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Research article

Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* strains in dairy farm wastewater in Chiang Mai

Prayuth Saekhow[1,*] and Chayaphon Sriphannam[2,3]

[1]Division of Veterinary Para clinic, Department of Veterinary Bioscience and Public Health, Faculty of Veterinary Medicine, Chiang Mai 50100, Thailand.
[2]Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok 65000 Thailand
[3]Center of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

Abstract

We investigated the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli strains in dairy farm wastewater in Chiang Mai, Thailand. We analyzed wastewater samples collected from 150 dairy farms and found that 88.7% of the farms (n = 133) were positive for ESBL-producing *E. coli*. Multiplex polymerase chain reaction (PCR) amplification was performed to characterize the presence of *bla CTX-M*, *bla TEM*, and *bla*SHV in ESBL-producing isolates. *blaCTX-M* was found in all isolates (n = 133), followed by *blaTEM* (80/133, 60.2%), whereas *bla*SHV was not detected in any isolate. *blaCTX-M* and *blaTEM* were present in 60.2% (80/133) of the isolates, and 39.8% (53/133) isolates carried *bla CTX-M* alone. Subgroup analysis showed that *CTX-M*-1 was the most prevalent subgroup among the isolates (129/133, 97.0%), followed by *CTX-M*-8 (2/133, 1.5%) and *CTX-M*-9 (2/133, 1.5%). The distribution of the phylogenetic groups was as follows: group A (100/133, 75.2%), followed by B1 (14/133, 10.5%), D (6/133, 4.5%), F (6/133, 4.5%), B2 (4/133, 3.0%), and E (3/133, 2.3%). Based on enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and dendrogram analysis, 24 isolates were classified into clades I (n = 21), II (n =1), and III (n =2). Minor genetic differences were found in all clade I isolates. Our data suggest that the circulating of ESBL-producing *E. coli* carried at least one *bla* gene strain distributed in dairy farm wastewater in Chiang Mai.

Keywords: Chiang Mai, Cow, Extended-spectrum beta-lactamase, *E. coli*, Wastewater

Corresponding author: Prayuth Saekhow, Division of Veterinary Para clinic, Department of Veterinary Bioscience and Public Health, Faculty of Veterinary Medicine, Chiang Mai 50100 Thailand, Email Address: chayaphons@nu.ac.th , Tel: 66 53948056, Fax: 66 53948065.

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INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing bacteria from the Enterobacteriaceae family have been frequently reported (Coque et al., 2008; Dhillon and Clark, 2012; Rawat and Nair, 2010; Anuchatkitcharoen et al., 2020). ESBLs are enzymes that can inactivate the third-generation cephalosporins cefotaxime and ceftazidime (M'Zali et al., 2000). The presence of ESBL-producing *Escherichia coli* in food-producing animals has created a public health problem (Madec et al., 2017; Pitout and Laupland, 2008). Several investigations have shown that food-producing animals serve as a reservoir for ESBL-producing *E. coli* (Madec et al., 2017; Michael et al., 2017; Umpiérrez et al., 2017). Improper antibiotic use in animal husbandry has been associated with the spread of ESBLs (Landers et al., 2012; Marshall and Levy, 2011; Mellata, 2013; Natthida Sooksai et al., 2019). In food-producing animal farms, antibiotics are used to treat and prevent bacterial diseases (Landers et al., 2012; Michael et al., 2017) and as a result affect the normal flora of the animals, including *E. coli*. Survival adaptations by these bacteria involve acquiring antibiotic-inactivating enzymes encoded by antimicrobial resistance genes (Courvalin, 1994; West et al., 2011). The genes encoding ESBL acquired by *E. coli* in food-producing animals could then spread to humans via the food supply chain, contaminated water, and healthy fecal carriers. These bacteria are normal flora in the animal intestinal tract (Mellata, 2013; Michael et al., 2017; Watson et al., 2012).

