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Molecular Epidemiology of Antibiotic Resistance among *Escherichia coli* Isolated from Broiler Chickens Sold at Selected Markets in Dar es Salaam, Tanzania

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Abstract

Unwarranted and improper uses of antibiotics in broiler farms contribute to the challenge of antibiotic resistance, making even previously treatable infections, difficult to treat. We conducted a cross sectional study from November 2021 to May 2022 from broiler chicken markets in four districts of Dar es Salaam to determine the extent of antibiotic resistance among *E. coli* isolates from broiler chickens. A total of 160 *E. coli* isolates recovered from cloacal swabs were identified by culture and biochemical tests and confirmed by Polymerase Chain Reaction (PCR) assays. Antimicrobial susceptibility testing, targeting seven classes of antibiotics, was performed by disk diffusion method and eleven representative antimicrobial resistance markers corresponding to each antibiotic class were screened by PCR. The highest resistance was found against trimethoprim (75%) and erythromycin (74.37%), while the most common resistance gene was *dfrA1* (74.37%) and bla_{TEM} (73.75%). The study also found a high prevalence of multidrug-resistant isolates (84.4%) from at least three antibiotic classes. The results highlight the significant contribution of poultry farming to the spread of antibiotic resistance, with potential consequences for both farmers and human health. Prompt measures are necessary to protect human and animal health.

Keywords: Antimicrobial resistance markers; *Escherichia coli*; Broiler chicken; Dar es Salaam; Tanzania.

Introduction

The world is challenged by the rise in resistance to antibiotics by both human and veterinary bacterial pathogens (Aslam et al. 2018). This has great negative impacts on public and animal health, jeopardizing milestones in sustainable development goals. Antimicrobials are increasingly being used inappropriately, which drives the maintenance and evolution of resistance to critically needed drugs (Andrew et al. 2020). In animal husbandry, antibiotics are used for therapeutic, growth promotion, or prophylactic purposes (Christian et al. 2018). When not properly managed, the quality of drugs and doses given are often below optimal standards, leading to the emergence and re-emergence of pathogens resistant to multiple drugs (Murray et al. 2022). The type of antibiotic resistance that spreads rapidly within diverse groups of pathogens is the one acquired by horizontal gene transfer mechanisms encoded on mobile genetic elements (Morrison and Zembower 2020).

Usually, these mobile genetic elements carry multiple resistance markers, making the host bacteria literary reservoirs of resistance genes (Murray et al. 2022).

Escherichia coli exist both in the environment and in the intestines of humans and animals as part of the normal flora (Allocati et al. 2013). Moreover, these bacteria are highly capable of acquiring foreign genes carrying advantageous traits, including resistance to multiple antibiotics (Aworh et al. 2021). When subjected to sub-optimal levels of antibiotics in their microenvironment, these bacteria expand the population of those with resistant phenotypes and are able to transmit these traits to other cohabiting bacteria (Morrison and Zembower 2020). Therefore, having resistant *E. coli* strains circulating among poultry poses a danger not only to the farmers for the potential economic losses but also to human health, as these may contaminate human food sources and cause infections in humans or transmit their resistance to human pathogens (Paitan 2018).

Dar es Salaam is the commercial capital of Tanzania, with an estimated population of 5.3 million people (NBS 2022). It is the largest consumer of meat of various origins, including poultry. The production-consumption deficit for chicken meat in Tanzania was estimated at 130,000 tons in 2017. Studies in Dar es Salaam have reported a very low level of farmer adherence to good clinical practice when it comes to protecting the health of their flocks (Kimera et al. 2020, Mgaya et al. 2021, Azabo et al. 2022). Most farmers, both in urban and peri-urban areas, access antimicrobials through over-the-counter prescriptions from non-trained veterinary drug dispensers (Sangeda et al. 2021). This, compounded with the unhygienic conditions of the farms and metaphylaxis, is a very good breeding ground and source of spread for antimicrobial resistant bacteria (Mgaya et al. 2021, Azabo et al. 2022).

