



ANTIBIOTIC SUSCEPTIBILITY PROFILES AND MULTIPLE ANTIBIOTIC RESISTANCE INDEX OF
SALMONELLA SPP AND *ESCHERICHIA COLI* FROM POULTRY DROPPINGS FROM SELECTED
FARMS IN SAMARU, ZARIA

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ABSTRACT

The poultry industry represents a critical source of affordable protein globally, yet intensive farming practices involving extensive antibiotic use have created environments favoring the emergence of antibiotic-resistant bacteria, posing significant public health threats. This study aimed to determine the prevalence and antibiotic susceptibility profiles of *Salmonella* species and *Escherichia coli* from poultry droppings collected from selected farms in Samaru, Zaria. Poultry droppings were collected from 10 different farms, and bacteria were isolated using culture techniques involving serial dilution and plating on MacConkey agar, followed by subculturing on Eosin Methylene Blue (EMB) and Bismuth Sulfite Agar (BSA). Isolates were identified through Gram staining and biochemical tests which include citrate utilization, urease, sulfide-indole-motility, methyl red, and Voges-Proskauer tests. Antibiotic susceptibility testing was performed using the disc diffusion method against six antibiotics. Multiple antibiotic resistance index (MAR index) and multidrug-resistant was determined. Thirty bacteria were isolated, comprising of 60% *E. coli* and 40% *Salmonella* species, identified through distinctive colony morphology and biochemical profiles. All isolates, i.e. 100% of the isolates, showed resistance to amoxicillin and linezolid, while ciprofloxacin showed the highest activity with 36.67% of isolates susceptible, followed by gentamicin at 33.33% susceptibility. All thirty isolates exhibited multidrug resistance and MAR index values exceeding 0.2, suggesting that the isolates originated from high-risk sources with significant antibiotic selective pressure. The study concludes that poultry serves as a significant reservoir for multidrug-resistant *Salmonella* species and *Escherichia coli*, with alarming resistance patterns to commonly used antibiotics, though isolates remained highly susceptible to ciprofloxacin and gentamicin, highlighting the urgent need for antimicrobial stewardship interventions in poultry production systems.

Keywords: Antibiotic-resistance, *Escherichia coli*, poultry, *Salmonella* species

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INTRODUCTION

The poultry industry represents one of the fastest-growing segments of global food production, providing affordable protein to billions of people worldwide [1]. Modern intensive poultry farming relies heavily on antibiotics to maintain animal health, prevent disease outbreaks, and enhance production efficiency [2]. However, this dependence has inadvertently created an environment conducive to the selection and amplification of antibiotic-resistant microorganisms, posing severe threats to public health [3]. The World Health Organization has identified antimicrobial resistance as one of the top ten global public health threats facing humanity, with agricultural antibiotic use playing a substantial contributory role [4].

Antibiotics have been used in poultry production since the 1950s, initially demonstrating remarkable benefits in reducing mortality rates and improving feed conversion efficiency [5]. The practice expanded rapidly as producers recognized economic advantages, leading to routine administration of sub-

therapeutic doses for growth promotion and prophylactic purposes [6]. Contemporary estimates suggest that approximately 73% of all antimicrobials sold globally are used in food-producing animals, with poultry accounting for a significant proportion of this consumption [7]. This massive antibiotic usage has created intense selective pressure within poultry production systems, favoring the survival and proliferation of resistant bacterial populations [8].

The connection between agricultural antibiotic use and human health consequences has become increasingly evident through epidemiological studies and molecular investigations [9]. Resistant bacteria emerging in poultry can colonize the human gastrointestinal tract, cause difficult-to-treat infections, and transfer resistance genes to human-associated microorganisms [10]. The transmission pathways are diverse and include consumption of undercooked poultry products, cross-contamination during food preparation, occupational exposure among poultry workers, and environmental spread through waste

disposal [11]. These multiple routes of exposure amplify the public health risks associated with antibiotic resistance in poultry production systems [12].

