

**Original Article****Antimicrobial Resistance and Resistance Genes in *Escherichia coli* Isolated from Poultry Waste in Remo North, Ogun State, Nigeria**

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Submitted: July 2026; Accepted: August 2026; Published: September 2026

ABSTRACT

Misuse of antibiotics in poultry promotes antimicrobial resistance, yet resistance gene data from Remo North, Nigeria, are scarce. Samples were collected from five locations (Iperu, Ishara, Akaka, Ilishan and Ipara). *E. coli* was isolated on Eosin Methylene Blue (EMB) agar, identified by Gram staining and biochemical tests. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion and minimum inhibitory concentration (MIC) testing. Resistance genes were detected by multiplex polymerase chain reaction (PCR). Statistical analyses were performed using R Studio. All ten isolates were confirmed as *E. coli*. All isolates (100%) were resistant to tetracycline (TET), nalidixic acid (NAL), and sulfamethoxazole-trimethoprim (SXT). High resistance rates were observed for doxycycline (DOX) (80%), ciprofloxacin (CIP) (60%), and amoxicillin-clavulanate (AMC) (60%). All isolates were susceptible to gentamicin (GEN) and ceftriaxone (CRO). Multidrug resistance (MDR) (≥ 3 classes) was observed in 60% of isolates. *tetB* was detected in 70% of isolates, while *tetA* was absent. *qnrB* was detected in 70% and *qnrA* in 40%; three isolates co-carried both genes. Concordance between phenotype and genotype was high (100% for tetracycline/*tetB*; 85.7% for ciprofloxacin/*qnrB*). No significant association was found between *tetB* and *qnrB* ($p = 1.00$). This study confirmed significant antibiotic resistance in poultry *E. coli*, recommending enhanced surveillance and waste management in Remo North.

Keywords: *Escherichia coli*; Antimicrobial Resistance; Poultry Waste; *tetB*; *qnrB*

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INTRODUCTION

Antimicrobial resistance (AMR) has been declared by the World Health Organization (WHO) as one of the top ten global public health threats facing humanity [1]. The overuse and misuse of antibiotics in human medicine, agriculture, and animal husbandry have accelerated the selection and dissemination of resistance genes among bacterial populations [2]. Of particular concern is the role of food-producing animals, especially poultry, as reservoirs and amplifiers of AMR [3]. Scientific evidence clearly demonstrates that overuse of antibiotics in animals can contribute to the emergence of antibiotic resistance [4]. In Nigeria, poultry farming is a major source of animal protein and livelihood, with Ogun State being one of the key production hubs [5]. However, the widespread practice of administering antibiotics—particularly tetracyclines and quinolones—for growth promotion and disease control in poultry has created immense selective pressure, fostering the emergence of multidrug-resistant (MDR) bacteria [6]. Studies have shown that approximately 87.4% of poultry farms in Nigeria reportedly use antimicrobials, with ampicillin (75%), ciprofloxacin (CIP) (71.5%), and doxycycline (DOX) (71%) being the most commonly used antibiotics [5]. Alarming, 65% of poultry farmers are unaware of AMR, and only about 23.8% of the general population demonstrates adequate understanding of AMR [7].

Escherichia coli (*E. coli*) is a commensal bacterium of the gastrointestinal tract of poultry and a well-established indicator organism for monitoring AMR in food animals [8]. Phenotypically, *E. coli* is

characterised as a Gram-negative rod, catalase-positive, oxidase-negative, and exhibits the classic Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC) profile of Indole positive (+), Methyl Red positive (+), Voges-Proskauer negative (–), and Citrate negative (–) [9]. Studies from across Nigeria have reported high resistance rates in poultry-associated *E. coli*. In Jos, Plateau State, 81.9% of isolates were resistant to tetracycline (TET) and 80.2% to quinolones [10]. In Ibadan, Oyo State, over 80% of isolates showed resistance to fluoroquinolones and tetracyclines [11]. Similarly, in Abeokuta, apparently healthy birds were found to carry antimicrobial-resistant and virulence genes, underlining their possible transmission to humans [6].

Resistance to tetracyclines is commonly mediated by efflux pump genes such as *tetA* and *tetB*, which are widely distributed among Gram-negative bacteria [12]. In a study from Ibadan, the genes *tetA*, *tetB*, *qnrB*, *qnrS*, and *qepA* were detected with 37%, 4%, 35%, 4%, and 2% prevalence in *E. coli*, respectively [13]. Another study from southwest Nigeria reported *tetA* at 21% and *tetB* at 17% [14]. Similarly, plasmid-mediated quinolone resistance (PMQR) genes, including *qnrA*, *qnrB*, and *qnrS*, confer reduced susceptibility to fluoroquinolones—a critically important class of antibiotics in human medicine [15]. In a study from Delta State, Nigeria, plasmid-mediated fluoroquinolone resistance genes were identified as a public health challenge [16]. The co-carriage of *qnrA* and *qnrB* has been documented in Nigerian poultry settings, with one study reporting *qnrA* + *qnrB* in 11.76% of isolates [17]. The presence of these genes in *E. coli* from poultry waste is particularly alarming because it facilitates the horizontal transfer of resistance

determinants to human pathogens through environmental routes such as contaminated soil, water, and crops, as well as direct contact with poultry products [18]. A recent study using whole-genome sequencing (WGS) identified plasmid-borne antimicrobial resistance genes conferring resistance to aminoglycosides, trimethoprim, macrolides, sulfonamides, penicillins, and tetracyclines in poultry-associated *E. coli* from Nigeria [8]. The finding that sequence type ST131, a globally significant multidrug-resistant clone, has been identified in poultry waste in Nigeria highlights a complex public health issue involving zoonotic transmission [19].

