



## Prevalence of Quinolone Resistance in *Salmonella* Isolated from Poultry Intestines

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### ABSTRACT

This study was designed to evaluate the prevalence of quinolone resistance in *Salmonella* spp. isolated from poultry intestines. A total of 25 poultry intestine samples were aseptically collected from poultry farms in Abakaliki metropolis using swab sticks and labeled accordingly. The samples were immediately taken to laboratory for microbiological analysis. Sample processing, isolation and identification of bacteria were carried out using standard procedures. The standard Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility patterns of the isolates and interpreted according to the guidelines of Clinical Laboratory Standards Institute. The results obtained revealed the presence of *Salmonella* species in poultry intestine. The result of antibiotic susceptibility patterns of the isolates showed that the highest sensitivity rate was associated with cefapime with 80% sensitivity to the *Salmonella* strains. Conversely, the highest resistance rate was associated with amoxicillin and ofloxacin both of which recorded 100% followed by levofloxacin, ciprofloxacin and norfloxacin all of which recorded 93.3%. Hence, the study has shown that *Salmonella* strains isolated from poultry intestine in Abakaliki harbours quinolone resistant genes.

### 1. INTRODUCTION

It has been established that poultry farming is very important in Nigeria because it contributes a lot towards the economic development of the country (Eriksson, 2012). For small-scale farmers across the African continent, poultry farming provides major source of income. Slaughtering of poultry continues to increase as a result of the increase in demand for meat and its products. This is because it provides us with proteins and serves as source of energy (Arogoet *al.*, 2001). Weeks et *al.* (2008) reported that poultry meat and its products contribute about “a third” of the energy that humans need. The poultry industry has made great strides in the last few decades in the country (Orji et *al.*, 2005). However, the industry is facing major problems such as lack of disease control programs mainly associated with poor handling of raw material from production to marketing. Increase in demand for meat without the infrastructure for proper sanitary handling may lead to transfer of pathogenic microorganisms from animals to the consumer (Aijazet *al.*, 2010). *Salmonella* is a rod-shaped, non-spore forming, gram Positivemotile enterobacter species salmonella is a flagellated organisms which grade in all directions (Fabrega and Vila, 2013). They are obtaining their energy from oxidation and reduction reactions using

organic sources, and are facultative anaerobes. *Salmonella* is closely related to the *E. coli* genus and are found worldwide in cold and warm-blooded animals in the environment. *Salmonella* are not destroyed by freezing (Jantschet *al.*, 2011). *Salmonella* is a major cause illnesses like typhoid fever and other food borne diseases (Fabrega and Vila, 2013).

According to the record of WHO over 16 million people worldwide are infected every year with typhoid fever (Cummings *et al.*, 2010). *Salmonella* can survive for weeks outside a living body and have been found in dried excrement after more than 2.5 years. *Salmonella* is the most frequently reported cause of food borne illness (Birhaneselassie and Williams, 2013). Most cases of food poisoning are caused by bacteria which arise from animal, human or environmental sources (Gastet *al.*, 2010). Contaminated meat is one of the main sources of food borne illnesses (Mershalet *al.*, 2010). Food borne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the intestines or edible organs (Alemayehuet *al.*, 2002). *Salmonella* infection in meat arises from intensive rearing practices and the use of contaminated feeds (Ejetaet *al.*, 2004). Food borne diseases remain a real and formidable problem in both developed and developing countries, causing great human suffering and significant economic losses. Moreover, an increase in the resistance of *Salmonella* to commonly used antimicrobials has been also noted in both public and private health sectors (Nandreet *al.*, 2014). Quinolones are widely used due to their broad-spectrum antimicrobial activity and strong antibacterial effects in both human and animal treatment of diseases (Li and Li, 2002). They are usually the antimicrobials of choice for treatment of severe or systemic human salmonellosis (Rotimiet *al.*, 2008). Quinolone use has led to increasingly resistant bacteria (Hooper, 2001), while the isolation rate of multiple serotype *Salmonella* strains resistant to quinolones and fluoroquinolones has increased every year (Malorny et *al.*, 1999). Taiwan, Japan, the Netherlands and the United States have all experienced outbreaks of *Salmonella* resistant to quinolones. The resistance mechanisms of *Salmonella* to the quinolones have become increasingly complicated and have drawn worldwide attention (Michael *et al.*, 2006). In order to quantify the risks associated with the harvesting of poultry animals that may harbour or shed *Salmonella*, estimates of the distribution and concentration of these pathogens in poultry intestine samples would be needed. The aim of this study was to determine the prevalence of quinolone resistance in *Salmonella* species isolated from poultry intestines within Abakiliki metropolis.

