

Antibiotic Resistance of *Escherichia coli* Strains Isolated from Fecal Samples of Poultry Farms in Ninh Binh, Vietnam

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ABSTRACT

Antibiotic resistance is a major global health threat, and antibiotic use in livestock farming contributes to this issue. The present study aimed to assess the prevalence and antibiotic susceptibility of *Escherichia coli* (*E. coli*) isolated from poultry fecal samples in Ninh Binh province, Vietnam. Between May and October 2025, 170 dropping fecal samples were randomly collected from 45 poultry farms in Ninh Binh province, Vietnam, including 24 broiler chicken farms (aged 2.5-3.0 months old) and 21 meat-oriented duck farms (aged 1.5-2.0 months old). Fecal samples were transferred to the laboratory and cultured in peptone buffer and on selective media, including MacConkey agar, eosin methylene blue agar, and triple sugar iron agar. Antibiotic susceptibility was assessed using the disk diffusion method on Mueller-Hinton agar. Suspected *E. coli* colonies were confirmed using Gram staining and biochemical tests such as citrate utilization, indole production, the methyl red test, and the Voges-Proskauer reaction. *Escherichia coli* was isolated from 134 (78.8%) fecal samples, including 69/85 (81.2%) chicken and 65/85 (76.5%) duck samples. The susceptibility of *E. coli* strains to 10 antibiotics was evaluated using the disk diffusion method. *Escherichia coli* strains isolated from chickens and ducks demonstrated the highest resistance to ampicillin (82.8%), followed by sulfonamides (79.9%), trimethoprim/sulfamethoxazole (74.6%), tetracycline (60.5%), and gentamicin (52.2%). Resistance to the remaining antibiotics, including norfloxacin, doxycycline, nalidixic acid, and cefotaxime, ranged from 14.6% to 38.1%; none of the isolates were resistant to meropenem. Among the isolates, 97 (72.4%) were multidrug-resistant; the proportion of multidrug-resistant isolates from chickens (60 strains, 87.0%) was higher than that from ducks (37 strains, 56.9%). *Escherichia coli* strains isolated from broiler chicken and duck farms in Ninh Binh, Vietnam, exhibited diverse resistance patterns to several antibiotics. Specifically, 34 and 36 resistance patterns were identified in chicken and duck isolates, respectively, with most isolates having multidrug resistance characteristics.

Keywords: Antibiotic resistance, Chicken, Duck, *Escherichia coli*, Poultry farm

INTRODUCTION

Antibiotics are widely used worldwide in livestock farming to prevent disease, treat infections, promote growth, and increase production (Pokharel et al., 2020; Rahman et al., 2022). Disease in livestock is a primary driver of antibiotic use (AMU), and global AMU is expected to rise by 8% by 2030 (Mulchandani et al., 2023). Over recent decades, antibiotic overuse in human and veterinary medicine has increased antibiotic resistance (AMR) and accelerated its spread among pathogenic and harmless bacteria (Moffo et

al., 2022; Salam et al., 2023). Additionally, AMU in livestock can foster AMR, jeopardizing the sustainability of the livestock industry by causing treatments to fail in animals (Mulchandani et al., 2023; Stevenson, 2023). AMR has become one of the most critical global health issues, and the World Health Organization (WHO) recognized AMR as one of the top ten public health threats (Bhargav et al., 2022). Antibiotic resistance is frequently referred to as a silent pandemic due to its deadly yet largely invisible impacts (Zhou et al., 2022).

Escherichia coli (*E. coli*) is among the most common microorganisms in the guts of warm-blooded animals, including mammals and chickens (Kaper et al., 2004; Kerek et al., 2025). While most *E. coli* strains are harmless, some are pathogenic, highly transmissible, and can form colonies. Because of their widespread presence and resistance to different antibiotics, these strains are found globally (Paitan, 2018). One important AMR mechanism in *Enterobacteriaceae*, including *E. coli*, is the production of Extended-spectrum β -lactamases (ESBLs) or β -lactamase type C (AmpC; Geser et al., 2012). With more than 600 variants, ESBLs/AmpC not only confer cephalosporin resistance but are also associated with resistance to many other antibiotics, including fluoroquinolones, trimethoprim-sulfamethoxazole, and tetracyclines (Woodford et al., 2004; Geser et al., 2012). Therefore, the WHO has included *E. coli* on the list of 12 priority AMR pathogens (Asokan et al., 2019).

Poultry industry of Vietnam ranks tenth globally in chicken production and second in waterfowl production, with approximately 584.4 million chickens annually, producing about 2.46 million tons of meat and 20.352 billion eggs, including 3.516 billion chicken eggs and 6.913 billion waterfowl eggs (Thanh Tra, 2025). The use of antibiotics in poultry farming for disease prevention, treatment, and growth promotion poses a significant risk of AMR bacteria emerging through genetic or non-genetic mechanisms, making poultry a major global reservoir of AMR *E. coli* (Osman et al., 2018; Liu et al., 2025). In response to AMR, Vietnam's Ministry of Agriculture and Rural Development (MARD) implemented a National Action Plan in 2017 to address AMR in livestock and aquaculture (MARD, 2017). The plan aimed to reduce non-therapeutic AMU, such as growth promotion, while strengthening surveillance and raising awareness. However, implementation of the AMR plan has faced challenges because poultry farms frequently use antibiotics for disease prevention, to boost productivity, and to prevent outbreaks (Carrique-Mas et al., 2015). To date, several studies have examined AMU and bacterial resistance on poultry farms in Vietnam (Carrique-Mas et al., 2015; Vounba et al., 2019; Nohrborg et al., 2024).

