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Isolation and Antimicrobial Susceptibility of *Enterobacteriaceae* Isolated from Wild Animals in Vietnam

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ABSTRACT

Antimicrobial resistance has increased globally among bacteria within the family *Enterobacteriaceae*. The present study aimed to identify *Enterobacteriaceae* species isolated from wild animals in Vietnam, including sika deer, chevrotain, bamboo rat, binturong, otter, palm civet, and wildcat, and to evaluate their antimicrobial susceptibility profiles. A total of 20 samples from wild animals were subjected to biochemical characterization and species identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Antimicrobial susceptibility was determined by the disc diffusion method according to Clinical and Laboratory Standards Institute guidelines. A total of 42 *Enterobacteriaceae* isolates were identified, including *Escherichia coli* (*E. coli;* 71.4%), *Enterobacter asburiae*, *Enterobacter bugandensis*, *Enterobacter hormaechei*, *Escherichia fergusonii*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Proteus mirabilis*. Most isolates were susceptible to norfloxacin (92.9%) and trimethoprim/sulfamethoxazole (88.1%), and no resistance to imipenem was detected. In contrast, resistance to ampicillin was the most common at 52.4%, followed by ciprofloxacin at 30.9%, cefotaxime at 26.2%, tetracycline at 23.8%, chloramphenicol at 21.4%, and streptomycin at 21.4%. Intermediate susceptibility was observed for doxycycline (9.5%), cefoxitin (9.5%), norfloxacin (7.1%), and gentamicin (4.8%). Among the isolates, 76.7% of *E. coli* and 91.7% of the remaining *Enterobacteriaceae* species demonstrated resistance to at least one antimicrobial agent. Seventeen distinct resistance patterns were identified among *E. coli*, whereas five patterns were observed among other species. Overall, 12 isolates (28.6%) were classified as multidrug-resistant, exhibiting resistance to three or more antimicrobial classes. The present findings indicated that wild animals in Vietnam carried different *Enterobacteriaceae* species, including multidrug-resistant strains. Consequently, wild animals could serve as a reservoir for antimicrobial-resistant bacteria, emphasizing the need for ongoing surveillance within a One Health approach.

Keywords: Antimicrobial resistance, *Enterobacteriaceae*, Multidrug resistance, Wild animal

INTRODUCTION

Members of the *Enterobacteriaceae* family are Gram-negative bacteria commonly found in the intestinal tracts of humans, domestic and wild animals (Octavia and Lan, 2014). There are several species within this family, including *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp., that act as opportunistic pathogens and can cause different infections, such as septicemia, urinary tract infections, and enteritis, in both humans and animals (Singh et al., 2015). Beyond their pathogenic potential, *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. bacteria are particularly concerning because of their ability to acquire and spread antimicrobial resistance (AMR) genes through horizontal gene transfer, mainly via plasmids, transposons, and integrons (Mancuso et al., 2021). Key resistance factors include β-lactamase genes (*bla*TEM, *bla*SHV, *bla*CTX-M, *bla*NDM), which confer resistance to β-lactam antibiotics, aminoglycoside-modifying enzyme genes (*aac*, *aph*, *aad*), tetracycline resistance genes (*tetA*, *tetB*), and sulfonamide/trimethoprim resistance genes (*sul1*, *sul2*, *dfrA*; Ejaz et al., 2021; Chaudhary et al., 2023). Class 1 integrons facilitate the capture and expression of multiple resistance gene cassettes, promoting multidrug resistance. The spread of antimicrobial-resistant *Enterobacteriaceae* species has become a serious global health challenge due to limited therapy options (O'Neill, 2016; Mancuso et al., 2021).

Wild animals are considered potential reservoirs and carriers of AMR bacteria (Li et al., 2024). Wild animals can acquire resistant bacteria through exposure to contaminated environments, domestic animals, or human sources (Laborda et al., 2022; Martak et al., 2024). *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. carrying AMR

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determinants can spread among ecosystems, connecting the environment, wildlife, livestock, and human populations. The interconnection between human, animal, and environmental reservoirs of AMR bacteria reflects the One Health concept, which emphasizes that the health of people, animals, and ecosystems are closely linked (Hassell et al., 2023; Ma et al., 2023). Therefore, monitoring AMR bacteria in wild animals is essential for understanding its ecological dynamics and preventing its transmission between humans, animals, and the environment (Doyle et al., 2025).