Several antimicrobial resistance genes have been reported in *E. coli* including *aadA, aac(3)* for aminoglycosides resistance; *dfrA and sul* for antimetabolites resistance; bla_{TEM}, bla_{CMY}, $bla_{OXA\text{-}1\text{-}like}$, bla_{SHV}, $bla_{CTX\text{-}M}$ for β-lactams resistance; *aac(6')-Ib-cr, oqxA, qepA, oqxB*, and *qnr* for fluoroquinolones resistance; *ermB* and *ere(A)* for macrolides resistance; *floR*, *catA1* and *cmLA* for phenicols resistance; and *tet(A-D, G-H, M, W)* for resistance to tetracyclines (Momtaz et al. 2012, Igwaran et al. 2018). Some of these resistance markers have been reported by studies in Dar es Salaam (Mgaya et al. 2021) and Karatu in Northern Tanzania (Sonola et al. 2022). The Dar es Salaam and Karatu studies reported on resistance markers belonging to only two and three classes of antibiotics, respectively (Mgaya et al. 2021, Sonola et al. 2022). To better understand the epidemiology of antimicrobial resistance in poultry, using *E. coli* as a model organism, this study expanded on the panel of resistance markers representing seven classes of antibiotics, for the survey of antimicrobial resistance in *E. coli* isolated from broiler chickens in Dar es Salaam.

Materials and Methods

Study area

The study took place in Dar es Salaam, the commercial capital of Tanzania, where chicken meat and eggs are the highest produced and consumed products. The study included six large poultry slabs located in four districts (Ilala, Kinondoni, Temeke, and Ubungo). These slabs had a daily slaughter capacity of 20,000 chickens and provide around 80% of the chicken consumed in Dar es Salaam.

Study design

This was a cross-sectional study conducted between November 2021 and May 2022 in four districts, which have the largest poultry slabs in Dar es Salaam. The slabs were Kisutu and Buguruni in Ilala District, Mwenge and Magomeni in Kinondoni District, Stereo in Temeke District, and Shekilango in Ubungo District. In this study, we targeted broilers because they are raised intensively in overcrowded environments and the use of antimicrobials for prophylaxis, growth promotion, and the management of infections is very high. Other types of

poultry, such as indigenous chickens, were excluded from the study.

Sample size

The minimum sample size (*n*) of 384 broiler chickens was obtained after assuming a prevalence (*p*) of 50% and a Z-score (*Z*) of 1.96 for a confidence interval of 95% within 5% error of estimation (d). The following formula "$(n) = Z^2Pq/d^2$" was applied, where $q = (1 - p)$.

Sample collection

Seven cloacal swabs were collected from ten randomly selected vendors for each of the six markets, for a total of 420 cloacal samples. All the swabs were collected aseptically and kept in separate sterile tubes containing 5 mL of tryptic soy broth (Oxoid, Basingstoke, UK). Immediately, the samples were transported to the University of Dar es Salaam, Department of Molecular Biology and Biotechnology Laboratory, and incubated for 24 hours at 37 °C.

Isolation and identification of *Enterobacteria*

E. coli was isolated and identified based on standard bacteriological procedures (ISO 2001). Overnight-cultured swabs were inoculated onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Three lactose-fermenting colonies that appeared pink or red were peaked and re-streaked on eosin methylene blue (EMB) agar and further incubated at 37 °C for 24 hours. Colonies with a metallic sheen appearance on EMB were considered to be *E. coli*. These colonies were further characterized by biochemical tests including catalase, oxidase, indole, methyl red, and the Voges-Proskauer test. Presumptive *E. coli* isolates were then enriched overnight in 4 mL nutrient broth, ready for DNA extraction. Aliquots of the enriched samples were preserved at -20 °C in nutrient broth containing 15% glycerol. The non-pathogenic *E. coli* ATCC 25922 was used as a control sample.

