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Antimicrobial Susceptibility Pattern of *E. coli* Isolated from Free Range Birds in Abia State, Nigeria

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Abstract: *Escherichia coli* is a major pathogen of worldwide importance in commercially produced poultry contributing significantly to economic losses in chickens, turkey and ducks. A study was conducted between September 2011 and February 2012 at 6 different farm sites located within 3 senatorial zones of Abia State, Nigeria to evaluate the antimicrobial succeptibility pattern of *E. coli* isolated from free ranged birds. Out of 127 samples (81.4%) of *E.coli* were isolated while in 28 samples (18.6%) no *E. coli* growth was observed. The isolates were screened for antimicrobial susceptibility using 8 commercially avaliable antibiotics by the disc diffusion method. Isolates from cockerels recorded 90, 80 and 70% sensitivity to gentamycine; kenamycin and ciprofloxacin, respectively. The least sensitive antibiotic were those of tetracycline, nalidixic acid and cotrimoxazole, presenting 0.5, 4.0 and 10%. Chloramphenicol and ampicillin presented partially intermediate sensitivity of 30 and 40%, respectively. *E. coli* isolates from local fowl recorded 80, 70 and 60% sensitivity against gentamycin, kenamycine and ciprofloxacin. The least sensitive were tetracycline, nalidixic acid and cotrimoxazole 0.0, 2.0 and 5%, respectively. For ducks, the values obtained were varied with gentanycin recording 70% while kenamycin and ciprofloxacine were 65 and 60%, respectively. The least sensitive were those of tetracycline, nalidixic acid and cotrimoxazole 0.0, 2.0 and 6.0%. Ampicillin and chloramphenicol presented 40 and 30%.

Key words: *E. coli*, anti-microbial sensitivity, cockerels, local fowls, ducks, Nigeria

INTRODUCTION

The level of antimicrobial sensitivity to *E. coli* and other normal intestinal flora of animals and even humans is likely to decrease as a result of excessive exposure to different types of antibiotics (Van de Bogaard and Stobberingh, 2000). The bacteria *E. coli* are normal flora but excessive amount when present in the chicken cause a disease described as collibacillosis and could be the basis for selective use of antibiotics to avoid prevalence of resistance by such bacteria (Murray, 1992).

Colonization of the intestine by *E. coli* usually occurs before infection and possession of more than one gene could be the factor responsible for occasional resistance (Lipstich and Samore, 2002). Antimicrobial treatment is given to animals suspected of being infected by *E. coli*. It has been known that such antimicrobial treatment alters the total population of *E. coli* in a given host (Halloran and Struchiner, 1991). There are evidences emerging from the use of antimicrobials in humans which may present a selective force for multi-drug resistance in enterobacteriaece in some developing countries which may have decrease antibiotic sensitivity in many countries (Kariuki *et al*., 1996). This view is opposite of what is obtained in the developed countries which suggest that anti-microbial use in agriculture is the major driving force in the selection and dissemination of poor bacterial sensitivity (Apley *et al*., 1998). Vaccination of birds are known to provide passive immunity to protect their offspring during the 1st 2 weeks of age (Melamed *et al*., 1991). It was reported that genetically modified live vaccine tend to prevent *E. coli* against broiler infections. The aim of the study is to know the level of prevalence of this organism in the local environment, the reason behind the occurrence and the most suitable antibiotics that could be used to control it or reduced it to the barest minimum.

MATERIALS AND METHODS

This study was carried out in Abia State, Nigeria. Abia State is located in the extreme part of the Southeastern region of Nigeria. The state has 17 different Local Government Areas (LGA). The LGAs are grouped

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into 3 senatorial zones namely: Abia North, Abia South and Abia Central, the state has been notable known for poultry production.

Identification and selection of sampling sites: The study was carried out between September 2011 and February 2012 at 6 selected urban and rural areas spread across the 3 senatorial zones of Abia State. The 6 sites include: Aba South and Ossisioma (Abia South senatorial zone), Umuahia South and Ikwuano (Abia Central senatorial zone) Ohafia and Bendel (Abia North senatorial zone). Random samplying of the poultry faces was collected using sterile swab stick (Silver Health Diagnostics) sterilized by ethylene oxide. The swab stick was introduced into the vent of each bird and sample collected and fixed back into the swab case. The farms were visited 3 times over a period of 6 months. It was ascertained that no earlier antibiotic therapy had been given to the birds since, this would hinder the sensitivity result. Samples were collected from cockerel, local fowl and ducks (Table 1).

