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Increased Occurrence of Antimicrobial Resistance and Virulence Genes in *Escherichia coli* from Broilers in the Central Highlands of Vietnam

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ABSTRACT

Antimicrobial resistance is becoming a major concern for the poultry industry, particularly as *Escherichia coli* strains are increasingly exhibiting multidrug resistance. This study aims to assess the antimicrobial resistance and virulence genes of *E. coli* isolated from broilers in the Central Highlands of Vietnam, providing a scientific basis for managing antimicrobial use in poultry production. A total of 370 rectal fecal samples were collected from 25 farms, including 250 samples from healthy broilers (67.6%) and 120 samples from diarrheal broilers (32.4%). *E. coli* isolates were identified using conventional biochemical methods and confirmed by 16S rRNA gene sequencing. In addition, isolates from diarrheal samples (n=120) were screened for seven virulence genes (*hlyE*, *iss*, *eaeA*, *ent*, *escV*, *stx1*, and *stx2*) using PCR. Data were statistically analyzed using R (version 4.4.1) to determine the prevalence of antimicrobial resistance, multidrug resistance (MDR), virulence genes, and AMR/MDR profiles. The results showed high resistance rates to most tested antimicrobial agents, particularly tetracycline and erythromycin (>90%), while ciprofloxacin exhibited the lowest resistance rate (31%–32%). The proportions of isolates resistant to at least one antimicrobial agent and those classified as MDR were 97.5% and 90.3%, respectively. Among the isolates from diarrheal samples, 94.2% carried at least one virulence gene. The genes *hlyE*, *iss*, and *stx2* were the most prevalent, whereas *ent*, *eaeA*, and *escV* were detected at lower frequencies, and *stx1* was not detected ($p<0.001$). The high prevalence of multidrug resistance, together with the frequent detection of *hlyE*, *iss*, and *stx2* suggests the co-occurrence of resistance and virulence traits, which may facilitate their dissemination within the intestinal microbiota.

Keywords: antimicrobial agents; broiler; diarrhea; multidrug resistance

INTRODUCTION

Escherichia coli is a Gram-negative bacterium belonging to the family Enterobacteriaceae, commonly present in the intestinal microbiota of poultry and plays an important role in the balance of the intestinal ecosystem (Ribeiro *et al.*, 2023). Although most *E. coli* strains are normal flora, some strains carry specific virulence factors capable of causing intestinal or systemic diseases, classified as Avian Pathogenic *E. coli* (APEC) (Hu *et al.*, 2022). These strains can cause diarrhea, enteritis, air sacculitis, sepsis, reduced weight gain, and increased mortality, especially in intensive farming systems with high density and short production cycles such as broilers (Nordin *et al.*, 2021).

Pathogenic *E. coli* is of global particular concern not only because of its ability to cause disease in poultry, but also because of its genetic diversity and ability to carry virulence genes related to adhesion (*eaeA*), invasion (*escV*), enterotoxin (*stx*), hemolysin (*hlyE*), or serum survival (*iss*) genes (LeStrange *et al.*, 2017). The simultaneous presence of multiple virulence genes is often associated with stronger pathogenicity, higher transmission potential, and a greater risk of causing severe intestinal mucosal damage (Rogers *et al.*, 2023). In particular, broilers' diarrhea is one of the important clinical symptoms reflecting an imbalance in the intestinal microflora, invasion of pathogenic microorganisms, or digestive disorders related to nutrition, farming environment, and management (Mora *et al.*, 2020; Wickramasuriya *et al.*, 2022). In addition to its pathogenicity, multidrug resistance (MDR) of *E. coli* strains isolated from poultry is becoming a major challenge for veterinary and public health. The prolonged use of antimicrobials for disease prevention, treatment, or growth promotion in poultry production systems has created strong selection pressure, promoting the spread of *E. coli* strains resistant to multiple groups of antimicrobial agents (Arbab *et al.*, 2022; Abreu *et al.*, 2023). Plasmids carrying MDR genes can be transferred horizontally between bacteria of the same or different species, forming a reservoir of drug-resistant genes in the livestock environment (Rodríguez-Beltrán *et al.*, 2021; Dimitriu, 2022).

