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**ANTIMICROBIAL RESISTANCE PROFILE OF AVIAN PATHOGENIC
ESCHERICHIE COLI ISOLATED FROM CHICKEN DIAGNOSED WITH
COLIBACILLOSIS IN ENUGU STATE, NIGERIA**

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ABSTRACT

Colibacillosis caused by avian pathogenic *E. coli* (APEC) has become difficult to treat due to antimicrobial resistance. Our objective was to determine the resistance profile of APEC from chicken to commonly used antimicrobials. Dead and moribund chickens from various farms across Enugu State submitted to the necropsy unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka between January 2011 and December 2013 were used for the study. At necropsy, liver, heart, spleen and oviduct samples from 182 chickens diagnosed of colibacillosis were collected and cultured on MacConkey agar, incubated at 37⁰C for 24hrs and pink colonies were sub-cultured on eosin methylene blue agar and incubated for 24hrs at 37⁰C. Colonies that produced greenish metallic sheen appearance were further confirmed as APEC strains following standard biochemical tests. Antibacterial resistance profile of the APEC strains was determined using the disc diffusion technique against 10 different antibacterial agents. APEC strains were isolated from 57 (31.3%) of the 182 chickens examined. The APEC strains were resistant to 98%, 93%, 86% and 84% tetracycline, streptomycin,

ciprofloxacin and ampicillin respectively while 5%, 21%, 33%, and 35% were resistant to ceftazidime, imipenem, ceftazidime and ceftazidime respectively. Sixty-three percent of the strains were resistant to gentamicin while 52% were resistant to amoxicillin/clavunanic acid. The APEC strains demonstrated 30 resistance patterns with TE-S-CN-CIP-AMP being the most predominant; occurring in 7 strains. Multi-drug resistance among *E. coli* strains were recorded which could be challenging for the treatment of both human and animal bacterial infections.

Keywords: Antibiotic resistance, chickens, colibacillosis, APEC

INTRODUCTION

Escherichia coli is a primary pathogen and an important causative agent of numerous bacterial infection in poultry collectively called colibacillosis [1]. Colibacillosis is the most frequently reported disease characterized by high morbidity and mortality and most often results in the condemnation of processed poultry meat [2]. Colibacillosis is a localized or systemic infection caused by avian pathogenic *Escherichia coli* (APEC), which include colisepticemia, air sac disease, cellulites, swollen-head syndrome, peritonitis, salpingitis, osteomyelitis or synovitis, panophthalmitis and yolk sac infection [3].

Antibiotics are widely used in animal husbandry including poultry production to control disease, treat infections and promote their growth. The use of antimicrobials has resulted in selection for antimicrobial-resistant *E. coli* in the commensal microbiota that could spread to humans through

consumption of contaminated meat [4]. Studies of broiler chickens have shown a high rate of antimicrobial multi-resistant *E. coli* [5]. Resistance to antimicrobial agents could lead to an increase in mortality as well as a longer duration of treatments [6]. Studies have also shown the presence of antimicrobial-resistant ExPEC in farm animals and in meat products [7, 8]. The use of antimicrobials in agriculture, farm management and in veterinary medicine including livestock and companion animals favours dissemination of antimicrobial resistance in animal reservoirs and the environment, making available multi-drug resistant bacteria which could infect humans with resultant difficulty in treatment and public health risks. This was therefore carried out to determine the antimicrobial resistance profile of APEC isolated from chickens diagnosed with colibacillosis in Enugu State, Nigeria.

MATERIALS AND METHODS

Sample collection, processing and preparation of the *E. coli* isolates

One hundred and eighty-two dead and moribund chickens from various farms across Enugu State were used for the study. At necropsy, liver, heart, spleen and oviduct samples from 182 chickens diagnosed of colibacillosis were collected. These samples were inoculated on MacConkey agar medium and incubated at 37°C for 24 hours. Pink colonies on the MacConkey agar which suggest lactose fermentation were further streaked onto eosin-methylene blue (EMB) agar and incubated overnight at 37°C. Colonies that showed greenish metallic sheen were subjected to further biochemical test for *E. coli* identification.