Additional studies are needed to fully understand the AMR characteristics of different bacteria, including *E. coli*, to inform AMR prevention strategies. Therefore, the present study aimed to determine the prevalence and antibiotic susceptibility of *E. coli* isolated from poultry fecal samples in Ninh Binh province, Vietnam.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in accordance with the animal welfare and safety guidelines of the Committee on Animal Research and Ethics at the Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam (Approval No. CARE-2025/06). The authors addressed farmers' ethical concerns and obtained their consent before conducting the study.

Sample size determination

The study sample size was calculated using the prevalence of *E. coli* to achieve a 95% confidence level and a 5% margin of error. For sample size determination, the present study used the following formula:

$$N = Z^2 \times P(1-P) / L^2$$

$$N = (1.96^2 \times 0.7105 \times 0.2895) / (0.05^2)$$

where N was the required sample size, Z was the Z-statistic for a 95% confidence level (1.96 for a two-tailed test), P was the prevalence of *E. coli*, and L was the margin of error (5%). In the present study, P was set at 71.05%, based on findings from Nguyen et al. (2021). Substituting these values into the formula yielded approximately 316 samples. However, due to budget constraints and fewer farms consenting to sampling than expected, the study collected 170 samples.

Sampling

In this cross-sectional study, 170 fresh fecal samples were randomly collected from 24 colored-feather broiler chicken farms and 21 meat-oriented duck farms in Ninh Binh Province, Vietnam, between May and October 2025. These farms were located between 20°23' and 20°44' North latitude and between 105°45' and 106°15' East longitude in Ninh Binh Province, Vietnam. The temperature in the poultry houses was maintained between 20°C and 25°C, humidity between 60% and 70%, and stocking density at 6-8 animals per m². Certified veterinarians selected broiler chicken and meat-oriented duck farms for sampling. These farms operated under a semi-closed husbandry model, specializing in raising a single breed of broiler chicken or meat-oriented duck, with flock sizes typically ranging from 2,000 to 3,000 chickens or ducks per farm. The colored-feather broiler flocks comprised indigenous breeds such as Luong Phuong, Ho, Mia, and Dong Tao (aged 2.5 to 3.0 months), while the meat-oriented duck flocks consisted of Super-meat, Grimaud, Cherry Valley, Bau, and Co breeds (aged 1.5 to 2.0 months). The flocks had been fully vaccinated against key diseases, including Newcastle disease at 5-7, 18-21, and 35-42 days of age, Gumboro disease at 10-14 and 21-25 days of age for chickens; and Duck viral hepatitis at 1-3 days of age, along with Duck plague at 7-10

and 35-40 days for ducks. Vaccines against Newcastle disease and Gumboro disease were typically supplied by companies such as Boehringer Ingelheim (Germany), Ceva Santé Animale (France), MSD Animal Health (USA), Zoetis (USA), Vaccindo (Indonesia), and Biovac (Israel). Vaccines against viral hepatitis in ducks and duck cholera were supplied by companies such as Hanvet, Navetco, and Vetvaco (Vietnam). Certified veterinarians conducted health checkups and clinical examinations to prevent external infectious diseases. All medications to prevent diseases were administered according to the veterinarians' recommendations. All poultry flocks were fed a balanced commercial poultry feed from well-known animal feed manufacturers, with diets tailored to each age stage's nutritional needs. At the time of sampling, the poultry flocks were healthy and demonstrated no clinical signs of disease, as evidenced by normal activity levels and average water and feed intake. Three to five samples of freshly voided poultry feces were collected from each flock in accordance with the [MARD \(2011\)](#) guidelines (QCVN 01-83:2011/BNNPTNT). Approximately 2-3 grams of fresh feces were collected from the pen floor using an autoclaved spoon. Each sample was placed in a separate sterile polyethylene bag, labeled, stored in a cool box with dry ice, and transported to the Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, for analysis within 24 hours.

Isolation of *Escherichia coli*

Escherichia coli were isolated from fecal samples using the method described by [Deng et al. \(2024\)](#), with some modifications. In the laboratory, approximately 1 g of each fecal sample was homogenized with buffered peptone water (BPW; Merck, Germany) at a 1:9 ratio. A loopful of the homogenate was streaked onto MacConkey agar (Merck, Germany) and incubated at 37°C under aerobic conditions for 24 hours. Pink colonies on MacConkey agar were then cultured onto eosin methylene blue agar (EMB; Merck, Germany) and incubated at 37°C under aerobic conditions for 24 hours. A typical colony exhibiting a green metallic sheen on EMB agar was streaked into a triple sugar iron (TSI; Merck, Germany) agar tube and incubated at 37°C under aerobic conditions for 24 hours. Colonies exhibiting typical TSI characteristics, including glucose and lactose fermentation with gas production and no hydrogen sulfide, were identified as *E. coli* by Gram staining and biochemical tests, including citrate utilization, indole production, the methyl red test, and the Voges-Proskauer reaction, performed in Simmons citrate agar (SCA; Merck, Germany), Tryptone broth (TB; Merck, Germany), and methyl-red

