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ORIGINAL RESEARCH

Multilocus sequence typing and bla_{ESBL} characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from healthy humans and swine in Northern Thailand

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Purpose: Here, we investigated the genetic relationships and characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) isolates from healthy hosts, humans in the community and swine among the livestock of Amphor Mueang, Lamphun Province, Thailand.

Patients and methods: Four hundred and nine rectal swabs were collected from healthy people and swine. A total of 212 ESBL-*E. coli* was isolated and phenotypically confirmed by a combination disk method. Putative ESBL-encoding genes, including bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, were examined by multiplex-PCR. Randomly selected 42 ESBL-*E. coli* isolates were whole genome sequenced to characterize the ESBL-encoding genes and identify additional antimicrobial resistance genes. The genetic relatedness of 212 ESBL-*E. coli* was investigated by multilocus sequence typing.

Results: Overall, bla_{CTX-M} was the dominant ESBL-encoding gene found in 95.75% of the isolates, followed by bla_{TEM} (60.85%) and bla_{SHV} (2.40%). While $bla_{CTX-M-55}$ was the most common bla_{ESBL} subgroup found in this study. Whole genome sequencing showed a total of 15 different antimicrobial resistance genes other than bla_{ESBL}, including *sul, qnr, aph(3')-Ia*, among the selected 42 ESBL-*E. coli* isolates. Over half of the ESBL-*E. coli* (56.60%) carried bla_{CTX-M} co-existing with bla_{TEM}. The most common sequence types (STs) identified from human isolates were ST131, ST101, and ST70 while those isolated from swine were ST10, ST48, and ST131. ST131 strains carrying bla_{CTX-M} were the major isolated ESBL-*E. coli* strains, supporting a previous study that considered this strain truly pathogenic. Noticeably, 66.51% of ESBL-*E. coli* strains shared 19 identical STs, including a host-restricted ST131 between humans and swine, suggesting that transmission between these two hosts might be possible.

Conclusion: Proof of a direct transfer of ESBL-*E. coli* from animals to humans, or vice versa, is required for further elucidation. The ESBL-*E. coli* isolated from both types of healthy hosts may serve as a reservoir for community-acquired antimicrobial resistance.

Keywords: ESBL-*E. coli*, bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, MLST, genetic relatedness

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Introduction

Antimicrobial resistant bacteria are a global problem, and there is a need for urgent surveillance and appropriate preventive strategies. According to the World Health Organization (WHO), the spread of antimicrobial resistant bacteria has increased in all regions of the world and become one of the major causes of death worldwide.

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Resistant bacteria lead to difficulty in treatment and infection control and may affect the treatment of other diseases.[1] The significant causes of the spread of antimicrobial resistance (AMR) in the community have been attributed to the overuse of antibiotics in households and in livestock, food-chain transmission, contamination of water environments or healthy faecal carriers.[2–5] All these different sources are defined as reservoirs that facilitate the silent spread of and colonization by antimicrobial resistant bacteria within different communities, even in individuals with no history of hospitalization.[6] These colonized bacteria probably present as normal flora; these bacteria may serve as a source of endogenous infections that occur after medical treatment or prolonged immune suppression.

Many recent works have reported the emergence of *Escherichia coli* resistant to beta-lactam antibiotics expressing extended-spectrum beta-lactamase (ESBL) enzymes. The extended-spectrum beta-lactamase-producing *E. coli* (ESBL-*E. coli*) are one of the leading pathogens that appear in both community and healthcare settings worldwide and are often resistant to other antimicrobial agents, including aminoglycosides, fluoroquinolones, and sulfonamides.[7] Infection caused by ESBL-*E. coli* can be associated with high mortality due to multi-resistance, weakening the efficacy of therapies. ESBL-*E. coli* prevalence has dramatically risen during the last decade. However, the majority of ESBL-*E. coli* are still reported from human clinical isolates. The occurrence of ESBL-*E. coli* in animals is also increasing in some countries.[7–9] A significant transmission of ESBL-*E. coli* along the pig production chain has occurred through pig compartments and abattoir waiting areas.[9]

