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Investigation of Antibiotic Resistance in *Escherichia coli* Isolated from Poultry Faeces

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ABSTRACT
Antibiotic resistance remains a major global challenge in the treatment of bacterial infections and has been strongly associated with the indiscriminate use of antibiotics. The unregulated administration of antibiotics by poultry farmers for the treatment and prevention of poultry diseases is widely recognized as a potential risk factor. This study, therefore, aimed to investigate antibiotic resistance in *Escherichia coli* isolated from poultry feces. A total of 0.5 g of poultry feces (PF) was collected from each of 20 purposively selected poultry farms into appropriately labeled sterile containers. The isolates were characterized morphologically and biochemically, and their susceptibility to antibiotics was determined using standard procedures. Morphological and biochemical analyses confirmed the presence of *Escherichia* species, with 12 (60%) out of the 20 PF samples testing positive for *E. coli*. The inhibition zones ranged from 8 to 28 mm, and several isolates exhibited resistance to amoxicillin, ofloxacin, streptomycin, gentamycin, and septrin. In conclusion, the findings indicate that the unregulated use of antibiotics in poultry production contributes significantly to the growing problem of antimicrobial resistance.

Keywords: Antibiotics, Resistance, Poultry, isolates, Infections

INTRODUCTION
The significance of antibiotics in modern medicine cannot be overstated. They have been universally recognized by health experts as life-saving agents and are regarded as one of the most outstanding medical discoveries of the twentieth century. Beyond their indispensable role in human health, antibiotics are extensively employed in animal production and husbandry for the treatment of infections and, in some cases, to enhance growth performance (Williams-Nguyen *et al*., 2016). However, the misuse and overuse of antibiotics, particularly in developing and underdeveloped nations, have facilitated the evolution of bacterial resistance mechanisms, enabling target organisms to evade the effects of these therapeutic compounds (CDC, 2023).

Antibiotic resistance occurs when bacteria continue to survive and proliferate in the presence of antibiotics that would ordinarily inhibit or kill them. This phenomenon has repeatedly undermined global efforts to preserve lives and resources, often resulting in increased morbidity and mortality (CDC, 2023). Livestock, which include cattle, goats, sheep, pigs, and poultry, are domesticated primarily for labour and the production of

meat, milk, eggs, leather, and wool. Among these, poultry comprising chickens, ducks, turkeys, and geese constitutes one of the fastest-growing livestock sectors worldwide and provides an affordable source of animal protein through meat and eggs (FAO, 2023). With this rapid expansion, poultry litter, a mixture of faeces, feathers, spilled feed, and bedding materials, has become an abundant agricultural by-product widely utilized as organic fertilizer due to its rich nutrient content, particularly nitrogen, phosphorus, and potassium (Ghaly and MacDonald, 2012).

Despite its agronomic value, poultry litter serves as a well-recognized reservoir of pathogenic and opportunistic microorganisms, including bacteria, fungi, parasites, and viruses. The extensive use of antibiotics in poultry farming for prophylaxis, therapy, and growth promotion has intensified concerns about the proliferation of multidrug-resistant (MDR) microorganisms within poultry environments (Gadde *et al.*, 2018). Consequently, poultry litter has emerged as an environmental hotspot for antimicrobial resistance (AMR) dissemination, posing significant risks to both animal and human health through direct contact, food contamination, and environmental runoff.

Among the bacterial species of concern, *Escherichia coli* (*E. coli*) stands out as a key indicator and reservoir of antimicrobial resistance in livestock production systems. *E. coli* is a Gram-negative, facultative anaerobe that typically resides in the intestinal tract of warm-blooded animals as a commensal organism. However, certain strains acquire virulence determinants located on plasmids, bacteriophages, or transposons, transforming them into pathogenic variants capable of causing both intestinal and extra-intestinal infections in animals and humans. These pathogenic lineages are frequently zoonotic, promoting cross-species transmission through contaminated food, water, or the environment (Kaper and Nataro, 2023).