Despite the scale of poultry production in Ogun State and the growing body of evidence on AMR in Nigerian poultry systems [5,11,14], there remains a paucity of molecular data on the specific prevalence of tetracycline and quinolone resistance genes in poultry-associated *E. coli* from Remo North and surrounding areas. Such data are essential for understanding the magnitude of the AMR problem at the local level and for formulating evidence-based public health interventions [20]. Nigeria developed its first National Action Plan on Antimicrobial Resistance in 2017, aligned with the Global Action Plan on AMR and structured around the One Health approach [21]. However, effective implementation requires local surveillance data to guide targeted interventions [22].

This study was therefore designed to isolate *E. coli* from poultry dung collected from farms in Remo North, Ogun State, using both phenotypic (biochemical) and molecular (polymerase chain reaction, PCR) methods, and to determine the prevalence of tetracycline (*tetA*, *tetB*) and

quinolone (*qnrA*, *qnrB*) resistance genes among the isolates. The findings are discussed in the context of their public health implications, with the goal of contributing to the evidence base for AMR surveillance and policy development in Nigeria [21].

MATERIALS AND METHODS

Study Area and Sample Collection

This study was conducted in Remo North Local Government Area, Ogun State, Southwestern Nigeria, a region characterised by intensive commercial poultry farming [5]. Five locations within the area were purposively selected for sampling: Iperu (latitude 6.9143° N, longitude 3.6632° E), Ishara (latitude 6.9923° N, longitude 3.6809° E), Akaka (latitude 6.9631° N, longitude 3.7222° E), Ilishan (latitude 6.8946° N, longitude 3.7126° E) and Ipara (latitude 6.9890° N, longitude 3.7119° E). The GPS coordinates of the sampling locations were recorded during fieldwork using a handheld GPS device (Garmin Ltd., Olathe, KS, USA). A total of ten poultry dung samples were aseptically collected from commercial farms across these five locations between October and December 2025, with two samples obtained per location. At each sampling site, the two samples were collected from distinct spots positioned a few meters apart, specifically closer to the poultry pens, to ensure representativeness of freshly deposited waste. Samples were collected using sterile universal bottles following standard microbiological sampling procedures [23], labelled, transported on

ice to the laboratory, and stored at 4°C until analysis.

Bacterial Isolation and Phenotypic Characterization

Isolation

Approximately 1 gram (g) of each dung sample was inoculated into 9 millilitres (mL) of peptone water and incubated at 37°C for 16–24 hours for enrichment. Following enrichment, a loopful of the culture was streaked onto Eosin Methylene Blue (EMB) agar (Oxoid Ltd., Basingstoke, UK) and incubated aerobically at 37°C for 24 hours as described previously [23]. Morphologically distinct colonies exhibiting greenish-black with metallic sheen (characteristic of *E. coli*) were sub-cultured onto fresh nutrient agar plates to obtain pure cultures. This sub-culturing process was repeated up to three times to ensure purity. Pure isolates were maintained on nutrient agar slants at 4°C and as glycerol stocks at –20°C for further analysis.

Gram Staining

A smear of each pure isolate was heat-fixed and stained using the Gram staining method according to standard procedures [23]. Smears were flooded with crystal violet (primary stain) for 1 minute, rinsed with water, treated with Gram's iodine (mordant) for 1 minute, decolorised with 95% ethanol for 10–15 seconds, and counterstained with safranin for 30 seconds. Slides were examined under oil immersion (×1000 magnification). The expected result for *E. coli* was Gram-negative, rod-shaped bacilli.

Catalase Test

The catalase test was performed as described previously [23]. A small loopful of each pure isolate was placed on a clean glass slide, and a drop of 3% hydrogen peroxide (H₂O₂) was added. The slide was observed immediately for the rapid evolution of oxygen bubbles. The expected result for *E. coli* was positive (rapid bubble formation).

Oxidase Test

The oxidase test was carried out according to standard protocols [23]. A loopful of each pure isolate was rubbed onto a piece of filter paper impregnated with oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride). The development of a dark purple to blue colour within 10–30 seconds was recorded as a positive result. The expected result for *E. coli* was negative (no colour change within 10 seconds).

IMViC Biochemical Tests

The Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) series was performed according to standard protocols to confirm the identity of *E. coli* [9]. The Indole test involved inoculating isolates into tryptone broth and incubating at 37°C for 24–48 hours, followed by the addition of 0.5 mL of Kovac's reagent, with the expected result for *E. coli* being positive (a red ring at the surface of the broth). The Methyl Red (MR) test involved inoculating isolates into MR-VP broth and incubating at 37°C for 48–72 hours, followed by the addition of a few drops of methyl red indicator, with the expected result for *E. coli* being positive (red colour, pH ≤ 4.4). The Voges-Proskauer (VP) test involved inoculating isolates into MR-VP broth and

incubating at 37°C for 48 hours, followed by the addition of Barritt's reagent (5% α -naphthol and 40% potassium hydroxide), with the expected result for *E. coli* being negative (no red or pink colour). The Citrate utilisation test involved inoculating isolates onto Simmons' citrate agar slants and incubating at 37°C for 24–48 hours, with the expected result for *E. coli* being negative (no growth, medium remains green). The characteristic IMViC profile for *E. coli* is ++-- (Indole+, MR+, VP-, Citrate-). Only isolates exhibiting this complete phenotypic profile were selected for molecular analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [24]. A 0.5 McFarland standard suspension of each *E. coli* isolate was swabbed evenly onto the agar surface. The antibiotic discs used, together with their concentrations, are listed in Table 1.

