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Antibiogram Characteristic of PCR-confirmed *Escherichia coli* Isolates from Cloacae of Chickens in selected Poultry in Abakaliki Metropolis, Ebonyi State, Nigeria

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Abstract

Background: Demand for poultry products has increased in recent years, as more people realize the nutritional and economic value of chicken and their products. Bacterial diseases associated with the consumption of poultry products remain a public health threat to the poultry industry. This study seeks to carry out the antibiotics susceptibility profiles of *Escherichia coli* isolated from chicken cloacal swabs in poultry farms in Abakaliki metropolis. **Methodology:** A total of nine samples were collected over a 3-month period using the random sampling technique, to investigate the presence of *E. coli* using the streak plate method ,on Eosine Methylene Blue Agar. Polymerase chain reaction technique was used to confirm the identity of the isolates. Susceptibility profile of the isolates was determined using the Kirby-Bauer disc diffusion assay containing varying concentrations (5 µg - 30µg) of selected antibiotics. **Results:** Twenty-six *E. coli* isolates were selected for the antibiotic susceptibility testing, from which the Multiple Antibiotic resistance profile(MARP) and Multiple Antibiotic resistance index (MARI) were determined. Counts of *E. coli* obtained across the sampling locations ranged from 150-517 CFU/g. The 26 isolates were resistant to Cefuroxime, Doripenemas, and other antibiotics in the order: 97% for Chloramphenicol, 96% each for Sulfamethoxazole-Trimethoprim and Tetracycline, and 73% each for Ciprofloxacin and Gentamycin. MARP ranged from 10 drugs (CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR) to 4 drugs (CXM/MRP/TE/NOR). All *E. coli* isolates had from 0.4-1.0. **Conclusion:** Fndings indicated a high prevalence of multiple drug-resistant *E. coli* in the cloacae of chickens, necessitating the need for better surveillance, improved hygiene practices, alternative therapy and monitoring of antibiotic resistance patterns in animal husbandry.

Keywords: Poultry, *Escherichia coli*. Antibiotics, Multidrug-resistance, Public-health.

1.Introduction

The term poultry refers to all domesticated birds kept for the purpose of egg or meat production. These include chickens (domesticated fowls), turkeys, ducks, geese, among others. Having a poultry farm is a very lucrative business and also an important economic component of the agro

industry. Aside being a source of nutrient to man, these food products serve as vehicles for the dissemination of pathogens to humans and animals (El-sharkawy *et al*., 2017).

Escherichia coli has been reported to be among the leading causes of infections from contaminated foods, and also a natural inhabitant of the gut in poultry and most other animals, as poultry remain one of the most preferable reservoirs for *E. coli*. The meat is thought to be the most common source of *E. coli*, accounting for about 40% of clinically reported cases (Smith *et al*., 2018). It is one of the most common causes of bacterial diseases in the poultry and avian species, resulting in significant economic losses and health challenges (Nolan *et al.*, 2013). Chicks can be infected with *E. coli* by vertical transmission through hatcheries, mating in contaminated hatcheries, transportation of equipment, feed and cloacal infection (Wales and Davies, 2020).

The use of antibiotics in poultry farming for growth promotion and disease treatment has been identified as a major driver of anti-microbial resistance (AMR) in bacterial pathogens, including *E. coli* (Silbergeld *et al*., 2008). AMR in *E. coli* can occur through several mechanisms, such as the acquisition of resistance genes via horizontal gene transfer and mutations in chromosomal genes (Bradley, 2012). Polymerase chain reaction (PCR) is a molecular biology technique that can amplify DNA sequences of interest and is widely used in the detection of antibiotic resistance genes in bacterial pathogens (Tang *et al*., 2017). It can be used to detect the presence of specific genes that are associated with antibiotic resistance in *E. coli* strains (Titilawo *et al*., 2015).The cloaca is the common opening for the urinary, digestive and reproductive tracts in birds. Cloacal swabs and chicken droppings have been shown to be useful samples for the isolation and characterization of *E. coli* in poultry, as *E. coli* has been established to be relatively abundant *Enterobacteriaceae species* in both cloacal swabs and litters, to provide valuable information on the prevalence and patterns of antibiotic resistance in poultry (Gupta *et al*., 2021).

Ebonyi is an agriculturally-based state. In addition to rice production, poultry rearing is one of the most lucrative businesses embarked upon by the inhabitants. Commercial poultry farms play a vital role in the economy of the state. In Abakaliki metropolis, the economic aspect of poultry diseases, and their mortality and morbidity owing to bacterial infections and concomitant increase in antibiotic resistance pattern, is a matter of great concern to livestock owners (Munonye *et al*., 2023). Therefore, the current study is aimed at isolating, identifying and elucidating the antibiotic susceptibility profile of *E. coli* from chicken cloacae from selected poultry in Abakaliki metropolis, Ebonyi state.

