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Prevalence of Multidrug-Resistant *Escherichia coli* from Poultry Farms in Nairobi and Kiambu Counties, Kenya

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Abstract

Antimicrobial resistance (AMR) is a critical global one-health issue affecting human, animal, and environmental health. Factors driving AMR include antibiotic misuse, poor disease prevention, agricultural antimicrobial waste, pollution, and the spread of drug-resistant bacteria via travel and trade. In low- and middle-income countries (LMICs), antibiotics are frequently added to animal feed in small, non-therapeutic doses to promote growth and prevent disease, with about 70% of antibiotics used annually for non-therapeutic purposes. *Escherichia coli (E. coli),* a common bacterium in the lower digestive tracts of warm-blooded animals, is part of the coliform bacteria group. It is a rod-shaped, facultatively anaerobic, Gram-negative bacterium and serves as an indicator for monitoring antibiotic-resistant bacteria in the environment. A significant number of antimicrobial-resistant (AMR) bacteria, including *E. coli*, have been isolated from chickens in Kenya, especially against antibiotics such as ampicillin, tetracycline, sulphamethoxazole, and co-trimoxazole. This study investigated the prevalence and antimicrobial resistance profiles of *Escherichia coli* isolated from poultry farms in Nairobi and Kiambu counties, Kenya. A total of 429 fecal samples were collected from which 92 *E. coli* isolates were confirmed through microbiological methods and MALDI-TOF. Antimicrobial susceptibility testing revealed widespread resistance to individual agents, with 30.4% of isolates meeting multidrug-resistance (MDR) criteria and a high rate of tetracycline resistance (55%). PCR analysis identified *blaTEM, tetA*, *strB*, and *sul2* as the most common resistance genes. Resistance and MDR were statistically comparable between Kiambu and Nairobi counties, indicating a shared regional AMR burden rather than a county-specific pattern, with resistance observed across multiple antibiotic classes. This highlights the urgent need for improved veterinary practices, stricter antibiotic regulations, and alternative

treatment approaches to curb the spread of AMR in poultry, which threatens both animal and public health.

Highlights

1. High prevalence of antimicrobial-resistant *E. coli* in poultry farms in Nairobi and Kiambu, Kenya.
2. 44.4% of resistant isolates (30.4% of all confirmed isolates) were multidrug-resistant.
3. Resistance and ARG prevalence were statistically comparable between Kiambu and Nairobi counties ($p > 0.05$).
4. *blaTEM, tetA,* and *sul2* were the most prevalent resistance genes found.

Background

The commercial poultry industry is a globally significant and thriving sector, producing 130 million tons of poultry meat annually and serving as the leading provider of animal protein worldwide. It is one of the fastest-growing livestock sectors globally, making a significant contribution to food and nutritional security. This industry is especially important to Kenya's population and economy, offering food and income (Agyare et al., 2019). Poultry and egg production are vital to Kenya's agriculture, making up to 30% of the national agricultural gross domestic product (GDP) and 7.8% of the total GDP. According to the Food and Agriculture Organization of the United Nations (FAO), Kenya's demand for chicken is expected to grow substantially over the next thirty years. Additionally, egg demand is projected to increase from about 89,000 tons in 2010 to 238,000 tons annually by 2030, and over 537,000 tons by 2050. Poultry meat production in Kenya is projected to increase substantially in response to rising demand for animal-source foods driven by population growth and urbanization. This growth is expected to position poultry and poultry products among the leading livestock commodities in the country by 2050 (Kiambi et al., 2021).

The poultry industry in Kenya faces challenges, including a high burden of poultry diseases such as colibacillosis, fowl cholera, and salmonellosis, which leads to a high demand for veterinary antimicrobials (Ndukui et al., 2021). In response to this demand, in Kenya, a mean of 14,594 ± 1,457 kilograms of antibiotics per year have been consumed in veterinary practice and livestock production (Waithiru et al., 2022). Furthermore, the practice of using veterinary antimicrobials for disease prevention and growth promotion, especially in dairy, poultry, and pig farming, has become increasingly common among veterinarians and farmers (Kiiti et al., 2021a). In low- and middle-income countries (LMICs), antimicrobial use in livestock

production is projected to increase by approximately 50–70% by 2030, primarily driven by rising demand for animal protein (Van Boeckel et al., 2019).

The widespread use of antimicrobials for disease prevention and growth promotion increases selection pressure on bacteria, promoting the emergence and spread of antimicrobial resistance (AMR) in both pathogenic and commensal microorganisms. These bacteria can be transmitted to humans through multiple pathways, including contaminated food products, environmental exposure, and direct contact with animals, highlighting the importance of a One Health approach. In Kenya, risks are amplified in peri-urban settings where slaughterhouse hygiene, waste disposal, and informal poultry markets may facilitate the dissemination of resistant pathogens (Ndukui et al., 2021).

Escherichia coli (*E.coli*) is a commensal bacterium commonly found in the gastrointestinal tract of humans and animals, but it also serves as an important indicator organism for monitoring antimicrobial resistance in the environment (Anjum et al., 2021). Because *E. coli* readily acquires and transfers resistance genes, it is widely used to assess the burden and dynamics of AMR in livestock systems. Studies have shown that *E. coli* strains from chickens exhibit significant levels of AMR to various drugs, which may be attributed to the improper use of antibiotics for non-intended purposes (Adelaide et al., 2008). Previous studies have highlighted resistance rates to frequently used antibiotics, such as ampicillin (76%), tetracycline (71%), and sulfamethoxazole (70%) (Kiambi et al., 2021).

Around 200 distinct resistance genes, including *tetA, tetB, strA,* and *strB,* have been identified in bacterial isolates in Kenya (Omar et al., 2024). Additionally, research indicates that antibiotic-resistant bacteria are found in Kenyan chickens (Kariuki et al., 2023). They found that the prevalence of antibiotic-resistant *E. coli* in chicken feces from broilers and layers was 57%, which differed from findings reported in similar studies (Kiambi et al., 2021). Reports

also suggest that even in local poultry varieties that receive minimal antibiotics, antimicrobial resistance (AMR) remains significant (Adelaide et al., 2008).

Despite growing evidence of AMR in poultry systems in Kenya, important knowledge gaps remain. There is limited research data on AMR prevalence in peri-urban and urban poultry systems, for example, in counties such as Nairobi and Kiambu. In addition, few studies have conducted phenotypic resistance profiling alongside molecular detection of resistance genes to better understand the mechanisms underlying AMR. According to Anjum et al. (2021)*E. coli* can serve as an indicator for monitoring antibiotic-resistant bacterial phenotypes in the environment. To increase our knowledge of the AMR problem in Kenyan farming systems, this study aimed to isolate *E. coli* from fecal samples obtained from poultry farms in Kiambu and Nairobi counties, determine the susceptibility of the isolates to selected commonly used antibiotics, investigate the presence of important resistance genes in these isolates, and look at the different antibiotic resistance patterns that are found in Kenyan poultry farms.

Materials and methods

Study design and study area

A cross-sectional study was carried out in Nairobi and Kiambu Counties, as shown in Figure 1, from 2019 to 2021. A total of 61 poultry farms were sampled. In Nairobi County, 21 farms were visited across the sub-counties of Kasarani, Dagoretti, Ruai, and Njiru; in Kiambu County, 40 farms were visited across the sub-counties of Thika, Lower Kabete, Juja, Gatundu South, and Ruiru. Farms were purposively selected within these sub-counties based on accessibility and willingness to participate as part of the parent biorepository collection (Gunathilake et al., 2024; Mhone et al., 2022) rather than through random or stratified sampling; consequently, prevalence estimates should be viewed as suggestive rather than population-representative since the sample may not be representative of all chicken farms in Nairobi and Kiambu counties. The poultry farms in the counties were georeferenced using a geographical positioning system, and the map was obtained using R software version 4.5.1, as shown in (Figure 1.)