The economic implications of antibiotic resistance extend beyond healthcare costs to encompass agricultural productivity losses and international trade restrictions [13]. Countries implementing stringent antibiotic use regulations may face competitive disadvantages against nations with less restrictive policies, creating tensions between food security objectives and public health protection [14]. Additionally, the pharmaceutical industry has reduced investment in new antibiotic development due to limited profitability, exacerbating concerns about diminishing treatment options for resistant infections [15].

The urgency of addressing antibiotic resistance in poultry production stems from projections indicating continued growth in poultry consumption and antibiotic use in developing regions [16]. Without coordinated global action, the burden of resistant infections will disproportionately affect low and middle-income countries with limited healthcare infrastructure [17]. This study aimed to determine antibiotic susceptibility profile and multidrug resistant index of *Salmonella* species and *Escherichia coli* from poultry droppings from selected poultry farms within Samaru, Zaria.

MATERIALS AND METHODS

Study area

This study was conducted in Samaru town, a ward in Sabon Gari local government of Kaduna state, Nigeria. The town is semi-urban area located on latitude 11° 09' 30.99" N and longitude 7° 38' 15.68 to 7° 39' 20.68" E.

Collection of poultry droppings

Poultry droppings were collected from 10 different poultry farms within Samaru, Zaria, Kaduna State. Samples collected from each of these poultries were dry feces inside the poultry cages. Using sterile gloves, litter was mixed and samples were collected in sterile universal sampling bottles and transported immediately to the laboratory for microbiological analysis.

Bacteria isolation from poultry droppings' samples via culture technique

One gram of poultry dropping matter was measured and dissolved in 9 ml of peptone water. The solutions were homogenized by vortexing at maximum speed prior to serial dilution. In the serial dilution step, an initial aliquot of 1 ml was drawn from the homogenized sample, serving as the starting stock for dilution. Hundred microliter was taken from the stock and introduced into 900 µl of normal saline, making a dilution of 10⁻¹. Following this, another 100 µL was taken from the 10⁻¹ dilution, yielding a 10⁻² dilution. This

stepwise dilution process was repeated eight times achieving dilutions ranging from 10⁻¹ – 10⁻⁸.

One (1) ml of the serial dilutions (dilution of 10⁻⁸) were inoculated onto MacConkey agar by spreading using a glass rod, and incubated aerobically at 37°C for 24 hours. A single pink/red colony and colorless colony were picked and sub-cultured on EMB and BSA media respectively and observed for growth after 24 hours of incubation at 37°C in an incubator [18].

Gram staining and biochemical identification of isolates

An overnight culture broth of poultry droppings bacterial isolates was then used to determine colonial morphology and for gram staining. The following biochemical tests were carried out according to Cheesbrough, [18] to identify the isolates: Citrate utilization test, urease test, SIM (sulfide, indole, motility), methyl red and Voges-Proskauer tests.

Antibiotic susceptibility test for poultry isolates

The isolates were screened for antibiotic susceptibility using modified protocol of Akond *et al.* [19]. All bacterial cultures used for testing were less than 24 hours old to ensure accurate outcomes. A few colonies from each fresh culture plate were transferred to a tube containing 3 ml of normal saline. The turbidity of these suspensions was adjusted to match a 0.5 McFarland standard by measuring the optical density at 625 nm [20].

Nutrient agar was prepared according to the manufacturer's instructions in Petri dishes and dried to minimize excess moisture for antibiotic test. The surface of the agar was uniformly swabbed with the bacterial suspension using a sterile cotton swab. Antibiotic-impregnated disks were carefully placed on the agar using sterile forceps to ensure adequate spacing and prevent overlap of inhibition zones. The inoculated plates were incubated at 35°C for 18 hours. Post-incubation, the diameters of the zones of inhibition around each antibiotic disk were measured using a ruler and recorded in millimeters and interpreted as sensitive, intermediate and resistant according to Clinical and Laboratory Standard Institute guidelines [21]. The following antibiotic discs were used: Amoxicillin (25 µg), Linezolid (30 µg), Sulfamethoxazole (25 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg).

