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Prevalence and Antimicrobial Susceptibility of *Escherichia coli* Isolated from Goats in the Mekong Delta, Vietnam

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ABSTRACT

Escherichia coli is one of the severe pathogens causing severe diarrhea and resistance to antibiotics in domestic animals, including goats. From April to June 2023, 122 fresh feces of hybrid Boer goats of different ages and genders were collected randomly in the small-scale farms in the Mekong Delta, Vietnam, to clarify the prevalence and antibiotic resistance of *E. coli* isolated from those feces. By the traditional culture method, of 122 samples, 87 fecal samples were positive for *E. coli* (71.31%). There were no statistically significant differences in the prevalence of *E. coli* among male or female goats and ages (< 6 months and ≥ 6 months). *E. coli* was detected in goats over 6 months and under 6 months at 76.56% and 65.52%, respectively, while 88.20% and 85.42% in male and female goats. The antimicrobial susceptibility of *E. coli* strains to 7 examined antibiotics was conducted using the Kirby-Bauer disk diffusion method. The results indicated that *E. coli* was sensitive 100% to colistin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefuroxime (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), and 87.50% to ampicillin (10 µg) and bactrim (trimethoprim/sulfamethoxazole, 1.25/23.75 µg), respectively. However, those *E. coli* strains were highly resistant to streptomycin (93.75%), and 93.67% of *E. coli* strains were resistant to one to three antibiotics. Among them, the resistant pattern of Ge+Sm (gentamycin + streptomycin) was the most frequent detection (43.75%). The prevalence rate of antibiotic resistance genes (*blaampC*, *tetA*, *qnrA*, *strA*, and *sulII*) in *E. coli* strains isolated from goat feces was detected by PCR. Among them, gene *blaampC* was the most predominant (96.88%), followed by *qnrA* (68.75%). Furthermore, 81.25% of *E. coli* strains harbored two to five antibiotic-resistance genes, and the gene pattern of *blaampC + tetA + qnrA* was the most popular (21.88 %). The antibiotic resistance and harbored antibiotic resistance genes in *E. coli* strains isolated from goat feces increase animal and public health concerns.

Keywords: Antibiotic resistance gene, Antimicrobial susceptibility, *E. coli*, Goat, Small-scale farm

INTRODUCTION

Goat farming has been increasingly developing and accounting for an increasing structure in the livestock industry in the Mekong Delta, Vietnam, because goats can adapt to climate change's effects (Van Thu, 2018). However, several pathogens can infect goats during raising, and diseases caused by *Escherichia coli* often occur frequently (Begum et al., 2016). If not treated promptly, animals susceptible to this pathogen could die or be stunted or grow slowly; from there, it reduces economic efficiency in livestock farming. *Escherichia coli* is a typical habitat in the mammalian gut flora, especially in the lower intestine of mammals. Meshram et al. (2009) reported that *E. coli* causing diarrhea was an opportunistic disease associated with sloppy environmental conditions, poor sanitation, and poor management practices. Islam et al. (2016) in Bangladesh recorded a positive rate of *E. coli* in the rectum of goats at 52.0%, and young goats were very susceptible. *E. coli* present in the feces of goats can become a source of animal disease and contamination for the farm environment.

On the other hand, previous studies revealed that livestock ruminants are the primary reservoirs of this crucial foodborne pathogen (Bosilevac et al., 2015; Al-Ajmi et al., 2020; Gonzalez and Cerqueira, 2020). Goats have also emerged as critical reservoirs of *E. coli* transmission into humans through food contamination by animal feces (La Ragione et al., 2009; Carlos et al., 2010; Al-Ajmi et al., 2020). In previous reports, *E. coli* was detected rapidly from skin leather, fecal samples, and meats in goats. More than 90% of goat meat samples in Tanzania have reported positive for *E. coli* (Mwanyika et al., 2016). The goat skin leather and fecal samples revealed the presence of *E. coli* contamination at the rates of 1.7% and 19.7%, respectively, in the United States and Mexico (Hanlon et al., 2018), while there was at least 2.4% in goat's feces in Saudi Arabia (Bosilevac et al., 2015).

