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Multi-drug Resistant *Escherichia Coli* from Poultry: A Potential Source of Antimicrobial Resistance Spread in Poultry Farm Environment

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Abstract

Background: *Escherichia coli* is known to inhabit the gastrointestinal tract of poultry and other animals. *E. coli* infection can cause major economic losses in poultry production. The development of resistance to the commonly used antimicrobials in the treatment of such infection can be a setback in the poultry sector.

Objective: This study was carried out to determine the antibiotic resistance profile of *E. coli* isolated from chickens in Imo State, Nigeria.

Materials and Methods: Twelve poultry farms were selected across the 3 senatorial zones of the state using purposive random sampling. A total of 120 cloacal samples were collected from chickens using sterile swabs. The samples were streaked onto MacConkey agar (MCA) and incubated at 37°C overnight. Pink colonies on the MCA were streaked onto eosin-methylene blue agar, incubated for 24 hours at 37°C, and confirmed by indole, methyl red, Voges-Proskauer, and citrate (IMVIC) tests. Antimicrobial susceptibility test was carried out using disc diffusion technique, and the inhibition zone diameter was measured and recorded as resistant or susceptible.

Results: Twenty isolates out of the 120 samples were identified as *E. coli*. Twenty isolates were highly resistant to 5 antibiotics out of 10 antibiotics used. Antibiotic resistance patterns were as follows: amoxicillin (AMP) (95%), cephalothin (CEP) (90%), nalidixic acid (NA) (85%), trimethoprim (SXT) (85%), cefoperazone (PEF) (80%), ampicillin (AMP) (70%) ofloxacin (OFX) (15%), ciprofloxacin (CIP) (10%), gentamicin (CN) (10%), and streptomycin (S) (10%). Sixteen resistance patterns were recorded with AMC-SXT-AMP-CEP-NA-PEF being the most prevalent.

Conclusion: This study shows that multi-drug resistant *E. coli* strains are present in poultry farms in Imo State.

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Background

Escherichia coli is the most frequently isolated microorganism that inhabits the digestive system of poultry, humans, and other animals.[1] Many *E. coli* strains were reported to be non-pathogenic and may serve as indicators of fecal contamination of food and water.[2] The likely source of *E. coli* in chickens may be some materials like litter, fecal droppings, wood shavings from poultry houses, rodent feces, water and feed.[3] The majority of *E. coli* strains are commensals which colonize the intestinal tracts of both humans and animals but some strains (e.g., O157:H7) are associated with the production of toxins which can cause acute infection[1]. Some normal non-pathogenic strains have been reported to cause infection in immune-suppressed hosts such as poultry, humans, and animals.[4]

Escherichia coli infection can cause major economic losses in poultry production by causing various diseases in chickens, resulting in mortality as well as condemnation of meat.[5] Most *E. coli* strains developing resistance to different antibiotics is worrisome globally.[6] In the study by Sharma et al on antimicrobial resistance of *E. coli* from food animals from Nigeria, multidrug-resistant *E. coli* strains were detected.[7] Humans can be infected with resistant *E. coli* from chicken by ingesting contaminated food particles. These resistant *E. coli* strains may colonize the intestinal tract and transmit drug resistance genes to non-pathogenic commensals.[8]

Different types of antimicrobials are incorporated in feeds for raising chickens in several countries including Nigeria.[9] This overuse of antimicrobials in human/veterinary medicine and agriculture results in

the development and dissemination of antimicrobial-resistant pathogens.[10] The abuse of such important antimicrobials in humans and poultry leads to an increase in antimicrobial resistance in different microorganisms including the normal flora of the digestive system.[11] Non-pathogenic *E. coli* may harbor antimicrobial resistance genes which can be transferred to other pathogens colonizing the animal host. This would result in poor management of the infection leading to economic losses and may be a means of transferring resistance genes to humans. Antimicrobial-resistant strains of *E. coli* pose a great challenge to public health since they might be transferred to humans through ingestion of contaminated food or direct contact with diseased chickens. These resistant *E. coli* isolates can transfer these antimicrobial resistance genes to other microorganisms.[12]

The indiscriminate use of antibiotics may be the cause of the growing incidence of antibiotic resistance in Nigeria because there is no strict adherence to regulations guiding the use of antibiotics in poultry and other livestock in Nigeria.[13-15] There is evidence of an increasingly high prevalence of antibiotic-resistant *E. coli* in Imo state and other parts of the country without any seasonal variation.[16,17] This supports the need for regular assessment of antibiotic susceptibility patterns of *E. coli* from farms in the study area to guide practitioners and policy-makers on the best way to curb the menace of antibiotic resistance in the environment. This study was therefore conducted to phenotypically isolate *E. coli* strains from selected poultry farms in different zones of Imo state, Nigeria, and determine their susceptibility to commonly used antibiotics.