Antimicrobial susceptibility testing

The susceptibility of *E. coli* to antimicrobials was tested using the standard Kirby-Bauer disk diffusion method with 9 antimicrobial agents belonging to 7 different classes. These selected agents represented classes of antibiotics commonly prescribed for human and animal bacterial infections caused by members of the *Enterobacteriaceae* family. The testing was performed following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). The procedure involved resuscitating isolates stored in glycerol with nutrient broth, incubating them at 37 °C for 24 hours, and using the culture to prepare a bacterial suspension that matched 0.5 McFarland standards. 150 µL of the suspension was then inoculated onto Mueller-Hinton agar and evenly distributed with a sterile swab stick, before antibiotic discs were dispensed on the agar plate using an antibiotic disc dispenser. The antibiotic discs used in this study were streptomycin (10 µg), gentamicin (10 µg), trimethoprim/sulfamethoxazole 1:19 (25 µg), trimethoprim (5 µg), ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), and tetracycline (30 µg). The *E. coli* ATCC 25922 was used as a control organism. After incubation at 37 °C for 16 to 18 hours, the diameter of growth inhibition was measured in millimetres and recorded as resistance, intermediate, or susceptible according to CLSI guidelines (Wayne 2018).

Polymerase Chain Reaction (PCR)

DNA extraction

Preserved presumptive *E. coli* isolates, along with *E. coli* ATCC 25922, were resuscitated from their storage in nutrient broth containing 15% glycerol. The resuscitation process involved incubating 4 mL of the broth at 37 °C for 24 hours. DNA extraction was performed using the simple boiling method, with slight modifications as described by Ahmed and Dablool (2017). To extract DNA, 1.5 mL of the overnight culture was first centrifuged at 13,000xg for 10 minutes to obtain the bacterial pellet. This

pellet was washed with sterile normal saline and centrifuged again, then re-suspended in 300 µL of sterile distilled water. The suspension was vortexed and heated at 100 ℃ for 10 minutes to lyse the cells, and the resulting cellular debris was removed by one final centrifugation at 13,000xg for 10 minutes. The resulting supernatant was transferred to a fresh sterile Eppendorf tube. The concentration and purity of the extracted DNA was measured using the NanoDrop One oneC (Thermo Fisher Scientific, USA). Finally, the DNA with a concentration of > 100 ng/µL was stored at -20°C for use in PCR assays.

Molecular detection of antibiotic resistant genes

All isolates exhibiting phenotypic resistance to any antimicrobial under study were screened for the respective resistance markers by PCR. The isolates were also confirmed to be *E. coli* isolates by PCR targeting the *uidA* gene as described by Igwaran et al. (2018). The list of markers and primers used for PCR are shown in Table 1.

Table 1: PCR primers used for detection of antibiotic resistance markers in *Escherichia coli*

Antibiotic	Gene	Primer sequence 5'-3'	Size (bp)	Ta (°C)
Streptomycin[a]	*aadA1*	F- TATCCAGCTAAGCGCGAACT	447	58
		R- ATTTGCCGACTACCTTGGTC		
Gentamicin[a]	*aac(3)-IV*	F- CTTCAGGATGGCAAGTTGGT	286	55
		R- TCATCTCGTTCTCCGCTCAT		
Beta-lactams[b]	bla_{TEM}	F- ACATGGGGGATCATGTAACT	421	52
		R- GACAGTTACAATGCTTACT		
	$bla_{CTX\text{-}M\text{-}1}$	F- GACGATGTCACTGGCTGAGC	499	55
		R- AGCCGCCGACGCTAATACA		
Chloramphenicol[a]	*cmLA*	F- CCGCCACGGTGTTGTTGTTATC	698	55
		R- CACCTTGCCTGCCCATCATTAG		
	catA1	F- AGTTGCTCAATGTACCTATAACC	547	55
		R- TTGTAATTCATTAAGCATTCTGCC		
Erythromycin[a]	*ere(A)*	F- GCCGGTGCTCATGAACTTGAG	419	52
		R- CGACTCTATTCGATCAGAGGC		
Fluoroquinolones[a]	*qnrA*	F- GGGTATGGATATTATTGATAAAG	670	50
		R- CTAATCCGGCAGCACTATTA		
Sulfonamides[a]	*sul1*	F- TTCGGCATTCTGAATCTCAC	822	47
		R- ATGATCTAACCCTCGGTCTC		
Tetracycline[a]	*tet(A)*	F- GGTTCACTCGAACGACGTCA	577	57
		R- CTGTCCGACAAGTTGCATGA		
	tet(B)	F- CCTCAGCTTCTCAACGCGTG	634	56
		R- GCACCTTGCTGATGACTCTT		
Trimethoprim[a]	*dfrA1*	F- GGAGTGCCAAAGGTGAACAGC	367	45
		R- GAGGCGAAGTCTTGGGTAAAAAC		

[a]adopted from Momtaz et al. 2012;
[b]Adopted from Swedan and Abu (2019) (bla_{TEM}) and Eguale et al. (2017) ($bla_{CTX\text{-}M\text{-}1}$).