2.Materials and Methods

2.1 Description of Study Areas

A cross-sectional study was conducted in three poultry farms in Abakaliki – Chi-boy farms (6.3208492°N; 8.0805964°E), NUC farms (6.3229°N; 8.1246°E) and Chizik farms (6.335443°N; 8.097797°E). These are farms where chickens are raised intensively in congested environments, and there is a high use of antibiotics for growth promotion and infection management.

2.2 Collection of cloacal swab samples

Cloacal swabs were collected from apparently healthy chickens from three different poultry farms. Triplicate sampling was carried out over a 3-month (January-March 2023) sampling duration. A total of nine (9) cloacal swab samples, comprising three each from three different locations using sterile swabs. Samples were transported on ice to the research laboratory of Alex Ekwueme Federal University, and processed immediately.

2. 3 Isolation of *E. coli*

All samples were processed and plated on Eosin Methylene Blue (EMB) medium. The samples were labelled into three groups (Sample IDs 1-3) and the culture plates were divided into three parts (locations A, B and C). After incubation for 24 h, distinctive metallic green sheen colonies were observed, counted and recorded as appropriate. Presumptive *E. coli* isolates were picked for molecular confirmation.

2.4 Molecular identification of *E. coli* isolates

2.5 DNA Extraction

DNA extraction was done using the conventional boiling method as described by (Levy *et al*., 2020). The mixture (2-3 colonies of *E. coli* colonies and 200 µl) was boiled at 100℃ for 15min, and centrifuged for 10minutes at 12,000 rpm. Thereafter, the supernatants containing the template DNA were carefully drawn and transferred into sterile Eppendorf tubes and stored at -20°C in the refrigerator. The template DNA was used for PCR amplification.

2.6 PCR amplification and Electrophoresis

PCR was performed to amplify *uid*A gene of *E. coli*. Two different primer pairs (F: 5'-AAAACGGCAAGAAAAAGCAG-3') and (R: 5'-ACGCTTAACAGTCTTGCG-3') were used as described (Schippa *et al.,* 2010). Amplification was carried out using PCR thermocycler (Applied Biosystems 2720 Thermal cycler). The cycling conditions were; initial denaturation 95°C for 3 minutes, and 35 cycles of 95°C for 45 s, 55°C for 45 s and 72°C for 60 s. The final extension was at 72°C for 5 min and held at 4°C. After that, the amplicons were run on 1.5% agarose, by gel electrophoresis, using Tris Borate EDTA (TBE) buffer at 100 V for 45 min. The gels were then stained with ethidium bromide and read on an electrophoresis power tank (EDVOTEX) UV light trans-illuminator, to confirm the amplifications of the *uid*A target region.

2.7 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed on the PCR-confirmed *E. coli* using the Kirby–Bauer disc diffusion assay (Kirby-Bauer *et al*., 1966). Conventionally used antibiotics , such as, Trimethoprim-sulfamethoxazole (25 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Tetracycline (30 µg), Meropenem (10 µg), Doripenem (10 µg), Gentamycin (10 µg), Amikacin

(30 µg), Chloramphenicol (30 µg) and Cefuroxime (30 µg) were used. Pure colonies of *E. coli* were transferred to a micro-centrifuge tube containing 2 ml of 0.85% physiological sterile saline and gently vortexed. The suspensions were modified to obtain turbidity corresponding to 0.5 McFarland standard solutions, homogenized unto Mueller-Hinton Agar plates using a sterile cell spreader, and incubated at 37 °C for 18 h. After incubation, the inhibition zone diameters of each antibiotic used, were measured using ruler, and results were interpreted following the CLSI standards (CLSI, 2021). An isolate was tagged as multidrug-resistant if resistant to three or more drugs from different classes of the antibiotics used (Titilawo *et al*., 2015).

2.8 Determination of Multiple Antibiotic Resistance phenotypes and index (MARP and MARI)

An isolate with a MARI value of > 0.2 indicates the presence of numerous antibiotic-resistant bacteria, as well as highly resistant bacteria. MARP of the isolates that showed resistance to three or more antibiotics were generated. (Krumperman *et al*., 1983).

MARI was calculated in order to determine the level of antibiotic resistance of each individual bacterial isolate. It was determined using the mathematical expression:

$$MAR_{index} = a/b$$

where "a" represents the number of antibiotics that will be successfully resistant and "b" represents the total number of antibiotics employed in the study.