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Furthermore, the overuse of antibiotics in livestock has caused an increase in antibiotic-resistant pathogens, especially *E. coli*. Even though humans could be infected with those *E. coli* strains through consuming contaminated animal products, a risk of transmission of drug-resistant genes between different strains is also presented (Mellata, 2013; Kumar et al., 2020; Rahman et al., 2021). *Escherichia coli* isolated from domestic animals could be resistant to several antibiotics, such as erythromycin, tetracycline, ampicillin, gentamicin, sulfamethoxazole/trimethoprim, chloramphenicol, kanamycin, and streptomycin (Yamamoto et al., 2014; Abbassi et al., 2017; Massé et al., 2021; Pascu et al., 2022). The resistance phenomenon causes economic loss for farmers because of costs incurred in treatment failure and a prolonged period of treatment of bacterial infections (Bengtsson and Greko, 2014; Wu et al., 2021). Moreover, multi-drug resistance has become a critical global issue, especially *E. coli* (Loayza et al., 2019; Abdalla et al., 2021). Manishimwe et al. (2021) reported that 5.9% of goat fecal samples containing *E. coli* and *Salmonella* strains exhibited a multidrug-resistant (MDR) phenotype in the east province of Rwanda, Africa. This reveals a significant potential hazard to the health of humans and animals in those provinces of Rwanda.

Therefore, the present study aimed to clarify the prevalence and antibiotic resistance of *E. coli* isolated from the feces of goats raised in small-scale farms in the Mekong Delta, Vietnam.

MATERIALS AND METHODS

Ethical approval

This study was conducted by collecting samples according to the guidelines outlined in the Helsinki Declaration and the animal welfare and safety procedures of Can Tho University, Vietnam.

Sample collection

A total of 122 healthy goat fecal samples of all ages and genders were collected randomly at a total of six small-scale goat meat farms (< 30 herds/farm) in O Mon district, Can Tho City, and Chau Thanh district, Hau Giang province in the Mekong Delta, Vietnam, from April to June 2023. Those goats were hybrid Boer goats, including male goats (n = 74) and female goats (n = 48). In addition, those goats were at < 6 months of age (n = 58) and ≥ 6 months of age (n = 64). Those goats were fed different diets on those farms, including fresh grass and commercial feed. In this cross-sectional study, feces (25 grams) were collected directly in the morning after goats had shed feces on the sterilized plastic covers put under cages when collecting samples. Those goats were randomly selected at each row of cages in those farms. After that, the feces samples were kept in separate sterilized plastic bags in cool conditions (4^{o}C) for transport to the laboratory to detect *E. coli* within 24 hours at the Veterinary Food Hygiene Lab, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Vietnam.

Insolation and identification

The isolation of *E. coli* was carried out according to Vietnamese National Standard TCVN 5155-90 and the guidelines of Barrow and Feltham (2003). The feces samples were incubated in buffered peptone water broth (BPW, Merck, Germany) to enrich *E. coli* in samples. After incubating at 37^{o}C for 24 h, one loop of enrichment broth of each sample was cultured on MacConkey agar (MC, Merck, Germany) for further incubation at 37^{o}C for 24 h. All suspicious colonies of *E. coli* were subculture on nutrient agar (NA, Merck, Germany) for further incubation at 37^{o}C for 24 h to examine biochemical tests following the guidelines of Barrow and Feltham (2003). Those confirmed *E. coli* strains were cultured on trypticase soy agar (TSA, Merck. Germany) and incubated at 37^{o}C for 24 h. Then, those *E. coli* strains were examined for antimicrobial susceptibility, and DNA was extracted for PCR to detect antibiotic-resistance genes.

Antimicrobial susceptibility

After identifying *E. coli*, 32 representative strains were selected to examine the antibiotic sensitivity. Those strains represented the genders and ages of goats in the surveyed farms. The Kirby-Bauer's agar plate diffusion method was used to examine the sensitivity of bacteria to antibiotics (Bauer et al., 1966). The results were compared with CLSI standards (2022) to evaluate the sensitivity level of bacteria to antibiotics. *Escherichia coli* ATCC 25922 was used as control quality, and the results were compared to the standards of CLSI (2022). Those strains, which were intermediate susceptibility, were accounted as susceptible strains.

