Research Article

Genotype–Phenotype Association of Antibiotic Resistance in *Escherichia coli* Isolates from Ducks

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Abstract | This study evaluated the phenotypic and genotypic characteristics of antibiotic resistance in *Escherichia coli* isolated from diseased duck flocks in Vinh Long province, Vietnam. A total of 40 *E. coli* strains were collected from clinically diseased ducks and tested using standard microbiological methods, antibiotic susceptibility testing, and PCR to identify five major resistance genes (*TetA, Sul1, TEM, SHV, aadA1)*. The isolates exhibited high resistance rates to Streptomycin (82.5%), Ampicillin (70%), and Trimethoprim/Sulfamethoxazole (62.5%), while lower resistance rates were observed for Doxycycline and Amoxicillin/Clavulanic acid (15% each). Multidrug resistance (MDR) was common, with 87.5% of strains resistant to at least two antibiotic classes and some strains resistant to six or seven antibiotics. Genotypic analysis revealed that TetA (72.5%) and Sul1 (70%) were the most common genes, followed by TEM (57.5%), aadA1 (45%) and SHV (22.5%). Complex resistance genotypes were common, with 67.5% of strains carrying two or more resistance genes and 22.5% of strains carrying five genes simultaneously. Strong genotype–phenotype correlations were observed for TetA–Tetracycline, TetA–Doxycycline, Sul1–Trimethoprim/Sulfamethoxazole and TEM–β-lactam pairs (75–80% similarity). However, the weak correlation between SHV–Ampicillin suggests that additional resistance mechanisms may be present. These findings demonstrate the high burden of multidrug-resistant *E. coli* in duck production systems and highlight the risk of transmission of resistance genes into the environment. Strengthening antibiotic stewardship, improving biosecurity and expanding genetic surveillance are needed to limit the spread of drug-resistant *E. coli*, protecting animal and public health.

Keywords | *Escherichia coli*, Ducks, Antimicrobial resistance, Multidrug resistance, Resistance genes, Genotype–phenotype correlation

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INTRODUCTION

Duck production is an important part of the livestock industry in Vietnam, especially in the Mekong Delta region, where free-range and semi-intensive farming models facilitate frequent contact between poultry, humans, and the environment. *Escherichia coli* (*E. coli*) is a common bacterium in the intestinal tract of poultry; however, some pathogenic strains (avian pathogenic *E. coli* – APEC) can cause severe systemic infections with symptoms such as diarrhea, sepsis, neurological signs, and high mortality, leading to major economic losses in poultry production (Watts and Wigley, 2024). The use and often overuse of antibiotics in poultry production for disease prevention and growth promotion has increased selection pressure, promoting the emergence of antibiotic-resistant (AMR) *E. coli* (Cuong *et al.*, 2018). As a result, multidrug-resistant (MDR) strains that are resistant to multiple classes of

antibiotics have become prevalent in poultry systems in Southeast Asia (Nhung *et al*., 2016).

Ducks, often raised in aquatic environments, act as important reservoirs and amplifiers of antibiotic-resistant bacteria. Their direct contact with water systems increases the risk of environmental dissemination of drug-resistant bacteria and antibiotic resistance genes (ARGs), potentially affecting other livestock, wildlife, and humans (Partridge *et al*., 2018). Previous studies have documented high rates of resistance of poultry *E. coli* to β-lactams, aminoglycosides, tetracyclines, and sulfonamides, often associated with mobile genetic elements such as plasmids, integrons, and transposons (Velhner *et al*., 2020; Olivia *et al*., 2023; Agusi *et al*., 2024). Important genes such as *TetA, Sul1, TEM, SHV*, and *aadA1* are commonly associated with tetracycline, sulfonamide, β-lactam, and aminoglycoside resistance (Gniadkowski *et al*., 1998; Madsen *et al*., 2000; Randall *et al*., 2004; Ahmed *et al*., 2009). However, there is still limited data on the phenotypic and genotypic antibiotic resistance characteristics of *E. coli* in duck populations in Vietnam. Therefore, this study was conducted to (1) characterize the antibiotic resistance phenotype of *E. coli* isolated from diseased ducks in Vinh Long province, (2) determine the prevalence of major antibiotic resistance genes, and (3) evaluate the correlation between genotype and phenotype. This integrated approach provides important insights into the dynamics of antibiotic resistance in duck production systems and highlights potential risks to animal and public health.