Several studies conducted in different countries have demonstrated the presence of *Enterobacteriaceae* species carrying extended-spectrum β-lactamase (ESBL) genes or exhibiting multidrug-resistant (MDR) profiles in wild animals in China (Wang et al., 2017), Tunisia (Sola et al., 2022), Gulf Cooperation Council countries (Hadi et al., 2023), Palestine (Ibaideya et al., 2024), and other regions (Doyle et al., 2025). The detection of AMR bacteria and resistance genes in wild animals suggests that these animals may serve as indicators of environmental contamination and as reservoirs of resistance determinants. There is focus on livestock, aquaculture, and pets, with limited investigation on the role of wild animals in maintaining and transmitting AMR bacteria in Vietnam (Phu et al., 2022). Nevertheless, data on the *Enterobacteriaceae* family and their antimicrobial susceptibility in wild animals remain scarce in Vietnam.

Vietnam is widely recognized as a biodiversity hotspot in Southeast Asia, supporting a wide range of wild species that often coexist with humans and domestic animals (Mitchell et al., 2020; Botterill-James et al., 2024). The emergence and spread of resistant bacteria among wild animals may be driven by increasing human activities, wildlife trade, and environmental factors (Glidden et al., 2021; Mitchell, 2023). Therefore, investigating the prevalence and antimicrobial resistance of *Enterobacteriaceae* species in wild animals is crucial for filling existing knowledge gaps and establishing a scientific basis for AMR surveillance in Vietnam. The present study aimed to characterize *Enterobacteriaceae* species isolated from wild animals in Vietnam and to evaluate their antimicrobial susceptibility patterns.

MATERIALS AND METHOD

Ethical approval

The present study was approved by the Committee on Animal Research and Ethics (CARE) of the Faculty of Veterinary Medicine, Vietnam National University of Agriculture (Approval No. CARE-2023/12), Vietnam. All procedures were carried out in accordance with CARE guidelines.

Sample collection

A total of 20 fecal samples were collected from healthy wild animals between February and March 2023. The samples were obtained from wildlife farms, a rescue center, and a national park across four Vietnamese provinces, including Binh Phuoc (n = 4), Dak Lak (n = 2), Dong Nai (n = 12), and Ha Tinh (n = 2; Table 1). Samples were collected shortly after defecation. To avoid contact with soil or bedding, a sterile plastic sheet was placed on the ground prior to defecation. Only the uncontaminated inner portion of the fecal mass was aseptically sampled using sterile gloves and instruments. Any visibly contaminated material (soil, bedding, or debris) was discarded. The mentioned procedures minimized environmental contamination and the risk of misidentification isolates.

Table 1. Distribution of fecal samples collected from different wild animal species and sources in Vietnam from February to March 2023

Wild animals	Sources (Number of samples)
Bamboo rat (*Rhizomys pruinosus*)	Farm in Dong Nai (2)
Binturong (*Arctictis binturong*)	Bu Gia Map National Park in Binh Phuoc (1)
Chevrotain (*Tragulus javanicus*)	Farm in Dong Nai (5)
Otter (*Aonyx cinereus*)	Bu Gia Map National Park Binh Phuoc (1)
Palm civet (*Paradoxurus hermaphroditus*)	Farm in Dak Lak (2) and Dong Nai (1)
Sika deer (*Cervus nippon*)	Farm in Dong Nai (3) and Ha Tinh (2) Wildlife rescue center in Dong Nai (1)
Wildcat (*Felis bengalensis Kerr*)	Bu Gia Map National Park in Binh Phuoc (2)

Isolation and identification of *Enterobacteriaceae*

Enterobacteriaceae species were isolated from fecal samples in accordance with a published protocol by Foti et al. (2018). Briefly, fecal samples were homogenized in buffered peptone water (BPW; Merck, Germany) and incubated at 37˚C for 24 hours. The enriched cultures were subsequently streaked onto MacConkey agar (MAC; Merck, Germany) and incubated under the same conditions for an additional 24 hours. Distinct colonies observed on MacConkey agar were selected and subculture on nutrient agar (NA; Merck, Germany) for further biochemical characterization and species identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS;