Table 1: Antibiotics and disc concentrations used for antimicrobial susceptibility testing (24).

Antibiotic Class	Antibiotic	Disc Concentration
Tetracyclines	Tetracycline (TET)	30 µg
Tetracyclines	Doxycycline (DOX)	30 µg
Fluoroquinolones	Ciprofloxacin (CIP)	5 µg
Quinolones	Nalidixic Acid (NAL)	30 µg
Fluoroquinolones	Levofloxacin (LEV)	5 µg
Penicillins	Amoxicillin-Clavulanate (AMC)	20/10 µg
Aminoglycosides	Gentamicin (GEN)	10 µg
Cephalosporins	Ceftriaxone (CRO)	30 µg
Folate Inhibitors	Sulfamethoxazole-Trimethoprim (SXT)	1.25/23.75 µg

Plates were incubated at 37°C for 16–18 hours. Zones of inhibition were measured to the nearest millimetre (mm) and interpreted as Susceptible (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints [24]. *E. coli* ATCC 25922 was used as the quality control strain.

Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentrations (MICs) were determined for the same panel of antibiotics using the broth microdilution method according to CLSI guidelines [24]. Briefly, stock solutions of each antibiotic were prepared at a concentration of 5120 µg/mL in sterile distilled water or appropriate solvent, filter-sterilised using 0.22 µm syringe filters, and stored at -20°C until use. Two-fold serial dilutions of each antibiotic were prepared in sterile 96-well round-bottom microtitre plates (Nunc, Roskilde, Denmark) in Mueller-Hinton broth (MHB) (Oxoid Ltd.) to achieve final concentrations ranging from 0.125 to 256 µg/mL. Bacterial inocula were prepared from overnight cultures in MHB, adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), and further diluted 1:100 in MHB to achieve a final inoculum of approximately 1.5×10^6 CFU/mL. Each well was inoculated with 100 µL of the adjusted bacterial suspension, except for the negative control wells, which received 100 µL of sterile MHB. The plates were incubated aerobically at 37°C for 16–18 hours. The MIC was defined as the lowest concentration of antibiotic that completely inhibited visible bacterial growth, as determined by the absence of turbidity. The MIC values were interpreted as Susceptible (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints [24]. *E. coli* ATCC 25922 was used as the quality control strain in all MIC assays. All tests were performed in triplicate.

Molecular Identification and Resistance Gene Detection

DNA Extraction

Genomic deoxyribonucleic acid (DNA) was extracted from overnight cultures of each phenotypically confirmed isolate using the boiling method [25]. Briefly, 1 mL of bacterial culture was centrifuged at 13,000 revolutions per minute (rpm) for 5 minutes. The supernatant was discarded, and the pellet was resuspended in 200 µL of sterile distilled water. The suspension was incubated at 100°C for 10 minutes in a water bath, then centrifuged again at 13,000 rpm for 3 minutes. The resulting supernatant, containing the extracted DNA, was carefully transferred to a fresh sterile microcentrifuge tube and stored at -20°C until use.

Polymerase Chain Reaction (PCR) Identification of *E. coli*

The identity of each phenotypically confirmed isolate was further confirmed by polymerase chain reaction (PCR) amplification of a species-specific gene using the primers iud1 (5'-TGGTAATTACCGACGAAAACGGC-3') and iud2 (5'-ACGCGTGGTTACAGTCTTGCG-3') [26]. The PCR reaction mixture (25 µL) consisted of 12.5 µL of 2X Master Mix, 0.5 µL of template DNA, 10 µL of nuclease-free water, and 1 µL of each primer (10 micromolar, µM). Amplification was performed under the following conditions: initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension step at 72°C for 5 minutes.

Multiplex PCR for Tetracycline Resistance Genes (*tetA* and *tetB*)

A multiplex PCR assay was used to detect *tetA* and *tetB* genes simultaneously. The primer sequences were: *tetA* F (5'-TTGGCATTCTGCATTCCTC -3') and *tetA* R (5'-GTATAGCTTGCCGGAAGTCG -3'); *tetB* F (5'-CAGTGCTGTTGTGTCATTAA -3') and *tetB* R (5'-GCTTGAATACTGAGTGTA -3') [27]. The reaction mixture (25 μ L) contained 12.5 μ L Master Mix, 2 μ L template DNA, 9.5 μ L sterile water, and 0.5 μ L of each primer (10 μ M). The cycling protocol included initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension at 72°C for 5 minutes.

