



## Multiple antimicrobial resistance of *Escherichia coli* and *Salmonella* species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria

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### Abstract

Pathogenic *Escherichia coli* and *Salmonella* species are the causative agents of various disease complexes in poultry such as colibacillosis, fowl typhoid, pullorum disease and salmonellosis. Some strains of *E. coli* and *Salmonella* spp. have been shown to be resistant to multiple antibiotics. We carried out a bacteriological investigation on 105 cloacal swabs from local and broiler chickens retailed along the roadside in Hanwa, Zaria for the occurrence of *E. coli* and *Salmonella* spp. by isolation through culture, and identification using biochemical and serotyping techniques. Serotyping of *E. coli* isolates was carried out with polyvalent *E. coli* O157:H7 antisera while *Salmonella* spp. isolates were serotyped using polyvalent antisera specific for all groups and type-factor for *Salmonella* spp. Presumptive isolates were subjected to antimicrobial susceptibility testing using 13 panels of antibiotics for both *E. coli* and *Salmonella* spp. Results showed that the overall isolation rate of *Salmonella* spp. was 12 (11.4%), broiler chickens had higher isolation rate 9 (12.0%) of *Salmonella* than local chickens. However, the isolation rate of *E. coli* from local chickens 15 (50.0%) was higher compared to broilers 6 (8.0%). Also, non-*E. coli* O157:H7 and *E. coli* O157:H7 were isolated from both broilers and local chickens at a frequency of 15 (14.3%) and 6 (5.7%) respectively. The overall isolation rate of *E. coli* was 21 (20.0%). Multiple antibiotic resistance was observed among local and broiler chickens. However, *E. coli* and *Salmonella* species were 100% susceptible to Enrofloxacin. We therefore concluded that *E. coli* and *Salmonella* species are prevalent in the cloacae of both broiler and local chickens retailed along the roadside in Hanwa, Zaria, Nigeria and could pose serious veterinary and public health risks.

**Keywords:** *Escherichia coli*, Isolation, *Salmonella*, Serotype, Zaria

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### Introduction

Broiler and local chickens serve as the major sources of poultry meat in Nigeria with consumption occurring independently of religious and ethnic backgrounds (Omodele & Okere, 2014). Broiler chickens (*Gallus gallus domesticus*) are gallinaceous domesticated fowl, bred and raised mainly for meat production while local or indigenous chickens are

free range gallinaceous fowl which are generally hardy and have the ability to adapt to adverse weather conditions and fluctuations in feed availability (Mwalusanya *et al.*, 2002; Ajayi, 2010). Broilers reach maturity in terms of size and weight faster than local chickens. Poultry production, especially broiler and local chickens, accounts for a

high percentage of quality protein and in addition serves as a source of revenue for farmers and traders in Nigeria (NAERLS, 2000; Sonaiya & Swan, 2004; Emaikwu *et al.*, 2011). However, chicken production is faced with a number of constraints. Notable among these are *Escherichia coli* and *Salmonella* species infections which can reduce overall production (Huang *et al.*, 2009). Moreover, strains of these bacteria are also zoonotic and have been described as the leading causes of food-borne illness worldwide (Panisello *et al.*, 2000; Ewers *et al.*, 2009).

*Escherichia coli* and *Salmonella* spp. are Gram negative bacteria belonging to the family *Enterobacteriaceae*. *Escherichia coli* are found in the environment, intestinal tracts of humans and animals. Most *E. coli* strains are harmless and are an important part of healthy human and animal intestinal tracts. However, some strains of *E. coli* have acquired virulence attributes and are called pathogenic *E. coli* (Hessain *et al.*, 2013; Javadi *et al.*, 2016). Pathogenic *E. coli* are categorized into six pathotypes (Raji *et al.*, 2007). Avian pathogenic *E. coli* is responsible for a disease syndrome referred to as Colibacillosis in chickens. Colibacillosis occurs as an acute fatal septicemia, fibrinous lesions (airsacculitis, peritonitis and pericarditis) salpingitis and swollen head syndrome (Raji *et al.*, 2007; Messai *et al.*, 2013).