Antibiotic susceptibility testing

Antibacterial susceptibility test was carried out on the isolated *E. coli* using the disc diffusion technique on Mueller Hinton agar according to the methods recommended by the Clinical and Laboratory Standards Institute [9]. Ten antibiotics were used and include the following: Cefoxitin (30µg); Imipenem (10µg); Tetracycline (30µg); Streptomycin (10µg); Ampicillin (10µg); Ciprofloxacin (5µg); Gentamycin (10µg); Ceftriaxone (30µg); Ceftazidime (30µg) and Amoxicillin/clavulanic acid (30µg). Colonies

of each test isolate was suspended in normal saline and turbidity of each suspension adjusted to correspond to 0.5 McFarland turbidity standards (approximately 10⁸cfu/ml). Each standardized broth culture was used to swab the surface of the Mueller-Hinton (MH) agar plates. Using a disc dispenser (Oxoid, Basingstoke, England), the antibiotic discs were placed on the surface of the inoculated agar plates and then incubated at 37°C for 24hrs. After incubation, the plates were examined for inhibition zones around each disc. The diameters of the zones were measured with a meter rule and recorded. Each test was conducted three times and the mean inhibition zone diameter recorded to the nearest whole millimeter. Each test isolate was classified as resistant, or sensitive to the test antibiotics in accordance with the criteria or guidelines given by the CLSI [9].

Data Presentation and Statistical Analysis

Data generated were presented in form of tables and percentages.

RESULTS

From the one hundred and eighty-two (182) samples that were examined from clinical cases of colibacillosis, a total of fifty-eight (57) APEC strains representing 31.3% of the total samples were obtained. The *E. coli* strains recorded high resistance of 98%, 93%, 86% and 84% against tetracycline,

streptomycin, ciprofloxacin and ampicillin respectively while low resistance of 5%, 21%, 33%, and 35% were recorded against ceftazidime, imipenem, ceftriaxone and ceftazidime respectively. The strains also showed 63% resistance against gentamicin and 52% resistance to amoxicillin/clavunanic acid.

The percentage antimicrobial resistance profile of the APEC strains from layers and broilers is presented in Figure 1. The *E. coli* strains recorded 96% resistance against both tetracycline and streptomycin followed by ciprofloxacin and ampicillin that showed 88% resistance. Ceftazidime had the lowest resistance of 4% against the strains isolated from layers. The APEC strains isolated from broiler chickens were 100%, 92%, 90% and 88% resistance against tetracycline,

ciprofloxacin, streptomycin and ampicillin respectively. The strains also showed lower resistance (6%) to ceftazidime.

Antibiotic resistance pattern of the APEC strains tested is shown in Table 1. The number of antibacterial agents which the test strains were resistant to ranged from 2 to 9 in number. From the table, majority of the strains were resistant to tetracyclines, streptomycin, ciprofloxacin and ampicillin. The strains were mostly sensitive to ceftazidime. The APEC strains demonstrated thirty (30) resistance patterns with TE-S-CN-CIP-AMP being the most predominant. This pattern occurred seven (7) times and was isolated from both broiler chickens and layers. Three of strains were resistant to nine (9) out of ten (10) antibiotics used.