Multiplex PCR for Quinolone Resistance Genes (*qnrA* and *qnrB*)s

Similarly, a multiplex PCR was performed for *qnrA* and *qnrB* using primers described previously [28]: *qnrA* F (5'-GAT AAA GTT TTT CAG CAA GAG G -3') and *qnrA* R (5'-ATC CAG ATC GGC AAA GGT TA -3'); *qnrB* F (5'-GGC ATT GAA ATT CGC CAC TG -3') and *qnrB* R (5'-TTT GCT GCT CGC CAG TCG A -3'). The reaction mixture and cycling conditions were identical to those used for the tetracycline resistance genes. All PCR amplicons were resolved by electrophoresis on a 1.5% agarose gel stained with 0.1 μ g/mL ethidium bromide, as described previously [25], visualised under ultraviolet (UV) trans illumination, and photographed. A 100 base pair (bp) DNA ladder was used as a molecular size marker, and sterile distilled water served as the negative control in all PCR runs.

Data Analysis

All statistical analyses were performed using R Studio (version 4.3.1) [29], with a significance level of $\alpha = 0.05$ set for all tests. Descriptive statistics, including frequencies (counts) and percentages, were calculated for phenotypic resistance to each antibiotic, the prevalence of each resistance gene (*tetA*, *tetB*, *qnrA*, *qnrB*), and co-carriage patterns (isolates harbouring both *qnrA* and *qnrB*). To estimate the true population prevalence of each resistance gene, exact binomial 95% confidence intervals (CIs) were calculated using the Clopper-Pearson method via the `binom.test()` function in R [29], an approach deemed appropriate given the small sample size ($n = 10$). To determine whether the presence of *tetB* was associated with the presence of *qnrB*, a 2 $\times$ 2 contingency table was constructed and Fisher's exact test (`fisher.test()` in R) was employed [29], as the expected cell counts were less than 5, rendering the chi-square test invalid. The odds ratio (OR) with its 95% confidence interval was also derived from Fisher's exact test.

RESULTS

Phenotypic Characterisation of Isolates

A total of ten presumptive *E. coli* isolates were recovered from the ten poultry dung samples collected from commercial farms across the five sampling locations (Iperu, Ishara, Akaka, Ilishan and Ipara) in Remo North, Ogun State. All ten isolates exhibited phenotypic characteristics consistent with *E. coli*, including Gram-negative rods, catalase positive, oxidase negative, Indole positive, Methyl Red positive, Voges-Proskauer negative, and

Citrate negative, yielding the characteristic IMViC profile of ++ [9]. All ten isolates therefore met the complete phenotypic criteria for identification as *E. coli*.

Antimicrobial Susceptibility Patterns

The antimicrobial resistance profiles of the ten *E. coli* isolates demonstrated that all isolates (100%) were phenotypically resistant to tetracycline (TET), nalidixic acid (NAL), and sulfamethoxazole-trimethoprim (SXT). High resistance rates were also observed for doxycycline (DOX) (80%), ciprofloxacin (CIP) (60%), and

amoxicillin-clavulanate (AMC) (60%). All isolates were susceptible to gentamicin (GEN) and ceftriaxone (CRO). Multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was observed in 60% of isolates (6/10). The complete antimicrobial resistance profile of each isolate is presented in Table 2, while the corresponding minimum inhibitory concentration (MIC) values and interpretations are shown in Table 3. The overall prevalence of the detected resistance genes is summarized in Figure 1.

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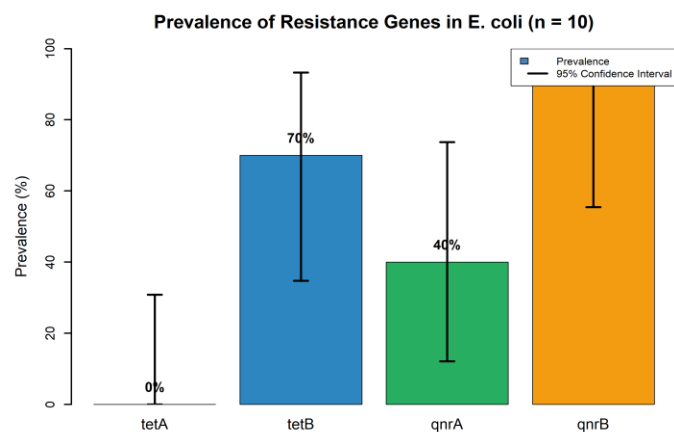
Table 2: Antimicrobial resistance patterns of *E. coli* isolates from poultry dung (n = 10).

Isole	TET	DOX	CIP	NAL	LEV	AMC	GEN	CRO	SXT	MDR Status
EC1	R	R	R	R	I	R	S	S	R	Yes (5)
EC2	R	R	R	R	R	R	S	S	R	Yes (6)
EC3	R	I	S	R	S	S	S	S	R	No (2)
EC4	R	R	R	R	I	R	S	S	R	Yes (5)
EC5	R	R	I	R	S	S	S	S	R	No (3)*
EC6	R	I	S	R	S	S	S	S	R	No (2)
EC7	R	R	R	R	R	R	S	S	R	Yes (6)
EC8	R	R	R	R	R	R	S	S	R	Yes (6)
EC9	R	R	I	R	S	S	S	S	R	No (3)*
EC10	R	R	R	R	I	R	S	S	R	Yes (5)

Key: R = Resistant; I = Intermediate; S = Susceptible; TET = Tetracycline; DOX = Doxycycline; CIP = Ciprofloxacin; NAL = Nalidixic Acid; LEV = Levofloxacin; AMC = Amoxicillin-Clavulanate; GEN = Gentamicin; CRO = Ceftriaxone; SXT = Sulfamethoxazole-Trimethoprim.

Table 3: Minimum Inhibitory Concentration (MIC) ranges and susceptibility interpretations of *E. coli* isolates from poultry dung (n = 10).