In Vietnam, the broiler industry has developed strongly in recent years. However, intensive farming

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conditions, high stocking density, the circulation of various pathogens, and the abuse of antimicrobials have contributed to the increase in *E. coli* infection levels and the risk of emerging MDR strains (Liang *et al.*, 2021; Nhung *et al.*, 2022a). However, findings of *E. coli* strains carrying virulence genes associated with multidrug resistance remain limited, hindering a comprehensive assessment of the prevalence risk of pathogenic and antimicrobial-resistant *E. coli* strains. Furthermore, although the Vietnamese government has banned the use of antimicrobials for prevention and growth promotion in poultry production since 2026, there is still a lack of comprehensive regulatory frameworks governing antimicrobial use in practice (Vietnam-Government, 2020). In the Central Highlands of Vietnam, where the agricultural economy is mainly based on poultry production, the limited regulatory controls of antimicrobial use, together with expanding intensive poultry production systems, may facilitate antimicrobial misuse and accelerate the emergence and spread of antimicrobial-resistant microorganisms, highlighting the need for regional surveillance studies.

Therefore, it is necessary to conduct this study to evaluate the levels of antimicrobial resistance and the distribution of virulence genes (*hlyE*, *iss*, *eaeA*, *ent*, *escV*, *stx1*, and *stx2*) in *E. coli* isolates from broilers in the Central Highlands of Vietnam. We hypothesize that *E. coli* isolates from broilers, particularly those from diarrheal chickens, exhibit a high prevalence of multidrug resistance and harbor multiple virulence genes, with a potential co-occurrence of resistance and virulence traits.

MATERIALS AND METHODS

Sample Collection

All procedures involving sample collection from broilers were conducted in accordance with the guidelines approved by the Animal Ethics Advisory Committee of Vietnam (Approval No: HUVN0059, 2025). The study took place from March to August, 2025. A total of 370 fecal samples from broiler rectum were collected from 25 broiler farms in three wards (Ea Kao, Khanh Xuan, and Cu Ebua) in Dak Lak Province, including 250 samples from healthy broilers and 120 samples from broilers with diarrhea (defined as loose or watery feces). After collection, the samples were placed into sterile plastic bags labeled with sample information and stored in a cold box (4–8 *°C)* and transported to the laboratory for analysis within 4 hours.

Isolation of *Escherichia coli*

Bacteria were isolated using standard culture and biochemical identification methods (Vietnam-Standard, 2011; Nhung *et al.*, 2022b). Each fecal sample yielded one representative *E. coli* isolate (one isolate per sample), resulting in a total of 370 isolates corresponding to 370 samples.

In brief, approximately 1 g of each fecal sample was enriched in Buffered Peptone Water (BPW, Oxoid, UK) at 37 °C for 24h. A sterile cotton swab was used to streak the enrichment onto MacConkey agar (Oxoid, UK) and incubated at 37 °C for 24 h. A single typical lactose-fermenting colony (pink) was selected and streaked onto Eosin Methylene Blue (EMB, Oxoid, UK) agar, followed by incubation at 37 *°C for 24 h.* Colonies showing metallic green sheen were selected and purified by subculturing on Nutrient Agar (NA, Oxoid, UK) for further analysis and storage.

For Gram staining, the bacteria were Gram-negative rods, non-spore-forming, and stained pink with safranin. Biochemical identification included IMViC (+, +, −, −), TSI (A/A, gas +, H_2S −), KIA (A/A, gas +, H_2S −), Urea broth (−), and LIA (alkaline/alkaline, H_2S −) (Oxoid, UK). *E. coli* ATCC 25922 was used as a quality control strain. All presumptive isolates were further confirmed by 16S rRNA gene sequencing (Li *et al.*, 2024).

Determination of Virulence Genes

Only *E. coli* isolates obtained from diarrheal samples (n=120) were screened for virulence genes using PCR (Table 1) (Wang *et al.*, 2010; Sun *et al.*, 2011; Bako *et al.*, 2017). In brief, (1) isolates were cultured on Luria-Bertani agar (LB, Oxoid, UK) at 37 °C for 24 h; (2) DNA template was prepared by boiling method, a single colony was suspended in 50–100 µL nuclease-free water, heated at 100 °C for 10 minutes, and centrifuged; (3) PCR reaction mixture (20 µL total volume) included Master Mix Phu Sa 2X (10 µL), forward primer (1 µL), reverse primer (1 µL), nuclease-free water (6 µL), and DNA template (2 µL); (4) PCR amplification was performed with initial denaturation at 95 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, annealing at 54–59 °C for 30 s (depending on primer), 72 °C for 1 min, and a final extension at 72 °C for 5 min (Table 1); (5) PCR products were analyzed by electrophoresis on 1.5% agarose gel prepared in 1X TAE buffer, stained and visualized under UV light. A 100 bp DNA ladder was used as a molecular marker (Figure 1).

