The Surveillance of Antimicrobial Susceptibility Pattern and $bla_{\text{CTX-M}}$ Gene Encoding in *Escherichia coli* Isolated from Healthy Goat Farms in Sai Yok District, Kanchanaburi Province, Thailand

Yupaporn Lanumtiang[1] Sineenard Jiemtaweeboon[2] Sivapong Sungpradit[3]
Arpron Leesombun[3] Sookruetai Boonmasawai[3]*

[1]Pasupalan livestock and wildlife hospital, Faculty of Veterinary Science, Mahidol University,
199 M.9 Lumsum, Sai Yok, Kanchanaburi, 71150 Thailand
[2]Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University
999 Phutthamonthon Sai 4 Road Salaya, Phutthamonthon Nakhon Pathom, 73170 Thailand
[3]Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University
999 Phutthamonthon Sai 4 Road Salaya, Phutthamonthon Nakhon Pathom, 73170 Thailand

*Corresponding author, E-mail address: sookruetai.boo@mahidol.edu

Received: 13 September 2022; Revised: 8 November 2022; Accepted: 8 November 2022

Abstract

This observation study aimed to investigate the antimicrobial susceptibility pattern and $bla_{\text{CTX-M}}$ gene encoding in *Escherichia coli* isolates collected from healthy goat farms in Sai Yok District, Kanchanaburi Province, Thailand. By collecting 92 samples from rectal swabs from goats in 7 subdistricts in Sai Yok District, the bacterial identification, antimicrobial susceptibility test, and detection of the presence of $bla_{\text{CTX-M}}$ genes by conventional polymerase chain reaction (PCR) techniques were performed. Based on the results of the study, *E. coli* prevalence was found in 72.8% of the samples (n = 67). The prevalence rate of *E. coli* isolated from the samples from 6 subdistricts (85.7%) was higher than or equal to 50%. One influencing factor of the high *E. coli* detection was the person administering antimicrobial agents to goats ($p < 0.001$). The number of *E. coli* isolated from goats drinking water from natural sources was higher than that isolated from goats drinking from the tap water system ($p < 0.038$). The antimicrobial susceptibility of amoxicillin/clavulanic acid, imipenem, fluoroquinolones, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole completely covered entire isolations. *E. coli* showed the highest resistance rate to cefotaxime (19.4%). The antimicrobial resistance (AMR) patterns were categorized by the number of antimicrobial agents into three patterns: one, two, and at least three AMRs (63%, 27%, and 10%, respectively). Every drug resistance pattern contained cefotaxime in all groups. The $bla_{\text{CTX-M}}$ gene was not found from all *E. coli* isolates. The study suggested the slightly high prevalence with the welling trend of AMR characteristics among the *E. coli* isolates collected from healthy goat farms in Sai Yok District, Kanchanaburi Province. Some influencing factors may be important for farm management to prevent the occurrence and spread of antimicrobial-resistant characteristics in goat farms.

Keywords: *Escherichia coli*, goat, Antimicrobial susceptibility, $bla_{\text{CTX-M}}$, Kanchanaburi

การสำรวจความไวต่อยาต้านจุลชีพ และการแสดงออกของยีนส์ *bla*$_{\text{CTX-M}}$ ในเชื้อ *Escherichia coli* ที่เพาะแยกได้จากฟาร์มแพะเนื้อสุขภาพดี ในอำเภอไทรโยค จังหวัดกาญจนบุรี ประเทศไทย

ยุภาภรณ์ ลาน้ำเที่ยง[1] สินีนาถ เจียมทวีบุญ[2] ศิวะพงษ์ สังข์ประดิษฐ์[3] อาภรณ์ ลี้สมบุญ[3] สุขฤทัย บุญมาไสว[3*]

[1]โรงพยาบาลปศุสัตว์และสัตว์ป่า ปศุปาลัน คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
199 หมู่ 9 ตำบลลุ่มสุ่ม อำเภอไทรโยค จังหวัดกาญจนบุรี 71150
[2]ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
999 ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170
[3]ภาควิชาปรีคลินิกและสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
999 ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