The antibiotic discs were used in this study, including ampicillin (Am, 10 µg), amoxicillin/clavulanic acid (Ac, 20/10 µg), cefuroxime (Cu, 30 µg), gentamycin (Ge, 10 µg), colistin (Co, 10 µg), streptomycin (Sm, 10 µg), doxycycline (Dx, 30 µg), ciprofloxacin (Ci, 5 µg), and trimethoprim/sulfamethoxazole (Bt, 1.25/23.75 µg). Those antibiotic discs were supplied by Nam Khoa Biotek Ltd., Vietnam.

Prevalence of antibiotic-resistance genes

Thirty-two *E. coli* strains were examined for antimicrobial susceptibility and used to detect antibiotic-resistance gene prevalence. The DNA of 32 *E. coli* strains was extracted using the heat-shock method and stored at -20^{o}C for use in this experiment (Ahmed and Dablool, 2017). The PCR reaction used Mastermix 2X (Bioline, Canada) in a total of 25 µl: Mastermix 2X (12.5 µl), forward primer (0.5 µl), reverse primer (0.5 µL), distilled water (9.5 µL), and DNA template (2.0 µL). The primer sequences and PCR conditions were conducted following the guidelines for *blaampC* (Caroff et al., 1999), *qnrA* (Cattoir and Nordmann, 2009), *tetA* (Randall et al., 2004), *strA* (Carattoli et al., 2002), and *sulII* (Sáenz et al., 2004). In this study, the negative control was distilled water without DNA and RNA, while the positive controls were *E. coli* strains, which harbored these genes, isolated from cattle (cows, beef cattle) previously in the Mekong Delta and kept in Veterinary Food Hygiene Lab., Faculty of Veterinary Medicine, College of Agriculture, Can Tho University.

Statistical analysis

The Chi-square test was used to determine the difference in the prevalence of *E. coli* in goats and antibiotic resistance among those strains. The Pearson chi-square statistic was used at the significance level of 95% in the Minitab 17.0 software (Minitab Pty Ltd, Australia).

RESULTS

Of 122 goat fecal samples (Table 1), *E. coli* was detected in 87 samples at a high rate (71.31%). Moreover, there were no significant differences in the prevalence of *E. coli* in goat feces between genders and ages ($p > 0.05$). *E. coli* was detected at 88.20% and 85.42% in male and female goats, respectively, while at 65.52% and 75.56% in goats under 6 months and over 6 months.

The antimicrobial susceptibility test indicated that those *E. coli* strains were still susceptible to most examined antibiotics (Table 2), such as amoxicillin-clavulanic acid (100%), cefuroxime (100%), doxycycline (100%), ciprofloxacin (100%) and colistin (100%). However, these *E. coli* strains showed significant resistance to aminoglycoside antibiotics, including streptomycin (93.75%) and gentamycin (43.75%). Of 32 examined *E. coli* strains, 93.67% resisted one to three antibiotics (Table 3). Among them, the pattern of Ge + Sm (gentamycin + streptomycin) was the most frequent (43.75%), and Sm was present in all antibiotic-resistance patterns.

Moreover, those *E. coli* strains harbored various antibiotic-resistance genes (Table 4). Gene *blaampC* (96.88%) was the most detected from those *E. coli* strains, followed by *qnrA* (68.75%), *tetA* and *sulII* (40.63%), and *strA* (18.75%). Of 32 *E. coli* strains, 81.25% harbored multiple antibiotic-resistance genes from two to five genes (Table 5). The *blaampC* + *tetA* + *qnrA* pattern was the most predominant (21.88%).