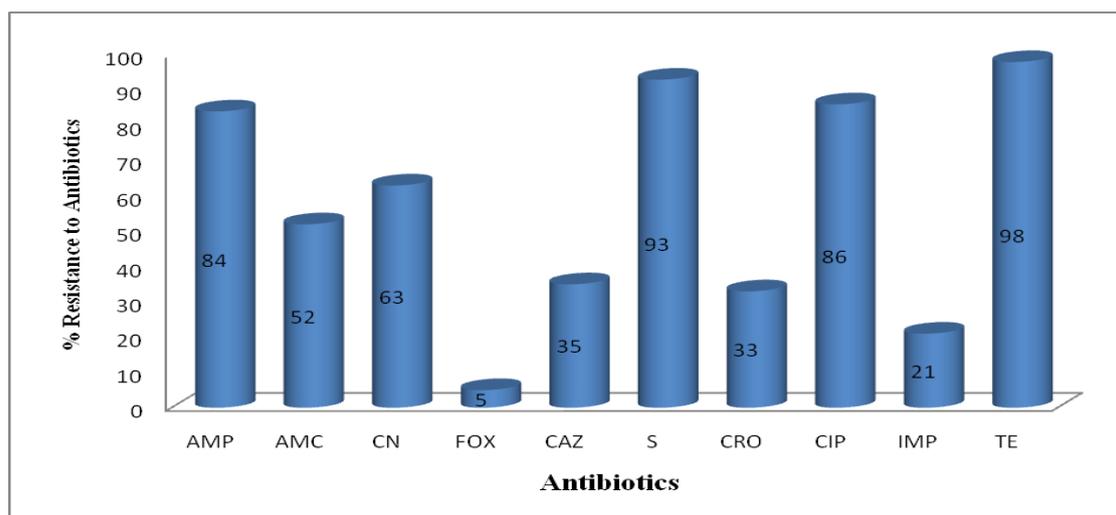


Figure 1: Antibacterial resistance profile of APEC strains isolated from chicken samples in Enugu State
AMP= ampicillin; CN = gentamicin; CAZ = ceftazidime; S = streptomycin; CRO = ceftriaxone; CIP = ciprofloxacin; TE = tetracycline;
AMC = amoxicillin/clavulanic acid; IPM = imipenem; FOX = ceftazidime

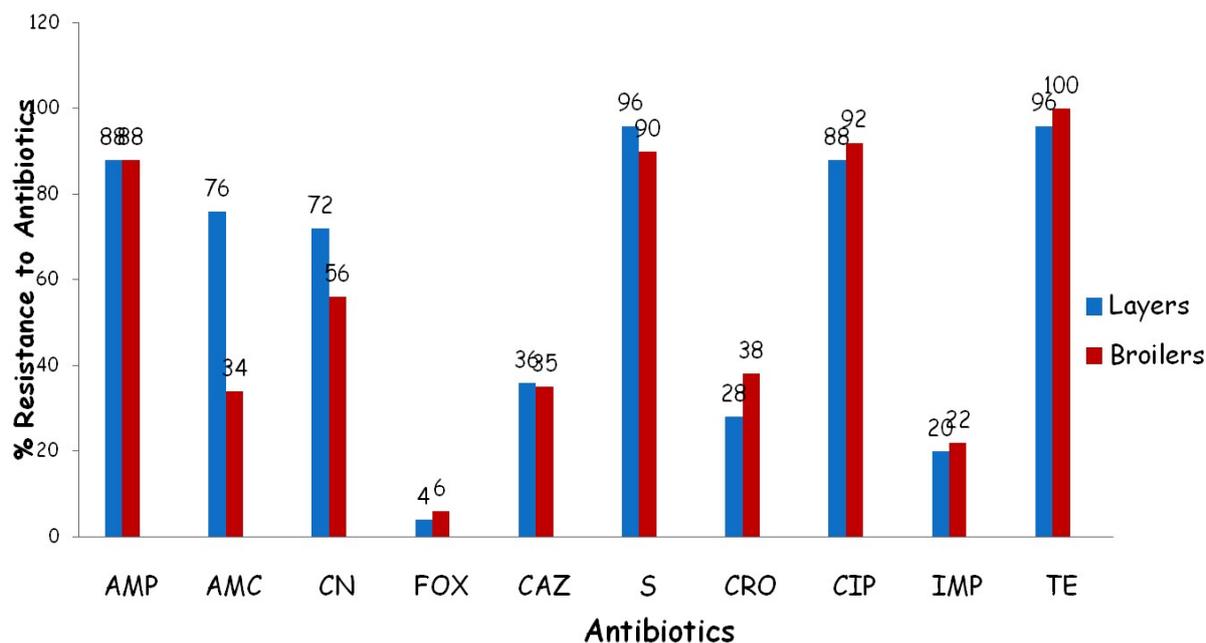


Fig. 2: Percentage antibacterial resistance in APEC strains from broilers and layers
 Secondly, the meanings of the abbreviations used in Figure 2 “AMP= ampicillin; CN = gentamicin; CAZ = ceftazidime; S = streptomycin; CRO = ceftriaxone; CIP = ciprofloxacin; TE = tetracycline; AMC = amoxicillin/clavulanic acid; IPM = imipenem; FOX = ceftiofur”