Since the discovery of the ESBL TEM-1 in 1965, various ESBLs have been identified in Enterobacteriaceae, particularly *E. coli* (Bar-Yoseph et al., 2016; McDanel et al., 2017). The most common ESBLs produced by *E. coli* are CTX-M, SHV, and TEM, which are encoded by the *bla* family genes bla_{CTX-M}, bla_{SHV}, and bla_{TEM}, respectively (Bar-Yoseph et al., 2016; Feizabadi et al., 2010; Seenama et al., 2019). CTX-M ESBLs are classified into various subgroups, such as CTM-X-1, CTM-X-2, CTM-X-8, CTM-X-9, and CTM-X-25 (Cantón et al., 2012; Livermore and Hawkey, 2005; Woerther et al., 2013). Over the past decade, the prevalence of CTM-X-15 has increased in many countries, including Thailand (Niumsup et al., 2018; Seenama et al., 2019; Watson et al., 2012).

The phylogenetic *E.coli* group was classified using quadruplex PCR to detect the presence or absence of genes, including *chuA*, *yjaA*, *TspE4.C2*, and *asp*. Therefore, seven phylogenetic groups were established: A, B1, B2, C, D, E, and F (Clermont et al., 2013). Phylogenetic group A was the most frequently isolated group in the mammalian intestinal tract, feces, and wastewater (Coura et al., 2015). Phylogenetic groups B2 and D were frequently isolated from extraintestinal *E. coli*, causing diseases such as mastitis and urinary tract infection (Horcajada et al., 2005; Le Gall et al., 2007; Liu et al., 2014).

ERIC-PCR generates DNA fingerprints using a pair of primers (Wilson and Sharp, 2006) and is an accurate, rapid, and reliable diagnostic approach (Meacham et al., 2003). The ERIC-PCR results and the dendrogram analysis of *E.coli* could be investigated for determination of genetic diversity and genetic source (Casarez et al., 2007; da Silveira et al., 2002; Leung et al., 2004).

In Chiang Mai, Thailand, ESBL-producing *E. coli* have been isolated from chicken and pig manure (Nuangmek et al., 2018; Rodroo et al., 2021). However, this is the first report of such isolates in dairy farm wastewater. ESBL-producing *E. coli* in wastewater can likely spread and contaminate inland water and natural environments.

This study aimed to investigate the prevalence, *bla* gene, phylogenetic group, and genetic diversity of ESBL-producing *E. coli* in wastewater collected from dairy farms in Chiang Mai, Thailand.

MATERIALS and METHODS

Sample collection

Samples were collected from 150 dairy farms located in three districts in Chiang Mai, Thailand: Mae On, Mae Wang, and Chai Prakan (n = 50 per districts). The wastewater samples were collected in sterile 50-mL sterilized bottles and stored on ice until use. The sample collection periods were as follows: Mae On in December 2019 and Mae Wang and Chai Prakan in January 2020. All farms were small holder farms with a dairy herd size of 10–50. The farm wastewater management system comprised wastewater collection in a manhole.

Bacterial isolation

E. coli strains were isolated by the direct plating method (Schauss et al., 2015). In brief, 10 mL of wastewater was inoculated in 90 mL of Luria–Bertani (LB) broth for 24 h at 37°C. The cultures were streaked on LB agar plates containing 2 mg/L cefotaxime and incubated for 24 h at 37°C. The resultant colonies were streaked on fresh LB agar plates and evaluated using the indole, methyl red, Voges–Proskauer, and citrate tests. After the biochemical tests, PCR was performed to detect a specific *E.coli* gene, *uspA*, using a specific pair of primers showed in Table 1.