In poultry, avian pathogenic *E. coli* (APEC) is the principal aetiological agent of colibacillosis, a systemic infection characterised by airsacculitis, septicaemia, peritonitis, and salpingitis (Guabiraba and Schouler, 2015). Colibacillosis remains one of the most significant bacterial diseases affecting poultry globally, leading to considerable economic losses arising from reduced productivity, high mortality rates, and increased antibiotic usage. The extensive and sometimes inappropriate administration of antimicrobials in poultry production systems has accelerated the emergence of resistant *E. coli* strains capable of transferring resistance genes horizontally (via conjugation, transformation, or transduction) and vertically to their progeny (Guabiraba and Schouler, 2015).

Antimicrobial resistance has, therefore, become a pressing global public health concern. According to the World Health Organization (WHO, 2024) and the Centers for Disease Control and Prevention (CDC, 2022), AMR is responsible for approximately 1.27 million deaths annually, with millions more infections becoming increasingly difficult to treat. The One Health approach underscores the interconnectedness of human, animal, and environmental health, highlighting the importance of integrated surveillance, stewardship, and control measures to curb AMR emergence and spread (FAO/WHO/OIE, 2023). Within this dynamic ecosystem, poultry farms play a pivotal role, where the continuous reuse of litter, inadequate waste management, and

indiscriminate antimicrobial use promote the persistence and transmission of resistant microorganisms (Chen *et al*., 2025).

Mechanistically, *E. coli* develops resistance through multiple pathways, including the production of extended-spectrum β-lactamases (ESBLs), carbapenemases, activation of efflux pumps, and enzymatic modification of antimicrobial agents. Resistant strains disseminate via contaminated litter, feed, water, and processing environments, facilitating environmental and foodborne transmission. In developing countries, where biosecurity measures and waste management practices are often suboptimal, the potential for AMR gene propagation from poultry to humans is particularly heightened. Studies across Africa and Asia have reported a high prevalence (up to 60%) of MDR *E. coli* in poultry litter and meat, underscoring the urgent need for continuous surveillance and evidence-based interventions (Dushayeva *et al*., 2025).

Given this background, the present study seeks to investigate and characterize the antimicrobial resistance profiles of *Escherichia coli* isolated from broiler litter. Furthermore, it aims to elucidate the relationship between phenotypic resistance and virulence gene carriage among these isolates, thereby providing insights into the role of poultry litter as a potential reservoir and transmission route for antimicrobial-resistant *E. coli*, with far-reaching implications for public health and biosecurity.

MATERIALS AND METHODS

Collection of samples

The purposive sampling technique was relied upon to collect 0.5 g of poultry faeces from each of 20 poultry farms selected for the study into appropriately labeled sterile containers.

Isolation of *Escherichia coli* from poultry faeces

The method described by Ajayi and Omoya (2017) was adopted, with slight modifications, for the isolation of *Escherichia coli* from poultry feces. Approximately 0.5 g of freshly collected poultry faeces was weighed into a sterile beaker, and 45 mL of sterile distilled water was added. The mixture was thoroughly homogenized for 90 seconds to obtain a uniform suspension. A ten-fold serial dilution was then prepared by transferring 1 mL of the suspension into 9 mL of sterile diluent at each step using a sterile pipette. From the final dilution (10^{-3}), 0.1 mL aliquots were aseptically inoculated onto sterile plates of Eosin Methylene Blue (EMB) agar for the selective enumeration of *E. coli*. The inoculated plates were incubated at 37 °C for 48 hours, after which characteristic colonies with a greenish metallic sheen were presumptively identified as *E. coli*.

Escherichia coli Count

The method described by Langata *et al.* (2019) was adopted to determine the Escherichia coli count. The colonies that developed on the plates were counted and expressed as colony-forming units per gram (cfu/g).