On the other hand, *Salmonella* spp., especially *Salmonella pullorum* and *Salmonella gallinarum* are responsible for systemic acute and chronic diseases of chicks and matured birds respectively. Salmonellosis and colibacillosis account for high economic losses from decreased egg production, mortality, morbidity, decreased feed conversion, decreased carcass weight, carcass condemnation and costs incurred from prevention, control and treatment (Messai *et al.*, 2013). Infection is generally enhanced by predisposing factors, such as stress, mycoplasma or viral infections, and adverse environmental conditions (Gomis *et al.*, 2001; Bopp *et al.*, 2005).

Studies in Nigeria have reported the isolation of *E. coli* and *Salmonella* spp. from broiler and local chickens in live bird markets and farms: Raji *et al.* (2007) reported, respective isolation rates of 4.7% and 7.5% of *E. coli* from hatcheries and farms in Zaria, Kaduna state of Nigeria. Adeyanju & Ishola (2014) reported prevalences of 33.0% and 43.4% for *Salmonella* spp. and *E. coli* respectively when isolating from retailed poultry in Oyo state, Nigeria. As these studies were conducted several years apart

and in different states of Nigeria, it is unclear if the disparity in these prevalences is due to geography or time as there is paucity of recent information on the isolation of *E. coli* and *Salmonella* from broilers and local chicken in Zaria, Nigeria.

The extensive use of antibiotics in poultry as growth promoters and most importantly for the control and treatment of diseases have been attributed as the cause of the emergence of bacteria with multidrug resistance associated with poultry. The emergence of resistance has the potential to impact on the treatment and management of infectious diseases in both animals and humans (Mamza *et al.*, 2010).

The aim of this study was to carry out isolation and antibiotic susceptibility testing of *E. coli* and *Salmonella* spp. from retailed broiler and local chickens along the roadside in Hanwa, Zaria metropolis and to determine if one source of chickens was potentially safer than the other.

## Materials and Methods

### Sample collection

A total of 105 cloaca samples were collected from broilers and local chickens sold in Hanwa, Zaria Metropolis. Samples were collected in duplicate, labelled and placed in sample bags and then transported on an ice pack to the Bacteria Zoonoses Laboratory, Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. Samples were processed within one hour of collection.

### Isolation of *E. coli* from cloacal sample

Cloacal swabs from broilers and local chickens were inoculated into buffered peptone water (Oxoid, Hampshire, UK) and incubated at 37°C for 18 – 24 hours. A loop full from the inoculated peptone water culture was transferred onto MacConkey agar (Oxoid, Hampshire, UK) plates and incubated at 37°C for 24 hours. Colonies typical of lactose fermenter (pinkish colonies) were harvested and sub-cultured on EMBA (Oxoid, Hampshire, UK) at 37°C for 24 hours. Presumptive identification of *E. coli* was based on colonial morphology and cultural appearance (greenish metallic sheen). Following identification, the colonies were sub-cultured on nutrient agar slant and stored at 4°C for further study.

### Isolation of *Salmonella* spp. from cloacal sample

Pre-enrichment was carried out by heating buffered peptone water at 37°C before inoculation with test sample (cloacal swabs), after inoculation it was incubated at 37°C for 24 hours (ISO 6579, 2002).

Enrichment was performed by inoculating a loop full from the buffered peptone water culture to transfer into Selenite "F" broth (Oxoid, Hampshire, UK) and incubation at 37°C for 24 hours. From the culture obtained in the enrichment, a loop full was taken using sterile wire loop and inoculated on to Xylose lysine deoxycholate agar (XLDA) (Oxoid, Hampshire, UK) and incubated at 37°C for 24 hours.

Presumptive identification of *Salmonella* spp. was based on colonial morphology and culture appearance.