MATERIALS AND METHODS

Sample collection and bacterial isolation

This study was carefully designed as a cross-sectional epidemiological survey to assess the prevalence and characteristics of *E. coli* infections in duck flocks from 240 different populations distributed in Vinh Long province, Vietnam. This research method allows for a comprehensive assessment of the prevalence of the bacteria in different geographical regions and farming systems. *E. coli* is a common bacterium in the intestinal tract of ducks; however, some strains can become pathogenic, leading to severe outbreaks. Therefore, this study focused on pathogenic *E. coli* strains, which are the main agents responsible for the clinical manifestations in affected duck flocks. In each survey area, intestinal samples were collected from ducks with clinical manifestations suspected of *E. coli* infection, including severe diarrhea, white or greenish-white feces, convulsions, torticollis, swollen leg joints, and watery eyes (Watts and Wigley, 2024). Samples were collected under sterile conditions, placed in sterile polyethylene bags, and then stored in an insulated box with ice to maintain bacterial viability during transport to the laboratory. All samples were stored under cold chain conditions and processed within 24 hours of collection to limit bacterial variation and ensure the reliability of microbiological results. Of the total 240 duck flocks surveyed, 40 flocks were clinically diagnosed with *E. coli* infection. In each infected duck flock, specimens were collected from organs outside the intestinal tract with obvious gross lesions, including the liver, spleen, lungs, air sacs, and heart. These are the organs commonly affected in systemic *E. coli* infections. The collected samples were isolated and identified according to standard microbiological procedures at the Microbiology Laboratory - Tra Vinh University. The entire procedure was performed in compliance with ISO 7251:2005, which is the internationally recognized standard for the detection and determination of *E. coli* in food and animal samples.

Antibiotic resistance phenotype testing

The antibiotic resistance phenotype of *E. coli* strains was determined by the agar disk diffusion method (Kirby–Bauer method) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, document VET01S, 2015. This standard procedure was applied to assess the sensitivity of bacterial strains to a group of antibiotics commonly used in veterinary and human medicine. Pure *E. coli* colonies were taken from fresh cultures and then suspended in sterile physiological saline solution. The turbidity of each suspension was adjusted to the equivalent of 0.5 McFarland standard (corresponding to approximately 1.5×10^8 CFU/mL) to ensure uniformity between samples. With a sterile cotton swab, the bacterial suspension was spread evenly on the surface of Mueller–Hinton agar (MHA) to form a uniform bacterial layer. After the agar surface had dried for 3–5 minutes, the antibiotic discs were placed on the agar surface using sterile forceps, ensuring adequate spacing between the discs to avoid overlapping inhibition zones. A total of eight antibiotics were tested, representing different antimicrobial classes and mechanisms of action: Ampicillin (Am), Streptomycin (Sm), Doxycycline (Do), Cefuroxime (Cu), Gentamicin (Ge), Trimethoprim/Sulfamethoxazole (Bt), Amoxicillin/Clavulanic acid (Ac), and Tetracycline (Te). The inoculated plates were incubated at 37°C for 18–24 hours under aerobic conditions. Following incubation, the diameters of the inhibition zones surrounding each antibiotic disk were measured in millimeters using a digital caliper. The results were interpreted according to the zone diameter interpretive standards established by CLSI (2015), classifying each isolate as Resistant (R), Intermediate (I), or Sensitive (S).

Identification of antibiotic resistance genes in bacterial isolates

A total of 40 *E. coli* strains were subjected to molecular screening to identify the presence of specific genes associated with antibiotic resistance. The detection of these

genes was performed using polymerase chain reaction (PCR) techniques, which allows amplification of target DNA fragments related to resistance determinants. The PCR protocol used in this study was developed based on previously published methods, ensuring consistency in thermal cycling parameters, including the initial denaturation, annealing and extension stages. The annealing temperature of each primer pair was optimized based on reference materials to ensure high specificity and amplification efficiency.

Each bacterial strain was tested separately for each target gene to avoid cross-contamination and ensure accurate identification results. The resistance genes analyzed in this study include TetA, which confers resistance to tetracycline antibiotics (Randall *et al*., 2004); Sul1, which confers resistance to sulfonamides (Ahmed *et al*., 2009); TEM and SHV, both of which are involved in the production of β-lactamases that confer resistance to β-lactam antibiotics (Gniadkowski *et al*., 1998; Ferreira *et al*., 2011); and aadA1, which confers resistance to aminoglycosides (Madsen *et al*., 2000).

After amplification, PCR products were subjected to agarose gel electrophoresis to visualize and determine the presence or absence of specific resistance genes. The resulting DNA bands were compared with molecular size scales and positive control strains to verify the accuracy of the amplification reaction. The presence or absence of each resistance gene was recorded for each *E. coli* strain, providing valuable molecular evidence of the distribution of antibiotic resistance determinants in the examined bacterial population.