Table 1. Prevalence of *Escherichia coli* isolated from feces of meat goats in small-scale farms in the Mekong Delta, Vietnam, from April to June 2023

Variable		No. of examined samples	No. of positive samples	Percentage
Gender	Male	74	66	88.20
	Female	48	41	85.42
				($p > 0.05$)
Age	< 6 months	58	38	65.52
	≥ 6 months	64	49	76.56
				($p > 0.05$)
Total		122	87	71.31

No: Number

Table 2. Antimicrobial susceptibility of *Escherichia coli* strains isolated from feces of meat goats in the Mekong Delta, Vietnam, from April to June 2023 (n = 32)

Antibiotic group	Antibiotic	Code	Sensitive		Resistant	
			No. of positive strains	Percentage	No. of positive strains	Percentage
Beta-lactam	Ampicillin	Am	28	87.50	4	12.50
	Amoxicillin-clavulanic acid	Ac	32	100.00	0	0.00
	Cefuroxime	Cu	32	100.00	0	0.00
Aminoglycoside	Gentamycin	Ge	18	56.25	14	43.75
	Streptomycin	Sm	2	6.25	30	93.75
Tetracycline	Doxycycline	Dx	32	100.00	0	0.00
Polypeptide	Colistin	Co	32	100.00	0	0.00
Quinolone	Ciprofloxacin	Ci	32	100.00	0	0.00
Sulfonamide	Bactrim*	Bt	28	87.50	4	12.50

*Trimethoprim/sulfamethoxazole; No: Number

Table 3. Antibiotic-resistance patterns of *Escherichia coli* isolated from feces of meat goats from April to June 2023 in Vietnam (n = 32)

No. of antibiotic	Pattern	No. of positive strains	Percentage
1	Sm	11	34.38
2	Am + Sm	1	3.13
	Ge + Sm	14	43.75
	Bt + Sm	1	3.13
3	Am + Bt + Sm	3	9.38
			($p < 0.05$)
Total		30	93.67

Am: Ampicillin; Bt: Bactrim; Ge: Gentamycin; Sm: Streptomycin; No: Number

Table 4. Prevalence of antibiotic-resistance genes in *Escherichia coli* isolated from feces of meat goats from April to June 2023 in Vietnam (n = 32)

Antibiotic group	Gene	No. of positive strains	Percentage
Beta-lactam	bla_{ampC}	31	96.88
Aminoglycoside	*strA*	6	18.75
Quinolone	*qnrA*	22	68.75
Tetracycline	*tetA*	13	40.63
Sulfonamide	*sulII*	13	40.63
			($p < 0.05$)

No.: Number

Table 5. Multiple antibiotic-resistance gene patterns of *Escherichia coli* strains isolated from feces of meat goats from April to June 2023 in Vietnam (n = 32)

No. of resistant genes	Pattern	No. of positive strains	Percentage
2	*blaampC+tetA*	5	15.63
	blaampC+sulII	1	3.13
	blaampC+qnrA	2	6.25
	blaampC+strA	1	3.13
3	*blaampC+tetA+qnrA*	7	21.88
	blaampC+tetA+sulII	5	15.63
4	*blaampC+tetA+qnrA+sulII*	4	12.50
5	*blaampC+tetA+qnrA+strA+sulII*	1	3.13
Total		26	81.25

No: Number

DISCUSSION

In this study, *E. coli* was isolated from goat feces in small-scale farms at a relatively high rate (71.31%). The previous reports indicated that *E. coli* was an enteropathogen frequently isolated from fecal samples of small ruminants, such as in sheep (34.7%) in Trinidad, goats in Germany and Egypt at 75.3% and 30.7%, respectively (Zschock et al., 2000; Adesiyun et al., 2001; Osman et al., 2013). Shabana and Al-Enazi (2020) also reported that *E. coli* was detected at a high rate (92.1%) in goat feces in Al-Madinah, Saudi Arabia. On the other hand, Adesiyun et al. (2001) and Shabana et al. (2017) reported that age was a significant factor affecting the occurrence of diarrhea caused by enteropathogens, including *E. coli*, and the prevalence of enteropathogens in young animals was higher than in older animals. However, this study showed that *E. coli* was present in the feces of meat goats and did not depend on gender or age. This difference could be due to the number of samples and the age of animals at the collecting times.

