



## Antibiotic Resistance and Pathogenicity of *Escherichia coli* Isolated from Cattle Raised in Households in the Mekong Delta, Vietnam

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### ABSTRACT

Of 127 cattle feces samples collected in the Mekong Delta from March to June 2023, 121 samples were positive for *E. coli* (95.28%). In those surveyed households, there was no statistically significant difference in the prevalence of *E. coli* in cattle of different ages, dairy or beef. The antimicrobial susceptibility test indicated that those *E. coli* strains were susceptible to ceftazidime (87.27%), cefuroxime (89.09%), amikacin, and doxycycline (90.91%). However, *E. coli* strains were remarkably resistant to ampicillin (81.82%), streptomycin and tetracycline (74.55%), colistin and chloramphenicol (70.91%), amoxicillin/clav.acid (65.45%). Moreover, 63.64% of examined *E. coli* strains were resistant to one to twelve antibiotics, and the resistant pattern of Am + Ac + Co + Sm + Te + Cl accounted for the highest rate (16.36%). By PCR method, the presence of genes encoding antibiotic resistance was determined, in which the *tetA* gene had the highest presence (69.09%), and the lowest was the *blaTEM* gene (18.18%). Most *E. coli* (34.55%) strains harbored two to four antibiotic resistance genes, and the phenotypes of *cat1* + *sulII* and *sulII* + *tetA* were the most common (5.45%). Using the PCR method to determine the presence of virulence genes, it was recorded that the *tsh* gene had the highest presence rate (54.55%), and the lowest was the *fyuA* gene (12.73%). Those *E. coli* strains (23.63%) could carry two to four virulence genes, and the most common pattern was *astA* + *tsh* (9.09%). Thus, the prevalence of pathogenic and antibiotic-resistant *E. coli* in cattle was critical to protect animal and human health.

**Key words:** Antibiotic resistance, Cattle, *E. coli*, Household, Pathogenic genes

### INTRODUCTION

Livestock manure contains microbial elements that can serve as a source of pathogenic microorganisms for animals and humans. *Escherichia coli* can be transmitted through the fecal-oral route and exists naturally in the intestinal tract of ruminants (Armstrong et al. 1996). Moreover, cattle are reservoirs of extraintestinal pathogenic *E. coli* (ExPEC), which could potentially lead to the transmission of diseases and the proliferation of antimicrobial resistance to humans (Bélanger et al. 2011). Pathogens, including *E. coli*, identified in manure samples might be resistant to antibiotics and zoonotic in nature (Argudin et al. 2017). Several epidemiological investigations have documented variations in the transmission of *E. coli* by cattle, which has significant implications for agriculture, medicine, and public health. The occurrence of *E. coli* super-shedding and super-

spreading in cattle is influenced by factors such as the specific microorganism, characteristics of the cattle, and environmental conditions (Stein and Katz 2017; Ezzat et al. 2023).

The current use of antibiotics has not been controlled in disease prevention and treatment for livestock, increasing the antibiotic resistance of bacterial strains. On the other hand, the overuse of antibiotics in treating human and animal diseases has led to antibiotic resistance or multi-resistance in bacteria, especially in *E. coli* (Wellington et al. 2013). The antibiotic resistance rate of *E. coli* has been increasing and multi-resistant to several antibiotic types. Gow et al. (2008) indicated that *E. coli* strains collected by rectal swabs from cattle in Western Canada harbored 23 resistance genes corresponding to 6 different antibiotic groups, and the most common antibiotic-resistance genes were *sulII* (48.3%), *tetB* (45.4%). Navajas-Benito et al. (2016) reported that 21.8% of *E. coli* isolated in dairy farms

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in Spain had high resistance to ampicillin, nalidixic acid, trimethoprim/sulfamethoxazole, and tetracycline. Moreover, those *E. coli* strains harbored several antibiotic-resistance genes, including *blaTEM-1*, *tetA*, *tetB*, *cmlA*, *floR*, *sul*, etc., and 14% of strains showed a multidrug-resistant phenotype.