Determination of Drug Resistance Phenotype

Antimicrobial susceptibility was determined using the Kirby–Bauer disk diffusion method. Briefly, Mueller-Hinton agar (MH, Oxoid, UK) plates were inoculated with *E. coli* isolates titrated at 0.5 McFarland standard (~1.5×10^8 CFU/mL). Antimicrobial disks were placed on the agar surface and incubated at 37 °C for 16–18 h. All tests were performed in triplicate, and inhibition zone diameters were measured and interpreted as susceptible (S), intermediate (I), or resistant (R) according to Clinical and Laboratory Standards Institute (CLSI, 2023). A total of 11 antimicrobials representing 8 classes were tested including tetracyclines (tetracycline 30 µg), penicillins (ampicillin 10 µg), aminoglycosides (streptomycin 10 µg, gentamicin 10 µg, kanamycin 30 µg), folate pathway antogonists (trimethoprim-sulfamethoxazole 1.25/23.75 µg), quinolones and fluoroquinolones (ciprofloxacin 5 µg, enrofloxacin 5 µg), macrolides (erythromycin 15 µg), cephems (cefotaxime 30 µg), and β-lactam combination agents (amoxicillin-clavulanate 20/10 µg). MDR was defined as resistance to at least one agent in three or more antimicrobial classes (Magiorakos *et al.*, 2012).

Table 1. Primer sequences, product sizes, and annealing temperatures used for PCR detection of *Escherichia coli* and virulence genes isolated from diarrheic broilers

Strain	Gene	Primer sequence (5′-3′)	Amplicon size (bp)	Annealing temperature	Source
Escherichia coli	E16S	F- ATCAACCGAGATTCCCCCAGT R- TCACTATCGGTCAGTCAGGAG	231bp	58 °C	Sun *et al.* (2011)
APEC	*hlyE*	F- ACGCCCGCAGCAATAGAATA R- AAAGCATCCGCCCAGAAAGA	283 bp	58 °C	Wang *et al.* (2010)
	iss	F- AACCGACAGCAGTAACAC R- TAGGGAGCCCAGAAGTA	274 bp	54 °C	
EPEC	*eaeA*	F- GACCCGGCACAAGCATAAGC R- CCACCTGCAGCAACAAGAGG	384 bp	59 °C	Bako *et al.* (2017)
	escV	F- GGCTCTCTTCTTCTTTATGGCTG R- CCTTTTACAAACTTCATCGCC	534 bp	55 °C	
	ent	F- TGGGCTAAAAGAAGACACACTG R- CAAGCATCCTGATTATCTCACC	629 bp	55 °C	
STEC	*stx1*	F- ATAAATCGCCATTCGTTGACTAC R- AGAACGCCCACTGAGATCATC	180 bp	56 °C	
	stx2	F- GGCACTGTCTGAAACTGCTCC R- TCGCCAGTTATCTGACATTCTG	255 bp	57 °C	

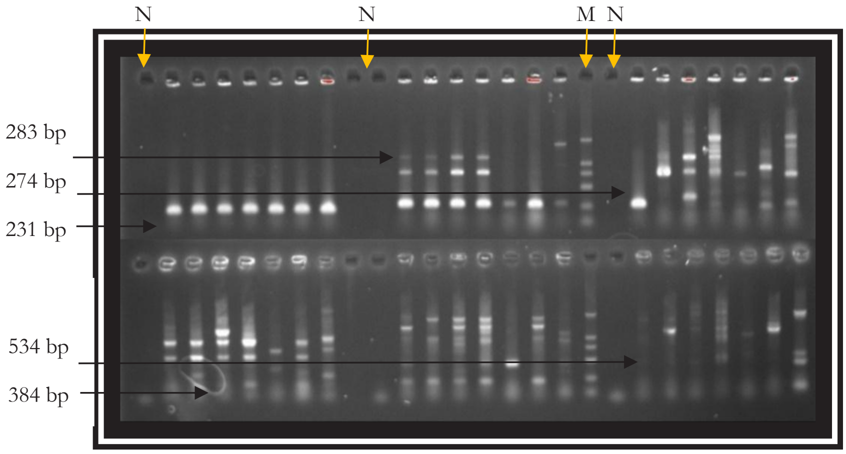

Figure 1. Amplified PCR products of the agarose gel electrophoresis process of *Escherichia coli* isolated from diarrheic broilers. N: negative control, M: DNA marker (gene ladder 100-bp), Other lanes: tested isolates.