Determination of multiple antibiotics resistant (MAR) index and multidrug resistance (MDR)

The MARI for each isolate was determined by using the a/b formula to determine the multiple antibiotic resistance index (MAR index) for each isolate, where "a" is the total number of antibiotics to which the isolated organism is resistant and "b" is the total number of test antibiotics that the isolated organisms were tested

for [22]. The isolates that showed resistance to antibiotics belonging to three or more classes were classified as multidrug resistant.

RESULTS AND DISCUSSION

Identification of poultry droppings isolates

Thirty bacteria were isolated. Pink/red and colorless colonies were observed on MacConkey agar. Confirmation of *E. coli* and *salmonella* isolation was achieved through subculture on EMB agar and BS agar

respectively, where the isolates displayed a distinct green metallic sheen and dark metallic sheen respectively, as presented in Figure 1. The distinctive cultural characteristics observed on MacConkey agar, EMB, and Bismuth Sulfite agar provided reliable preliminary identification markers consistent with established microbiological protocols for enterobacterial differentiation [18]. The result for biochemical identification of the poultry isolates is presented in Table 1 below.

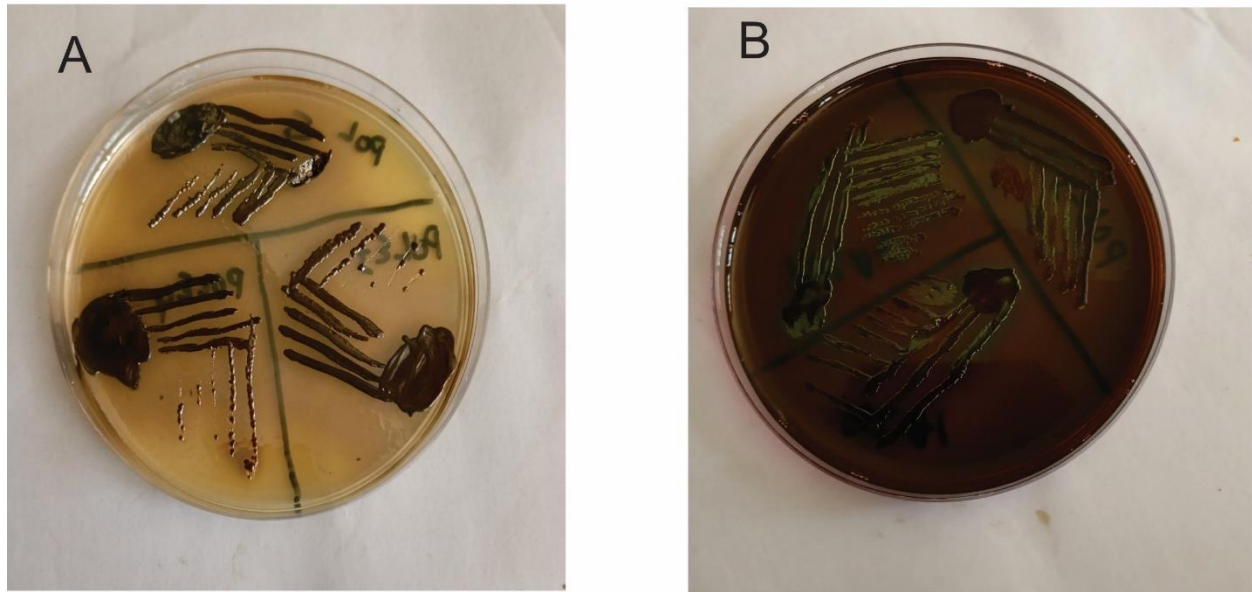


Figure 1: Colonial morphology of poultry Droppings’ isolates on media. (A): Colonial morphology of *Salmonella* spp. on bismuth sulfite agar. (B): colonial morphology of *Escherichia coli* on EMB

Table 1: Cultural and biochemical characteristics of thirty poultry droppings bacterial isolates