Most studies about *E. coli* in cattle focused on Shiga-toxin-producing *E. coli*. However, ExPEC can cause disease for hosts via excreting toxins, such as *astA*, *fuyA*, *tsh*, and *traT*. These toxins cause diarrhea, hemorrhage, kidney failure, pneumonia, urinary tract infections, meningitis in newborns, sepsis, and pneumonia (Johnson et al. 2007). Kirtikliene et al. (2022) reported that ExPEC was the main cause of human bloodstream infections in Lithuania, and genes *fuyA* and *traT* were detected from those strains. In other research, Shoaib et al. (2023) examined the presence of virulence genes in *E. coli* isolated from cattle in Xinjiang, China, and found that the *ompA* gene was the most common (86.69%), followed by *ibeB* (85.0%), *traT* (84.91%), and *fyuA* (23.1%). These genes were considered the main cause of animal mastitis and transmitted to humans through contaminated milk, meat, surface water, and agricultural crops. Fathy et al. (2019) also reported that gene *tsh* was detected from *E. coli* strains isolated from diarrhea newborn calves in Egypt. Thus, it was revealed that those pathogenic genes could cause diseases in cattle and humans.

In the Mekong Delta, Vietnam, cattle have been raised mainly in households with small herds. Therefore, hygiene and disease management have been unsatisfied, and they can affect animal and human health. This study aimed to clarify the prevalence of antibiotic resistance and pathogenicity of *E. coli* isolated from cattle. The results are distributed for epidemiological management of diseases caused by *E. coli* in cattle and humans in this region.

## MATERIALS AND METHODS

### Sample collection from cattle

This study was conducted following the guidelines outlined in the Helsinki Declaration. The Animal Ethic Committee of Can Tho University, Can Tho City, Vietnam, accepted animal experiment procedures.

In this study, the experiment was done randomly and 127 cattle feces were collected at all genders, ages, and dairy or beef cattle at the households (<10 herds/household) in Tra Vinh province, Ben Tre province, and Can Tho City from March to June 2023 in the Mekong Delta, Vietnam.

Feces (25 grams) were collected directly in the morning after cattle had shed on the floor barns. After that, the inside of the feces was taken and kept in separate sterilized plastic bags in cool conditions (4-8°C) for transport to the laboratory to detect *E. coli* within 24 hours.

### Isolation and identification of *E. coli* in cattle feces

In the laboratory, *E. coli* was detected following the guidelines of the Vietnamese National Standard TCVN 5155-90 and Barrow and Feltham (2003). The feces (25g) samples were incubated in 250mL of buffered peptone water broth (BPW, Merck, Germany) to enrich *E. coli* in the samples. After incubating at 37°C for 24h, one loop of

enrichment broth of each sample was streaked on MacConkey agar (MC, Merck, Germany) and further incubated at 37°C for 24h.

All suspicious colonies of *E. coli* were picked up and subcultured on nutrient agar (NA, Merck, Germany) for further incubation at 37°C in 24h to examine biochemical tests following the guidelines of Barrow and Feltham (2003). Then, those confirmed *E. coli* strains were cultured on trypticase soy agar (TSA, Merck, Germany) for incubation at 37°C for 24h to conduct other experiments.

### Antimicrobial susceptibility of *E. coli* isolated from cattle

Of 121 *E. coli* positive samples, 55 *E. coli* strains, which were representative of households, ages, and cattle types, were selected to examine the antimicrobial susceptibility of thirteen antibiotics. The sensitivity of bacteria to antibiotics was assessed using the Kirby-Bauer diffusion method (Bauer et al. 1966) and compared to CLSI standards (2022). *Escherichia coli* ATCC 25922 served as the quality control. Bacteria showing intermediate susceptibility were classified as susceptible strains.

The antibiotic discs were used in this study, including ampicillin (Am, 10µg), amoxicillin/clavulanic acid (Ac, 20/10µg), cefuroxime (Cu, 30µg), ceftazidime (Cz, 30µg), gentamycin (Ge, 10µg), colistin (Co, 10µg), streptomycin (Sm, 10µg), amikacin (Ak, 30µg), doxycycline (Dx, 30µg), tetracycline (Te, 30µg), chloramphenicol (Cl, 30µg), ofloxacin (Of, 5µg), levofloxacin (Lv, 5µg), and trimethoprim/sulfamethoxazole (Bt, 1.25/23.75µg) supplied by Nam Khoa Biotek Ltd. (Vietnam).

