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Antibiotic Resistance of *Escherichia coli* Isolated from Ducks Suspected of Colibacillosis in Vietnam

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ABSTRACT

The extensive application of antibiotics within the livestock sector has led to a rise in bacterial resistance, especially against *Escherichia coli* (*E. coli*), complicating the treatment of bacterial infections in poultry. The present study aimed to determine the antibiotic resistance of the *E. coli* isolated from Cherry Valley ducks exhibiting signs of colibacillosis in northern Vietnam. From March to May 2025, 21 Cherry Valley duck flocks raised on 15 duck farms in Ha Nam Province, Vietnam, experienced disease outbreaks characterized by clinical signs such as anorexia, reduced mobility, abnormal movement, and respiratory distress, including sneezing, gasping, and nasal discharge, which were suspected of being caused by *E. coli* infection. A total of 63 ducks suspected of colibacillosis were necropsied for sampling and pathological observation. Heart, liver, and lung samples were collected simultaneously from all ducks for *E. coli* isolation. The isolated *E. coli* strains were examined for antibiotic susceptibility by the agar diffusion method. The *E. coli* strains demonstrated the highest susceptibility to levofloxacin (82.9%), followed by norfloxacin (78.0%), gentamicin (73.2%), and colistin (70.7%). The highest resistance rates were observed for ampicillin (75.6%) and tetracycline (73.2%), whereas resistance to ciprofloxacin, streptomycin, and doxycycline ranged from 31.7% to 48.8%. Of the 41 *E. coli* strains, 82.9% were resistant to at least one antibiotic; these strains exhibited 17 distinct antibiotic resistance patterns. Additionally, 63.4% isolated strains were identified as multi-drug resistant (MDR). The current results emphasized the high resistance of *E. coli* strains isolated from ducks to multiple antibiotics.

Keywords: Antibiotic resistance, Duck, Colibacillosis, Diffusion method

INTRODUCTION

Escherichia coli (E. coli) is a widely recognized bacterium within the *Enterobacteriaceae* family, commonly found in the intestinal tracts of poultry and other animal species (Darwish et al., 2015; Luo et al., 2023). Nevertheless, certain strains of *E. coli* carry virulence-associated genes that enable them to induce diarrhea and intestinal hemorrhage in poultry (Darwish et al., 2015).

As in other Asian countries, duck farming is highly popular in Vietnam. However, during the breeding process, some infectious diseases in ducks, such as pasterellosis, salmonellosis, riemerellosis, and colibacillosis, have been recorded in the Mekong Delta (Thuan and Khai, 2018; Thu et al., 2019) and northern Vietnam (Vui and Tiep, 2016). Ducks can be infected with *E. coli* at different ages, but the disease typically manifests in ducks between 2 and 6 weeks old. Infected ducks have a high mortality rate, reaching up to 43.5% (Roshdy et al., 2012). Since 1961, the pathogenic role of *E. coli* in poultry has been recognized; however, the disease has not gained much attention in many countries so far (Dziva and Stevens, 2008). Prevention and treatment of bacterial diseases in poultry still rely on using antibiotics. However, the frequent use of antibiotics results in continuous exposure of the intestinal microbiota, thereby developing antibiotic resistance (Kissinga et al., 2018; Yanestria et al., 2022).

Antibiotic resistance is a major public health threat and hinders the treatment of bacterial diseases in poultry. Nevertheless, the management of colibacillosis depends on antibiotics globally (Azam et al., 2019). The widespread use of antibiotics in animal livestock has increased the antibiotic resistance in pathogenic bacteria, including *E. coli*, and can cause public health problems (Magiorakos et al., 2013; Luo et al., 2023). Therefore, the present study aimed to examine the antibiotic resistance of *E. coli* strains isolated from ducks suspected of colibacillosis raised on duck farms in Ha Nam, Vietnam.

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MATERIALS AND METHODS

Ethics approval

The present study was conducted by collecting samples in accordance with the guidelines outlined in the Helsinki declaration and the animal welfare and safety procedures of the Committee on Animal Research and Ethics (CARE), Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam (Approval No. CARE-2025/02).