[*]ผู้รับผิดชอบบทความ E-mail address: sookruetai.boo@mahidol.edu

Received: 13 September 2022; Revised: 8 November 2022; Accepted: 8 November 2022

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์ เพื่อสำรวจหาความชุก รูปแบบความไวต่อยาต้านจุลชีพ และการแสดงออกของยีนส์ *bla*$_{\text{CTX-M}}$ ในเชื้อ *Escherichia coli* ที่เพาะแยกได้จากฟาร์มแพะเนื้อสุขภาพดีในอำเภอไทรโยค จังหวัดกาญจนบุรี ประเทศไทย โดยการเก็บตัวอย่างจากไม้ที่ป้ายจากทวารหนัก (rectal swab) ทั้งหมด 92 ตัวอย่าง จาก 7 ตำบล ในอำเภอไทรโยค นำมาจำแนกชนิดของเชื้อ *E. coli* เพื่อทดสอบความไวต่อยาต้านจุลชีพ โดยวิธี disc diffusion method และการแสดงออกของยีนส์ *bla*$_{\text{CTX-M}}$ ด้วยเทคนิคการเพิ่มปริมาณสารพันธุกรรม จากผลการศึกษา พบความชุกของ *E. coli* ร้อยละ 72.8 (67 ตัวอย่าง) อัตราความชุกของของเชื้อ *E. coli* 6 ตำบล จากทั้งหมด 7 ตำบล (ร้อยละ 85.7) มีค่าสูงกว่าหรือเท่ากับร้อยละ 50 ปัจจัยที่มีอิทธิพลต่อการพบเชื้อ *E. coli* คือ บุคคลผู้ให้ยาต้านจุลชีพเพื่อรักษาแพะ ($p < 0.001$) แพะที่ดื่มน้ำจากแหล่งธรรมชาติ พบเชื้อ *E. coli* จำนวนมากกว่าแพะที่ดื่มน้ำจากระบบท่อประปา ($p < 0.038$) ผลความไวต่อยาต้านจุลชีพ พบว่าแบคทีเรียทั้งหมดมีความไวต่อยา amoxicillin/clavulanic acid imipenem fluoroquinolones piperacillin/tazobactam และ trimethoprim/sulfamethoxazole และพบ เชื้อ *E. coli* ดื้อต่อยา cefotaxime มากที่สุด (ร้อยละ 19.4) รูปแบบการดื้อยาต้านจุลชีพที่พบมี 3 รูปแบบ คือ ดื้อต่อยาต้านจุลชีพ 1 ชนิด 2 ชนิด และอย่างน้อย 3 ชนิด (ร้อยละ 63 ร้อยละ 27 และร้อยละ 10 ตามลำดับ) โดยรูปแบบการดื้อยาดังกล่าว พบการดื้อยา cefotaxime ในทุกกลุ่ม จากการทดสอบการแสดงออกของยีนส์ *bla*$_{\text{CTX-M}}$ ไม่พบการแสดงออกของยีนส์ดังกล่าว ใน *E. coli* ที่เพาะแยกได้ทั้งหมด จากการทดลองนี้ทำให้ทราบว่า มีอัตราความชุกของ *E. coli* ค่อนข้างสูงในแพะที่เลี้ยงในเขตอำเภอไทรโยค จังหวัดกาญจนบุรี และมีแนวโน้มความไวต่อยาต้านจุลชีพที่ดี บางปัจจัยที่มีอิทธิพลในการจัดการฟาร์ม อาจจะมีความสำคัญในการช่วยป้องกันการพบเชื้อแบคทีเรีย และการแพร่กระจายของลักษณะดื้อยาในฟาร์มแพะได้

คำสำคัญ : เอสเชอริเชีย โคไล แพะเนื้อ ความไวต่อยาต้านจุลชีพ *bla*$_{\text{CTX-M}}$ กาญจนบุรี

Journal of Applied Animal Science 2022; 15(2): 9-24.

Introduction

Escherichia coli is a common aerobic bacterium of the mammalian gut microbiome, especially in the lower intestine of mammals (Blount 2015). This versatile pathogenic bacterium can cause a variety of gastrointestinal and extraintestinal infections, such as hemolytic-uremic syndrome, urinary tract infections, and septicemia (Riley 2020). Many studies revealed that livestock ruminants are the major reservoirs of this important foodborne pathogen (Gonzalez and Cerqueira 2020). Pathogenic strains of *E. coli* are commonly isolated from foodborne illness patients who consumed contaminated dairy products (Elzhraa et al., 2021).

Goats have also emerged as important subclinical carriers 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). *E. coli* contamination can be found in various food products from these small domestic ruminants, such as goat milk and cheese (Ioanna et al., 2018). In Tanzania, more than 90% of goat meat samples have tested positive for *E. coli* (Mwanyika et al., 2016). The goat skin leather and fecal samples collected in the United States and Mexico revealed the presence of *E. coli* contamination at the rates of 1.7% and 19.7%, respectively (Hanlon et al., 2018), and the contamination rate of skin leather and fecal samples from Saudi Arabia was at least 2% (Bosilevac et al., 2015). The household ownership of goats is a factor associated with the detection of pathogenic *E. coli* in children, which causes negative and long-term outcomes of illnesses (Lambrecht et al., 2021). Moreover, 5.9% of goat fecal samples from Rwanda exhibited a multidrug-resistant (MDR) phenotype (Manishimwe et al., 2021). The problem of antimicrobial resistance (AMR) transmission between humans and livestock should be of concern. Given the continuous evolution of AMR genes, *E. coli* strains and phylogenetic results of AMR genes showed random spread patterns between livestock farms in each country (Leekitcharoenphon et al., 2021), especially the extended spectrum β-lactamase (ESBL) *E. coli* which is a major phenotype contaminating pigs, poultry, and small ruminants (Miltgen et al., 2022). ESBLs have been classified into several families such as the TEM-, SHV-, IRT-, CMT- and CTX-M- type ESBLs. Several variants of CTX-M, which is the most widespread ESBL type, commonly found worldwide (Castanheira et al., 2021). The understanding of animal reservoirs and transmission patterns leading to the acquisition of ESBL-producing Enterobacterales between humans and livestock is urgently required. However, studies on the derived surveillance of ESBL *E. coli* infection in small ruminants in Thailand are limited.