Voges-Proskauer broth (MR-VP; Merck, Germany), respectively. All isolated strains were cultured in brain-heart infusion broth (BHI; Merck, Germany) under aerobic conditions for 16-18 hours. Then, 0.5 mL of the bacterial culture was transferred to an Eppendorf tube containing 0.5 mL of glycerol (Merck, Germany). The mixture was shaken thoroughly to ensure homogeneity, incubated for an additional 2-3 hours at 37°C, and finally stored at -80°C for the following experiment.

Antibiotic susceptibility testing

The agar disk diffusion method was performed on Mueller-Hinton agar (MHA; Merck, Germany) following the methodology of [Bauer et al. \(1966\)](#). Ten antibiotics manufactured by Oxoid, United Kingdom, from seven antibiotic classes were tested, including penicillins (ampicillin, 10 µg), cephalosporins (cefotaxime, 30 µg), carbapenems (meropenem, 10 µg), aminoglycosides (gentamicin, 10 µg), quinolones (nalidixic acid, 30 µg; norfloxacin, 10 µg), tetracyclines (doxycycline, 30 µg; tetracycline, 30 µg), and sulfonamides (sulfonamides, 300 µg; trimethoprim/sulfamethoxazole, 1.25/23.75 µg). Briefly, *E. coli* colonies were suspended in nutrient broth (Merck, Germany), and the suspension's turbidity was adjusted to the 0.5 McFarland standard. A sterile swab was dipped into the standardized bacterial suspension, pressed against the tube wall to remove excess liquid, and then streaked across prepared MHA plates. The inoculated plates were left to stand for 10-15 minutes, after which sterile forceps were used to place five antibiotic discs onto the agar surface. The plates were incubated at 37°C under aerobic conditions for 16-18 hours ([Bauer, 1966](#)). After incubation, the zone of inhibition around each plate was measured to the nearest millimeter with a ruler, recorded, and classified as resistant, intermediate, or susceptible according to the Clinical and Laboratory Standards Institute guidelines ([CLSI, 2024](#)). The *Escherichia coli* ATCC 25922 strain, provided by the Department of Pharmacology, Internal Medicine, and Toxicology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, was used as a quality control strain. An *E. coli* strain was classified as resistant when it was confirmed resistant to at least one tested antibiotic, and as multidrug-resistant when it exhibited resistance to at least one antibiotic from each of three antibiotic classes ([Magiorakos et al., 2012](#)).

Data analysis

Data on *E. coli* isolation and antimicrobial susceptibility testing were recorded and edited in a Microsoft Excel spreadsheet. A two-tailed Fisher's exact test was used to assess differences in prevalence and antibiotic susceptibility

between *E. coli* isolated from chicken and duck fecal samples. P values less than 0.05 were considered significant.

RESULTS

Of 170 samples collected, 134 (78.8%) tested positive for *E. coli*. Specifically, *E. coli* was detected in 69 of 85 chicken fecal samples (81.2%) and in 65 of 85 duck fecal samples (76.5%; Table 1). Overall, *E. coli* strains isolated from chicken and duck fecal samples were resistant to nine antibiotics tested (Table 2). Among the 134 *E. coli* strains, resistance was highest for ampicillin (82.8%), followed by sulfonamides (79.9%), trimethoprim/sulfamethoxazole (74.6%), tetracycline (60.5%), and gentamicin (52.2%). Resistance rates were lower for norfloxacin (16.4%), doxycycline (20.9%), nalidixic acid (30.6%), and cefotaxime (38.1%); none of the isolates exhibited resistance to meropenem. Resistance rates for ampicillin did not differ significantly ($p = 0.398$) between *E. coli* isolates from chicken and duck samples (85.5% versus 80.0%). Similarly, resistance rates to trimethoprim/sulfamethoxazole was not significantly different ($p = 0.549$) between chicken and duck isolates (76.8% versus 72.3%). Cefotaxime (43.5% versus 32.3%; $p = 0.183$) and nalidixic acid (33.3% versus 27.7%; $p = 0.479$) demonstrated no significant differences. Doxycycline resistance was comparable between *E. coli* isolates from chicken and duck (21.7% versus 20.0%; $p = 0.805$). Norfloxacin resistance did not differ significantly ($p = 0.148$) between *E. coli* isolates from chicken and duck samples (21.7% versus 10.8%). However, Sulfonamide resistance was significantly higher in chicken

isolates ($p = 0.012$; 88.4% versus 70.8%). A similar pattern was observed for tetracycline, with chicken isolates demonstrating a significantly higher ($p = 0.001$) resistance rate than duck isolates (78.3% versus 41.5%). Gentamicin resistance was significantly higher ($p = 0.039$) in isolates from chicken fecal samples than in duck isolates (60.9% versus 43.1%).