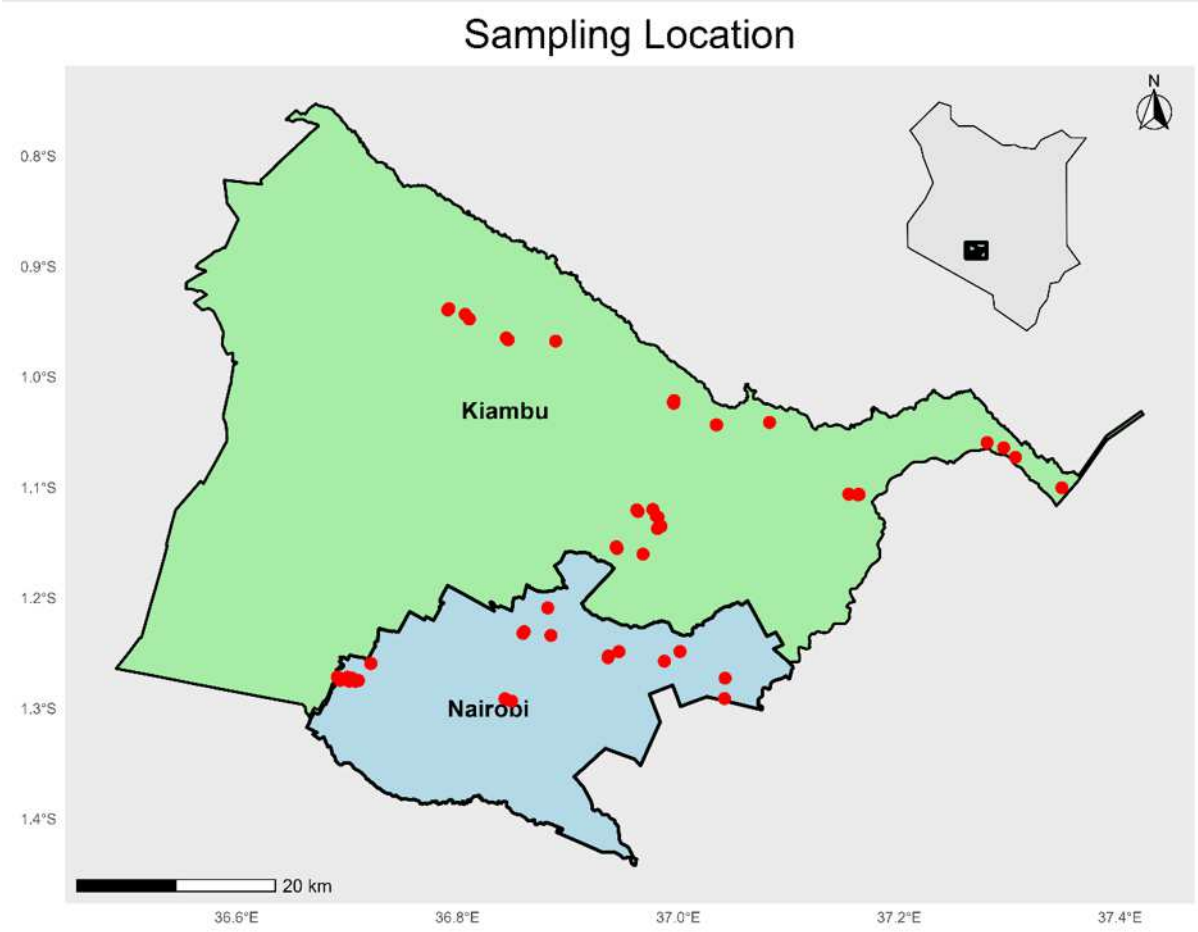

Figure 1. Location of the study area and sampled poultry farms in Nairobi and Kiambu Counties, Kenya.

Sample collection

A total of 429 archived poultry fecal samples collected from 61 poultry farms in Nairobi and Kiambu counties, stored at -80°C, were retrieved from the ILRI Biorepository in a biosafety level 2 laboratory for antimicrobial resistance analysis (Mhone et al., 2022;Gunathilake et al., 2024).

Escherichia coli isolation and identification

A total of 5 mL of enrichment media (Buffered Peptone Water; Oxoid, UK; cat. # CM0509) was inoculated with 1 g of chicken feces and incubated overnight at 37 °C. The samples were then placed on MacConkey agar (Oxoid Ltd., Ireland, cat. 3132377) and incubated overnight at 37

°C. Afterwards, donut-shaped, dry, and pink-to-dark-pink colonies were selected as suspected *E. coli* and further subcultured on MacConkey agar to obtain pure cultures. The pure cultures were further identified using Matrix-Assisted Laser Desorption Ionization-time of Flight Mass Spectrometry (MALDI-TOF) (Bruker MALDI Biotyper Identification Machine) (Chen et al., 2021). The positive E. coli ATCC 25922 strain was used as a positive control. The results were interpreted using score values, with 2.00-3.00 interpreted as high-confidence identification, 1.70-1.99 as low-confidence identification, and 0.00-1.69 as no organism identification possible.

Antimicrobial susceptibility testing

Antimicrobial sensitivity testing was performed on all confirmed-positive *E. coli* isolates (n = 92) using the disk diffusion method on Mueller-Hinton agar, as described by (Gajic et al., 2022). A standard bacterial suspension was prepared by selecting isolated *E. coli* colonies and adjusting the turbidity to match the 0.5 McFarland standard. A sterile cotton swab dipped into the standardized suspension was used to evenly swab the entire surface of the Mueller-Hinton agar, which was then allowed to dry for 5 minutes. Sterilized forceps were used to place the antibiotic disks on the agar surface, ensuring they were spaced at least 24 mm apart to avoid overlapping. The plates were incubated at 37°C for 18 hours. Interpretation was based on the Clinical and Laboratory Standards Institute's (CLSI) guidelines. Outcomes were recorded as zone sizes for each antibiotic and compared with relevant interpretation standards. Results were classified as susceptible, intermediate, or resistant based on breakpoints (Humphries et al., 2019). *E. coli* ATCC strains were used to control quality. The antibiotics tested included cefoxitin (FOX) 30 µg, ampicillin (AMP) 10 µg, streptomycin (ST) 30 µg, amoxicillin/clavulanic acid 30 µg, trimethoprim/sulfamethoxazole (SXT) 25 µg, ciprofloxacin (CIP) 5 µg, tetracycline (TET) 30 µg, gentamicin (GEN) 10 µg, nalidixic acid (NA) 30 µg, cefepime 30 µg, aztreonam 30

µg, cefpodoxime 30 µg, meropenem 30 µg, ceftazidime (CAZ) 30 µg, ceftriaxone (CRO) 30 µg, kanamycin (K) 30 µg, neomycin (N) 30 µg, and penicillin G (P) 10 µg.

Detection of Antimicrobial Resistance Genes (ARGs)

The DNA of the 92 *E. coli* isolates was extracted using the Promega kit (cat. No 0000054672). Antimicrobial resistance genes (ARGs) were detected through conventional PCR. For each sample, a 25 µL PCR mixture was prepared, containing 1 µL of each forward and reverse primer for the target genes (Table 1), 12.5 µL of Taq master mix, 2 µL of *E. coli* DNA, and 11.5 µL of nuclease-free water. DNA amplification was carried out in a thermocycler with conditions including initial denaturation, denaturation, annealing, extension, and final extension, following the protocol of Jaja et al. (2019). The target ARGs included *tetA, tetB, tetC, tetM, aadA1, aadA2, strA, strB, aacC, cat1, cat2, dfrl, floR, sul2, blaZ, blaOXA-1*, and *blaTEM* (Messele et al., 2017). Nuclease-free water served as a negative control. After PCR, a 1.5% agarose gel was used for gel electrophoresis to verify the expected band size, which was visualized with a gel reader. Primer sequences, expected amplicon sizes, and target resistance genes are summarized in Table 1. All primers were adopted from previously published studies and synthesized commercially (Messele et al., 2017).

