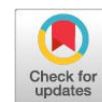


Research Article



Antibiotic Resistance of *Escherichia coli* Strains From Dog and Cat Fecal Samples Collected at Veterinary Clinics in Hanoi, Vietnam

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Abstract | Pets can be reservoirs and hosts for resistant bacteria, raising concerns about the risks to both human and animal health. This study aimed to investigate the prevalence and antibiotic resistance phenotypes of *E. coli* in fecal samples of dogs and cats from twelve veterinary clinics in Hanoi, Vietnam. *E. coli* were identified in 59.6% (96/161) of dog and cat fecal samples; of which, *E. coli* was more prevalent in diarrhea than in healthy fecal samples, 64.4% and 55.7%, respectively. The highest resistance rate was observed for ampicillin (68.8%), followed by streptomycin (65.6%), tetracycline (47.9%), and sulfonamides (44.8%). The remaining antibiotics, i.e., norfloxacin, nalidixic acid, gentamicin, doxycycline, and trimethoprim/sulfamethoxazole were resistant at lower rates (ranging from 11.5 - 32.3%); and the lowest resistance rate (4.2%) was observed for meropenem. In overall, the antibiotic resistance rates were significantly higher in *E. coli* obtained from dogs and cats with diarrhea than in healthy dog and cat samples ($p < 0.05$). In total, 85.4% of the *E. coli* strains exhibited resistance to at least one antibiotic. Of which, 62.5% of the strains were identified as multidrug-resistant (MDR). The *E. coli* strains from dogs and cats with diarrhea exhibited higher MDR rates as compare to those from healthy dogs and cats (87.2% and 38.8%, respectively; $p < 0.0001$). These results highlight the need to monitor and manage antibiotic use in veterinary clinics to limit antibiotic resistance and the spread of antibiotic-resistant bacterial strains.

Keywords | *Escherichia coli*, Antibiotic resistance, Multidrug resistance, Veterinary clinic, Dog, Cat

Received | March 06, 2026; **Accepted** | April 29, 2026; **Published** | May 15, 2026

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Citation | Huong CTT, Phuong NV, Oanh TL, Tuong NM, Thinh NH, Giap NV, Thai TH (2026). Antibiotic Resistance of *Escherichia coli* strains from dog and cat fecal samples collected at veterinary clinics in Hanoi, Vietnam. Adv. Anim. Vet. Sci., 14(5):1091-1099.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2026/10.5.1091.1099>

ISSN (Online) | 2307-8316



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INTRODUCTION

Antibiotic resistance is a serious threat to public health worldwide, highlighting the health crisis associated with the global increase in infections caused by pathogens resistant to late-generation antibiotics (Antimicrobial Resistance Collaborators, 2022). In this sense, companion animals are frequently exposed to antibiotics due to treatment or prevention of infections or through the consumption of food products (Guardabassi *et al.*, 2004;

Ramos *et al.*, 2022), increasing selective pressure and leading to the development of multidrug resistance (MDR) in their commensal bacteria (Wu *et al.*, 2019). Thus, pets can be reservoirs and hosts for resistant bacteria, raising concerns about the risks to both human and animal health (Carvalho *et al.*, 2021; Yuan *et al.*, 2024).

Escherichia coli (*E. coli*) is one of the most common microorganisms in the gut microbiota and is frequently subjected to antibiotic selective pressure. Therefore, testing

for antibiotic resistance in *E. coli* strains isolated from fecal samples is a good indicator for assessing the level of drug resistance (Marchetti *et al.*, 2021). *E. coli* are highly susceptible to antibiotic pressure and have a very high potential for developing antibiotic resistance, leading to an increase in the number of MDR strains and even those that are almost completely resistant to all antibiotics. MDR is defined as insensitivity to at least one agent in three or more antibiotic classes (Magiorakos *et al.*, 2012). MDR pathogens can cause more severe, prolonged infections and ultimately have the potential to cause global pandemics (Cummins *et al.*, 2021).