Among the isolates, 97 (72.4%) were multidrug-resistant. The proportion of multidrug-resistant isolates from chickens (60 strains, 87.0%) was significantly higher ($p < 0.001$) than that from ducks (37 strains, 56.9%; Figure 1). All 69 *E. coli* strains isolated from chicken fecal samples were resistant to at least one antibiotic and exhibited 34 distinct resistance patterns (Table 3). The most common resistance pattern was ampicillin - gentamicin - tetracycline - trimethoprim/sulfamethoxazole - sulfonamides pattern, observed in 9 strains (13.04%). The second most frequent resistance pattern was ampicillin - cefotaxime - gentamicin - trimethoprim/sulfamethoxazole - sulfonamides - nalidixic acid - norfloxacin, observed in 5 strains (7.25%). Of the 65 *E. coli* strains isolated from ducks, 4 were susceptible to all 10 antibiotics, and the remaining 61 exhibited 36 distinct resistance patterns (Table 4). The most common resistance patterns were ampicillin - trimethoprim/sulfamethoxazole and ampicillin - trimethoprim/sulfamethoxazole - sulfonamides, observed in 6 (9.23%) and 7 (10.77%) strains, respectively. Additionally, the ampicillin alone and ampicillin - gentamicin - trimethoprim/sulfamethoxazole - sulfonamides resistance patterns were each detected in 4 strains (6.15%).

Table 1. Isolation rate of *Escherichia coli* from poultry fecal samples in Ninh Binh, Vietnam

Sources	Sample	Samples (number)	Positive isolates number (%)	95% confidence interval
Chicken		85	69 (81.2)	(71.24 - 88.84)
Duck		85	65 (76.5)	(66.03 - 85.00)
Total		170	134 (78.8)	(71.81 - 84.71)

Table 2. Antibiotic resistance rates among *Escherichia coli* strains isolated from poultry fecal samples in Ninh Binh, Vietnam

Antibiotic classes	Antibiotics	Number and resistance rate (%) of <i>Escherichia coli</i> strains				
		Chicken (n = 69)	Duck (n = 65)	P-value	Total (n = 134)	95% confidence interval
Penicillin	Ampicillin	59 (85.5) ^a	52 (80.0) ^a	0.398	111 (82.8)	(75.4 - 88.8)
Cephalosporin	Cefotaxime	30 (43.5) ^a	21 (32.3) ^a	0.183	51 (38.1)	(29.8 - 49.8)
Carbapenems	Meropenem	0 (0.0) ^a	0 (0.0) ^a	nd	0 (0.0)	(0.0 - 0.2)
Aminoglycosides	Gentamicin	42 (60.9) ^a	28 (43.1) ^b	0.039	70 (52.2)	(43.4 - 60.9)
Quinolones	Nalidixic acid	23 (33.3) ^a	18 (27.7) ^a	0.479	41 (30.6)	(22.9 - 39.1)
	Norfloxacin	15 (21.7) ^a	7 (10.8) ^a	0.148	22 (16.4)	(10.6 - 23.8)
Tetracyclines	Doxycycline	15 (21.7) ^a	13 (20.0) ^a	0.805	28 (20.9)	(14.4 - 28.8)
	Tetracycline	54 (78.3) ^a	27 (41.5) ^b	0.001	81 (60.5)	(51.6 - 68.8)
Sulfonamides	Sulfonamides	61 (88.4) ^a	46 (70.8) ^b	0.012	107 (79.9)	(72.1 - 86.3)
	Trimethoprim/ Sulfamethoxazole	53 (76.8) ^a	47 (72.3) ^a	0.549	100 (74.6)	(66.4 - 81.7)

n: number of samples, nd: Not detected. ^{a,b} Mean different superscript letters on the same row indicate statistically significant differences at p-value less than 0.05.

Table 3. Multidrug-resistance profiles of *Escherichia coli* strains isolated from chicken fecal samples in Ninh Binh, Vietnam

AMR pattern	Number of AMR	Number of classes	Number of strains (%)
AMP	1	1	1 (1.45)
TET	1	1	1 (1.45)
AMP, TET	2	2	1 (1.45)
STX, SUL	2	1	1 (1.45)
AMP, STX, SUL	3	2	3 (4.35)
AMP, TET, SUL	3	3	3 (4.35)
TET, STX, SUL	3	2	1 (1.45)
CTX, DOX, TET	3	2	1 (1.45)
CTX, TET, SUL	3	3	2 (2.90)
AMP, GEN, STX, NAL	4	4	1 (1.45)
AMP, GEN, STX, SUL	4	3	1 (1.45)
AMP, TET, STX, SUL	4	3	3 (4.35)
AMP, CTX, GEN, TET	4	4	1 (1.45)
CTX, DOX, TET, SUL	4	3	1 (1.45)
AMP, GEN, DOX, TET, STX	5	4	1 (1.45)
AMP, DOX, TET, STX, SUL	5	3	2 (2.90)
AMP, CTX, TET, STX, SUL	5	4	2 (2.90)
AMP, GEN, TET, STX, SUL	5	4	9 (13.04)
AMP, TET, STX, SUL, NAL	5	4	3 (4.35)
CTX, GEN, TET, SUL, NOR	5	5	1 (1.45)
AMP, GEN, DOX, TET, STX, SUL	6	4	3 (4.35)
AMP, CTX, GEN, STX, NAL, NOR	6	5	1 (1.45)
AMP, CTX, GEN, TET, STX, SUL	6	5	4 (5.80)
AMP, GEN, TET, STX, SUL, NAL	6	5	2 (2.90)
AMP, CTX, TET, STX, SUL, NAL	6	5	2 (2.90)
AMP, CTX, GEN, SUL, NAL, NOR	6	5	2 (2.90)
GEN, TET, STX, SUL, NAL, NOR	6	4	1 (1.45)
CTX, GEN, DOX, TET, SUL, NOR	6	5	1 (1.45)
AMP, CTX, GEN, DOX, TET, STX, SUL	7	5	3 (4.35)
AMP, GEN, DOX, TET, STX, SUL, NAL	7	5	1 (1.45)
AMP, CTX, GEN, STX, SUL, NAL, NOR	7	5	5 (7.25)
AMP, GEN, DOX, TET, SUL, NAL, NOR	7	5	1 (1.45)
AMP, CTX, GEN, DOX, TET, STX, SUL, NAL	8	6	1 (1.45)
AMP, CTX, GEN, TET, STX, SUL, NAL, NOR	8	6	3 (4.35)