Statistic Analysis

Data were analyzed using R language software (version 4.4.1). Categorical variables such as fecal conditions, positive/negative results, and drug resistance status were standardized to a binary form. Positive rates were calculated using the Wilson method with 95% confidence intervals. Differences between groups (region, fecal conditions, virulence genes, etc.) were assessed using the Chi-square or Fisher's exact test. Pairwise comparisons were performed using the proportion test (prop.test or Fisher), and p-values were adjusted using the Benjamini–Hochberg method to control for multiple testing bias. Antimicrobial resistance (AMR), multidrug resistance (MDR), virulence gene rates, and AMR/MDR profiles were summarized and visualized using bar charts and heatmaps to describe the distribution trends, antimicrobial resistance, and virulence levels between groups. Results are reported with sample size (n/N), proportions (%), 95% CI, and corresponding p-values or adjusted p-values (p_adj), where applicable.

RESULTS

Prevalence and Multidrug Resistance of *E. coli* in Areas

Of the total 370 rectal fecal samples of broilers collected at farms in the three areas of Ea Kao, Khanh Xuan and Cu Ebua, Dak Lak Province, 360/370 samples were positive for *E. coli* (97.3%, 95% CI: 95.1%–98.6%) (Table 2). The results showed that the prevalence of *E. coli* in diarrheal samples was 120/120 (100%, 95% CI: 97.0%–100%), which was significantly higher than in healthy samples (240/250; 96%, 95% CI: 92.7%–97.9%) (p=0.03). The prevalence in Ea Kao, Khanh Xuan, and Cu Ebua was 95.8% (95% CI: 89.7%–98.4%), 96% (95% CI: 88.9%–98.6%), and 96.2% (95% CI: 89.5%–98.7%), respectively, with no statistically significant differences between areas (p>0.05). The overall multidrug resistance (MDR) rate was 325/360 (90.3%, 95% CI: 86.7%–93.1%), with no significant difference between areas (X^2=2.267; df=2; p=0.322).

Resistance rates were high for most antimicrobials tested across the three areas (Figure 2). In Eakao, resistance rates were >80% for tetracycline, ampicillin, streptomycin, gentamicin, enrofloxacin, erythromycin, and amoxicillin-clavulanate. In Khanh Xuan, this pattern was observed for tetracycline, ampicillin, streptomycin, and erythromycin (>80%), while in Cu Ebua, high resistance was observed for tetracycline, ampicillin, streptomycin, enrofloxacin, and erythromycin. The results showed that resistance rates were highest for tetracycline and erythromycin (>90%), and exceeded 50% for most antimicrobials tested. The lowest resistance rate was recorded for ciprofloxacin (31%–32%) (Table 3). Susceptibility rates ranged from 0.6% to 28.3%, with the lowest for erythromycin and the highest for gentamicin (23.6%) and trimethoprim-sulfamethoxazole (28.3%) (Table 3).

Table 2. Prevalence of *Escherichia coli* and multidrug resistance (MDR) isolates from diarrheic and normal broilers in different sampling locations

Isolates	Sampling location			Total
	Ea Kao	Khanh Xuan	Cu Ebua	
Diarrheal fecal samples				
No. of samples	50	35	35	120
No. of isolates positive	50	35	35	120
% of isolates positive	100	100	100	100^{a}
Normal fecal samples				
No. of samples	95	75	80	250
No. of isolates positive	91	72	77	240
% of isolates positive	95.8	96	96.2	96^{b}
MDR (n=360)				
No. of MDR isolates	128	93	104	325
% of MDR isolates	90.8	86.9	92.9	90.3