S/N	ISOLATE	U	C	S	I	M	M	V	TS	GS	MAC	EMB	BSA	SO
O	S	I				R	P	I	R					
1	POLA1	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metalli c sheen	NA	<i>E. coli</i>
2	POLA2	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metalli c sheen	NA	<i>E. coli</i>
3	POLA3	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metalli c sheen	NA	<i>E. coli</i>
4	POLA4	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metalli c sheen	NA	<i>E. coli</i>
5	POLC1	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metalli c sheen	NA	<i>E. coli</i>

6	POLC2	-	+	+	-	+	+	-	K/ A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
7	POLC3	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
8	POLC4	-	+	+	-	+	+	-	K/ A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
9	POLD1	-	+	+	-	+	+	-	K/ A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
10	POLD2	-	+	+	-	+	+	-	K/ A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
11	POLD3	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
12	POLD4	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
13	POLE1	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
14	POLE2	-	+	+	-	+	+	-	K/ A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
15	POLE3	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
16	POLE4	-	+	+	-	+	+	-	K/ A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
17	POLE5	-	+	-	+	+	+	-	A/ A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
18	POLF1	-	+	+	-	+	+	-	K/ A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
19	POLF2	-	+	+	-	+	+	-	K/ A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.

20	POLF3	-	+	+	-	+	+	-	K/A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
21	POLF4	-	+	+	-	+	+	-	K/A + Gas + H ₂ S	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
22	POLI1	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
23	POLI2	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
24	POLI3	-	+	+	-	+	+	-	K/A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
25	POLI4	-	+	+	-	+	+	-	K/A + Gas	-	Colorless	NA	Black, metallic sheen	<i>Salmonella</i> spp.
26	POLI5	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
27	POLJ1	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
28	POLJ2	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
29	POLJ3	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>
30	POLJ4	-	+	-	+	+	+	-	A/A + Gas	-	Pink	Green, metallic sheen	NA	<i>E. coli</i>

Key: GSR: Gram staining reaction, S: sulfide test I: indole test, MR: methyl red test, VP: Voges Proskauer test, Ci: citrate utilization test, U: urease test, M: motility test, TSI: triple sugar iron (sugar fermentation test), MA: MacConkey agar, EMB: Eosin methylene blue, BSA: bismuth sulfite agar, A/A: acid slant/acid butt, K/A: alkaline slant/acid butt, H₂S: hydrogen sulphide, SO: suspected organism, +: positive, -: negative.

The biochemical tests results showed that all the isolates were gram negative, VP negative, Urease negative. It also showed that all isolates were positive for citrate utilization, motility, and MR tests. The differential indole production distinguished *E. coli* isolates (indole-positive) from *Salmonella* species (indole-negative), a classical biochemical distinction that has been employed in clinical microbiology for decades [18]. These findings corroborate previous investigations by Akond *et al.* [19], who reported similar biochemical profiles in poultry-derived enterobacteria from Bangladesh. The consistent biochemical patterns across isolates suggest stable

phenotypic characteristics within these bacterial populations, despite their different farm origins.

Antibiotic susceptibility test against thirty poultry droppings bacterial isolates

All the isolates showed resistance against amoxicillin (AML), and linezolid (LZD), while ciprofloxacin showed the highest activity against them. Additionally, all the isolates exhibited multi-drug resistance property. It was observed that 11 isolates were susceptible to ciprofloxacin which represents 36.67% of all isolates, and 10 isolates were susceptible to gentamicin which is

33.33% of all the isolates (Table 3). The result of the multiple antibiotic resistance index showed that all the isolates had MAR index values greater than 0.2, indicating that all isolates originated from environments with high antibiotic usage and selective pressure. The distribution of MAR index values in this study reveals a concerning pattern, with 46.67% of isolates (14 out of 30) showing MAR index values of 0.83 or higher, and 13.3% (4 isolates) demonstrating complete resistance across all tested antibiotics (MAR index = 1.00). These extremely high MAR index values indicate not merely multidrug resistance, but approaching extensively drug-resistant phenotypes that severely limit therapeutic options. Several recent studies from Nigeria have documented similar patterns of high MAR index values in poultry systems. A study examining *E. coli* and *Salmonella* contamination in chicken meat from traditional markets reported MAR index values ranging from 0.3 to 0.7, indicating high disease risk [23]. All the