The total number of *Escherichia coli* strains isolated from chicken fecal samples was 69. AMP: Ampicillin, CTX: Cefotaxime, DOX: Doxycycline, GEN: Gentamicin, NAL: Nalidixic acid, NOR: Norfloxacin, SUL: Sulfonamides, TET: Tetracycline, SXT: Trimethoprim/Sulfamethoxazole

Table 4. Multidrug-resistance profiles of *Escherichia coli* strains isolated from duck fecal samples in Ninh Binh, Vietnam

AMR pattern	Number of AMR	Number of classes	Number of strains (%)
Susceptible	0	0	4 (6.15)
AMP	1	1	4 (6.15)
STX	1	1	1 (1.54)
TET	1	1	1 (1.54)
AMP, STX	2	2	6 (9.23)
AMP, TET	2	2	1 (1.54)
STX, SUL	2	1	3 (4.62)
AMP, STX, SUL	3	2	7 (10.77)
AMP, CTX, SUL	3	3	1 (1.54)
AMP, SUL, NAL	3	3	1 (1.54)
AMP, TET, SUL	3	3	1 (1.54)
TET, STX, SUL	3	2	1 (1.54)
TET, SUL, NAL	3	3	1 (1.54)
AMP, GEN, STX, SUL	4	3	4 (6.15)
AMP, GEN, TET, STX	4	4	1 (1.54)
AMP, TET, STX, SUL	4	4	1 (1.54)
AMP, CTX, GEN, STX, SUL	5	4	1 (1.54)
AMP, CTX, GEN, STX, NAL	5	5	1 (1.54)
AMP, CTX, TET, STX, SUL	5	4	1 (1.54)
AMP, GEN, TET, STX, SUL	5	4	2 (3.08)
AMP, GEN, TET, SUL, NAL	5	5	1 (1.54)
AMP, CTX, DOX, TET, STX, SUL	6	4	3 (4.62)
AMP, GEN, DOX, TET, STX, SUL	6	4	1 (1.54)
AMP, CTX, GEN, STX, SUL, NAL	6	5	3 (4.62)
AMP, CTX, GEN, TET, STX, SUL	6	5	1 (1.54)
AMP, GEN, TET, STX, SUL, NAL	6	5	1 (1.54)
AMP, CTX, GEN, DOX, TET, SUL	6	5	1 (1.54)
AMP, GEN, DOX, TET, SUL, NAL	6	5	1 (1.54)
CTX, GEN, STX, SUL, NAL, NOR	6	4	1 (1.54)
AMP, CTX, GEN, DOX, TET, STX, SUL	7	5	1 (1.54)
AMP, CTX, GEN, STX, SUL, NAL, NOR	7	5	1 (1.54)
AMP, CTX, GEN, DOX, TET, SUL, NAL	7	6	1 (1.54)
AMP, CTX, GEN, DOX, TET, STX, SUL, NAL	8	6	1 (1.54)
AMP, GEN, DOX, TET, STX, SUL, NAL, NOR	8	5	1 (1.54)
AMP, CTX, GEN, TET, STX, SUL, NAL, NOR	8	6	1 (1.54)
CTX, GEN, DOX, TET, STX, SUL, NAL, NOR	8	5	1 (1.54)
AMP, CTX, GEN, DOX, TET, STX, SUL, NAL, NOR	9	6	2 (3.08)

The total number of *Escherichia coli* strains isolated from duck fecal samples was 65. AMP: Ampicillin, CTX: Cefotaxime, DOX: Doxycycline, GEN: Gentamicin, NAL: Nalidixic acid, NOR: Norfloxacin, SUL: Sulfonamides, TET: Tetracycline, SXT: Trimethoprim/Sulfamethoxazole