In Vietnam, current research on antibiotic resistance mainly focuses on food-producing animals, such as livestock and poultry. However, due to close contact between companion animals and humans, bacteria including *E. coli*, *Salmonella* spp., etc., isolated from dogs and cats have a high degree of similarity to strains isolated from humans, increasing the potential for interspecies transmission of antibiotic-resistant *E. coli* and posing a potential threat to human health (Derakhshandeh *et al.*, 2018). To date, data on antibiotic resistance levels in bacteria isolated from pets and companion animals, such as *E. coli* and *Salmonella* spp., is very limited in Vietnam. This study aimed to investigate the prevalence and phenotype of antibiotic resistance in *E. coli* strains obtained from dogs and cats at veterinary clinics in Hanoi, Vietnam.

MATERIALS AND METHODS

SAMPLE COLLECTION

In this cross-sectional study, from November 2024 to December 2025, one-hundred and sixty-one dog (n=92) and cat (n=69) fecal samples were randomly collected from twelve veterinary clinics in Hanoi, Vietnam. Healthy dogs and cats are those brought to the veterinary clinic for vaccinations, grooming, and deworming. Sick dogs and cats are those suffering from gastrointestinal illness or diarrhea and receiving treatment at the veterinary clinic. The samples were collected according to the QCVN 01-83:2011/BNNT guidelines of the Ministry of Agriculture and Rural Development (2011). Briefly, fecal samples were carefully collected using the rectal swab sampling with sterile cotton swabs. Each sample was placed in a separate sterile sample bag, labeled, stored at 4 °C in a dry ice box, and immediately transported to the Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture for analysis within 24 hours.

ESCHERICHIA COLI ISOLATION

The isolation and identification of *E. coli* was performed as previous studies described with some modification

(Mustapha *et al.*, 2021; Yuan *et al.*, 2024). Briefly, at the laboratory, each feces sample was homogenized with buffer peptone water (BPW; Merck, Germany). Next, a loopful of the homogenized cultures was streaked onto MacConkey agar (Merck, Germany) and incubated at 37°C for 24 hours. Subsequently, the pink colonies (Figure 1) were cultured 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 (Figure 2) were streaked into 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, (Figure 3), were identified as *E. coli* by Gram staining and biochemical tests, such as indole production, methyl red, Voges-Proskauer, and citrate utilisation reactions, with the expected IMViC result being “++--” (Figure 4). All isolates were kept in brain heart infusion broth (BHI; Merck, Germany) plus 50% glycerin at minus 20 °C for the next experiment.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

A total of 96 *E. coli* strains from positive samples were selected for antibiotic susceptibility analysis, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). Agar diffusion method (Figure 5) was performed on Mueller-Hinton agar (MHA, Merck, Germany) following Bauer *et al.* (1966). Eleven different antibiotic agents (Oxoid, UK) belonging to seven classes were used, including penicillins (ampicillin, 10 µg) cephalosporin (cefotaxime, 30 µg), carbapenem (meropenem, 10 µg), tetracyclines (doxycycline, 30 µg; tetracycline, 30 µg), aminoglycosides (gentamicin, 10 µg; streptomycin, 30 µg), quinolones (nalidixic acid, 30 µg; norfloxacin, 10 µg) and sulfonamides (sulfonamides, 300 µg; trimethoprim/sulfamethoxazole, 1.25/23.75 µg). The *Escherichia 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 MDR strain upon confirmation of resistance to at least one antibiotic from three different antibiotic classes (Magiorakos *et al.*, 2012).

DATA ANALYSIS

Data on *E. coli* isolates and antimicrobial susceptibility tests were recorded in a Microsoft Excel spread sheet where edits were done. The Fisher's exact test was used to assess differences in prevalence and antibiotic susceptibility of *E. coli* isolates between the sample types, and values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

In the current study, *E. coli* was identified in 59.6% of the

fecal samples (Table 1). There was no difference in the isolation rates of *E. coli* between dog and cat fecal samples, 59.8% in dog fecal samples and 59.4% in cat fecal samples ($p > 0.90$). However, *E. coli* was more prevalent in diarrheal compared to healthy dog and cat samples, with rates of 64.4% and 55.7% ($p = 0.3331$), respectively. Overall, the isolation rates of *E. coli* were lower than that in West Indies, European countries and Zimbabwe (Amandi *et al.*, 2019; Joosten *et al.*, 2020; Matope *et al.*, 2024), reported that 93.0 - 98.6% of the feces samples collected from dogs and cat were *E. coli* -positive. Moreover, *E. coli* was identified in 78.2% and 91.9% of the feces samples from cat and dogs in Australia, respectively (Bourne *et al.*, 2019). However, this rate could comparable to those in previous studies in China (Tong *et al.*, 2023; Zhou *et al.*, 2021), reported that *E. coli* were identified in 63.75-63.92% of the dog and cat fecal samples. The high prevalence of *E. coli* observed in feces samples of dogs and cats demonstrates the reason why *E. coli* is often selected to determine antibiotic resistance in both human and veterinary medicine (Joosten *et al.*, 2020; Matope *et al.*, 2024).

