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Antimicrobial Susceptibility of *Enterobacteriaceae* Isolated from Farmed Sambar Deer (*Rusa unicolor*) in Vietnam

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ABSTRACT

Antimicrobial-resistant (AMR) bacteria have been reported worldwide in both livestock and wildlife. However, information on AMR bacteria in captive wild animals, including sambar deer (*Rusa unicolor*) in Southeast Asia, is still limited. This study was conducted to identify and determine the antimicrobial susceptibility of *Enterobacteriaceae* isolated from the feces of sambar deer in Vietnam. Bacteria were isolated from fecal samples of 30 healthy sambar deer at wildlife farms and wildlife rescue centers in Dak Lak, Dong Nai, Nghe An, and Ha Tinh provinces in 2023. After isolation, bacterial identification was performed using MALDI-TOF MS and biochemical tests. The disk diffusion method was used for antimicrobial susceptibility testing. Nine species belonging to the family *Enterobacteriaceae* were identified, including *Citrobacter freundii*, *Enterobacter asburiae*, *Enterobacter bugandensis*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Escherichia coli* (*E. coli*), *Klebsiella variicola* (*K. variicola*), *Pseudocitrobacter faecalis*, and *Raoultella ornithinolytica*, of which *E. coli* accounted for the highest proportion (83.6%), followed by *K. variicola* (4.92%). The *Enterobacteriaceae* isolates showed high susceptibility rates to norfloxacin (95.08%), sulfamethoxazole/trimethoprim (93.44%), and gentamicin (91.80%). Whereas 65.57% of isolates were resistant to at least one antimicrobial, the highest resistance rates to ampicillin (34.43%) and cefotaxime (29.51%) were observed. The *Enterobacteriaceae* isolates had diverse antimicrobial resistant phenotypes; 17 resistant phenotypes of *E. coli* and 5 resistant phenotypes of other bacterial species, especially 6 multidrug-resistant *E. coli* isolates were observed. The present study indicates that it is necessary to improve awareness and practice regarding antibiotic use in captive wild animals, thereby minimizing antibiotic resistance.

Keywords: Antimicrobial susceptibility, *Enterobacteriaceae*, Multidrug resistance, *Rusa unicolor*, Vietnam.

INTRODUCTION

Vietnam is one of the countries where the traditional use of wildlife products for food, medicine, furniture, and fashion has been prevalent for a long time (Nguyen et al. 2021; Green et al. 2022). Therefore, breeding, raising, and trading of captive wildlife are common practices in Vietnam, especially for some profitable species such as crocodiles, palm civets, porcupines, and sambar deer (Van Thu et al. 2023). However, the lack of detailed practice guidelines on husbandry techniques, farm construction, and biosecurity for captive wildlife facilities has made farm management more difficult than conventional livestock farming and has increased the risk of disease transmission (Van Thu et al. 2023; Chan nuoi Vietnam 2024).

The sambar deer (*Rusa unicolor*) is an even-toed ungulate species classified in group II of the list of endangered, rare and precious species, according to Decree No. 84/2021/ND-CP issued by the Vietnamese Government (The Government of Vietnam 2021). Sambar deer is one of the most common wildlife species raised in Vietnam for meat consumption and to obtain antlers,

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with 4,654 individuals in 1,013 captive facilities in 32 provinces across the country in 2021, mainly concentrated in Dak Lak, Dong Nai, and Nghe An provinces (Van Thu et al. 2023). With the large population of captive sambar deer, disease prevention and treatment have become major concerns for farmers. Captive deer in Vietnam usually suffer from bloat, diarrhea, pneumonia, and hemorrhagic septicemia (Nguyen et al. 2015; Chan nuoi Vietnam 2023; Palmer et al. 2025). As a result, antimicrobials might be used for both treatment and prevention. However, this can lead to the risk of development and spread of antimicrobial-resistant (AMR) bacteria through contaminated feces, which could potentially transmit AMR genes into the environment and humans through farming, transport, slaughter, processing, and meat consumption (Udikovic-Kolic et al. 2014; Samtiya et al. 2022). Currently, AMR has become a crucial global health threat in both human and veterinary medicine, with an estimated 5 million AMR-related infection deaths in 2019 (Antimicrobial Resistance Collaborators 2022).