PCR was performed in a final volume of 25 µL containing 3 µL (100–200 ng/µL) of extracted DNA as a template, 12.5 µL of Taq 2X Master Mix (New England Biolabs), 1 µL (10 pmol) of each primer set and 7.5 µL of nuclease-free water (BioConcept). Each PCR amplification cycle consisted of an initial denaturation step at 95 °C for 10 minutes, followed by 35 cycles of denaturation at 95 °C for the 30 s, annealing for 30 s at a temperature appropriate for each primer pair used, extension for 1 minute at 72 °C and a final extension at 72 °C for 10 minutes. The amplified PCR products were verified by electrophoresis at 100 V for 45 min in 1.5% agarose gels stained with safe view™ classic dye. Gel visualization and documentation was done in the Gel. LUMINAX Gel documentation system (BioZen Labs/Zenith). GeneRuler I kb Plus DNA ladder (Thermo Scientific™) and Quick-Load 100 bp DNA ladder (NEB) were used as molecular weight markers.

Data analysis

The data were entered into Microsoft Excel; proportions of isolates showing phenotypic resistance and carriage of respective genetic marker were analysed by Chi-square test. A *p*-value ($\leq$ 0.05) was considered to be statistically significant. Descriptive statistical analysis was constructed using IMB SPSS Statistics software (version 20).

Results

Prevalence of antibiotic resistant *E. coli* isolated from broiler chickens

A total of 420 cloacal swab samples were collected from six selected markets in Dar es Salaam, and all 420 samples grew colonies that were presumptively identified as *E. coli* through culture and biochemical methods. Of these, 160 isolates were randomly selected from each district, with 40 isolates from each market (20 from Ilala and 20 from Kinondoni) and confirmed to be *E. coli* through PCR. Antimicrobial susceptibility testing was performed on all the 160 isolates and the results showed the highest resistance to trimethoprim (75.0%), followed by erythromycin (74.5%), ampicillin (73.75%), tetracycline (73.13%), trimethoprim-sulfamethoxazole 1:19 (72.5%), ciprofloxacin (38.75%), chloramphenicol (33.75%), gentamicin (19.38%) and streptomycin (15.0%) (Table 2). Out of the 160 isolates screened 135 (84.4%) were resistant to at least three different classes of antibiotics (Table 3).

Table 2: Antimicrobial resistance profiles of *Escherichia coli* isolated from broiler chickens

Antibiotic name	Total *E. coli* isolates (N = 160)	
	Susceptible: n (%)	Resistant: n (%)
Trimethoprim	40 (25)	120 (75)
Erythromycin	41 (25.63)	119 (74.5)
Ampicillin	42 (26.25)	118 (73.75)
Tetracycline	43 (26.88)	117 (73.13)
Trimethoprim-sulphamethoxazole	44 (27.5)	116 (72.5)
Ciprofloxacin	98 (61.25)	62 (38.75)
Chloramphenicol	106 (66.25)	54 (33.75)
Gentamicin	129 (80.63)	31 (19.38)
Streptomycin	136 (85)	24 (15.06)

Table 3: Pattern of antimicrobial resistance by antibiotic classes

MDR *E. coli* isolates: n (%)	Classes of antibiotics: n (%)				
	3	**4**	**5**	**6**	**7**
135 (84.4)	63 (39.4)	18 (11.3)	14 (8.8)	17 (10.6)	23 (14.4)

Prevalence of antibiotic resistant markers among *E. coli* isolated from broiler chickens