### Prevalence of antibiotic-resistance genes in *E. coli* isolated from cattle

Fifty-five *E. coli* strains were examined for antimicrobial susceptibility and used to detect antibiotic-resistance gene prevalence. The DNA of those *E. coli* strains was extracted using the heat-shock method (Ahmed and Dablood 2017) and stored at -20°C for use in this experiment.

The PCR reaction used Mastermix 2X (Bioline, Canada) in a total of 25µL: Mastermix 2X (12.5µl), forward primer (0.5µl), reverse primer (0.5µL), distilled water (9.5µL) and DNA template (2.0µL).

The antibiotic-resistance genes were chosen due to the high resistance of *E. coli* in the antimicrobial susceptibility test. The primer sequences and PCR conditions were conducted following the guidelines for *blaTEM* (Jouini et al. 2007), *cat1* (Santos et al. 2014), *mcr-1* (Arcilla et al. 2016), *strB* (Han et al. 2004), *tetA* (Randall et al. 2004), and *sullI* (Sáenz et al. 2010). In this study, the negative control was distilled water, while the positive controls were *E. coli* strains, which harbored these genes, isolated from cattle previously in the Mekong Delta.

### Prevalence of pathogenic genes in *E. coli* isolated from cattle

Fifty-five *E. coli* strains were examined to detect four pathogenic genes. The PCR assays were conducted following similar steps to detect antibiotic-resistance genes. The primer sequences and PCR conditions were performed following the guidelines for *astA* (Kimata et al. 2005), *fuyA* (Schubert et al. 1998), *tsh* (Nikpiran et al. 2018), and *traT*

(Książczyk et al. 2021). In this study, the negative control was distilled water, while the positive controls were *E. coli* strains, which harbored these genes, isolated from domestic animals previously in the Mekong Delta.

**Statistical Analysis**

Statistical analysis was used to clarify the difference in the prevalence of *E. coli* in cattle and antibiotic resistance and pathogenic genes among those strains. The Pearson Chi-square test was used at the significance rate of 95% in the Minitab 17.0 software.

**RESULTS**

**Prevalence of *E. coli* isolated from cattle in the households in the Mekong Delta, Vietnam**

Of 127 feces samples, 121 were positive for *E. coli* at a high rate (95.28%). However, there were no significant differences at all ages, and beef or dairy cattle in those households (P>0.05). *E. coli* was detected in beef and dairy cattle at 95.10% and 96.00%, while at > 12 months and ≤ 12 months at 98.41% and 92.19%, respectively (Table 1).

**Antimicrobial susceptibility of *E. coli* strains isolated from cattle**

Of 121 *E. coli*-positive samples, 55 *E. coli* strains were selected to examine the antimicrobial susceptibility to 13 antibiotics (Table 2). The results indicated that those strains were highly sensitive to several antibiotics such as doxycycline (90.91%), amikacin (90.91%), cefuroxime (89.09%), ceftazidime (87.27%), ofloxacin (78.18%), and levofloxacin (74.55%). However, those *E. coli* strains were remarkably resistant to ampicillin (81.82%), streptomycin (74.55%), tetracycline (74.55%), colistin (70.91%), chloramphenicol (70.91%), and amoxicillin/clavulanic acid (65.45%). The results also revealed that *E. coli* strains resistant to antibiotics in one antibiotic group were different.

Moreover, those *E. coli* strains (63.64%) could be multi-resistant to two to twelve examined antibiotics (Table 3). The most common resistance pattern was Am+Ac+Co+Sm+Te+Cl (16.36%). One *E. coli* strain was particularly resistant to 12/13 examined antibiotics with the pattern of Am+Ac+Cz+Cu+Co+Ge+Sm+Te+Dx+Cl+Lv+Of (1.81%).

