and as growth promoters. As the use of antimicrobials as growth promoters in intensive animal production increases, the exposure of zoonotic microbial pathogens to antibiotics and the associated resistance increases. Given fairly limited veterinary and extension services, antibiotic abuse in intensive agricultural practice in Nigeria is a potent possibility with all the associated challenges. These are poorly investigated and reported. This work was implemented to investigate incidence of fluoroquinolone resistant *Salmonella* spp in intensively reared poultry and pigs in Nsukka, an area representative of the emerging trend in intensive animal farming in Nigeria.

## MATERIALS AND METHODS

### Study Area

Nsukka Local Government Area is located in Enugu State, South East Nigeria. It is situated within the longitude 7°1300 - 7°3530 and an elevation of 1,810ft and (Federal Republic of Nigeria Official Gazette, 2007). Intensive pig and poultry farming is a rapidly growing agricultural industry in this region on account of favourable climate and state policy incentives, and the existence in Nsukka of a large land-grant-type university with extensive and active extension and farmer support services. This study was carried out over a period of 9 months (July to March) covering the peak of the rainy season and all of the dry season. A total of 12 large scale farms including private farms and the University of Nigeria, Veterinary Medicine demonstration farm were selected for this study. The University of Nigeria is a major source of regular and quality veterinary and agricultural extension services to farmers in the study area.

### Sample Collection

A total of 120 rectal swab samples used for the study were randomly collected from 60 apparently healthy pigs in 6 pig farms and 60 apparently healthy mature broilers in 6 poultry farms (Olateru *et al*., 2018). The birds were reared in deep litter systems while pigs were reared on concrete floor pens.

Samples were collected only from farms with no history of antibiotics use during the preceding 8 weeks (for pigs) and 4 weeks (for poultry) for treatment, prophylaxis or as nutritional supplement. Each piggery held a minimum of 200 animals of the age of interest while each poultry farm held a minimum of 2000 birds of the age of interest. Swab samples were transported (on ice packs) to the laboratory for analysis within two hours of collection.

**Isolation and Characterization of *Salmonella* spp**

Isolation was done according to the procedure of the International Organization for Standardization (ISO 6579, 2002). Swabs were inoculated into 9 ml of nutrient broth (Oxoid, England) and incubated at 37°C for 16 to 20hours for the purpose of non-selective pre-enrichment. A 0.1ml of the nutrient broth culture was inoculated into 10 ml of Rappaport-Vassiliadis (RV) broth (Oxoid, England) and incubated at 42°C for 18 to 24hours for selective enrichment of salmonella. A loop full from RV broth culture was streaked on Salmonella-Shigella agar (SSA) (Titan, India). After 24hours incubation at 37°C two to three colourless colonies with or without dark centres were randomly picked from each SSA plate and streaked on MacConkey Agar (MCA) (Oxoid, England). Inoculated plates were incubated at 37°C for 24hours to confirm colonies as non-lactose fermenters. Suspect colonies from MCA were purified by repeated sub-culture onto sterile MCA. Pure cultures were inoculated onto nutrient agar slants as working and stock cultures and maintained by repeated sub-culture stored at 4°C in the refrigerator. Non-lactose fermenting colonies were subjected to biochemical tests identification as described in General Guidance on Methods for the Detection of *Salmonella,* (ISO, Geneve, Switzerland (ISO 6579, 2002). The tests include Urease test, Simon citrate test, TSI test, Methyl red test and Indole test. A *Salmonella galinarium* reference isolate included for comparison was obtained from the collection of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka.

**Antimicrobial Susceptibility Testing**

Susceptibility of isolates to fluoroquinolones was conducted using disc diffusion techniques (Bauer *et al.,* 1966) on Mueller-Hinton agar (Oxoid, England) incubated at 37°C for 24hours. Five fluoroquinolone drugs used for this test were ciprofloxacin 5µg, ofloxacin 5µg, levofloxacin 1µg, norfloxacin 10µg and enrofloxacin 5µg (Oxoid, UK). Susceptibility to other commonly used non-fluoroquinolone antibiotics including extended-spetrum beta-lactam antibiotics (cefotaxime 30µg, ceftriaxone 30µg and ceftazidime 30µg), aminoglycosides (gentamicin 10µg, amikacin 30µg and streptomycin 10µg), tetracycline 30µg, chloramphenicol 30µg and amoxicillin/clavulanic acid 20/10µg, (Oxoid, UK) was also performed for comparison. Sensitivity of each isolate to the antimicrobial agents used was tested in triplicates and the means taken. Results were interpreted using CLSI antimicrobial susceptibility testing standards (CLSI, 2018).