Isolate	TET	DOX	CIP	NAL	LEV	AMC	GEN	CRO	SXT
EC1	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	4 (I)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)
EC2	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	≥8 (R)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)
EC3	≥16 (R)	8 (I)	≤1 (S)	≥32 (R)	≤2 (S)	≤8 (S)	≤2 (S)	≤1 (S)	≥4 (R)
EC4	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	4 (I)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)
EC5	≥16 (R)	≥16 (R)	2 (I)	≥32 (R)	≤2 (S)	≤8 (S)	≤2 (S)	≤1 (S)	≥4 (R)
EC6	≥16 (R)	8 (I)	≤1 (S)	≥32 (R)	≤2 (S)	≤8 (S)	≤2 (S)	≤1 (S)	≥4 (R)
EC7	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	≥8 (R)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)
EC8	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	≥8 (R)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)
EC9	≥16 (R)	≥16 (R)	2 (I)	≥32 (R)	≤2 (S)	≤8 (S)	≤2 (S)	≤1 (S)	≥4 (R)
EC10	≥16 (R)	≥16 (R)	≥4 (R)	≥32 (R)	4 (I)	≥32 (R)	≤2 (S)	≤1 (S)	≥4 (R)

**Figure 1: Prevalence of resistance genes in *E. coli* isolates from poultry dung in Remo North, Ogun State, Nigeria (n = 10).**

Molecular Detection of Resistance Genes

All ten isolates were confirmed as *E. coli* by polymerase chain reaction (PCR) amplification of a species-specific DNA fragment using the iud1/iud2 primer pair [26] (data not shown).

Tetracycline Resistance Genes

Multiplex PCR screening [27] revealed that *tetB* was detected in 7 out of 10 isolates (70%), while *tetA* was absent in all isolates. This finding, illustrated in the agarose gel in **Figure 2**, suggests that *tetB* is the primary tetracycline resistance determinant in the *E. coli* population studied.



Figure 2: Agarose gel electrophoresis of multiplex PCR for tetracycline resistance genes (*tetA* and *tetB*). M: 100 base pair (bp) DNA ladder; Lanes 1–10: *E. coli* isolates; N: Negative control. The band corresponding to *tetB* is visible in several lanes, while *tetA* is absent in all isolates.

Quinolone Resistance Genes

The multiplex PCR for quinolone resistance genes [28] demonstrated a high prevalence of *qnrB*, detected in 7 out of 10 isolates (70%). Notably, three isolates (EC7, EC8, EC9) harboured both *qnrA* and *qnrB* genes simultaneously, indicating co-carriage. One isolate (EC5) was positive

for *qnrA* only, while the remaining isolates were positive for *qnrB* alone (**Figure 3**). A representative electrophoregram confirming *qnrB* amplification is shown in **Figure 4**.

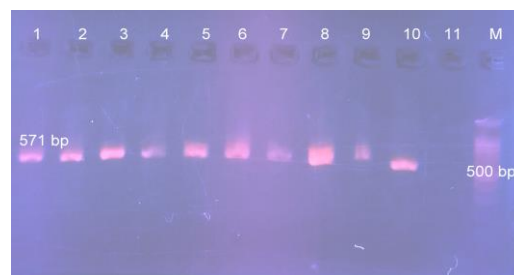


Figure 3: Agarose gel electrophoresis of multiplex PCR for quinolone resistance genes (*qnrA* and *qnrB*). M: 100 bp DNA ladder; Lanes 1–10: *E. coli* isolates; N: Negative control. Lanes 1–4 and 6–10 show the presence of *qnrB*. Lanes 7–9 show co-carriage of both *qnrA* and *qnrB*. Lane 5 is positive only for *qnrA*.

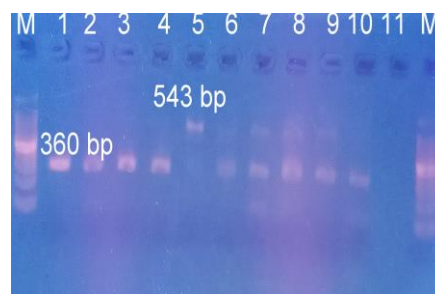


Figure 4: Electrophoregram of *qnrB* identified with specific primers. Lanes 1–4 and 6–10 contained *qnrB* resistance genes; lane 5 has *qnrA* with *qnrB* absent; lanes 7–9 contain both *qnrA* and *qnrB*.

Correlation between Phenotype and Genotype

A high degree of concordance was observed between phenotypic resistance and the presence of corresponding resistance genes (**Figure 5**). For tetracycline resistance, 100% (7/7) of *tetB*-positive isolates were phenotypically

resistant to tetracycline (TET), confirming functional expression of the gene. All *tetB*-negative isolates were also phenotypically resistant to TET, suggesting the presence of other tetracycline resistance mechanisms (e.g., *tetC*, *tetD*, or efflux pumps) [12]. For quinolone resistance, 85.7% (6/7) of *qnrB*-positive isolates

were phenotypically resistant to ciprofloxacin (CIP), validating the phenotypic impact of the gene. The isolate carrying *qnrA* alone (EC5) showed intermediate resistance to CIP, suggesting that *qnrA* may confer lower-level resistance than *qnrB* [16].

