Prevalence and antibiotic resistance of *Escherichia coli* isolated from the respiratory tract of goats in Can Tho city, Vietnam

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ABSTRACT

A total of 319 nasal swab samples were collected to clarify the prevalence and antimicrobial susceptibility of *E. coli* in the respiratory tract of goats in Can Tho, Vietnam. It indicated that *E. coli* was detected at a relatively minor rate (8.46%), and their prevalence in male goats and dairy goats was higher than that in others. However, the ages and health conditions of goats did not affect the presence of *E. coli* in those goats. Those *E. coli* strains were still susceptible to seven examined antibiotics, but the resistance was recorded in ampicillin (25.93%) and bactrim (25.93%) in this study. Those *E. coli* strains (33.33%) could resist one to seven antibiotics with several patterns; the pattern of ampicillin + amoxicillin/clavulanic acid (7.41%) was more common than others. Moreover, *E. coli* strains harbored antibiotic-resistance genes, and *blaampC* was detected at the highest rate (92.11%), followed by *sulII* (43.86%), *tetA* (24.56%), and *qnrA* (5.26%). Of those *E. coli* strains, 22.81% harbored two to four examined genes with several patterns of antibiotic-resistance genes; the most detected pattern was *blaampC* + *sulII* + *tetA* (7.89%). Thus, controlling the prevalence of antibiotic-resistant *E. coli* in the respiratory tract of goats will protect animal and public health.

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1. Introduction

Small ruminants, especially goats, have increased in the Mekong Delta, Vietnam, because of their behavior, which is suitable for raising the climate change conditions in this area. Goats have become crucial in the economy on small-scale farms. They supply meat and milk, and their nature is to replicate and grow rapidly. However, goat diseases have been a concern; thus, keeping livestock healthy is a significant challenge. Respiratory illness affects the survival of small

ruminants because it can cause prolonged impact and depression for the rest of life (Besser et al., 2012). The diseases occur due to the interaction of infectious pathogens (bacteria, viruses, and fungi), host defense, environmental factors, and stress. Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals, or secondary to several conditions that cause immunosuppression (Yesuf et al., 2012).

Escherichia coli could cause intestinal and extra-intestinal colibacillosis in humans and animals. Pathogenic *E. coli* not only had a severe impact on the breeding industry but also posed a threat to public health. Two major groups have been proposed: intestinal pathogenic *E. coli* (IPEC) and extra-intestinal pathogenic *E. coli* (ExPEC). For animals, ExPEC infects pigs, poultry, and cattle, causing septicemia, mastitis, and respiratory diseases (Russo et al., 2003; Zhang et al., 2018; Ma et al., 2021). Detecting *E. coli* in the respiratory tract of goats is essential for controlling diseases. In previous reports, *E. coli* was isolated from healthy or pneumonia goats in China and India at 9.98% to 44.16% (Puvarajan et al., 2020; Yun et al., 2022). However, there was little understanding of the characteristic prevalence of *E. coli* in the respiratory tract of goats in the Mekong Delta and Vietnam.

Furthermore, the continuous use and abuse of antibiotics in livestock have caused an increase in antibiotic-resistant bacteria, especially *E. coli*. Antimicrobial-resistant *E. coli* and multiple drug-resistant (MDR) strains have been frequently identified. These *E. coli* strains might be transmitted to humans through consuming contaminated animal products, and a risk of transmission of drug-resistant genes between different strains was also presented (Aarestrup et al., 2001; Mellata, 2013). This reveals a significant potential hazard to the health of humans and animals. Algammal et al. (2020) showed that *E. coli* isolated from pneumonia cattle in Egypt was significantly resistant to erythromycin, gentamycin, streptomycin, and trimethoprim/sulphamethoxazole. In India, Singh et al. (2019) reported that *E. coli* strains isolated from pneumonia goats harbored antibiotic-resistance genes, such as *blaTEM* (70.60%) and *blaSHV* (1.70%). Therefore, assessing the antimicrobial resistance profile of E. coli in goats is necessary to contribute to the overall picture of the abuse of antibiotics in the Mekong Delta.

Therefore, this study aimed to clarify the prevalence and antimicrobial susceptibility of *E. coli* isolated from the respiratory tract of goats in Can Tho, Vietnam. These results will be a valuable reference for treating and controlling respiratory diseases caused by *E. coli* in this region and the Mekong Delta, Vietnam.