Table 1 Primers for detect *blaCTX-M*, *blaTEM*, *blaSHV*, and *usp*A gene internal control

Gene	Primer	Sequence (5′ to 3′)	Product Size (bp)	References
bla_{CTX-M}	CTX-M-U1	ATGTGCAGACCAGTAAGATGGC	593	Monstein et al., 2007
	CTX-M-U2	TGGGTAATAGTACCAGAACAGCGG		
bla_{TEM}	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein et al., 2007
	TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT		
bla_{SHV}	SHV.SE	ATGCGTTATATTCGCCTGTG	747	Monstein et al., 2007
	SHV.AS	TGCTTTGTTATTCGGGCCAA		
uspA	uspA-up	CCGATACGCTGCCAATCAGT	884	Chen and Griffiths, 1998
	uspA-down	ACGCAGACCGTAGGCCAGAT		

Screening for ESBL-producing *E. coli*

ESBL-producing *E. coli* were screened using a standard double-disc synergy test according to Clinical Laboratory Standard Institute recommendations (CLSI, 2012). Briefly, bacterial suspensions were normalized to McFarland 0.5 turbidity standard in normal saline. Mueller–Hinton agar plates were inoculated with the bacterial suspensions and overlaid with disks containing ceftazidime (30 µg), ceftazidime (30 µg) + clavulanic acid (10 µg), cefotaxime (30 µg), and cefotaxime (30 µg) + clavulanic acid (10 µg). ESBL production was indicted by a growth inhibition zone of ≥5 mm around the combination drug disks versus the single-drug disks. CLSI recommends performing phenotypic confirmation of potential ESBL-producing *E. coli* isolates by testing both cefotaxime and ceftazidime, alone and in combination with clavulanic acid.

Genomic DNA extraction

In this study, we performed genomic DNA extraction using bacterial culture boiling in media as previously described (Madico et al., 1995). ESBL-producing *E. coli* were cultured on LB agar at 37°C for 24 h, and then a single colony was cultured in LB broth at 37°C for 24 h. A 200-µL aliquot of bacterial culture was added to 800 µL of distilled water, boiled at 95° for 10 min, and clarified by centrifugation at 12,000 rpm for 5 min. Then, the supernatant was collected and stored at −20°C.

Detection of blaTEM, blaSHV, blaCTX-M, and CTX-M subgroups

Multiplex polymerase chain reaction (PCR) was performed to detect the presence of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ in 133 ESBL-producing *E. coli* isolates. All CTX-M ESBL-producing isolates were then classified using multiplex PCR with subgroup-specific primers for CTM-X-1, CTM-X-2, CTM-X-8, CTM-X-9, and CTM-X-25. The primers used in this study are shown in Tables 1 and 2. PCR consisted of an initial enzyme activation step at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and final extension at 72°C for 7 min. The multiplex PCR reaction mixture contained 10 µL of 2× HS Quick Taq Dye Mix (including Taq polymerase; Toyobo, Osaka, Japan), 0.2 µL of each primer, 8.2 µL of DNase-free water, and 1 µL of bacterial DNA.

The detection of CTM-X-1, CTM-X-2, CTM-X-8, CTM-X-9, and CTM-X-25 was performed using the same method used to characterize *bla*.

Table 2 Primers for CTX-M subgrouping (Woodford et al., 2005)

Primer	CTX-M Subgroup	Sequence (5′ to 3′)	Product Size (bp)
CTX-M-1-F	Group 1	AAA AAT CAC TGC GCC AGT TC	415
CTX-M-1-R		AGCTTATTCATCGCCAC TT	
CTX-M-2-F	Group 2	CGACGCTACCCCTGCTAT T	552
CTX-M-2-R		CCAGCGTCAGATTTTTCAGG	
CTX-M-9-F	Group 9	CAAAGAGAGTGCAACGGATG	205
CTX-M-9-R		ATTGGAAAGCGTTCATCACC	
CTX-M-8-F	Group 8	CGCGTTAAGCGGATGATGC	666
CTX-M-25-F	Group 25	GCACGATGACATTCGGG	237

Phylogenetic grouping

The distribution of phylogenetic groups of ESBL-producing *E. coli* isolates was examined using a previously described protocol (Clermont et al., 2013). The *E. coli* strains could be classified into the following phylogenetic groups: A, B1, B2, C, D, E, and F. To examine the phylogenetic group of ESBL-producing *E. coli* isolates, we used a quadruplex PCR method based on the presence or absence of the *chuA*, *yjaA*, TspE4.C2 and arpA genes. ESBL-producing *E. coli* isolates, identified as phylogenetic group E or C isolates by quadruplex PCR, were further subjected to phylogenetic grouping by specific primers for further investigation of phylogenetic groups E and C. A list of the primers is provided in Table 3. PCR consisted of an initial enzyme activation step at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 3 min; and final extension at 72 °C for 7 min.