## 2. MATERIALS AND METHODS

**2.1** The study area of this research was Abakiliki metropolis. Abakiliki is the capital of Ebonyi State, Nigeria. This city is located in longitude 8°05'15"E. It has a population of 149,683. The inhabitants of this area are predominantly Igbo speaking people. The occupation of the people includes farming, trading and civil service. Abakiliki is a center of agricultural trade in such products as yams, cassava, rice, palm oil, palm kernels as well as livestock. It is also known for its local lead, zinc, salt, and limestone mining. Abakiliki is blessed with natural water sources like lakes, rivers and streams. This study is restricted to chicken samples bought from poultry farms within Abakiliki metropolis.

## 2.2 METHODOLOGY

### 2.2.1. Collection and processing of Samples

Samples of poultry intestine were collected from poultry farms in Abakiliki metropolis. A total of 25 samples were aseptically collected using swab sticks and labeled accordingly. The samples were transported immediately to Applied Microbiology Laboratory Unit of Ebonyi State University, Abakiliki

for microbiological analysis. The intestine samples were cultured in *Salmonella*- *Shigella* Agar Petri dishes according to the method described by Doyles (2008). Trypton broth, Mueller Hinton agar, Nutrient Agar and Shigella-Salmonella Agar used in this work were prepared according to the manufacturer's instructions. 0.5 McFarland turbidity standards was prepared and used to adjust and compare the turbidity of the test bacteria in order to obtain a confluent growth on culture plate when performing Susceptibility Testing (Cheesbrough, 2006). The bacteria isolate identification was based on Gram reaction, Colony morphology, cultural characteristics and biochemical tests such as Indole test, Catalase test and oxidase test as described by Cheesbrough (2006). Once the bacterium was isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates. Bacterial inoculum was prepared by suspending the freshly grown bacteria in 4–5 mL sterile nutrient broth and the turbidity adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium against Cefepime (FEP) 30µg, Amoxicillin/clavulanic acid (AMC) 30µg, Norfloxacin (NOR) 10µg, Levofloxacin (LEV) 5µg, Nalidixic acid (NA) 30 µg, Ciprofloxacin (CIP) 5 µg and Ofloxacin (OFX) 5µg. The plates were then incubated at 37°C for 18–24 h. This was carried out bearing in mind the diameter of the disc (6mm). Using a meter-rule on the underside of each plate, the inhibition zone diameter (IZD) of each antibiotic disc (marked by clear zones around each disk) was observed, measured and recorded after 18–24 h incubation. The diameter of the disk (6 mm) was subtracted from the total inhibition zone diameter of each disk and accurately recorded as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2006).

#### 4. RESULTS

**4.1:** Table 1 presents the morphological, microscopic and biochemical characteristics of *Salmonella* species isolated from poultry intestines. The data indicate the presence of *Salmonella* species.

