



Multidrug resistant *Salmonella* sp isolated from chicken

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Abstract

Antibiotic resistance has been a major problem in combatting *Salmonella* in poultry, this research was designed to determine the antibiotic resistance level of *Salmonella* isolated from freshly dead chicken. A total of 107 freshly dead chicken were collected from 23 different farms, birds were necropsied, liver and trachea were collected, *Salmonella* were recovered from the samples using peptone water, Rappaport-Vassiliadis R10 Broth and *Salmonella-Shigella* agar. Pure culture were identified using cultural, morphological and biochemical characteristics. The pure isolates were subjected to antibiotic test using disc diffusion method. Sixteen isolates of *Salmonella* were recovered, 3 of which were from the trachea while 13 were recovered from the liver. All of the *Salmonella* isolates were resistant to Amoxicillin 100% while Nitrofurantoin was least resisted with only 37.5% of the *Salmonella* isolates showing resistance. The antibiotic resistant pattern often observed in this study were AMP, AUG, TLY with 75% (12); AMP, AUG, TLY, CPR, ENR, 50% (8); while 6.3% (1) was resistant to all of the antibiotics tested. This result showed that there is an emergence of multi-resistance *Salmonella* in poultry, therefore it is important to carry out sensitivity test before administration of antibiotics in order to control poultry salmonellosis.

Keywords: *Salmonella*; Poultry Feed; Resistance; Salmonellosis.

1. Introduction

In animal production systems, antibiotics are used for both therapeutic and non-therapeutic purposes. Non-therapeutically, antibiotics are used as growth promoters in livestock and poultry (Johnson, 2001; McEwen and Fedorka-Cray, 2002). Antibiotics are also used to improve the general hygiene in barns. This non-therapeutic use of antibiotics in feed may lead to increased levels of antibiotic resistance in both the pathogens and fecal micro flora of poultry (Witte 2000; Van-Veen *et al.*, 2001).

The development of resistance in poultry pathogens may undermine the efficacy and utility of antibiotics used to control these infections. Infections of domestic poultry with *Salmonella* are expensive both for the poultry industry and for society as a whole (Amand *et al.*, 2013). Some *Salmonella* serovars can affect multiple host species and it makes a serious problem according to the food chain (Jung *et al.*, 2011). There is evidence that eggs and poultry meat are two of the most important sources of *Salmonella* associated with human infection (Arsenault *et al.*, 2007).

Salmonella species isolated from clinical and environmental sources has shown an increased resistance to antibiotics since it has developed a number of elaborate mechanisms for acquiring and disseminating plasmids, transposons, phages, and other genetic determinants (Harts and Kaariuki, 1998). Therefore, this study focuses on the resistance of *Salmonella* species isolated from freshly dead chicken.

2. Materials and methods

2.1. Sample collection

One hundred and seven (107) freshly dead chicken were collected from 23 different farms in Ondo and Ekiti State (South Western,

Nigeria) between January and June 2015. The samples were conveyed to the laboratory for analysis within 2 hours of collection.

2.2. Bacteriology

The Freshly dead chicken were necropsied, the liver and trachea were collected. Swabs were collected aseptically from the trachea and the liver for bacteria isolation. The swabs collected was activated in buffered peptone water for 1 hour at 37°C. A loop full of the activated organisms in the buffered peptone water was inoculated into a selective enrichment medium Rappaport-Vassiliadis R10 Broth. Thereafter, a loop full of the activated organism in the Rappaport-Vassiliadis broth was inoculated on *Salmonella-Shigella* agar and nutrient agar (Biomark) and incubated for 24 hours at 37°C temperature in an incubator (Royalcare England. DNP 9022A) (Ramya *et al.*, 2012). Further confirmation of *Salmonella* was done by Gram reaction, motility, catalase, oxidase, nitrate, urease, indole, methyl red, Voges-Proskauer, and citrate tests (Atere *et al.*, 2015a).

2.3. Antibiotic Susceptibility test

In vitro susceptibility of the identified *Salmonella* isolates against antimicrobial agents was determined by the standard disk diffusion procedure. The organisms were standardized using McFarland standard at the absorbance of 450nm. The samples were inoculated on Muller-Hinton agar. The following antimicrobial agents were tested: Ceftazidime (CAZ 30µg), Cefuroxime (CRX 30µg), Gentamicin (GEN 10µg), Ciprofloxacin (CPR 5µg), Ofloxacin (OFL 5µg), Nitrofurantoin (NIT 300µg), Ampicillin (AMP 10µg), Amoxicillin/Clavulinate (AUG 30µg), Enrofloxacin (ENR 10µg), Furasol (FUR 10µg), Tylosin (TLY 10µg) and Doxycycline (DOX 10µg). Following the application of antimicrobial discs, the plates were incubated at 37 °C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition

were measured (millimetres) and were compared to internationally accepted standard to determine the susceptibility or resistance of the isolate (Atere *et al.*, 2015a).

3. Results

Salmonella was recovered from 12 of the 23 farms. A total of 16 *Salmonella* isolates were recovered, 3 of the isolates was isolated from the trachea while 13 were from the liver of the birds. The cultural characteristics of the isolates were small round pale convex colony on nutrient agar and black spot colony on *Salmonella-Shigella* agar. They are gram negative rods, catalase positive,

methylred positive and are motile. The isolates were negative to indole, citrate, oxidase, urease and voges-proskauer.