Table 3 Primers for detect phylogenetic grouping (Clermont et al., 2013)

Type of PCR	Target	Primer	Sequence (5′ to 3′)	Product Size (bp)
Quadruplex	*Chu*	chuA.1b	ATGGTACCGGACGAACCAAC	288
		chuA.2	TGCCGCCAGTACCAAAGACA	
	yjaA	yjaA.1b	CAAACGTGAAGTGTCAGGAG	211
		yjaA.2b	AATGCGTTCCTCAACCTGTG	
	TspE4.C2	TspE4C2.1b	CACTATTCGTAAGGTCATCC	152
		TspE4C2.2b	AGTTTATCGCTGCGGGTCGC	
	arpA	AceK.f	AACGCTATTCGCCAGCTTGC	400
		ArpA1.r	TCTCCCCATACCGTACGCTA	
Group E	*arpA*	ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	301
		ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG	
Group C	*trpA*	trpAgpC.1	AGTTTTATGCCCAGTGCGAG	219
			TCTGCGCCGGTCACGCCC	

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

ERIC-PCR was performed to assess the genetic diversity of eight ESBL-producing *E. coli* isolates per district. The ERIC primers used were 5′-ATGTAAGCTCCTGGGGATTCAC-3′ and 5′-AAGTAAGTGACTGGG-GTGAGCG-3′ (Wilson and Sharp, 2006). The cycling conditions included an initial enzyme activation step at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 7 min; and final extension at 72°C for 7 min.

The amplified products were separated using 1% agarose gel electrophoresis with RedSafe™ (Scientifix, NSW, Australia), and the green bands were visualized under ultraviolet light. The banding pattern was used as the principal clade determinant by PyElph (Pavel and Vasile, 2012).

Biosafety protocols

The biosafety protocols (number CMUIBC A-076202) were approved by the Institutional Biosafety Committee of the Faculty of Veterinary Medicine, Chiang Mai University.

Statistical analysis

Poisson regression was performed to evaluate the statistical significance of bla_{CTM-X} and bla_{TEM} co-expression and bla_{CTX-M} expression alone.

RESULTS

Prevalence of ESBL-producing *E. coli* in dairy farm wastewater

Wastewater samples from a total of 150 dairy farms were screened. The prevalence of the ESBL-producing *E. coli* isolates in each district were as follows: 30.8% (n = 41) were from Mae On, 35.3% (n = 47) were from Mae Wang, and 33.8% (n = 45) were from Chai Prakan. The results showed that 88.7% of farms (n = 133) were positive for ESBL-producing *E. coli*.

bla and CTX-M subgroup distribution

Genetic analysis was performed to characterize the 133 isolates for bla_{CTX-M}, bla_{SHV}, and bla_{TEM} using multiplex PCR with specific primers. bla_{CTX-M} was found in every isolate (133/133, 100%), followed by bla_{TEM} in 60.2% of isolates (80/133). bla_{SHV} was not detected in any isolate. bla_{CTX-M} co-existing with bla_{TEM} were carried by 60.1% (80/133) of the ESBL-producing E. coli isolates, and 39.8% (53/133) of the isolates carried bla_{CTX-M} alone. Poison regression analysis indicated that the likelihood of bla_{CTX-M} co-expessing with bla_{TEM} was highly significant (Z = 26.81).

CTX-M subgroup analysis of all 133 ESBL-producing *E. coli* isolates revealed that 97.0 % (129) belonged to the CTX-M-1 subgroup, followed by CTX-M-8 (n = 2, 1.5%) and CTX-M-9 (n = 2, 1.5%). CTX-M-1 was the most prevalent subgroup in all study areas. Two isolates of CTX-M-9 were found in Mae Wang, and two isolates of CTX-M-8 were found in Chai Prakan.