**Table 1: Morphological, Microscopic and Biochemical Characteristics of the *Salmonella* species Isolated from Poultry Intestines**

|     |           |                               |        | Biochemical Tests |               |              |             |                   |
|-----|-----------|-------------------------------|--------|-------------------|---------------|--------------|-------------|-------------------|
|     |           | Morphological characteristics |        | Gram staining     | Catalase test | Oxidase test | Indole test |                   |
| S/N | Sample No | Shape                         | Colour |                   |               |              |             | Probable Organism |
| 1   | P1        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 2   | P2        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 3   | P3        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 4   | P4        | Rods                          | Black  | -                 | +             | -            | +           | Salmonella spp.   |
| 5   | P5        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 6   | P6        | Rods                          | Black  | -                 | +             | -            | +           | Salmonella spp.   |
| 7   | P7        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 8   | P8        | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 9   | P9        | Rods                          | Black  | -                 | +             | -            | +           | Salmonella spp.   |
| 10  | P10       | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 11  | P11       | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 12  | P12       | Rods                          | Black  | -                 | +             | -            | +           | Salmonella spp.   |
| 13  | P13       | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 14  | P14       | Rods                          | Black  | -                 | +             | -            | -           | Salmonella spp.   |
| 15  | P15       | Rods                          | Black  | -                 | +             | -            | +           | Salmonella spp.   |

Key: + = Positive  
- = Negative

#### 4.2: Frequency of bacterial isolation from poultry intestine

Table 2 below shows the frequency of bacterial isolation from the chicken intestine examiner. Out of the 25 samples collected, 15 (60 %) had salmonella strains while 40 percent were non salmonella. The results indicate that chicken intestine were contaminated with salmonella species which posse danger to both the chicken handlers, public and environment.

**Table 2: Frequency of bacterial isolation from poultry intestine**

| Sample Examined | Positive  | Negative  |
|-----------------|-----------|-----------|
| 25              | 15 (60 %) | 10 (40 %) |

#### 4.3: Inhibition zone diameter of *Salmonella* species Isolated from Poultry Intestines

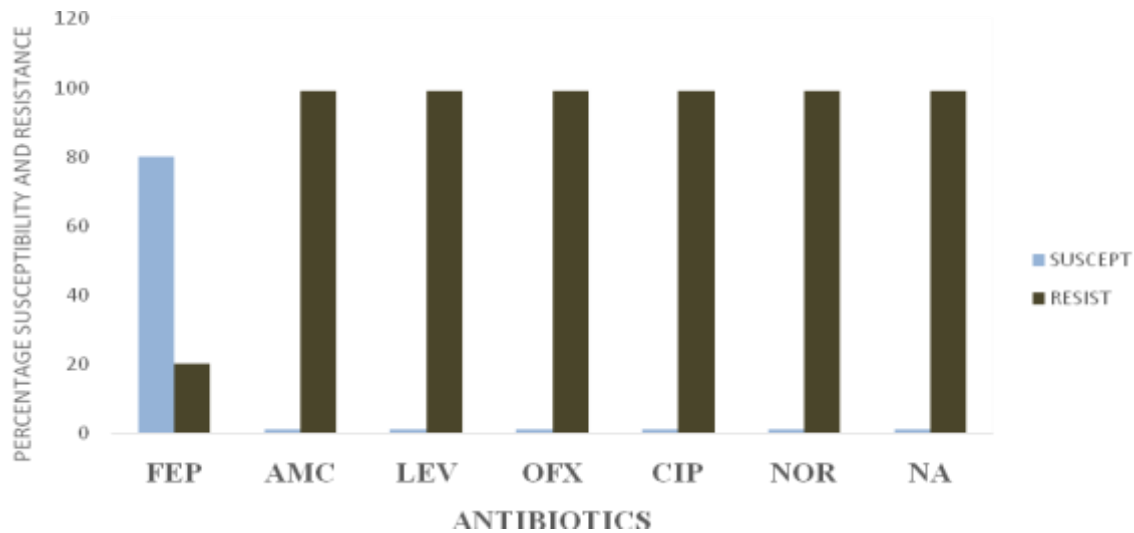
Table 3 shows the inhibition zone diameter of *Salmonella* species isolated from poultry intestines. It reveals varying levels of inhibition zones diameter as obtained from the result using Kirby-Bauer disk diffusion with little modification.