Thus, this study aimed to survey the prevalence, antimicrobial susceptibility pattern, and the encoding of $bla_{\text{CTX-M}}$ gene in *E. coli* isolated from healthy goat farms in Sai Yok District, Kanchanaburi Province, Thailand. The data from the small ruminant farm must fill the gaps in our knowledge to solve the AMR problem via a health system.

Materials and Methods

Sample collection

The study protocol was approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC no. MUVS-2017-10-47) and Veterinary Science-Institutional Biosafety Committee (IBC/MUVS-B-003-2561). A total of 92 rectal swab samples were randomly collected from healthy female goats (7 months to 2 years of age) living in seven local goat farms. Each farm was located in each subdistrict of Sai Yok District, Kanchanaburi Province, Thailand (Figure 1 and Table 1).

Bacterial isolation and identification

The rectal swab samples were collected using the Cary-Blair transport medium (EUROTUBO®) and then inoculated onto sheep blood and MacConkey agar plates (Clinical diagnostics LTD., Thailand). A loopful of each culture was streaked on eosin methylene blue-selective agar (Oxoid LTD., England), and the plates were incubated at 37 °C for 24 h. In addition, biochemical tests were performed for further confirmation, including gram staining, triple sugar iron, MIO-motility indole, urease, oxidase and catalase tests (Oxoid LTD., England) (Md Bashir Uddin, 2022). *E. coli* ATCC25922 was used as the control strain. Then, confirmations of microorganism species were performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis at the Kamphaeng Saen Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand.

Antimicrobial Susceptibility Test

Disc diffusion method

The susceptibility of the isolates to antimicrobial agents was determined by the disc diffusion method using the following antimicrobials: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), imipenem (10 µg), meropenem (10 µg), cefotaxime (30 µg), cefpodoxime (30

Figure 1. Modify geographic map of goat farms from 7 subdistricts in Sai Yok District, Kanchanaburi Province, Thailand. (Google, 2022)

Table 1. Prevalence of *E. coli* isolated from goats in livestock farms from 7 subdistricts in Sai Yok District, Kanchanaburi Province, Thailand

Subdistrict	Location of goat farms	No. of rectal swab (n=92)	No. of *E. coli* isolations (n=67)	Prevalence of isolated *E. coli* (%)
Sai Yok	14°28'08.2"N 98°50'32.3"E	8	8	100
Lumsum	14°05'14.8"N 99°07'54.0"E	14	12	85.7
Si Mongkhon	13°59'32.6"N 99°15'25.3"E	14	12	85.7
Tha Sao	14°19'07.1"N 98°58'36.8"E	14	12	85.7
Sing	14°06'54.9"N 99°12'19.6"E	14	11	78.6
Wang Krachae	14°15'31.5"N 98°56'13.4"E	14	7	50.0
Bongti	14°05'08.4"N 99°00'18.1"E	14	5	35.7

µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), enrofloxacin (5 µg), sulfonamides (25 µg), gentamycin (10 µg), amikacin (30 µg), nitrofurantoin (100 µg), and piperacillin/tazobactam (100/10 µg) (Oxoid LTD., England). The zone of inhibition (mm) was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018). *E. coli* ATCC25922 was used as the control strain.

Screening of the ESBL-producing *E. coli* phenotype

The isolates showing resistance characteristics to cefotaxime (the zone of inhibition: less than 27 mm) and ceftriaxone (the zone of inhibition: less than 25 mm) were screened for ESBL production using a modified double-disc synergy test (MDDST). A lawn culture of the organisms was made on a Mueller-Hinton agar plate, as was recommended by CLSI (CLSI, 2018). The amoxicillin/clavulanate (20/10 µg) was placed at the center of the plate along with four cephalosporins: third-generation cephalosporins (cefotaxime, ceftriaxone, and cefpodoxime) and a fourth-generation cephalosporin (cefepime). The discs of third- and fourth-generation cephalosporin were placed 15 and 20 mm apart (Kaur et al., 2013).

bla$_{\text{CTX-M}}$ **gene encoding in *E. coli* isolates by polymerase chain reaction (PCR) technique DNA extraction**

The genomic DNA of *E. coli* isolates was extracted from a single colony following the boiling method (Bai et al., 2010). Briefly, a single colony was picked and boiled in 100 µl RNase-free water (Thermo Fisher Scientific, USA) in a 95 °C block heater for 10 min and then centrifuged at 1,300 rpm for 10 min to remove the cell debris. The supernatant was transferred to a new tube, whose concentration and purity were measured by a Nanodrop One (Thermo Fisher Scientific, USA) at 260/280 and 260/230 ratios, respectively, and kept at -20 °C until use.