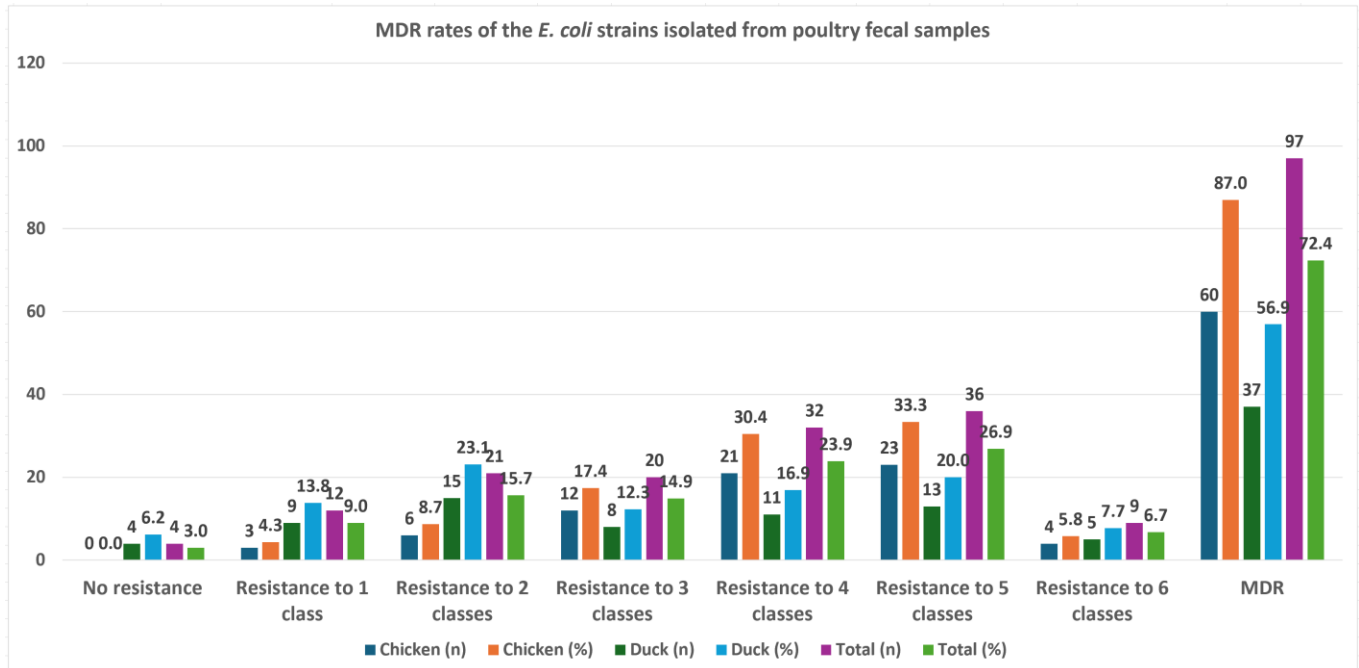


Figure 1. Multidrug resistance pattern of *Escherichia coli* strains isolated from poultry fecal samples in Ninh Binh, Vietnam

DISCUSSION

The *E. coli* isolation rate from poultry fecal samples in the current study was comparable to the 86.39% observed in broiler chickens fecal samples in China (Liu et al., 2025) but higher than the 36.7%-66.7% rate documented in broiler chicken fecal samples from Kenya (Langata et al., 2019), Egypt (Badr et al., 2022), Iraq (Hussein et al., 2025), Nigeria (Ugbo et al., 2025), South Africa (Gumede et al., 2026), and Pakistan (Khan et al., 2026). Notably, previous studies in Nigeria (Agusi et al., 2025), Bangladesh (Ibrahim et al., 2023), and India (Patel et al., 2023) have reported higher *E. coli* isolation rates from broiler chicken fecal samples, ranging from 86.4% to 99.44%. *Escherichia coli* isolation rates varied across studies due to differences in sampling methods, sample sizes, isolation methods, study sites, and farm hygiene conditions (Roy et al., 2025).

The 82.8% ampicillin resistance rate observed in the present study was predictable, given the widespread and long-term use of this antibiotic in human and veterinary medicine in Vietnam (Nga-do et al., 2014; Nohrborg et al., 2024). Similarly, high ampicillin resistance rates in avian-derived *E. coli* strains, ranging from 78.3% to 87.8%, have been reported in studies from India (Patel et al., 2023), Kenya (Deng et al., 2024), and China (Afayibo et al., 2022; Liu et al., 2025). However, the ampicillin resistance rate of *E. coli* strains in the current study was lower than

E. coli strains isolated from broiler chickens in Egypt (Awad et al., 2016; Badr et al., 2022), Algeria (Barour et al., 2022), Poland (Racewicz et al., 2022), Bangladesh (Ibrahim et al., 2023), Nepal (Bhattarai et al., 2024), and South Africa (Gumede et al., 2026), where resistance rates ranged from 92.9% to 100%. Conversely, lower rates of ampicillin resistance, ranging from 45.5% to 75.5%, were observed in broiler chicken-derived *E. coli* strains in Nigeria (Agusi et al., 2024; Adetunji et al., 2025; Ugbo et al., 2025), Tanzania (Mtemisika et al., 2022), and Pakistan (Khan et al., 2026). Notably, chicken- and duck-derived *E. coli* strains from Canada demonstrated lower rates of ampicillin resistance than those in the present study, ranging from 4.17% to 15.92% (Varga et al., 2019).