**Prevalence of antibiotic-resistance genes in *E. coli* strains isolated from cattle**

Of six examined antibiotic-resistance genes (Table 4), gene *tetA* (69.09%) was detected at the highest frequency, followed by *catI* (36.36%), *strB* (27.27%), *mcr-1* (23.64%), *sulIII* (21.82%), and *blaTEM* (18.18%). Those genes' prevalence was inconsistent with the antimicrobial susceptibility test results in some antibiotic groups.

Those *E. coli* strains (34.55%) could carry multiple antibiotic-resistance genes from two to four genes (Table 5). Among patterns, the patterns of *catI*+*mcr-1* and *sulIII*+*tetA* (5.45%) were more common than others.

**Prevalence of pathogenic genes in *E. coli* strains isolated from cattle**

Of four examined pathogenic genes, gene *tsh* (54.55%) was found at the highest rate in those *E. coli* strains (Table 6), followed by *traT* (29.09%), *astA* (25.45%), and *fyuA* (12.73%).

**Table 1:** Prevalence of *E. coli* isolated from cattle in households of the Mekong Delta, Vietnam

Factor	No. of examined samples	No. of positive samples	Percentage (%)
Beef cattle	102	97	95.10
Dairy cattle	25	24	96.00
>12 month-age	63	62	98.41
≤12 month-age	64	59	92.19
Total	127	121	95.28

**Table 2:** Antimicrobial susceptibility of *E. coli* strains isolated from cattle (n=55)

Antibiotics	Sensitivity		Resistance	
	No. of strains	Percentage (%)	No. of strains	Percentage (%)
Ampicillin	10	18.18	45	81.82
Streptomycin	14	25.45	41	74.55
Tetracycline	14	25.45	41	74.55
Colistin	16	29.09	39	70.91
Chloramphenicol	16	29.09	39	70.91
Amoxicillin/clavulanic acid	19	34.55	36	65.45
Gentamycin	34	61.82	21	38.18
Levofloxacin	41	74.55	14	25.45
Ofloxacin	43	78.18	12	21.82
Ceftazidime	48	87.27	7	12.73
Cefuroxime	49	89.09	6	10.91
Amikacin	50	90.91	5	9.09
Doxycycline	50	90.91	5	9.09

**Table 3:** The antibiotic-resistance patterns of *E. coli* isolated from cattle (n=55)

Resistant antibiotics (n)	Resistant pattern	Strains #	%
2	Co+Ge	4	7.27
3	Co+Ak+Lv	1	1.81
	Ac+Cu+Co	2	3.63
4	Ge+Te+Lv+Of	2	3.63
	Am+Sm+Te+Cl	1	1.81
	Am+Co+Ge+Sm	1	1.81
5	Am+Ac+Co+Sm+Te	2	3.63
	Am+Ac+Sm+Te+Cl	3	5.45
	Am+Cz+Sm+Te+Cl	2	3.63
	Am+Ge+Cl+Lv+Of	2	3.63
	Am+Ac+Ge+Sm+Te	2	3.63
6	Am+Ac+Co+Sm+Te+Cl	9	16.36
7	Am+Ac+Co+Ge+Sm+Te+Cl	3	5.45
12	Am+Ac+Cz+Cu+Co+Ge+Sm+Te+Dx+Cl+Lv+Of	1	1.81
		Total	35 63.64

Co: colistin; Cu: cefuroxime; Te: tetracycline; Ge: gentamycin; Sm: streptomycin; Am: ampicillin; Cl: chloramphenicol; Ac: amoxicillin/clavulanic acid; Dx: doxycycline; Cz: ceftazidime; Of: ofloxacin; Lv: levofloxacin; Ak: amikacin

**Table 4:** Prevalence of antibiotic-resistance genes in *E. coli* strains isolated from cattle in the Mekong Delta (n=55)

Antibiotics	Gene	Positive strains #	%
Tetracycline	<i>tetA</i>	38	69.09
Phenicol	<i>catI</i>	20	36.36
Aminoglycosides	<i>strB</i>	15	27.27
Colistin	<i>mcr-1</i>	13	23.64
Sulfonamid	<i>sulIII</i>	12	21.82
Beta-lactam	<i>blaTEM</i>	10	18.18

Those *E. coli* strains (23.63%) harbored multiple pathogenic genes from two to four examined genes (Table 7). The pattern of *astA*+*tsh* (9.09%) was the most detected from those strains, followed by *astA*+*tsh*+*traT* (7.27%).