Amplification and detection of beta-lactamase gene

The supernatant DNA of boiled bacterial colonies was used as a template to amplify 543 base pairs (bp) of the *bla*$_{\text{CTX-M}}$ gene using the CTX-M universal primers: MA1; 5'-SCSATGTGCAGYACCAGTAA-3' and

MA2; 5'-CCGCRATATGRTTGGTGGTG-3' (Macrogen laboratory, South Korea) (Saladin et al., 2002). The PCR reaction was set up as follows: 12.5 µl Taq polymerase TopTaq master mix (Qiagen., Hilden, Germany) and 0.5 µl each of forward and reverse primers (10 µm), 2.5 µl CoralLoad Concentrate, 2 µl DNA template (50 ng/µl), and up to 25 µl molecular biology grade water in a PCR tube. The CTX-M1 ESBL-producing *E. coli* was used as a positive control (Lugsomya et al., 2018). Amplifications were conducted in a T100TM Thermal Cycler. The thermocycling conditions were set up as follows: one 5 min step at 94 ^{o}C followed by 35 cycles of 30 s at 94 ^{o}C, 30 s at 54 ^{o}C, and 60 s at 72 ^{o}C with a final extension step of 10 min at 72 ^{o}C. Amplicons were electrophoresed on 2.0% agarose gels, stained with GelRed^{TM}(Biotium, USA), and visualized using a c150 UV transilluminator (Azure Biosystems, USA). To verify the DNA quality, we conducted 16S rRNA amplifications using 12.5 µl Taq polymerase TopTaq master mix 2.5 µl coralLoad concentrate, 0.5 µl forward primer (10 µm) (16S rRNA-F: 5'-AGAGTTTGATCCTGGCTCAG-3'), 0.5 µl reverse primer (10 µm) (16S rRNA-R: 5'-CTTGTGCGGGCCCCC GTCAATTC-3') (Macrogen laboratory, South Korea) (Yadav et al., 2009; Magray et al., 2011), 2 µl DNA template (50 ng/µl), and up to 25 µl molecular biology-grade water. The thermocycling conditions were set up as follows: one 3 min at 94 ^{o}C followed by 30 cycles of 30 s at 94 ^{o}C, 30 s at 55 ^{o}C, and 60 s at 72 ^{o}C with a final extension step of 10 min at 72 ^{o}C. The PCR products (758 bp) were examined, stained, and visualized as described (Yadav et al., 2009).

Statistical analysis

The IBM SPSS Statistics 21 was used to analyze the statistical differences in the collected data. The influencing factors of goat farm operations were also analyzed by Chi-square test. All significant levels were justified at *p*-value < 0.05

Results

Prevalence of *E. coli* isolates from healthy goat farms in Sai Yok District, Kanchanaburi Province

A total of 67 *E. coli* isolates (72.8%) were identified from 92 swab samples. These *E. coli* isolates can be found in all goat farms in Sai Yok District. Table 1 showed the prevalence of *E. coli* from each subdistrict. The prevalence rates of the isolated samples from 6 subdistricts (85.7%) were higher than or equal to 50%.

Factors related to the prevalence of *E. coli*

The data of influencing factors related to farming operations were collected and classified (Table 2). The results showed that all 92 goats received antimicrobial agents at least 3 months before the sample collection, and 84.8% of the goats were given drugs by the owner. The number of *E. coli* isolates from these groups was significantly higher than that in the goats that received antimicrobial agents from a veterinarian ($p < 0.001$). The *E. coli* isolated from goats drinking water from natural source nearly livestock farms showed a significant difference compared with those found in goats drinking from the tap water system ($p < 0.038$). The method of farming and type of animal feed did not affect the number of *E. coli* observed.

Table 2. Farming operation factors categorized with the prevalence of *E. coli* isolated from goats in livestock farms from 7 subdistricts in Sai Yok District, Kanchanaburi Province, Thailand.

Factors	Category	No. of rectal swab (%) n=92	No. of *E. coli* isolations (%) n=67	p-value
Method of farming	Intensive rearing	78 (84.8)	55 (82.1)	0.239
	Semi-Intensive rearing	14 (15.2)	12 (17.9)	
Animal feed	Roughage	64 (69.6)	48 (71.6)	0.479
	Roughage and Concentrated	28 (30.4)	19 (28.4)	
Source of water	Tap water system	50 (54.3)	32 (47.8)	0.038*
	Natural sources	42 (45.7)	35 (52.2)	
Previous antimicrobial use	Ever	92 (100)	67 (72.8)	N/A
	Never	-	-	
Person giving antimicrobials	Owners	78 (84.8)	62 (92.5)	0.001*
	Veterinarian	14 (15.2)	5 (7.5)	

N/A=Not applicable, *Significance was determined when $p < 0.05$.

