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Research article

Prevalence of antimicrobial resistance and integrons in *Escherichia coli* isolated from feces of dairy goats in Nong Chok, Bangkok, Thailand

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Abstract

Antimicrobial resistance is recognized as a growing public health problem. Antimicrobial use and misuse in animal farms have boosted antimicrobial resistance among bacteria in the animal habitat and may be transferred to humans. Therefore, this study was to determine the prevalence of antimicrobial resistance, integrons and their association in *Escherichia coli* isolated from dairy goats in Nong Chok, Bangkok. Ninety-four fecal samples from dairy goats were collected by rectal swab between April 2019 and May 2019. Of 180 *E. coli* isolates, 141 were resistant to at least one antimicrobial agent by disc diffusion method. The most frequent *E. coli* resistance was to streptomycin 65.6% (118/180), followed by tetracycline 30.0% (54/180), kanamycin 21.7% (39/180), and sulfamethoxazole/trimethoprim 21.7% (39/180). Furthermore, the percentage of multidrug resistant (MDR) *E. coli* was 23.9% (43/180). Thirty-nine antimicrobial resistance profiles were found in this study and the most common resistance profiles were STR 23.3% (42/180), STR-TET-SXT 10.0% (18/180) and KAN-STR 6.7% (12/180). All of the 180 *E. coli* isolates were detected class 1 and 2 integrons by multiplex PCR. The results revealed 22.2% (40/180) were positive for integrons including resistant isolates 92.5% (37/40) and susceptible 7.5% (3/40). Moreover, *E. coli* isolates resistant to streptomycin, tetracycline, enrofloxacin and sulfamethoxazole/trimethoprim were significantly associated with the presence of integrons ($P < 0.05$). The data of this study indicated that dairy goats in farms could be a reservoir and possible spread of resistant isolates to farmers and consumers via animals and their products.

Keywords: Antimicrobial resistance, Dairy goats, *Escherichia coli*, Integrons

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INTRODUCTION

Antimicrobial resistance is increasingly being recognized as a serious public health problem. The overuse and misuse of antibiotics in animal farms have stimulated antimicrobial resistance among bacteria in the animal habitat (Baragona, 2015; Ketkhao et al., 2019). Antibiotics are regularly used in animal farms for three main purposes: as growth promoters; prophylactic, metaphylactic treatment to prevent diseases and therapeutic purposes to treat diseases (Barton, 2014). In contrast, the negative effects of using antibiotics in agriculture may be partly responsible for the emergence of antimicrobial resistant microorganisms (Marshall and Levy, 2011). Goats are well known to harbor antimicrobial resistant *E. coli* strains and can pass these strains not only to animals but also to humans (Shabana and Al-Enazi, 2020). Not only does the presence of *E. coli* in animal feces permit it to enter the food chain via fecal contamination, but also animal-human contact has a key role in infectious transmission (Lim et al., 2010). In the past 10 years, dairy goat population in Thailand was markedly increased due to the increase in market demand of goat milk consumption in Thailand, especially in the community of Thai Muslims people (Nakavisut and Anothaisinthawee, 2014). Bangkok is the area that has the most dairy goat population in Thailand. Nong Chok is the district of Bangkok where ranked the top three of dairy goat population (Division of Livestock Extension and Development, 2019) and 75% of population is Thai Muslims. People in Nong Chok have a semi-rural lifestyle. Farmers and animals are in close contact resulting in a high risk of antimicrobial resistance infection. However, information regarding antimicrobial resistance of *E. coli* isolated from dairy goats in Thailand is rare and more research needs to be done especially in Nong Chok.

The dissemination of antibiotic resistant genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria (Barlow et al., 2004), especially integrons that have been shown to play an important role in the evolution and dissemination of multidrug resistance in Gram-negative bacteria. There are 3 main classes of integrons mainly associated with antimicrobial resistance in the clinical samples (Deng et al., 2015). Class 1 and 2 integrons were detected in high a percentage of *E. coli* isolates that were obtained from animals and humans (Kheiri and Akhtari, 2016). Currently, there is a lack of published data on antimicrobial resistance and integrons among the enteric bacteria of dairy goats in Thailand. Consequently, the spread of antimicrobial resistance determinants and integrons of *E. coli* in dairy goats are required to be demonstrated. Therefore, the current study aimed to investigate the prevalence of antimicrobial resistance and integrons and their association of *E. coli* isolated from feces of dairy goats in Nong Chok, Bangkok, Thailand.