Enterobacteriaceae is a large family of Gram-negative bacteria that are commonly found in the gastrointestinal tract of human and animals, and easily spread into the environment (Kumar 2012; Sanz et al. 2021). *Escherichia coli* (*E. coli*), a member of the family, is considered as an important indicator bacterium and used to monitor AMR changes in different ecosystems (Anjum et al. 2021). AMR of *Enterobacteriaceae* isolated from wild animals has been reported worldwide (Sarker et al. 2019; Elsby et al. 2022; Russo et al. 2022; Sabença et al. 2024); however, previous studies have mainly focused on free-range wildlife, and little is known about farmed wild animals. In addition, information on the antimicrobial susceptibility of bacteria from both free-range and captive wild animals, including sambar deer, is still limited in Vietnam. Therefore, the present study was designed to evaluate the antimicrobial susceptibility of *Enterobacteriaceae* isolated from the feces of sambar deer.

MATERIALS AND METHODS

Sample collection

Thirty fecal samples of healthy sambar deer were collected from wildlife farms and a wildlife rescue center in four provinces (Fig. 1), including Dak Lak (n=10), Dong Nai (n=4), Nghe An (n=12), and Ha Tinh (n=4) between February and March 2023. The samples were kept in a cool box and then transported to the Laboratory of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, VNUA for analysis.

Bacterial isolation and identification

The isolation of *Enterobacteriaceae* in fecal samples was carried out according to the method described in a previous study (Foti et al. 2018). Briefly, samples were homogenized in buffer peptone water (BPW, Merck, Germany), then incubated at 37°C for 24h. The enrichment was streaked onto MacConkey agar (MAC, Merck, Germany) and incubated at 37°C for 24h. All types of colonies on MAC were selected and subcultured on Nutrient agar (NA, Merck, Germany) for biochemical tests and species identification using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) as previously described (Wang et al. 2021). Fresh colonies on NA were transferred onto target polished steel plates. After drying at room temperature, one drop of 1µL of saturated α-cyano-4-hydroxycinnamic acid matrix solution was applied, and the target plates were placed into a Bruker MALDI-TOF MS for measurements. In addition, biochemical tests were performed for all isolates, including Gram staining, catalase, indole, methyl red, Voges-Proskauer, growth on Simmons citrate agar, and Triple Sugar Iron agar.

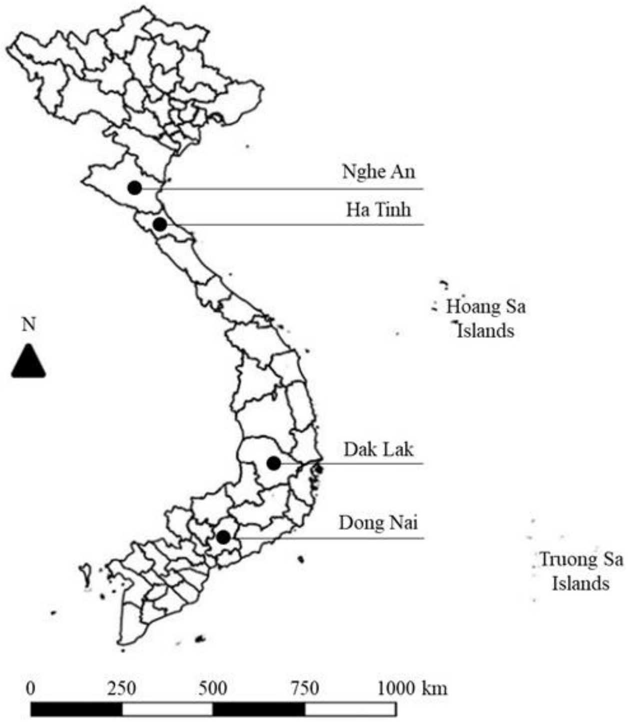

Fig. 1: Locations of the study areas (black circles).

Antimicrobial susceptibility tests

Antimicrobial susceptibility testing of all bacterial isolates was performed using the disk diffusion method on Mueller Hinton agar (MHA, Merck, Germany), and the results were interpreted based on Clinical and Laboratory Standards (CLSI) guidelines (CLSI 2024). Susceptibility to 12 antimicrobial agents were tested, including ampicillin (AMP, 10µg), cefotaxime (CTX, 30µg), cefoxitin (FOX, 30µg), chloramphenicol (CHL, 30µg), ciprofloxacin (CIP, 5µg), doxycycline (DOX, 30µg), gentamicin (GEN, 10µg), imipenem (IPM, 10µg), norfloxacin (NOR, 10µg), streptomycin (STR, 10µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75µg) and tetracycline (TET, 30µg) (all Oxoid, Basingstoke, UK). *E. coli* ATCC 25922 was used for quality control for routine testing.