**Table 5:** The antibiotic-resistance genotypes of *E. coli* strains isolated from cattle (n=55)

Genes #	Patterns	Strains #	%
2	<i>blaTEM</i> + <i>tetA</i>	2	3.64
	<i>catI</i> + <i>sulIII</i>	2	3.64
	<i>catI</i> + <i>strB</i>	2	3.64
	<i>catI</i> + <i>mcr-1</i>	3	5.45
	<i>catI</i> + <i>tetA</i>	2	3.64
	<i>sulIII</i> + <i>strB</i>	1	1.82
	<i>sulIII</i> + <i>tetA</i>	3	5.45
	<i>strB</i> + <i>mcr-1</i>	1	1.82
3	<i>mcr-1</i> + <i>sulIII</i> + <i>strB</i>	1	1.82
	<i>catI</i> + <i>mcr-1</i> + <i>strB</i>	1	1.82
4	<i>blaTEM</i> + <i>catI</i> + <i>strB</i> + <i>tetA</i>	1	1.82
		Total 19	34.55

**Table 6:** Prevalence of pathogenic genes in *E. coli* strains isolated from cattle in the Mekong Delta (n=55)

Gene	Positive strains (n)	%
<i>astA</i>	14	25.45
<i>fyuA</i>	7	12.73
<i>tsh</i>	30	54.55
<i>traT</i>	16	29.09

**Table 7:** The combined pathogenic genes of *E. coli* strains isolated from cattle (n=55)

Genes #	Patterns	Strain #	%
2	<i>astA</i> + <i>tsh</i>	5	9.09
	<i>fyuA</i> + <i>tsh</i>	2	3.64
3	<i>astA</i> + <i>tsh</i> + <i>traT</i>	4	7.27
	<i>astA</i> + <i>fyuA</i> + <i>tsh</i>	1	1.82
4	<i>astA</i> + <i>fyuA</i> + <i>tsh</i> + <i>traT</i>	1	1.82
		Total 13	23.63

## DISCUSSION

In this study, *E. coli* was detected at a high rate (95.28%), and there was no difference between beef and dairy cattle in the households of the Mekong Delta, Vietnam. These households had similarities in livestock farming practices, breeding techniques, disease prevention procedures and non-guaranteed hygiene; therefore, these cattle could harbor or be infected with *E. coli* at the same rate. Bako et al. (2017) surveyed cow dung samples collected in Ouagadougou, Burkina Faso also showed a high presence rate of *E. coli* (95.00%). Ribeiro et al. (2019) detected *E. coli* in 128 feces samples (80.00%) and indicated cattle feces as important contamination sources of pathogenic *E. coli* in non-technified dairy farms, causing cross-contamination among feces, water, and raw milk. In addition, although *E. coli* permanently exists in the digestive tract of most animals (Fairbrother and Nadeau 2006), the infection of *E. coli* depends on environmental conditions and the animal's resistance (Kaper et al. 2004). The research of Hassan et al. (2014) showed that *E. coli* was present in 75% of rectal swabs taken from healthy cattle regardless of their age, gender, breed, or management system. In contrast, Mir et al. (2015) stated that animal age was a significant factor influencing STEC prevalence in cattle. Further studies could be carried out on cattle in the Mekong Delta, Vietnam, to clarify the factors that could affect the prevalence of *E. coli* there.

In this survey, antibiotics were not commonly used in the treatment of cattle in those households; however, those *E. coli* strains showed significant resistance to several antibiotics belonging to groups of beta-lactam,