Assessment of the correlation between genotypic and phenotypic characteristics

To comprehensively evaluate the relationship between antibiotic resistance at the genotypic and phenotypic levels, the raw data were systematically reorganized to ensure scientific accuracy and reasonable consistency with the processed summary tables. Each *E. coli* strain was constructed as a separate dataset, consisting of two main components: (1) a phenotypic antibiotic resistance profile, expressed in three levels - resistant (R), intermediate (I), and susceptible (S) - to the eight tested antibiotics; and (2) a genotypic profile, expressed as positive (+) or negative (–) for the five target antibiotic resistance genes. For the purpose of analysis and to better understand the overall antibiotic resistance potential of the bacterial population, strains showing intermediate (I) resistance were reclassified as resistant (R). This binary classification (resistant/susceptible) allows for a more precise comparison between phenotype and genotype, thus allowing for a more rigorous statistical assessment of the correlation between the two. The correspondence between phenotypic and genotypic results was assessed by comparing the pattern of antibiotic susceptibility (susceptible vs. resistant) with the presence or absence of the corresponding resistance genes. This comparison helps to clarify how genetic factors manifest the observed antibiotic resistance behavior in *E. coli*.

The expected associations between specific genes and the corresponding antibiotic class were considered as follows: TEM gene- associated with resistance to β-lactam antibiotics, including Ampicillin (Am), Amoxicillin–Clavulanic acid (Ac) and Cephalexin (Cu); Gene tetA - associated with resistance to tetracycline antibiotics, including Tetracycline (Te) and Doxycycline (Do); Gene sul1- associated with resistance to the Trimethoprim/Sulfamethoxazole combination (Bt); Gene aadA1- associated with resistance to aminoglycoside antibiotics, such as Streptomycin (Sm) and Gentamicin (Ge). This integrative evaluation, based on the methodology proposed by Feldgarden *et al*. (2019), provided a comprehensive understanding of the genetic basis underlying the observed phenotypic antibiotic resistance patterns in the studied *E. coli* isolates.

Statistical analysis

Statistical analyses were performed to evaluate differences in antibiotic resistance proportions among *E. coli* isolates and to assess the association between phenotypic resistance and corresponding resistance genes. Differences in resistance frequencies among antibiotics and in the distribution of resistance genes were analysed using the Chi-square (χ^2) test. When expected cell counts were small, Fisher's exact test was applied to ensure statistical validity. The relationship between phenotypic resistance (resistant vs. susceptible) and genotypic characteristics (presence vs. absence of resistance genes) was assessed using Fisher's exact test, which is appropriate for categorical data and small sample sizes. For genotype–phenotype comparisons, isolates classified as having intermediate susceptibility were grouped with resistant isolates to allow binary analysis and improve interpretability. All statistical analyses were conducted using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA). A *P*-value of <0.05 was considered statistically significant. Reported *P*-values indicate the strength of association between resistance phenotypes and underlying genetic determinants and were interpreted to elucidate the relationship between molecular resistance mechanisms and phenotypic expression in the tested *E. coli* isolates.

RESULTS AND DISCUSSION

Phenotypic antibiotic resistance profiles

The phenotypic antibiotic resistance profiles of the 40 isolated *E. coli* strains are presented in detail in Table 1.

Table 1: The phenotypic antibiotic resistance profiles of *E. coli* isolates against individual antibiotics (n=40).

Antibiotics	Resistant		Intermediate		Susceptible	
	Number of bacterial strains	Ratio (%)	Number of bacterial strains	Ratio (%)	Number of bacterial strains	Ratio (%)
Ampicillin (Am)	28.00	70.00	5.00	12.50	7.00	17.50
Streptomycin (Sm)	33.00	82.50	2.00	5.00	5.00	12.50
Doxycycline (Do)	6.00	15.00	17.00	42.50	17.00	42.50
Cefuroxime (Cu)	22.00	55.00	7.00	17.50	11.00	27.50
Genetamicin (Ge)	14.00	35.00	7.00	17.50	19.00	47.50
Trimethoprim/sulfamethoxazole (Bt)	25.00	62.50	4.00	10.00	11.00	27.50
Amoxicilin/clavulanic acid (Ac)	6.00	15.00	11.00	27.50	23.00	57.50
Tetracycline (Te)	13.00	32.50	14.00	35.00	13.00	32.50

All 40 *E. coli* isolates from ducks exhibited variable resistance patterns across the eight antibiotics tested, representing multiple antimicrobial classes. The highest resistance was recorded against Streptomycin (82.5%), followed by Ampicillin (70.0%), and Trimethoprim/Sulfamethoxazole (62.5%). Moderate resistance levels were observed for Cefuroxime (55.0%), whereas lower resistance rates were detected for Gentamicin (35.0%), Tetracycline (32.5%), Amoxicillin/Clavulanic acid (15.0%), and Doxycycline (15.0%). The proportion of isolates showing intermediate resistance ranged from 5.0% (Streptomycin) to 42.5% (Doxycycline), indicating varying degrees of antimicrobial tolerance. The highest susceptibility rates were observed for Amoxicillin/Clavulanic acid (57.5%) and Doxycycline (42.5%), suggesting these antibiotics may still retain partial therapeutic efficacy against *E. coli* of duck origin.