MATERIALS and METHODS

Sample collection

Ninety-four rectal fecal samples were collected from 13 dairy goat local farms (7-8 samples per farm) in Nong Chok, Bangkok, Thailand between April 2019 and May 2019 (Fig. 1). The rectal swab samples were collected by using the Cary-Blair transport medium. All samples were kept in an ice-box and immediately transferred to the laboratory at Mahanakorn University of Technology within 8 h. The animal ethics clearance was approved by Institutional Animal Care and Use Committee of Mahanakorn University of Technology (ACUC-MUT), Protocol No. 2021/001.

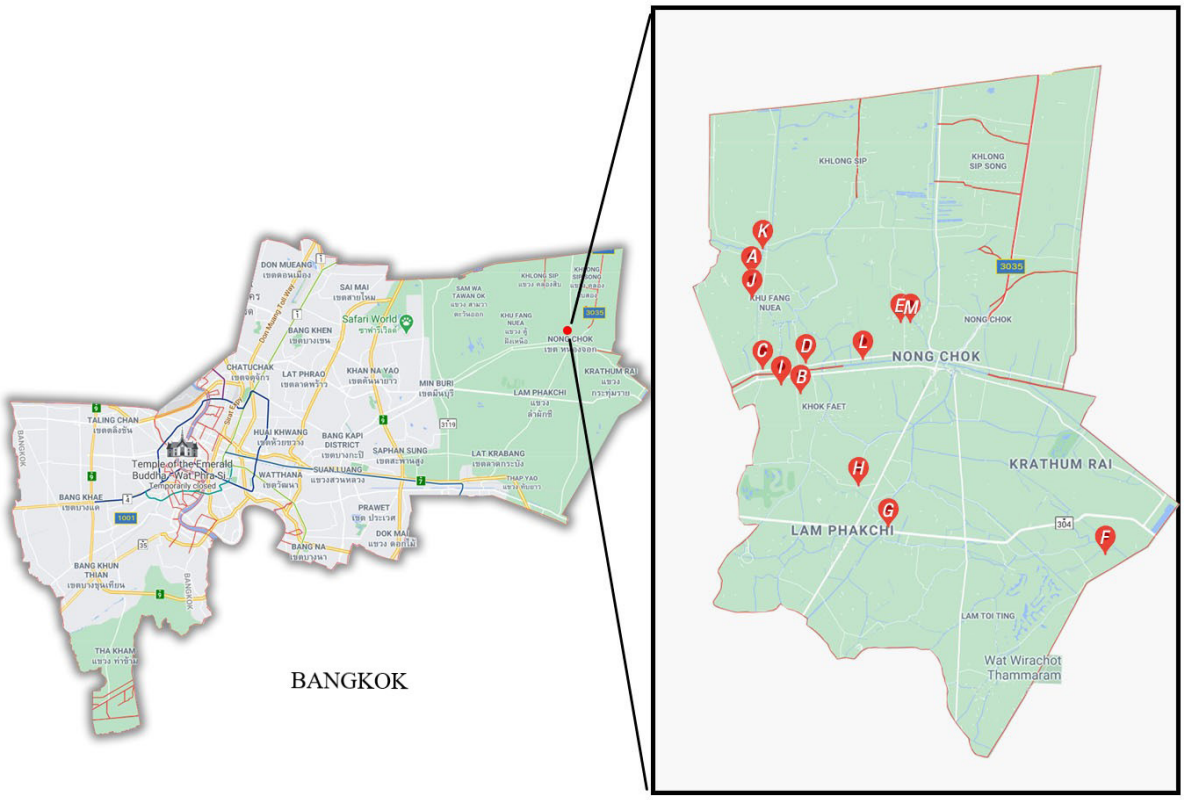

Figure 1 Geographic distribution of the 13 dairy goat farms (A-M) in Nong Chok, Bangkok, Thailand.