To date, more than 350 different ESBL variants have been characterized and classified into nine distinct families based on their amino acid sequence comparisons.[10] ESBL is found in a wide range of gram-negative bacteria, but the great majority hosts belong to the family *Enterobacteriaceae*, including *E. coli, Klebsiella* spp., *Enterobacter* spp., and *Salmonella* spp. The four, most common ESBLs, namely, CTX-M, TEM, SHV, and OXA, are found among *Enterobacteriaceae* species.[8] Previous studies performed throughout diverse regions in the world showed variable epidemiology of these ESBLs. While TEM predominated in China, SHV was the leading group of ESBLs in Canada. However, reports from South America, Spain, New York, the United Kingdom, and several parts of India revealed CTX-M as the predominant ESBL.[10] The CTX-M types of β-lactamases are the most prevalent family of ESBLs in *E. coli*, in which the CTX-M-15 ESBL is widely distributed across the world and often disseminated with the ST131 *E. coli* uropathogenic clone.[11]

This study aimed to connect data of ESBL-*E. coli* transmission among healthy humans in the community and swine among the livestock of Amphor Mueang, Lamphun Province, Thailand. Multiplex-PCR and whole genome sequencing (WGS) were performed to characterize the common ESBL-encoding genes of the isolates, while the genetic relatedness was investigated by multilocus sequence typing (MLST).

Materials and methods

Bacterial samples used in this study

Nonduplicated rectal swabs of 223 healthy people (living in the local community) and 186 healthy swine (living among the livestock) in Amphor Mueang, Lamphun Province, Thailand were collected between October and November of 2013. Each rectal swab was inoculated onto MacConkey agar (Oxoid, Baskingstoke, England) supplemented with ceftriaxone (Oxoid, Baskingstoke, England) (4 mg/L) in order to screen for ESBL-*E. coli* strains. Samples were incubated at 35°C for 24 hrs. All isolated colonies were identified by conventional biochemical tests. The *E. coli* isolates were stored at −80°C until use. Written informed consent was obtained from all participating people, whose rectal swabs were collected. The study was ethically approved by the Human Research Protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University (exemption number 0517.071/EC).

Antimicrobial susceptibility testing and ESBL detection

Antimicrobial susceptibility was performed using the Kirby-Bauer disk diffusion method according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) 2016 guidelines.[12] Antimicrobial agents (Oxoid, Baskingstoke, England) used in this study included ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefoxitin (30 μg), imipenem (10 μg), ertapenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), and trimethoprim/sulfamethoxazole (1.25/23.75 μg). Phenotypic confirmation tests for ESBL production were conducted with a combination disk method, using ceftazidime (30 μg)

versus ceftazidime/clavulanic acid (30/10 µg) and cefotaxime (30 µg) versus cefotaxime/clavulanic acid (30/10 µg) placed on Mueller-Hinton agar (Oxoid, Baskingstoke, England) and incubated at 35°C for 24 hrs. ESBL production was indicated if there was a difference in the inhibition zone of 5 mm between cephalosporin/beta-lactamase inhibitor disks compared with cephalosporin alone (12). *E. coli* ATCC25922 was used as a control strain.

Genomic DNA extraction

ESBL-*E. coli* isolates were grown on Tryptic soy agar at 35°C for 24 hrs. A single colony of each ESBL-*E. coli* was regrown in Tryptic soy broth at 35°C for 24 hrs. Two hundred microliters of each cell suspension was diluted in 800 µL of sterile distilled water and boiled at 95°C for 10 min followed by a 5-min centrifugation at 12,000 rpm. The supernatant was used as DNA template for PCR amplification.