Note: ^{a,b} Different letters within a column are statistically significant

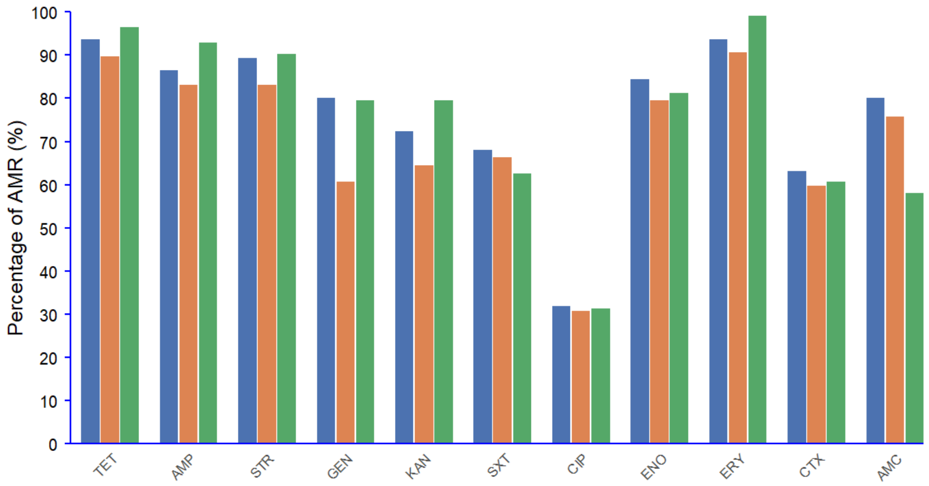

Figure 2. Percentage of antimicrobial resistance (AMR) in regions. TET: tetracycline, AMP: ampicillin, STR: streptomycin, GEN: gentamicin, KAN: kanamycin, SXT: trimethoprim-sulfamethoxazole, CIP: ciprofloxacin, ENO: enrofloxacin, ERY: erythromycin, CTX: cefotaxime, AMC: amoxicillin-clavulanate. 🟦 Ea Kao; 🟧 Khanh Xuan; 🟩 Cu Ebua.

Table 3. *Escherichia coli* resistance towards the tested antimicrobials

Antimicrobial agents	Resistance status (%)		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Tetracycline	93.3	4.7	1.9
Ampicillin	87.5	11.1	1.4
Streptomycin	87.8	6.4	5.8
Gentamicin	74.2	2.2	23.6
Kanamycin	72.2	12.8	15.0
Trimethoprim-sulfamethoxazole	65.8	5.8	28.3
Ciprofloxacin	31.4	55.5	13.1
Enrofloxacin	81.9	10.6	7.5
Erythromycin	94.4	5.0	0.6
Cefotaxime	61.4	22.2	16.4
Amoxicillin-clavulanate	71.9	19.7	8.3

Multidrug Resistance Profiles of *E. coli* Isolates

A total of 325/360 isolates (90.3%, 95% CI: 86.7%–93.1%) exhibited multidrug resistance (MDR). It was found that the most common MDR phenotypes were TET-AMP-STR-GEN-KAN-SXT-CIP-ENO-ERY-CTX-AMC (27.3%), TET-AMP-STR-GEN-KAN-SXT-ENO-ERY-CTX-AMC (14.1%), while other MDR patterns were observed at frequencies <6% (Figure 3).

Prevalence of Virulence Genes and Virulence Profiles of *E. coli*

Among isolates from diarrheal samples (n=120), the most prevalent virulence genes were *hlyE* (93.3%), *iss* (54.2%), and *stx2* (31.7%). In contrast, *ent*, *eaeA*, and *escV* were detected at low frequencies (1.7%–3.3%), and *stx1* was not detected (0%) (Table 4). The differences in gene prevalence were statistically significant (p<0.001). The occurrence of *hlyE* and *iss* genes was significantly higher than expected (standardized residuals – SR: +17.8, +7.3), while *eaeA*, *ent*, *escV*, and *stx1* were significantly lower (SR: from –6.2 to –7.1). Pairwise comparisons with Benjamini–Hochberg (BH adjusted) indicated that most gene pairs differed significantly (p_adj<0.05), confirming a heterogeneous distribution of virulence genes.