2. Materials and Methods

2.1. Sample collection

From March to September 2023, 319 nasal swab samples were collected from goats of all ages and genders in small-scale farms (< 50 heads/farm) in Can Tho, Vietnam. These goats included meat goats (n = 232) and dairy goats (n = 87). Before nasal samples were collected, these goats were examined for clinical symptoms of respiratory disease, such as coughing, nasal discharges, breath, fever, etc. The outside of the goat's nose was cleaned with sterilized cotton tissues. Then, sterilized swabs were used to collect the nasal samples and were put in the Cary Blair medium tubes (Merck, Germany). Those Cary Blair tubes were cleaned outside and put in separately sterilized zip bags with the code. They were kept in cool conditions

(cool box, 2 - 8°C with dry ice) and sent to the laboratory to isolate pathogens.

When collecting samples, this study followed the animal welfare and safety procedures and guidelines of Can Tho University, Vietnam. Samples were collected and directly sent to the laboratory for analysis within 24 h.

2.2. Isolation and identification of *E. coli* in the nasal samples

The isolation method of *E. coli* in goat nasal fluid was carried out and modified according to the guidelines of the Vietnam Standards - TCVN 8400-16:2011 (VS, 2011) and Barrow and Feltham (2003).

The nasal swabs were incubated in buffered peptone water broth (BPW, Merck, Germany) to enrich *E. coli* in samples. After incubating at 37°C for 24 h, one loop of enrichment broth of each sample was cultured on MacConkey medium (MC, Merck, Germany) for further incubation at 37°C for 24 h. Then, all suspicious colonies of *E. coli* were collected and subcultured on nutrient agar (NA, Merck, Germany) for further incubation at 37°C in 24 h to examine biochemical tests following the guidelines of Barrow and Feltham (2003).

Then, those *E. coli* strains were confirmed to subculture on trypticase soy agar (TSA, Merck, Germany) at 37°C for 24 h for other experiments.

2.3. Antimicrobial susceptibility of *E. coli* strains isolated from goats

Positive *E. coli* strains (one strain/positive sample) were examined for antimicrobial susceptibility using the disc fusion method followed by Bauer's guidelines (Bauer et al., 1966). There were seven antibiotics used in this study, including ampicillin (Am, 10 µg), amoxicillin/clavulanic acid (Ac, 20/10 µg), ceftazidime (Cz, 30 µg), gentamycin (Ge, 10 µg), doxycycline (Dx, 30 µg), ciprofloxacin (Ci, 5 µg), trimethoprim/sulfamethoxazole (Bt, 1.25/23.75 µg). Those antibiotics were purchased by Nam Khoa Biotek Ltd. (Vietnam).

Escherichia coli ATCC 25922 was used as control quality, and the results were compared to the standards of Clinical and Laboratory Standards Institute (CLSI, 2022). Those strains, which were intermediate susceptibility, were accounted as susceptible strains.

2.4. Determination of antibiotic-resistance genes in *E. coli* isolated from goats

The DNA of *E. coli* strains was extracted using Ahmed and Dablool's heat-shock method (Ahmed & Dablool, 2017) and stored at -20°C for use in this experiment. A total of 114 *E. coli* isolates from 27 positive samples were used in this experiment.

The single PCR assay was used to detect four antibiotic-resistance genes representative of beta-lactam (*blaampC*), tetracycline (*tetA*), sulfonamide (*sulII*), and quinolone (*qnrA*). The PCR conditions and primer sequences followed the description of Randall et al. (2004), Cattoir & Nordmann (2009), and Sáenz et al. (2010). The MyTaq Mix 2X (BIO25042, Bioline, Meridian Bioscience, USA) was used in those experiments. The PCR reaction was a total of 25 µL, including Mastermix 2X (12.5 µL), forward primer (0.5 µL), reverse primer (0.5 µL), distillation water (9.5 µL), and DNA template (2 µL). Thermal cycle was modified as follows: 94°C - 5 min; 30 cycles: 94°C - 1 min, 58°C - 45 sec, 72°C - 1 min; 72°C - 10 min. The *E. coli* strains, previously isolated from cattle in the Mekong Delta, were used as a control.