Statistical Analysis

Descriptive statistics were used to describe the antimicrobial susceptibility profiles and resistance patterns of bacterial isolates.

RESULTS

Identification of *Enterobacteriaceae* isolates

In total, 61 *Enterobacteriaceae* isolates were found in 30 samples (Table 1), of which *E. coli* (51 isolates) was the most dominant species. Other species, including *Citrobacter freundii* (*C. freundii*), *Enterobacter asburiae* (*E. asburiae*), *Enterobacter bugandensis* (*E. bugandensis*), *Enterobacter hormaechei* (*E. hormaechei*), *Enterobacter kobei* (*E. kobei*), *Klebsiella variicola* (*K. variicola*), *Pseudocitrobacter faecalis* (*P. faecalis*), and *Raoultella ornithinolytica* (*R. ornithinolytica*) were also identified.

Table 1: *Enterobacteriaceae* isolates from sambar deer's feces included in this study (n=61)

Species	No. of isolates	Percentage
Citrobacter freundii	1	1.64
Enterobacter asburiae	1	1.64
Enterobacter bugandensis	1	1.64
Enterobacter hormaechei	1	1.64
Enterobacter kobei	1	1.64
Escherichia coli	51	83.60
Klebsiella variicola	3	4.92
Pseudocitrobacter faecalis	1	1.64
Raoultella ornithinolytica	1	1.64

Antimicrobial susceptibility test

All 61 *Enterobacteriaceae* isolates were tested for antimicrobial susceptibility (Fig. 2). Most of the isolates were susceptible to norfloxacin, trimethoprim/sulfamethoxazole, and gentamicin (95.08, 93.44 and 91.8%, respectively). Whereas, resistance to ampicillin (34.43%) was the most frequent, followed by cefotaxime (29.51%), tetracycline (18.03%), ciprofloxacin (14.75%), streptomycin (13.11%), chloramphenicol (9.84%), cefoxitin (8.2%) and trimethoprim/sulfamethoxazole (6.56%). The lowest resistance rates (3.28%) to doxycycline, gentamicin, and norfloxacin were observed.

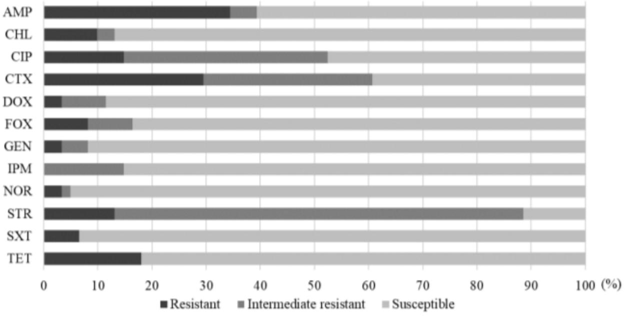

Fig. 2: Antimicrobial susceptibility profiles of *Enterobacteriaceae* isolates; Ampicillin: AMP; Cefotaxime: CTX, Cefoxitin: FOX, Chloramphenicol: CHL; Ciprofloxacin: CIP, Doxycycline: DOX; Gentamicin: GEN; Imipenem: IPM; Norfloxacin: NOR; Streptomycin: STR; Trimethoprim/sulfamethoxazole: SXT; Tetracycline: TET.

Resistance pattern analysis revealed that 30 out of 51 *E. coli* isolates (58.82%) and all other bacterial species exhibited resistance to at least one of the tested antimicrobials (Table 2 and 3). The resistance patterns varied among *Enterobacteriaceae* isolates; seventeen resistant phenotypes of *E. coli* and five resistant phenotypes of other bacterial species, particularly 6 multidrug-resistant (MDR) *E. coli* isolates (resistant to at least three classes of antimicrobials) were found.

Table 2: Resistance patterns of *E. coli* isolates (n=30)

No.	Patterns	No. of isolates	Sources	Percentage (%)
1	AMP	4	Ha Tinh, Nghe An	13.33
2	CIP	2	Dong Nai, Nghe An	6.67
3	CTX	5	Dak Lak, Dong Nai, Nghe An	16.67
4	STR	2	Ha Tinh, Nghe An	6.67
5	TET	2	Ha Tinh, Nghe An	6.67
6	AMP+CTX	2	Dong Nai, Nghe An	6.67
7	CHL+TET	1	Dak Lak	3.33
8	CIP+CTX	2	Dak Lak, Nghe An	6.67
9	GEN+TET	1	Nghe An	3.33
10	SXT+TET	1	Nghe An	3.33
11	AMP+CIP+CTX	2	Dak Lak, Nghe An	6.67
12	AMP+SXT+TET	1	Dong Nai	3.33
13	AMP+CHL+CTX+STR+TET	1	Ha Tinh	3.33
14	CHL+DOX+NOR+STR+TET	1	Nghe An	3.33
15	AMP+CHL+CTX+GEN+STR+TET	1	Nghe An	3.33
16	AMP+CHL+CIP+DOX+STR+SXT+TET	1	Nghe An	3.33
17	AMP+CHL+CIP+CTX+NOR+STR+SXT+TET	1	Nghe An	3.33