Of the 120 isolates, 113/120 (94.2%, 95% CI: 88.6%–97.3%) carried at least one virulence gene, while 7/120 (5.8%) carried none (p<0.001). The most common virulence profiles were *hlyE* alone (29.2%), *hlyE-iss* (31.7%), *hlyE-iss-stx2* (18.3%), and *hlyE-stx2* (7.5%), whereas other combinations were observed at frequencies <3% (Figure 4).

DISCUSSION

This study showed that the antimicrobial resistance of *E. coli* isolated from diarrheal stool samples of broilers was at a high level. High resistance rates were found in most of the tested antimicrobials, indicating a concerning pattern of resistance in intensive poultry production systems. The antimicrobials tested in this study were based on the list of drugs permitted for use in animal production in Vietnam and antimicrobials

Table 4. Distribution of virulence genes among Escherichia coli isolated from diarrheic broilers (n=120)

Virulence gene	Positive isolates	Virulence isolates (%)
hlyE	112	93.3
iss	65	54.2
ent	2	1.7
eaeA	3	2.5
escV	4	3.3
stx1	0	0
stx2	38	31.7

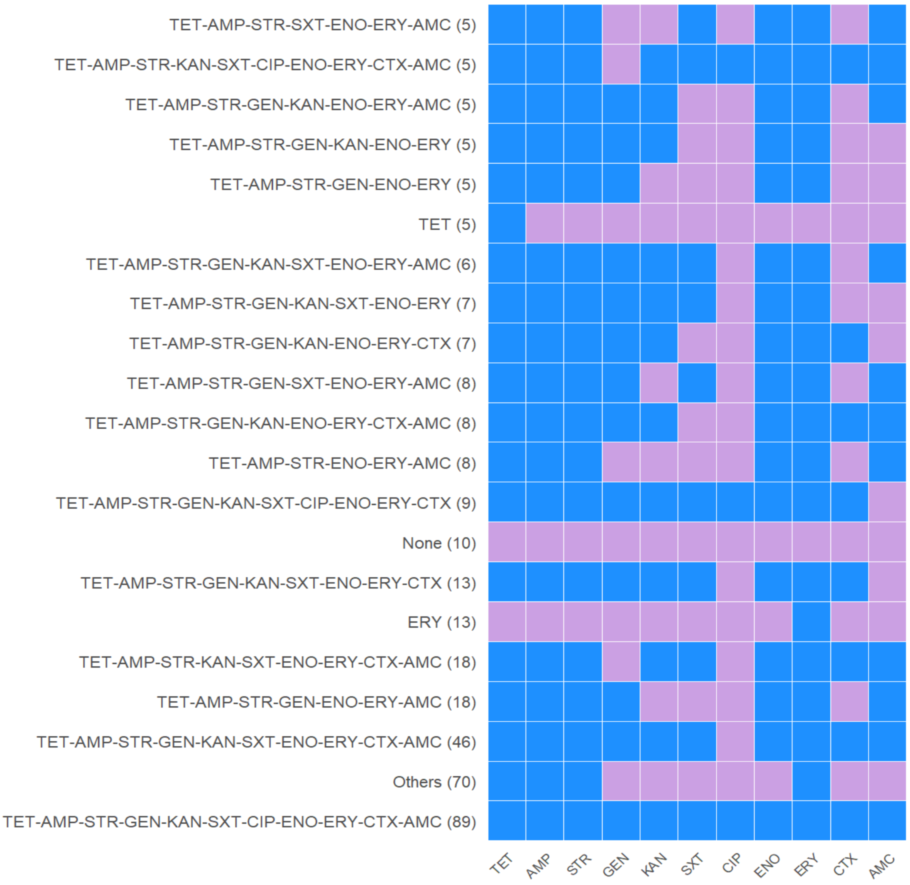

Figure 3. Antimicrobial resistance patterns of *Escherichia coli* isolates. TET: tetracycline, AMP: ampicillin, STR: streptomycin, GEN: gentamicin, KAN: kanamycin, SXT: trimethoprim-sulfamethoxazole, CIP: ciprofloxacin, ENO: enrofloxacin, ERY: erythromycin, CTX: cefotaxime, AMC: amoxicillin-clavulanate. 🟪 Non-R; 🟦 R.