isolates were classified as multidrug resistant (Table 2) which implies that all isolates (100%) were resistant to at least three or more antibiotics. The antibiotic susceptibility testing conducted in this investigation revealed alarming patterns of multidrug resistance across all thirty isolates. The complete resistance observed against amoxicillin and linezolid represents a critical finding with substantial clinical implications. Amoxicillin, a beta-lactam antibiotic widely employed in both veterinary and human medicine, showed zero efficacy against all tested strains [24]. This universal resistance pattern likely stems from extensive antibiotic usage in poultry production systems, where beta-lactams are commonly administered for therapeutic and prophylactic purposes [25]. The selective pressure exerted by continuous antibiotic exposure facilitates the emergence and dissemination of resistance determinants, particularly beta-lactamase genes that enzymatically inactivate beta-lactam compounds [25].

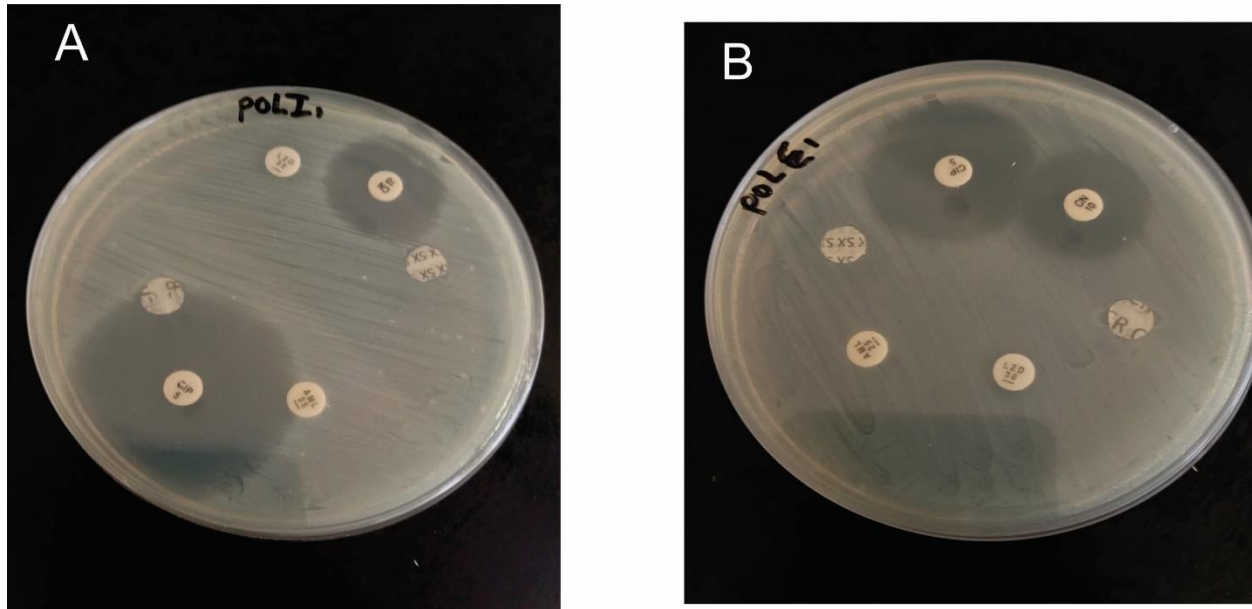


Figure 2: Antibiotic susceptibility test against poultry droppings bacterial isolates. (A): antibiotic susceptibility test against isolate POLI1. (B): antibiotic susceptibility test against isolate POLE1

Table 2: Antibiotic resistance pattern and MAR index of the thirty poultry droppings bacterial isolates