2.5. Statistical analysis

Statistical analysis was used to clarify the difference in the prevalence of *E. coli* in goats and antibiotic resistance among those isolates. The Chi-square method was used at the significance rate of 95% in the Minitab 17.0 software.

3. Results and discussions

3.1. Prevalence of *E. coli* in the respiratory tract of goats in Can Tho city, Vietnam

Of 319 nasal swab samples, *E. coli* was detected in 27 samples (8.46%) (Table 1). Although *E. coli* was present at a low rate, it indicated that *E. coli* could survive and multiply in the respiratory tracts of goats. It could become an opportunistic pathogen causing respiratory diseases in goats. In previous reports, *E. coli* belonging to the ExPEC group can infect and cause several diseases, including respiratory diseases in domestic animals (Logue et al., 2017; Zhang et al., 2018; Ma et al., 2021). De Oliveira et al. (2016) isolated *E. coli* from healthy and diseased animals with respiratory signs at a low proportion (4.22%), while another report indicated the significance of *E. coli* in bronchopneumonia (DebRoy et al., 2008). Most goat farms in this study were small and unclean; therefore, those goats could also transmit pathogens, including *E. coli,* in the husbandry environment. Pelczar et al. (1986) suggested that *E. coli,* which is usually harmless in its normal habitat, could cause pulmonary and urogenital infections. It should control this pathogen even though it is not frequently prevalent in goats.

Table 1. Prevalence of *E. coli* in the respiratory tract of goats

Factor	No. examined samples	No. of positive samples	Percentage (%)
Meat goats	232	15	6.47
Dairy goats	87	12	13.79
			($P < 0.05$)
Male	96	13	13.54
Female	233	14	6.01
			($P < 0.05$)
Under 6 month-age	108	13	12.04
Upper 6 month-age and under 1 year-age	109	6	5.50
Upper 1 year-age	102	8	7.84
			($P > 0.05$)
Respiratory goats	175	16	9.14
Healthy goats	144	11	7.64
			($P > 0.05$)
Total	319	27	8.46

This study showed that the prevalence of *E. coli* in the respiratory tract of goats did not depend on age and health conditions ($P > 0.05$). It indicated that *E. coli* could be present in goats of all ages and a common pathogen that could be dominant in the respiratory tract. However, there was a significant difference in the prevalence of *E. coli* in genders and meat or dairy goats ($P < 0.05$). This study was randomized and did not have to raise model data of meat and dairy farms. Therefore, this result could be crucial due to the difference in the number of goats examined in those farms. In previous reports, no studies have been published on the difference between raising models of meat goats and dairy goats and how this will affect the transmission of pathogens. However, Zhou et al. (2023) clarified the correlation of pathogens in the respiratory tract of cows in Northeast China. It noted that all cattle (raised for meat or milk) were equally susceptible to pathogens. Thus, other research should be conducted to clarify the exact reasons for this difference in Can Tho and the Mekong Delta.

3.2. Antimicrobial susceptibility of *E. coli* isolated from the respiratory tract of goats

Of 27 positive samples, 27 *E. coli* strains were selected from 114 identified *E. coli* strains (one strain/positive sample) to examine antibiotic resistance. The results showed that all *E. coli* strains were still susceptible to seven examined antibiotics (Table 2). Among antibiotics, those *E. coli* strains were the most sensitive to ceftazidime (96.30%), followed by doxycycline (88.89%), ciprofloxacin (88.89%), and the minor sensitive to ampicillin (74.07%). *E. coli* isolates from ruminants generally demonstrated lower resistance trends than those from poultry and swine (Lei et al., 2010; Abbassi et al., 2017). In this study, antibiotics were used less in goats because of the high natural resistance of goats to diseases, and no disease outbreaks occurred in surveyed farms in Can Tho. It indicated that those antibiotics were still effective in treating respiratory diseases in goats here.