The *E. coli* strains were resistant to ampicillin, streptomycin and tetracycline at the rates of 68.8%, 65.6%, and 47.9% (Table 2), respectively. Previous studies in China have reported high resistance rates in dog and cat-derived *E. coli* strains to these antibiotics (Zhou *et al.*, 2022; Yuan *et al.*, 2024). Additionally, moderate resistance rates ranging

from 32.6% to 50.5% have been observed in *E. coli* strains in previous studies in Argentina (Marchetti *et al.*, 2021), Indonesia, and Timor-Leste (Nugroho *et al.*, 2024). Conversely, the rate of antibiotic resistance was lower in *E. coli* strains in previous studies in Australia (Bourne *et al.*, 2019), European countries (Joosten *et al.*, 2020), Canada (Leonard *et al.*, 2012; Awosile *et al.*, 2018), Argentina (Marchetti *et al.*, 2021), South Korea (Moon *et al.*, 2023) and Brazil (Feitosa *et al.*, 2024). The high resistance of *E. coli* strains to these antibiotics is not surprising, given their widespread use worldwide in both human and veterinary medicine.

In the current study, doxycycline resistance (26.0%) was higher than the rates of 11.0% and 14.8% reported in similar studies in Canada and South Korea (Leonard *et al.*, 2012; Moon *et al.*, 2023). However, this antibiotic was resistant to 45.19% of the canine-derived *E. coli* strains with diarrhea in China (Yuan *et al.*, 2024). The isolated *E. coli* strains showed resistance to gentamicin at a rate of 16.7%, lower than the 28.89% reported in a similar study in China (Yuan *et al.*, 2024). Notably, resistance to this antibiotic was only observed in 0.8 - 8.0% of *E. coli* strains isolated from dogs and cats in several countries such as Canada (Leonard *et al.*, 2012), the West Indies (Amandi *et al.*, 2019), South Korea (Moon *et al.*, 2023), Peru (Ventura *et al.*, 2024), Germany (Frenzer *et al.*, 2024), and Brazil (Feitosa *et al.*, 2024). The proportion of trimethoprim/

Table 1: Prevalence of *E. coli* in the fecal samples of dogs and cats at veterinary clinics.

Animal condition	Dog fecal samples			Cat fecal samples			Total		
	n	Positive n (%)	Negative n (%)	n	Positive n (%)	Negative n (%)	n	Positive n (%)	Negative n (%)
Healthy	50	28 (56.0)	22 (44.0)	38	21 (55.3)	17 (44.7)	88	49 (55.7)	39 (44.3)
Diarrhea	42	27 (64.3)	15 (35.7)	31	20 (64.5)	11 (35.5)	73	47 (64.4)	26 (35.6)
Total	92	55 (59.8)	37 (40.2)	69	41 (59.4)	28 (40.6)	161	96 (59.6)	65 (40.4)

Table 2: Antibiotic resistance of the *E. coli* strains isolated from dog and cat fecal samples.