3.Results

3.1 Enumeration of *E. coli*

The *E. coli* colonies were counted on Eosin methylene blue agar plates and recorded (Table 1). While the highest counts (517CFU/g) were obtained in sample ID 1 (Location A), lowest (150CFU/g) was in sample ID 2 (Location B).

Table 1: Counts of *E. coli* colonies on EMB agar plates

Sample ID	Location A	Location B	Location C
1	517	190	411
2	210	150	TNTC
3	TNTC	Nil	Nil

TNT3. C; Too numerous to count.

3.2 PCR confirmation of *E. coli*

PCR confirmation of *E. coli* isolates was performed. The figure 1 below shows the representative gel electrophoresis image of the *E. coli* isolates.

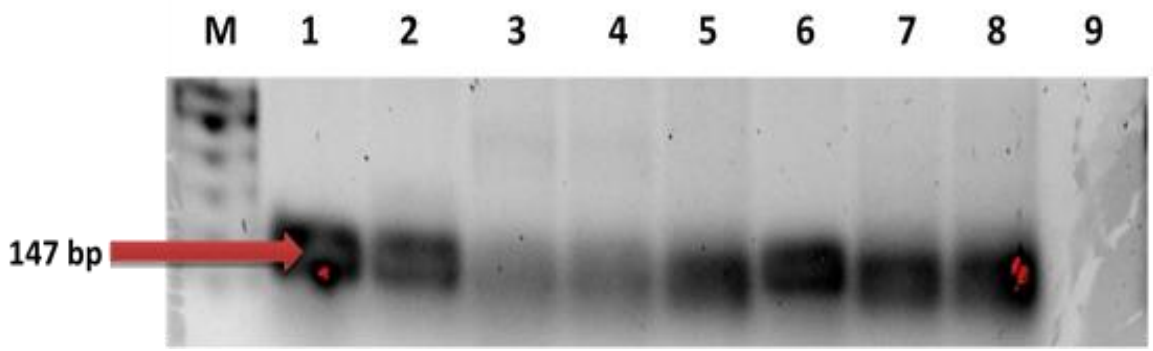

Figure 1. PCR confirmation of *E. coli* isolates from cloaca of chicken through the amplification of *uid*A gene (147bp). Lanes 1: positive control, Lane 2 to 8: representative positive isolate; Lane 9: negative control; Lane M: molecular weight maker (100bp).

3.3 Antibiotic susceptibility testing

Interestingly, all isolates were resistant to Cefuroxime. Others were variously resistant in the order: Trimethoprim-sulfamethoxazole (96.15%), Tetracycline (96.15%) and Ciprofloxacin (92.31%). Only Amikacin and Gentamycin showed efficacy against *E. coli* strains with 23.01% and 19.23% susceptibilities respectively. Similarly, isolates 14, 18, 20 and 25 were resistant to all the antibiotics screened (Figure 2).