Bruker Daltonics, Bremen, Germany), following the methodology outlined by Wang et al. (2023). From each fecal sample, multiple colonies sharing identical morphology were selected, and every isolate was identified. To account for non-independence, isolates of the same species originating from the same sample were considered as a single data point for statistical analysis. Fresh colonies grown on NA were transferred onto polished steel target plates, air-dried at room temperature, and overlaid with 1 µL of a saturated α-cyano-4-hydroxycinnamic acid matrix solution. The target plates were then analyzed using a Bruker MALDI-TOF MS system. In addition, all isolates were subjected to standard biochemical tests, including Gram staining, catalase, indole, methyl red, Voges-Proskauer, and growth on Simmons citrate agar and triple sugar iron (TSI) agar.

Antimicrobial susceptibility tests

The antimicrobial susceptibilities of all isolates were evaluated by disk diffusion on Mueller-Hinton agar (MHA; Merck, Germany). After incubation, the diameters of the inhibition zones were measured in millimeters (mm) using a digital caliper, and the results were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2024). A total of twelve antimicrobial disks (Oxoid, Basingstoke, UK) were tested, including ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), doxycycline (DOX, 30 µg), gentamicin (GEN, 10 µg), imipenem (IPM, 10 µg), norfloxacin (NOR, 10 µg), streptomycin (STR, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), and tetracycline (TET, 30 µg). *Escherichia coli* ATCC 25922 was used as the quality control strain for routine susceptibility testing.

Statistical analysis

Descriptive statistical analysis was conducted to indicate the antimicrobial susceptibility profiles and resistance patterns of the bacterial isolates.

RESULT

Identification of *Enterobacteriaceae* isolates

A total of 42 *Enterobacteriaceae* isolates were identified from 20 fecal samples, with all samples testing positive (Table 1). *Escherichia coli* was the predominant species, accounting for 30 isolates. Additionally, other species were detected, including *Enterobacter asburiae* (2/42, 4.8%), *Enterobacter bugandensis* (1/42, 2.4%), *Enterobacter hormaechei* (1/42, 2.4%), *Escherichia fergusonii* (2/42, 4.8%), *Klebsiella aerogenes* (1/42, 2.4%), *Klebsiella pneumoniae* (1/42, 2.4%), *Klebsiella variicola* (3/42, 7.1%), and *Proteus mirabilis* (1/42, 2.4%; Table 2).

Table 2. Distribution of *Enterobacter*, *Escherichia*, *Klebsiella*, and *Proteus* species isolated from wild animal fecal samples in Vietnam from February to March 2023

Species	**Number of isolates**	**Sources (Number of isolates)**	**Percentage**
Enterobacter asburiae	2	Sika deer (2)	4.8
Enterobacter bugandensis	1	Sika deer (1)	2.4
Enterobacter hormaechei	1	Chevrotain (1)	2.4
Escherichia coli	30	Bamboo rat (3), Binturong (1), Chevrotain (6), Otter (2), Palm civet (6), Sika deer (9), Wildcat (3)	71.4
Escherichia fergusonii	2	Palm civet (2)	4.8
Klebsiella aerogenes	1	Palm civet (1)	2.4
Klebsiella pneumoniae	1	Otter (1)	2.4
Klebsiella variicola	3	Sika deer (2), Bamboo rat (1)	7.1
Proteus mirabilis	1	Otter (1)	2.4

Antimicrobial susceptibility test

Analysis of resistance patterns indicated that 23 out of 30 *E. coli* isolates (76.7%) and the remaining isolates (11/12) were resistant to at least one antimicrobial test (91.7%; Table 3 and Table 4). The resistance patterns were different among *Enterobacteriaceae* isolates, with 17 resistant phenotypes identified in *E. coli* and five in the other species. A total of 12 of 42 isolates (28.6%) were MDR, characterized by resistance to at least three classes of antimicrobial agents. All 42 *Enterobacteriaceae* isolates were tested for antimicrobial susceptibility (Table 5 and Figure 1). The majority of isolates were susceptible to norfloxacin and trimethoprim/sulfamethoxazole, with susceptibility rates of 92.9% and 88.1%, respectively. No isolate exhibited resistance to imipenem. The highest resistance rate was observed for ampicillin (52.4%), followed by ciprofloxacin (30.9%), cefotaxime (26.2%), tetracycline (23.8%), chloramphenicol (21.4%), and streptomycin (21.4%). Low resistance rates to trimethoprim/sulfamethoxazole (11.9%), doxycycline (9.5%), cefoxitin (9.5%), norfloxacin (7.1%), and gentamicin (4.8%) were observed (Table 5).