Detection of ESBL genotypes by multiplex-polymerase chain reaction (multiplex-pcr)

All the 212 ESBL-*E. coli* isolates (111 isolates from humans and 101 isolates from swine) were examined for the presence of ESBL encoding genes, including $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes, by multiplex-PCR. A *uspA* gene, which is specific to *E. coli* species, served as an internal control. A list of primers used for the multiplex-PCR is shown in Table 1. The multiplex-PCR amplification was performed in a total volume of 25 µL, containing 60 ng of template DNA, 10 pM each forward and reverse primer, 20 mM deoxynucleotide triphosphates (dNTPs), 1.5 mM magnesium chloride and 0.5 U *Taq* polymerase (Qiagen, Valencia, CA, USA) in 1x PCR standard buffer. The multiplex-PCR conditions were as follows: initial denaturation at 95°C for 15 min; 30 cycles of 94°C for 30 sec, 60°C for 40 sec and 72°C for 2 min; followed by a final extension step at 72°C for 10 min. In this study, the *E. coli* EC137 strain harbouring $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} (as confirmed by WGS from a previous study and kindly provided by Associate Professor Dr. Methee Chayakulkeeree, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand) was used as a positive control.[13] *E. coli* ATCC25922 was used as a negative control. Multiplex-PCR products were analyzed by 1.0% gel electrophoresis containing RedSafe™ (Scientifix, NSW, Australia) under UV light. Randomly selected multiplex-PCR products were confirmed by

Table 1 Primers used for multiplex-PCR amplification

Gene	Primer	Sequence (5′ to 3′)	Product Size (bp)	Reference
uspA	uspA-up	CCGATACGCTGCCAATCAGT	884	14
	uspA-down	ACGCAGACCGTAGGCCAGAT		
$bla_{\text{CTX-M}}$	CTX-M-U1	ATGTGCAGACCAGTAAGATGGC	593	15
	CTX-M-U2	TGGGTAATAGTACCAGAACAGCGG		
bla_{TEM}	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	15
	TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT		
bla_{SHV}	SHV.SE	ATGCGTTATATTCGCCTGTG	747	15
	SHV.AS	TGCTTTGTTATTCGGGCCAA		

Sanger sequencing. The sequence reads were compared to gene sequences as described in the NCBI database (Blast search http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Whole genome sequencing analysis

Randomly selected genomic DNA of 42 confirmed ESBL-*E coli* isolates (27 isolates from humans and 15 isolates from swine) were whole genome sequenced and analyzed by the Pathosystems Resource Integration Center (PATRIC), Chicago, IL, USA. In brief, sequencing was carried out using a NextSeq 500 platform. The raw data was assembled using SPAdes (version 3.9). Whole genome sequencing data were used for genotypic characterization of bla_{ESBL} subgroups and other antimicrobial resistance genes.

Multilocus sequence typing and phylogenetic analysis

To investigate the phylogeny of clonal spread versus ESBL resistance genes and bacterial hosts, a sequence type (ST) analysis of 212 ESBL-*E. coli* isolates was performed according to the protocols described by Tartof et al.[16] Seven housekeeping genes, including *adk, fumC, gyrB, icd, mdh, purA*, and *recA*, of each isolate were amplified and sequenced. For amplification, PCR reactions of individual genes were separately performed in a total volume of 100 µL, containing 50 ng of template DNA, 20 pM each primer, 200 µM dNTPs, and 5 U *Taq* polymerase in 10 µl of 10x PCR standard buffer. The PCR conditions were as follows: initial denaturation at 95°C for 2 min; 30 cycles of 95°C for 1 min, 54°C (*adk, fumC, icd*, and *purA*) or 58°C (*recA*) or 60°C (*gyrB* and *mdh*) for 1 min and 72°C for 2 min; followed by a final extension step at 72°C for 5 min. The PCR products were sent to Bioneer Sequencing Service Co., Ltd., South Korea, to perform a sequence analysis by conventional Sanger method, using an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data were imported to the *E. coli* MLST database, http://mlst.warwick.ac.uk/mlst/dbs/Ecoli, for MLST type determination.[17] Phylogenetic analysis was performed by BioNumerics version 7.6 software (Applied Maths, Sint-Martens-Latem, Belgium).