Sampling

The present study was conducted on 21 commercially-raised Cherry Valley duck flocks, aged 3-6 weeks, that experienced disease outbreaks on 15 farms in Ha Nam Province, Vietnam, from March to May 2025. Affected ducks exhibited clinical signs suggestive of colibacillosis, including anorexia, moodiness, reduced mobility, impaired movement, bloody diarrhoea, and lesions such as an inflamed pericardium, swollen liver, and pneumonia (Punnoose et al., 2021). Sixty-three ducks suspected of colibacillosis underwent postmortem examination and necropsy for sampling and pathological observations in accordance with the QCVN 01-83:2011/BNNPTNT guidelines of the Ministry of Agriculture and Rural Development (MARD, 2011). Several samples of heart, liver, and lung were collected simultaneously from each of sixty-three affected ducks. The samples were placed in sterile bags, put in a dry-ice container, and transported to the laboratory of the Faculty of Veterinary Medicine, Vietnam National University of Agriculture, for further analysis within 24 hours.

Escherichia coli isolation

Isolation of *E. coli* was carried out according to the protocol of Roshdy et al. (2012) with some modifications. Each sample was streaked onto MacConkey agar (Merck, Germany) and incubated at 37°C for 24 hours. Subsequently, the pink colonies from MacConkey agar were subculture onto eosin methylene blue agar (EMB; Merck, Germany) and incubated at 37°C for 24 hours. Then, a typical colony exhibiting the green metallic sheen on EMB agar was selected, streaked onto a triple sugar iron (TSI; Merck, Germany) agar tube, and incubated at 37°C for 24 hours. Colonies exhibiting a typical TSI profile, including glucose and lactose fermentation with gas production and no H2S, were identified as *E. coli* by Gram staining and biochemical tests, such as citrate utilization, indole production, the methyl red, and Voges-Proskauer reactions. All isolates were kept in brain heart infusion broth (BHI; Merck, Germany) supplemented with 50% glycerol at-20°C for subsequent experiments.

Antibiotic susceptibility testing

A total of 41 *E. coli* strains from positive ducks were selected for antibiotic susceptibility analysis, in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2024). Antibiotic susceptibility was determined using the agar disc diffusion method on Mueller-Hinton agar (MHA; Merck, Germany), as described by Bauer et al. (1966). Twelve different antibiotics (Oxoid, UK) were tested, including ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), ciprofloxacin (5µg), colistin (10µg), doxycycline (30µg), florfenicol (30µg), gentamicin (10µg), kanamycin (30µg), levofloxacin (5µg), norfloxacin (10µg), streptomycin (10µg), and tetracycline (30µg). The *E. coli* ATCC 25922 strain was used for quality control. One *E. coli* strain was classified as a resistant isolate following the confirmation of resistance to at least one tested antibiotic. It was further designated as a multidrug-resistant (MDR) strain upon confirmation of resistance to at least one antibiotic from three different antibiotic classes (Magiorakos et al., 2012).

Data analysis

Isolation and antibiotic resistance rates of *E. coli* were compared using the Chi-Square test in EpiInfo version 7.2.3.1. The P-value was calculated and considered significantly less than 5% ($p < 0.05$).

RESULTS

The *E. coli* isolation rates for each type of sample are shown in Table 1. The *E. coli* was detected in 41 of the 63 duck samples (65.1%). Isolation rates ranged from 44.4% to 65.1% across different types of samples. The highest rate of *E. coli* isolation was observed in liver samples (65.1%), followed by heart (58.7%) and lung (44.4%).

The antibiotic susceptibility of the 41 *E. coli*-positive strains are presented in Table 2. The selected isolates demonstrated the highest susceptibility to levofloxacin (82.9%), followed by norfloxacin (78.0%), gentamicin (73.2%), and colistin (70.7%). Higher susceptibility (≥ 50%) was observed for florfenicol (68.3%), amoxicillin-clavulanate acid (65.9%), and kanamycin (61.0%). Meanwhile, 75.6% and 73.2% of the isolates were resistant to ampicillin and tetracycline, respectively. Resistance to ciprofloxacin, streptomycin, and doxycycline was lower, ranging from 31.7% to

48.8%. The antibiotic resistance patterns of the *E. coli* isolates are presented in Table 3. The *E. coli* strains exhibited 17 different antibiotic resistance patterns. The current results indicated that 34 of the 41 *E. coli* isolates (82.9%) were resistant to at least one antibiotic, of which 26 (63.4%) were MDR.

