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Full Length Research Paper

Prevalence of Multidrug Resistance *E. coli* among the Poultry Birds of Elele, Nigeria

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Antibiotic resistance has been recognized as a worldwide emerging problem in both human and animals. To investigate the prevalence and antibiotic susceptibility pattern of *E.coli* among the birds at Madonna University poultry, fresh droppings were streaked on MacConkey and EMB agar plates and incubated for 24h at 37ºC. Emergent colonies were identified and characterized as *E.coli* by using a standard bacteriological techniques and serotype i.e. *E. coli* 0157 Latex agglutination. Disk diffusion method was used to evaluate the susceptibility of the isolates against 8 antibiotics. Among the samples collected from 100 birds (25% old layers, 30% new layers, 25% main layers and 20% cockrells), *E.coli* was isolated from 95 comprising 21.1% old layers, 31.6% new layers, 24.3% main layers and 21.1% broilers. All the tested strains were found resistant to tetracycline, nalidixic acid, chloramphenicol, co-trimaoxazole, ceftriazole, cefpirome and cefoxitin while 10% were susceptible to ciprofloxacin. The distribution of *E.coli* 0157:H7 showed 10%, 20%, 33.3% and 30% occurrence in old layers, new layers, main layers and broilers respectively. This abnormal antibiotics resistance observed in this study indicates that poultry is a major source of antibiotics resistance traits.

Keywords: *E. coli*, poultry, multidrug resistance.

INTRODUCTION

Frequent usage of antibiotics are considered as most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both human and human animals (Nsofor and Iroegbu 2012; Nsofor and Iroegbu 2013; Writt, 1998; Neu, 1992). Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals, animals or humans (Nsofor and Iroegbu, 2013). Antibiotics are used in animals as in humans for therapy and control of bacterial infections. In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals. In addition, antimicrobial agents may be continuously fed to food animals such as broilers and turkeys as antimicrobial growth promoters. This practice is believed to enhance selection of resistant bacteria more than the therapeutic use of antibiotics in response to clinical diseases (CDC, 1999), and it may contribute to induced antibiotics resistance in human pathogen because they acquired these antibiotics and resistance endogenous flora through the human food chain (Casewell et al., 2003). Therefore the antibiotic selection pressure for resistant bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria (Van den Bogaard, and Stobberingh, 1999)

One strategy to minimize this problem that has been recommended is to minimize the use of antibiotics needed for human treatment as feed additives (WHO,1997), but there is argument whether, and to what extent feed additives contribute to the development of resistance in human bacterial pathogens (Casewell et al., 2003). If the use of antibiotics is an important factor for the development of antibiotics resistance, it could be

hypothesized that the pattern of antibiotic resistance in different animal population vary according to the types and quantity of the antibiotics used. In this study, the antibiotics resistance pattern of *E coli* isolates from the poultry birds of Elele, Nigeria were investigated.

MATERIALS AND METHODS

Sample collection, cultivation and identification of *E. coli*

Fresh chicken droppings were collected by using a plastic vial and taken immediately to the Laboratory for culturing. A sterile inoculation loop was used to stab fecal sample and inoculated directly by streaking on MacConkey agar. The plates were incubated for 24h at 37ºC. A pink colony was picked and sub-cultured on EMB agar (Oxoid). Colonies with metallic green sheen on EMB (characteristic of *E. coli)* were later confirmed by biochemical tests. Only isolates confirmed to be *E.coli* were selected for antibiotics susceptibility testing.

Antibiotics susceptibility testing

The susceptibility pattern of *E. coli* against the eight antibiotics Viz: Tetracycline (30µg), Nalidixic acid (30µg), Co-trimaoxazole (25µg), Cefoxitin (30µg), Ceftraixone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), and Cefpirome(30µg) Oxoid was evaluated. The bacterium inoculum was inoculated from the standardized culture by using sterile cotton wool stick. The culture was standardized using 0.5McFarland standard. The antibiotics disks were then picked with a sterile pin and placed on the inoculated plates, 25mm apart. The inoculated plates were incubated at 37ºC for 16-18 hours, the inhibition zone diameter (IZD) was measured and interpreted based on Clinical Laboratory Standard Institute breakpoints (CLSI, 2006). *E. coli* (ATCC 25922) was used as control strain.