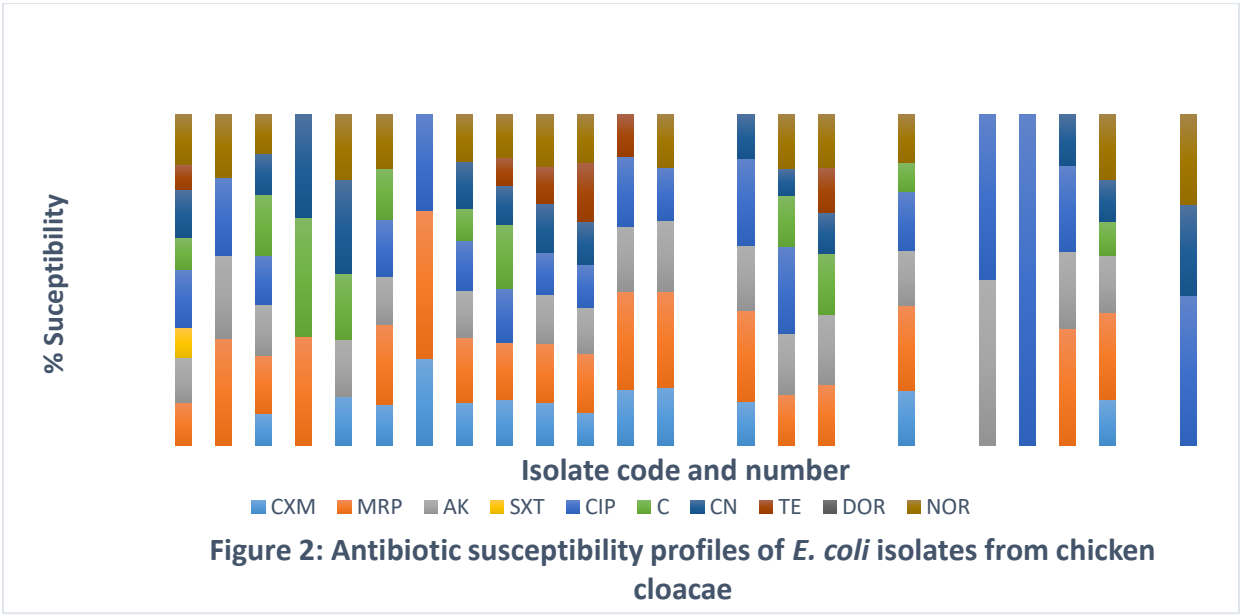

Figure 2: Antibiotic susceptibility profiles of *E. coli* isolates from chicken cloacae

Cefuroxime (CXM); Meropenem (MRP); Doripenem (DOR); Amikacin (AK); Trimethoprim sulfamethoxazole (SXT); Ciprofloxacin (CIP); Chloramphenicol (C); Gentamycin (CN); Tetracycline (TE); Norfloxacin (NOR).

3.4 Multiple antibiotic resistance phenotypes (MARP) and multiple antibiotic resistance index (MARI)

All the *E. coli* isolates were multi-drug resistant. The highest MARP was 10 drugs (CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR), while the least MARP was 4 drugs (CXM/MRP/TE/DOR). *E. coli* isolates exhibited the lowest MARI at (0.4), and highest T(1.0). (Table 2).

Table 2: Determination of MARP and MARI of *E. coli* isolates from cloacal samples

MARP	MARI
CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR	1.0
CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/MRP/SXT/CIP/C/CN/TE/DOR/NOR	0.9
CXM/MRP/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/MRP/AK/SXT/CIP/C/TE/DOR/NOR	
CXM/AK/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/AK/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/SXT/CIP/C/CN/TE/DOR/NOR	0.8
CXM/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/SXT/CIP/C/CN/TE/DOR/NOR	
CXM/MRP/SXT/CIP/CN/TE/DOR/NOR	
CXM/MRP/SXT/CIP/C/TE/DOR/NOR	
CXM/MRP/AK/SXT/CIP/C/TE/DOR	
CXM/SXT/CIP/C/CN/TE/DOR	0.7
CXM/SXT/CIP/C/CN/TE/DOR	
CXM/MRP/SXT/CIP/TE/DOR/NOR	
CXM/MRP/SXT/CIP/C/TE/DOR	
CXM/MRP/AK/SXT/CIP/TE/NOR	
CXM/AK/SXT/CIP/TE/DOR/NOR	
CXM/SXT/CIP/CN/TE/DOR	0.6
CXM/MRP/SXT/CN/TE/DOR	
CXM/AK/SXT/CIP/C/DOR	
CXM/MRP/TE/DOR	0.4

Cefuroxime (CXM); Meropenem (MRP); Doripenem (DOR); Amikacin (AK); Trimethoprim-sulfamethoxazole (SXT); Ciprofloxacin (CIP); Chloramphenicol (C); Gentamycin (CN); Tetracycline (TE); Norfloxacin (NOR).

Discussion

The current study was aimed at investigating antibiogram characteristics of PCR-confirmed *E. coli* isolated from the cloacal swabs of chickens. *E. coli* is one of the most common causes of bacterial diseases in the poultry and avian species, resulting in significant health challenges and economic losses (Nolan *et al.*, 2013).

In this study, *E. coli* counts obtained, ranged between 517CFU/g and 150CFU/g. The population of positive culture samples in this study were relatively higher than study findings in Ethiopia that reported only 140CFU/g of culture positive samples (Bushen *et al.*, 2021). The finding also disagrees with the report of Lee *et al*. (2017), who had lower counts in their chicken cloacal

samples. However, this result is consistent with other studies that have reported high counts of *E. coli* in chicken cloacal samples, as observed in the repot of (Nakamura *et al*., 2015). Differences in prevalence rate in various studies as compared, could be due to sample collection and processing methods, method of isolation, animal management practices, hygienic conditions as well as variations in the chicken production (Conner *et al*., 2003; Bushen *et al.*, 2021).