#### Biochemical identification of *E. coli* and *Salmonella*

Catalase test, sugar fermentation using TSI (LAB M, Heywood/Lancashire, UK), mannitol test, hydrogen sulphide test, motility test, methyl red, urea utilization test, indole test, Voges Proskauer (VP) and Gram staining were carried out for both *Salmonella* spp. and *E. coli* (Raji *et al.*, 2007; Adeyanju & Ishola, 2014).

#### Serotyping of *Salmonella* and *E. coli* isolates

Serotyping of the obtained *Salmonella* isolates was carried out by means of a commercially available polyvalent *Salmonella* antisera kit (Denka Seiken Co. Ltd. Tokyo, Japan) specific for all group and type-factor *Salmonella* antigens. A loop full from *Salmonella* isolates was emulsified with one drop of normal saline (0.85% NaCl) on a microscope glass slide. The preparation was gently stirred and observed for auto-agglutination. If there was no self-agglutination, a drop of the polyvalent antisera was added and gently agitated by rocking back and forth for about three minutes and observed for agglutination. Those that showed agglutination were considered to belong to the genus *Salmonella*.

Polyvalent *E. coli* O157:H7 antisera kit was used for serotyping of *E. coli* on a microscope glass slide. A loop full of *E. coli* isolates was emulsified with a drop of normal saline followed by a drop of *E. coli* O157:H7 polyvalent antisera. It was rocked gently for

about 3 minutes and observed for agglutination. Those that showed agglutination were considered as *E. coli* O157:H7.

#### Antimicrobial susceptibility testing

Antibiotic sensitivity was determined by disc diffusion method (Bauer *et al.*, 1966) on solid Mueller-Hinton medium according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010).

Susceptibility was tested against the following antibiotics discs: ampicillin (AM 10 µg), amoxicillin (AML, 25 µg), chloramphenicol (C, 30 µg), streptomycin (S, 10 µg), neomycin (N, 30 µg), enrofloxacin (ENR, 5 µg), trimethoprim/sulfamethoxazole (CO, 25 µg), norfloxacin (NOR, 10 µg), cefuroxime (CXM, 30 µg), ceftazidime (CAZ, 10 µg), ofloxacin (OX, 1 µg), erythromycin (E, 15 µg) and tetracycline (TE, 30 µg). Commercial antibiotic disks were purchased from Oxoid, UK.

The plates were incubated for 24 h at 37°C and the diameters of inhibition zones were measured in millimetre and interpreted by referring to the reading table of Enterobacteria as recommended by CLSI (2010).

#### Results

##### Isolation of *E. coli* and *Salmonella* from retailed chickens at Hanwa, Zaria

The total isolation rate of *Salmonella* species from local and broiler chickens sold along the roadside in Hanwa, Zaria was 12 (11.4%). Local chickens had 3(10.0%) while broilers had 9 (12.0%). The overall isolation rate of *E. coli* was 20.0% (21/105) of which *E. coli* O157:H7 was 6 (5.7%) and *E. coli* non O157:H7 was 15 (14.3%). Out of the 30 local chickens sampled 12 (40%) had *E. coli* non O157:H7 and 4.0% had *E. coli* O157: H7. Also, out of the 75 broiler chickens sampled, 3 (4.0%) each had *E. coli* non O157:H7 and *E. coli* O157:H7 (Table 1).

**Table 1:** Isolation of *Salmonella* species and *Escherichia coli* from broilers and local chickens.

S/No	Chicken type	Number	<i>Salmonella</i> spp.	<i>E. coli</i>	<i>E. coli</i> non O157:H7	<i>E. coli</i> O157:H7
1	Local	30 (28.6)	3 (10.0)	15 (50.0)	12 (40.0)	3 (10.0)
2	Broilers	75 (71.4)	9 (12.0)	6 (8.0)	3 (4.0)	3 (4.0)
	Total	105 (100.0)	12 (11.4)	21 (20.0)	15 (14.3)	6 (5.7)

the number of isolates from each bacterial class and selected strains are shown with the percentage (%) in parenthesis