The high resistance rates to Ampicillin and Streptomycin are consistent with previous studies that have reported widespread resistance to β-lactam and aminoglycoside antibiotics in *E. coli* isolates from poultry (Nhung *et al.*, 2016; Agusi *et al.*, 2024). This high resistance level may reflect the long-term and widespread use of these antibiotics in duck and poultry production for disease prevention and growth promotion purposes (Cuong *et al.*, 2018). The resistance levels to Trimethoprim/Sulfamethoxazole (62.5%) and Cefuroxime (55.0%) are also consistent with global reports of sulfonamide and cephalosporin resistance in *E. coli* from poultry (Kerta *et al.*, 2024). This suggests the potential for the spread of sul1 and β-lactamase (TEM/SHV) genes in duck-associated *E. coli* populations. Notably, sulfonamide and β-lactam resistance genes are often located on mobile genetic elements such as plasmids and integrons, which facilitate horizontal gene transfer between commensal and pathogenic bacterial strains (Olivia *et al.*, 2023). The moderate resistance levels to Gentamicin (35.0%) and Tetracycline (32.5%) remain a concern, due to the widespread occurrence of tetA and aadA1 genes in poultry *E. coli* strains (Nawaz *et al.*, 2025). Previous studies have demonstrated that *E. coli* from ducks and chickens often carry multiple resistance genes, contributing to the formation of multidrug-resistant (MDR) phenotypes (Velhner *et al.*, 2020; Elmorsy *et al.*, 2025). Although the current study did not include fluoroquinolones or macrolides, the observed resistance patterns suggest significant selective pressure on commensal *E. coli* strains in the duck farming environment. Encouragingly, Amoxicillin/Clavulanic acid (15.0% resistant; 57.5% susceptible) and Doxycycline (15.0% resistant; 42.5% susceptible) exhibited lower resistance rates than the other antibiotics. This result is consistent with previous reports that drug combinations containing β-lactamase inhibitors retained partial efficacy against *E. coli* strains from poultry (Harris *et al.*, 2015; Kadry *et al.*, 2022). The efficacy of these antibiotics may be due to the β-lactamase enzyme inhibitory effect of clavulanic acid, which restored *E. coli* sensitivity to amoxicillin. However, caution is needed overuse of even these relatively effective antibiotics may promote the emergence of extended-spectrum β-lactamase producing or tetracycline-resistant strains. The findings of this study demonstrate high levels of phenotypic resistance in duck *E. coli* isolates, with resistance patterns suggesting the potential for multidrug resistance (MDR). This level of resistance has important implications for both veterinary treatment efficacy and public health safety, as resistant bacteria can spread through the food chain or the environment (Nhung *et al.*, 2016; Cuong *et al.*, 2018). Therefore, these results emphasize the urgent need for appropriate antibiotic stewardship programs in duck farming, enhanced drug use controls, and continuous monitoring of resistance trends. In addition, the adoption of alternative practices such as the use of herbal essential oils, probiotics, and improved farm biosecurity may help to reduce the spread of antibiotic resistance in poultry farming environments.

Phenotypic patterns of multidrug antibiotic resistance

The phenotypic patterns of multidrug antibiotic resistance (MDR) in *E. coli* isolates are summarized in Table 2.

Table 2: Phenotypic patterns of multidrug antibiotic resistance in *E. coli* isolates (n=40).

Number of antibiotics	Number of multidrug-resistant patterns	Multidrug-resistant phenotype	Number of resistant bacterial strains	Ratio (%)	Total number of resistant strains	Total ratio (%)
2.00	4.00	Sm-Bt	1.00	12.50	8.00	20.00
		Am-Sm	4.00	50.00		
		Cu-Bt	2.00	25.00		
		Sm-Ge	1.00	12.50		
3.00	8.00	Am-Sm-Bt	2.00	22.22	9.00	22.50
		Am-Sm-Ge	1.00	11.11		
		Am-Sm-Te	1.00	11.11		
		Am-Sm-Cu	1.00	11.11		
		Am-Cu-Bt	1.00	11.11		
		Am-Cu-Te	1.00	11.11		
		Cu-Ge-Bt	1.00	11.11		
		Sm-Cu-Bt	1.00	11.11		
4.00	4.00	Am-Sm-Cu-Bt	3.00	50.00	6.00	15.00
		Sm-Cu-Bt-Te	2.00	16.67		
		Am-Sm-Ge-Bt	1.00	16.67		
5.00	2.00	Am-Sm-Cu-Ge-Bt	1.00	50.00	2.00	5.00
		Am-Sm-Do-Cu-Ge	1.00	50.00		
6.00	4.00	Am-Sm-Ge-Bt-Ac-Te	1.00	20.00	5.00	12.50
		Am-Sm-Do-Ge-Bt-Te	1.00	20.00		
		Am-Sm-Do-Cu-Bt-Te	1.00	20.00		
		Am-Sm-Cu-Bt-Ac-Te	2.00	40.00		
7.00	3.00	Am-Sm-Cu-Ge-Bt-Ac-Te	2.00	40.00	5.00	12.50
		Am-Sm-Do-Cu-Ge-Bt-Te	2.00	40.00		
		Am-Sm-Do-Cu-Ge-Bt-Ac	1.00	20.00		