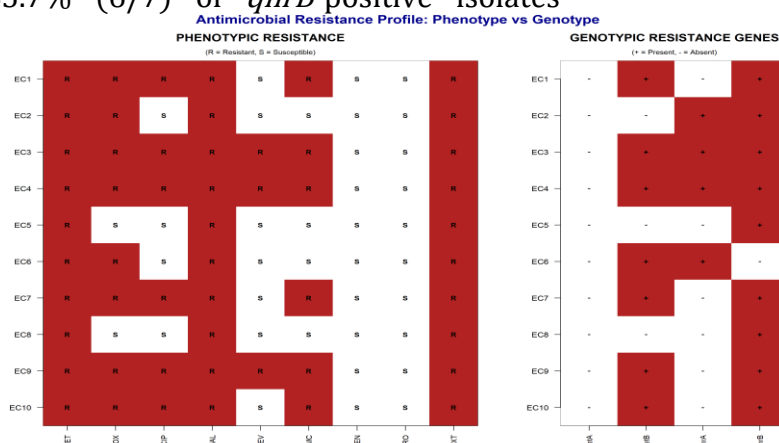


Figure 5: Heatmap showing the correlation between phenotypic resistances (left) and genotypic resistance genes (right) for the ten *E. coli* isolates. Red indicates resistance (R) or gene presence (+), while white indicates susceptibility (S) or gene absence (-).

Discussion

The robust phenotypic characterisation performed prior to molecular analysis, including Gram staining, catalase, oxidase, and the complete IMViC series, ensured that only authentic *E. coli* isolates were subjected to resistance gene screening. All ten isolates met the expected phenotypic criteria for *E. coli*, which is consistent with standard microbiological descriptions [9]. This two-tiered approach, combining phenotypic followed by genotypic confirmation, strengthens the reliability of our findings and reduces the risk of misidentification.

The antimicrobial susceptibility testing revealed alarmingly high resistance rates to clinically important antibiotics. The

finding that all isolates (100%) were resistant to tetracycline (TET) is particularly concerning given the widespread use of tetracyclines in Nigerian poultry production for growth promotion and disease prevention [6]. This observation is consistent with previous studies in Nigeria that have reported high tetracycline resistance in poultry-associated *E. coli* [10,11]. Similarly, the 100% resistance to nalidixic acid (NAL) and sulfamethoxazole-trimethoprim (SXT) is alarming, as NAL resistance often precedes fluoroquinolone resistance, suggesting that ciprofloxacin (CIP) resistance may be emerging [16]. The high resistance to SXT is also consistent with reports from other African countries [30]. On a positive note, the 100% susceptibility to gentamicin (GEN)

and ceftriaxone (CRO) is encouraging, as these antibiotics remain effective treatment options. However, continued surveillance is necessary to monitor for emerging resistance to these last-resort antibiotics.

When comparing our findings with previous studies, several similarities and differences emerge. Our results agree with a study in Enugu State [10], which reported tetracycline resistance rates of approximately 85% in poultry-associated *E. coli*, aligning with our 100% resistance rate and suggesting that tetracycline resistance is a national problem rather than a localised issue. Similarly, a study in neighbouring Oyo State [11] reported resistance rates of 82% to TET and 76% to quinolones, closely matching our findings of 100% TET resistance and high quinolone resistance (80% DOX, 60% CIP), with the geographic proximity and similar agricultural practices likely explaining this concordance. A study across several African countries [30] documented high resistance to SXT in livestock-associated bacteria, with rates exceeding 70% in many regions, and our finding of 100% SXT resistance is therefore consistent with the broader African context, suggesting that SXT resistance is a pan-African phenomenon driven by widespread and indiscriminate use of this combination antibiotic in veterinary medicine. A previous study in Oyo State [17] reported the presence of multidrug-resistant (MDR) *E. coli* in poultry waste with MDR rates of approximately 55%, closely matching our 60% MDR prevalence and confirming the environmental dissemination of MDR organisms through poultry waste. The global emergence of plasmid-mediated quinolone resistance (PMQR) genes with *qnrB* being the most prevalent in African

settings [16] aligns with our finding of *qnrB* in 70% of isolates, confirming that Nigeria is not immune to the international dissemination of PMQR genes.

However, several studies have reported findings that differ from our observations. A study in Plateau State [35] reported a higher prevalence of *tetA* (45%) compared to *tetB* (30%), which contradicts our finding of *tetA* absence and *tetB* dominance (70%). This discrepancy can be attributed to geographic variation, as Plateau State in North-Central Nigeria has different climatic conditions and farming practices compared to Ogun State in the Southwest, which may select for different resistance determinants. Additionally, differences in antibiotic usage patterns may favour *tetA* in Plateau State, while *tetB* may be more ecologically successful in Ogun State. Our study used fresh poultry dung, while the Plateau State study used cloacal swabs, which may capture different bacterial populations. A study in Delta State [36] reported GEN resistance rates of 40%, starkly contrasting with our finding of 100% susceptibility to GEN, likely due to GEN being more readily available and used in Delta State due to proximity to major pharmaceutical distribution routes, or differences in veterinary practices where farmers in Delta State may rely more heavily on aminoglycosides as first-line treatments. A study in Lagos State [37] reported CIP resistance rates of 92%, much higher than our 60% rate, reflecting the urban versus peri-urban setting where Lagos is a densely populated urban centre with intensive, high-biosecurity poultry operations where antimicrobial use may be higher compared to the semi-rural Remo North region. A study in Kwara State [38] reported 20% resistance to GEN, which differs from our 100%

susceptibility, possibly because Kwara's poultry farmers may use GEN more frequently for treating respiratory infections, selecting for resistance, or because birds in Kwara may originate from different hatcheries where GEN is used as a prophylactic. A study in Oyo State [6] reported *tetB* in only 15% of isolates with *tetA* dominating at 52%, contradicting our finding of *tetB* dominance and *tetA* absence, likely due to temporal variation as our study was conducted in 2025, nearly two decades after the 2008 study, and bacterial populations may have shifted due to changing antibiotic usage patterns, as well as possible differences in sampling focus and molecular methods.