## RESULTS AND DISCUSSION

**Incidence of Salmonella in Broilers and Pigs**

In this study the occurrence and the antibiogram of *Salmonellae* from intensively reared pigs and chickens in Nsukka agricultural zone of Enugu State, Nigeria was investigated. Out of the 60 pigs sampled, 3 (5%) were positive for salmonellae (two isolates from one farm) while two (3%) of the 60 broiler chickens (from different farms) were positive for *Salmonella*. The antibiogram of the salmonella isolates to fluoroquinolone is shown in Table 1 while the resistance profile of the isolates to the non-fluporoquinolone antibiotics is shown in Table 2. Two (40%) of the five isolates were resistant to all the five fluoroquinolones used in this study. Two of the five isolates were resistant to chloramphenicol and ceftazidime while another two showed intermediate susceptibility to cefotaxime and tetracycline.

Two isolates (OP 005a and LC 009) were more susceptible to fluoroquinolones than to non fluoroquinolones while three of the isolates (AP 005a, AP 005b and VC 001) were more susceptible to non fluoroquinolones than to fluoroquinolones. Two different multidrug resistance patterns were exhibited by the *Salmonella* isolates (Table 3). Generally the *Salmonella* isolates exhibited a higher degree of resistance to fluoroquinolones than to non fluoroquinolones. This is of concern considering that fluoroquinolones constitutes an important class of antimicrobial agents used in the treatment of a variety of severe human bacterial infections and very importantly, the last-line-of-defence status of fluoroquinolones in that regard.

Table 1. Susceptibility/ Resistance of isolates to Fluoroquinolones

| Isolates | CIP | OFX | LEV | NOR | ENR |
|---|---|---|---|---|---|
| AP005a | R (0)* | R (0) | R (0) | R (0) | R (8.33) |
| AP 005b | S (29.0) | S (27.0) | S (22.0) | S (26.3) | S (26.0) |
| VC 001 | R (0) | R (0) | R (0) | R (0) | R (0) |
| OP 005 | S (23.6) | S (21.7) | S (16.3) | S (21.3) | S (20.3) |
| LC 009 | S (36.3) | S (32.0) | S (28.7) | S (30.3) | S (33.7) |
| *S.gallinarum* | S (25.0) | S (19.0) | S (15.7) | S (19.3) | S (21.3) |

CIP- Ciprofloxacin 5µg, OFX- Ofloxacin 5µg, LEV- Levofloxacin 1µg, NOR- Norfloxacin 10µg, ENR- Enrofloxacin 5µg; R-Resistant; S-Susceptible

* Numbers in parentheses: diameter of zones of inhibition (in mm), mean of triplicates taken from three plates

Table 2. Resistance profile of *Salmonella* isolates to non–fluoroquinolones antibiotics

| Isolates | CN | S | CTX | AMC | C | CAZ | CRO | TE | AK |
|---|---|---|---|---|---|---|---|---|---|
| AP 005a | I(14.3)* | S(21) | S(36) | S (33.3) | R(0) | S(33) | S(41) | I(13.7) | S (31.7) |
| AP 005b | S(21.3) | S(22) | S(33.7) | S(19.7) | S(31) | S(31.3) | S(32.3) | S(28.3) | S(25) |
| VC 001 | S(17.3) | S(17.3) | I(16.3) | S(30.7) | R(0) | S(26.7) | S(19.7) | I(13) | S(32) |
| OP 005 | S(14.7) | S(21.3) | S(27) | R(10.7) | S(29) | I(14.3) | S(29) | R(10.3) | S(25) |
| LC 009 | S27.3) | S(33) | I(14.7) | S(28.7) | S(28.3) | R(0) | R(12) | S(42.3) | S(33.3) |
| *S. gallinarum* | S(28.3) | S(30) | S(36) | S(41) | S(21.3) | S(20.7) | S(28.3) | S(35.3) | S(25.3) |

CN- Gentamicin 10µg; S- Streptomycin 10µg; CTX- Cefotaxine 3010µg; AMC- Amoxycillin/ Clavulanic acid 30µg; C- Chloramphenicol 30µg; CAZ- Ceftazidime 30µg; CRO- Ceftraxone 30µg; TE- Tetracycline 30µg; AK- Amikacin 30µg; R-Resistance; S-Sensitive; I-Intermediate

* Numbers in parentheses: diameter of zones of inhibition (in mm), mean of triplicates taken from three plates

Table 3. Multidrug Resistance Pattern Exhibited by Different *Salmonella* isolates

| Drug resistance pattern | Frequency | Isolate name |
|---|---|---|
| R-CIP-OFX-LEV-ENR-C | 2 | AP 005a and VC 001 |
| R-AMC-CAZ-TE | 1 | OP 005 |
| Total | 3 | |

CIP- Ciprofloxacin; OFX- Ofloxacin; LEV- Levofloxacin; NOR- Norfloxacin; ENR- Enrofloxacin; C- Chloramphenicol; AMC-Amoxycillin/Clavulanic acid; CAZ- Ceftazidime; TE- Tetracycline.