Am: Ampicillin; Sm: Streptomycin; Do: Doxycycline; Cu: Cefuroxime; Ge: Genetamicin; Bt: Trimethoprim/sulfamethoxazole; Ac: Amoxicilin/clavulanic acid; Te: Tetracycline

Of the 40 strains tested, 35 (87.5%) exhibited multidrug resistance, defined as resistance to at least two different classes of antibiotics. A total of 25 different MDR phenotypes were identified, ranging from resistance to two to seven antibiotics. Strains resistant to two antibiotics accounted for 20%, while those resistant to three, four, five, six, and seven antibiotics accounted for 22.5%, 15%, 5%, 12.5%, and 12.5%, respectively. The most common dual resistance patterns included Am–Sm (Ampicillin-Streptomycin, 50%), followed by Cu–Bt (Cefuroxime–Trimethoprim/ Sulfamethoxazole, 25%) and Sm–Bt (Streptomycin–Trimethoprim/Sulfamethoxazole, 12.5%). Among the triple resistance patterns, Am–Sm–Bt (22.2%) was the most common. Among the quadruple resistance patterns, Am–Sm–Cu–Bt (50%) appeared with the highest frequency. Among the pentadrug-resistant strains, two patterns, Am–Sm–Cu–Ge–Bt and Am–Sm–Do–Cu–Ge, were recorded with similar frequency (50%). Notably, strains with very high levels of resistance were also detected: five strains (12.5%) were resistant to six antibiotics, and another five strains (12.5%) were resistant to seven antibiotics, with common extended resistance phenotypes such as Am–Sm–Do–Cu–Ge–Bt–Te and Am–Sm–Do–Cu–Ge–Bt–Ac.

The high diversity and prevalence of multidrug resistance (MDR) phenotypes observed in *E. coli* strains isolated from ducks indicate the alarming prevalence of antibiotic resistance in the avian microbial community. The predominance of resistance patterns related to Ampicillin (Am) and Streptomycin (Sm) suggests persistent selective pressure due to the overuse of β-lactam and aminoglycoside antibiotics in duck and poultry farming. Similar resistance patterns have also been observed in several studies in Asia and Europe, confirming the global nature of this problem (Nhung *et al*., 2016; Cuong *et al*., 2018; Agusi *et al*., 2024).

The emergence of strains resistant to six or seven antibiotics including combinations of β-lactams, aminoglycosides, cephalosporins, tetracyclines, and sulfonamides - suggests the emergence of extensively drug-resistant (XDR) *E. coli* strains. These phenotypes have also been documented in Hungary, where *E. coli* isolated from ducks showed 79.6% MDR and 28.7% XDR resistance, with high levels of

resistance to Neomycin (88.9%), Florfenicol (58.3%), and Amoxicillin (46.3%) (Kerek and Szabó, 2025). Similarity in resistance patterns across geographically distant regions suggests the global circulation of resistance genetic elements, possibly mediated by mobile genetic elements such as plasmids, integrons, and transposons (Olivia *et al*., 2023). The coexistence of multiple resistance mechanisms within the same bacterial strain as demonstrated by complex phenotypes such as Am–Sm–Do–Cu–Ge–Bt–Te is of particular concern. These strains are difficult to control in treatment, as effective antibiotic options are very limited. Their persistence also raises concerns about the potential for horizontal transfer of resistance genes from commensal *E. coli* in ducks to strains that cause disease in humans or other animals (Velhner *et al*., 2020; Elmorsy *et al*., 2025; Nawaz *et al*., 2025).

From a one health perspective, the high prevalence of MDR and XDR *E. coli* in duck populations represents a significant threat to both animal and public health. Poultry-derived *E. coli* have been shown to share resistance genes and mobile genetic elements with human clinical isolates, indicating potential zoonotic transmission pathways through the food chain, contaminated water, or occupational exposure (Kerta *et al*., 2024). The persistence of such highly resistant strains in duck farming environments may therefore contribute to the broader environmental resistome and undermine the effectiveness of critically important antimicrobials.