Groups/classes	Antibiotics	From healthy dogs and cats n (%)	From diarrhea dogs and cats n (%)	P-value	95% CI	Total n (%)
Penicillin	Ampicillin	22 (44.9)	44 (93.6)	≤ 0.0001	0.01 - 0.22	66 (68.8)
Cephalosporin	Cefotaxime	14 (28.6)	24 (51.1)	0.0445	0.15 - 0.96	38 (39.6)
Carbapenem	Meropenem	0 (0.0)	4 (8.5)	0.0591	0.00 - 1.41	4 (4.2)
Tetracyclines	Doxycycline	8 (16.3)	17 (36.2)	0.0445	0.11 - 0.99	25 (26.0)
	Tetracycline	15 (30.6)	31 (66.0)	0.0036	0.09 - 0.58	46 (47.9)
Aminoglycosides	Gentamicin	3 (6.1)	13 (27.7)	0.0128	0.03 - 0.70	16 (16.7)
	Streptomycin	27 (55.1)	36 (76.6)	0.0445	0.14 - 0.98	63 (65.6)
Quinolones	Nalidixic acid	3 (6.1)	13 (27.7)	0.0128	0.03 - 0.70	16 (16.7)
	Norfloxacin	3 (6.1)	8 (17.0)	0.1168	0.05 - 1.46	11 (11.5)
Sulfonamides	Sulfonamides	16 (32.7)	27 (57.4)	0.0430	0.14 - 0.89	43 (44.8)
	Trimethoprim/ Sulfamethoxazole	5 (10.2)	26 (55.3)	≤ 0.0001	0.02 - 0.30	31 (32.3)

E. coli isolated from healthy dogs and cats (n=49); from diarrhea dogs and cats (n=47); and Total (n=96); CI= Confidence Interval.

sulfamethoxazole-resistant *E. coli* strains (32.3%) was consistent with those (25.7% and 25.8%), reported in similar studies in Peru (Ventura *et al.*, 2024) and Brazil (Feitosa *et al.*, 2024), respectively. However, these rates are lower than those observed in *E. coli* strains from diarrheal dogs in China (Yuan *et al.*, 2024), and healthy dogs and cats in Zimbabwe (Matope *et al.*, 2024). In contrast, the *E. coli* strains showed antibiotic resistance of 5.0% to 12.0%, and 14.6% in the previous studies conducted in West Indies, Germany, and South Korea (Amandi *et al.*, 2019; Moon *et al.*, 2023; Frenzer *et al.*, 2024), respectively. The relatively high resistance rates of *E. coli* strains to these three antibiotics may be related to their widespread use for treating infections in dogs and cats at veterinary clinics in Vietnam.

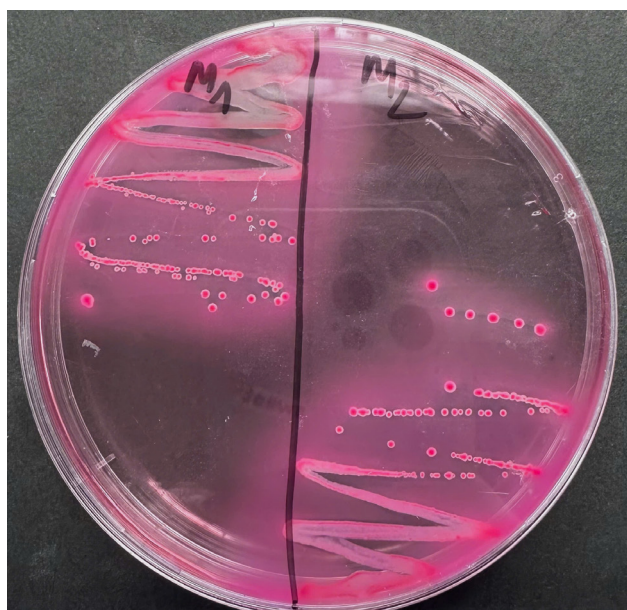


Figure 1: *E. coli* colonies isolated from dog and cat feces exhibited pink colour on MacConkey agar.

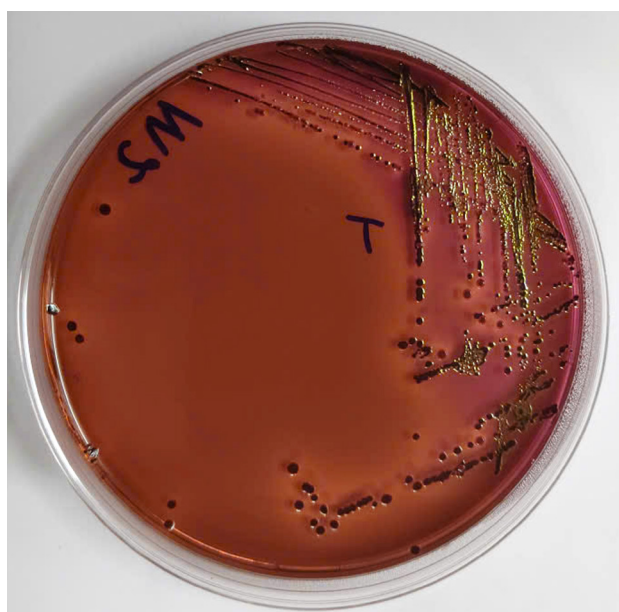


Figure 2: *E. coli* colonies isolated from dog and cat feces exhibited green metallic sheen on EMB agar.

