Susceptibility Pattern to Common Antibiotics of Intestinal Escherichia Coli from Slaughtered Commercially Grown Chickens

Ihuoma Flora Odoemene[1], Oguamanm Okezie Enwere[2]

ABSTRACT

Introduction: Use of antibiotics, especially its irrational use, is a critical factor that promotes emergence of antibiotic resistant bacteria organisms, as well as its spread among farm animals and humans. This study aimed to investigate the prevalence and antibiotic sensitivity pattern of intestinal Escherichia coli isolates from slaughtered commercially grown broiler and layer chickens in Imo State, Nigeria.
Material and Methods: Swab samples from 200 table size chickens (100 broilers and 100 layers) were collected from the inner part of the intestine after it was emptied of faecal matter. *E. coli* was isolated after culture on eosine methylene blue (EMB) agar and later subcultured on MacConkey agar. The isolates were then analyzed by agar disc diffusion to determine their susceptibility patterns.
Results: A total of 79 (39.5%) slaughtered chickens had *E. coli* isolated (37 broilers and 42 layers) from a total number of 200 samples. The isolates showed the following pattern of antibiotic resistance against ten commonly used and available antibiotics in Nigeria: all isolates (100%) were resistant to both co-amoxiclav and cotrimoxazole; chloramphenicol 60(75.9%), amoxacillin 58(73.4%), streptomycin 57(72.2%), sparfloxacin 53(67.1%), gentamycin 35(44.3%), pefloxacin 25(31.6%), ofloxacin 21(26.6%) and ciprofloxacin 16(20.3%).
Conclusion: Prevalence of *E.coli* in slaughtered chickens intestine was 39.5% with all resistant to both co-amoxiclav and cotrimoxazole. The organism was less resistant to the quinolones especially ciprofloxacin.

Key words: *E. coli*, Isolates, Chickens, antibiotics, sensitivity, resistance

INTRODUCTION

Commercially grown chickens (broilers and layers) are widespread and quite popular in Nigeria for their cheap and acceptable source of meat and eggs. E. coli and other organisms have been observed to contaminate poultry meat of retail market in Nigeria, with E. coli contamination accounting for 43.4% of chicken meat and 39.1% of turkeys.[1] However, evidence of a growing and varied presence of antibiotic resistant Escherichia coli *(E. coli)* has been observed, especially in Imo state and other parts of the country without any seasonal variation.[2,3] This antibiotic resistance can be found among both pathogenic and non-pathogenic strains of *E. coli*[4,5] in commercially available broilers.

Majority of the strains of *E. coli* do not cause disease, but the virulent strains can cause diseases such as gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis and Crohn's disease in humans. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and gram-negative pneumonia.[6] Some common signs and symptoms of infection by *E. coli* include severe abdominal cramps, vomiting, diarrhea, hemorrhagic colitis and sometimes fever. Very young children are most susceptible to develop severe illness such as hemolytic uremic syndrome (HUS), however healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli.*[6]

Indiscriminate and irrational use of antibiotics, as whole flocks are most times treated with antibiotics rather than sick individual birds, is largely responsible for the widespread development of antibiotic resistance[7,8] with a progressive reduction in antibiotic efficacy in the treatment of bacterial infections in both humans and animals. This increases antibiotic selection pressure for resistance in bacteria among poultry and, consequently observation of a relatively high proportion of resistant bacteria among their bacterial flora.[9]

In animal farms such as poultry, drugs are used for both therapeutic and prophylactic purposes to the whole herd; and in many cases, in the earlier stage of life, as growth promoters, in sub-therapeutic concentrations. In humans, antibiotics are administered to sick individuals only.

Most of the common infections that should otherwise be easily treatable in people with limited economic resources may now require expensive medical care, invariably making treatment unavailable to this large population of people.[10]

A good knowledge of antibiotic resistance patterns among pathogens is a prerequisite for effective medical treatment of humans as well as in farm animals.