Organism/Antibiotics	Genes/Target	Primers 5'-3'	Product size
Beta-lactam	*blaTEM*	Fw 5' CATTTCCGTGTCGCCCTTAT 3'	690
		Rev 5' TCCATAGTTGCCTGACTCCC 3'	
	blaZ	Fw 5'ACTTCAACACCTGCTGCTTTC 3'	490
		Rev 5''TGACCACTTTTATCAGCAACC 3'	
	blaOXA	Fw 5' ATATCTCTACTGTTGCATCTCC 3'	619
		Rev 5'AAACCCTTCAAACCATCC 3'	
Tetracycline	*tetA*	Fw 5' GCTACATCCTGCTTGCCTTC 3'	201
		Rev 5' CATAGATCGCCGTGAAGAGG 3'	
	tetB	Fw 5' TTGGTTAGGGGCAAGTTTTG 3'	359
		Rev 5' GTAATGGGCCAATAACACCG 3'	

	tetC	Fw 5′ CTTGAGAGCCTTCAACCCAG 3′	418
		Rev 5′ ATGGTCGTCATCTACCTGCC 3′	
	tetM	Fw 5′AGTGGAGCGATTACAGAA 3′	158
		Rev 5'CATATGTCCTGGCGTGTCTA3'	
Sulphonamide	*sul2*	Fw 5′ CGGTCCGGCATCCAGCAATCC3′	625
		Rev 5′ CGAGAGCCACGACCGCGCC 3′	
Streptomycin	*aadA1*	Fw 5′ TATCAGAGGTAGTTGGCGTCAT 3′	525
		Rev 5′ GTTCCATAGCGTTAAGGTTTCATT 3′	
	aadA2	Fw 5′ TGTTGGTTACTGTGGCCGTA 3′	420
		Rev 5′ GATCTCGCCTTTCACAAAGC 3′	
	aacC	Fw 5′ GGCGCGATCAACGAATTTATCCGA 3′	428
		Rev 5′ CCATTCGATGCCGAAGGAAACGAT 3′	
	strA	Fw 5′ AGCAGAGCGCGCCTTCGCTC 3′	348
		Rev 5′ CCAAAGCCCACTTCACCGAC 3′	
	strB	Fw 5′ ATCGTCAAGGGATTGAAACC 3′	509
		Rev 5′ GGATCGTAGAACATATTGGC 3′	
Chloramphenicol	*cat1*	Fw 5′ CTTGTCGCCTTGCGTATAAT 3′	508
		Rev 5′ ATCCCAATGGCATCGTAAAG 3′	
	cat2	Fw 5′ CCGGATTGACCTGAATACCT 3′	572
		Rev 5′ TCACATACTGCATGATGAAC 3′	
	dfrI	Rev 5′ ACCCTTTTGCCAGATTTGGTAACT 3′	470
		Fw 5′ AACCCGCCCTCTGGATCAAGTCAA 3′	
	floR	Fw 5′ GTGAAACTATCACTAATGGTAGCT 3′	548
		Rev 5′ CAAATCACGGGCCACGCTGTATC 3′	

Table 1. List of antimicrobial resistance genes (ARGs) tested in this study, including their target antibiotic classes, primer sequences (forward and reverse), and the expected PCR amplicon sizes.

Results

E. coli distribution

To better understand the burden of AMR in poultry farms, especially in *E. coli*, we sampled farms in Kiambu and Nairobi Counties (Figure 1). The selection of these counties is based on the fact that Kiambu has the largest broiler poultry population, with over 533,562 broilers in 2022, accounting for 30% of Kenya's total broiler population. This makes it a key area for studying broiler farming practices in the country. Additionally, it is located in the peri-urban area of Nairobi City County, which has a human population of 5.5 million (World Population Review, 2024), and serves as a major market hub for poultry products. Nairobi County also has a high number of chickens per household, with approximately 65% of households in Nairobi reported to be raising chickens (Langata et al., 2019).

From these areas, a total of 429 samples were analyzed, and 119 lactose fermenters were initially identified on MacConkey plates, suggesting that these bacteria could be *E. coli*. Further confirmation was done using MALDI-TOF, which validated 92 of these isolates as *E. coli*. The demographic details of the positive *E. coli* samples are shown in Table 2. A slightly higher percentage of positive *E. coli* was found in Kiambu (21.5%, 56/260) than in Nairobi (21.3%, 36/169), and among breeds, layers showed the highest proportion of positive *E. coli* cases (23.1%, 28/121), followed by broilers (21.1%, 57/270) and indigenous chickens (18.4%, 7/38) (Table 2*).* However, neither difference was statistically significant (county: $\chi^2 < 0.001$, df = 1, $p > 0.99$; breed: $\chi^2 = 0.43$, df = 2, $p = 0.81$), indicating that *E. coli* isolation was broadly comparable across counties and poultry breeds in this study.

Characteristic	N=429	Positive *E. coli*	%
E. coli		92	21.4
County			
Nairobi	169	36	21.3
Kiambu	260	56	22.0
Breed (Chicken)			
Broiler	270	57	21.1
Layer	121	28	23.1
Indigenous	38	7	18.4

Table 2. Demographic characteristics of poultry farms sampled in Nairobi and Kiambu Counties, showing the distribution of positive E. coli isolates by county and chicken breed.

Phenotypic resistance to antibiotics

The highest resistance among all tested antibiotics was towards penicillin G (92/92, 100%) and erythromycin (91/92, 99%) (Figure 2). Penicillin G was included for comparative screening purposes; however, *E. coli* is intrinsically resistant to penicillin, and therefore these results should be interpreted with caution. Relatively low resistance levels were observed for kanamycin, nalidixic acid, ciprofloxacin, cefoxitin, cefpodoxime, and chloramphenicol compared with tetracycline, erythromycin, and ampicillin. All 92 *E. coli* isolates were susceptible to ceftriaxone, ceftazidime (30 μg), cefepime, aztreonam, meropenem, gentamicin, and amoxicillin-clavulanic acid (Figure 2).