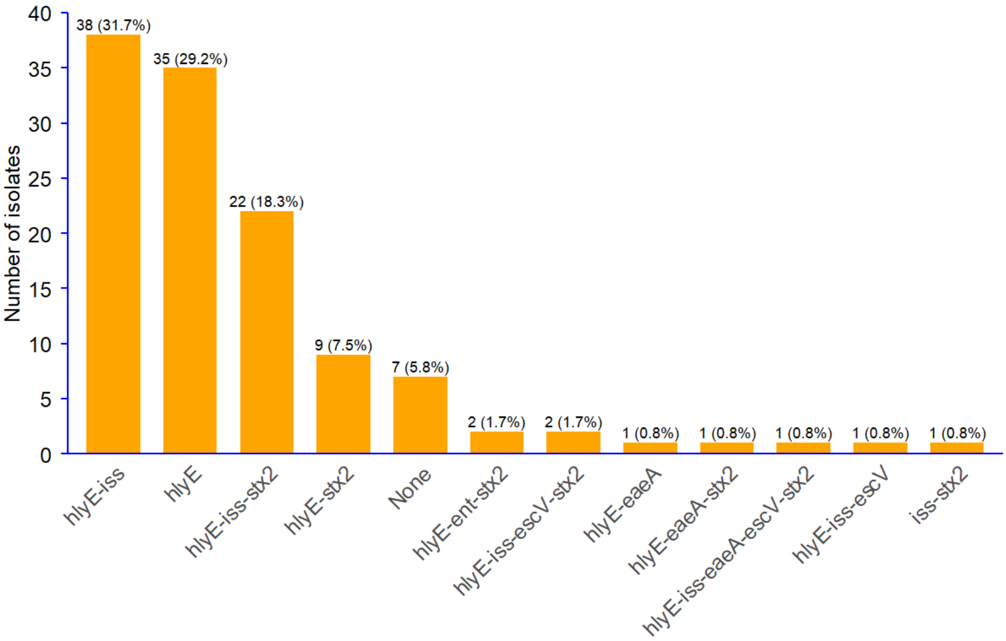

Figure 4. Virulence gene profile of *Escherichia coli* isolated from diarrheic broilers

sold at local veterinary drug stores. Overall, the findings indicate widespread antimicrobial resistance and multidrug resistance (MDR) in the surveyed poultry farms. The frequent and repeated use of antimicrobials in treatment may contribute to the selection of resistant strains (Molia *et al*., 2025).

One important mechanism explaining this phenomenon is the selective pressure exerted by prolonged antimicrobial use in animal husbandry, which facilitates the survival and spread of antimicrobial-resistant bacterial strains within the gut microbiota. Additionally, many antimicrobial resistance genes are often located on plasmids or mobile genetic elements, allowing them to be transferred horizontally between different microorganisms, thereby accelerating the dissemination of resistance within microbial populations (Pitout & Chen, 2023).

In this study, high resistance rates (>70%) were observed for tetracycline, ampicillin, streptomycin, enrofloxacin, and erythromycin, while most other antimicrobials also showed resistance rates above 50%. A previous study in southern Vietnam reported high resistance rates of *E. coli* to gentamicin (42.2%), ciprofloxacin (73.3%), and ampicillin (97.8%) (Nguyen *et al*., 2016). Another recent study in southern Vietnam also reported high resistance rates to amoxicillin (68.8%), doxycycline (75.9%), and oxytetracycline (77.2%) (Nhung *et al*., 2022a). These findings are consistent with reports from other countries, such as resistance to ampicillin (99.4%), intermediate resistance to enrofloxacin (92%) (Bhattarai *et al*., 2024), resistance to erythromycin (91.8%), and tetracycline (100%) (Roy *et al*., 2025).

High resistance to tetracycline and ampicillin may be related to their widespread overuse in poultry husbandry, leading to strong selective pressure on bacteria carrying resistance genes such as *tet* and *bla*. These genes are often located on plasmids or integrons, allowing them to spread rapidly within the bacterial population and create MDR (Roy *et al*., 2025). Additionally, co-selection can occur when multiple different resistance genes coexist on a single plasmid, meaning that the use of one antimicrobial may simultaneously maintain resistance to others (Bhattarai *et al*., 2024).

In this study, the proportion of isolates resistant to at least one antimicrobial agent was very high (97.5%). This result is comparable to a previous study in Vietnam from 2017 to 2019, where the rate reached 99% (Tuat *et al*., 2021). A recent study also reported that 96.7% of *E. coli* isolates carried at least one AMR gene (Laopiem *et al*., 2025).