All PCR products amplified were of the sizes expected per genetic marker of resistance except *tet(A)* which showed a product twice the expected size (Table 1 and Figure 1). Overall, the most frequently detected resistance marker was *dfrA1* for trimethoprim (74.37%) (Table 4) followed by bla_{TEM} for β-lactam (73.75%), for tetracycline *tetA* and *tetB,* either one or both together (73.13%), *sul*1 for sulfamethoxazole (71.25%), and *cmLA* and *catA1,* either one or both together (26.88%) for chloramphenicol. The least detected resistance markers were *qnrA* for ciprofloxacin (1.25%) and *ere(A)* for erythromycin (0.63%). The $bla_{CTX\text{-}M\text{-}1}$ marker for β-lactam antibiotics and *aac(3)-IV* for gentamycin resistance were not detected in this study.

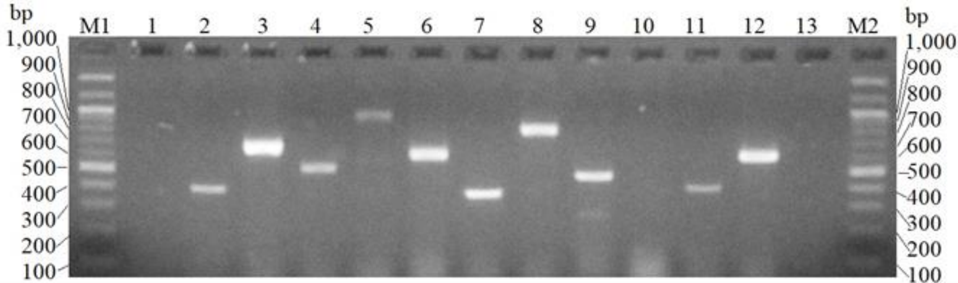

Figure 1: Representative agarose gel electrophoresis of PCR analysed antibiotic resistance markers. Lane M1 and M2: 100 bp DNA marker; lane 1: $bla_{CTX\text{-}M\text{-}1}$ (499 bp); lane 2: bla_{TEM} (421 bp); lane 3: *cmLA* (698 bp); lane 4: *catA*1 (547 bp); lane 5: *tet(A)* (1,000 bp instead of 577 bp); lane 6: *tet(B)* (634 bp); lane 7: *dfrA*1 (367 bp); lane 8: *sul*1 (822 bp); lane 9: *aadA*1 (447 bp); lane 10: *aac(3)*-IV (286 bp); lane 11: *ere(A)* (419 bp); lane 12: *qnrA* (670 bp); Lane 13: negative control.

Table 4: Prevalence of genetic resistance markers among isolates under study

Antibiotic	Phenotypic resistance	Genetic resistance marker		Genotypic/ Phenotypic agreement
Ampicillin	118 (73.75%	$bla_{CTX\text{-}M\text{-}1}$	0 (0%)	100%
		bla_{TEM}	118 (73.75%)	
		Total	118 (73.75%)	
Chloramphenicol	54 (33.75%)	*cmLA*	11 (6.88%)	79.63%
		catA1	18 (11.25)	
		cmLA and catA1	14 (8.75%)	
		Total	43 (26.88%)	
Tetracycline	117 (73.13%)	*tet(A)*	19 (11.88%)	100%
		tet(B)	16 (10%)	
		tetA + tetB	82 (51.25%)	
		Total	117 (73.13%)	
Trimethoprim	120 (75.0%)	*dfrA1*	119 (74.37%)	99.16%
Sulphamethoxazole	116 (72.25%)	*sul1*	114 (71.25%)	98.61%
Streptomycin	24 (15.0%)	*aadA*1	24 (15.0%)	100%
Gentamycin	31 (19.37%)	*aac(3)*-IV	0 (0%)	0.0 %
Erythromycin	119 (74.37%)	*Ere(A)*	2 (1.25%)	1.68 %
Ciprofloxacin	62 (38.75%)	*qnrA*	1 (0.63%)	1.61%

Discussion

The ongoing use of antimicrobial compounds for treatment, prophylaxis, and metaphylaxis of animals in livestock keeping increases the selective pressure for the emergence of antimicrobial resistance and multidrug resistance (MDR) organisms (Karczmarczyk et al. 2011, Kimera et al. 2020). Most of the antimicrobial agents used belong to classes similar to human drugs and have broad-spectrum activities, including β-lactams and new cephalosporins, fluoroquinolones, chloramphenicol, and some aminoglycosides (Kimera et al. 2020). There is growing evidence that *E. coli* infections in both animals and humans in Tanzania are becoming increasingly difficult to treat due to the rise in antimicrobial resistance (Kimera et al. 2020), posing major threats to human and veterinary health as *E. coli* is associated with many animal and human infections (Paitan 2018).