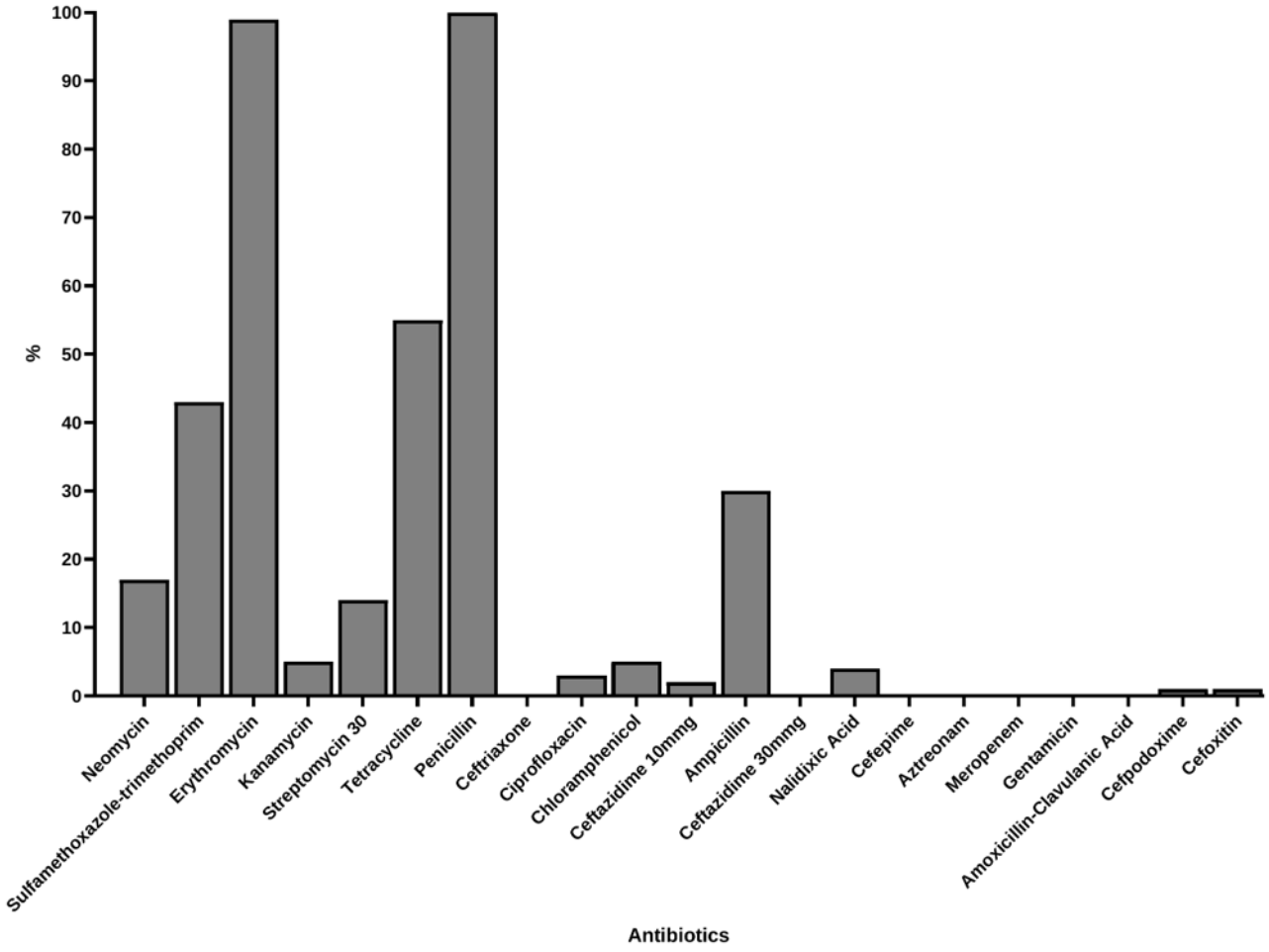

Figure 2. Antimicrobial resistance profile of *E. coli* isolates from poultry farms in Nairobi and Kiambu Counties.

Multidrug resistance of the *E. coli* isolates

Of the 92 *E. coli* isolates, 63 (68.5%) isolates showed resistance to one or more classes of antibiotics. Of the 63 isolates, 25 (39.7%) were from Nairobi and 38 (60.3%) from Kiambu. In Nairobi, 8 of 25 isolates (32%) were resistant to two classes of antibiotics, 6 (24%) to three, and 5 (20%) to four, as shown in Figure 3. In Kiambu County, out of the 38 *E. coli* isolates, 9 (23.7%) were resistant to two classes of antibiotics, 8 (21%) were resistant to three classes of antibiotics, and 9 (23.7%) were resistant to four different classes of antibiotics, as shown in (Figure 4). In this study, we defined multidrug resistance (MDR) as resistance to three or more antibiotic classes (Magiorakos et al., 2012). This gave us a prevalence of 44.4% MDR in the two

counties. Because *E. coli* is intrinsically resistant to penicillin G, this agent was excluded from the antibiotic-class count used to classify isolates as MDR, so as not to artificially inflate the MDR profile. Of the 63 isolates that showed resistance to at least one antibiotic class, 28 (44.4%) met the criteria for MDR; expressed as a proportion of all 92 confirmed *E. coli* isolates, the overall MDR prevalence was 30.4% (28/92). MDR prevalence did not differ significantly between the two counties (Nairobi: 11/36, 30.6%; Kiambu: 17/56, 30.4%; Fisher's exact test, p = 1.00).

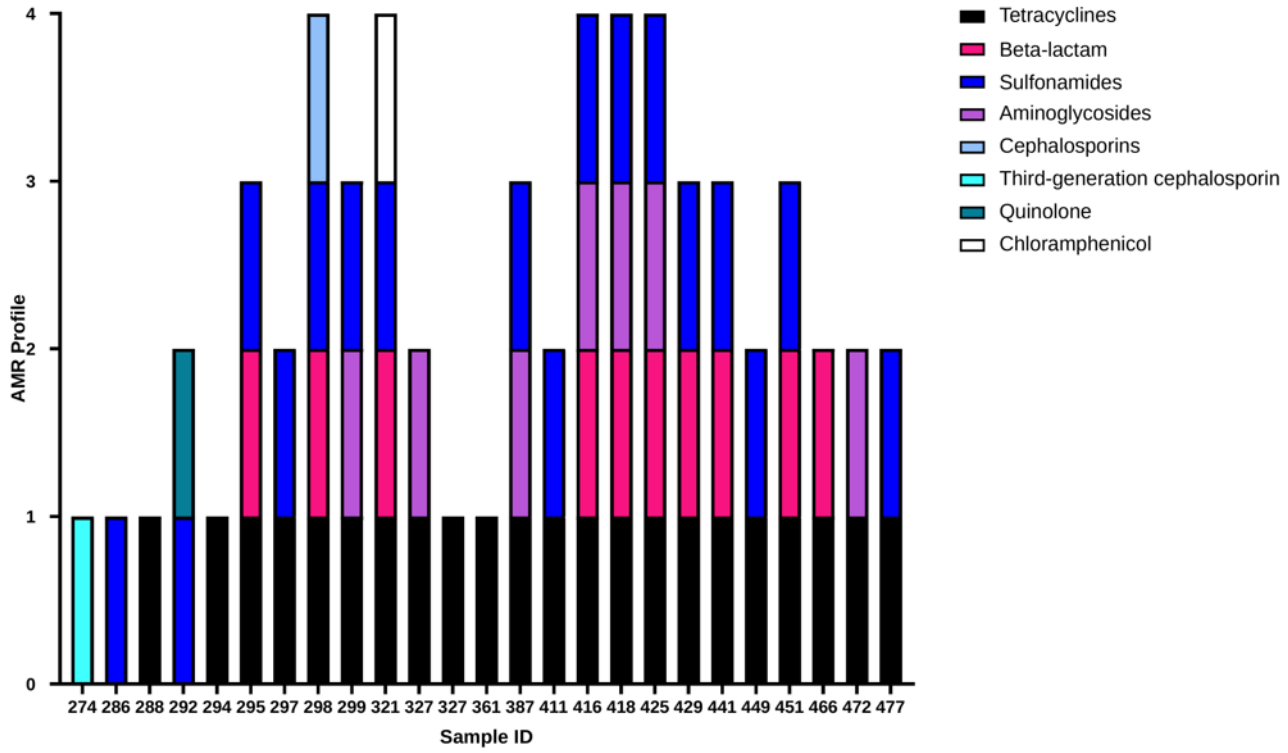

Figure 3. Multidrug resistance (MDR) profile of *E. coli* isolates from Nairobi County.