The cefotaxime resistance rate (38.1%) in the current study was comparable to the 29.27% and 38.3% rates observed in *E. coli* isolates from broiler chickens in Slovakia (Sciberras et al., 2019) and Nigeria (Adetunji et al., 2025). However, some studies have reported higher cefotaxime resistance rates of *E. coli* isolates from broiler chickens, including 59.09% in Bangladesh (Khan et al., 2026) and 52.6-80.12% in China (Afayibo et al., 2022; Liu et al., 2025). In contrast, previous studies from Bangladesh (Ibrahim et al., 2023), Iraq (Hussein et al., 2025), and Spain (Sevilla-Navarro et al., 2022) reported lower cefotaxime resistance rates of 3.0%, 6.6%, and 9.0% among *E. coli* isolates from broiler chickens. Gumede et

al. (2025) observed that all *E. coli* strains from poultry farms in South Africa were susceptible to cefotaxime.

All *E. coli* strains in the present study were susceptible to meropenem, consistent with findings in *E. coli* from chickens and ducks in Canada (Varga et al., 2019), broiler chickens in South Africa (Gumede et al., 2026), and Nigeria (Adetunji et al., 2025). Nonetheless, meropenem resistance was reported at higher rates in *E. coli* from broiler chickens, including 3.2% in Tanzania (Mtemisika et al., 2022) and 9.09% in Pakistan (Khan et al., 2026). These differences may reflect variations in management practices and AMU, which can influence the development of meropenem resistance in livestock isolates.

In the current study, 52.2% of *E. coli* strains were resistant to gentamicin, a widely used antibiotic in livestock and veterinary medicine in Vietnam (Nga-do et al., 2014). Previous studies in China (Liu et al., 2025), Nepal (Shrestha and Banjara, 2025), and Slovenia (Sciberras et al., 2019) reported higher resistance rates of *E. coli* strains from broiler chickens, at 62.2%, 65.38%, and 75.6%, respectively. In contrast, lower resistance rates, ranging from 22.0% to 44.7%, were observed in *E. coli* strains isolated from broiler chickens in Bangladesh (Ibrahim et al., 2023), India (Khan et al., 2026), Tanzania (Mtemisika et al., 2022), Nepal (Bhattarai et al., 2024), China (Afayibo et al., 2022), Poland (Racewicz et al., 2022), and Nigeria (Adetunji et al., 2025). Notably, studies from Kenya and South Africa (Langata et al., 2019; Gumede et al., 2026) did not detect gentamicin-resistant *E. coli* isolates derived from broiler chickens, whereas Varga et al. (2019) reported a low resistance rate of 6.42% in *E. coli* strains from chicken and duck sources in Canada.

The resistance rate to nalidixic acid (30.6%) among isolated *E. coli* strains was lower than that reported in other poultry-related studies, such as 78.7% reported in Nigeria (Adetunji et al., 2025), 90.0% documented in Bangladesh (Ibrahim et al., 2023), 94.44% identified in India (Patel et al., 2023), and 97.96% found in Algeria (Barour et al., 2022). Conversely, poultry-derived *E. coli* strains in Canada (Varga et al., 2019) and Kenya (Langata et al., 2019) exhibited lower nalidixic acid resistance rates, ranging from 2.0 to 4.17%. The present study observed 16.4% resistance to norfloxacin, lower than the 56.76% recorded in *E. coli* strains isolated from chickens and ducks in Canada (Varga et al., 2019), and the 66.1% and 91.7% recorded in *E. coli* strains isolated from broiler chickens in Kenya (Badr et al., 2022) and Iraq (Hussein et al., 2025), respectively. The relatively low resistance levels to nalidixic acid and norfloxacin observed in the

present study may reflect less frequent use of these antibiotics on poultry farms in Vietnam compared with other antibiotics (Carrique-Mas et al., 2015).

The 60.5% tetracycline resistance rate in the present study aligns with the antibiotic's widespread global use. Numerous studies have documented high rates of tetracycline resistance in broiler chicken-derived *E. coli* isolates, such as 97.22% in India (Patel et al., 2023), 78.7–81.92% in Nigeria (Agusi et al., 2024; Adetunji et al., 2025), 81.1–100% in Nepal (Bhattarai et al., 2024; Shrestha and Banjara, 2025), 87.4% in Tanzania (Mtemisika et al., 2022), 84.6–95.2% in China (Afayibo et al., 2022; Liu et al., 2025), 92.68% in Slovakia (Sciberras et al., 2019), 93.1–100% in Egypt (Awad et al., 2016; Badr et al., 2022), 98.0% in Bangladesh (Ibrahim et al., 2023), 100% in Algeria (Barour et al., 2022), and South Africa (Gumede et al., 2026). However, some studies reported lower tetracycline resistance rates than those in the present study, such as 12.0% and 38.0% resistance rates in broiler chicken-derived *E. coli* isolates in Kenya (Langata et al., 2019) and Spain (Sevilla-Navarro et al., 2022), respectively, and 41.67–43.3% resistance rates in broiler chicken- and duck-derived *E. coli* strains reported in Canada (Varga et al., 2019). The doxycycline resistance rate (20.9%) among *E. coli* strains in the current study was comparable to the 27.0% observed in *E. coli* isolates from broiler chickens in Spain (Sevilla-Navarro et al., 2022). Higher rates of doxycycline resistance have been reported in *E. coli* isolates from broiler chickens, such as 39.42% and 76.4% in Nepal (Bhattarai et al., 2024; Shrestha and Banjara, 2025), 62.6% and 93.9% in China (Afayibo et al., 2022; Liu et al., 2025), and 88.0% in Poland (Racewicz et al., 2022).