Sample collection, culture and isolation: A total of one hundred and fifty samples were collected via cloaca swab (Ten sample at a time) using sterile swab sticks (Aritee R). The samples were taken to the Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike for processing. Each fecal swab were streaked directly on MCA and incubated at 37°C overnight for 24 h. The streaking technique is that described by (Cruickshank *et al*., 1983). The MCA was prepared according to the manufacturer's instructions. The colonies that grow on the MCA plates were about 2-4 mm in character, opaque and convex at the entire edge. The colonies were subculture into Eosin Methylene Blue (EMB) and incubated overnight 37°C. Marked green metallic sheen isolates were confirmed by specific biochemical tests (Bauer *et al*., 1974).

Antimicrobial susceptibility testing: All 127 isolates of *E. coli* were tested against 10 commercial antibiotics. The antimicrobial sensitivity test was performed according to Bauer Kirby Disc Diffusion Method (Bauer *et al*., 1960).

The streaking technique used was that recommended by the National Committee for Clinical Laboratory Standards Guideline (NCCLS, 2002). This was done by streaking the nutrient agar plates surface evenly with the organism and later exposing them to the disc. The disc is already inbuilt with known concentration of antibiotic substances available 8 commercial antibiotics were used via disc diffusion method and this includes: ampicillin, kanamycin, gentamycin, chloramphenicol, ciprofloxacin, nalidixic acid, tetracycline and cotrimoxazole. The sensitivity data were recorded by measuring the diameter using meter rule in millimeter. The zones of inhibition for each isolate were interpreted as sensitive or resistant based on the size of the diameter. For the purpose of this study, the isolates with intermediate sensitivity were grouped as resistance (Table 2).

Table 1: Fecal samples from different free ranged birds

Bird type	Number of sample	Sample (%)
Cockerels	70	46.7
Local fowls	50	33.3
Ducks	30	20.0
Total	150	100.0

Table 2: Number of isolates and their percentages

Isolates	Values	Percentage
No. of isolates positive	122	81.4
No. of isolates negative	28	18.6
Total isolate sample	150	100.0

RESULTS AND DISCUSSION

Antimicrobial sensitivity of *E. coli* isolates from cockerels. *E. coli* isolates from cockerels (Fig. 1) recorded 90 and 80% sensitivity to gentamycin and kenamycin while ciproflaxin has 70% sensitivity. Furthermore, nalidixic acid, tetracycline and cotromoxazole recorded 0.5, 2.0 and 5.0% sensitivity, respectively.

Anti-microbial sensitivity of *E. coli* isolates from local fowl: The mean percentage of *E. coli* strain isolated from local fowls as shown in Fig. 2. Gentamycin, kanamycin and ciprofloxacin presented 80, 70 and 60% sensitivity while tetracycline, nalidixic acid and cotrimoxazole recorded 0.5, 2.0 and 5.0% sensitivity, respectively.

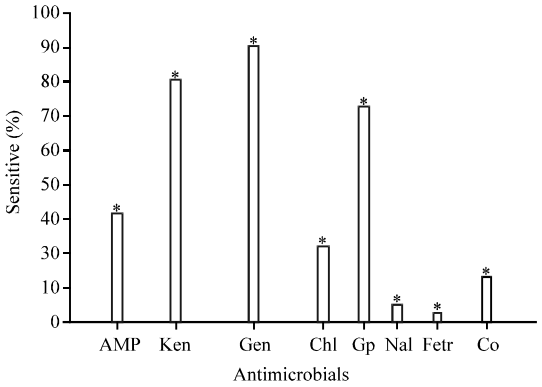

Fig. 1: Antimicrobial sensitivity frequencies of *E. coli* isolates from cockerel. Amp: Ampicillin; Gen: Gentamycin; Ken: Kenamycin; Cip: Ciprofloxacin; Chlo: Chlorophenicol; Nal: Nalidixic acid; Tetr: Tetracyclin; Co: Cotrimoxazole