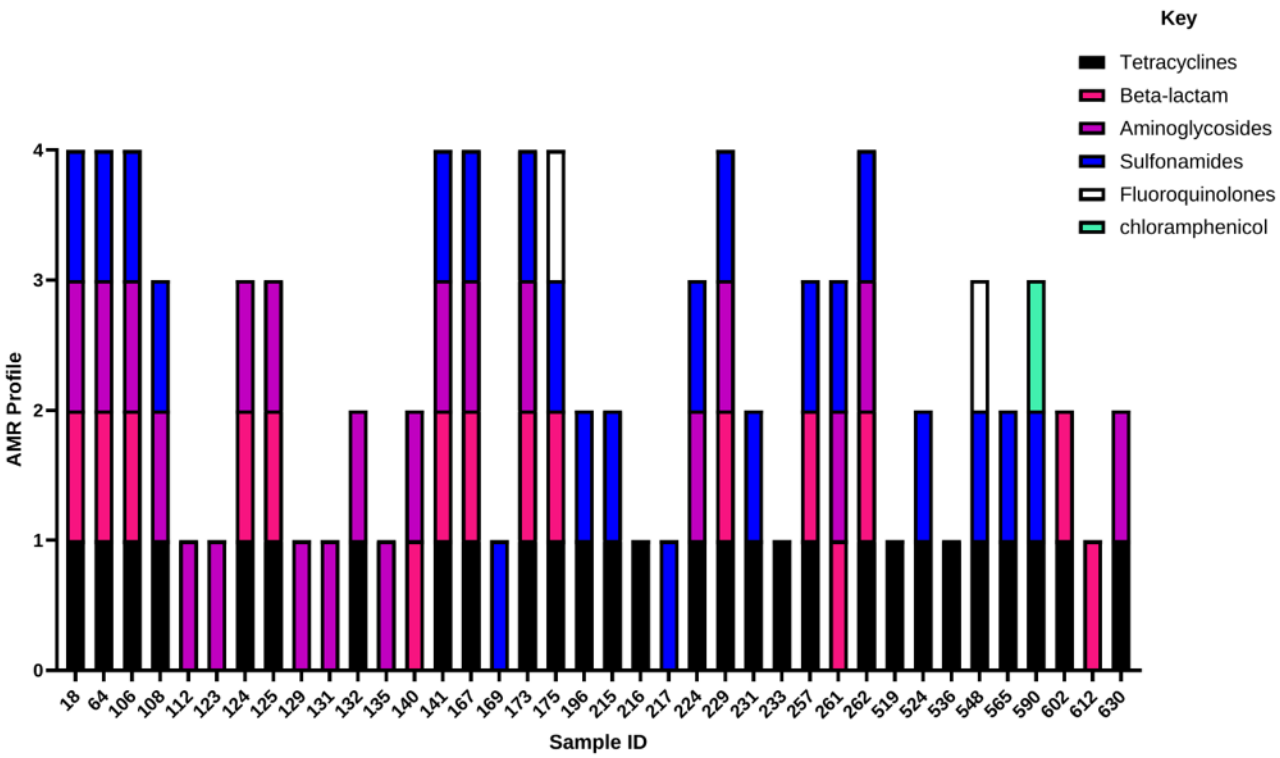

Figure 4. Multidrug resistance (MDR) profile of *E. coli* isolates from Kiambu County.

Resistance patterns among *E. coli* isolates from Nairobi and Kiambu Counties.

An UpSet plot (Figure 5) was generated to show the distribution of *E. coli* isolates resistant to different antibiotic classes. From this analysis, tetracyclines and sulfonamides have the highest prevalence among all antibiotic classes. The highest combination had 11 isolates, showing MDR patterns. Notably, multiple isolates show simultaneous resistance to beta-lactamases, aminoglycosides, and tetracyclines, suggesting extensive multidrug resistance (MDR).

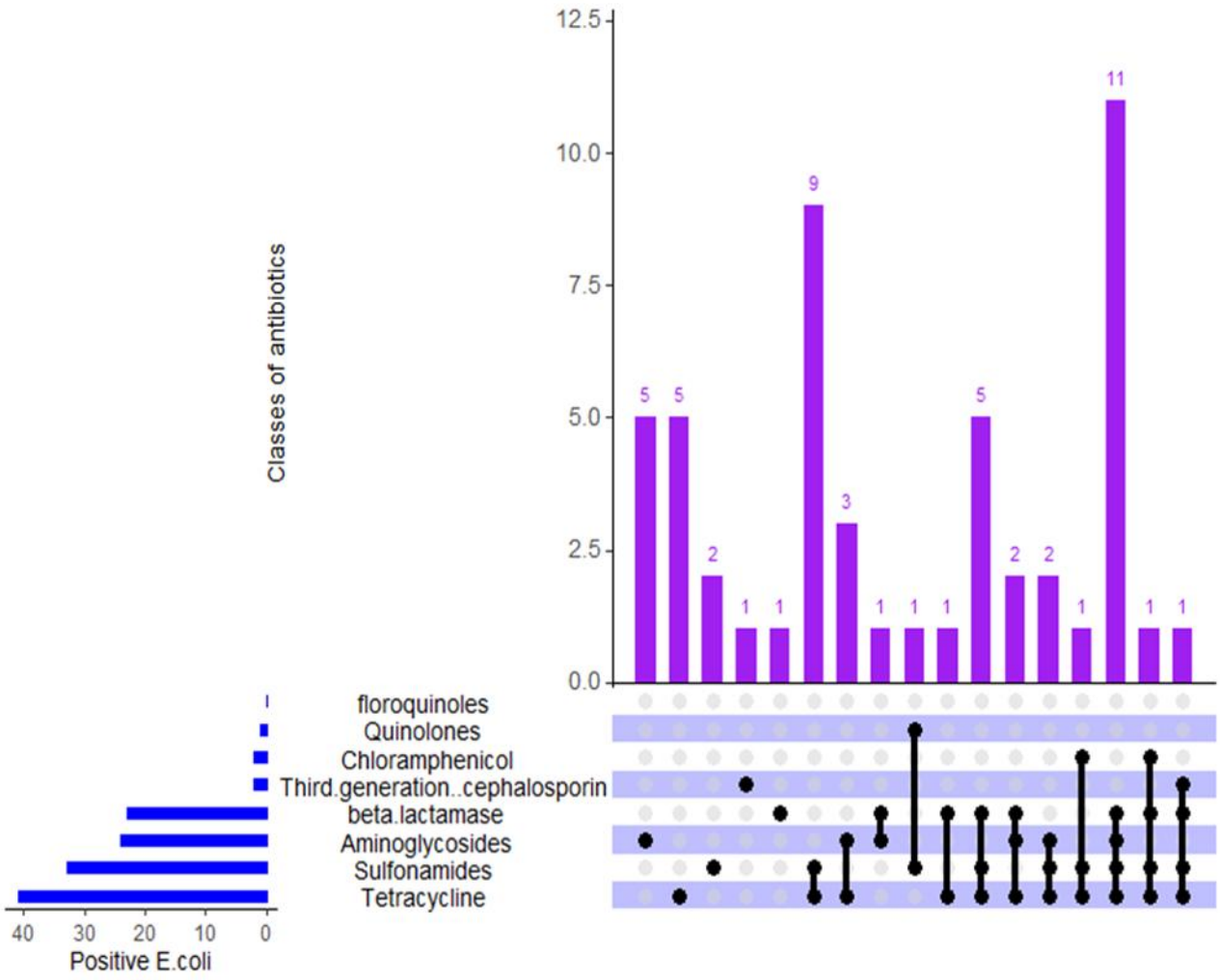

Figure 5. Resistance patterns among *E. coli* isolates from Nairobi and Kiambu Counties.

Prevalence of antibiotic-resistant genes.