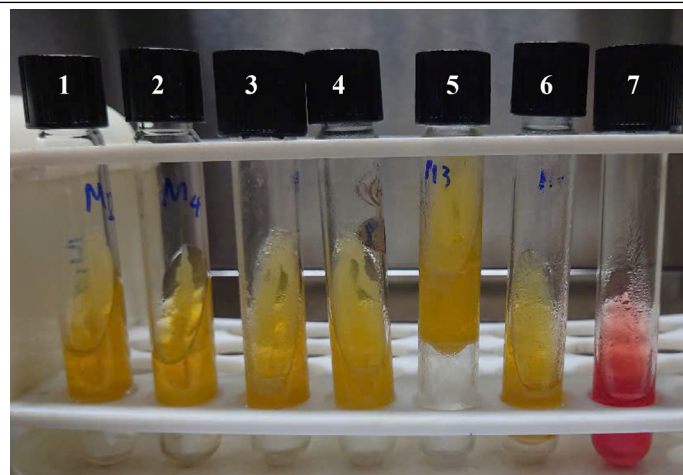


Figure 3: *E. coli* strains isolated from dog and cat feces on TSI agar.

Tubes 1-6: The *E. coli* strains exhibited fermentation and gas production; Tube 7: negative control.

The isolated *E. coli* strains exhibited resistance to norfloxacin and nalidixic acid at rates of 11.5% and 16.7%, respectively. These rates are lower than those reported in a previous study in Peru (Ventura *et al.*, 2024), which reported resistance rates of 31.4% and 54.3%, respectively. Specifically, norfloxacin resistance rates were identified at 17.3% and 21.9% in *E. coli* strains originating from dog and cat feces in Thailand (Sattasathuchana *et al.*, 2024) and Poland (Rzewuska *et al.*, 2015). Information and levels of resistance to these antibiotics are crucial because they are primarily used in humans and veterinary medicine (Antimicrobial Resistance Collaborators, 2022). The resistance rate of cefotaxime (39.6%) is consistent with the resistance rate (33.3%) of *E. coli* isolated from dogs with diarrhea in China (Yuan *et al.*, 2024). However, a lower resistance rate (20.0%) was observed in *E. coli* isolated from dogs in Peru (Ventura *et al.*, 2024). Notably, none and 1.0% of dog and cat-derived *E. coli* strains were resistant to this antibiotic in West Indies (Amandi *et al.*, 2019) and European countries (Joosten *et al.*, 2020), respectively. In this study, approximately 4.2% of the isolated *E. coli* strains were suspected to be meropenem-resistant. However, the determination of meropenem resistance using the agar diffusion method is not recommended by the Clinical Laboratory Standards Institute (CLSI) unless it is a clinical *E. coli* strain (CLSI, 2020). Therefore, further studies are needed to definitively determine whether these strains are resistant to meropenem by determining the minimum inhibitory concentration (MIC) and/or molecular resistance mechanisms through screening of carbapenemase resistance genes (bla_{NDM} , bla_{KPC} , bla_{OXA-48} , bla_{IMP} , bla_{VIM}). A similar resistance rate of meropenem was observed in the *E. coli* strains isolated from dogs in Peru (Ventura *et al.*, 2024). However, the *E. coli* strains exhibited higher resistance rate (10.1%) to meropenem in previous studies in China (Tong *et al.*, 2023). Notably, *E.*

coli strains isolated from cat feces in Thailand exhibited a very low resistance rate to meropenem (Sattasathuchana *et al.*, 2024); and all *E. coli* strains isolated from dogs and cats in European countries were susceptible to this antibiotic (Joosten *et al.*, 2020). Although the resistance rates of *E. coli* to carbapenems and cephalosporins remains relatively low, this is still concerning because these are antibiotic classes considered last-resort treatments for human infections.