Materials and Methods

A total of 120 cloacal samples were collected from apparently healthy chickens using sterile swabs. Twelve poultry farms were selected using purposive sampling from 3 senatorial zones of Imo State. Forty samples were collected from 4 poultry farms in each zone using simple random technique. The samples were labelled and transferred in cold chain to the laboratory immediately for processing and identification.

Isolation and Identification of Escherichia coli

Each sample was streaked onto MacConkey agar (MCA) (Oxoid, England) and incubated at 37°C for 24 hours. Pink colonies on MCA were sub-cultured on Eosin Methylene Blue (EMB) agar (Oxoid, England) and incubated at 37°C for 24 hours. Colonies with a greenish metallic sheen appearance on the EMB agar were considered presumptive *E. coli* and were further identified by biochemical tests (indole, methyl red, and Voges-Proskauer tests) (HiMedia, India). All isolates that were indole and methyl red positive and Voges-Proskauer negative were confirmed as *E. coli* and evaluated for antimicrobial resistance.

Antimicrobial Susceptibility Testing

Disc diffusion technique on Mueller-Hinton agar (Oxoid, England) was used as recommended by Clinical and Laboratory Standard Institute (CLSI) document M100-S15.[18] The antibiotics used in this study included ampicillin (AMP), amoxicillin clavulanic acid (AMC), cephalothin (CEP), ciprofloxacin (CIP), gentamicin (CN), nalidixic acid (NA), cefoperazone (PEF), ofloxacin (OFX), trimethoprim (SXT), and streptomycin (S) (Oxoid, England). The inhibition zone diameter around each antibiotic disc was measured in millimeters after incubation and recorded for each isolate. The measured inhibition zone diameters were interpreted using the breakpoints recommended by the CLSI[18] and recorded as resistant or susceptible.

All data generated were presented using tables, percentages, and pie chart.

Results

Twenty (16.67%) out of the 120 fecal samples of chicken collected from various farms in the zones of the state harbored *E. coli*. The prevalence rates of *E. coli* among samples from Owerri, Okigwe, and Orlu zones of Imo State were 8 (40%), 6 (30%), and 6 (30%), respectively (Figure 1).

All the 20 *E. coli* isolates from the study area showed high resistance to AMP (90%), CEP (90%), NA (85%), SXT (85%) PEF (80%), and AMP (70%). Low resistance was shown to CN (30%), CIP (25%), OFX (15%), and streptomycin (10%) (Table 1).

E. coli isolates showed 16 different resistance patterns as displayed in Table 2. The most predominant resistance pattern was AMC-SXT-AMP-CEP-NA-PEF with a frequency of 5.

The number of antibiotics to which the *E. coli* isolates were resistant ranges from 2 to 10 (Table 3). Out of 20 isolates tested, 2 (10%) were resistant to two antibiotics, 3

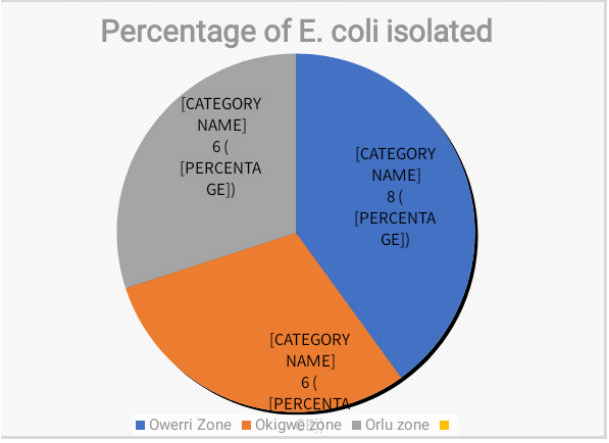

Figure 1. Distribution of *Escherichia coli* Isolated From Chickens in Different Zones of Imo State