Table 3. Resistance patterns of *Escherichia coli* isolates in Vietnam from February to March 2023

Patterns	Number of isolates	Sources
CIP	1	Sika deer
CTX	3	Chevrotain, Palm civet, Sika deer
AMP + CTX	2	Bamboo rat, Otter
CIP + CTX	2	Palm civet, Sika deer
CIP + NOR	2	Chevrotain, Otter
CTX + STR	1	Chevrotain
DOX + TET	1	Sika deer
CIP + CTX + STR	1	Sika deer
AMP + CIP + STR + SXT	1	Chevrotain
AMP + CHL + CIP + CTX	1	Bamboo rat
AMP + CHL + CIP + TET	1	Wildcat
AMP + CHL + STR + TET	1	Palm civet
AMP + CHL + CIP + DOX + TET	1	Palm civet
AMP + CHL + CIP + STR + TET	1	Chevrotain
AMP + CHL + GEN + SXT + TET	2	Palm civet, Wildcat
AMP + CHL + CIP + DOX + NOR + SXT + TET	1	Wildcat
AMP + CHL + CIP + CTX + DOX + STR + SXT + TET	1	Bamboo rat

AMP: Ampicillin, CTX: Cefotaxime, FOX: Cefoxitin, CHL: Chloramphenicol, CIP: Ciprofloxacin, DOX: Doxycycline, GEN: Gentamicin, IPM: Imipenem, NOR: Norfloxacin, STR: Streptomycin, SXT: Trimethoprim/Sulfamethoxazole, TET: Tetracycline

Table 4. Resistance patterns of *Enterobacteriaceae* isolates except *Escherichia coli* in Vietnam from February to March 2023

Patterns	Species	Number of isolates	Sources
AMP	*Klebsiella variicola*	3	Bamboo rat, Sika deer
AMP	*Klebsiella pneumoniae*	1	Otter
STR	*Proteus mirabilis*	1	Otter
AMP + FOX	*Enterobacter asburiae*	2	Sika deer
AMP + FOX	*Enterobacter bugandensis*	1	Sika deer
AMP + FOX	*Klebsiella aerogenes*	1	Palm civet
AMP + STR	*Escherichia fergusonii*	1	Palm civet
AMP + STR + TET	*Escherichia fergusonii*	1	Palm civet

AMP: Ampicillin, CTX: Cefotaxime, FOX: Cefoxitin, CHL: Chloramphenicol, CIP: Ciprofloxacin, DOX: Doxycycline, GEN: Gentamicin, IPM: Imipenem, NOR: Norfloxacin, STR: Streptomycin, SXT: Trimethoprim/Sulfamethoxazole, TET: Tetracycline

Table 5. Antimicrobial susceptibility profiles of *Enterobacteriaceae* isolates in Vietnam from February to March 2023

Antimicrobials	Resistant		Intermediate		Susceptible	
	Number	Percentage	Number	Percentage	Number	Percentage
AMP	22	52.4	6	14.3	14	33.3
CHL	9	21.4	1	2.4	32	76.2
CIP	13	31.0	12	28.6	17	40.5
CTX	11	26.2	12	28.6	19	45.2
DOX	4	9.5	3	7.1	35	83.3
FOX	4	9.5	5	11.9	33	78.6
GEN	2	4.8	12	28.6	28	66.7
IPM	0	0	8	19	34	81
NOR	3	7.1	0	0	39	92.9
STR	9	21.4	18	42.9	15	35.7

SXT	5	11.9	0	0	37	88.1
TET	10	23.8	0	0	32	76.2

AMP: Ampicillin, CTX: Cefotaxime, FOX: Cefoxitin, CHL: Chloramphenicol, CIP: Ciprofloxacin, DOX: Doxycycline, GEN: Gentamicin, IPM: Imipenem, NOR: Norfloxacin, STR: Streptomycin, SXT: Trimethoprim/Sulfamethoxazole, TET: Tetracycline. The total number of *Enterobacteriaceae* isolates was 42.