Pathogenic *E. coli* cause serious food poisoning in humans and occasionally are responsible for product recalls due to contamination of food.[11] In the United States, it is estimated that *E. coli* 0157:H7 makes over 73,000 people/year sick, with an annual estimated 2,100 hospitalizations and 61 E. coli-related deaths.[10]

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E. coli is sensitive to many antibiotics. However, isolates from poultry are frequently resistant to one or more antibiotics, especially when widely used in poultry industry over a long period.[12,13] Contamination of meat and fecal contamination of eggs is the most common form of transmission of pathogenic *E. coli* from birds to humans. But concerns exist that resistant strains of *E. coli* may be transmitted to humans; while there may not appear to be a common source for multiple drug resistance in the strains from avian or human origin, the genes encoding resistance are thought to be similar in most cases.[14]

In the review by Hammerum and Heuer, several examples of humans colonized by antimicrobial resistant commensal *E. coli* from food animals were widely observed, thereby presenting antimicrobial resistance burdens, with the high possibility of limiting therapeutic options.[15] Also, van den Bogaard et al demonstrated the presenece of multi-drug resistant clones and resistance plasmids of E. coli among were commonly transmitted from broilers and turkeys to the farmers and those who slaughter these animals.[16]

It is very important to monitor changing antibiotic resistance patterns of *E. coli* isolated from animals in various areas making it easier to choose appropriate antibiotics for treatment of human infections when necessary.

Most commercial poultries are located in the urban and in semi-urban areas, while most poultries in rural areas are involved in smaller scale production of poultry meat and eggs. Poultry markets, especially in Nigeria, are avenues for sale of birds and eggs in wholesale and retail outlets, as well as slaughter of table size chickens of various varieties, including broilers and old layers.

This study, conducted in August and September 2017, was aimed at investigating the prevalence and antibiotic sensitivity pattern of intestinal *E. coli* isolates from slaughtered commercially grown broiler and layer chickens in Imo State, Nigeria.

MATERIAL AND METHODS

Sample collection: In August 2017, 200 table-size chicken GIT swab samples (100 broilers and 100 layers) were collected from a popular chicken market in Owerri, Imo state. A sterile swab stick (AntecR) was used to collect the sample from the inner wall of the chicken large intestine after the chicken was slaughtered and the intestinal contents emptied.

Bacterial isolation: Each swab sample was smeared onto the Eosine methylene blue (EMB) agar and incubated for 24 hours at 37°C. After incubating for 24hours, green metallic sheen colonies indicative of E. coli were sub cultured in the MacConkey agar (MCA) (Fluka BioChemicaR), which was prepared according to manufacturer's instruction, and incubated overnight at 37°C. Growths on the MCA plates suggestive of *E. coli* colonies were 2-4mm in diameter, opaque and convex with entire edge and, rose pink on account of lactose fermentation.

Susceptibility testing: The isolated *E. coli* were screened for anti-microbial resistance profile using the disc diffusion method in line with recommended standards.[17,18] This was done by streaking the surface of nutrient agar plates uniformly with the organisms and subsequently exposing them to antibiotic impregnated discs with known concentrations of antimicrobial substances.

Commercial antibiotics discs used in the study were 10 in number and included those commonly available in Nigeria: Co-amoxiclav (CA) 15µg, Gentamycin (CN) 10µg, Pefloxacin (PER) 10µg, Ofloxacin (OFX) 30µg, Streptomycin (S) 30µg, Septrin (SXT) 30µg, Chloramphenicol (CH) 30µg, Sparfloxacin (SP) 10µg, Ciprofloxacin (CPX) 10µg, Amoxacillin (AM) 30µg.

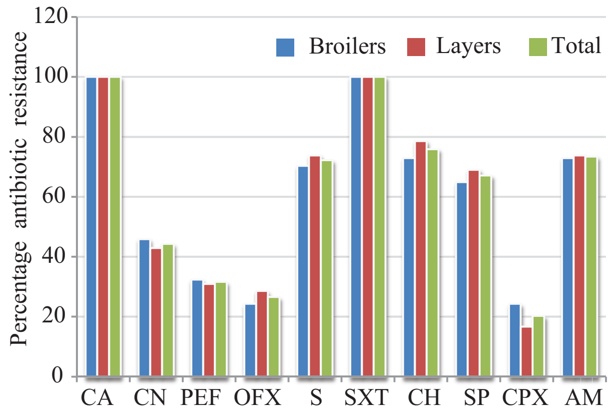
The disc was placed directly in the centre of the agar surface on which the inoculum has been evenly spread and incubated for 24hours at 37°C. After 24hours, the plates were examined for sensitive and resistant antibiotic patterns. The zones of inhibitions were observed and their diameters measured in millimeters.