Table 1. Resistance and Sensitivity of *Escherichia coli* Isolates from Various Zones of Imo State

Antibiotics	Resistant (%)	Susceptible (%)
Amoxicillin	18 (90)	2 (10)
Cephalothin	18 (90)	2 (10)
Trimethoprim	17 (85)	3 (15)
Nalidixic Acid	17 (85)	3 (15)
Cefoperazone	16 (80)	4 (20)
Ampicillin	14 (70)	6 (30)
Gentamicin	6 (30)	14 (70)
Ciprofloxacin	5 (25)	15 (75)
Ofloxacin	3 (15)	17 (85)
Streptomycin	2 (10)	18 (90)

Table 2. Multidrug Resistance Pattern of *Escherichia coli* Isolated from Various Zones in Imo State

Resistance Pattern	No (%) of isolates
AMC – NA	1 (5)
NA – PEF	1(5)
AMC – SXT – CEP – NA	1(5)
AMC – SXT – AMP – CEP	1(5)
AMC – SXT – CEP – PEF	1(5)
AMC – AMP – CEP – NA – PEF	1(5)
AMC – SXT – CEP – NA – PEF – CN	1(5)
AMC – SXT – AMP – CEP – NA – PEF	5(25)
AMC – SXT – CEP – OFX –NA – PEF	1(5)
CIP –SXT – AMP – CEP – NA – PEF	1(5)
AMC – CIP – SXT – AMP – CEP – NA – PEF	1(5)
AMC – SXT – AMP – CEP – NA – PEF – CN	1(5)
AMC – SXT – S – AMP – CEP – NA – CN	1(5)
AMC – CIP – SXT – AMP – CEP – PEF CN	1(5)
AMC – CIP – SXT – AMP – CEP – OFX – NA – PEF – CN	1(5)
AMC – CIP – SXT – S – AMP – CEP - OFX – NA – PEF – CN	1(5)
Total	20 (100)

Ampicillin (AMP), Amoxicillin clavulanic acid (AMC), Cephalothin (CEP), Ciprofloxacin (CIP), Gentamicin (CN), Nalidixic Acid (NA), Cefoperazone (PEF), Ofloxacin (OFX), Trimethoprim (SXT), and Streptomycin (S).

Table 3. Number of Antibiotics to Which *Escherichia coli* Isolates Were Resistant

No. of Antibiotics	No (%) of Resistant *Escherichia coli* isolates
1	0 (0)
2	2 (10)
3	0 (0)
4	3 (15)
5	1 (5)
6	8 (40)
7	4 (20)
8	0 (0)
9	1 (5)
10	1 (5)

(15%) to 4 antibiotics, 8 (40%) to 6 antibiotics, 4 (20%) to 7 antibiotics, and 1 (5%) isolate was resistant to 5, 9, and 10 antibiotics (Table 3).

Discussion

Escherichia coli is a commensal organism of the intestine of chickens, humans, and other livestock. In immunocompressed hosts, non-pathogenic *E. coli* strains may become pathogenic and cause disease or infections in chickens, humans, and other livestock.[19] The prevalence of *E. coli* in the 3 zones of Imo State studied was found to be 16.67%. This may be due to management practices because most of the farms administer antibiotics as growth promoters and for prophylaxis. This finding is lower than the finding by Odoemene and Enwere[20] who reported a prevalence of 39.5% in Imo State. This finding is also lower than 83.3% isolation rate from poultry faeces and meat reported by Al-Salauddin et al[21] but it is similar to the finding of Glasner et al[22] who reported a prevalence of 16.8% in Greece. The isolation rate of *E. coli* from faecal material might be high.[1] The difference in the studies might be due to different management practices, general hygiene, environmental sanitation, feeding habits, absence of underlying infections, and antibiotics applied in various farms or locations. The phenotypic method of identification used in this study is simple and easily adaptable in most rural settings where access to more modern methods is unavailable and it is recommended to be employed for monitoring of antimicrobial resistance in farms in rural areas.