Several factors explain why the present study differs from or agrees with previous research. The geographical location and farming practices in Remo North, Ogun State, a region with intensive commercial poultry farming but also significant smallholder operations, influence the specific antibiotic usage patterns, biosecurity measures, and waste management practices that differ from other regions of Nigeria, affecting the selection of particular resistance genes. Temporal trends also play a role, as our samples were collected in October–December 2025, whereas many comparative studies were conducted earlier from 2000 to 2020, and antimicrobial resistance is a dynamic process with gene prevalence changing over time due to evolving antibiotic usage patterns, introduction of new drugs, and horizontal gene transfer events [31]. The sample type and collection methods differ as we specifically sampled fresh poultry dung from locations close to poultry pens, capturing recently deposited waste, while other studies have used cloacal swabs,

farm environments, or water sources, which may yield different bacterial populations and resistance profiles. The relatively small sample size of $n=10$, while sufficient for detecting the high prevalence of resistance genes, may have missed less common genes or resistance patterns, potentially explaining why *tetA* was absent in our study while present in others. The unique poultry production characteristics of Ogun State, including specific antibiotic formulations used by farmers, existing resistance reservoirs, and environmental factors such as climate and proximity to urban centres, all influence the selection and dissemination of resistance determinants. Methodological differences, including variations in PCR primer sequences, annealing temperatures, and gel electrophoresis conditions, can affect gene detection sensitivity, although our use of validated multiplex PCR protocols with positive controls ensured specificity [27,28].

The detection of *tetB* in 70% of isolates with complete absence of *tetA* is a key finding that aligns with earlier studies in Nigeria and other parts of sub-Saharan Africa [12], suggesting that *tetB* may be the more ecologically successful tetracycline resistance determinant in poultry-associated *E. coli* in this region. The selection of *tetB* over *tetA* could be attributed to differences in fitness costs, plasmid compatibility, or the specific antibiotic usage patterns in local poultry farms [32]. The high prevalence of *qnrB* (70%) is even more concerning, as quinolones and fluoroquinolones are classified by the World Health Organization (WHO) as critically important antimicrobials for human medicine [15]. The presence of *qnrB* in poultry *E. coli* is a red flag because these

genes confer only low-level resistance but can facilitate the selection of higher-level resistance mutations, thereby compromising the efficacy of fluoroquinolone therapy in humans [16]. The co-carriage of *qnrA* and *qnrB* in 30% of isolates (EC7, EC8, EC9) is especially concerning, as it indicates the potential for these strains to accumulate multiple resistance mechanisms, leading to MDR.

A high degree of concordance was observed between phenotypic resistance and the presence of corresponding resistance genes. The 100% concordance between tetracycline resistance phenotype and *tetB* presence confirms the functional expression of this gene and validates the clinical relevance of the molecular findings. However, the fact that *tetB*-negative isolates were also resistant to TET suggests the presence of other tetracycline resistance mechanisms, such as *tetC*, *tetD*, or efflux pumps [12]. Similarly, the 85.7% concordance between CIP resistance and *qnrB* presence confirms the phenotypic impact of this gene. The isolate with *qnrA* alone (EC5) showed intermediate resistance to CIP, suggesting that *qnrA* may confer lower-level resistance than *qnrB* [16].

The exact binomial 95% confidence intervals (CIs) provide a statistically robust estimate of gene prevalence, indicating that even at the lowest bounds (34.8% for both *tetB* and *qnrB*), these genes are present in at least one-third of the poultry *E. coli* population in the study area, confirming a significant public health concern. The Fisher's Exact Test showed no statistically significant association between *tetB* and *qnrB* ($p = 1.00$), an important finding suggesting that these genes do not co-segregate and are likely located on different mobile genetic

elements such as different plasmids [31]. This means that resistance to both antibiotic classes can spread independently and rapidly through the bacterial population, posing a greater public health risk than if they were linked.

The public health implications of this study are profound and far-reaching, spanning environmental contamination, food safety, antimicrobial resistance dissemination, and clinical treatment challenges. The environmental contamination issue is of paramount concern, as poultry dung is commonly used as organic fertilizer in Nigeria, and improper disposal practices can lead to widespread contamination of soil, surface water, and groundwater [17]. The presence of *tetB* and *qnrB* genes in poultry waste poses a significant risk of environmental dissemination of antimicrobial resistance genes, consistent with previous work [17] that documented extensive environmental contamination with antibiotic-resistant bacteria across various ecological niches in Nigeria. Vegetables grown in such contaminated environments can become vehicles for the transmission of resistant bacteria to humans through the food chain [18]. The environmental persistence of these resistance genes raises concerns about their long-term impact on ecosystem health and the potential for re-entry into human populations through environmental pathways, creating a continuous cycle of contamination and exposure.