A significant finding of this study is the low level of occurrence of (asymptomatic) *Salmonella* in healthy intensively reared pigs and chickens, with a total of 5 isolates from 120 animals (4.17% incidence) randomly sampled in 12 medium to relatively large commercial farms.

Similar low incidence of salmonella in the ranges of 4.0% to 9.1% and 0% to 23.1% were reported in goats and cattle respectively by Felix *et al.* (2012) in Ogun state, South West Nigeria. The low incidence of salmonella obtained in this study is consistent with incidence rates reported by Felix *et al.* (2012) in well managed large scale farms. The finding of this work is also consistent with the low incidence of salmonella (in the range of 0.5 to less that 10%) in chicken and pigs reported from various locations (Molla *et al.,* 2003; Margaret *et al*., 2007; Akoachere *et al.,* 2009; Ishihara *et al* 2009; Viott *et al* 2013). On the contrary, high incidence of *Salmonella* in chickens taken from poultry farms (White *et al*., 2001; Mayrhofer *et al*., 2004; Zhao *et al*., 2006) and carcasses (Ojo *et al.,* 2011) have variously been reported. Understandably, the choice of carcasses in the work of Ojo *et al*. (2011) could have skewed the result since the cause of death may have been an outbreak of salmonella infection in the poultry. Previous studies have shown that *Salmonella* and *E. coli* were common causes of morbidity and mortality in poultry (Ogunleye *et al.,* 2008; Agbaje *et al.,* 2010). Abdoulaye (2012) reported a *Salmonella* prevalence of 15% in apparently healthy slaughtered adult local free range chickens in a study that showed a significant association between markets and infection. A significantly high association between free range chicken and *Salmonella* spp would again seem understandable, given the nature of exposure of such birds. Reports on *Salmonella* prevalence in chicken samples from supermarkets and butcheries in the United Arab Emirates revealed a total absence of *Salmonella* from those obtained from supermarkets while high rate of occurrence (100%) was obtained in butcheries. This contrast was attributed to the unsanitary and unhygienic conditions of these butcheries.

Various unit operations in a poultry farm and piggeries could either amplify or reduce bacterial contamination (Munawwar *et al.,* 2010). Contaminations by Salmonella are reported from poultry manure, workers, equipment, poultry environment and water (Okoli *et al.* 2006; Djim-adjim, 2013). The relative resistance to desiccation may justify the high probability of isolating *Salmonella* from litter. According to Davies and Breslin (2003) high contamination of caged flocks is due to failure to properly clean and disinfect the poultry house or to an insufficient sanitary control. Several studies suggest that measures to limit vertical and horizontal transmissions include ensuring Salmonella free feed and water, effective cleaning and disinfection of the farm and applying appropriate measures against animate and inanimate vectors (Humphery, 2008; Wales *et al.,* 2007). *Salmonella* contamination of poultry has been reported to be seasonal with higher prevalence in spring, summer and autumn than winter (Wallace *et al.,* 1997; Erol, 1999; CDC, 2001; Logue *et al*., 2003; Iseri and Enol 2010) suggesting a correlation between environmental temperature and *Salmonella* isolation rate, although in some other reports no seasonal effect on *Salmonella* prevalence are obvious (Wedderkopp *et al.,* 2001; Jordan *et al.,* 2006). The current study was unable to establish any seasonal effects given the low overall incidence even though sampling covered most of the wet season and all of the hot weather.

It is possible that the low incidence of *Salmonella* in poultry and pigs in this study is due to enhanced operational hygiene in the farms. This is to be expected given the level of farm hygiene that usually obtains in medium to large farms (that may retain animal health and production professionals) relative to the smaller cottage facilities. The availability of quality veterinary and extension services provided by the Veterinary Teaching Hospital and Extension Services as part of the university's community service could also have impacted the performance of these farms located in a university town. The result of this study is also consistent with the low incidence of salmonella reported in pigs by other workers (Molla *et al.,* 2003; Akoachere *et al.,* 2009; Ishihara *et al.,* 2009; Viott *et al*., 2013).

In comparing results of surveys however, several factors such as differences in origin, time, age of samples, sampling procedure, contamination level of animals, farm sanitation, cross contamination as well as differences in isolation methodology applied to detect the pathogen must be taken into consideration (Eriksson and Aspan, 2007). Reports by Olufemi and Adeyemi (2009) showed that placing specimen in Selenite F immediately after collection and holding for 24hours increased the rate of isolation; and that the use of XLD in addition to DCA enhanced chances of isolation. The use of MSRV (Modified Semisolid Rappaport Vasiliadis) agar showed high detection rate of 86.8%, followed by DIA (diagnostic Semisolid *Salmonella* Agar) and SMS (Simple Method *Salmonella* Agar) with detection rate of 85.3% then RV (Rappaport Vassiliadis) broth, RVS (Rappaport Vassiliadis Soya) broth and MKTTN (Mueller Kauffmann Tetrathionate Novobiocin) broth with detection rates of 58.8%, 54.4% and 50.0% respectively (Houf *et al.,* 2011). These discrepancies point to the need for standardization of detection and isolation procedures and media as essential for effective comparison of isolation data. It is important however, that reports of very high incidence/ isolation of salmonella from farm animals in non-disease outbreak situations be examined closely.