These findings underscore the urgent need to strengthen antimicrobial stewardship in duck production systems. Excessive or inappropriate use of antibiotics for disease prevention or growth promotion is likely a key driver of MDR and XDR emergence. Implementation of evidence-based antimicrobial stewardship programs focusing on veterinary prescription, susceptibility guided therapy, and restriction of over the counters antibiotic use is essential to reduce selective pressure. In parallel, improvements in farm biosecurity, hygiene management, and waste handling can reduce infection pressure and limit environmental dissemination of resistant bacteria. Importantly, alternative strategies such as the use of probiotics, herbal essential oils, vaccination, and improved animal husbandry practices should be promoted as sustainable approaches to reduce antibiotic dependence and mitigate the spread of MDR and XDR *E. coli* in poultry farming environments.

Occurrence frequency of antibiotic resistance genes

The frequency of antibiotic resistance genes detected in duck *E. coli* isolates is shown in Table 3. Among the five resistance genes examined, TetA (72.5%) and Sul1 (70.0%) were the most commonly detected, followed by TEM (57.5%), aadA1 (45.0%) and SHV (22.5%). The high detection rate of TetA gene was closely correlated with the resistance phenotype to Tetracycline, with 32.5% of strains showing resistance and 35% showing intermediate resistance. Similarly, the frequency of Sul1 gene also correlated with the phenotypic resistance rate to Trimethoprim/Sulfamethoxazole, with 62.5% of strains showing resistance. This close association suggests that TetA and Sul1 are the main genetic factors conferring resistance to tetracycline and sulfonamide antibiotics, respectively, in the studied *E. coli* populations. The TEM gene, detected in 57.5% of the strains, and the aadA1 gene, detected in 45%, represent the two main genetic mechanisms conferring resistance to β-lactams and aminoglycosides. Specifically, TEM encodes β-lactamase enzymes, capable of hydrolyzing Ampicillin and other β-lactam antibiotics such as Cefuroxime and Amoxicillin/Clavulanic acid (Jacoby and Carreras, 1990). In parallel, aadA1 encodes the aminoglycoside adenyltransferase enzyme, responsible for inactivating Streptomycin and Gentamicin (Madsen *et al*., 2000). The high frequency of these genes is consistent with the observed phenotypic results, especially the high resistance rates to Ampicillin (70%) and Streptomycin (82.5%). The SHV gene was detected at a lower frequency (22.5%), but its presence is still noteworthy because SHV β-lactamases are often associated with extended-spectrum β-lactamase (ESBL) activity, which can confer resistance to many important β-lactam antibiotics.

Table 3: Occurrence frequency of antibiotic resistance genes identified in *E. coli* isolates (n=40).

Antibiotic resistance genes	Occurrence frequency	Ratio (%)
TetA	29.00	72.50
Sul1	28.00	70.00
TEM	23.00	57.50
aadA1	18.00	45.00
SHV	9.00	22.50

Genotypic profiles of multidrug-resistant

Genotypic analysis showed that 27 out of 40 (67.5%) *E. coli* strains isolated from ducks carried two or more antibiotic resistance genes (ARGs), indicating a high prevalence of multiresistant genotypes in the surveyed population (Table 4). Four major genotype combinations were detected. The 5-gene combination (TEM + SHV + TetA + Sul1 + aadA1) was the most common, recorded in 9 strains (22.5%), followed by the 4-gene combination (TetA + Sul1 + TEM + SHV) in 7 strains (17.5%), the 2-gene combination (TetA + Sul1) in 6 strains (15%), and the 3-gene combination (TetA + Sul1 + TEM) in 5 strains (12.5%). Overall, β-lactamase genes (TEM, SHV), tetracycline resistance genes (TetA), sulfonamide

resistance genes (Sul1), and aminoglycoside resistance genes (aadA1) were frequently co-expressed at high frequencies, suggesting the existence of multiple resistance mechanisms in the same bacterial genome. These results demonstrate that a significant proportion of *E. coli* strains carry complex multidrug resistance genotypes, covering many different antibiotic classes.

Table 4: Results of the genotypic survey of multidrug antibiotic resistance in *E. coli* (n = 40).

Number of resistance genes	Resistance genotype pattern	Number of positive genotypes	Ratio (%)
2.00	TetA + Sul1	6.00	15.00
3.00	TetA+Sul1+TEM	5.00	12.50
4.00	TetA+Sul1+TEM+SHV	7.00	17.50
5.00	TEM + SHV + TetA + Sul1 + aadA1	9.00	22.50
Total		27.00	67.50

The observation that more than two-thirds of *E. coli* strains carried two or more antibiotic resistance genes (ARGs) suggests that the genetic architecture of antibiotic resistance in duck-associated bacterial populations is complex. The repeated co-occurrence of TetA and Sul1 genes the most common gene combination suggests that these genes may be located on the same mobile genetic element such as a plasmid or transposon, allowing them to be transferred simultaneously between bacteria. This linkage facilitates the phenomenon of "co-selection" of antibiotic resistance, whereby the use of one antibiotic (e.g., tetracycline) may inadvertently maintain resistance to other antibiotics (e.g., ampicillin or trimethoprim/sulfamethoxazole) if their corresponding genes are genetically related (Pouwels *et al*., 2019). This co-selection mechanism plays a key role in the maintenance and spread of multiple antibiotic resistance (MDR), even when some antibiotics are not used directly in the environment.