Antimicrobial susceptibility of *E. coli* isolates

The *E. coli* isolates showed a high susceptibility to almost of the antimicrobial agents tested (Table 3). The total samples did not show resistance characteristics to amoxicillin/clavulanic acid, fluoroquinolones, and trimethoprim/sulfamethoxazole. A moderate susceptibility to antimicrobial agents was found with the administration of cephalosporin (35.8%-55.2%) and amikacin (55.2%). The highest rate of resistance was observed with cefotaxime, but the value was less than 20%.

Among the 67 *E. coli* (38.8%) isolates from healthy goats, 26 exhibited the AMR patterns categorized by the number of antimicrobial agents in Table 4. Most of the *E. coli* showed resistance to one agent followed by two and at least three agents (63%, 27%, and 10%, respectively). Group 1 showed the highest number of *E. coli* isolates with cephalosporin resistance. Meanwhile, 57.1% of group 2 and 100% of group 3 also exhibited resistance to cephalosporin.

Screening of the ESBL-producing *E. coli* phenotype and *bla*$_{\text{CTX-M}}$ gene expression

Out the 67 *E. coli* isolates (65.67%), 44 showed positive phenotypic results of MDDST. Thus, the conventional PCR technique was performed and screened the isolates to determine the *bla*$_{\text{CTX-M}}$ gene expression in the total *E. coli* isolates using the universal primers. The results revealed that the *bla*$_{\text{CTX-M}}$ gene expression could not be found in *E. coli* isolated (Figure 2 and 3) from the goat farms in Sai Yok District, Kanchanaburi Province.

Table 3. The antimicrobial susceptibility of *E. coli* isolated from goats (n=67).

Antimicrobials		The antimicrobial susceptibility (%)	
		S	**R**
Penicillins	AMP	92.5 (62)	7.5 (5)
	AMC	100 (67)	-
Carbapenems	IPM	100 (67)	-
	MEM	89.6 (60)	10.4 (7)
Cephalosporins	CRO	95.5 (64)	4.5 (3)
	CPD	97.0 (65)	3.0 (2)
	CTX	80.6 (54)	19.4 (13)
Fluoroquinolones	NOR	100 (67)	-
	CIP	100 (67)	-
	ENR	100 (67)	-
Sulfonamides	STX	100 (67)	-
Aminoglycosides	CN	83.6 (56)	16.4 (11)
	AK	98.5 (66)	1.5 (1)
Other	F	97.0 (65)	3 (2)
	TZP	100 (67)	-

Abbreviation for table 3-4: S=Sensitivity, I=Intermediate, R=Resistant, AMP=Ampicillin, AMC=Amoxicillin/clavulanic acid, IPM=Imipenem, MEM=Meropenem, CRO=Ceftriaxone, CPD=Cefpodoxime, CTX=Cefotaxime, CIP=Ciprofloxacin, NOR=Norfloxacin, ENR=Enrofloxacin, STX=Trimethoprim/sulfamethoxazole, AK=Amikacin, CN=Gentamicin, F=Nitrofurantoin, TZP=Piperacillin/tazobactam

Table 4. The pattern of antimicrobial-resistant of *E. coli* isolated from goats (n=27). Group 1: resistance to 1 antimicrobial agent, Group 2: resistance to 2 antimicrobial agents, Group 3: resistance to more than 3 antimicrobial agents.

By group	By pattern of resistance	The number of resistant *E. coli* (%)	
		Pattern	**Group**
1	CTX	6 (35.3)	17(63)
	CN	4 (23.5)	
	AMP	3 (17.6)	
	CRO	1 (5.9)	
	CPD	1 (5.9)	
	F	1 (5.9)	
	MEM	1 (5.9)	
2	CN+MEM	2 (28.5)	7 (27)
	CN+APM	1 (14.3)	
	CTX+AMP	1 (14.3)	
	CTX+MEM	1 (14.3)	
	CTX+AK	1 (14.3)	
	CTX+CN	1 (14.3)	
≥ 3	CTX+CPD+F	1 (33)	3 (10)
	CTX+CRO+CN+MEM	2 (67)	