Statistical analysis

To evaluate the correlation between antimicrobial resistant phenotypes or genotypes and bacterial host types, a Fisher's exact test (IBM SPSS 17.0 statistical package, Chicago, IL) was used. p-values ≤ 0.05 were considered statistically significant.

Results

Antimicrobial susceptibility testing

There were 115 and 103 *E. coli* strains isolated from 223 people and 186 swine, respectively, in this study. Antibiotic susceptibility testing revealed that all the isolates (100%) from both hosts were resistant to ampicillin, ceftriaxone, and cefotaxime. On the other hand, none of these isolates showed resistance against carbapenems, including imipenem, ertapenem, and meropenem. The *E. coli* isolated from the swine, compared to those isolated from humans, were significantly more resistant to amoxicillin/clavulanic acid (41.74% vs 14.78%), ceftazidime (93.20% vs 51.30%), and gentamicin (82.52% vs 35.65%) (p-value <0.05). However, ciprofloxacin resistance was significantly more common in human isolates (54.78%) rather than in swine isolates (26.21%) (p-value <0.05). There was no significant difference in resistance to cefoxitin, amikacin, and trimethoprim/sulfamethoxazole between *E. coli* strains isolated from both hosts (p-value >0.05). Interestingly, multidrug-resistant *E. coli* were predominately found in both humans (84.35%) and swine (85.44%). Resistance percentages against antimicrobial agents of *E. coli* in this study are presented in Table 2. Among 212 of the 218 isolated *E. coli*, ESBL-producing strains were detected by a combination disk method. These included 111 out of the 115 (96.52%) human isolates and 101 out of the 103 (98.06%) swine isolates.

ESBL gene characterization

Multiplex-PCR amplification assay was designed for $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} detection in ESBL-*E. coli*. The amplified products were separated by 1% agarose gel electrophoresis. Multiplex-PCR generated bands for the *usp*A gene used as an internal control (884 bp) and $bla_{\text{CTX-M}}$ (593 bp), bla_{TEM} (455 bp), and bla_{SHV} (747 bp) (Figure 1). All the 212 ESBL-*E. coli* isolates harboured *usp*A gene and at least one *bla* gene. The $bla_{\text{CTX-M}}$ gene was the most predominantly observed in ESBL-*E. coli* isolated from both hosts. There were 95.75% (203 isolates) harbouring $bla_{\text{CTX-M}}$, followed by 62.74% (133 isolates) and 2.36% (5 isolates) harbouring bla_{TEM} and bla_{SHV}, respectively. The majority of the ESBL-*E. coli* (56.60%; 120 isolates) carried $bla_{\text{CTX-M}}$ co-existing with bla_{TEM}, while 36.79% (78 isolates), 4.25% (9 isolates), 1.89% (4 isolates), and 0.47% (1 isolates) carried $bla_{\text{CTX-M}}$ alone, bla_{TEM} alone, $bla_{\text{CTX-M}}$+bla_{TEM}+bla_{SHV} and $bla_{\text{CTX-M}}$+bla_{SHV}, respectively. Interestingly, the presence of bla_{SHV} alone was not observed among the strains. Statistical analysis revealed that there was no significant differences (p-value >0.05) in the distribution of

Table 2 An overview of the resistance percentages of *E. coli* isolated from healthy people (n=115) and swine (n=103) against antimicrobial agents

Antimicrobial agent	Resistance percentages (%)	
	Healthy people (n=115)	Healthy swine (n=103)
Ampicillin	100.00	100.00
Amoxicillin/clavulanic acid[a]	14.78	41.74
Ceftriaxone	100.00	100.00
Cefotaxime	100.00	100.00
Ceftazidime[a]	51.30	93.20
Cefoxitin	3.48	2.91
Imipenem	0.00	0.00
Ertapenem	0.00	0.00
Meropenem	0.00	0.00
Ciprofloxacin[a]	54.78	26.21
Gentamicin[a]	35.65	82.52
Amikacin	2.61	0.00
Trimethoprim/sulfamethoxazole	62.61	60.19
MDR	84.35	85.44

Notes: [a]Antimicrobial agents with significant differences in resistance percentages between *E. coli* isolated from humans and swine (p-value <0.05).
Abbreviation: MDR, Multidrug-resistant.