Ampicillin: AMP; Cefotaxime: CTX; Chloramphenicol: CHL; Ciprofloxacin: CIP, Doxycycline: DOX; Gentamicin: GEN; Norfloxacin: NOR; Streptomycin: STR; Trimethoprim/sulfamethoxazole: SXT; Tetracycline: TET.

Table 3: Resistance patterns of other *Enterobacteriaceae* isolates except *E. coli* (n=10)

No.	Patterns	Species	No. of isolates	Sources
1	AMP	*K. variicola*	3	Dak Lak, Nghe An
	AMP	*R. ornithinolytica*	1	Nghe An
2	AMP+FOX	*E. kobei*	1	Dak Lak
3	CIP+CTX	*P. faecalis*	1	Dong Nai
4	FOX+STR	*C. freundii*	1	Dak Lak
5	AMP+CTX+FOX	*E. asburiae*	1	Nghe An
	AMP+CTX+FOX	*E. bugandensis*	1	Dong Nai
	AMP+CTX+FOX	*E. hormaechei*	1	Nghe An

Ampicillin: AMP; Cefotaxime: CTX, Cefoxitin: FOX; Ciprofloxacin: CIP; Streptomycin: STR.

DISCUSSION

There have been several publications worldwide on the identification of *Enterobacteriaceae* in wild animals; however, studies on captive wild animals are limited. Moreover, a few bacterial species of the *Enterobacteriaceae* family were isolated in previous studies. In this study, the identification results showed that among the isolated bacterial species, *E. coli* accounted for the highest proportion (83.61%), similar to the findings of Gnat et al. (2015), who revealed that *E. coli* was the most abundant species among *Enterobacteriaceae* isolates from 120 fecal samples of wild red deer (*Cervus elaphus*) in Poland, Hungary and Slovakia, followed by *Enterobacter cloacae*, *Providencia rustiganii*, *Rahnella aquatilis*, and *Enterobacter amnigenus*. A study of Laaksonen et al. (2017) reported that Shiga toxin-producing *E. coli* were found in 32.6% of samples, while none of *Salmonella* spp. were detected in 470 fecal samples of reindeer (*Rangifer tarandus tarandus*) slaughtered in Finland and Norway. Another study in the UK reported that the prevalences of *E. coli* carrying the *astA* gene and *Salmonella* spp. isolated from wild, farmed, park, and zoo deer feces were 61.2% and 1.6%, respectively (Pearce et al. 2023).

Some of the other bacterial species detected in this study might be considered as potential causes of diseases in animals and humans. *C. freundii* was reported to cause an outbreak of septicemia and encephalitis in sheep in central China in 2014 (Liu et al. 2018). In human, *C. freundii* is an opportunistic pathogen frequently isolated from urinary tract infections (UTIs), surgical wounds, respiratory infections, and gastrointestinal infections (Jabeen et al. 2023). Similarly, *K. variicola* has been associated with respiratory tract infections and UTIs in humans worldwide (Rodriguez-Medina et al. 2019). On the other hand, bloodstream infections and outbreaks of neonatal sepsis caused by *K. variicola* were described previously (Rodriguez-Medina et al. 2019). In addition, *K. variicola* was isolated from horses with respiratory diseases and bovine mastitis (Podder et al. 2014; Mondo et al. 2021). *E. hormaechei* was found to cause respiratory diseases in calves and sheep (Wang et al. 2020; Shi et al. 2022). Whereas, *E. bugandensis* and *E. kobei* have been isolated from outbreaks of neonatal sepsis (Doijad et al. 2016; Manandhar et al. 2022).