The *uid*A gene, which encodes beta-glucuronidase enzyme, was used to confirm the identity of the presumptive *E. coli* isolates from chicken cloacal samples. About 90% of the isolates obtained were *uid*A positive. The gene has been employed by various researchers to establish the effectiveness of PCR-based methods for identifying *E. coli* isolates from different sources, including environmental samples (Titilawo *et al*., 2015; Pabst *et al*., 2016; Logue *et al*., 2018),
Furthermore, this study elucidated antibiotic susceptibility profiles of *E. oli* isolated from the cloaca of chickens. All the isolates were resistant to cefuroxime and doripenem. (Langata *et al*.(2019), stated that Omoya and Ajayi (2016) and Ajayi *et al*.(2017), had reported high rates of resistance to these antibiotics in *E. coli* isolated from chicken cloaal samples. Similarly, the study revealed high rate of resistance against tetracycline (96.2%) and trimethoprim-sulfamethoxazole (96.2%). These confirm the resistance rate reported by Bushen *et al.* (2021) and Racewicz *et al.* (2022) at 75% and 70.8% respectively, of both antibiotics. The high resistance against tetracycline is an indication of its frequent usage to treat bacterial infections in livestock (Borghi and Palma, 2014).

In the same vein, highest susceptibilities recorded in this study were to gentamicin (73%) and norfloxacin, (58%). These findings corroborate the report of Smith *et al*.(2018). That high susceptibility rates to these antibiotics in *E. coli* isolated from chicken samples exist.The variations could be attributed to various factors, including geographical location, farm management practices and the use of antibiotics in poultry production (Sandelin *et al*., 2022).
The multiple antibiotic resistance profile (MARP), and multiple antibiotic resistance index (MARI) are widely used measures of antibiotic resistance in bacterial isolates. The MARP measures the percentage of antibiotics to which a bacterium is resistant, while the MARI calculates the number of antibiotics to which the bacterium is resistant, relative to the total number of antibiotics tested (Krumperman *et al.* 1983).

Further, most of the *E. coli* isolates exhibited MARI greater than 0.4. Other studies have reported similar results, with high levels of antibiotic resistance observed in *E. coli* isolates from different sources. Onanuga and Oyekunle (2018), reported MARI values ranging from 0.4 to 1.0 in *E. coli* isolates from chicken cloacal samples in Abeokuta, SouthWestern Nigeria. Gholami *et al*. (2019) had MARI ranging from 0.6 to 0.8 in *E. coli* isolates from poultry faeces in Iran. The highest MARP was 10 drugs (CXM/MRP/AK/SXT/CIP/C/CN/TE/DOR/NOR),which is in line with the report of Tadesse *et al*. (2012), whose MARP was 8, indicating a high level of antibiotic resistance in *E. coli* isolates from chicken carcasses, highlighting the emergence of multi-drug resistant *E. coli* in chickens, which is a public health issue. The least MARP was 4 drugs (CXM/MRP/TE/DOR), which is in conformity with the report of (Katakweba *et al.*, 2012)

Conclusion

The study investigated the antimicrobial susceptibility, and PCR-confirmation of E. *coli* isolated from chicken cloacal swabs. The results of this study revealed that *E. coli* was present in a significant proportion in the samples, indicating a high prevalence of the bacterium in the chicken population. The antimicrobial susceptibility testing revealed resistance to several classes of antibiotics, including tetracycline, and ciprofloxacin, which are commonly used in poultry farming. Furthermore, the PCR-based detection revealed the presence of *uid*A gene, which are associated with pathogenicity, and can potentially cause infections in humans. These findings suggested that bird (avian) farms may serve as reservoirs of antibiotic-resistant bacteria that can infect humans directly by contact with chicken droppings or indirectly through food chain. Hence, the need for emphasis on the usage of antibiotics like tetracycline in poultry farm has to be reconsidered and a comprehensive surveillance program to monitor hygiene practices and responsible use of antibiotics in poultry farming.

Recommendation

Based on the findings of this study, judicious management and use of antibiotics necessary, to prevent microbial resistance, regular monitoring of microbial resistance to identify the emergence and spread of resistant strains of *E. coli* Improved hygiene practices should be implemented to reduce the risk of infection and contamination.

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Ethical Clearance

The study did not require ethical permission

Competing Interest

The authors declare no competing interest.

Author's Contribution

Study conception and design: Titilawo, O.Y.; Ali, C.M.; Udechukwu Festus, A.

Collection of Samples: Udechukwu Festus, A.

Provision of funds: All Authors

Performance of experiment: Udechukwu Festus, A.; Titilawo, O.Y.; Ali, C.M.; Akindele, K.I.; Adeoye, O.O.

Data analysis and manuscript drafting: Titilawo, O.Y.; Ali, C.M.; Udechukwu Festus, A.; Ugwuocha, S.C.; Victor-Ekwebelem, M.O.

Proof reading of the manuscript: All Authors

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