All 92 *E. coli* isolates, regardless of their phenotypic results and whether they were resistant or susceptible to the antibiotic disk diffusion method, were screened for resistance determinants associated with antibiotic resistance using PCR. Among the 36 *E. coli* samples isolated from Nairobi County, *strB*, *tetA*, *blaTEM*, and *sul2*, conferring resistance to streptomycin, tetracycline, ampicillin, and sulfamethoxazole-trimethoprim, respectively, had the highest prevalence. The resistance genes *aacC*, *cat2*, and *floR*, which confer resistance to aminoglycosides, chloramphenicol, and fluoroquinolones, respectively, showed the lowest rates of resistance. In contrast, the *tetC* gene was absent in all the isolates (Figure 6). Of the

56 *E. coli* isolates from Kiambu County, the *blaTEM* and *sul2* ARGs showed the highest prevalence of 86% and 80%, respectively. On the contrary, *cat2*, *aacC*, and *tetM* showed the lowest ARG prevalence, whereas *floR* and *tetC* were absent from all isolates (Figure 7).

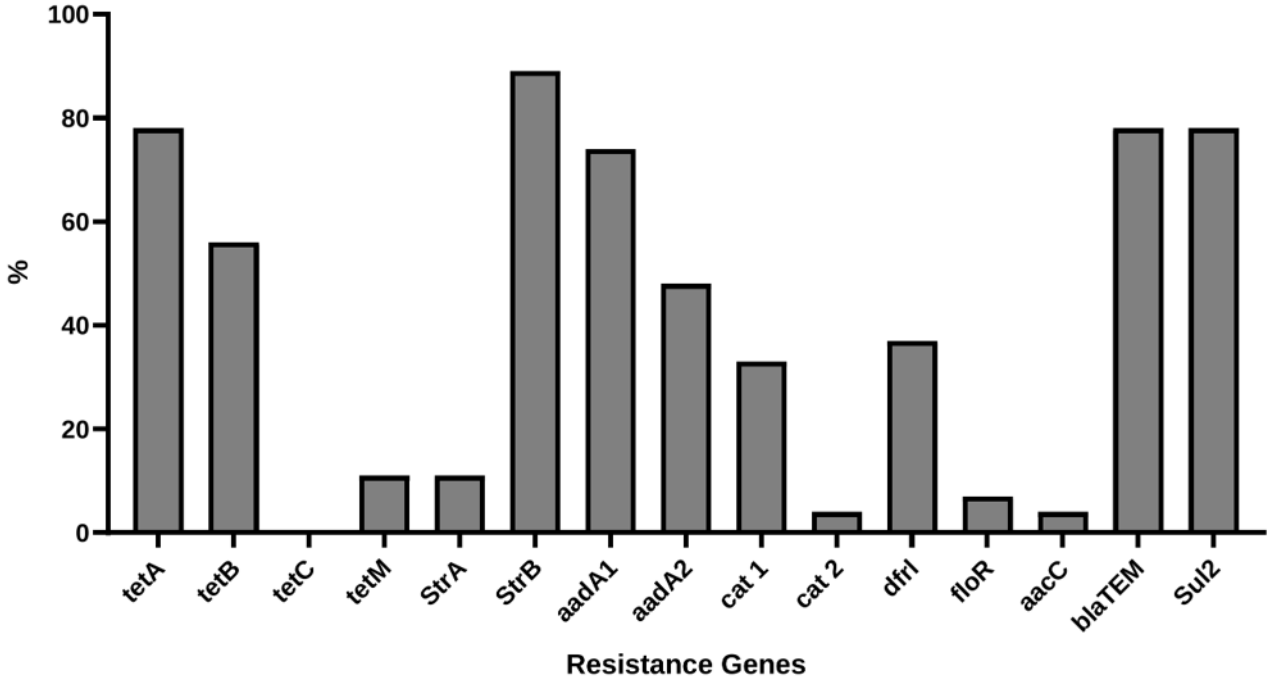

Figure 6. Prevalence of antimicrobial resistance genes in *E. coli* isolates from Nairobi County.

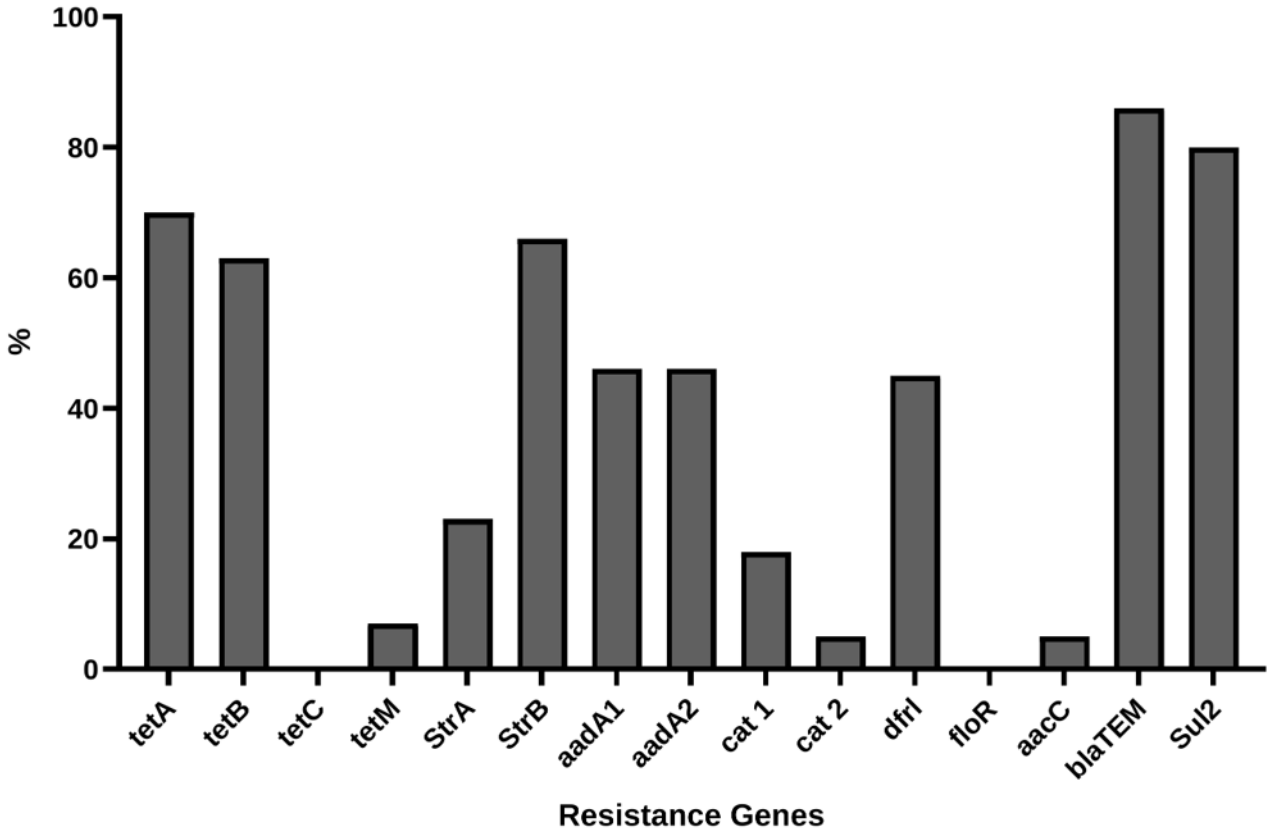

Figure 7. Prevalence of antimicrobial resistance genes in *E. coli* isolates from Kiambu County.