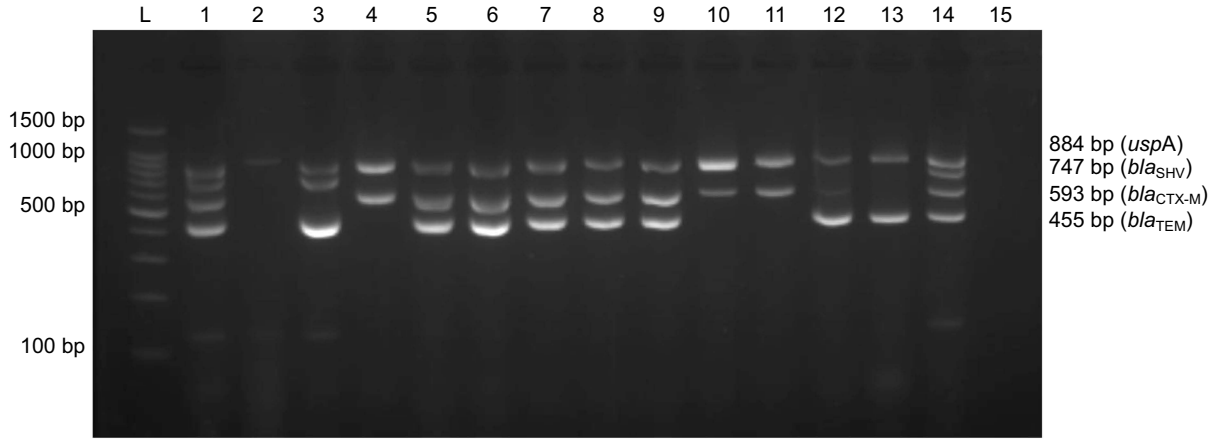

Figure 1 Multiplex-PCR banding patterns of bla_{CTX-M} (593 bp), bla_{TEM} (455 bp), bla_{SHV} (747 bp) and *usp*A (884 bp) genes of *E. coli* generated by gel electrophoresis. L: standard DNA ladder 100 bp. Lane 1: a positive control (*E. coli* EC137 strain) generated 4 bands of bla_{CTX-M}, bla_{TEM}, bla_{SHV} and *usp*A genes. Lane 2: *E. coli* ATCC25922 generated 1 band for the *usp*A gene. Lanes 3–14: *E. coli* isolated from the samples. Lane 15: a negative control.

ESBL genes in *E. coli* isolated from humans and swine. Table 3 shows a summary of the ESBL-*E. coli* gene distribution in humans and swine in this study.

WGS revealed that $bla_{CTX-M-55}$ (56.10%; 23 isolates) was the most prevalent bla_{CTX-M} in those selected 42 ESBL-*E. coli* isolates, followed by $bla_{CTX-M-14}$ (43.90%; 18 isolates). However, $bla_{CTX-M-14}$ was significantly predominate in isolates from humans (13 isolates) rather than those from swine (4 isolates), while there was no significant difference in $bla_{CTX-M-55}$ found in ESBL-*E. coli* isolated from both hosts. All the bla_{TEM} found among the 42 ESBL-*E. coli* isolates were only bla_{TEM-1B} (100%; 5 isolates) (Table 4).

Detection of additional antimicrobial resistance genes

Apart from the bla_{ESBL} subgroup, WGS revealed 15 additional different antimicrobial resistance genes among the 42 ESBL-*E. coli* isolates. These included *sul*1, *sul*2 and *sul*3 (encoding resistance to sulfonamides), *aad*A22 and *aph(3')-Ia* (encoding

Table 3 Distribution of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} in ESBL-*E. coli* isolated from healthy people (n=111) and swine (n=101)