In this study, resistance to more than one class of antibiotics was detected among the isolates tested. This is in line with the results of studies conducted by Adenipekun et al[23] and Ejeh et al[24] who reported that resistance to more than two classes of antibiotics is mostly seen among multidrug-resistant *E. coli* strains. This might be due to the abuse of antimicrobials by farmers in the study area. The indiscriminate use of antibiotics in veterinary medicine for prophylaxis has been reported to increase the emergence and dissemination of antimicrobial-resistant microorganisms.[25] The level of resistance to these antimicrobial classes found in this study and other reports may be the result of inappropriate or continuous use of the same drugs in poultry farms.[26] A recent study shows that most of the farms sampled in the study area often or always use tetracycline, macrolide, aminoglycoside, and penicillin classes of antibiotics for disease prevention, treatment, and growth promotion.[27] This could lead to the development of resistance among commensal organisms which could be transferred to pathogenic organisms through plasmids in conjunction with other genetic factors and increase resistance to these antimicrobials.[28,29] This will make the bacterium liable to acquire resistance genes through conjugation or transformation.[30,31]

Escherichia coli showed high level of resistance to AMP in the study area. This is partly due to the fact that AMP is not a well-established antibiotic for animal therapy. As reported by Breijyeh et al[31] and Krishnamoorthy et al,[32] it can be concluded that *E. coli* is basically resistant to the curative dose of penicillin G due to its outer membrane barrier and various classes of antibiotics with obvious mechanisms of action. The rate of resistance to AMP was higher than the one reported by Ibrahim et al.[33] The level of resistance to CN reported in this study is low and similar to the low resistance level reported by Bywater et al.[34] However, CN as a relatively old antimicrobial agent is not mostly used in the study area. This is mostly because it is presently available only in injectable form not in sachet or oral forms and is usually administered by veterinarians. The low level of resistance to CN observed in *E. coli* isolates from chickens may be due to the use of unapproved drugs or the spread of resistant isolates that originated from a single progeny as suggested by Kijima-Tanaka et al.[35]

The levels of antimicrobial resistance reported in this study are similar to those documented for coliforms isolated from fecal and non-fecal samples by McKeon et al[36]. The increase in the resistance rate recorded in this study may be due to the indiscriminate use of antibiotics in Imo State for prophylaxis and therapeutic purposes in poultry production. The inappropriate use of antibiotics for sub-therapeutic and therapeutic purposes in poultry and other livestock and humans promotes the spread of resistant bacteria with plasmids. Nsofor and Iroegbu[37] reported that pathogenic as well as non-pathogenic *E. coli* strains that are resistant to antibiotics may be passed from chickens to humans through food.

The possibility of the transmission of antimicrobial resistance from intestinal bacteria from food animals to the human population is of great importance. Exposure to food animals and their feces and ingestion of foods of animal origin are the major means of spreading resistance from food-producing animals to humans. The antimicrobial resistance and sensitivity results of this study revealed that chickens in Imo State harbor *E. coli* strains which are resistant to various antimicrobials commonly used in veterinary and human medicine. The way chickens and other farm animals are kept may be a predisposing factor for the establishment of infection thereby requiring antibiotic use for the prevention and management of the infection and thus contributing to the antimicrobial resistance of organisms to these agents.

The very low rate of resistance to CN, OFX, and streptomycin indicates that these antimicrobial agents can be used for therapeutic purposes when the disease occurs among chickens in the study area.

Conclusion

Escherichia coli strains that were resistant to many antimicrobials were isolated from chickens in farms in the 3 zones of Imo State and this may be a threat to poultry, livestock, and public health. This is because they can serve as reservoirs for the transmission of antimicrobial resistance attributes to pathogenic bacteria. The multi-drug resistant *E. coli* could also pose a problem of drug failure with the resultant rise in the cost of treatment and possible increased mortality in cases of infections with *E. coli* and other enteric organisms. Therefore, there is a need for regular assessment of the antibiotic susceptibility profile of *E. coli* from chickens in the study area to guide the use of appropriate antimicrobials in case of enteric infections and reduce the risk of developing resistance caused by wrong or indiscriminate use of antibiotics by farmers and animal health practitioners.

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Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Approval

Not Applicable.

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