Serotyping

E.coli 0157:H7 latex kit (Oxoid) was used to serotype the isolates to investigate the prevalence of *E.coli* 0157:H7 among the isolated strains. On a sterile tile card, a smear of the test organism was made with a loop full of normal saline and mixed with a drop of the antisera. The mixture was rocked gently for 60 seconds to observe for agglutination. *E.coli* (ATCC 25922) was used for quality control

Statistical analysis

Comparative resistance rates for *E. coli* strains from the different types of birds were statistically analyzed by T-test and results were considered significant at 95% confidence level Agwunglefah (2007)

RESULTS AND DISCUSSION

The occurrence of *E.coli* among the birds at Madonna University Poultry is high; samples from New layers, Main layers and Cockrell pens showed 100% while that of Old layers' pen has 90% occurrence (Table 1). Among the isolates subjected to antibiotics susceptibility testing, very high resistance was observed against most of the antibiotics evaluated Table 2. The distribution of *E.coli* 0157:H7 serotype among the isolated strains is shown in Table 3. Main layers have the highest incidence of *E.coli* 0157:H7 strain (10%) while the least incidence is seen in Old layers (2**%).** Statistical analysis showed that average number of resistance phenotypes per isolate was not significantly different in any of the sampled birds (P<0.05).

In present study, antibiotics susceptibility pattern in *E. coli* isolated from poultry pens at Madonna University Elele was investigated. The results of the study revealed that, the occurrence of *E.coli* was highest in Main layers, New layers, and Cockrell, while Old layers had the least occurrence. The high occurrence may be as a result of overcrowded of the chickens in those pens while that observed in Old layers may be as a result of few number of chicken in the pen, thus the chances of contamination are higher in the former. Overcrowding and poor sanitary condition has assorted with transmission of *E.coli* in poultry (Van den Bogaard, 1997).

The abnormal high resistance against the tested antibiotics reported in this study may be due to wide spread and lengthy use of these antibiotics. Since most of them especially tetracycline and cephalosporins are naturally derived compounds, bacteria can be exposed to these agents in nature and sometimes, they are used as additives to the poultry feeds for growth (Troy et al., 2002). Two groups of chicken (Main layers and New layers) were compared for pattern susceptibility based on their on their location and frequency of occurrence. It was observed that the critical value of t was higher than the calculated value, showing that there is no significant difference between the susceptibility pattern of Main layers and that of New layers. This may be due to the fact that, they have common sources of resistant bacteria. The presence of *E.coli* 0157:H7 among the isolated strains showed that the most pathogenic strains

Table 1: Occurrence of *E. coli* in fecal samples collected from the Poultry birds.

Sample Source	Number Analyzed	Number of positive Sample (%)
Old layers	25	20(80%)
New layers	30	30(100%)
Main layers	25	25(100%)
Cockrell	20	20(100%)
Total	100	95

Table 2: Antibiotics susceptibility pattern of *E. coli* isolated from the Poultry birds.

Source	D R U G S															
	CIP		TE		NA		C		SXT		CTX		FOX		CPO	
	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S
Old layers	18	2	20	0	20	0	20	0	20	0	20	0	20	0	20	0
New layers	28	2	30	0	30	0	30	0	30	0	30	0	30	0	30	0
Main layers	21	4	25	0	25	0	25	0	25	0	25	0	25	0	25	0
Cockrell	18	2	20	0	20	0	20	0	20	0	20	0	20	0	20	0

Key:
CIP: Ciprofloxacin **TE:** Tetracycline, **NA:** Nalidixic acid,
C: Chloramphenicol, **SXT:** Cotrimaoxazole, **CTX:** Cefotaxime,
FOX: Cefoxitin, **CPO:** Cefpirome
%R: Percentage Resistance, **%S:** Percentage sensitive

Table 3: The distribution of *E. coli* 0157:H7 Serotypes Among the Poultry Birds.

Sample Source	Number of Isolates	Number of Positive Samples
Old layers	10	01
New layers	15	03
Main layers	15	05
Cockerels	10	03
Total	50	12

of *E.coli* with respect to diarrhea is prevalent in the poultry.

In conclusion, this study showed high antibiotics resistance among the *E.coli* isolates from Madonna University Poultry, Elele. This may be due to resistance factors that are readily retained by *E.coli*, or significant sources of resistant bacteria not captured by this study. The collection of more bacteria isolates from different sources and the addition of genetic analysis will provide more information on the dynamics of the introduction and spread of resistant bacteria in the farm. Also, this study has shown that overcrowding of the pens contribute to a large extent, to the cross contamination of the birds, and also the use of antibiotics as feed additives has contributed so much to high resistance of the birds to these antibiotics. As a result of these, the incidence of *E.coli* 0157:H7 strain, which is the most pathogenic, causing bloody diarrhea is high, thus, posing a big threat to the health of the birds. Therefore, we recommend that the number of birds per pen should be reduced and the use of antibiotics as feed additives should be brought to the barest minimum.

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