Bacterial isolation, identification and DNA extraction

The rectal swab sample was inoculated onto MacConkey agar (Difco, Detroit, MI) then incubated at 37˚C for 24 h. Colonies positive for lactose fermentation were picked and confirmed by the triple sugar iron and indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests (Adams and Moss, 2000). The identified *E. coli* isolates were stocked in 20% of glycerol and stored at -80°C for further analysis. Genomic DNA extraction, E. coli isolates were cultured in Luria-Bertani (LB) broth at 37˚C for 15-18 h and DNA of the *E. coli* isolates were extracted using Qiagen's QIAamp® DNA mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

Antimicrobial susceptibility test

The antimicrobial susceptibility to nine antimicrobial agents from six classes (Oxoid Ltd., Basingstoke, United Kingdom), i.e., amoxicillin/clavulanic acid (AMC), ceftazidime (CAZ), cefotaxime (CTX), enrofloxacin (ENR), gentamicin (GEN), kanamycin (K), streptomycin (STR), sulfamethoxazole/trimethoprim (SXT), tetracycline (TE) was carried out by using disc diffusion method on Mueller-Hinton agar (Oxoid Ltd., Basingtoke, UK) according to Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018). *E. coli* ATCC 25922 was used as a quality control. Multidrug resistance was defined as acquired resistance to at least one antimicrobial agent in three or more antimicrobial classes (Magiorakos et al., 2012).

Detection of class 1 and 2 integrons

All isolates were detected for the presence of class 1 and 2 integrons by multiplex PCR modified from Su et al. (2006) (Table 1). The 25 µl PCR mixture contained 1x buffer solution (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.4 µM of each primer, 1 U of *Taq* polymerase (Promega, Madison, WI, USA) and 2 µl of DNA template. The reaction parameters were 1 cycle for pre-incubation at 94˚C for 5 min; 30 cycles at 94˚C for 30 sec, 52˚C for 30 sec, 72˚C for 2 min and a final cycle at 72˚C for 7 min. The amplicons were visualized under ultraviolet light using Gel document after ethidium bromide staining.

Table 1 Primers used for PCR amplification of class 1 and 2 integrons

Primer	Sequence (5' to 3')	Amplicon (bp)	Targets	Reference
IntM1-U IntM1-D	5'ACGAGCGCAAGGTTTCGGT3' 5'GAAAGGTCTGGTCATACATG3'	565	*IntI 1*	Su et al., 2006
IntM2-U IntM2-D	5'GTGCAACGCATTTTGCAGG3' 5'CAACGGAGTCATGCAGATG3'	403	*IntI 2*	Su et al., 2006

Statistic analysis

The data were analyzed by using SPSS software (version 18.0. SPSS Inc) and the P-value was calculated using a Chi-square test to find any significant relationship. A P-value less than 0.05 was considered statistically significant.

RESULTS

Bacterial isolation and antimicrobial resistance phenotypes

A total of 180 *E. coli* isolates were obtained from 94 fecal samples of dairy goats. The antimicrobial susceptibility of the 180 *E. coli* isolates was tested by disc diffusion method. Resistance isolates to at least one antimicrobial agent were found 78.3 % (141/180) and susceptible isolates were found 21.7% (39/180) in *E. coli* isolates. The highest proportion showed resistance to streptomycin 65.6% (118/180) followed by tetracycline 30% (54/180), sulfamethoxazole/trimethoprim 21.7% (39/180), kanamycin 21.7% (39/180), amoxicillin/clavulanic acid 12.2% (22/180), cefotaxime 6.1% (11/180), ceftazidime 6.1% (11/180), enrofloxacin 5.6% (10/180) and gentamicin 2.8% (5/180), respectively (Fig. 2, Table 2).