Phylogenetic groups

Phylogenetic group analysis was performed for all 133 isolates of ESBL-producing *E. coli*. The majority of isolates belonged to phylogenetic group A (100/133, 75.2%), followed by B1 (14/133, 10.5%), D (6/133, 4.5%), F (6/133, 4.5%), B2 (4/133, 3.0%), and E (3/133, 2.0%). Phylogenetic group C was not detected. The geographic distribution of these phylogenetic groups is shown in Table 4. Six phylogenetic groups of ESBL-producing *E. coli* were found in Mae On and Mae Wang (A, B1, B2, D, E, and F), but only two groups (A and F) were found in Chai Prakan.

Table 4 The geographic distribution and phylogenetic groups

	Districts																		
	Mae On						Mae Wang						Chai Parkarn						Total
Phylogenetic group bla genes	A	B1	B2	D	E	F	A	B1	B2	D	E	F	A	B1	B2	D	E	F	
CTXM	8	1	1	ND	1	ND	16	2	1	1	2	ND	16	1	ND	ND	ND	3	53
	(6.0%)	(0.8%)	(0.8%)		(0.8%)		(12.0%)	(1.5%)	(0.8%)	(0.8%)	(1.5%)		(12.0%)	(0.8%)				(2.3%)	(39.8%)
CTXM+TEM	19	5	2	2	1	1	18	4	ND	1	1	1	23	1	ND	ND	ND	1	80
	(14.3%)	(3.8%)	(1.5%)	(1.5%)	(0.8%)	(0.8%)	(13.5%)	(3.0%)		(0.8%)	(0.8%)	(0.8%)		(0.8%)				(0.8%)	(60.2%)

ERIC-PCR

To determine the genetic diversity of ESBL-producing *E. coli*, we performed ERIC-PCR fingerprinting using the ERIC-1 and ERIC-2 primers. The selection criteria to investigate the genetic diversity of ESBL-producing *E. coli* strains included the phylogenetic group and the district. The first criteria selected was phylogenetic group A as this strain was predominant in both this and other studies. As the second criteria, we randomly selected each of the eight ESBL-producing *E. coli* strains per district from Mae On, Mae Wang, and Chai Prakan. Dendrogram analysis of the 24 isolates showed three putative clades: clade I, clade II, and clade III (Figure 1). Twenty-one isolates belonged to clade I (Mae On, 8; Mae Wang, 6; Chai Prakan, 7). ESBL-producing *E. coli* strains in clade I showed a minor genetic differences. A single isolate from Mae Wang belonged to clade II, and the remaining isolates belonged to clade III one each from Mae Wang and Chai Prakan.