In this study, we analysed the prevalence of antimicrobial resistance in *E. coli* isolates from broiler chickens sold at selected markets in Dar es Salaam city. Our findings indicate a high resistance to trimethoprim (75%), followed by erythromycin (74.4%), ampicillin (73.5%), tetracycline (73.1%), and sulfamethoxazole (72.5%) (Table 2). These results are similar to what has been reported elsewhere, which is in line with the overuse of these drugs to treat bacterial diseases (Karczmarczyk et al. 2011, Khairy et al. 2020, Murray et al. 2022). In Tanzania, high resistances to tetracyclines, sulfamethoxazole, and ampicillin have been reported among food animals (Katakweba et al. 2018, Mgaya et al. 2021). Tetracycline is highly popular among veterinarians, farmers, and importers due to its broad antibacterial activity and affordability (Mgaya et al. 2021). Furthermore, soluble tetracyclines are used as growth promoters (Sangeda et al. 2021). Sulfonamides and trimethoprim are also frequently used in animal farming for controlling coccidiosis and poultry colibacillosis (Murray et al. 2022).

The study found moderate to low resistance to ciprofloxacin (38.8%), chloramphenicol (33.8%), gentamycin (19.4%) and streptomycin (15%) in *E. coli* isolates from broiler chickens sold in Dar es Salaam. This can be attributed to the reduced use of these drugs in poultry, with some no longer being used, such as streptomycin and gentamycin (Krause et al. 2016, Kimera et al. 2020, Sangeda et al. 2021). The study also showed a high level of multidrug-resistant (MDR) *E. coli* isolates among poultry (84.4%) (Table 3) higher than previously reported by Mgaya et al. (2021).

A major mechanism for the maintenance and spread of antimicrobial resistance among bacteria is horizontal transfer of resistance genes between closely related and even to unrelated bacterial species (Murray et al. 2022). The continuous presence of sub-optimal levels of antibiotics within the microbial niches provides the selection pressure required to promote resistance (Igwaran et al. 2018). Intensive use of antibiotics in poultry, by people with limited knowledge and skills on how to apply these antibiotics, on top of unregulated dispensing of drugs to farmers from local unregistered vendors, makes the rise of resistance almost inevitable (Kumar et al. 2013, Khan et al. 2020).

We analysed genetic markers of resistance to representative members of antibiotic classes used in both human and livestock disease management. Results showed that all *E. coli* isolates resistant to ampicillin harboured the bla_{TEM} gene, and none had the $bla_{\text{CTX-M-1}}$ gene (Figure 1 and Table 4). This is in contrast to a previous report in Tanzania, where bla_{TEM} was not detected but $bla_{\text{CTX-M-1}}$ was detected (Mgaya et al. 2021). In support of our findings, studies in Mwanza and northern Tanzania reported a higher frequency of TEM genes compared to CTX-M among isolates from chicken and drinking water sources (Lyimo et al. 2016, Kiiti et al. 2021, Sonola et al. 2022).

Resistance markers for chloramphenicol (*cmLA* and *catA*1) were detected among 79.63% of chloramphenicol resistant isolates (Table 4). A study in Bangladesh found the prevalence of the two markers for chloramphenicol resistance to be 24.87% for *cmLA* and 8.63% for *catA*1 (Azad et al.