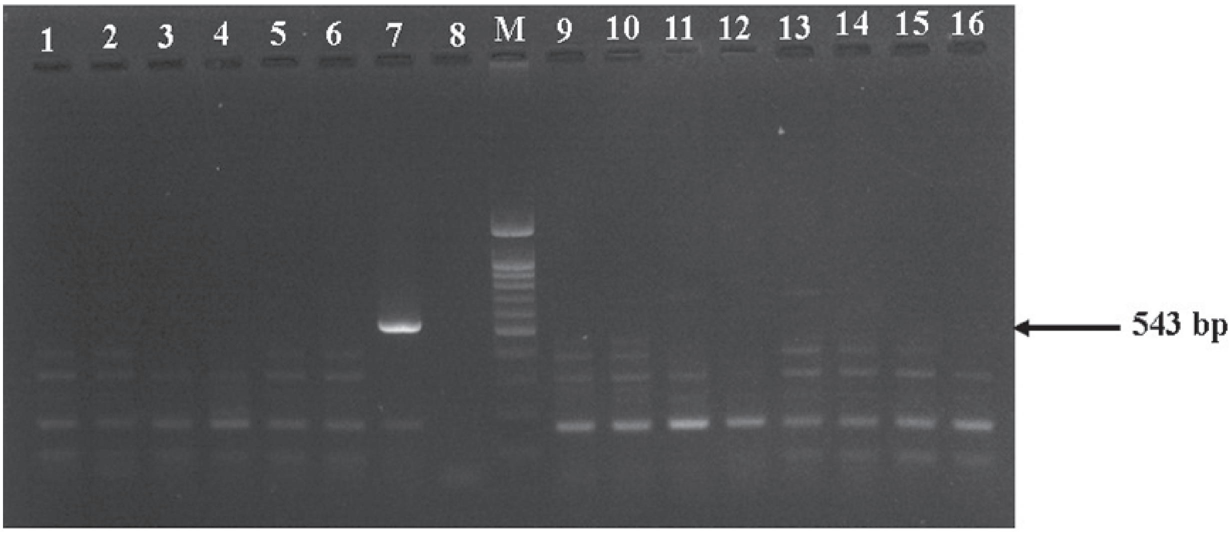

Figure 2. PCR amplification of $bla_{\text{CTX-M}}$ gene of *E. coli* on 2% agarose gel electrophoresis. Lane M DNA ladder, MW 100 bp ladder. Lanes 7 showed a typical band size of 543 bp corresponding to $bla_{\text{CTX-M}}$ of positive isolate using universal CTX primers (MA1 and MA2). Lanes 1-6 and 9-16 were the examples of isolated samples. Lane 8 was the negative control (nuclease-free water).

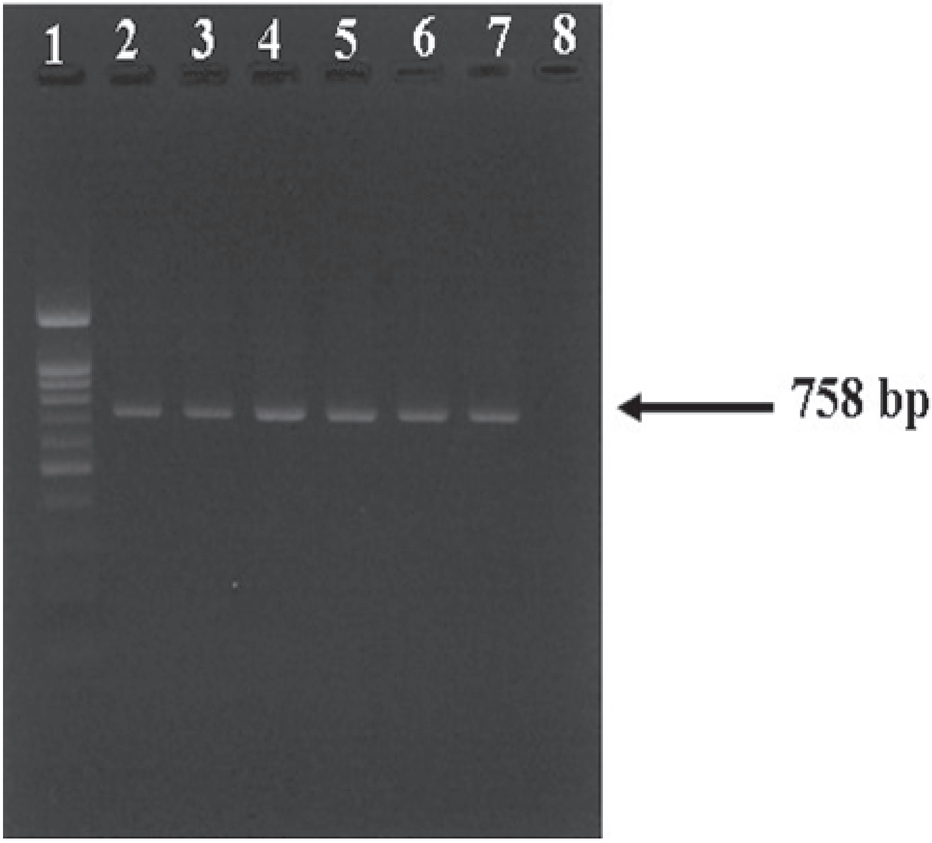

Figure 3. PCR amplification of 16S rRNA gene of *E. coli* on 2% agarose gel electrophoresis. Lane 1 DNA ladder, MW 100 bp ladder. Lane 2 positive control (CTX-M1 ESBL-producing *E. coli*). Lanes 3-7 showed a typical band size of 758 bp corresponding to 16S rRNA of the selected isolates using 16S rRNA primers. Lane 8 was the negative control (nuclease-free water).