In the current study, 79.9% of isolated *E. coli* strains were resistant to sulfonamides, comparable to the 84.3% reported in China (Afayibo et al., 2022) but higher than the 53.2% and 66.0% documented in Nigeria (Agusi et al., 2024; Adetunji et al., 2025). Furthermore, 74.6% of isolates were resistant to trimethoprim/sulfamethoxazole, consistent with previous studies reporting high resistance rates of *E. coli* isolates derived from broiler chicken samples in Nepal (53.0%; Shrestha and Banjara, 2025), Egypt (58.6%–82.1%; Badr et al., 2022; Awad et al., 2026), Spain (66.0%; Sevilla-Navarro et al., 2022), Tanzania (69.8%; Mtemisika et al., 2022), and in South Africa (76.0%; Gumede, 2026). However, resistance levels in broiler chicken-derived *E. coli* strains were reported as 84.0% in Poland (Racewicz et al., 2022), 90.74% in India (Patel et al., 2023), and 100% in Algeria (Barour et al., 2022). Conversely, *E. coli* isolates collected

from poultry samples in Canada (Varga et al., 2019) and Kenya (Langat et al., 2019) exhibited much lower resistance to trimethoprim/sulfamethoxazole, at 8.38-16.67% and 26.0%, respectively.

The isolated *E. coli* strains exhibited a broad spectrum of AMR patterns, with 34 in chicken-derived strains and 36 in duck-derived strains. Overall, 72.4% of isolates were multidrug-resistant, representing 87.0% of chicken isolates and 56.9% of duck isolates. The worldwide rise of multidrug-resistant *E. coli* strains poses a noticeable challenge for choosing effective antibiotics. The prevalence of multidrug-resistant *E. coli* strains in this study aligns with rates of 74.0% reported in Nigeria (Adetunji, 2025) 74.0% in South Africa (Gumede et al., 2025), and 81.1% in Poland (Racewicz et al., 2022), but exceeded the multidrug-resistance rates of 12.5-19.55% and 35.3% observed in poultry-origin *E. coli* strains in studies from Canada (Varga et al., 2019) and Nigeria (Ugbo et al., 2025), respectively. Notably, very high rates of multidrug-resistance have been documented in *E. coli* isolates from broiler chickens, including 86.3% in Tanzania (Mtemisika et al., 2022), 90.38% in Nepal (Shrestha and Banjara, 2025), 96.45-100% in China (Afayibo et al., 2022; Liu et al., 2025), 100% in Algeria (Barour et al., 2022), 100% in Spain (Sevilla-Navarro et al., 2022), and 100% in Bangladesh (Ibrahim et al., 2023). In the current study, multidrug-resistant *E. coli* demonstrated co-resistance to some antibiotics such as ampicillin, tetracycline, sulfonamide, trimethoprim/sulfamethoxazole, and gentamicin, as well as to newer antibiotics. These resistance patterns indicated that *E. coli* strains on poultry farms are frequently exposed to different antibiotics because of drug-use practices by farm owners and technical staff during rearing.

CONCLUSION

The present study confirmed the presence of AMR *E. coli* strains in colored-feather broiler chicken and meat-oriented duck farms in Ninh Binh, Vietnam, with most isolates exhibiting multidrug-resistance patterns. The isolated *E. coli* strains demonstrated high resistance to several commonly used antibiotics, including ampicillin, sulfonamides, tetracycline, trimethoprim/sulfamethoxazole, gentamicin, and cefotaxime. However, due to the small sample size and the lack of investigation into poultry drug-use practices in the study area, the study did not establish a link between *E. coli* prevalence and specific poultry breeds or between AMU and the AMR rate of the isolated *E. coli* strains. The

relationship between the phenotypic and genotypic profiles of these strains remains unclear. Therefore, further studies should examine how farm hygiene and medication practices contribute to AMR in poultry.

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Authors' contributions

Chu Thi Thanh Huong and Nguyen Van Giap designed the study and methodology and contributed to the manuscript. Truong Lan Oanh and Nguyen Hoang Thinh conducted the experiments and analyzed the data. Truong Ha Thai supervised the study, provided methodological support, and wrote the manuscript. All authors revised and approved the final edition of the manuscript.

Availability of data and materials

All data are included in the submitted paper and will be available upon reasonable request from the corresponding author.

Competing interests

The authors have no conflicts of interest.

Ethical considerations

This article was originally written without copying from published articles and books. No AI was used in this study. The authors take full responsibility for the originality of the present study data before submission and after that, during the peer review process.

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