2019). Many countries have banned the use of chloramphenicol in food animals due to its several adverse effects in humans but enforcing this law is difficult because of the easy accessibility to the drug and the myriad of diseases cured by this drug in animals (McCubbin et al. 2021). Moreover, in this study, all isolates resistant to tetracycline (73.13%) had either the *tet(A)* (11.88%) or *tet(B)* (10%) resistant marker or both (51.25%) (Table 4). Our results translate to a frequency of 63.13% for *tetA* and 61.25% for *tetB*, which is comparable to the findings of the study in northern Tanzania which reported a frequency of 60% for *tetA* (Sonola et al. 2022). Our results are also similar to those reported from Western Cameroon, where the frequency of *tetA* and *tetB* genes was found to be 59.94% and 64.71% respectively (Marbou et al. 2020). Further, our results show that these resistance markers do occur together more commonly than they do singly, findings also supported by a study from Nigeria (Perewari et al. 2022).

The study also found 74.37% and 71.25% of all the PCR confirmed *E. coli* isolates had the *dfrA1* and *sul1* resistant markers, respectively. Moreover, 99.16% of all the trimethoprim resistant isolates carried the *dfrA1* gene, whereas 98.61% of those resistant to sulphamethoxazole had the *sul1* gene (Table 4). A closely similar result was reported in Bangladeshi where *dhfrA1* prevalence was reported at 65.5% (Azad et al. 2019). The prevalence of *aadA1* gene was 15%, whereas all streptomycin resistant *E. coli* isolates had this gene (Table 4). This marker was reported at 88.25% in Bangladeshi (Azad et al. 2019), 81% in Vietnam (Van et al. 2008), 70.6% and in Portugal (Costa et al. 2009). None of the gentamycin resistant isolates in our study had the *aac(3)-IV* gene (Table 4). Similar lack of detection of the *aac(3)-IV* marker was reported in Ethiopia (Messele et al. 2017) and Iran (Momtaz et al. 2012).

The current study observed a 74.37% and 38.75% of erythromycin and ciprofloxacin resistant *E. coli* isolates, but the respective resistance markers were only found at 1.68% (*ereA*) and 1.61% (*qnrA*). Similar to our results, a study in Ethiopia did not detect *ereA* gene in all the 27 *E. coli* isolates resistant to erythromycin (Messele et al. 2017). With respect to ciprofloxacin resistance, the study from Northern Tanzania reported the *qnrA* gene to be present in 6 out of 12 isolates analysed (Sonola et al. 2022). Nonetheless, our results agree with a recent study in Dar es Salaam which also, did not detect *qnrA* gene among isolates analysed (Mgaya et al. 2021).

This study found that there was a strong agreement between genotypic and phenotypic resistance to some antibiotics (Table 4). However, there was no agreement between resistance to gentamycin, erythromycin and ciprofloxacin with their tested markers. The lack of agreement may be due to resistance as a result of genes not tested in the study, resistance mechanisms against other antibiotics, point mutations in important antibiotic metabolism or penetration genes, or other factors (Murray et al. 2022). In the future, more resistance markers, especially for gentamycin, erythromycin, and ciprofloxacin, should be tested and isolates that are phenotypically resistant to particular drugs but have no known markers of resistance should be sequenced.

Conclusion

This study has shown a widespread incidence of resistance and multidrug resistance to antibiotics commonly used in broiler chicken farming, which could have negative effects on human bacterial treatment. The results of the resistance profile suggest that there are certain patterns that reflect the use and misuse of antibiotics in poultry, with those antibiotics that are used less frequently, such as gentamicin and streptomycin, showing low resistance prevalence. It is therefore important to increase monitoring in food animals to gain a better understanding of the situation and enhance both animal and human safety in relation to diarrheal outbreaks. Additionally, the presence of specific resistance markers for certain antibiotics can be used for efficient screening and monitoring of antimicrobial resistance in communities.

Recommendation

We recommend strict adherence to existing regulations for antibiotic use in poultry, training to farmers and to veterinary shops drug dispensers. Extension services should also be improved in order to guide farmers on better ways of raising their poultry which would reduce the demand for more antimicrobial use in poultry. Further studies should be done especially to check the correlation of antimicrobial resistance patterns between isolates from poultry and those from humans. Such studies should include more markers, in order to determine which markers are more predictive of phenotypic resistances in the community.

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Conflicts of Interest: The authors declare to have no conflicts of interest.

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