Prevalence of antimicrobial resistance genes in different poultry breeds

The analysis of ARGs in *E. coli* isolates from indigenous (Kienyeji), broiler, and layer chicken breeds reveals varying levels of gene prevalence**.** The *tetA* and *strB* ARGs are highly prevalent in 72% and 82% of indigenous and broiler *E. coli* isolates, respectively. Both the *blaTEM* and *sul2* genes are highly prevalent in all groups, but they are most prevalent in broilers (*blaTEM*, 91%; sul2, 29.5%) and indigenous chickens (blaTEM, 82%; sul2, 27.8%). Layers show a different pattern of ARGs predominance, with *aadA1* (65%), *strB* (75%), *tetA* (75%), and *tetB* (70%). The *aadA1* and *aadA2* genes are moderately prevalent in *E. coli* isolates (52% in indigenous chickens and 55% in broilers, respectively; 46% overall). The *dfrl* gene has a moderate prevalence among *E. coli* isolates (44% in indigenous chickens and 55% in broilers). The incidence of *dfrl* (30%) and *aadA2* (55%) is moderate in layers. Low-prevalence genes

include *tetM* (8% in indigenous chickens and 14% in broilers), *strA* (16% in indigenous chickens, 18% in broilers), and various *cat* genes. These genes are less prevalent yet present in all groups. Interestingly, the prevalence of *tetC*, *blaZ*, and *blaOXA-1* genes is null in all three groups, suggesting that current farming practices do not select for these resistance genes (Figure 8). Overall, these findings indicate a substantial burden of antimicrobial resistance among *E. coli* isolates, with a high proportion exhibiting multidrug resistance and carrying clinically relevant resistance genes.

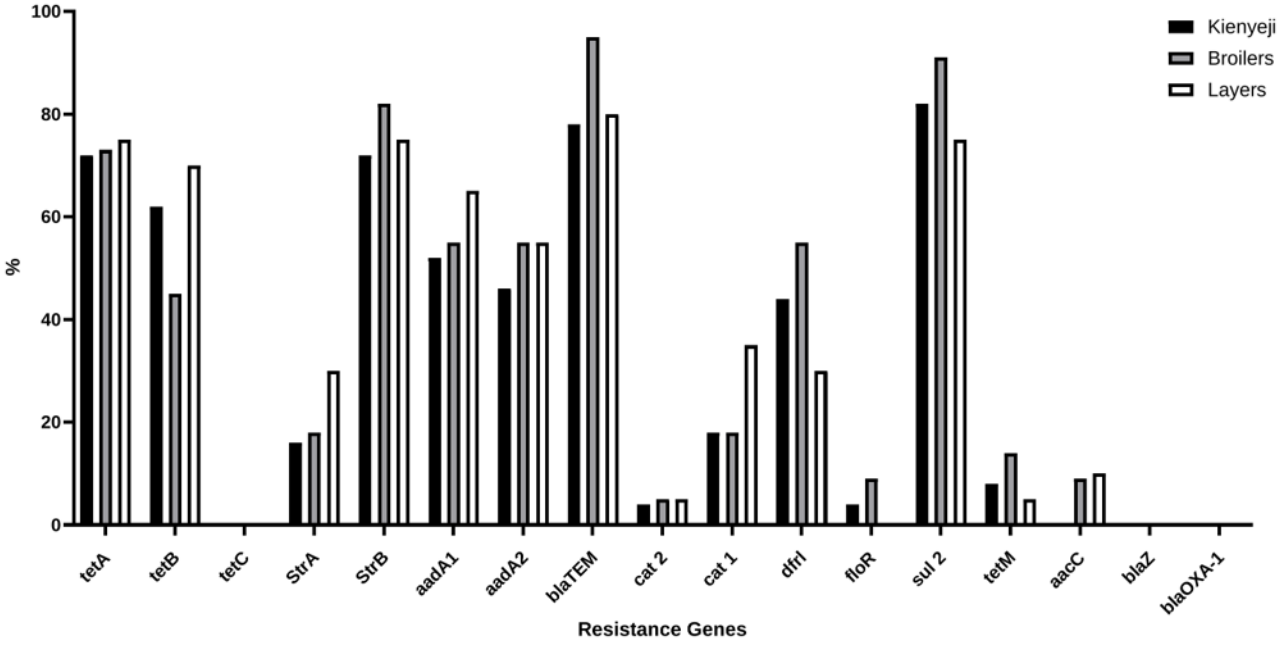

Figure 8. Distribution of antimicrobial resistance genes in *E. coli* isolates from different poultry breeds

Discussion

This study found a high burden of antimicrobial resistance in *E. coli* isolates from poultry farms in Nairobi and Kiambu counties, with 44.4% of resistant isolates (28/63), equivalent to 30.4% of all confirmed isolates (28/92), meeting the criteria for multidrug resistance (MDR). This prevalence is lower than that reported in Ghana, where MDR was found in 57.1% of *E. coli* from fecal samples (Mensah et al., 2022). However, it is comparable to other findings from Kenya, as another study observed 40% of the isolates showing MDR to three antibiotic classes (Waithiru et al., 2022a). This variation may potentially reflect differences in farming systems, access to antimicrobials, or biosecurity practices, although antimicrobial usage data were not collected in this study. This level of resistance is concerning and shows the threat to public health that the poultry sector in Kenya is facing. Overall, the substantial proportion of resistant isolates meeting MDR criteria (30.4% of all confirmed isolates) indicates a notable AMR burden in poultry systems, posing a risk to both animal and human health.

The high level of resistance found to popular antibiotics, including ampicillin, tetracycline, and sulfamethoxazole-trimethoprim, is in accordance with other research conducted in Kenya, for example, Waithiru et al., (2022) reported resistance in *E. coli* strains from Kiambu County with high resistance to tetracycline, ampicillin, and sulfamethoxazole-trimethoprim, which may suggest selection pressure associated with antimicrobial exposure in poultry systems. These results point to sustained selection pressure within the production systems, which encourages resistant bacterial populations to spread and increase.

Interestingly, we observed resistance to some antibiotics that are not commonly used on poultry farms, such as ciprofloxacin and streptomycin. This has also been reported in Ghana (Agyare et al., 2019). This can be attributed to horizontal gene transfer of ARGs or to co-

selection due to multidrug exposure (Loayza et al., 2020). Our study recorded significantly lower levels of ciprofloxacin resistance (3%) compared with the study by (Adelaide et al., 2008), which reported 19%.

The most prevalent antibiotic patterns found in this study were between tetracycline and sulfonamides, which is similar to those of other studies done (Adelaide et al., 2008). The widespread patterns observed in this analysis indicate that antibiotic use practices on poultry farms may be similar across places and may promote the development of antibiotic resistance. Overall, the findings from this study provide evidence that *E. coli* from poultry farms exhibit a high level of AMR and can serve as an indicator of AMR dissemination in poultry.

The detection of resistance genes, such as *blaTEM* and *tetA*, supports previous studies indicating that genetic elements, including integrons and plasmids, contribute to the spread of AMR genes among *E. coli* strains in poultry environments. Resistance levels and ARG prevalence were numerically similar between Kiambu and Nairobi, and this comparability was supported statistically, with neither county-level *E. coli* prevalence ($p = 0.99$) nor county-level MDR proportion ($p = 1.00$) reaching statistical significance. We do not see county as a driver of the resistance patterns found here. Shared regional customs rather than county-specific ones might provide a more convincing explanation: The average yearly consumption of antimicrobials in Kenya's veterinary sector is 14,594 ± 1,457 kg. (Waithiru et al., 2022), and the use of antimicrobials for disease prevention and growth promotion has become increasingly common among poultry farmers and veterinarians across production systems, not only in one county(Kiiti et al., 2021). Both Nairobi and Kiambu sit within the same peri-urban poultry corridor, where informal drug access, over-the-counter antimicrobial purchase, and limited veterinary oversight have been documented(Ndukui et al., 2021), which may explain why resistance profiles converge across the two counties rather than diverge by

farming intensity. Without farm-level antimicrobial usage records, the specific drivers of resistance at each site cannot be disentangled in this study, underscoring the need for future surveillance to pair AMR data with detailed AMU and farm-management records. The prevalence of *blaTEM* and *sul2* in this study, especially in Kiambu, is consistent with the study by (Messele et al., 2017), where *blaTEM* and *sul2* genes were among the most detected resistance genes. Our study showed *blaTEM* in 85.7% of isolates from Kiambu and *sul2* in 80.35%, figures close to (Messele et al., 2017b) findings. This highlights the continued presence and spread of resistance genes in poultry farming environments in Kenya.