RESULTS

From the one hundred (100) samples obtained from the broilers following the biochemical tests, the number of pure *E. coli* isolates obtained from sub-cultures was 37 (37%) as shown in table 1. Of the 100 samples collected from layers, 42 (42%) pure isolates were identified (table 2). Being 79 (39.5%) samples had *E. coli* isolated from the 200 chicken samples collected.

Sensitivity pattern of *E. coli* isolates from broilers

Out of the 37 *E. coli* isolates (Table 1) from broilers, antibiotic susceptibility showed that all organisms (100%) were resistant to both Co-amoxiclav and Septrin; followed by Chloramphenicol 27(72.9%) and Amoxacillin 27(72.9%), Streptomycin 26(70.3%), Sparfloxacin 24(64.9%), Gentamycin 17(45.9%), Pefloxacin 12(32.4%), Ofloxacin 9(24.3%) and Ciprofloxacin 9(24.3%). *E. coli* was most sensitive to Ofloxacin 28(75.7%) and Ciprofloxacin 28(75.7%); followed by Pefloxacin 25(67.6%), and



CA= Co-amoxiclav, CN= Gentamycin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin, SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxacillin.

Figure-1: Comparative and total antibiotic resistance pattern of *E. coli* isolates from broilers and layers

	CA	CN	PEF	OFX	S	SXT	CH	SP	CPX	AM
1	0	12	20	16	0	0	0	0	24	0
2	0	14	28	28	18	0	12	0	28	0
3	0	0	20	20	0	0	0	0	0	0
4	0	0	0	12	0	0	0	0	14	0
5	0	0	20	18	0	0	0	14	22	10
6	0	12	20	20	0	0	24	12	26	0
7	0	0	0	0	0	0	0	0	14	0
8	0	12	0	0	12	0	0	0	14	0
9	0	0	20	14	0	0	0	0	24	0
10	0	0	0	16	0	0	0	0	0	0
11	0	14	22	20	0	0	0	20	24	10
12	0	13	25	14	14	0	16	0	25	0
13	0	15	0	20	0	0	0	13	20	16
14	0	0	28	0	16	0	24	16	0	0
15	0	0	0	16	10	0	20	0	0	0
16	0	12	20	12	0	0	0	0	24	10
17	0	0	24	10	0	0	0	20	14	0
18	0	14	0	20	0	0	0	10	0	12
19	0	0	20	0	0	0	12	0	16	0
20	0	12	26	0	12	0	0	0	0	0
21	0	14	0	22	0	0	0	0	20	0
22	0	0	18	18	0	0	0	0	20	0
23	0	14	26	20	0	0	0	0	20	0
24	0	0	30	18	0	0	0	0	28	0
25	0	13	0	0	0	0	0	10	26	14
26	0	0	28	20	18	0	12	0	26	0
27	0	12	25	0	0	0	0	0	24	0
28	0	16	0	18	0	0	0	0	0	0
29	0	0	20	26	0	0	22	16	30	13
30	0	16	20	16	0	0	20	11	27	0
31	0	16	28	16	0	0	0	12	14	10
32	0	0	0	23	15	0	0	0	0	0
33	0	0	26	0	13	0	0	0	18	0
34	0	16	22	0	0	0	0	0	24	16
35	0	0	22	20	0	0	0	14	0	10
36	0	14	24	26	18	0	14	0	28	0
37	0	18	0	14	12	0	0	20	27	0
Total resistance (%)	37 (100%)	17 (45.9%)	12 (32.4%)	9 (24.3%)	26 (70.3%)	37 (100%)	27 (72.9%)	24 (64.9%)	9 (24.3%)	27 (72.9%)

Key: CA= Co-amoxiclav, CN= Gentamycin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin, SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxacillin.