The antibiotic resistance of bacteria, including *E. coli*, was a considerable challenge in treating and preventing diseases in animals and humans (Bengtsson and Greko, 2014; Loayza et al., 2019; Abdalla et al., 2021; Wu et al., 2021). Although *E. coli* strains isolated from meat goats in this study were still sensitive to several antibiotics, they showed significant resistance to streptomycin (93.75%) and gentamycin (43.75%). Those antibiotics were commonly used to treat goat diseases in those examined farms, and the farmers used antibiotics mainly depending on their experiences. Besides, the hygiene status in those surveyed small-scale farms was poorly managed, the feces were not cleaned up, and

other animals could enter the farms. Pathogens, including antibiotic-resistant bacteria from feces or the environment, such as *E. coli*, could contaminate and spread in those farms. Pehrsson et al. (2016) reported that antibiotic-resistant *E. coli* and resistance genes could be transmitted between pathogens and benign microbes from diverse habitats through environments contaminated with feces. Obaidat et al. (2017) reported that *E. coli* isolated from sheep and goat farms in Jordan highly resisted tetracycline (45.5%), ampicillin (35.4%), and streptomycin (32.30%) but were sensitive to gentamycin (93.80%). *E. coli* strains isolated from goats in Bangladesh showed high resistance to ampicillin (65.38%), amoxicillin-clavulanic acid (60.26%), trimethoprim–sulfamethoxazole (52.56%), tetracycline (51.28%), streptomycin (47.44%), and gentamicin (37.18%) (Islam et al., 2016). Ndegwa et al. (2019) reported that most *E. coli* strains isolated from pastured goats in Virginia (USA) were resistant to tetracycline (51.00%), streptomycin (30.00%), and they also resisted ampicillin (19.00%) which had never been used on the farm. Moreover, *E. coli* isolated from goats in this region was highly resistant to tetracycline because of a history of previous use of tetracycline for treatment on the farm. Thus, the difference in antibiotic resistance levels could be due to the characteristics of using antibiotics in husbandry in each region.

Moreover, this study exhibited that *E. coli* strains isolated from the feces of meat goats in small-scale farms of the Mekong Delta could be multi-drug resistant to three antibiotics used frequently in this region. The pattern of Ge + Sm (gentamycin + streptomycin) was the most popular, consistent with the high resistance performance to gentamycin and streptomycin in the antimicrobial susceptibility test in this study. This could be due to the frequency of using those antibiotics in goats in the surveyed farms; thus, *E. coli* strains have established a high resistance to those examined antibiotics. The multi-drug resistance phenomenon of *E. coli* isolated from small ruminants was recorded in previous research in other regions. *E. coli* strains, originating from goats in Bangladesh, were multidrug-resistant to three to eight subclasses of antimicrobials (Islam et al., 2016). Obaidat et al. (2017) found that approximately one-third of both *E. coli* and *Salmonella enterica* isolated from small ruminant herds of rural Jordan, which were less used antimicrobials, were also multidrug resistant. Nsofor and Iroegbu (2012) indicated that the average number of antibiotic-resistance phenotypes of *E. coli* was significantly higher for goat and poultry than for cattle and swine. It revealed a significant public health concern in Southeast Nigeria that multidrug-resistant *E. coli* strains might become a potential reservoir of resistance genes to be transferred to other pathogenic bacteria. Prapasawat and Intarapuk (2021) showed that *E. coli* isolated from the feces of dairy goats in Thailand were resistant to at least one antimicrobial agent by disc diffusion method, especially streptomycin (65.6%), and 23.9% of those isolated *E. coli* strains were multidrug resistant. In addition, dairy goats in farms could become a reservoir and possibly spread antibiotic-resistant isolates to farmers and consumers via animals and their products in Thailand.