Table 1. Incidence of *Escherichia coli* isolated from Cherry Valley ducks suspected of colibacillosis collected from Northern Vietnam

Order	Kind of sample	Positive, n (%)
1	Heart	37 (58.7)
2	Liver	41 (65.1)
3	Lung	28 (44.4)

n: Number

Table 2. The antibiotic resistance rates of the *Escherichia coli* isolated from Cherry Valley ducks suspected of colibacillosis collected from Northern Vietnam

Kind of antibiotics	Susceptibility n (%)	Intermediate n (%)	Resistance n (%)
Ampicilin	6 (14.6)	4 (9.8)	31 (75.6)
Amoxicillin/clavunalic acid	27 (65.9)	3 (7.3)	11 (26.8)
Colistin	29 (70.7)	12 (29.3)	0 (0.0)
Doxycyline	19 (46.3)	2 (4.9)	20 (48.8)
Ciprofloxacin	18 (43.9)	10 (24.4)	13 (31.7)
Florfenicol	28 (68.3)	4 (9.8)	9 (22.0)
Gentamicin	30 (73.2)	2 (4.9)	9 (22.0)
Levofloxacin	34 (82.9)	7 (17.1)	0 (0.0)
Streptomycin	18 (43.9)	4 (9.8)	19 (46.3)
Tetracycline	11 (26.8)	0 (0.0)	30 (73.2)
Norfloxacin	32 (78.0)	4 (9.8)	5 (12.2)
Kanamycin	25 (61.0)	5 (12.2)	11 (26.8)

Table 3. The antibiotic resistance patterns of the *Escherichia coli* isolated from Cherry Valley ducks suspected of colibacillosis collected from Northern Vietnam

Order	Antibiotic resistance pattern	resistant antibiotics (Number)	n (%)
1	All susceptible	0	7 (17.1)
2	TET	1	3 (7.3)
3	AMP, STR	2	2 (4.9)
4	AMP, DOX, TET	3	3 (7.3)
5	AMP, FLO, TET	3	1 (2.4)
6	AMP, STR, TET	3	5 (12.2)
7	AMC, AMP, CIP, STR	4	2 (4.9)
8	AMP, DOX, CIP, TET	4	2 (4.9)
9	AMP, DOX, FLO, TET	4	3 (7.3)
10	AMP, DOX, CIP, FLO, TET	5	1 (2.4)
11	AMP, DOX, FLO, GEN, TET	5	1 (2.4)
12	AMC, AMP, DOX, STR, TET, KAN	6	1 (2.4)
13	AMC, AMP, GEN, STR, TET, KAN	6	1 (2.4)
14	AMP, DOX, CIP, FLO, TET, KAN	6	1 (2.4)
15	AMC, AMP, DOX, FLO, STR, TET, KAN	7	1 (2.4)
16	AMP, DOX, CIP, GEN, STR, TET, KAN	7	1 (2.4)
17	AMC, AMP, DOX, CIP, FLO, GEN, STR, TET, KAN	9	1 (2.4)
18	AMC, AMP, DOX, CIP, GEN, STR, TET, NOR, KAN	9	5 (12.2)

No: Number; AMP: Ampicillin; AMC: Amoxicillin/clavunalic axit; CIP: Ciprofloxacin; DOX: Doxycycline; FLO: Florfenicol; GEN: Gentamicin; KAN: Kanamycin; NOR: Norfloxacin; STR: Streptomycin, and TET: Tetracycline.

DISCUSSION

The prevalence of *E. coli* found during the present study (44.4-65.1%) aligns with findings of Majumder et al. (2017) in Bangladesh (43.33%) and Bariha et al. (2019) in India (55%). However, the isolation rates in the present study were lower than those reported by Thu et al. (2019) in the Mekong Delta, southern Vietnam, who reported an 78.3% isolation rate from liver and 71.8% from lung samples. The differences in isolation rates are likely due to factors such as farming practices, regions, duck ages, sampling, and isolation methods. Previous studies from Bangladesh (Singh et al., 2012) and Nepal (Kissinga et al., 2018) reported *E. coli* isolation rates of 100% and 91.0%, respectively. Postmortem findings, including fibrinous perihepatitis, pericarditis, enteritis, and pneumonia, confirmed systemic dissemination of *E. coli* to multiple organs, including the liver, lungs, and heart, which led to septicemia and subsequent death (Kabir, 2010).