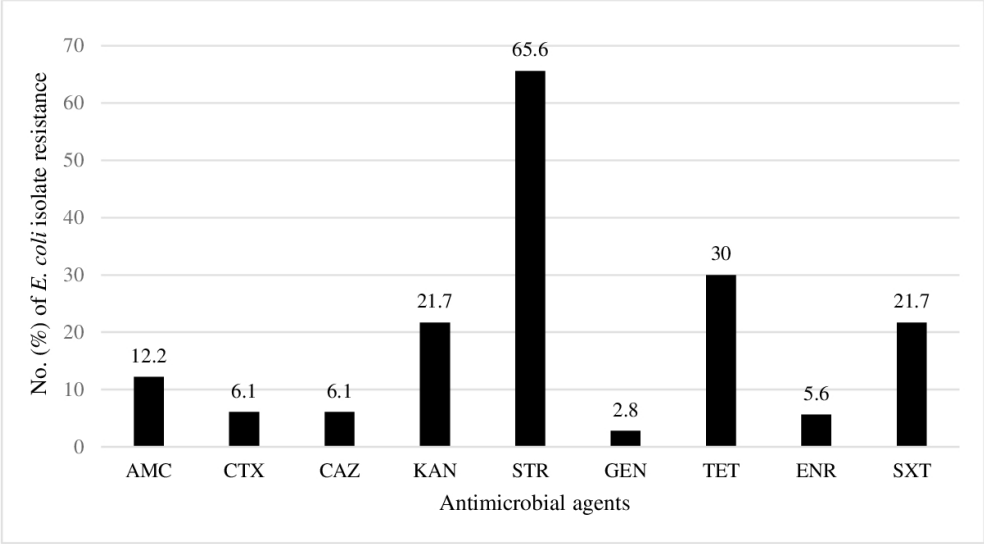

Figure 2 Antimicrobial resistance in *Escherichia coli* isolated from fecal samples of dairy goats. AMC: Amoxicillin/clavulanic acid, CTX: Cefotaxime, CAZ: Ceftazidime, KAN: Kanamycin, STR: Streptomycin, GEN: Gentamicin, TET: Tetracycline, ENR: Enrofloxacin, SXT: Sulfamethoxazole/trimethoprim.

Table 2 Association between antimicrobial resistance and integrons in 180 *Escherichia coli* isolates from fecal samples of dairy goats

Antimicrobial classes	Antimicrobial agents	No.(%) of *E. coli* isolates resistance	No. (%) Integrons-positive			P-value
			int 1	*int 2*	Total	
β-lactamase inhibitor combinations	AMC	22 (12.2)	2 (9.1)	5 (22.7)	7 (31.8)	0.276
Cephalosporins	CTX	11 (6.1)	2 (18.2)	1 (9.1)	3 (27.3)	0.71
	CAZ	11 (6.1)	2 (18.2)	1 (9.1)	3 (27.3)	0.71
Aminoglycosides	KAN	39 (21.7)	4 (10.3)	3 (7.7)	7 (17.9)	0.468
	STR	118 (65.6)	16 (13.6)	19 (16.1)	35 (29.7)	0.001*
	GEN	5 (2.8)	0	2 (40.0)	2 (40.0)	0.308
Tetracyclines	TET	54 (30.0)	16 (29.6)	17 (31.5)	33 (61.1)	<0.001*
Fluoroquinolones	ENR	10 (5.6)	5 (50.0)	2 (20.0)	7 (70.0)	0.001*
Sulfonamides	SXT	39 (21.7)	13 (33.3)	16 (41.0)	29 (74.4)	<0.001*

*Significantly different (χ^2; $P < 0.05$)
β-lactamase inhibitor combinations; Amoxicillin/clavulanic acid (AMC)
Cephalosporins; Cefotaxime (CTX), Ceftazidime (CAZ)
Aminoglycosides; Kanamycin (KAN), Streptomycin (STR), Gentamycin (GEN)
Tetracyclines; Tetracycline (TET)
Fluoroquinolones; Enrofloxacin (ENR)
Sulfonamides; Sulfamethoxazole/trimethoprim (SXT)

Integrons and their association with antimicrobial resistant phenotype

All of 180 *E. coli* isolates were determined for the presence class 1 and 2 integrons (*int1*, and *int2*) by multiplex PCR amplification (Fig. 3). The result showed 22.2% (40/180) were positive for class 1 or class 2 integrons. Among 40 integron-positive isolates in this study, 52.5% (21/40) carried class 1 integron and 47.5% (19/40) carried class 2 integron. Of these integron-positive isolates, 92.5% (37/40) were detected in resistant isolates including class 1 integron 45% (18/40) and class 2 integron 47.5% (19/40), whereas susceptible isolates were found only class 1 integron 7.5% (3/40). The proportion of integron-positive in resistant isolates was significantly higher than those of susceptible isolates ($P<0.05$). Furthermore, the highest percentage of integron-positive isolates was found in SXT-resistance isolates 74.4% (29/39) followed by ENR-resistant isolates 70% (7/10) and TET-resistant isolates 61.1% (33/54) (Table 2). Association between antimicrobial resistance and integrons were analyzed in this study. As expected, *E. coli* isolates resistant to streptomycin, tetracycline, enrofloxacin and sulfamethoxazole/trimethoprim were significantly associated with the presentce of integrons ($P <0.05$) (Table 2).