Antimicrobials are widely used for the treatment and control of animal diseases, which leads to the development and increase of AMR bacteria. On the other hand, wild animals and captive wildlife in the environments shared with humans and domesticated animals also contribute to the spread of AMR bacteria (Dolejska and Literak 2019; Baros Jorquera et al. 2021). In this study, among 12 antimicrobial agents tested, *E. coli* isolates showed the highest resistance rates to cefotaxime (27.45%), ampicillin (25.49%), and tetracycline (21.57%). This finding is consistent with Elsby et al. (2022), who reported the same resistance rate of *E. coli* isolated from wild deer in Scotland toward tetracycline (21.8%). Similarly, an investigation of AMR *E. coli* isolated from the feces of captive musk deer (*Moschus berezovskii*) in Sichuan, China, revealed that the majority of the strains were resistant to sulfamethoxazole (85.99%), ampicillin (26.11%), tetracycline (24.84%), and cefotaxime (24.2%) (Liu et al. 2023). Whereas, higher resistance rates of *E. coli* isolated from fecal samples of wild deer were observed in a previous study in Bangladesh, with 90.63% of *E. coli* isolates being resistant to ampicillin and sulfamethoxazole, 87.5% were resistant to tetracycline, and 53.13% were resistant to chloramphenicol (Sarker et al. 2019). In addition, another study conducted in Northeastern China revealed that all *E. coli* isolates from farm-raised sika deer (*Cervus nippon*) showed resistance to at least one of the antimicrobials tested, while the most frequently resistant antimicrobials were sulfadiazine (89.5%), sulfamethazine (82.7%), tetracycline (80.0%), ampicillin (71.4%), amoxicillin (67.7%), and chloramphenicol (65.0%) (Li et al. 2013).

Our results showed that 11.76% (6/51) *E. coli* isolates were MDR. This is inconsistent with the findings of Sarker et al. (2019) and Liu et al. (2023), who reported higher rates of MDR *E. coli* isolates in wild deer in Bangladesh (96.88%) and in captive musk deer in China (24.2%), respectively.

According to Circular No. 10/2016/TT-BNNPTNT of the Ministry of Agriculture and Rural Development in 2016, chloramphenicol and ciprofloxacin have been banned for use in terrestrial animals. However, resistance of bacteria against these two antibiotics was found in this study to be 9.84% and 14.75%, respectively. Furthermore, resistance to cefoxitin (a second-generation cephalosporin) and cefotaxime (a third-generation cephalosporin) was also observed. Similarly, the study of Liu et al. (2023) revealed that 10.19% and 15.92% of *E. coli* isolates from captive musk deer in China were resistant to chloramphenicol and ciprofloxacin, respectively. In a study conducted in northeastern Ohio, USA, Ballash et al. (2022) reported that cephalosporin-resistant Enterobacterales were detected in 10.1% of fecal samples from wild, free-ranging white-tailed deer (*Odocoileus virginianus*). The World Health Organization (WHO) has updated the list of medically important antimicrobials, in which third-generation cephalosporins and quinolones are defined as the highest priority critically important antimicrobials (Tompkins and Van Duin 2021; WHO 2024). Thus, the moderate resistance rates to cefotaxime and ciprofloxacin raise concerns about the source and dissemination of resistant bacteria in sambar deer farming in Vietnam.

The study, however, had some limitations, as the small sample size may not represent the whole picture of AMR status in sambar deer farms in Vietnam. Moreover, information on the use of antimicrobials was not collected, and AMR genes were not analyzed in this study.

Conclusion

The study demonstrated high rates of AMR among *Enterobacteriaceae* isolates from the feces of farmed sambar deer with diverse resistance patterns. Such findings highlights the potential risk posed by wildlife-associated farming practices in contributing to the spread of AMR. Various factors including improper management of waste and manure could lead to the spread and propagation of AMR bacteria into the surrounding environment, posing a threat to public health. To address these concerns, it is essential to implement effective antimicrobial stewardship programs and enhance waste management practices in sambar deer farms. Moreover, future research should focus to investigate the genetic mechanisms of AMR as well as the role of environmental contamination in sambar deer farms.

DECLARATIONS

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Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability: The data presented in this study are available from the corresponding author upon reasonable request.

Ethics Statement: This study received approval from the Committee on Animal Research and Ethics (CARE) of the Faculty of Veterinary Medicine, Vietnam National University of Agriculture (Approval No. CARE-2023/12). All procedures were conducted in compliance with the CARE guidelines.

Author's Contribution: Thi Thi Tra Vu and Thomas Alter designed the study. Van Hieu Dong and Phuc Pham-Duc collected samples. Thi Thu Tra Vu, Thi Thu Ha Hoang, Khanh Chau Tran, Thi Hoa Nguyen, Thi Thu Ha Cam analyzed samples. Thi Hoang Yen Nguyen and Tran Anh Dao Bui analyzed the data. Thi Thu Tra Vu wrote the manuscript. All authors read and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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