aminoglycoside, tetracycline, polymyxin, and chloramphenicol. The use of antibiotics is limited in ruminant farming to avoid affecting the cattle's rumen microflora; thus, this resistance could be bacterial nature-resistance or due to exposure to antibiotic-resistant agents in the surrounding environment (Heydari et al. 2020). Antibiotic resistance emerges through intricate interactions, as resistance is either generated through spontaneous mutation during clinical antibiotic exposure or commonly acquired through the assimilation of mobile genes that have gradually evolved within bacteria in the surrounding environment (Wellington et al. 2013). In Europe, Chantziaras et al. (2014) revealed that the significantly high coefficients in antibiotic resistance were linked to the use and the resistance levels found in commensal *E. coli* isolated from pigs, poultry, and cattle. Poirel et al. (2018) reported that the development of colistin resistance in *E. coli* was primarily linked to the widespread use of colistin in veterinary medicine worldwide. Srinivasan et al. (2007) showed that *E. coli* strains isolated from cattle in New York State, USA, were resistant to antibiotics used in veterinary medicine, including ampicillin (98.40%), streptomycin (40.30%), sulfisoxazole (34.10%), tetracycline (24.80%). Adenipekun et al. (2015) isolated *E. coli* from food animals, including cattle, in Lagos, Nigeria, and found that those *E. coli* strains exhibited the highest resistance to tetracycline (58.8%), trimethoprim/sulfamethoxazole (39.8%), and ampicillin (34.1%), but susceptibility to amikacin, cefepime, ceftazidime.

Moreover, those *E. coli* strains isolated from cattle in this study showed multiple resistance to several antibiotics. It indicated a critical issue in preventing and treating *E. coli* infection in cattle and human health in the Mekong Delta. The effects of the uncontrolled use of antibiotics, not according to the manufacturer's and veterinarian's regulations in disease treatment, are one of the leading causes of antibiotic resistance in animal husbandry. The increase in multidrug resistance phenotypes may be due to the accumulation of genes encoding antibiotic resistance on bacterial chromosomes or plasmids (Yamamoto et al. 2013). Benedict et al. (2015) stated that some resistances in bacterial populations are interconnected biologically, leading to their persistence even when exposed to selective pressures. This persistence is not solely due to exposure to antimicrobial drugs but rather due to their relationship with other resistances, as indicated by the outcomes in the multivariate model. Yamamoto et al. (2014) recorded that *E. coli* isolated from beef cattle manure in Japan was simultaneously resistant to 9-11 types of antibiotics. Thus, managing *E. coli* multi-drug resistance is essential to protect animal and human health.

Although *E. coli* strains isolated from cattle showed a high resistance in the antimicrobial susceptibility tests, the antibiotic-resistance genes were detected at a relatively low rate, except for gene *tetA*, in this study. It meant that gene *tetA* could be commonly present or a natural part of genomes in those strains. Shin et al. (2015) suggested that the significant occurrence of tetracycline-resistant *E. coli* isolates in beef cattle in Korea could be attributed to the transferability of tetracycline resistance genes among *E. coli* populations that have withstood the selective pressure

induced by the administration of antimicrobial agents. Thuan et al. (2022) conducted research on Shiga-toxin-producing *E. coli* isolated from cattle in the Mekong Delta and found that gene *tetA* (51.28%) was the most predominant in those strains. Yamamoto et al. (2013) proposed the potential scenario that resistance strains and genes of *E. coli* isolated from healthy cattle in different areas of Japan were indirectly transmitted among cattle through the farming environment, including their food and drink. Moreover, it was plausible that these resistance strains and genes spread when the growing cattle were sold and relocated to various regions. Massé et al. (2021) found that the resistance genes in *E. coli* isolated from dairy farms in Canada included several genes, including *tet(A)*, *tet(B)*, *sul1*, *sul2*, *sul3*, *aph(3'')-Ib (strA)*, *aph(6)-Id (strB)*, *aadA1*, *aadA2*, and *aadA5*. Furthermore, certain mobile genetic elements found in those *E. coli* strains could be demonstrated to enable the preservation of resistance even in the absence of antibiotic selection pressure. Therefore, further research could be done to examine and determine the characteristics of antibiotic-resistance genes distributed in *E. coli* strains isolated from cattle in the Mekong Delta.