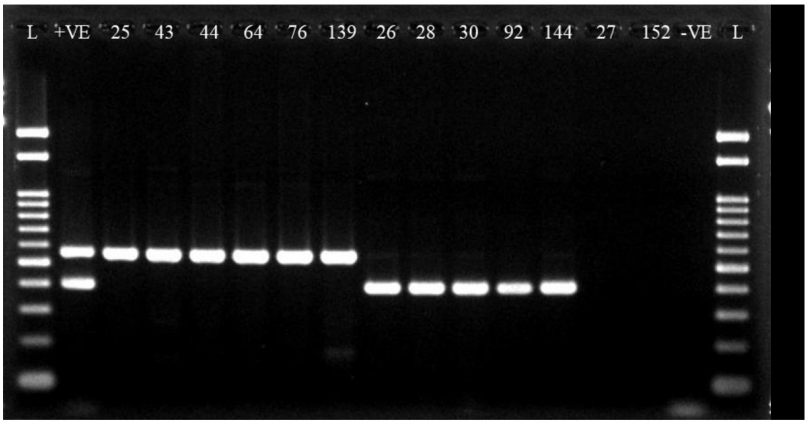

Figure 3 Detection of class 1, and 2 integrons by multiplex PCR method using primer sets specific for integrase (*IntI*) Lane L: Ladder, Lane +VE: Positive control (class 1 integron (565 bp) and class 2 integron (403 bp)), Lane 1-13: the tested *E. coli* isolates; EC-25, EC-43, EC-44, EC-64, EC-76, EC-139, EC-26, EC-28, EC-30, EC-92, EC-144, EC-27, EC- 152, Lane –VE: Negative control (no DNA template), Lane L: Ladder.

Multidrug resistance

Of the 180 *E. coli* isolates, 43 (23.9%) were identified as multidrug resistant (MDR; resistance to at least three antimicrobial classes). Moreover, this study showed 8 categories of antimicrobial resistance patterns ranging from one to eight antimicrobial agents. In all 8 categories of antimicrobial resistance patterns, there were 39 antimicrobial resistance patterns. One-drug resistance was identified 31.7% (57/180), two-drug resistance patterns 20% (36/180), three-drug resistance patterns 16.7% (30/180), four-drug resistance patterns 5% (9/180), five-drug resistance patterns 1.7% (3/180), six-drug resistance patterns 2.2% (4/180), seven-drug resistance patterns 0.6% (1/180) and eight-drug resistance patterns 0.6% (1/180). The most frequent of multidrug resistance pattern was STR-TET-SXT 10% (18/180). One *E. coli* isolate (0.6%) was resistant to 8 tested antimicrobial agents. (Table 3). Moreover, the proportion of integron-positive in multidrug resistance isolates was detected 72.1% (31/43) that included class 1 integron 45.2% (14/31) and class 2 integron 54.8% (17/31). This result obviously demonstrated that integrons-positive isolates were found the highest percent in STR-TET-SXT resistance patterns of MDR (Table 3).

Table 3 Antimicrobial resistance profile of *Escherichia coli* isolated from fecal samples of dairy goats

Antimicrobial resistance patterns	*E. coli* isolates (n=180)	No. (%) of *E. coli* isolates Integrons-positive		
		int 1	*int 2*	Total
MDR	43 (23.9)	14 (32.6)	17 (39.5)	31 (72.1)
One-drug	57 (31.7)	1 (1.8)	0	1 (1.8)
AMC	4 (2.2)	0	0	0
CAZ	3 (1.7)	0	0	0
CTX	1 (0.6)	0	0	0
KAN	7 (3.9)	0	0	0
STR	42 (23.3)	1 (2.4)	0	1 (2.4)
Two-drugs	36 (20.0)	3 (8.3)	1 (2.8)	4 (11.1)
AMC-KAN	1 (0.6)	0	0	0
AMC-STR	2 (1.1)	0	0	0
CTX-CAZ	1 (0.6)	0	0	0
CTX-STR	3 (1.7)	0	1 (33.3)	1 (33.3)
KAN-STR	12 (6.7)	1 (8.3)	0	1 (8.3)
KAN-SXT	1 (0.6)	0	0	0
STR-SXT	2 (1.1)	0	0	0
STR-TET	14 (7.8)	2 (14.3)	0	2 (14.3)
Three-drugs	30 (16.7)	9 (30.0)	11 (36.7)	20 (66.7)
AMC-CTX-STR	1 (0.6)	0	0	0
AMC-KAN-STR	1 (0.6)	0	0	0
AMC-KAN-SXT	1 (0.6)	0	0	0
AMC-STR-ENR	1 (0.6)	0	0	0
CTX-KAN-SXT	1 (0.6)	0	0	0
KAN-STR-TET	3 (1.7)	0	1 (33.3)	1 (33.3)
KAN-TET-ENR	1 (0.6)	0	0	0
KAN-TET-SXT	1 (0.6)	1 (100.0)	0	1 (100.0)

Table 3 Cont.