Table 2. Antimicrobial susceptibility of *E. coli* strains isolated from goats (n = 27)

Antibiotic group	Antibiotic	Susceptibility		Resistance	
		No. of strains	Percentage (%)	No. of strains	Percentage (%)
Beta-lactam	Ampicillin	20	74.07	7	25.93
	Amoxicillin-clavulanic acid	22	81.48	5	18.52
	Ceftazidime	26	96.30	1	3.70
Aminoglycoside	Gentamycin	22	81.48	5	18.52
Tetracycline	Doxycycline	24	88.89	3	11.11
Quinolone	Ciprofloxacin	24	88.89	3	11.11
Sulfonamide	Bactrim*	20	74.07	7	25.93

**Trimethoprim/sulphamethoxazole.*

However, *E. coli* strains seemed resistant to ampicillin (25.93%) and bactrim (25.93%). Those antibiotics have been usually used in treating colibacillosis in domestic animals for a long time. Additionally, according to our observations, the hygiene in those surveyed farms was not clean; therefore, *E. coli* in the husbandry environment could form and transfer resistance to those antibiotics. Pehrsson et al. (2016) reported that antibiotic-resistant *E. coli* and resistance genes might be transmitted through environments contaminated with feces, especially in developing countries. In other research, Khalifa et al. (2021) showed that *E. coli*, isolated from the respiratory tract of diseased sheep and goats in Egypt, had very high resistance levels for different beta-lactam antibiotics, including ampicillin (78.9%), amoxicillin-clavulanic acid (39.5%), and cefoxitin (34.2%). *Escherichia coli* isolates recovered from animal sources in the USA were resistant to tetracycline, streptomycin, sulfonamide, and ampicillin (Tadesse et al., 2012).

Table 3. Antibiotic-resistance patterns of *E. coli* strains (n = 27)

No. of resistant antibiotics	Pattern	No. of strains	Percentage (%)
1	Am	1	3.70
	Ci	1	3.70
2	Am + Ac	2	7.41
	Am + Bt	1	3.70
	Ge + Bt	1	3.70
3	Am + Ge + Bt	1	3.70
6	Am + Ac + Ge + Dx +Ci + Bt	1	3.70
7	Am + Ac + Cz + Ge + Dx +Ci + Bt	1	3.70
Total		9	33.33

Am: ampicillin, Ac: amoxicillin/clavulanic acid, Cz: ceftazidime, Ge: gentamycin, Dx: doxycycline, Ci: ciprofloxacin, Bt: bactrim (trimethoprim/sulfamethoxazole).

Although those *E. coli* strains were still sensitive to all antibiotics in this study, a few exhibited multi-drug resistances (33.33%) (Table 3). Significantly, there were *E. coli* strains resistant to six or seven examined antibiotics. A more significant proportion of multi-drug resistant *E. coli* isolates was recovered from animals in the USA; it found that the pattern of tetracycline and streptomycin was the most common co-resistance phenotype (30.00%), followed by tetracycline and sulfonamide (29.00%) (Tadesse et al., 2012). In previous research, this will favor the dissemination of the strains among animals and humans by co-selection (Belanger et al., 2011), and animal-derived *E. coli* can eventually infect humans through animal products or farm wastes, which seriously endanger public health safety (Fricke et al., 2008; Hannah et al., 2009). This study revealed that those *E. coli* strains could concern animal and human health in Can Tho. Thus, the use of antibiotics in goats should be controlled to prevent the formation and spread of multi-drug resistant *E. coli* strains and to guarantee human and animal health.

3.3. Prevalence of antibiotic-resistance genes in *E. coli* strains

Of 27 positive samples, all 114 *E. coli* strains were collected after being examined in biochemical tests. Those strains determined the prevalence of four common antibiotic-resistance genes using PCR assay. The results were shown in Table 4.

Table 4. Prevalence of antibiotic-resistance genes in *E. coli* strains isolated from goats (n = 114)

Antibiotic group	Gene	No. of positive strains	Percentage (%)
Beta-lactam	*blaampC*	105	92.11
Tetracycline	*tetA*	28	24.56
Sulfonamide	*sulII*	50	43.86
Quinolone	*qnrA*	6	5.26
			($P < 0.001$)