Discussion

The monitoring system for the AMR patterns in ESBL-producing bacteria recently focused on relevant information from cattle, pigs, and poultry and linked the summarized data to human healthcare (Murphy et al., 2018; Schrijver et al., 2018). The prevalence *E. coli* isolates harbored $bla_{\text{CTX-M}}$ genes trend to rise even in newborn and young dairy cattle (Waade et al., 2021; Nüesch-Inderbinen et al., 2022). The study of van Hoek showed that the rapid dissemination of ESBL-*E. coli* in broiler farm most likely occurred by the horizontal transfer of plasmid carrying $bla_{\text{CTX-M-1}}$ (van Hoek AHAM et al., 2018). The high level of ESBL-*E. coli* carriage in veterinary healthcare workers due to occupational contact with animals might be important source of ESBL-*E. coli*. (Meijs et al., 2021).

Domestic ruminants, including goats, can be natural reservoirs by carrying *E. coli* in their intestine and excreting microorganisms through feces (Dulo et al., 2015). The digestive tract of goats is a natural habitat for *E. coli*. However (Kannan et al., 2021), the prevalence of *E. coli* in small domestic ruminants, especially goats, is less reported than that in cattle. The samples from healthy farm animals in Tunisia revealed that the highest number of *E. coli* isolated from goats (27.7%) was higher than those from sheep (20%) and cattle (14.2%) (Bessalah et al., 2021). The prevalence rate of *E. coli* isolated from healthy goats in this study (72.8%) was lower than that in studies in Spain (79%-86%) and South Africa (80.2%) (Malahlela et al., 2022). These values were higher than prevalence rates in Vietnam (38.5%) (Vu-Khac et al., 2008) and Greece (37.5%) (Tsilipounidaki et al., 2022). Moreover, *E. coli* can be found in petting zoos (20.0%) by rectal swabs (Göttling et al., 2022). Some previous reports revealed that healthy goats can be reservoirs of pathogenic *E. coli*, including the Shiga toxin-producing and atypical enteropathogenic types. *E. coli* isolates were found in goat carcass, milk, feces, and farm environment, including feed pellets and water (Dulo et al., 2015; Álvarez-Suárez et al., 2016).

All *E. coli* samples from this study had a high susceptibility to almost all of the tested antimicrobial agents, including amoxicillin/clavulanic acid, imipenem, fluoroquinolones, and trimethoprim/sulfamethoxazole. The samples also showed a moderate susceptibility to cephalosporin and amikacin (more than 50%). The highest rate of resistance was observed with cefotaxime, but the value was not more than 20% (19.4%). The various data on AMR characteristics and gene expression were revealed from a previous study. Our results differed from those in studies in Tunisia and USA, where the *E. coli* isolated from goats had the highest resistance rates to tetracycline (44.4% and 51%, respectively) (Bessalah et al., 2021). Ndegwa et al. (2019) showed that *Tet*B and bla-TEM were the most detected from drug-resistant *E. coli*. Most isolates in this study were resistant to only one antimicrobial agent (63%). Similar to the *E. coli* isolated from a goat farm in Bangkok, the resistance rate to at least one antimicrobial agent was 78.3%. However, the highest AMR was observed with streptomycin (65.6%) (Prapasawat and Intarapuk 2021). According to the definition by Magiorakos et al. (2012) MDR phenotypes are non-susceptible to at least one agent in three or more antimicrobial categories; the MDR *E. coli* isolates from this study (7%) had a lower rate than those obtained from another dairy cattle farm (37.1%) (Obaidat et al., 2018).

The recent emergence of MDR *E. coli* threatens worldwide public health. The existence of various antimicrobial gene-encoded β-lactamases poses a concern. The ESBLs are encoded by specific ESBL genes, including bla_{TEM}, bla_{KPC}, and bla_{CTX}. ESBL-harboring *E. coli* were found in 50% of the fecal samples from healthy food-producing animals in Pakistan. Among the ESBL genes, $bla_{\text{CTX-M}}$ was the most prevalent group detected (98.66%) (Shafiq et al., 2022). The $bla_{\text{CTX-M}}$ is commonly detected in porcine (72.0%) and poultry farms (34.2%) (Balázs et al., 2021). In contrast to our results, which were in agreement with those of a previous study by Abdalhamed et al. (2021), $bla_{\text{CTX-M}}$ expression could not be detected from the *E. coli* isolates from goats by rectal swabs. The highest rate of bla_{TEM} expression (25.9%) was found more than that of $bla_{\text{CTX-M}}$. In addition, our study performed the screening of $bla_{\text{CTX-M}}$ gene using the universal primers (MA1/MA2) to indicate at least one of $bla_{\text{CTX-M}}$ genes if the PCR result showed positive. (Ramadan et al., 2019). However, the CTX-M universal primer might not amplify $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-32}}$, $bla_{\text{CTX-M-55}}$, and $bla_{\text{CTX-M-65}}$, which have been reported in sheep, the whole-genome sequencing should be used to explore the multiple CTX-M type (Atlaw et al., 2021).