**Table 3: Inhibition zone diameter of *Salmonella* species Isolated from Poultry Intestines.**

| S/N | SAMPLES | FEP | AMC | LEV | OFX | CIP | NOR | NA |
|-----|---------|-----|-----|-----|-----|-----|-----|----|
| 1   | P1      | 30  | 7   | 11  | 6   | 6   | 6   | 6  |
| 2   | P2      | 26  | 6   | 9   | 6   | 6   | 6   | 6  |
| 3   | P3      | 29  | 6   | 16  | 10  | 6   | 6   | 6  |
| 4   | P4      | 18  | 6   | 6   | 6   | 6   | 6   | 6  |
| 5   | P5      | 31  | 6   | 6   | 6   | 8   | 6   | 6  |
| 6   | P6      | 27  | 7   | 12  | 9   | 9   | 6   | 6  |
| 7   | P7      | 22  | 6   | 13  | 10  | 6   | 6   | 6  |
| 8   | P8      | 17  | 6   | 8   | 6   | 6   | 6   | 6  |
| 9   | P9      | 31  | 6   | 9   | 6   | 6   | 6   | 6  |
| 10  | P10     | 25  | 6   | 10  | 6   | 6   | 6   | 6  |
| 11  | P11     | 29  | 6   | 20  | 12  | 18  | 14  | 6  |
| 12  | P12     | 21  | 6   | 9   | 6   | 10  | 6   | 6  |
| 13  | P13     | 19  | 6   | 13  | 6   | 9   | 6   | 6  |
| 14  | P14     | 19  | 7   | 12  | 6   | 9   | 6   | 6  |
| 15  | P15     | 18  | 8   | 10  | 7   | 6   | 6   | 6  |

Key: FEP = Cefapime, AMC = Amoxicillin/clavulanic acid, LEV = levofloxacin, OFX = ofloxacin, CIP = ciprofloxacin, NOR = norfloxacin, NA = nalidixic acid.

The percentage antibiotic susceptibility pattern of *Salmonella* species isolated from poultry intestine samples by disc diffusion method is shown in fig 1. It was indicated that out of 15 strains of *Salmonella* species tested with cefapime, 80 % were sensitive and 20 % resistant. Out of 15 strains of *Salmonella* species tested with levofloxacin, 1 % was sensitive and 99 % were resistant. Out of 15 strains of *Salmonella* species tested with ofloxacin, 1 % was sensitive and 99 % resistant. Out of 15 strains of *Salmonella* species tested with ciprofloxacin 1 % was sensitive and 99 % were resistant. Out of 15 strains of *Salmonella* species tested with norfloxacin, 1 % was sensitive and 99% resistant. Finally, out of 15 strains of *Salmonella* species tested with nalidixic acid, 1 % was sensitive and 99 % resistant.



**Fig 1: Antibiotics susceptibility pattern of *Salmonella* species isolated from Poultry intestine**

Key: FEP = Cefapime, AMC = Amoxicillin/clavulanic acid, LEV = levofloxacin, OFX = ofloxacin, CIP = ciprofloxacin, NOR = norfloxacin, NA = nalidixic acid.