Food chain transmission represents a direct pathway for human exposure to resistant bacteria, as poultry workers, handlers, and consumers of poultry products are at immediate risk of exposure to these resistant organisms.

This finding corroborates previous work [33], which documented the significant human health consequences of antimicrobial use in food animals. The presence of MDR *E. coli* in poultry waste poses a substantial food safety risk through cross-contamination during processing, handling, and consumption of poultry products [18]. The potential for transmission along the food chain underscores the interconnectedness of agricultural practices and human health outcomes, highlighting the need for improved food safety measures from farm to fork.

The phenomenon of horizontal gene transfer amplifies the public health threat considerably, as the environmental dissemination of these resistance genes is particularly dangerous because it introduces them into the broader community microbiome where they can be readily acquired by pathogenic bacteria through horizontal gene transfer [31]. This finding aligns with previous work [31], which documented horizontal gene transfer as a major mechanism of AMR dissemination in microbial communities. The mobility of these genetic elements means that resistance can spread rapidly across bacterial species, including clinically significant human pathogens, thereby accelerating the AMR crisis. The implications of this genetic promiscuity are alarming, as resistance genes can move from environmental or commensal bacteria into pathogenic strains, rendering previously treatable infections increasingly difficult to manage [20].

Limited treatment options represent a direct clinical consequence of the observed resistance patterns, as the presence of MDR strains in 60% of the isolates, with resistance to multiple

antibiotic classes including critically important fluoroquinolones, could significantly complicate the treatment of common *E. coli* infections such as urinary tract infections, sepsis, and other invasive infections [20]. This finding is consistent with the global concern that AMR is progressively reducing the effectiveness of antibiotics, rendering once-treatable infections increasingly difficult to manage [1]. The erosion of treatment options has profound implications for patient outcomes, healthcare costs, and the overall burden of infectious diseases. The presence of resistance to fluoroquinolones, considered critically important antimicrobials for human medicine by the WHO [15], is particularly concerning as it limits therapeutic options for severe infections.

This study has several limitations that should be acknowledged. The sample size was relatively small (n=10), limiting the generalisability of the findings. Future studies should incorporate larger sample sizes and whole-genome sequencing (WGS) to better understand the genetic context of these resistance determinants and their transmission dynamics.

Despite these limitations, the findings highlight an urgent and compelling need for public health action across multiple interconnected domains. First, antibiotic stewardship represents a critical imperative, with an urgent need for enhanced antibiotic stewardship in poultry production including the phasing out of medically important antibiotics as growth promoters [22]. This recommendation is consistent with the global call for action on AMR [1] and aligns with international efforts to preserve the efficacy of critically important antibiotics for human medicine. Second, farmer

education is essential for promoting responsible antibiotic use and waste management practices, as farmers should be educated on proper antibiotic use, dosage regimens, withdrawal periods, and waste management practices to minimise environmental contamination [7]. Third, routine surveillance of AMR in poultry environments should be established as part of Nigeria's national AMR action plan [21], aligning with WHO guidelines on AMR surveillance [1] and providing the data necessary for evidence-based policy making and intervention design. Fourth, a One Health approach integrating human, animal, and environmental health is essential to effectively combat the silent threat of AMR [34], recognising that human health is inextricably linked to animal health and environmental integrity. Finally, policy implementation should be strengthened at the national and state levels, with the Nigerian government strengthening implementation of the National Action Plan on Antimicrobial Resistance [21] by allocating adequate resources, training personnel, and establishing robust monitoring and evaluation mechanisms [22].

Addressing the silent threat of AMR in poultry systems requires coordinated action across multiple sectors and levels of governance, from individual farmers to national policymakers. The four pillars of antibiotic stewardship, farmer education, routine surveillance, and a One Health approach are mutually reinforcing and should be implemented in an integrated manner. The time for action is now, before the window of opportunity to preserve the effectiveness of antibiotics closes further. The findings of this study serve as a wake-up call, underscoring the urgent need for comprehensive and sustained public health interventions to safeguard the

health of the Nigerian population and future generations.

Conclusion

This study confirms that poultry dung from farms in Remo North, Ogun State, harbours phenotypically and molecularly confirmed *E. coli* carrying clinically relevant tetracycline (*tetB*) and quinolone (*qnrB*) resistance genes at high prevalence (70%). A high degree of concordance was observed between phenotypic resistance and genotypic gene presence, validating the clinical relevance of these findings. Statistical analysis using exact binomial 95% confidence intervals (CIs) confirms the significance of these findings, while Fisher's exact test indicates independent dissemination between *tetB* and *qnrB*. The presence of multidrug-resistant (MDR) isolates (60%) is alarming and underscores the urgent need for antibiotic stewardship, farmer education, routine surveillance, and a robust One Health approach to safeguard public health in Nigeria.

Declarations

Ethics Approval and Informed Consent

Not applicable. This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

Authors' Contributions

OSL conceptualised the study, designed the methodology, conducted the investigation, wrote the original draft, and created the visualisations. OMO contributed to methodology,

investigation, and data curation. HOA contributed to methodology and formal analysis. AOS contributed to investigation and validation. AAS contributed to formal analysis and software. ISJE supervised the study and reviewed and edited the manuscript. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

None.

Disclosure of Funding

The study did not receive any external funding.

Data Availability Statement

The data supporting the findings of this study are available within the manuscript. Raw data are available from the corresponding author upon reasonable request.

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