In petting zoos, only one sample of *Escherichia fergusonii*, not *E. coli*, carried a $bla_{\text{CTX-M-1}}$-encoded ESBL phenotype (Göttling et al., 2022). CTX-M genotypes are associated with different geographical regions. The reports of isolates producing CTX-M remain sporadic in Asia (Hawkey and Jones 2009). In addition, the prevalence of ESBL-producing *E. coli* possessing the $bla_{\text{CTX-M}}$ gene in healthy animals is usually lower than those in diarrheic animals (Shabana and Al-Enazi, 2020).

The contamination by drug-resistant *E. coli* in the farm environment may play a role in the acquisition of resistant bacteria shedding from feces of pastured goats (Ndegwa et al., 2019; Nichols et al., 2021). The *E. coli* prevalence in intensively reared goats in this study was not significantly different from that in semi-intensively reared goats. However, intensive management was the influencing factor of prevalence and AMR of *E. coli* isolates from goats in Jordan (Novotna et al., 2005). From the report of Gutta et al. (2009), goats receiving concentrated feed had a higher level of *E. coli* in the rectum than hay-fed goats. These results differ from the findings of this study. The number of *E. coli* isolated from goats drinking water from the natural sources near livestock farms was significantly higher than that from goats drinking from the tap water system ($p < 0.038$). The antibiotic-resistant *E. coli* can be found from natural sources of water, such as stream, inflow rivers (Ma et al., 2022), and groundwater environments (Tropea et al., 2021). These water sources, which are composed of bed and bank sediments, can be a reservoir of fecal indicator bacteria, including *E. coli*, because of human and livestock fecal contamination (Brinkmeyer et al., 2015; Smolders et al., 2015; Salam et al., 2021). Some plasmid-mediated antibiotic-resistant *E. coli* were collected from riverbed sediments near aquaculture farms and almost had ampicillin, amoxicillin, and streptomycin resistance characteristics (Lihan et al., 2021). These MDR *E. coli* isolates from water samples near aquaculture farms showed high resistance rates to penicillin, amoxicillin, ampicillin, tetracycline, sulfamethoxazole, sulfisoxazole, chloramphenicol, florfenicol and rifampin (Liao et al., 2021). Moreover, other environmental contaminations may be the sources of MDR *E. coli* gene contaminating water sources. The samples from swine feces revealed several drug-resistant genes in *E. coli*, such as *clm*A, *gry*B, *tet*O, *tet*M, *fex*A, *erm*A, *qnr*S, *sul*1, *mcr1*, etc., that can be transmitted horizontally from animal feces to the surrounding environments of farms (Peng et al., 2021).

This study revealed the high prevalence of *E. coli* isolated from the goats that received antimicrobial agents from the farm owner ($p < 0.001$). The integration of work, monitoring, and data sharing system between veterinarians and farmers positively influence the animal health, biosecurity, production management, and livestock AMR problems (Magouras et al., 2017). As the antimicrobial agents dispensed without a veterinary prescription can aggravate the drug resistance problem in livestock farms, effective veterinary services are required to minimize the risk of prevalence and contamination by drug-resistant bacteria (Magnusson et al., 2021). Due to the complex association between the usage of antibiotics on farms and the human health risk (Samreen et al, 2021), the major aspects of future research in goat farm are to investigate of more covering data that may contribute to prevalence and transmission dynamics on goat farms. One Health system may be hopeful strategies for developing the food-animal farming management and managing the challenge of AMR.

In conclusion, the study showed the prevalence of *E. coli* isolated from healthy farm goats in Sai Yok District, Kanchanaburi Province, Thailand (72.8%, 67 isolates from a total of 92 swab samples). This finding implied the healthy goats can be natural reservoirs by carrying *E. coli* in their intestine. The influencing factors of *E. coli* prevalence in this area were the sources of drinking water and person providing the antimicrobial agents. The *E. coli* isolates had a high susceptibility to almost all of the antimicrobial agents tested. All isolates were susceptible to commonly used antimicrobials, such as amoxicillin/clavulanic acid, fluoroquinolones, and trimethoprim/sulfamethoxazole. The *E. coli* isolates showed a moderate susceptibility to cephalosporin (35.8%-55.2%) and amikacin (55.2%). The highest resistance rate was found *E. coli* isolates exposed to cefotaxime (19.4%). Most of the drug-resistant *E. coli* resisted only one antimicrobial followed by two and at least three antimicrobials (63%, 27%, and 10%, respectively). In addition, 7% of the *E. coli* isolates showed the MDR phenotype. The $bla_{\text{CTX-M}}$ gene could not be found from any of the *E. coli* isolates. Although the study revealed the slightly low level of MDR phenotype and non-expression of $bla_{\text{CTX-M}}$ gene in the goat farms, appropriate management and monitoring strategies should be continuously carried out to protect the animal and human health, welfare, and food safety as public concern issues.

Acknowledgements

We thank Assoc. Prof. Dr. Nuvee Prapasarakul and Kittitat Lugsomya, Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, for supporting the original material of ESBL-producing *E. coli* and Asst. Prof. Sakdichod Kimsakulvech for the assistance with the statistical analysis. The study was funded (2018) by the Faculty of Veterinary Science, Mahidol University, Thailand.

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