The extensive use of conventional antibiotics as growth promoters and for prophylaxis in intensive animal production has led to dramatic rise in antimicrobial resistance amongst domestic animal pathogens and zoonotic bacteria. This in turn led to a gradual shift to use of fluoroquinolones in animal production. As a result, there has been a gradual but steady selection of fluoroquinolone resistant pathogens in intensively reared animals and poultry (Alo and Ojo, 2007). The pattern of antibiotic resistance obtained in this work corroborates European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food (EFSA, 2010) which revealed that *Salmonella* from fowls, pigs, cattle and meat were commonly resistant to tetracyclines, showed low resistance to third generation cephalosporins and moderate to high level resistance to ciprofloxacin (a fluoroquinolone). High level resistance of various bacterial (salmonella and others) isolates from poultry to fluoroquinolone has also been reported in Nigeria. Ojo *et al.* (2011) in Nigeria reported that out of 200 isolates, 81 (40.5%) were resistant to ciprofloxacin, 42 (21.0%) to enrofloxacin, 19 (9.5%) to nalidixic acid and 88 (44.0%) to norfloxacin. High level resistance to quinolones by poultry (Chen *et al*., 2004; Agbaje *et al.,* 2010; Mine *et al*., 2010; Moon, 2011; Djim-ajim *et al*., 2013) and commensal (Ajayi *et al.,* 2010) isolates of *Salmonella* has also been reported. These have been attributed to extensive use of such antibiotics in intensive poultry and animal production (Alo and Ojo, 2007; Ogunleye *et al.,* 2008) which creates a selection pressure that favours the emergence of fluoroquinolone resistant bacteria (Philips *et al*., 2004; Goldmann and Kearns, 2011).

Importantly, since the modes of action of fluoroquinolones are similar, resistance to one may induce resistance to others. This was observed in 2 isolates from this study (AP 005a and VC 001). Both isolates were resistant to all 5 fluoroquinolones. Also, one isolate (LC 009) exhibited resistance to ceftazidime and ceftraxone and an intermediate resistance to cefotaxime; all 3 drugs belonging to third generation cephalosporins. Similar patterns of resistance have been reported for isolates from day old chicks, poultry meat, domestic animals, healthy cattle, goats and dogs (Olufemi and Adeyemi, 2009; Anyawu *et al.,* 2010; Munawwar *et al.,* 2010; Felix *et al*., 2012). On the other hand however, Zhao *et al*. (2006; 2008) reported that Salmonella isolates from retail foods of animal origin were resistant to tetracycline, streptomycin, ampicillin, sulfamethoxazole, cefoxitin and ceftiofur but susceptible to ciprofloxacin although 3% of isolates were resistant to nalidixic acid.

This resistance pattern has been attributed to the fact that fluoroquinolones are relatively new group of antibiotics and being quite expensive have not been abused in animal production compared to other drugs like tetracycline, ampicillin and chloramphenicol.

## CONCLUSION

Bacterial pathogens of human and animal origins are becoming increasingly resistant to most frontline antimicrobials including expanded spectrum cephalosporins, aminoglycosides and even fluoroquinolones. Antimicrobial resistance traits in bacteria are often resident within transmissible mobile elements which can be shared among bacteria (Lee *et al*., 2006). Exchange of resistance genetic material is particularly common among enteric bacteria. This contributes significantly to the persistence, spread and over all prevalence of antimicrobial resistance among bacteria within a community (Ojo *et al*., 2011). The lion's share of these antimicrobial resistant phenotypes is gained from extra chromosomal genes that may impart resistance to an entire antimicrobial class. Fluoroquinolones remain very potent antimicrobial agents against a wide range of pathogenic organisms, but its introduction into veterinary medicine has led to an increase in bacterial resistance. Newer and more potent fluoroquinolones are under development. These agents have improved activity against Gram positive organisms and some Gram negatives. Emergence of resistance to the newer fluoroquinolones will still be of concern. The challenge is to be better stewards in the use of these compounds. Caution should be exercised in the use of these antibiotics as growth promoters in intensive animal production in order to preserve their efficacy in treatment of diseases. To do otherwise will have serious implications for the treatment and prevention of infectious diseases in both humans and animals. The widespread acquisition of quinolone resistance may limit the prophylactic or sub-therapeutic use of fluoroquinolones and may severely compromise animal production and global food security.

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