Table-1: Measurement (in millimeters) of the antibiotic zones of inhibition of E. coli isolates from broilers

Gentamycin 20(54%).

Sensitivity pattern of *E. coli* isolates from Layers

All the 42 *E. coli* isolates from the layers (Table 2) were resistant to Septrin and Co-amoxiclav. The varying levels of resistance of the organism observed were: Chloramphenicol 33(78.6%), Streptomycin 31(73.8%) and Amoxacillin 31(73.8%); Sparfloxacin 24(69%), Gentamycin 18(42.9%), Pefloxacin 13(30.9%), Ofloxacin 12(28.6%), and Ciprofloxacin 7(16.7%). Ciprofloxacin 35(83.3%) was the most sensitive against isolated E.coli, followed by Ofloxacin 30(71.4%), Pefloxacin 29(69%), and Gentamycin 24(57.1%).

Overall sensitivity of isolated *E. coli*:

The overall antibiotic susceptibility pattern is as follows (figure 1); all the 79 isolates (100%) from 200 birds, were resistant to both Co-Amoxiclav and Septrin. Other proportions of *E. coli* resistance were: Chloramphenicol 60(75.9%), Amoxacillin 58(73.4%), Streptomycin 57(72.2%), Sparfloxacin 53(67.1%), Gentamycin 35(44.3%), Pefloxacin 25(31.6%), Ofloxacin 21(26.6%), Ciprofloxacin 16(20.3%). Therefore, the organism was most sensitive to Ciprofloxacin 63(79.7%), followed by Ofloxacin 58(73.4%), Pefloxacin 54(68.4%), and Gentamycin 44(55.7%).

DISCUSSION

Antibiotic resistance of isolated pathogenic organisms from farm and food animals is an important health care parameter that needs to be constantly monitored as this can affect the approach of drug treatment in established illness involving

S/N:	CA	CN	PEF	OFX	S	SXT	CH	SP	CPX	AM
1	0	18	28	0	12	0	0	0	0	0
2	0	0	20	20	0	0	0	0	26	10
3	0	0	0	0	0	0	16	0	0	10
4	0	14	26	0	16	0	0	20	20	0
5	0	14	22	14	0	0	0	16	14	0
6	0	0	0	20	0	0	0	0	28	0
7	0	0	22	16	0	0	0	20	25	0
8	0	14	20	20	0	0	0	14	30	0
9	0	14	28	28	18	0	12	0	26	0
10	0	0	0	14	0	0	0	0	0	18
11	0	12	0	0	0	0	0	0	0	0
12	0	18	22	20	0	0	0	12	28	10
13	0	14	26	21	0	0	26	20	30	16
14	0	0	0	0	0	0	0	0	10	0
15	0	12	12	10	16	0	0	0	18	0
16	0	0	18	10	10	0	0	0	24	0
17	0	0	0	16	0	0	0	0	18	0
18	0	14	22	26	0	0	0	18	26	10
19	0	16	25	24	14	0	0	16	28	13
20	0	14	20	22	0	0	0	0	25	0
21	0	0	18	18	0	0	15	0	16	0
22	0	0	0	23	0	0	0	13	0	0
23	0	18	23	0	17	0	20	0	22	0
24	0	0	0	20	0	0	0	0	24	16
25	0	0	12	25	0	0	0	0	14	0
26	0	0	30	0	18	0	0	19	0	0
27	0	13	0	26	0	0	0	0	25	0
28	0	16	28	0	0	0	20	0	25	0
29	0	12	28	0	0	0	0	0	20	0
30	0	13	20	18	0	0	0	0	27	0
31	0	14	28	28	18	0	12	0	27	0
32	0	0	0	17	0	0	0	0	18	0
33	0	0	0	13	0	0	0	0	0	0
34	0	18	22	19	0	0	0	14	24	10
34	0	16	23	20	0	0	24	16	28	0
36	0	0	0	0	0	0	0	0	12	0
37	0	12	10	0	14	0	0	0	16	0
38	0	0	19	12	10	0	0	0	24	0
39	0	0	0	16	0	0	0	0	18	0
40	0	14	22	24	0	0	0	18	26	10
41	0	12	28	14	0	0	0	0	16	0
42	0	14	20	0	0	0	12	0	20	12
Total resistance (%)	42 (100%)	18 (42.9%)	13 (30.7%)	12 (28.6%)	31 (73.8%)	42 (100%)	33 (78.6%)	13 (83.3%)	7 (16.7%)	31 (73.8%)