The *E. coli* strains isolated from dog and cat feces exhibited various resistance phenotypes to the antibiotics tested. For the isolates obtained from healthy dogs and cats (Table 3), thirteen strains were susceptible to all antibiotics; fifteen strains were resistant to 1-2 antibiotic agents. Meanwhile, resistance to 3-4 and 5-6 antibiotic agents were observed in thirteen and seven strains, respectively; only one of the isolated strains shown resistant to eight antibiotic agents. The *E. coli* strains exhibited twenty-one antibiotic-resistant phenotypes. The most common resistance phenotype was AMP (five isolates), followed

by AMP+CTX+STM+SUL and AMP+STM, detected in four and three out of the isolates, respectively. The remaining resistance phenotypes were determined in from one to two out of the isolated *E. coli* strains. Among the isolates obtained from dogs and cats with diarrhea (Table 4), only one strain was susceptible to all antibiotics. Five out of the isolated strains were found to be resistant to two antibiotic agents; resistance to 3-4 antibiotic agents and 5-6 antibiotic agents were observed in fifteen and thirteen out of the isolated strains, respectively. Nine out of the isolated strains were found to be resistant to 7-8 antibiotic agents. Notably, resistance to 9 - 10 antibiotic agents was identified in three isolated strains. The *E. coli* strains were found to possess a total of thirty-six antibiotic resistance patterns. Furthermore, the most common resistance pattern was AMP+STX+DOX+TET+CTX+STM+SUL and AMP+TET+CTX+STM, which was found in three and four out of the isolated strains, respectively. The remaining resistance patterns were usually found in one or two out of the *E. coli* isolates. The *E. coli* strains from dogs and cats with

Table 3: Multidrug resistance of the *E. coli* isolated from fecal samples of healthy dogs and cats (n=49).

Order	Antibiotic resistance patterns (n)	No. of antibiotics	No. of antibiotic classes
1	Susceptible (n=13)	0	0
2	AMP (n=5)	1	1
3	STM (n=2)	1	1
4	TET (n=2)	1	1
5	AMP, STM (n=3)	2	2
6	CTX, SUL (n=1)	2	2
7	STM, SUL (n=2)	2	2
8	AMP, CTX, STM (n=1)	3	3
9	AMP, STM, SUL (n=1)	3	3
10	DOX, TET, STM (n=2)	3	2
11	TET, CTX, STM (n=2)	3	3
12	TET, STM, SUL (n=1)	3	3
13	AMP, CTX, STM, SUL (n=4)	4	3
14	AMP, TET, STM, SUL (n=1)	4	4
15	DOX, TET, CTX, STM (n=1)	4	3
16	AMP, STX, CTX, STM, SUL (n=2)	5	4
17	AMP, STX, TET, GEN, NAL, NOR (n=1)	6	5
18	AMP, DOX, TET, NAL, NOR, STM (n=1)	6	4
19	DOX, TET, GEN, CTX, STM, SUL (n=1)	6	4
20	AMP, STX, DOX, TET, CTX, STM, SUL (n=1)	7	5
21	AMP, STX, DOX, TET, GEN, STM, SUL (n=1)	7	5
22	AMP, DOX, TET, NAL, CTX, NOR, STM, SUL (n=1)	8	6

AMP: Ampicillin; CTX: Cefotaxime; DOX: Doxycycline; GEN: Gentamicin; NAL: Nalidixic acid; NOR: Norfloxacin; STM: Streptomycin; STX: Trimethoprim/Sulfamethoxazole; SUL: Sulfonamides; TET: Tetracycline.

Table 4: Multidrug resistance of the *E. coli* isolated from fecal samples of diarrhea dogs and cats (n=47).