The detection of bacterial strains carrying up to five resistance genes (TEM + SHV + TetA + Sul1 + aadA1) suggests the existence of genotypes with high resistance and a broad spectrum of resistance. These strains are likely to possess integrons or conjugative plasmids, which facilitate horizontal gene transfer between bacteria, thereby promoting the evolution of a multidrug-resistant (MDR) phenotype (Cabot *et al*., 2012).

The coexistence of resistance genes to β-lactams, tetracyclines, sulfonamides, and aminoglycosides reflects strong selective pressure due to the frequent and sometimes uncontrolled use of antibiotics in duck farming. Similar patterns of multidrug resistance have also been observed in *E. coli* strains isolated from poultry and pigs in Vietnam as well as in many other Asian countries, suggesting that the integrated livestock environment is becoming a hotbed for the exchange of antibiotic resistance genes (ARGs) (Nhung *et al*., 2016).

These findings also suggest that *E. coli* strains with complex resistance genotypes possess a strong evolutionary advantage in antibiotic-rich environments. These strains can persist for long periods, spread through horizontal gene transfer, and act as reservoirs of resistance genes (ARGs) that can potentially be transmitted to humans through the food chain or environmental contamination (Partridge *et al*., 2018). The accumulation of multiple resistance genes in the same bacterial strain poses a major public health concern. Ducks, which are raised in aquatic environments, can act as vectors for the spread of bacteria and resistance genes into water systems, where they can interact with bacteria that cause human and environmental diseases. The discovery of *E. coli* strains carrying multiple antibiotic resistance genes underscores the need for appropriate antimicrobial stewardship programs and regular genetic surveillance in duck production systems. To prevent the spread of these resistance genes, evidence-based antibiotic use restrictions, improved farm hygiene, and enhanced biosecurity measures are needed to reduce the selection pressure leading to multiple resistance. Without timely intervention, such resistance gene combinations could continue to spread, seriously affecting the effectiveness of antibiotic treatment in both veterinary and human medicine.

Correlation between phenotypic and genotypic antimicrobial resistance

Correlation analysis between key antibiotic resistance genes and their corresponding resistance phenotypes showed high concordance in most of the gene–antibiotic pairs (Table 5). The TetA–Tetracycline (Te) pair showed the strongest concordance, with 95% concordance ($P<0.001$), followed by Sul1–Trimethoprim/Sulfamethoxazole (Bt) with 97.5% concordance ($P<0.001$). Similarly, the TetA–Doxycycline (Do) pair also showed high concordance, reaching 85% ($P<0.001$). For the β-lactamase genes, the TEM–Ampicillin (Am), TEM–Cephalexin (Cu), and TEM–Amoxicillin/Clavulanic acid (AC) pairs had 75%, 75%, and 80% concordance, respectively (all $P\leq0.002$). The SHV gene showed a moderate degree of concordance, with SHV–Cephalexin and SHV–Amoxicillin/Clavulanic acid having 62.5% and 67.5% concordance, respectively ($P<0.05$). However, the SHV–Ampicillin pair showed a low concordance (57.5%) and was not statistically significant ($P=0.072$), suggesting a weak or unreliable association between SHV and Ampicillin resistance. Overall, the results showed that most gene–antibiotic pairs were significantly correlated, except for the SHV–Ampicillin pair, which did not reach statistical significance.

Table 5: Correlation between genotype and phenotype for key antibiotic–gene pairs (n=40).

Correlation pair	Number of matching strains	Matching percentage (%)	Number of non-matching strains	Non-matching percentage (%)	P value
TetA-Do	34.00	85.00	6.00	15.00	<0.001
TetA-Te	38.00	95.00	2.00	5.00	<0.001
Sul-Bt	39.00	97.50	1.00	2.50	<0.001
SHV-Am	23.00	57.50	17.00	42.50	0.072
SHV-Cu	25.00	62.50	15.00	37.50	0.036
SHV-Ac	27.00	67.50	13.00	32.50	0.031
TEM-Am	30.00	75.00	10.00	25.00	0.001
TEM-Cu	30.00	75.00	10.00	25.00	0.002
TEM-AC	32.00	80.00	8.00	20.00	<0.001