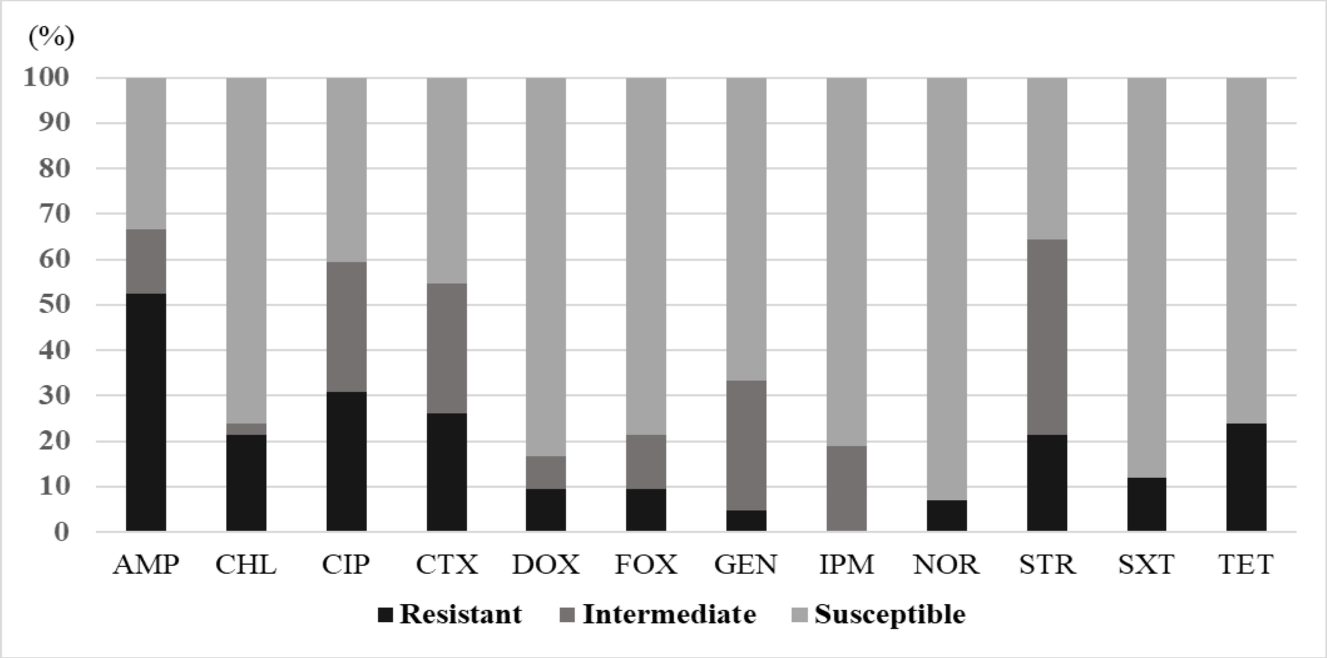

Figure 1. Antimicrobial susceptibility profiles of *Enterobacteriaceae* isolates (n = 42) collected in Vietnam from February to March 2023. Bars represent the number of susceptible, intermediate, and resistant isolates for each antimicrobial agent, as determined by the disc diffusion method according to CLSI guidelines. AMP: Ampicillin, CTX: Cefotaxime, FOX: Cefoxitin, CHL: Chloramphenicol, CIP: Ciprofloxacin, DOX: Doxycycline, GEN: Gentamicin, IPM: Imipenem, NOR: Norfloxacin, STR: Streptomycin, SXT: Trimethoprim/Sulfamethoxazole, TET: Tetracycline

DISCUSSION

According to the current findings, environmental contamination in Vietnam was attributed to antibiotic residues and resistant bacteria originating from livestock and aquaculture productions (Bordier et al., 2018; Phu et al., 2022). The high prevalence of *E. coli* among isolates in the present study is consistent with findings from wildlife and environmental studies worldwide (Murphy et al., 2021; Lagerstrom et al., 2024). This is probably because *E. coli* is commonly found as a gut commensal in both animals and humans, and it is widely used as an indicator organism for fecal contamination and antimicrobial resistance (Ramos et al., 2020). Furthermore, the detection of other clinically important species, including *Klebsiella pneumoniae*, *Enterobacter hormaechei*, and *Proteus mirabilis*, highlighted the diversity of *Enterobacteriaceae* species transmission among wild animals and their potential impacts on both animal and human health (Palusiak, 2022; Asokan et al., 2025).