Order	Antibiotic resistance patterns (n)	No. of antibiotics	No. of antibiotic classes
1	Susceptible (n=1)	0	0
2	AMP, CTX (n=1)	2	2
3	AMP, STM (n=1)	2	2
4	AMP, TET (n=2)	2	2
5	STM, SUL (n=1)	2	2
6	AMP, STX, MEM (n=1)	3	3
7	AMP, CTX, SUL (n=1)	3	3
8	TET, CTX, STM (n=1)	3	3
9	AMP, STX, CTX, SUL (n=1)	4	3
10	AMP, STX, STM, SUL (n=2)	4	3
11	AMP, DOX, TET, STM (n=1)	4	3
12	AMP, NAL, CTX, STM (n=1)	4	4
13	AMP, TET, CTX, STM (n=4)	4	4
14	AMP, TET, STM, SUL (n=3)	4	4
15	AMP, STX, CTX, STM, SUL (n=1)	5	4
16	AMP, STX, DOX, TET, STM (n=1)	5	4
17	AMP, STX, GEN, NAL, SUL (n=1)	5	4
18	AMP, STX, TET, CTX, STM (n=1)	5	5
19	AMP, DOX, TET, CTX, SUL (n=1)	5	4
20	AMP, TET, CTX, STM, SUL (n=1)	5	5
21	STX, DOX, TET, STM, SUL (n=1)	5	4
22	AMP, STX, DOX, TET, GEN, STM (n=2)	6	4
23	AMP, STX, GEN, NAL, CTX, MEM (n=1)	6	6
24	AMP, STX, GEN, NAL, STM, SUL (n=1)	6	5
25	AMP, STX, NAL, NOR, STM, SUL (n=1)	6	4
26	AMP, TET, NAL, NOR, STM, SUL (n=1)	6	5
27	AMP, STX, DOX, TET, CTX, STM, SUL (n=3)	7	5
28	AMP, STX, DOX, TET, GEN, STM, SUL (n=1)	7	5
29	AMP, STX, DOX, TET, STM, MEM, SUL (n=1)	7	6
30	AMP, STX, GEN, NAL, CTX, NOR, STM (n=1)	7	5
31	AMP, STX, DOX, TET, GEN, CTX, STM, SUL (n=1)	8	5
32	AMP, STX, DOX, TET, GEN, NAL, NOR, STM (n=1)	8	5
33	AMP, STX, TET, NAL, CTX, NOR, STM, SUL (n=1)	8	6
34	AMP, STX, DOX, TET, GEN, NAL, CTX, STM, SUL (n=1)	9	6
35	AMP, STX, DOX, TET, GEN, NAL, CTX, NOR, STM, SUL (n=2)	10	6
36	AMP, DOX, TET, GEN, NAL, CTX, NOR, STM, MEM, SUL (n=1)	10	7

AMP: Ampicillin; CTX: Cefotaxime; DOX: Doxycycline; GEN: Gentamicin; MEM: Meropenem; NAL: Nalidixic acid; NOR: Norfloxacin; STM: Streptomycin; STX: Trimethoprim/Sulfamethoxazole; SUL: Sulfonamides; TET: Tetracycline

diarrhea exhibited higher MDR rates as compared to the isolated strains from healthy dogs and cats (87.2% and 38.8%, respectively; $p < 0.0001$). Resistance to five or six antibiotic groups was found in 36.2% (17/47) and 8.2% (4/49) of the diarrhea and healthy dog and cat isolates, respectively. Only one out of the *E. coli* strains obtained from diarrhea fecal samples exhibited resistance to seven

antibiotic classes.

In the present study, approximately 85.4% of the *E. coli* strains exhibited resistance to at least one antibiotic (Table 5). Of these, 62.5% of the strains were identified as MDR. These rates were comparable to the previous studies in China (Zhou *et al.*, 2022; Yuan *et al.*, 2024), reported that

Table 5: Number of the *E. coli* isolates exhibited the MDR.

Order	No. of antibiotic resistance classes	Healthy (n=49)		Diarrhea (n=47)		Total (n=96)	
		n	%	n	%	n	%
1	No resistance	13	26.5	1	2.1	14	14.6
2	Resistance to 1-2 classes	17	34.7	5	10.6	22	22.9
3	Resistance to 3-4 classes	15	30.6	23	48.9	38	39.6
4	Resistance to 5-6 classes	4	8.2	17	36.2	21	21.9
5	Resistance to ≥ 7 classes	0	0.0	1	2.1	1	1.0



Figure 4: The IMViC tests for *E. coli* confirmation (from left). Tube 1: The *E. coli* strain exhibited fermentation and gas production on TSI agar; Tube 2: Indole (+); Tube 3: Methyl red (+); Tube 4: Voges-Proskauer (-); Tube 5: Simmons citrate (-)