**Table 2:** Antimicrobial susceptibility pattern for the *Escherichia coli* isolates (n = 21) from this study

Antibiotics ( $\mu$ g)	Sensitive (%)	Intermediate (%)	Resistance (%)
Sulphamethoxazole/ Trimethoprim, RL (25)	0 (0)	3 (14.3)	18 (85.7)
Ampicillin, AMP (10)	0 (0)	0 (0)	21 (100)
Enrofloxacin, ENR (5)	18 (85.7)	3 (14.3)	0 (0)
Norfloxacin, NOR (10)	6 (28.6)	9 (42.9)	6 (28.6)
Neomycin, N (30)	9 (42.9)	3 (14.3)	9 (42.9)
Cefuroxime, CXM (30)	0 (0)	0 (0)	21 (100)
Erythromycin, E (15)	0 (0)	3 (14.3)	18 (85.7)
Streptomycin, S (10)	3 (14.3)	3 (14.3)	15 (71.4)
Tetreacyclin, TET (30)	6 (28.6)	3 (14.3)	12 (57.1)
Ceftaxidime, CAZ (10)	0 (0)	0 (0)	21 (100)
Oxacillin, OX (1)	0 (0)	0 (0)	21 (100)
Chloramphenicol, C (30)	9 (42.9)	6 (28.6)	6 (28.6)
Amoxicillin, (25)	3 (14.3)	6 (28.6)	9 (42.9)

**Table 3:** Antimicrobial susceptibility pattern for *Salmonella* spp. (n = 12)

Antibiotics ( $\mu$ g)	Sensitive (%)	Intermediate (%)	Resistance (%)
Sulphamethoxazole/ Trimethoprim, RL (25)	0 (0)	0 (0)	12 (100)
Ampicillin, AMP (10)	0 (0)	0 (0)	12 (100)
Enrofloxacin, ENR (5)	9 (75)	3 (25)	0 (0)
Neomycin, N (30)	6 (50)	3 (25)	3 (25)
Cefuroxime, CXM (30)	0 (0)	0 (0)	12 (100)
Erythromycin, E (15)	0 (0)	6 (50)	6 (50)
Streptomycin, S (10)	6 (50)	3 (25)	3 (25)
Tetracycline, TET (30)	0 (0)	3 (25)	9 (75)
Ceftaxidime, CAZ (10)	0 (0)	0 (0)	12 (100)
Oxacillin, OX (1)	0 (0)	0 (0)	12 (100)
Chloramphenicol, C (15)	0 (0)	0 (0)	12 (100)
Amoxicillin, AML	3 (25)	3 (25)	6 (50)

#### *Antimicrobial susceptibility pattern of E. coli and Salmonella spp. isolated from local and broiler chicken*

The highest levels of resistance by *E. coli* were against Ampicillin (100%), Cefuroxime (100%), Ceftaxidime (100%), Oxacillin (100%), Erythromycin (85.7%) and Sulphamethoxazole-trimethoprim (85.7%). Low levels of resistance of *E. coli* were recorded for Norfloxacin (28.6%) and Chloramphenicol (28.6%) (Table 2). While all *E. coli* isolates were sensitive to Enrofloxacin (Table 2).

Antimicrobial susceptibility pattern for *Salmonella* spp. showed highest resistance against sulphamethoxazole-trimethoprim (100%), ampicillin (100%), cefuroxime (100%), ceftazidime (100%), oxacillin (100%) and chloramphenicol (100%). Highest level of susceptibility of *Salmonella* were to enrofloxacin (100%), neomycin (75.0%) and streptomycin (75.0%) (Table 3). Multiple resistances were observed in all of the isolates.

#### **Discussion**

In this study, the cloacal carriage of *E. coli* and *Salmonella* spp. were investigated amongst local and broiler chickens sold along the roadside in Hanwa, Zaria. We observed a higher isolation rate (50.0%) of *E. coli* from local chickens than from broilers (8.00%). A possible explanation for this difference may be due to the increased use of antibiotics for treatment and as growth promoter in broiler chickens (White *et al.*, 2001; Nwankwo *et al.*, 2014). The high occurrence of *E. coli* in local chickens in Hanwa, Zaria may be due to the fact that local chickens in Nigeria are commonly reared under free range conditions with minimal care, as chickens scavenge for food and water (Geidam *et al.*, 2012); hence are exposed to environmental contaminants and bacterial infections. Amadi *et al.* (2015) further explained that local chickens in Nigeria are neither vaccinated nor given any antibiotic medication and their sources of feed include food remains, grasses, maggots from cow dungs and other environmental waste which

may expose them to pathogenic bacteria including *E. coli*.