Colonial Morphology Identification

The method described by Cappuccino (2014) was adopted for colonial morphology identification. Presumptive identification of bacterial colonies was carried out by

observing their distinct morphological characteristics such as shape, color, elevation, edge, surface texture, consistency, and overall appearance on the respective culture media. Colonies exhibiting a characteristic metallic green sheen on Eosin Methylene Blue (EMB) agar were noted as presumptive *Escherichia coli*

Purification and Preservation of Bacterial Isolates

The procedure outlined by Langata *et al.* (2019) was used for the purification and preservation of bacterial isolates. Discrete colonies were carefully picked using a sterile wire loop and streaked onto freshly prepared nutrient agar slants to obtain pure cultures. The inoculated slants were incubated at 37°C for 24 hours. Following incubation, the pure cultures were stored in McCartney bottles and preserved in a refrigerator at 4°C for subsequent analyses.

Gram staining

The Gram staining technique described by Cheesbrough (2010) was employed to differentiate the bacterial isolates. A thin smear of each isolate was prepared on a clean glass slide, air-dried, and heat-fixed. The fixed smears were then stained with crystal violet for 60 seconds and rinsed with water. They were subsequently flooded with Lugol's iodine for 60 seconds, followed by rinsing. Decolorization was carried out using acetone-alcohol for 10 seconds, after which the slides were immediately washed with water. The smears were then counterstained with safranin for 2 minutes, rinsed again with clean water, and air-dried. The prepared slides were examined under ×40 and ×100 oil immersion objectives using a light microscope. Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared pink to red.

Biochemical Characterization of Bacterial Isolates

The biochemical characteristics of the bacterial isolates were determined according to the standard procedures described by Cheesbrough (2010) and Ochei and Kolhatkar (2010), with slight modifications. The biochemical tests conducted included catalase, oxidase, citrate utilization, indole production, coagulase, motility, and sugar fermentation tests. These tests were performed to aid in the presumptive identification and differentiation of the bacterial isolates.

Catalase Test

The catalase test was performed following the method of Cheesbrough (2010). About 3 mL of freshly prepared hydrogen peroxide (3%) was placed in a clean test tube. Using a sterile wooden applicator stick, a colony of the test organism was transferred into the hydrogen peroxide solution. The immediate production of effervescence (bubbles) indicated the liberation of oxygen, signifying a catalase-positive reaction. The absence of bubble formation indicated a catalase-negative result.

Oxidase Test

The oxidase test was carried out as described by Ochei and Kolhatkar (2010). A piece of sterile filter paper was placed in a Petri dish, and 3 drops of freshly prepared oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) were added. Using a sterile glass rod, a colony of the test organism was smeared on the reagent-soaked paper. The development of a deep purple-blue color within 10 seconds indicated an oxidase-positive reaction, while the absence of color change indicated a negative result.

Citrate Utilization Test
The citrate utilization test was performed using Simmons citrate agar, following the method of Cheesbrough (2010). The medium was inoculated with the test organism using a sterile inoculating loop and incubated at 37°C for 48 hours. The appearance of visible growth with a deep blue coloration of the medium indicated a positive result, while the absence of growth and retention of the original green color indicated a negative result.

Indole Test
The indole test was performed according to Ochei and Kolhatkar (2008). The test organism was inoculated into peptone water broth and incubated at 37°C for 48 hours. After incubation, 0.5 mL of Kovac's reagent was carefully added to the culture. The formation of a pink to red-colored ring at the interface indicated a positive indole reaction, while the absence of color change denoted a negative result.

Motility Test
Motility testing was carried out as described by Cheesbrough (2010). A semi-solid nutrient agar medium (0.4% agar concentration) was prepared and dispensed into sterile test tubes. The medium was inoculated by stabbing the center with a sterile straight wire containing a colony of the test organism and incubated at 37°C for 24 hours. Diffuse, hazy growth radiating from the line of inoculation indicated motility, while growth restricted to the stab line indicated non-motility.