From a One Health perspective, the high prevalence of MDR observed in the two counties poses a significant risk to human health. In both urban and peri–urban areas, close

contact between humans and poultry, poor management, and informal slaughterhouses may facilitate the transmission of resistant bacteria. These challenges can be addressed by following measures such as enhancing the AMR surveillance system and encouraging antimicrobial stewardship among poultry farmers.

Conclusion

This study provides baseline evidence that shows AMR is common among the *E. coli* isolates recovered from poultry farms in Nairobi and Kiambu counties, including high resistance to tetracycline, sulfamethoxazole-trimethoprim, and ampicillin, which could jeopardize not only animal but also human health. The presence of resistance genes, particularly *blaTEM* and *sul2*, suggests that other mechanisms of resistance might also be present in poultry farms. The widespread presence of these genes aligns with findings from similar studies across Kenya and other regions, indicating that AMR is a nationwide challenge. Based on our findings, we recommend promoting veterinary-guided prescribing, increasing regulation and oversight of antibiotic use in chicken raising, and evaluating alternate disease-prevention tactics, such as enhanced biosecurity. These findings, which are based on a baseline, cross-sectional analysis of archived samples from two counties, must be corroborated by prospective, farm-level studies that integrate AMR surveillance with antibiotic usage data before being more widely applied to Kenya's chicken sector. Several methodological limitations should be considered when interpreting these findings. As part of the parent biorepository collection, sampling was conducted throughout accessible sub-counties in Nairobi and Kiambu; hence, the sample may not be representative of all poultry farms in either county, and the results should be interpreted cautiously. Only 92 out of 429 archived faecal samples (21.4%) yielded a confirmed *E. coli* isolate; this relatively low recovery is likely caused by the selective nature of MacConkey-based culture, reduced organism viability after prolonged storage at -80°C and freeze-thaw cycling, and the selective nature of MacConkey-based culture, rather than a genuine lack of *E. coli* in the original samples. As all resistance and MDR estimates are based on this smaller recovered subset rather than the full 429 samples, they should be interpreted as indicative of circulating resistance among viable isolates rather than as a precise

population-level prevalence estimate. Other limitations included the study's limited geographic coverage, the use of archival samples, and the lack of thorough farm-level antimicrobial usage data. Furthermore, because just a portion of resistance genes were studied, the total genetic diversity of antimicrobial resistance may be underestimated. The comparisons at the county and breed levels presented here should be interpreted as descriptive rather than as evidence of actual regional or breed-based differences because they were not statistically significant. This is because associations between resistance patterns and antimicrobial use practices could not be directly established because farm-level antimicrobial usage data were not analysed.

Declarations.

Abbreviations

AMR, antimicrobial resistance; AMP, ampicillin; ARG, antibiotic resistance gene; ATCC, American Type Culture Collection; CGIAR, Consultative Group on International Agricultural Research; CIP, ciprofloxacin; CLSI, Clinical and Laboratory Standards Institute; DNA, deoxyribonucleic acid; *E. coli*, Escherichia coli; FAO, Food and Agriculture Organization of the United Nations; GDP, gross domestic product; IDRC, International Development Research Centre; ILRI, International Livestock Research Institute; LMICs, low- and middle-income countries; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight mass spectrometry; MDR, multidrug-resistant; NACOSTI, National Commission for Science, Technology and Innovation; PCR, polymerase chain reaction; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

Ethics approval and consent to participate

Institutional ethics approvals and the Kenyan government's National Commission for Science, Technology, and Innovation (NACOSTI) approvals were obtained: license numbers ILRI-IREC2019-08/1, ILRI-RCO12/IBC/012/CR, and NACOSTI/P/19/94777/28167. Permission to collect the faecal specimens was obtained from the County governments of Nairobi and Kiambu, and from the owners of each participating poultry farm, who gave informed consent before sampling. No additional permits were required, as the study involved only the non-invasive collection of faecal droppings from poultry, with no handling of birds beyond routine farm access, and no protected or endangered species were sampled. The specimens were collected as part of a parent biorepository collection(Mhone et al., 2022 ; Gunathilake et al., 2024) and were archived at the ILRI Biorepository at -80 °C until used in the present study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article. The datasets generated and analysed during the study (isolate-level antimicrobial susceptibility results and resistance gene PCR results) are available from the corresponding author. No DNA or RNA sequence data were generated in this study, as isolates were identified by MALDI-TOF mass spectrometry.

Competing interests

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contributions

J. O.: designed and performed laboratory experiments, conducted field sampling activities, analyzed the data, and wrote the manuscript. **A.M.**: supervised laboratory experiments, conducted field sampling activities, and reviewed the manuscript. **L. G.**: performed laboratory experiments, isolated and typed the *E. coli* strains, and conducted field sampling activities. **R.N.**: provided inputs to the conceptualization of experiments and to the analyses of data and reviewed the manuscript. **N.S.**: conceptualized and designed experiments, supervised microbiology experiments and field sampling activities, provided inputs to the analyses of data, acquired funding, and wrote the manuscript. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. Location of the study area and sampled poultry farms in Nairobi and Kiambu Counties, Kenya.

Map showing Nairobi County (urban) (blue) and Kiambu County (peri-urban) (green), with the inset indicating their position within Kenya. Georeferenced locations of the sampled poultry farms (red dots) in Nairobi and Kiambu, mapped using R version 4.5.1

Figure 2. Antimicrobial resistance profile of *E. coli* isolates from poultry farms in Nairobi and Kiambu Counties.

Percentage of *E. coli* isolates (n = 92) resistant to each antibiotic tested by disk diffusion. All isolates were susceptible to ceftriaxone, ceftazidime (30 µg), cefepime, aztreonam, meropenem, gentamicin, and amoxicillin-clavulanic acid. Penicillin G was included for comparison only, because *E. coli* is intrinsically resistant to it.

Figure 3. Multidrug resistance (MDR) profile of *E. coli* isolates from Nairobi County.

Each bar represents one isolate (sample ID on the x-axis) that was resistant to at least one antibiotic class (n = 25). Bar height shows the number of antibiotic classes to which the isolate was resistant (AMR profile), and colours identify the individual classes. Isolates resistant to three or more classes were classified as MDR; penicillin G was excluded from the class count.

Figure 4. Multidrug resistance (MDR) profile of *E. coli* isolates from Kiambu County.

Each bar represents one isolate (sample ID on the x-axis) that was resistant to at least one antibiotic class (n = 38). Bar height shows the number of antibiotic classes to which the isolate was resistant (AMR profile), and colours identify the individual classes. Isolates resistant to three or more classes were classified as MDR; penicillin G was excluded from the class count.

Figure 5. Resistance patterns among *E. coli* isolates from Nairobi and Kiambu Counties.

UpSet plot of the antibiotic classes to which isolates were resistant. Vertical bars show the number of isolates with each combination of classes, the connected dots below indicate the classes in each combination, and the horizontal bars at the left show the number of resistant isolates for each class.

Figure 6. Prevalence of antimicrobial resistance genes in *E. coli* isolates from Nairobi County.

Percentage of isolates (n = 36) in which each resistance gene was detected by PCR. All isolates were screened regardless of their phenotypic resistance.

Figure 7. Prevalence of antimicrobial resistance genes in *E. coli* isolates from Kiambu County.

Percentage of isolates (n = 56) in which each resistance gene was detected by PCR. All isolates were screened regardless of their phenotypic resistance.

Figure 8. Distribution of antimicrobial resistance genes in *E. coli* isolates from different poultry breeds.

Percentage of isolates carrying each resistance gene among indigenous chickens (Kienyeji), broilers, and layers.