Of particular importance was the isolation of *E. coli* O157:H7 from both local and broiler chickens. *E. coli* O157:H7 is also known as verotoxigenic *E. coli* (VTEC). It was first recognised as a pathogen in 1983 during an investigation into an outbreak of haemorrhagic colitis associated with consumption of hamburgers in a restaurant (Riley *et al.*, 1983).

The isolation of *E. coli* O157:H7 was consistent with the report of Heuvelink *et al.* (1999) who reported the isolation of *E. coli* O157:H7 from poultry and characterised verotoxin – producing *E. coli*. Doyle & Schoeni (1987) also isolated *E. coli* O157:H7 from retailed poultry.

Pathogenic *E. coli* such as *E. coli* O157:H7 isolated from cloacal swabs of chickens sold along the roadside in Hanwa, Zaria may serve as sources of infection to the public and farmers who use poultry droppings as fertilizer. Others at risk of infection include people who consume raw vegetables, because wastes from poultry farms are used for irrigation and fertilizer in vegetable farms in Nigeria. Pathogenic *E. coli* from the gut could contaminate chicken meat and this may constitute serious public health concern.

*Salmonella* spp. are regarded as important causes of foodborne infections worldwide (CDC, 2016). In this study, we observed that *Salmonella* spp. were isolated from both local and broiler chickens retailed along the roadside at Hanwa, Zaria. The isolation rate was 10.0% from local chickens and 12.0% from broilers, suggesting there was no difference in the isolation rates from differentially sourced chickens in this study. Our findings were similar to the report of Rodriguez *et al.* (2015) who reported a prevalence of 17.41% of *Salmonella* spp. in broilers at Ibaguè, Colombia, and of Al-Khayat & Khammas (2016) who reported a prevalence of 10.4% of *Salmonella* from layers and broilers in Baghdad. However, Yhiler & Bassey (2015) reported an isolation rate of 59.1% of *Salmonella* from poultry in Calabar, Nigeria.

The isolation rate of *Salmonella* spp. obtained in this study was lower compared with the result of Yhiler & Bassey (2015), who reported an isolation rate of 59.1% of *Salmonella* spp. from cloacal swab of poultry in Calabar Cross River state, Nigeria. Also Umeh & Enwuru (2014) reported 52.5% isolation rate of *Salmonella* spp. from chicken faecal samples in Owerri metropolis Imo state, Nigeria. However, Garcia *et al.* (2011) reported a lower isolation rate of *Salmonella* from laying hens in Aquidauana, Brazil. The results obtained in this study were consistent

with the reports of other studies carried out in other parts of the world (Akond *et al.*, 2012).

It was observed in this study that multiple antibiotic resistance was common among *E. coli* and *Salmonella* spp. These results are in agreement with previous reports in Nigeria (Raji *et al.*, 2007; Olonitola *et al.*, 2015). There was complete resistance of *E. coli* and *Salmonella* to ampicillin, oxacillin, cefuroxime, ceftazidime, erythromycin and sulphamethoxazole/trimethoprim. This level of resistance to multiple antibiotics may be due to excessive and uncontrolled use of antibiotics in poultry (Velge *et al.*, 2005; Nsofor *et al.*, 2013; Adeyanju & Ishola, 2014). Salihu *et al.* (2014) further explained that the excessive use of antibiotics in poultry results from being freely available and readily affordable. The resistance observed in *E. coli* and *Salmonella* spp. isolated from local chickens has been observed to have resulted from the transfer of resistance gene(s) from another host in the same production environment (Salihu *et al.*, 2014).