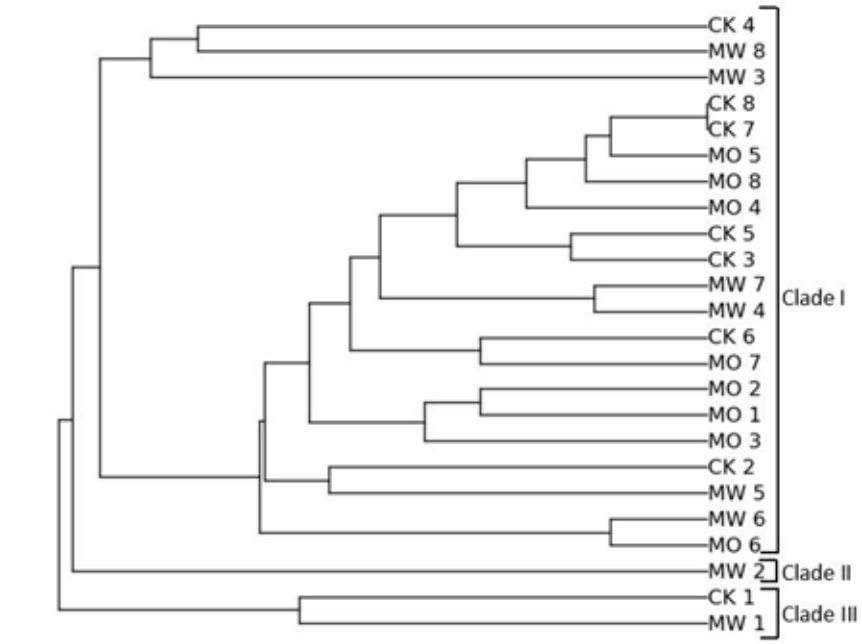

Figure 1 A dendrogram was generated based on ERIC-PCR profiles with 24 of ESBL-producing *E. coli* isolates. Among 24 ESBLs-producing *E.coli* isolates were classified into 3 clades named as, clade I, II, and III. The dendrogram was constructed by PyElph program using unweighted pair group method with arithmetic mean. (MO = Mae On, MW = Mae Wang and CK = Chai Prakan).

DISCUSSION

We investigated the prevalence of ESBL-producing *E. coli* isolated from wastewater samples collected from dairy cattle farms in Chiang Mai as these sites may be the origin of contaminants that leach into natural water sources. The waste management of dairy farms usually involves the collection of wastewater in manholes. The land surrounding the farm is arable and has irrigation systems. Irrigation systems use natural water as reservoirs to store water. The reservoirs are used for human consumption and cultivation (Ngammuangtueng et al., 2019) This irrigation system might be the link between the dairy farms and the natural water sources.

ESBL-producing *E. coli* were identified in 88.7% of wastewater samples in three districts: Mae On, 30.8%; Mae Wang, 35.3%; and Chai Prakan, 33.8%. The prevalence of ESBL-producing *E. coli* in this study may be explained by the improper use of antibiotics. Although this study did not investigate the use of antibiotics in dairy farms, a survey was previously conducted in Mae On showing that livestock farmers lack understanding of the negative effects of antibiotic use. (Sooksai et al., 2019; Suriyasathaporn et al., 2012). There is also a causal relationship between antibiotic use and the development of antimicrobial resistance. ESBL-producing *E. coli* are resistant to many commonly used antibiotics (Hardy, 2002; Landers et al., 2012). Multidrug-resistant *E. coli* has been reported in Chiang Mai and in the central region of Thailand (Boonyasiri et al., 2014). ESBL-producing *E. coli* strains have been isolated in the cattle intestinal tract and the farm environment, including pen floors, collecting yards, and slurry ponds (Boonyasiri et al., 2014). Our data suggest that farm wastewater, where cow feces and water are collected, is a reservoir of ESBL-producing *E. coli*.

There are many ESBLs, such as CTX-M, SHV, TEM, OXA, PER, VEB, BES, GES, SFO, TLA, and IBC (Rawat and Nair, 2010). CTX-M, SHV, and TEM are the most prevalent in ESBL-producing *E. coli* worldwide (Bonnedahl et al., 2009; Cantón et al., 2012; Coque et al., 2008; Sasaki et al., 2010; Watson et al., 2012). In this study, $bla_{CTX\text{-}M}$ was found in 100% (n = 133) of samples, 60.2% of which (n = 80) also carried bla_{TEM}. These findings are consistent with prior reports of $bla_{CTX\text{-}M}$ as the dominant *bla* gene in dairy cattle in many parts of the world (Cormier et al., 2016; Rehman et al., 2017; Tamang et al., 2013). Similar to a study of ESBL-producing *E. coli* in Thailand, $bla_{CTX\text{-}M}$ has been reported as the dominant *bla* gene in clinical isolates, human feces, pig feces, farm waste, and canal water (Boonyasiri et al., 2014; Bubpamala et al., 2018; Niumsup et al., 2018; Nuangmek et al., 2018; Runcharoen et al., 2017; Sasaki et al., 2010). A study conducted in Lamphun, which neighbors Chiang Mai, showed a predominance of $bla_{CTX\text{-}M}$ and bla_{TEM} co-expression in human feces and pig feces (Seenama et al., 2019). The co-expression of $bla_{CTX\text{-}M}$ with bla_{TEM} was significant (z = 26.81), as determined using Poison regression. Therefore, our data suggested that at least two antibiotic resistance genes in ESBL-producing *E. coli* provide best-fit adaptation.