No of Isolates (%)	Antibiotic Pattern	Resistant	MAR Index	MDR
4 (13.33)	LZD, AML, C		0.5	+
2 (6.67)	LZD, AML, SX		0.5	+
2 (6.67)	GN, LZD, AML, SX		0.67	+
8 (26.67)	LZD, AML, SX, C		0.67	+
1 (3.33)	GN, LZD, AML, CIP, SX		0.83	+
3 (10.0)	LZD, AML, CIP, SX, C		0.83	+
6 (20.0)	GN, LZD, AML, SX, C		0.83	+
4(13.33)	GN, LZD, AML, CIP, SX, C		1.0	+

Key: GN=gentamicin, LZD=linezolid, AML=amoxicillin, CIP=ciprofloxacin, SX=sulfamethoxazole, C=chloramphenicol, MDR=multidrug-resistance, MAR Index=multiple antibiotic resistance index

Table 3: Antibiotic susceptibility profile of the bacterial isolates obtained from poultry droppings collected from selected farms in Samaru, Nigeria

S/NO	Antibiotic	Number of Susceptible Isolates (%)	Number of Intermediate Isolates (%)	Number of Resistance Isolates (%)
1	GN	10 (33.33)	6 (20)	14 (46.67)
2	LZD	0 (0)	0 (0)	30 (100)
3	AML	0 (0)	0 (0)	30 (100)
4	CIP	11 (36.67)	12 (40)	7 (23.33)
5	SX	3 (10)	1 (3.33)	26 (86.67)
6	C	4 (13.33)	1 (3.33)	25 (83.33)

Key: GN=gentamicin, LZD=linezolid, AML=amoxicillin, CIP=ciprofloxacin SX=sulfamethoxazole, C=chloramphenicol

The documentation that all thirty isolates exhibited multidrug resistance, defined as non-susceptibility to at least three different antibiotic classes, represents a critical public health concern [26]. Multidrug-resistant bacteria complicate treatment protocols, increase healthcare costs, and contribute to elevated morbidity and mortality rates [27]. The agricultural origin of these resistant strains emphasizes potential transmission pathways from food animals to humans through direct contact, environmental contamination, or foodborne routes [3]. Epidemiological investigations by Manyi-Loh et al. [3] have traced multidrug-resistant *E. coli* and *Salmonella* from poultry farms through the food chain to human clinical cases, establishing clear zoonotic transmission links.

The emergence and persistence of multidrug resistance in poultry bacteria reflect complex interactions between antibiotic usage practices, bacterial genetics, and environmental factors [28]. Many poultry operations incorporate antimicrobials into feed or water for growth promotion and disease prevention, creating constant selective pressures across entire flocks [2]. Additionally, intensive farming conditions with high animal densities facilitate bacterial transmission and genetic exchange through conjugation, transformation, and transduction mechanisms [29].

The multidrug resistance patterns observed in poultry isolates from this study is closely similar to findings from other Nigerian research groups. Raufu et al. [30] documented extensive multidrug resistance in poultry *E. coli* and *Salmonella* isolates from Southwest Nigeria, with resistance profiles remarkably similar to those reported here. The consistency of findings across different regions of Nigeria suggests systemic issues in antimicrobial stewardship within Nigerian poultry production systems, warranting coordinated interventions [28]. Internationally, studies from Asia have reported similar trends in poultry antimicrobial

resistance. Van et al. [25] investigated *E. coli* and *Salmonella* from Vietnamese poultry farms, documenting resistance rates to common antibiotics exceeding 80% for several classes. Their work emphasized the role of intensive farming practices and prophylactic antibiotic use in driving resistance emergence [2]. The global nature of this problem underscores the universal relevance of discoveries like those reported in this study, where novel antimicrobials from endophytic sources could address worldwide challenges [31]

CONCLUSION

Our study has demonstrated that poultry is a significant reservoir for both *Salmonella* species and *Escherichia coli*. All thirty isolates were multidrug resistant, with 100% of isolates resistance to both amoxicillin, and linezolid. However, 36.67% of the isolates were susceptible to ciprofloxacin, and 33.33% of the isolates were susceptible to gentamicin.

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