Antimicrobial resistance patterns	*E. coli* isolates (n=180)	No. (%) of *E. coli* isolates Integrons-positive		
		int 1	*int 2*	Total
STR-GEN-TET	1 (0.6)	0	0	0
STR-TET-SXT	18 (10.0)	7 (38.9)	10 (55.6)	17 (94.4)
TET-ENR-SXT	1 (0.6)	1 (100.0)	0	1 (100.0)
Four-drugs	9 (5.0)	1 (11.1)	5 (55.6)	6 (66.7)
AMC-CAZ-KAN-STR	1 (0.6)	0	0	0
AMC-CTX-KAN-STR	1 (0.6)	0	0	0
AMC-STR-TET-SXT	3 (1.7)	0	3 (100.0)	3 (100.0)
CAZ-STR-GEN-ENR	1 (0.6)	0	1 (100.0)	1 (100.0)
KAN-STR-TET-SXT	2 (1.1)	0	1 (50.0)	1 (50.0)
STR-TET-ENR-SXT	1 (0.6)	1 (100.0)	0	1 (100.0)
Five-drugs	3 (1.7)	2 (66.7)	1 (33.3)	3 (100.0)
AMC-CTX-STR-TET-ENR	1 (0.6)	1 (100.0)	0	1 (100.0)
AMC-KAN-STR-TET-SXT	1 (0.6)	0	1 (100.0)	1 (100.0)
CTX-CAZ-STR-TET-SXT	1 (0.6)	1 (100.0)	0	1 (100.0)
Six-drugs	4 (2.2)	2 (50.0)	1 (25.0)	3 (75.0)
AMC-KAN-STR-TET-ENR-SXT	1 (0.6)	1 (100.0)	0	1(100.0)
AMC-STR-GEN-TET-ENR-SXT	1 (0.6)	0	1 (100.0)	1 (100.0)
CAZ-KAN-STR-TET-ENR-SXT	1 (0.6)	1 (100.0)	0	1 (100.0)
CTX-CAZ-KAN-STR-TET-SXT	1 (0.6)	0	0	0
Seven-drugs	1 (0.6)	0	0	0
AMC-CAZ-KAN-STR-GEN-TET-SXT	1 (0.6)	0	0	0
Eight-drugs	1 (0.6)	0	0	0
AMC-CAZ-KAN-STR-GEN-TET-ENR-SXT	1 (0.6)	0	0	0

The bold text determined MDR of *E. coli* isolates.

DISCUSSION

Increased antibiotic use for therapeutic purposes, growth promotion and disease prevention in animal production is the major causes of emergence of resistant bacteria (Barton, 2014). Goats have been identified as major reservoirs, and food contaminated by their fecal material is a common source for human infections (Kiranmayi et al., 2010). The prevalence of antimicrobial resistance in goats have been reported in many countries, i.e., Kenya 26% (14/54) (Njoroge et al., 2013) Bangladesh 65.38% (51/78) (Islam et al., 2016), Jordan 67.7% (128/189) (Obaidat et al., 2017). This current study showed prevalence of antimicrobial resistance in *E. coli* from dairy goats 78.3 % (141/180) that is slightly higher than those from previous reports. The prevalence may vary depending on several factors, such as geographical area, sampling methods, sample size, collection period, and antimicrobial use, etc. (Tanomsridachchai et al., 2021). Tetracyclines and streptomycin have been used commonly in animal productions, and their residues have been persisted in animal farms (Graslund and Bengtsson, 2001). The high resistance to tetracycline and streptomycin was recorded in different animals (Hariharan et al., 2004; Zou et al., 2018), i.e., swines (>80%) (Prapasawat et at., 2017), broilers (>75%) (Rodroo et al., 2020), rams (97%), goats (71%) (Adefarakan et al., 2014). This study also showed high resistance rates for tetracycline and streptomycin. These presence of antimicrobial-resistant organisms appeared to be due to the uncontrolled use of tetracycline, and streptomycin and the spread of antimicrobial resistant *E. coli* in animal farms.