In this study, *E. coli* strains isolated from the feces of meat goats harbored genes *blaampC* and *qnrA* at a high rate. However, those *E. coli* strains did not show much resistance to beta-lactam and quinolone antibiotics in the antimicrobial susceptibility test. Whereas *E. coli* strains had high resistance to aminoglycoside antibiotics, *strA* was detected at the most minor rate (18.75%). Thus, the antibiotic resistance performance of *E. coli* strains might be affected by other factors, such as the pressure of using antibiotics, a combination of several genes, environmental factors, etc. (Bengtsson-Palme et al., 2017; Chen et al., 2019; Liu et al., 2020). Resistance genes could be acquired through natural mutations and transferred to the next generation or due to conjugation, transduction, or mutation of resistance genes between bacteria species (Sommer et al., 2017). Moreover, antibiotic-resistance genes are silent resistance genes that do not usually express or express at low levels, even when exposed to antibiotics (Stasiak et al., 2021). Hasan et al. (2014) reported that *E. coli* harbored antimicrobial-resistant genes, could transmit among species, and confer resistance to common antibiotics like penicillins, tetracycline, gentamicin, cephalosporins, and carbapenems. Shabana and Al-Enazi (2020) indicated that genes *rmtB*, *CTX-M*, and *qnr* were detected in healthy, diarrheic sheep and goats in Saudi Arabia. Those genes were present in all aminoglycoside-resistant *E. coli*, ESBL-producing *E. coli*, and fluoroquinolone-resistant *E. coli*, respectively.

In addition, those *E. coli* strains isolated from the feces of meat goats in this study also harbored multiple antibiotic-resistance genes. It indicated that those strains could highly resist several antibiotics and combine antibiotic-resistant effects, such as beta-lactam and quinolone antibiotics. Moreover, the multiple antibiotic resistance of *E. coli* strains could cause failure in treatment for goats and humans in the Mekong Delta. Even though bacterial populations that are exposed to various antibiotics can develop tolerance to these antibiotics, it is possible for one antibiotic in a combination to counter resistance to another antibiotic and offer effective treatment. However, if tolerance has already emerged for one antibiotic, the combination may inadvertently facilitate the spread of resistance to the partner antibiotic (Liu et al., 2020). Zhao et al. (2020) also reported that certain antibiotics, such as cephalexin, chloramphenicol, kanamycin, sulfamethazine, and tetracycline, enhanced the co-selection of antibiotic-resistance genes related to other antibiotic classes. Tenover (2006) indicated that multidrug resistance could be due to combined multiple resistance mechanisms. Multidrug resistance could be expressed in pathogens due to continuous exposure to an antibiotic or the acquisition of

genetic resistance elements through plasmids or transposons. The antibiotic resistance of *E. coli* via resistance genes can be obtained through horizontal gene transfer to make multi-drug resistant strains (Huddleston, 2014). The research of Van Hoek et al. (2023) showed that Shiga-toxin-producing *E. coli* (STEC) isolated from dairy goats and sheep farms in the Netherlands could harbor various antibiotic-resistance gene patterns, including genes *blaTEM*, *sul*, *aadA1*, *strA*, *tetA*, and *dfrA*. A few strains shared the same genetic pattern with STEC isolates from humans; it revealed that those *E. coli* strains could infect and cause severe diseases in humans.

CONCLUSION

The prevalence of *E. coli* in the feces of meat goats in small-scale farms in the Mekong Delta, Vietnam, was high and has no relation to gender and age. Although *E. coli* strains were still susceptible to most examined antibiotics, those isolated *E. coli* strains showed significant resistance to aminoglycoside antibiotics, and several antibiotic-resistant patterns were obtained. Moreover, those *E. coli* strains isolated from goat feces harbored antibiotic-resistance genes, especially *blaampC*, at a high rate. Further research should be conducted to clarify the antibiotic-resistance characteristics of *E. coli* in goats of the study area and control multi-drug-resistant *E. coli* strains to protect animals and public health.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Thuan K. Nguyen, Binh C. Tran, and Trung T. Truong conceptualized, designed, and supervised the research. Thuan K. Nguyen critically reviewed the study. Binh C. Tran, Vy L.P. Nguyen, and Trung T. Truong collected samples and processed the data. Binh C. Tran analyzed and interpreted the data generated. All authors revised and approved the final edition of the manuscript.

Availability of data and materials

The authors of this article confirm that all data supporting the findings of this research are available upon reasonable request.

Ethical considerations

The authors considered farmers' ethical concerns and consent before conducting the study. This article was written originally without any copy from data of published articles and books.

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