It was observed in this study that *Salmonella* spp. isolates were 100% resistant to chloramphenicol. However, only 28% of *E. coli* were resistant to this drug, this variation in resistance of *Salmonella* and *E. coli* from the same environment may be due to genetic variation between the two pathogens as they are of different genera and other factors not considered in this study.

The high susceptibility of *E. coli* to chloramphenicol in this study was not in agreement with earlier study in Zaria (Raji *et al.*, 2007) and may have resulted from an increase in the use of the drug in poultry production in recent time. Chloramphenicol is widely used for the treatment of salmonellosis in both veterinary and human medicine over a long period of time and this could be responsible for the high prevalence of resistance of *Salmonella* observed in this study. It was explained that resistance of *Salmonella* to chloramphenicol may have resulted from selection of chloramphenicol-resistant strains (Cannon *et al.*, 1990; Nogrady *et al.*, 2005).

Our findings demonstrated high susceptibility of *Salmonella* spp. and *E. coli* to enrofloxacin and these data agree with Scur *et al.* (2014) who reported the occurrence and antimicrobial resistance of *Salmonella* serotypes isolates recovered from poultry of Western Parana, Brazil. Tebiyan *et al.* (2014) also reported low resistance of *E. coli* isolated from chickens in Iran. The high susceptibility of both *E. coli* and *Salmonella* to the quinolones observed in this study is of particular important since quinolones especially enrofloxacin are commonly used for

treatment and chemoprophylaxis of fowl typhoid and other bacterial diseases in Nigeria. Quinolones are widely used in veterinary medicine since their introduction in the late 1980s and early 1990s. This class of antibiotics offers the advantage of oral administration, high potency against Gram negative organisms and low toxicity (Lee *et al.*, 2004). However, Manic *et al.* (2016) observed that enrofloxacin is not an effective drug in the treatment of salmonellosis of birds since *Salmonella* colonize the intestine after stoppage of the drug. It was also reported that the use of enrofloxacin can induce resistance in *Campylobacter* species (Velhner *et al.*, 2013).

Antibiotic resistance is a global problem in both human and veterinary medicine, and varieties of factors have been identified as the cause of bacteria resistance. Van den Bogaard *et al.* (2001) explained that usage of antibiotics was the most significant factor responsible for antimicrobial resistance in bacteria. This view may also explain the reason for emerging antibiotic resistance in Nigeria, since antibiotics are used excessively in poultry and livestock in Nigeria without regulation (Raji *et al.*, 2007; Geidam *et al.*, 2012; Olonitola *et al.*, 2015). Van den Bogaard *et al.* (2001) further observed that other factors that select for antimicrobial resistance in bacteria include overcrowding and poor sanitation.

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Important findings in the current research were the occurrence of *E. coli* O157:H7 in both local and broiler chickens sold along the roadside in Hanwa, Zaria since *E. coli* O157:H7 is pathogenic and can cause serious public health concern that can result in haemorrhagic colitis, kidney failure and sometimes death. Another important observation is the occurrence of susceptibility of *E. coli* and *Salmonella* spp. to the quinolones especially Enrofloxacin. Enrofloxacin is widely used in poultry for the treatment of fowl typhoid and other diseases of bacterial origin. We also discovered that *Salmonella* spp. are less commonly isolated from local chickens than from broilers.

In conclusion, *E. coli* and *Salmonella* spp. pose serious public health and economic risks and have been isolated from both local and broiler chickens retailed along the roadside in Hanwa, Zaria with multiple antibiotic resistance. *Escherichia coli* and *Salmonella* spp. were observed to be susceptible to enrofloxacin. Indiscriminate use of antimicrobials has led to multidrug resistant strains of these bacteria. There is a need to improve the stewardship of the use of enrofloxacin to avoid the emergence of resistance. Importantly, people purchasing either broiler or local chickens should ensure correct handling, preparation and cooking as there is a considerable risk of multidrug resistant bacteria being present from both sources, some of which could cause severe disease.

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