High resistance of the *E. coli* strains to common antibiotics, such as ampicillin, tetracycline, and streptomycin, has been reported in previous studies in northern Vietnam (Vui and Tiep, 2016) and in the Mekong Delta in southern Vietnam (Trang et al., 2017; Sang et al., 2017; Thu et al., 2019). This pattern of resistance has been observed in many countries worldwide, including China (Dou et al., 2016; Afayibo et al., 2022), Malaysia (Adzitey et al., 2013), Tanzania (Kissinga et al., 2018), and Egypt (Reham et al., 2023). This is likely because these antibiotics have been used extensively in livestock farming in these countries for a long time (Thu et al., 2019; Afayibo et al., 2022). The doxycycline resistance rate among *E. coli* strains in the current study was 48.8%; a rate that fell within the wide range (20.3-77.14%) reported in earlier studies from southern Vietnam (Sang et al., 2017; Thuan and Khai, 2018; Thu et al., 2019). In contrast, amoxicillin resistance was markedly lower (26.0%) than the high rates documented in Egypt (66.7%; Reham et al., 2023) and China (90%; Luo et al., 2023). This discrepancy may be attributed to the widespread use of doxycycline and amoxicillin for disease prevention and treatment in poultry farming in China and Vietnam (Thu et al., 2019; Luo et al., 2023). Based on the present findings, gentamicin resistance was 22.0%, consistent with previous studies in Vietnam (Vui and Tiep, 2016; Thu et al., 2019). The florfenicol resistance rate among *E. coli* strains in the present study (22.0%) was higher than that reported in Mekong Delta provinces, Vietnam (16.5%; Thu et al., 2019), but lower than the 41.9% found in Dong Thap province (Sang et al., 2017). Additionally, quinolone-resistant *E. coli* strains were observed in the current study. The isolated *E. coli* strains exhibited ciprofloxacin resistance at 31.7%. This resistance may stem from the ready availability of ciprofloxacin in the retail market, despite its official prohibition for veterinary purposes in Vietnam. The observed norfloxacin resistance rate of 12.2% was consistent with previous reports on *E. coli* from southern Vietnam (Thu et al., 2019) and Egypt (Abdel Rahman et al., 2020). Colistin is a highly effective antibiotic against *E. coli* and has been widely used for the prevention and treatment of colibacillosis in animals. However, none of the E.coli strains were resistant to colistin in the present study. This may be due to the antibiotic's poor absorption in the poultry gastrointestinal tract after oral administration, making it rarely used in ducks for treatment and prevention (Thu et al., 2019).

In the current study, 63.4% of *E. coli* strains were identified as MDR. The prevalence of MDR *E. coli* in the present study was consistent with rates of 60.0-65.5% reported in southern Vietnam (Thuan and Khai, 2018) and similar to findings in Korea (Jeong et al., 2021). Notably, 100% of the *E. coli* isolates from China were found to be MDR (Afayibo et al., 2022). The emergence of MDR indicates that livestock facilities serve as critical hotspots for antibiotic resistance, and livestock themselves function as substantial reservoirs of antibiotic-resistant genes and microorganisms (Kissinga et al., 2018; Yanestria et al., 2022). A critical factor for the spread of antibiotic resistance in the environment is intensive animal husbandry (Karwowska, 2024). Moreover, the high rates of antibiotic resistance among *E. coli* strains isolated from affected ducks in the current study indicated that antibiotics were frequently used to prevent and treat infections on duck farms. This resulted in a very high rate of antibiotic resistance among bacterial strains isolated from duck farms in Vietnam.

CONCLUSION

The current study highlighted the high resistance of *E. coli* strains to multiple antibiotics isolated from ducks in the study area. The studied antibiotics are generally used in treating and preventing diseases in human and veterinary medicine. The presence of MDR *E. coli* in ducks highlighted a significant public health concern, as these strains can facilitate the spread of antibiotic resistance. Controlling antibiotic use in poultry is therefore essential to mitigate risks within the food chain.

DECLARATIONS

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Authors' contributions

Chu Thi Thanh Huong designed the investigation and methodology and contributed to the manuscript. Vu Thi Thu Tra and Truong Lan Oanh participated in doing the experiments and analysing the data. Truong Ha Thai was responsible for supervision, methodological support, and primarily for writing 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 requests from the corresponding author.

Ethical considerations

The authors considered farmers' ethical concerns and consent before conducting the study. This article was written originally without any copy from published articles and books. No AI was used to conduct the current study.

Competing interests

The authors declared no conflict of interest.

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