Table 1: Antibiotic resistance pattern of the APEC strains

S/No	Resistant Pattern	No of Isolates
1	TE-S	1
2	TE-S-CIP	3
3	TE-S-AMP	1
4	TE-S-CAZ-IPM-AMC	1
5	TE-S-CRO-FOX	1
6	TE-S-CN-CIP-AMC	1
7	TE-S-CIP-AMP	3
8	TE-S-CIP-AMP-AMC	2
9	TE-S-CN-AMP	1
10	TE-S-CN-AMP-AMC	1
11	TE-CN-CIP-AMP	1
12	TE-S-CN-CIP-AMP	7
13	TE-S-CN-CIP-AMP-AMC	5
14	TE-S-CAZ-CIP-AMP	1
15	TE-S-CAZ-CIP-AMP-AMC	2
16	TE-S-CIP-AMP-IPM	1
17	TE-S-CRO-CIP-AMP-AMC	1
18	TE-AMP-CRO-CIP-IPM	1
19	TE-AMP-CN-S-CAZ-CIP	3
20	TE-AMP-CN-S-CRO-CIP	1
21	TE-AMP-CN-S-CRO-CIP-AMC	3
22	TE-AMP-CN-S-CIP-IPM-AMC	2
23	TE-AMP-S-CAZ-CRO-CIP-AMC	2
24	TE-AMP-S-CAZ-FOX-CIP	1
25	TE-AMP-CN-CRO-CIP-IPM	1
26	TE-AMP-CN-S-CAZ-CRO-CIP-AMC	5
27	TE-AMP-CN-S-CRO-CIP-IPM	1
28	TE-AMP-CN-S-CAZ-CIP-IPM-AMC	1
29	TE-AMP-CN-S-CAZ-CRO-CIP-IPM-AMC	2
30	TE-FOX-CN-S-CAZ-CRO-CIP-IPM-AMC	1

AMP= ampicillin; CN = gentamicin; CAZ = ceftazidime; S = streptomycin; CRO = ceftriaxone; CIP = ciprofloxacin; TE = tetracycline; AMC = amoxicillin/clavulanic acid; IPM = imipenem; FOX = ceftiofur

DISCUSSION

Resistance to antibiotics seen among *E. coli* strains is now a global problem [10]. *E. coli* resistance to many antibacterial agents has been reported in many countries including Nigeria [11, 12]. The APEC strains in this study demonstrated high resistance rates to the commonly available antibacterial agents (tetracycline, streptomycin, ciprofloxacin and ampicillin). Surprisingly, a high rate of resistance was also demonstrated against ciprofloxacin, an important fluoroquinolones used in the treatment of human infections. Resistance to commonly available antimicrobial may be a reflection of their indiscriminate use in poultry production. The fluoroquinolones (enrofloxacin and ciprofloxacin in form of conflux[®]) are now widely prescribed and used in veterinary medicine [13]. This wide indiscriminate application may also explain the emergence of resistance to this class of antimicrobial agents. Cefoxitin, ceftazidime, ceftriaxone and imipenem are also not used in veterinary practice in the study area. This may explain why the APEC strains were least resistant to these antimicrobial agents.

The APEC strains in this study showed 84% and 52% resistance to ampicillin and amoxicillin-clavulanic acid respectively. Resistance rate to ampicillin

found in this study was higher than those findings of Rahimi, [14] and Akond *et al.* [15] who reported 66.2% and 58% resistance, respectively, in *E. coli* strains from poultry. The finding in this study is similar to 95% resistance to ampicillin reported in *E. coli* isolates from broilers in Malaysian [16]. Ampicillin is commonly used by poultry farmers and its widespread use in the treatment of diseases could have led to emergence of ampicillin-resistance among these *E. coli* strains.

Tetracyclines are commonly used for treatment of animals before the antibiotic susceptibility of a pathogen is determined. They are the most frequently used antimicrobial in animal therapeutics in Nigeria. This may explain the reason for the high resistance to this drug recorded in this study. It is also the most widely used antibiotic by poultry farmers in Nigeria and other developing countries [12, 17]. The high resistance recorded in this study is in agreement with the findings of Salehi and Bonab [18] in Iran and Geidam *et al.*, [19]. in Nigeria who reported high resistance to tetracycline by *E. coli* strains tested.