Coagulase Test
The coagulase test was conducted according to the method of Cheesbrough (2010). A drop of sterile distilled water was placed on each end of a clean glass slide. A colony of the test organism was emulsified in each drop to form thick suspensions. To one of the suspensions, a loopful of plasma was added and mixed gently. The appearance of visible clumping within 10 seconds indicated a coagulase-positive reaction, whereas the absence of clumping indicated a negative result.

Sugar Fermentation Test
Sugar fermentation was determined using Triple Sugar Iron (TSI) agar, following the method described by Cheesbrough (2020). The test organism was inoculated into the TSI slant by stabbing the butt and streaking the slant surface, followed by incubation at 37°C for 24 hours. The resulting color changes and gas/H_2S production were recorded. Yellow slant and butt indicated glucose, lactose, and/or sucrose fermentation; red slant and yellow butt indicated glucose-only fermentation; blackening indicated hydrogen sulfide (H_2S) production; and cracks or bubbles in the medium indicated gas production.

Antibiotic resistance patterns of Escherichia coli isolated from poultry feces
The disc diffusion method described by the Clinical and Laboratory Standards Institute (2020) was employed to determine the antibiotic resistance patterns of *Escherichia coli* isolates obtained from poultry feces. The antibiotic susceptibility profiles of the isolates were evaluated using standard disc diffusion assays on Mueller–Hinton agar (MHA).

Antibiotic discs containing the following agents were used: tarivid (10 µg), riflacine (10 µg), ciproflox (10 µg), augmentin (30 µg), gentamicin (10 µg), streptomycin (30 µg), ceporex (10 µg), nalidixic acid (30 µg), septrin (30 µg), and ampicillin (30 µg). The discs were aseptically placed on the surface of MHA plates previously inoculated with bacterial suspensions adjusted to 0.5 McFarland turbidity standard. The plates were then incubated at 37°C for 24 hours. Following incubation, the diameters of the inhibition zones around each antibiotic disc were measured in millimeters, and the results were interpreted as sensitive or resistant based on the manufacturer's interpretive zone diameter standards in accordance with CLSI (2020) guidelines.

Data Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 20.0 for Windows (IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation (SD); mean separation was performed using the least significant difference (LSD) test. Statistical significance was established at $p < 0.05$.

RESULTS

The morphological and biochemical characteristics of *Escherichia coli* isolated from poultry faeces are presented in Table 1. The organism appeared as pinkish, convex, Gram-negative rods. It tested positive for catalase and motility, but negative for oxidase, indole, citrate, and coagulase. The sugar fermentation test further indicated the absence of hydrogen sulphide production.

Table 1: Morphological and Biochemical characteristics of *Escherichia* coli from Poultry Feces

								Sugar fermentation test				
Morphological Characteristics	Gram reaction	Oxidase test	Indole test	Catalase test	Citrate test	Coagulase test	Motility test	S	B	G	H_2S	Possible organism
Pinkish, convex mucoid colonies	Gram-negative rods	-	-	+	-	-	+	R	Y	+	-	*Escherincia specie*

S = colour of slope, **B** = colour of butt **G**= Gas production, **H_2S**= Hydrogen sulphide production

R= Reddish colouration (alkaline production) **Y**=Yellow coloration (Acidic production)

Figure 1 is a bar chart showing the percentage occurrence of Escherichia coli in poultry feces. It indicates that of the 20 poultry fecal samples collected, 12 (60%) were positive for E. coli, while the remaining 40% were negative.