Based on genetic differences, CTX-M can be classified into five groups: CTM-X-1, CTM -X-2, CTM-X-8, CTM-X-9, and CTM-X-25, and each group is composed of a number of variants (Lewis et al., 2007; Livermore and Hawkey, 2005; Sasaki et al., 2010; Tamang et al., 2013; Watson et al., 2012). CTX-M

subgroups can be characterized using multiplex PCR with specific primers or nucleotide sequences (Tamang et al., 2013; Woodford et al., 2005). In this study, CTX-M-1 was found to be the most prevalent type of ESBL in all the study areas, with CTX-M-2 and CTX-M-25 in far fewer locations. We were unable to investigate the most common CTX-M-1 variant, CTX-M-15, because of limited resources and facilities. Further studies will be needed to explore the enzyme kinetics of $bla_{CTX\text{-}M}$ espectailly CTM-X-1 with bla_{TEM} *in vitro* and *in vivo*.

A quadruplex PCR amplification of the genes chuA, yjaA, TspE4. C2, and asp was performed for phylogenetic classification (Clermont et al., 2013). *E. coli* phylogenetic groups differ in their phenotypic and ecological characteristics (Duriez et al., 2001). Phylogenetic group A are the most well adapted to different environments and are the most frequently isolated group in the mammalian intestinal tract, feces, and wastewater (Coura et al., 2015). In contrast, phylogenetic groups B2 and D are frequently isolated from extraintestinal *E. coli*, which cause diseases such as mastitis and urinary tract infection (Horcajada et al., 2005; Le Gall et al., 2007; Liu et al., 2014). In this study, ESBL-producing *E. coli* isolates predominantly belonged to phylogenetic group A (75.2%), followed by B1 (10.5%), D (4.5%), F (4.5%), B2 (3%), and E (2%). The predominance of groups A and B1 is consistent with reports of these groups in household wastewater investigations conducted worldwide (Figueira et al., 2011). The variability in *E. coli* antibacterial resistance and virulence genes among phylogenetic groups is considered a significant concern. ESBL-producing *E. coli* strains from cow mastitis has been associated with phylogenetic group A (Dogan et al., 2012; Goldstone et al., 2016; Tomazi et al., 2018) and *E. coli* phylogenetic group B1 isolates carrying eae were isolated from diarrheic calves (Coura et al., 2017). Importantly, the most prevalent ESBL-producing *E. coli* isolate in this study belonged to phylogenetic group A or B1, and these groups can be disseminated to natural water sources and thus reach human communities that consume the contaminated water.

In this study, ERIC-PCR and dendrogram analysis were used to classify 24 group A ESBLs-producing *E. coli* isolates into clades I, II, and III. Slight genetic differences were found in clade I isolates (Mae On, 8; Mae Wang, 6; and Chai Prakan, 7). These findings suggest that phylogenetic group A ESBL-producing *E. coli* were distributed in dairy farms in Chiang Mai. Bacteria constantly undergo adaptation and exchange genetic materials with other bacteria via plasmids, integration, and bacteriophages (Médigue et al., 1991). These genetic modifications provide survival advantages and increase population fitness, leading to the emergence of clones (Brisson-Noël et al., 1988; Price et al., 2008).

CONCLUSION

We studied the prevalence of ESBL-producing *E. coli* isolates in dairy cattle farm wastewater in Chiang Mai. The results showed a high prevalence of ESBL-producing *E. coli* isolates. We also studied the distribution of antimicrobial resistance genes, phylogenetic groups, and genetic diversities of the isolated strains. Further studies should assess whether ESBL-producing *E. coli* strains isolated from dairy cattle are a source of inland water contamination or human infection.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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