Although ciprofloxacin showed the lowest resistance rate (31%–32%), the high intermediate proportion is also a concern, indicating an increasing resistance trend. Similar increasing trends in fluoroquinolone resistance have been reported in other countries (Garcia & Zavala-Cerna, 2024; Ruiz-Lievano *et al*., 2024).

The emergence of fluoroquinolone resistance may be associated with mutations in the *gyrA* and *parC* genes, leading to structural changes in the DNA gyrase and topoisomerase IV, which are the primary targets of fluoroquinolones. Additionally, mechanisms such as efflux pumps or reduced membrane permeability may also contribute to decreased antimicrobial efficacy (Ruiz-Lievano *et al*., 2024).

The susceptibility rates observed in this study were generally low (0.6%–28.3%). Erythromycin showed the lowest susceptibility (0.6%), while gentamicin and trimethoprim–sulfamethoxazole showed relatively higher values (23.6% and 28.3%, respectively), although these levels remain limited in clinical relevance. These findings suggest that the selection of effective antimicrobials for treating *E. coli*-associated diarrhea in broilers is increasingly challenging and requires continuous monitoring and reassessment of treatment strategies (Ribeiro *et al*., 2023).

Regarding MDR, the study recorded a high prevalence (>90%). A previous study showed that the MDR rate of 89% in Vietnam during 2017–2019 (Tuat *et al.,* 2021). Similar trends have been reported in other countries, indicating substantial antimicrobial selection pressure in intensive livestock systems (Zhou *et al.,* 2022; Tongkamsai *et al.,* 2024). In the current study, the most common MDR phenotypes involved resistance to 9–11 antimicrobial agents, reflecting complex resistance patterns.

The increasing complexity of MDR phenotypes may result from the accumulation of multiple resistance genes on plasmids or integrons, facilitating horizontal gene transfer between bacterial strains. This is particularly relevant in high-density farming systems, where bacteria can rapidly exchange genetic material and disseminate resistance traits within the gut microbiota (Tongkamsai *et al.,* 2024).

In this study, *E. coli* isolates showed a high prevalence of virulence genes, particularly *hlyE*, *iss*, and *stx2*, while *ent*, *eaeA*, and *escV* were detected at low frequencies, and *stx1* was not detected. This is consistent with a recent study in Vietnam, which reported a higher prevalence of *stx2* (90.5%) compared to *stx1* (9.5%) (Duc *et al.,* 2024).

The co-occurrence of virulence and antimicrobial resistance genes may reflect genetic linkage, as many plasmids or pathogenicity islands can carry both types of determinants (Zhou *et al.,* 2022). Such co-occurrence may enhance bacterial fitness under antimicrobial pressure while also contributing to pathogenic potential (Duc *et al.*, 2024).

The current study showed a high prevalence of virulence genes in isolates from diarrheal broilers ($p<0.001$). This is consistent with the known pathogenic mechanisms of APEC and strains carrying *stx2* or genes related to adhesion and invasion (Castro *et al.* 2025; Van Nederveen & Melton-Celsa, 2025).

Furthermore, the association between resistance phenotypes and the presence of certain virulence genes may indicate that pathogenic strains may harbor multiple adaptive traits that support persistence in poultry production systems (Castro *et al.,* 2025). This underscores the importance of simultaneously monitoring resistance and virulence determinants in epidemiological studies of *E. coli*.

The findings of the study provide additional evidence on the coexistence of antimicrobial resistance and virulence traits in *E. coli* and contribute to a better understanding of their potential role in diarrheal conditions in broilers. These results may support the development of improved disease management strategies and antimicrobial stewardship in poultry production, particularly in Vietnam.

CONCLUSION

This study showed a high prevalence of antimicrobial resistance and multidrug-resistant phenotypes of *E. coli* isolates. The common prevalence of *hlyE*, *iss*, and *stx2* was found in diarrheal fecal samples. These findings revealed the circulation of potentially resistant and pathogenic *E. coli* strains in broiler production systems. The study provides important baseline data for antimicrobial resistance surveillance and supports the development of more effective antimicrobial stewardship strategies in poultry production.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

Authors declare that the use of generative AI and AI-assisted technologies in the writing process is only to improve the readability and language of the work.

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