## 5. Discussion

It is widely accepted that *Salmonella* contaminations in poultry and poultry products at various stage of production are one of the major factors leading to food borne illnesses in humans and animals (Laxmee and Hudaa, 2015). The global increase in the prevalence of *Salmonella* strains with reduced susceptibility to quinolones constitutes a major concern, since these pathogens have been associated with a significant burden of hospitalization and mortality and with clinical failures of therapy (Chiu *et al.*, 2004; Renukaet *al.*, 2005). In our study, salmonella was isolated and characterized using standard microbiological techniques as shown in table 1. Also, the frequency of salmonella isolation is high in this present study as revealed in Table 2. This observation is consistent with the study carried out by Laxmee and Hudaa (2015) who reported the isolation of *Salmonella* species from the gut, egg, intestine and litter of poultry. From Table 3, the highest sensitivity rate was associated with Cefepime which recorded 80 % sensitivity to the *Salmonella* strains. Conversely, the highest resistance rate was associated with amoxicillin and ofloxacin both of which recorded 100% followed by levofloxacin, ciprofloxacin and norfloxacin all of which recorded 93.3%. Hadaset *al.* (2007) reported that *Salmonella* strains showed 90% resistance to nalidixic acid, among *Salmonella* serovars in Israel. They opined that active efflux mechanisms resulting in a decreased quinolone accumulation may also contribute to the elevated resistance.

In a related study on the characterization of quinolone resistance in *Salmonella enterica* serovar Indiana from chickens, Yan *et al.* (2015) reported that the resistance of the *Salmonella* Indiana strains to nalidixic acid, levofloxacin, norfloxacin, and ciprofloxacin were 100, 73.1, 71.2 and 82.7% respectively. The trend is consistent with the result of the present study. The results obtained in this study are at variance with those found by Berchieriet *al.* (1987), who reported an antimicrobial susceptibility of 100%. In the same vein, the result of this study contradicts the study by Arvanitidouet *al.* (1998) who reported 1.6 % prevalence of resistance by salmonella isolated from faeces, water, scalding water,

chicken carcass and animal feeds. The differences in levels of resistance and susceptibilities between the results of the present study and those of other researchers may be explained when several factors such as differences in origin, time of sample collection, sample source and sampling procedure are taken into account (Bryan et al., 1995). In another related study (San et al., 2005), out of 94 *Salmonella* spp. strains isolated, 39 strains were resistant to flumequine, nalidixic acid, and oxolinic acid simultaneously, while only two strains were resistant to nalidixic acid and oxolinic acid. In the study all the strains were sensitive to ciprofloxacin and enrofloxacin. Some authors have observed an increase in quinolone resistance in *Salmonella* (Molbak et al., 2002). Quinolone resistance is chromosomally mediated thus allowing an increase of *Salmonella* quinolone-resistant in humans or animals. In Germany, the incidence of quinolone resistance between 1986 and 1998 in *Salmonellae* isolated from cattle, poultry and pigs increased in the period following the licensing of the drug (Malorny et al., 1999). One likely explanation for the high resistance to amoxicillin and ofloxacin could be because of their low cost, ready availability and ease of administration, rendering them more prone to misuse. The indiscriminate use of antimicrobials in livestock farming have resulted in increased resistance and these have been transmitted to humans via the food chain and thus have been the major cause of drug resistance in humans. Also, resistant strains not only hinder treatment, but are also found to cause more severe illnesses in humans (Holmberg et al., 1984).

This study has shown that *Salmonella* strains were present in the intestines of poultry animals in different poultry farms in Abakaliki metropolis. Analysis of the drug sensitivity results showed that the isolated *Salmonella* strains were generally resistant to several frequently used quinolones but were sensitive to Cefepime. It is necessary to closely monitor the hygienic practices prevailing in farms and the food production systems to reduce the risk of antibiotic-resistant bacteria in the food chain. Given the occurrence of quinolone resistance in *Salmonella* strains worldwide, the development of vigilance programs in veterinary medicine at the government level in Nigeria is recommended, in order to keep track of phenotypical and genotypical aspects of antimicrobial resistant strains. The levels of antibacterial resistance found in the present study imply that antibiotics must be used judiciously, based on previous resistance tests and on the determination of appropriate doses by minimal inhibitory concentration (MIC). These goals can be achieved by continuous training of medical professionals and the acquisition of the in-depth knowledge of the pharmacokinetics of antibiotics, and the microbiology of *Salmonella*.

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