MDR phenotypes of *E. coli* isolates from goats have been detected in several countries (Lanz et al., 2003), i.e., Bangladesh 39.7% (31/78) (Islam et al., 2016), Jordan 33.3% (63/189) (Obaidat et al., 2017) and Nigeria 81.7% (61/82) (Adefarakan et al., 2014). Our study demonstrated the prevalence of MDR 23.9% (43/180) and STR-TET-SXT patterns was the commonly found. The prevalence of MDR in *E. coli* is strong association with antimicrobials use (Barton, 2014). MDR not only affects animal but also has an impact on the environment by contaminated soil, water, and human through the transfer of resistant organisms via food chain and/or direct contact of farm personnel (d de la Torre et al., 2015). One of the most important factors in the development of resistance to antibiotics is the remarkable ability of bacteria to share genetic resources via lateral gene transfer (LGT) including plasmid, transposon, and integron (Stokes and Gilling, 2011).

Integrons are able to disseminate antibiotic resistant genes by horizontal or vertical transfer and have been shown to play an important role in the evolution and dissemination of multidrug resistance in Gram negative bacteria (Fluit and Schmitz, 2004). Integrons of *E. coli* are commonly found in clinical and commensal isolates from livestock, companion animals, and exotics (Goldstein et al., 2001). Previously studies have been reported prevalence of integrons 55% (49/89) in giant panda, China (Zou et al., 2018), and 90% in waterfowls, China (Zhang et al., 2019). In this study, the result revealed that 22.2 % (40/180) were integrons-positive isolates including, class 1 integron 52.5% (21/40) and class 2 integrons 47.5% (19/40). Similar results have previously been reported in Thailand that reported class 1 integron in swine 11.5% and in chicken 10.8% (Trongjit et al., 2016). The prevalence of integron detection in animal farms

might be due to the type of animals, the farm size and amount or frequency of antimicrobials used in the farms (Prapasarakul et al., 2010; Prapasawat et al., 2017). Moreover, the high detection rate of integrons indicated the ability of dissemination of antimicrobial resistant genes between bacteria and could be transferred to humans.

Association between the presence of integrons and MDR in clinical *Enterobacteriaceae* isolated from people is described in several studies (White et al., 2001). This study also found that the proportion of integrons-positive isolates detected in resistance isolates 92.5% (37/40) was significantly higher than susceptible isolates 7.5% (3/40). Moreover, we found that the resistance to streptomicin, tetracycline, enrofloxacin, and trimethoprim/ sulfamethoxazole was significantly more common in the isolates carrying integrons ($P < 0.05$). This finding agreed with the reports by Stalder et al. (2012) and Kheiri and Akhtari (2016) who recorded the *E. coli* isolated from human and animals containing the streptomycin resistant gene (*aadA*) and trimethoprim/ sulfamethoxazole resistant gene (*dfrA*). This association was explained by the play role of integrons in the evolution and dissemination of multidrug resistance in Gram negative bacteria, while the resistance to amoxicillin/clavulanic acid, cefotaxime, ceftazidime, kanamycin, gentamycin was not associated with the isolates carrying integrons. The similar percentage of association between antimicrobial resistance and integrons-positive as well as integrons-negative were also found in *E. coli* isolated from swines and farmers (Phongpaichit et al., 2007). Although integrons play a major role in the spread of resistance genes, the resistance is not spread through integrons but rather through other exchange mechanisms such as plasmids, transposons, and bacteriophages (Kang et al., 2005). Moreover, this study revealed 72.1% (31/43) of multi-antimicrobial resistance isolates carrying integrons. These data suggest that food-producing animals might be simultaneously considered as a reservoir of clones and integrons carrying multi-antimicrobial resistance genes. However, the results of these isolates in present study should be further investigated focusing on gene cassettes encoding resistance to class 1 and 2 integrons.

CONCLUSION

In summary, this is the first study to investigate the prevalence of antimicrobial resistance and integrons in *E. coli* isolates from dairy goats in Nong Chok, Bangkok, Thailand. This study reveals the high level of antimicrobial resistance emergence in dairy goats. Moreover, the high detection rate of integrons indicated the ability of dissemination of antimicrobial resistance in dairy goat farms. These findings could be of concern to farmers and consumers, especially animal-human contact and unpasteurized goat milk consumption

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CONFLICT ON INTEREST

The authors declare no conflicts of interest.

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