The high degree of genotype-phenotype similarity for the TetA–Tetracycline, TetA–Doxycycline and Sul1–Trimethoprim/Sulfamethoxazole pairs confirms the important functional role of these genes in mediating tetracycline and sulfonamide resistance in *E. coli* isolated from ducks. TetA, encoding an efflux pump, is widely recognized as the most common tetracycline resistance mechanism in the Enterobacteriaceae family (Belaynehe *et al*., 2018), while Sul1 is the well-characterized sulfonamide resistance determinant (Ola, 2000). The high level of gene–phenotype similarity observed in this study is consistent with previous reports in poultry and waterfowl, where TetA and Sul1 are frequently co-expressed and strongly predict the resistance phenotype (Jahantigh *et al*., 2020). This consistency reinforces the value of the above genes as reliable molecular markers for monitoring antibiotic resistance in duck production systems. A similar strong association was also observed for the TEM gene, which showed moderate to high levels of similarity (75–80%) with Ampicillin, Cephalexin, and Amoxicillin/Clavulanic acid resistance. TEM group β-lactamases are among the earliest and most common enzymes involved in penicillin resistance in *E. coli* (Bush and Bradford, 2016). The level of concordance observed in this study mirrors results from *E. coli* associated with livestock production, where TEM remains the predominant β-lactamase despite the increasing prevalence of CTX-M enzymes (Drugea *et al*., 2025).

While these findings demonstrate strong genotype–phenotype concordance for several resistance determinants, notable discrepancies were also identified and warrant further discussion. In contrast to TEM, the correlation between SHV and Ampicillin resistance was weak and not statistically significant, suggesting that SHV is not a reliable predictor of Ampicillin resistance in this *E. coli* population. Several factors may explain this observation. First, SHV variants appear to be less prevalent and less functionally dominant in avian *E. coli*, where TEM and CTX-M families are more commonly associated with β-lactam resistance (Liu *et al*., 2020). Second, Ampicillin resistance may be mediated by additional resistance determinants not examined in the present study, including other plasmid-encoded β-lactamase genes such as *CTX-M*, *OXA*, or *PSE*, or by chromosomal mechanisms such as alterations in penicillin-binding proteins, reduced outer membrane permeability, or enhanced efflux pump activity (Tang *et al*., 2014). The presence of nonfunctional or weakly expressed SHV variants may also explain cases in which the gene was detected without corresponding phenotypic resistance.

Discrepancies between genotype and phenotype, including both "gene present but phenotypically susceptible" and "gene absent but phenotypically resistant" profiles, underscore the complex and multifactorial nature of antimicrobial resistance. Similar inconsistencies have been widely reported in Enterobacteriaceae and may result from gene silencing, regulatory mutations affecting gene expression, compensatory chromosomal adaptations, or resistance mechanisms not captured by targeted PCR screening (Partridge, 2015; Rogers *et al*., 2021). These findings highlight the limitations of relying solely on selected resistance genes for resistance prediction and emphasize the importance of broader molecular surveillance approaches. Future studies employing expanded resistance gene panels or whole-genome sequencing would provide a more comprehensive understanding of β-lactam resistance mechanisms in duck-associated *E. coli* populations (Feldgarden *et al*., 2019).

CONCLUSIONS

This study showed a very high prevalence of antibiotic resistance in *E. coli* strains isolated from ducks, especially to Streptomycin, Ampicillin and Trimethoprim/Sulfamethoxazole. Multidrug resistance was common, and some strains carried complex resistance genotypes with up to five resistance genes. Strong genotype-phenotype correlations were observed for TetA, Sul1 and

TEM, confirming their important role in tetracycline, sulfonamide and β-lactam resistance. However, the discordance between genotype and phenotype suggests that other resistance mechanisms are still unexplored. These findings suggest that duck production systems may be an important reservoir of multidrug-resistant *E. coli*, posing a risk to both animal and public health.

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NOVELTY STATEMENT

This study presents the first integrated assessment of antibiotic resistance phenotypes and genotypes in *E. coli* from diseased ducks in Vinh Long Province, revealing complex resistance patterns including strains carrying up to five resistance genes. The strong genotype–phenotype correlations for TetA, Sul1, and TEM alongside notable SHV discrepancies uncover previously unrecognized resistance mechanisms in duck-associated *E. coli*. These findings offer novel insights into antimicrobial resistance dynamics in waterfowl and provide a critical foundation for surveillance and antibiotic management in duck production systems.

AUTHOR'S CONSTRIBUTION

NVV and LVD conceived and designed the experiments. NVV and HMH performed the experiments and analysed the data. LVD contributed materials. NVV, NTL and HMH wrote the paper. All authors reviewed and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors confirm that ChatGPT was used solely to improve the manuscript's English grammar. All content was carefully reviewed, revised, and validated by the authors to guarantee accuracy and originality.

Conflict of interest

The authors have declared no conflict of interest.

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