The results of this study also demonstrated that those *E. coli* strains (34.55%) could harbor multiple antibiotic-resistance genes. Although the number of *E. coli* strains was low, they could be resistant to several antibiotics used in animal husbandry, including beta-lactam, polymyxin, and aminoglycoside. Most multi-resistance phenomena are due to the combination of mobile genetic elements such as plasmids, transposons, and integrins contributing to the spread of antibiotic-resistant genes (Bradford 2001; Carattoli 2009). Gupta et al. (2017) reported that 100% of *E. coli* isolated from cattle in Bangladesh were found resistant to tetracycline and sulfamethoxazole. Moreover, 34% of those *E. coli* strains were resistant to more than two antimicrobials, and they were commonly present in cattle of different management systems. Bag et al. (2021) also clarified the antibiotic resistance of *E. coli* strains isolated from mastitis cattle in Bangladesh and reported that the antimicrobial resistance profile of *E. coli* strains was linked to the antimicrobial agents utilized on the farms. The presence of multidrug-resistant *E. coli* strains might lead to treatment failures and pose a risk for the transmission and emergence of antibiotic resistance in humans. Therefore, the prevalence of multi-antibiotic resistance to *E. coli* strains in cattle in the Mekong Delta should be controlled to prevent the spreading of those strains to husbandry environments and public health.

In this study, the *E. coli* strains harbored pathogenic genes that caused infection in cattle and humans outside the intestine, and gene *tsh* (54.55%) was the most detected from *E. coli* strains. Moreover, a few *E. coli* strains could harbor multi-pathogenic genes examined, and the pattern of *astA* + *tsh* was frequent (9.09%). Previous studies identified gene *tsh*, which encodes a temperature-sensitive hemagglutinin, as one of the prominent virulence factors for bacteria adhesion and colonization in the small bowel and the intestinal secretion of fluids (Welch 2006). Akiyama et al. (2015) found diarrheic genes, including *astA*, in several *E. coli* groups, such as Shiga-toxin-producing *E. coli* and Diarrheagenic *E. coli*

from cattle in Japan. Recently, Martins et al. (2022) found that the serum resistance-related genes (*traT*, *ompT*) could be used as biomarkers to investigate ExPEC isolates from umbilical infections in calves. Other reports showed that *E. coli* strains isolated from cattle in Lithuania, China, and Egypt could harbor those pathogenic genes, including *fuyA*, *traT*, and *tsh*. These strains were considered the main cause of sepsis and mastitis in humans and cattle (Fathy et al. 2019; Kirtikliene et al. 2022; Shoab et al. 2023). Furthermore, Bacciu et al. (2004) indicated that an intergeneric transfer of virulence genes via an insertion sequence from *E. coli* to other pathogens and horizontal transfer of virulence factors between bacterial genera occurred in nature. Veilleux and Dubreuil (2006) also stated that outbreaks of food-borne and water-borne diarrhea in humans could be encountered with contamination from *E. coli* encoding gene *astA* associated with farm animals. Those results indicated that *E. coli* harboring pathogenic and multi-pathogenic genes isolated from cattle in the Mekong Delta was a significant issue to animal and human health.

### Conclusion

The cattle in the Mekong Delta excreted *E. coli* at a high rate in their feces at all ages, whether beef or dairy cattle. Furthermore, those *E. coli* strains were remarkably resistant or multi-resistant to several antibiotics used in animal husbandry, with various resistant patterns. Those strains also harbored diverse antibiotic resistance and pathogenic genes, which could cause severe diseases and multi-drug resistance for cattle and humans in this region. Therefore, further research should be carried out to clarify the characteristics and origins of those antibiotic-resistant and pathogenic genes circulating in cattle, and rigorous management should be conducted to protect animals' and humans' health.

### Author's contribution

Conceptualization, Minh T.L. Bui, Thuong T. Nguyen, Thuan K. Nguyen, Khai T.L. Ly; methodology, Thuong T. Nguyen, Thuan K. Nguyen, Hieu C. Nguyen, Minh T.L. Bui; formal analysis, Thuan K. Nguyen, Hieu C. Nguyen, Khai T.L. Ly; writing—original draft preparation, Thuong T. Nguyen, Thuan K. Nguyen, Minh T.L. Bui, Hieu C. Nguyen; writing—review and editing, Minh T.L. Bui, Thuan K. Nguyen. All authors have read and agreed to the published version of the manuscript.

### Data availability statement

The data supporting this study's findings are available on request from the corresponding author.

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