***bla* genes**	**Number of isolates**		
	Healthy humans (n=111)	**Healthy swine (n=101)**	**Total (n=212)**
bla_{CTX-M}	46	32	78
bla_{CTX-M} +bla_{SHV}	1	0	1
bla_{CTX-M} +bla_{TEM}	57	63	120
bla_{CTX-M}+bla_{TEM}+bla_{SHV}	3	1	4
bla_{TEM}	4	5	9

Table 4 Summary of the bla_{ESBL} subgroup identified in randomly selected ESBL-*E. coli* isolates from healthy people (n=27) and swine (n=15)

Subgroup of bla_{ESBL} genes	**Number of isolates**		
	Healthy humans (n=27)	**Healthy swine (n=15)**	**Total (n=42)**
$bla_{CTX-M-14}$	5	3	8
$bla_{CTX-M-14}$+bla_{TEM-1B}	8	2	10
$bla_{CTX-M-55}$	9	10	19
$bla_{CTX-M-55}$+bla_{TEM-1B}	4	0	4
bla_{TEM-1B}	1	0	1

resistance to aminoglycosides), *str*A, *str*B, *aad*A2 and *aad*A5 (encoding resistance to streptomycin), *qnr*S1 (encoding resistance to fluoroquinolones), *tet*A, *tet*B and *tet*D (encoding resistance to tetracyclines), *arr*2 (encoding resistance to rifampicin), and *mef*B (encoding resistance to macrolides) (Table 5). *qnr*S1 was the dominant antimicrobial resistance gene other than bla_{ESBL} found in 61.90% (26 isolates) of 42 ESBL-*E. coli* isolates, followed by *sul* subgroup (45.24%, 19 isolates). Although, there was no correlation between the significant pattern of those resistance genes and the ST of 42 ESBL-*E. coli* isolates. Several whole genome sequenced isolates carried multiple antimicrobial resistance genes, as summarized in Table 5.

Multilocus sequence typing analysis

A total of 7 housekeeping genes, including *adk, fum*C, *gyr*B, *icd, mdh, pur*A, and *rec*A were amplified and sequenced for each ESBL-*E. coli* isolate. Sixty-six unique sequence types (STs) were identified among the 212 ESBL-*E. coli* isolates. Among these, only 25 STs (n=132) clustered into 16 clonal complexes (CCs) (Table 6). The five, most common CCs were CC10 (n=48), CC131 (n=24), CC23 (n=14), CC155 (n=14), and CC101 (n=9). In total, ST131 (n=24, 11.32%) was the most predominant ST, followed by ST10 (n=18, 8.49%), ST48 (n=17, 8.02%), ST34 (n=9, 4.25%), and ST101 (n=9, 4.25%). An MLST dendrogram of these isolates was constructed with BioNumerics software. Figure 2 demonstrates the ESBL-*E. coli* STs isolated from human and swine sample sources. In the MLST dendrogram, each circle represents an ST, and the size of a circle is proportional to the number of ESBL-*E. coli* isolates belonging to this ST.

Interestingly, 141 out of 212 (66.51%) ESBL-*E. coli* shared identical STs between human and swine isolates. All these isolates belonged to 19 shared STs, for example, ST10 (CC10), ST23 (CC23), ST34 (CC10), ST48 (CC10), ST58 (CC155), ST101 (CC101), and ST131 (CC131). In the human source, a total of 47 STs were identified, and ST131 (n=17) was the most prevalent ST, followed by ST101 (n=8), ST70 (n=6), ST48 (n=6), and ST10 (n=5). There were 28 out of 47 STs that were identified in only human isolates, including ST55 and ST56 (CC155), ST93 (CC168), ST142 (CC95), and ST648 (CC648). In the swine source, a total of 38 STs were identified, and ST10 (n=13) was the most prevalent, followed by ST48 (n=11) and ST131 (n=7). There were 19 of 38 STs that were identified in only swine isolates, including ST218 (CC10), ST155 (CC155), ST168 (CC168), and ST405 (CC405).