The results exhibited that *E. coli* strains could harbor most of the examined antibiotic-resistance genes at relatively high rates. Among them, gene *blaampC* was detected at the highest rate (92.11%), followed by *sulII* (43.86%), *tetA* (24.56%), and gene *qnrA* was the lowest (5.26%). It indicated *E. coli* strains isolated from the respiratory tract of goats could remarkably resist beta-lactam antibiotics. However, in the antimicrobial susceptibility test, *E. coli* strains showed a low resistance to beta-lactam antibiotics (Table 2). It was like the resistance performance of bactrim and the prevalence of gene *sulII*. In contrast, the less resistance of doxycycline and ciprofloxacin seemed to correspond with the infrequent presence of genes *tetA* and *qnrA* in those *E. coli* strains. Thus, the antibiotic resistance performance of those strains might be affected by different factors. Resistance genes in bacteria could be acquired through natural mutations and transferred to the next generation or due to conjugation, transduction, or mutation of resistance genes between diverse bacteria (Sommer et al., 2017). Moreover, some antibiotic-resistance genes seem like silent resistance genes that are usually not expressed or expressed at low levels, even when exposed to antibiotics (Stasiak et al., 2021). In a previous report, *E. coli* strains isolated from pneumonia goats harbored antibiotic-resistance genes, such as *blaTEM* (70.60%) and *blaSHV* (1.70%) (Singh et al., 2019). Whereas *E. coli* isolated from goats' fecal samples in India showed multiple drugs related to a high presence of gene *blaCTXM-1*, *blaSHV*, *blaTEM*, *blaCMY-6*, *blaCITM*, *qnrB*, *qnrS*, *tetA*, *tetB*, and *sul1* (Banerjee et al., 2022).

Furthermore, those *E. coli* strains (22.81%) in this study also harbored multiple antibiotic-resistance genes (Table 5). Those strains could harbor two to four examined antibiotic-resistance genes; it showed that *E. coli* strains isolated from the respiratory tract of goats could resist many antibiotics. It could become a challenge in treating respiratory diseases in goats in Can Tho. Tenover (2006) reported that multidrug resistance often occurred with not just one but a combination of multiple resistance mechanisms. Multidrug resistance can occur in bacteria if exposed to a type of antibiotic continuously or due to the acquisition of genetic resistance elements through plasmids or transposons.

Table 5. Multi antibiotic-resistance gene patterns of *E. coli* strains (n = 114)

No. of genes	Gene patterns	No. of strains	Percentages (%)
2	*blaampC* + *sulII*	6	5.26
	blaampC + *qnrA*	3	2.63
	blaampC + *tetA*	2	1.75
	sulII + *tetA*	3	2.63
3	*blaampC* + *sulII* + *tetA*	9	7.89
4	*blaampC* + *sulII* + *tetA* + *qnrA*	3	2.63
	Total	26	22.81

In other research on *E. coli* isolated from fecal samples, multidrug-resistant phenotypes were found in 72.7% of the examined strains (Martínez-Vázquez et al., 2021). Among those phenotypes was the presence of genes *tet* (*tetA* or *tetB*), *strA*, and *aadA*. Obaidat and Gharaibeh (2022) reported that 93.5% and 96.3% of ESBL and AmpC *E. coli* isolated from sheep and goat milk were resistant to ≥ 1 another antimicrobial class, and 44.5% and 44.4% were resistant to ≥ 3 another antimicrobial class, respectively. Antibiotic resistance to *E. coli* is an increasingly severe problem, and resistance genes can be acquired through horizontal gene transfer to make *E. coli* multi-drug resistant strains (Huddleston, 2014). Although the *E. coli* strains in this study showed a low resistance rate, a high prevalence of antibiotic-resistant genes was detected. It again revealed that the management of using antibiotics was essential to prevent multi-drug resistance *E. coli* outbreaks in animal husbandry and public health in Can Tho and the Mekong Delta, Vietnam.

4. Conclusions

Escherichia coli was present at a relatively minor rate in the respiratory tract of goats in Can Tho, Vietnam. However, that revealed a potential ability to cause diseases in the respiratory tract by *E. coli*. Further study should be conducted to explain the high prevalence of *E. coli* in male goats and dairy goats than others. In addition, those *E. coli* strains could resist various antibiotics and harbor antibiotic-resistance genes, incredibly high to *blaampC*, with several multi-drug patterns. It indicated that those *E. coli* strains could become an emergency in treating diseases in goats and other domestic animals here. It is necessary to control the prevalence of antibiotic-resistant *E. coli* in the respiratory tract of small ruminants, including goats, to protect animals and public health in Can Tho and the Mekong Delta.

Conflict of interest

The authors have no conflicts of interest to declare.

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