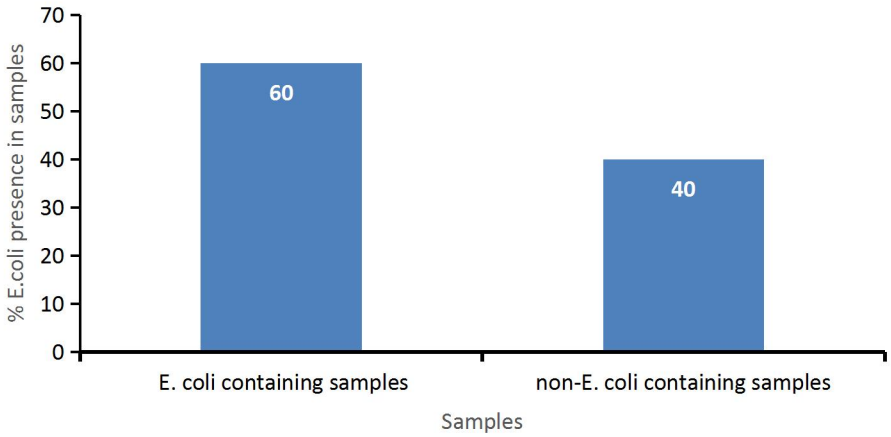

Figure 1: Percentage appearance of *E. coli* in poultry feces samples

Table 2 shows the antibiotic susceptibility pattern of Escherichia coli from the poultry feces samples. The recorded zones of inhibition ranged from 8 mm to 28 mm, with some isolates showing resistance to antibiotics such as amoxicillin, ofloxacin, streptomycin, gentamicin, and Septrin.

Table 2: Escherichia coli Sensitivity to Antibiotics

Isolate	**Antibiotics**							
E. coli	CPX	CN	PEF	E	S	AMX	OFX	SXT
EC 1	-	-	25	14	22	-	24	-
EC 2	22	26	8	22	22	24	-	22
EC 3	26	20	10	18	18	-	-	10
EC 4	12	16	20	24	-	26	24	-
EC 5	16	12	24	26	-	-	14	-
EC 6	18	20	22	24	-	-	-	24
EC 7	12	-	20	-	14	-	-	-
EC 8	-	14	8	12	9	13	-	28
EC 9	16	20	10	18	18	-	-	20
EC 10	28	16	28	24	-	20	22	-

EC 11	16	12	04	26	-	-	14	-
EC 12	18	10	22	24	-	8	12	24

Key: CPX (Ciprofloxacin), CN (Gentamycin), PEF (Peflacine), E (Erythromycin), S (Streptomycin), AMX (Amoxicillin), OFX (Ofloxacin), SXT (Septrin).

DISCUSSION

Primarily, Escherichia coli is domiciled within the lower intestine of humans. It also survives in other warm, moist, nutrient-rich environments and can be found in soil, water, and food as a result of fecal contamination. The presence of *Escherichia coli* in poultry faeces samples confirms that bacteria are naturally present within the enteric flora of poultry birds. This finding aligns with Islam *et al.* (2014), who reported the isolation and identification of *Escherichia coli* and *Salmonella* from poultry. Similarly, Langata *et al*. (2019) isolated *Salmonella* and *E. coli* from chicken droppings in Nairobi, Kenya.

The observed resistance of *E. coli* to ofloxacin, streptomycin, gentamicin, and septrin may be attributed to the indiscriminate use of antibiotics in poultry rearing. This is consistent with Naturinda (2020), who reported that *E. coli* isolates from poultry faeces exhibited resistance to tetracycline, ampicillin, chloramphenicol, and nalidixic acid. In a similar study, Ajayi and Omoya (2016) observed that bacterial isolates from free-range chickens and nine commercial chicken farms in Akure, Ondo State, Nigeria, were resistant to a wide range of antibiotics, including Augmentin, ceftriaxone, nitrofurantoin, amoxicillin, cotrimoxazole, tetracycline, chloramphenicol, gentamicin, ciprofloxacin, ofloxacin, and streptomycin.

CONCLUSION

The study investigated the antibiotic resistance of *E. coli* isolated from poultry feces, revealing that *E. coli* isolated from poultry can resist the bactericidal activity of amoxicillin, ofloxacin, streptomycin, gentamicin, and septrin.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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