Sixty three percent resistance to gentamicin in this study was similar to 65% reported by Bass *et al*, [20] and comparable to 52% and 54% reported by Rahimi, [14]

and Daini and Adesemowo, [21]. Gentamycin which usually comes as injectable, is often not used for chicken treatment in the study area which makes the link between usage and development of resistance not probable in this instance. However, its resistance could be as a result of inclusion in Marek's vaccine administered to almost all poultry in ovo [22]. Resistance to streptomycin (93%) in this study was very high but similar to 97% reported by Bass *et al.* [20]. However, it is higher than 59% reported by Daniel *et al.* [23]. It is not clear if the *E. coli* resistance streptomycin in this study has anything to do with use of this antimicrobial for chicken therapeutics. However, streptomycin could be adjudged one of the most abused antimicrobial among farmers in Nigerian especially in large animals. Rahimi, [14] reported 52.6% gentamicin resistance among APEC strains isolated from broilers with colibacillosis in Iran. Saberfar *et al.* [24] reported low rate of resistance (12%) to gentamicin in *E. coli* isolated from cases of colibacillosis in broiler chickens in Iran. Daini and Adesemowo [21] reported 54% resistance of *E. coli* strains to gentamicin in Nigeria.

There is also growing evidence of proliferation of quinolone resistance in Nigeria including detection of horizontally

transmitted resistance traits [25, 26]. In the developing countries like Nigeria, quinolones (enrofloxacin, ciprofloxacin, ofloxacin etc) are less commonly used in veterinary medicine, they account for 1.7% of all antibiotics used in poultry [27]. In this study, the APEC strains recorded 86% resistance to ciprofloxacin. High resistance to ciprofloxacin observed in this study is in conformity with earlier reports of resistance to ciprofloxacin (79% and 90%) in *E. coli* reported by Hanchun *et al.*, [28] and Saenz *et al.*, [29] respectively. However, the level of resistance to ciprofloxacin of poultry *E. coli* strains observed in this study was higher than that (16.7%) observed in northern Nigeria by Mamza *et al.*, [30].

The occurrence of antibiotic resistance among *E. coli* strains isolated from these chickens may be related to the use of different antibiotics in the different farms in the study area. The findings in this study confirms the significant increase in the incidence of antimicrobial resistance among *E. coli* strains in Nigeria which is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases. The resistance of *E. coli* isolates in these chickens is of great concern and may result in contamination of the environment by resistant strains from

poultry. The method of processing poultry meat in Nigeria where sometimes involve slaughtering of chicken in households could endanger household members and aid the spread of these multi-drug resistant strains in the community.

The study showed that all the APEC strains were resistant to 2 or more antibacterial agent which shows that they were multidrug resistant. This makes the treatment of colibacillosis difficult in chicken and it may pose a serious danger to poultry and the entire populace. The resistance genes mediated by plasmid can make the resistance spread among other bacteria. These bacteria can then obtain resistance genes easily through this mechanism and thus, produce multiple resistances to antibiotics [31, 32]. This mechanism of antibiotic resistance in which resistance genes directly code enzymes results in damage of antibiotic effect when administered [33]. The antibiotic resistance acquired by a microorganism in one ecosystem can easily be transferred among other organisms in different ecosystems. This may facilitate the spread of bacteria and their genes around the world [34]. Environmental bacteria in different ecological niches have been shown to be a reservoir of antibiotic resistance genes and a potential source of novel resistance genes in

clinical pathogens [35]. This may in turn be responsible for epidemic and endemic spread of multi-drug resistance [36, 37].

A total of 30 resistance patterns were observed in this study. This shows that the resistance to antibacterial agents was caused by different attributes as almost all the APEC strains were unique in their resistance to antibacterial agents. This implies that the pressure from the environment which imposes resistance to these bacteria is very high. The drug of choice in the treatment of poultry infection caused by APEC strains in the study area is cefoxitin, followed by imipenem, ceftriaxone and ceftazidime.

Although these drugs are mostly in human preparation, there is need for alternative treatment or prophylaxis in poultry production. Antimicrobial use should be reduced to a minimum and alternative approaches should be undertaken to limit the spread of antimicrobial resistance in animals and humans.

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