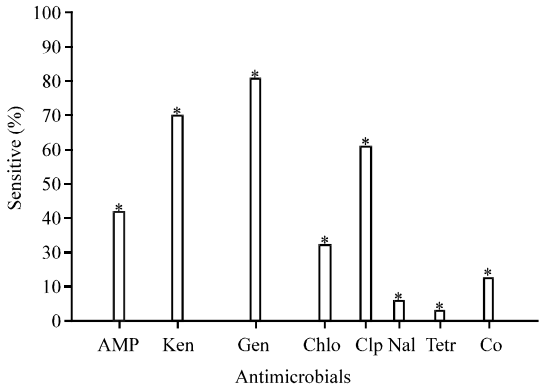

Fig. 2: Antimicrobial sensitivity frequencies of *E. coli* form local fowls. Amp: Ampicillin; Ken: Kenamycin; Chlo: Chloramphenicol; Cip: Ciprofloxacin; Nal: Nalidixic acid; Tetr: Tetracycline; Co: Cotrimoxazole

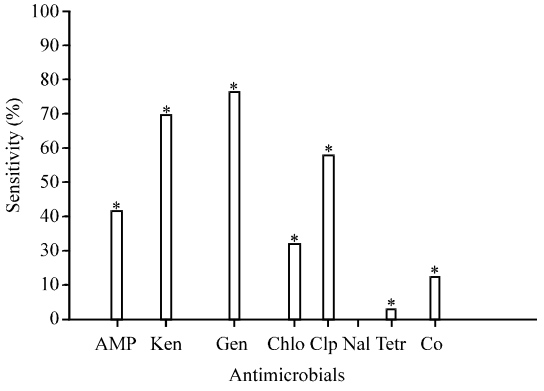

Fig. 3: Anti-microbial sensitivity frequencies of ducks. Amp: Ampicillin; Ken: Kenamycin; Chlo: Chloromphenicol; Cip: Ciprofloxacin; Nal: Nalidixic acid; Tetra: Tetracycline; Co: Cotrimoxazole

Antimicrobial sensitivity of *E. coli* isolates from ducks: Figure 3 shows that ducks yielded *E. coli* organism that were highly sensitive was record against nalidixic acid.

Antibiotic usage is one of the most important factors that promotes the emergence, selection and spread of antibiotic resistant microorganisms in livestock. In poultry flocks, overdosage of antibiotics as well as use of antibiotics as growth promotes in feed may result in high antibiotic selection pressure. From this study, gentamycin is the most sensitive of all the antibiotics. This results is supported by the findings of Salehi and Bonab (2006). The antibiotic sensitivity test result obtained from this study of kenamycin and enrofloxocin of about 70 and 60%, respectively is in agreement with the study reported by Tabatabaci and Nasiran (2003) that the sensitivity of drug is due to less use in the farms. Furthermore, there are difficulties in poultry farmers using gentamycin for treatment in Abia State, Nigeria. This is as a result of availability of the drug only in injection form and this could be stressful to the farmer and the birds hence most farmers resort to using the other range of antibiotic like enrofloxacin which is equally effective. The efficacy of the fluoroquinolones group enrofloxacin in the treatment of local fowl, cockerels and duck has also been reported in broilers (Chansiripornchai, 2009; Chansiripornchai and Susiprecyajan, 2004). Local fowls and ducks generally scarcely receive the attention of veterinarians, they do maintain close contact via various ways with organisms originating from other important host in their environment such as humans and exotic chicken that had been earlier exposed to various antibiotics. A good example is the commercial town of Aba and Umuahia in Southeastern Nigeria where indiscriminate urination and defeacation and other unhygienic methods of excreta disposal create avenues for these free ranged chickens to be exposed to *E. coli* infection during feeding. Furthermore, the need to make use of antibiotic sensitive to this organism is very important as shown in this research. This is in agreement with the report from which stated that selective use of antibiotics in other hosts in the same environment is very necessary.

CONCLUSION

This shows the pattern of antibiotic sensitivity to this bacterial is clinically important for the poultry industry and should be closely monitored.

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