The antimicrobial susceptibility results demonstrated that most isolates were susceptible to norfloxacin, trimethoprim/sulfamethoxazole, and imipenem, whereas resistance was notably high to ampicillin, ciprofloxacin, and cefotaxime. The AMR patterns identified in the present study were similar to those reported in wild birds and mammals from different regions, where resistance to β-lactams and fluoroquinolones was commonly detected (Radhouani et al., 2013; Alonso et al., 2016; Regassa et al., 2023). The relatively high resistance to ampicillin (52.4%) might be due to the widespread presence of ampicillin-resistant *Enterobacteriaceae* species in the environment; however, the specific resistance mechanisms were not investigated during the present study. While none of the isolates exhibited resistance to imipenem, the presence of resistance to third-generation cephalosporins in 26.2% of isolates was concerning, suggesting the possible emergence of ESBL-producing organisms within wild animals (Husna et al., 2023).

Additionally, the detection of MDR isolates (28.6%) in the present study highlighted the potential role of wild animals as reservoirs of AMR genes. Comparable populations of MDR *Enterobacteriaceae* species have been observed in wild birds across several countries, supporting the idea that wild birds may serve as biological vectors of resistance bacteria. This finding raised concerns regarding the potential role of wild birds in spreading AMR genes and the subsequent effects on public health (Loucif et al., 2022; Zhang et al., 2022; Cocoş et al., 2025). Wild animals residing near human settlements or consuming human waste may consequently be exposed to resistant microorganisms (Beckman et al., 2022; Wierucka et al., 2023).

The high proportion of *E. coli* isolates resistant to at least one antimicrobial agent (76.7%) indicated persistent environmental selective pressure, despite the absence of direct antimicrobial exposure. The diversity of resistance phenotypes among *E. coli* and other *Enterobacteriaceae* species indicated the multiple origins or transmission pathways for resistance factors. Recently, additional mechanisms, including horizontal gene transfer via plasmids, integrons, or transposons, have been shown to play a crucial role in spreading resistance among bacterial populations across different ecological systems (Vinayamohan et al., 2022).

From a One Health perspective, the present study confirmed the presence of MDR *Enterobacteriaceae* species in wild animals, indicating a potential connection among the environmental, animal, and human sources of AMR (Höcketstaller et al., 2025). Wild animals can serve as indicators and carriers of environmental pollution, facilitating the spread of resistant bacteria across wide geographic regions through migration and overlapping habitats (Esposito et al., 2024).

CONCLUSION

The present study identified antimicrobial-resistant (AMR) and multidrug-resistant (MDR) *Enterobacteriaceae* species (28.6%) among bacterial isolates obtained from wild animals in Vietnam. Resistance was most frequently observed to β-lactams and fluoroquinolones, suggesting possible environmental selective pressures affecting wildlife-associated bacteria. The current findings highlighted the importance of including wild animals in AMR surveillance within a One Health framework. This framework would enhance understanding of environmental AMR transmission and its potential implications for public and environmental health. The limited sample size and focus on particular wild animal species and locations might have restricted the present findings. Furthermore, molecular characterization of resistance genes was not conducted, which could have provided further insights into the genetic mechanisms and potential mobility of AMR factors. Future studies should incorporate molecular analyses, larger sample sizes, broader geographic coverage, and investigation of environmental sources to elucidate the transmission pathways of resistant *Enterobacteriaceae* species in wild animals in Vietnam.

DECLARATIONS

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Authors' contributions

Thi Thi Tra Vu and Phuc Pham-Duc designed the study. Van Hieu Dong and Phuc Pham-Duc collected samples. Thi Thu Tra Vu, Thi Thu Ha Hoang, and Thanh Trung Nguyen analyzed samples. Thi Thi Tra Vu, Ha Thai Truong, and Thanh Trung Nguyen analyzed the data and wrote the manuscript. All authors read and approved the final edition of the manuscript.

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Competing interests

The authors declared no conflict of interest.

Ethical considerations

The present study was originally written by the authors and has not been published elsewhere. The authors checked the article for plagiarism and confirmed that the study was written based on their original scientific results. The authors did not use any AI applications to prepare the data, write, or revise the draft of the present study.

Availability of data and materials

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

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