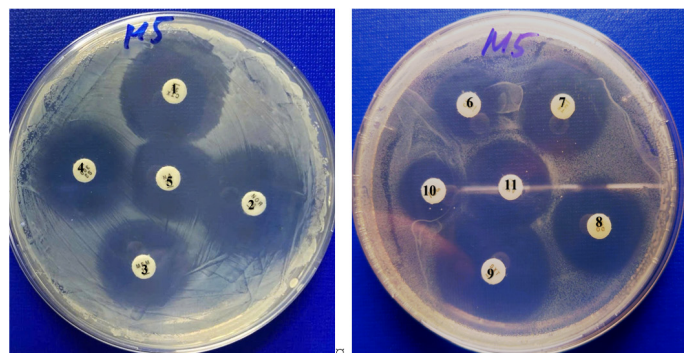


Figure 5: Antibiotic susceptibility testing of 11 antibiotics against *E. coli* strains isolated from dog and cat feces.

(1): Cefotaxime; (2): Norfloxacin; (3): Meropenem; (4): Sulfonamides; (5): Nalidixic acid; (6): Gentamicin; (7): Tetracycline; (8): Doxycycline; (9): Trimethoprim/Sulfamethoxazole; (10): Streptomycin; (11): Ampicillin.

the MDR was identified in 54.81-63.7% of the *E. coli* isolates. Furthermore, another previous study in China documented that 72.25% of the *E. coli* isolated from dogs as MDR strains (Tong *et al.*, 2023). Nevertheless, MDR rates were determined in 13.3-14.5%, 18.0%, and 20.9-34.9% of the *E. coli* strains in previous studies in South

Korean (Moon *et al.*, 2023), Zimbabwe (Matope *et al.*, 2024) and Canada (Awosile *et al.*, 2018), respectively. The detection of multidrug-resistance isolates is serious concern and requires attention (Bourne *et al.*, 2019; Tong *et al.*, 2023). These multidrug-resistant bacterial strains can pose significant challenges to antibiotic therapy in livestock, such as complicating antibiotic selection, reducing treatment efficacy, and increasing veterinary service costs (Awosile *et al.*, 2018; Zhou *et al.*, 2022). Therefore, raising awareness of antibiotic resistance transmission in humans, animals, and the environment is crucial (Poirel *et al.*, 2018; Feitosa *et al.*, 2024; Yuan *et al.*, 2024). Antibiotic resistance can also be controlled by providing education to raise public awareness and understanding of antibiotic resistance, aiming to changing people's behaviour towards rational antibiotic use and good environmental hygiene practices (Stålsby Lundborg and Tamhankar, 2014; Ahmed *et al.*, 2024).

CONCLUSIONS

In summary, this study shows that the majority of isolated *E. coli* strains were resistant to at least one antibiotic, with various resistance patterns observed in the isolates. Notably, multidrug-resistant *E. coli* strains were more commonly found in fecal samples from dogs and cats with diarrhea compared to strains from healthy dogs and cats. These results suggest that veterinary clinics in Hanoi city need to use antibiotics correctly, following manufacturer guidelines and legal regulations to limit antibiotic resistance and the spread of antibiotic-resistant bacterial strains. Furthermore, further molecular studies are needed to identify pathogenic and antibiotic resistance genes in the isolated bacterial strains.

ACKNOWLEDGEMENTS

The authors would like to thank the students for transporting samples and the local veterinarian for their excellent technical assistance.

NOVELTY STATEMENT

This study provides information on the prevalence of *E. coli* in fecal samples from dogs and cats collected from veterinary clinics in Hanoi, Vietnam. The results indicated that the isolated *E. coli* strains were resistant to many antibiotics, including important ones such as cefotaxime and meropenem, which are used in human medicine. Furthermore, information on the antibiotic resistance of bacteria helps raise awareness of the safe and prudent use of antibiotics in animal husbandry and veterinary medicine.

CTTH: study design, investigation and methodology, and manuscript writing. NVP, TLO, NMT: experimentation and data analysis. NVG and NHT: laboratory research design, sample testing supervision, and manuscript editing. THT: conceptualization, supervision, writing, review and editing, project management.

FUNDING INFORMATION

The present study received no external funding. The authors contributed to this study independently.

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-2024/06).

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

AI-generated tools were not used to create any scientific content. Any AI assistance was limited to minor language editing, and all ideas, interpretations, and conclusions remain entirely the authors.

STATEMENT OF CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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