The antibiogram of the *Salmonella* isolates showed that all showed 100% resistance to Amoxicillin while Nitrofurantoin was least resisted with only 37.5% of the *Salmonella* isolates showing resistance (Table 1). All the isolates of *Salmonella* recovered in this study were resistant to at least four antibiotic. Only one of the isolates showed resistance to only 4 antibiotics while all others were resistant to between 5 and 12 antibiotics. One of the *Salmonella* isolates showed resistance to all the antibiotics used in this research (Table 2). The pattern of antibiotic resistance showed that 75% of the isolates had resistance pattern AMP, AUG, TLY while 50% had AMP, AUG, TLY, CPR, ENR pattern.

Table 1: Percentage (%) Antibiotic Resistant of *Salmonella* Sp. Isolated from Chicken

Isolates	AMP	AUG	OFL	TLY	CPR	ENR	DOX	FUR	GEN	NIT	CAZ	CRX
Trachea (n=3)	100 (3)	100 (3)	33.3 (1)	100 (3)	100 (3)	33.3 (1)	66.7 (2)	66.7 (2)	33.3 (1)	33.3 (1)	100 (3)	66.7 (2)
Liver (n=13)	92.3 (12)	100 (13)	76.9 (10)	76.9 (10)	61.5 (8)	92.3 (12)	92.3 (12)	61.5 (8)	84.6 (11)	38.5 (5)	53.8 (7)	53.8 (7)
Total n=16	93.8 (15)	100 (16)	68.8 (11)	81.2 (13)	68.8 (11)	81.2 (13)	87.5 (14)	62.5 (10)	75.0 (12)	37.5 (6)	62.5 (10)	56.3 (9)

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Ceftazidime (CAZ) and Cefuroxime (CRX).

Table 2: Pattern of Antibiotic Resistance in *Salmonella* Isolates

Antibiotics	No of Isolates	Percentage %
AMP, AUG, TLY	12	75.0
CAZ, CRX, GEN	6	37.5
NIT, OFL, GEN	4	25.0
DOX, FUR, ENR, TLY	5	31.3
AMP, AUG, TLY, CPR, ENR	8	50.0
FUR, ENR, CPR, TLY	4	25.0
GEN, CRX, NIT, CAZ	3	18.8
AMP, AUG, OFL, TLY, CPR, ENR, NIT, GEN, CRX, DOX, CAZ, FUR	1	6.3

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Ceftazidime (CAZ) and Cefuroxime (CRX).

4. Discussion

Salmonellosis is an infectious disease of humans and animals caused by *Salmonella* sp a facultative intracellular pathogen causing localized or systemic infections, in addition to a chronic asymptomatic carrier state. Infection of *Salmonella* in poultry is often referred to as fowl typhoid, which affect birds of all ages (Ramya *et al.*, 2012). *Salmonella* is most found in the gastro intestinal tract of birds, however, because of their invasive mechanism, they get their way into tissues of the host. In infected birds, the liver serves as the resting place for the organism (Johnston, 2001). Since *Salmonella* infection could be systemic, isolating *Salmonella* from the trachea of the birds is possible. In previous reports *Salmonella* had been isolated from the tissue, lungs, liver gastro-intestinal tract of the infected birds (Hernandez *et al.*, 2005).

Salmonella in poultry is traceable to the poultry feeds and water, as well as the hygiene of the poultry (environmental factor) and sometimes from humans (Roy *et al.*, 2006). Atere *et al.* (2015a) reported isolating *Salmonella* from the poultry feeds, this is an indication that the type of feed or the hygiene of the feeds can have a great effect on the microbial contamination of the poultry which can later transfer an effect of economic importance on the farmer.

The antibiotic susceptibility of the isolates of *Salmonella* in this research showed multiple resistance where they resist at least four antibiotics. In other research where samples are collected from poultry, the resistance is not as high as observed in this study. Nchawa and Bassey (2015) reported the antibiotic susceptibility of *Salmonella* isolates in poultry where it was found that 72.9% resisted AMP, AUG, 29.4% and CAZ 19.5%, this result is lower to what was observed for this study. Oluyeye and Oyinloye (2013) also reported the antibiotic resistance of *Salmonella* isolated from poultry as AUG 73.5%, OFL 20.9% and CPR 35.3% which is also lower compare to what was observed in this research. The high level of resistance observed in this research may have been as a

result of the type of sample collected and the effect of misuse of antibiotics by the farmer. Another possible reason for the multiple resistance of *Salmonella* could be attributed to the proliferation of fake or sub-standard drug in Nigeria (Dashe *et al.*, 2013). The high sensitivity of the isolated organisms to Nitrofurantoin, could be related to less frequent usage of this drug for therapeutic purposes, therefore reducing the chance of resistance to develop (Mohammad *et al.*, 2012).

The major factors responsible for antimicrobial resistance in bacteria is misuse of antibiotic, crowding and poor sanitation. These three factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in *Salmonella* of poultry origin (Van-den Bogaard, *et al.*, 2000). The source of the resistance may also have come from the poultry feeds consumed, since antibiotics are used as feed additives to improve feed efficiency and weight gain (Tabatabaieci and Nasirian, 2003; Helmuth and Hensel, 2004). Many antibiotics are also used in feed and water to control disease. Indiscriminate use of antibiotics has provided selective pressure for the emergence of drug resistant strains of bacteria associated with poultry products. In a research carried out by Atere *et al.* (2015b), a similar report was given on the antibiotic susceptibility of *Escherichia coli* which is far higher in poultry (freshly dead chicken) than in the samples isolated from environment. The reason for the high level of antibiotic resistance was attributed to misuse of antibiotics before clinical reports.

In conclusion, there is a need to create more awareness among the poultry farmers in Nigerian about the proper use of antibiotics, importance of clinical and laboratory test before administration of antibiotics, proper hygiene and effect the type of feed adopted can have on their birds. Since *Salmonella* is zoonotic, it is of public health importance because these resistance gene can easily be transferred to human population.

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