Key: CA= Co-amoxiclav, CN= Gentamycin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin, SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxacillin.

Table-2: Measurement (in millimeters) of the antibiotic zones of inhibition of E. coli isolates from layers.

these organisms as E.coli. It is not uncommon for the pattern of antibiotic resistance to change over the course of several years. This study demonstrated that *E.coli* is very present in chicken samples and each of the isolates was resistant and susceptible to at least a particular antibiotic. Several studies have shown that *E. coli* is present in different fresh chicken samples.

We observed that the overall resistance patterns to antibiotics of E. coli isolates from the broilers and layers was similar (figure 1). While the number of E. coli isolates was slightly higher than that from broilers, the organisms isolated from them demonstrated similar antibiotic sensitivity/resistance patterns. It is generally known that layers are slaughtered at a much older age compared to broilers and are likely to be infected with E. coli.

All isolates were resistant to co-amoxiclav and septrin; with very high resistant rates to chloramphenicol (75.9%), amoxicylin (73.4%), streptomycin (72.2%) and sparfloxacin (67.1%). Just as we observed, E.coli isolates have been demonstrated to be highly resistant to the β-lactam penicillins, septrin and chloramphenicol.[1,2,19,20] While we observed that our isolates were resistant at varying levels to all the commonly available antibiotics tested, multidrug resistant (MDR) E. coli isolates from poultry are frequently observed, especially if they have been widely used in poultry industry over a long period.[1,12,13,16] This observation of MDR E. coli have been observed in other parts of the country[1,21] implying that this is a country-wide problem and not specific to any region. The emergence of MDR E. coli is significantly highest in the food animal industry, acting as reservoir for intra- and interspecific exchange and, a source for spread of MDR determinants through contaminated food to humans; a public health concern that requires monitoring in the future.[22]

The high rate of antibiotic resistance of this organism to some commonly used antibiotics, especially co-amoxiclav and septrin, renders it a difficult to manage this organism in case of any epidemic outbreak among the chickens. Also, infected humans are unlikely to be successfully treated with co-amoxiclav and septrin since similar antibiotic resistant clones are observed among farmers and slaughterers[16] and by extension, consumers of infected products.

The isolates showed a lower rate of antibiotic resistance to the fluoroquinolones; pefloxacin (31.6%), ofloxacin (26.6%) and ciprofloxacin (20.3%). But the observed rate of resistance to the quinolones is considerably high compared to that made by Okoli et al which demonstrated no resistance of E. coli isolated from layers to the quinolones in 2005, in the same state[19] and Adeyanju et al who demonstrated a resistance rate of 16% to ofloxacin in Oyo state in 2014[1] Perhaps this is an observed rise in antibiotic resistance to the quinolones in poultry animals over a period of 13years. The implications are obvious; though the quinolones are most effective antibiotic, a cautionary approach must be instituted to avoid a rising situation of E. coli resistance in the future rendering them ineffective.

CONCLUSION

Out of 200 chicken samples, 79 were positive for *Escherichia coli* pure isolates. In comparison, the number of E. coli isolates from layers was slightly higher than that of broilers but there was a similarity between their antibiotic susceptibility patterns.

The quinolones Pefloxacin, Ofloxacin and Ciprofloxacin were the most effective antibiotics against the isolates while Septrin and Augmentin had no effect on E. coli from the chickens. There's a need for continuous monitoring of antibiotic susceptibility patterns in order to influence antibiotic use among farm animals by farmers and protect presently effective antibiotics used against E. coli infections.

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