A relationship between the MLST-based dendrogram and the distribution of ESBL genes in *E. coli* is illustrated in Figure 3. Almost all human and swine isolates carried bla_{CTX-M}, either alone or in combination with bla_{TEM} and/or bla_{SHV}. Only 4 human isolates, including ST221 (n=2), ST1193 (n=1), and ST3376 (n=1), and 5 swine isolates, including ST10 (n=2), ST48 (n=1), ST58 (n=1), and ST224

Table 5 Distribution of bla_{ESBL} subgroups and additional antimicrobial resistance genes identified by WGS in randomly selected ESBL-*E. coli* isolates from healthy people (n=27) and swine (n=15)

Sequence types	Number of isolates			
	Resistance genes	Healthy humans (n=27)	Resistance genes	Healthy swine (n=15)
ST 10	$bla_{CTX-M-14}$, *sul*2, *qnr*S1	1	$bla_{CTX-M-55}$, *sul*2	1
	$bla_{CTX-M-55}$, bla_{TEM-1B}, *sul*3, *qnr*S1	1	$bla_{CTX-M-55}$	1
	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*2, *sul*3, *qnr*S1	1	$bla_{CTX-M-14}$, *sul*1, *tet*A, *aph(3')-Ia*	1
			$bla_{CTX-M-55}$, *sul*1, *aad*A2, *aph(3')-Ia*	1
			$bla_{CTX-M-55}$, *sul*2, *sul*3, *qnr*S1, *str*A, *str*B, *tet*A, *mef*B	1
ST 23	-	-	$bla_{CTX-M-55}$	1
ST 34	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*2, *qnr*S1	1	-	-
ST 48	-	-	$bla_{CTX-M-55}$, *sul*3, *aad*A2, *aph(3')-Ia*	1
ST 70	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*3, *qnr*S1, *str*A, *str*B, *aad*A2	1	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*1, *tet*D, *aad*A5	1
	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*1, *sul*2, *str*A, *tet*A, *aad*A5	1		
ST 93	$bla_{CTX-M-55}$, *qnr*S1, *str*B, *arr*2	1	-	-
ST 101	$bla_{CTX-M-14}$, *sul*2, *str*A, *str*B	1	-	-
	$bla_{CTX-M-55}$, *sul*2, *aad*A22, *qnr*S1	1		
	$bla_{CTX-M-55}$, bla_{TEM-1B}, *sul*2, *qnr*S1, *str*A, *str*B, *tet*A	1		
	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*3, *qnr*S1, *tet*A, *aph(3')-Ia*	1		
ST 117	$bla_{CTX-M-55}$, *qnr*S1, *str*A, *tet*A	1	$bla_{CTX-M-55}$	1
	$bla_{CTX-M-55}$, *qnr*S1	1		
ST 131	$bla_{CTX-M-55}$, *qnr*S1, *mef*B	1	-	-
	$bla_{CTX-M-55}$, bla_{TEM-1B}, *str*A, *str*B, *tet*A	1		
	$bla_{CTX-M-55}$, *sul*3, *aad*A22, *qnr*S1	1		
ST 155	-	-	$bla_{CTX-M-14}$, bla_{TEM-1B}, *sul*2, *qnr*S1, *tet*D	1
ST 221	bla_{TEM-1B}, *sul*2, *aad*A22, *qnr*S1	1	-	-
ST 224	-	-	$bla_{CTX-M-55}$	1
ST 410	-		$bla_{CTX-M-55}$, *qnr*S1	1
		-	$bla_{CTX-M-55}$, *qnr*S1	1
ST 457	-	-	$bla_{CTX-M-55}$, *sul*3	1

(*Continued*)

Table 5 (Continued).

Sequence types	Number of isolates			
	Resistance genes	**Healthy humans (n=27)**	**Resistance genes**	**Healthy swine (n=15)**
ST 617	$bla_{CTX\text{-}M\text{-}14}$, *sul*2, *qnr*S1 $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1B}$, *sul*2, *aad*A22, *qnr*S1	1 1	-	-
ST 648	$bla_{CTX\text{-}M\text{-}14}$, *sul*3, qnrS1, *tet*B, *aad*A2	1	-	-
ST 685	$bla_{CTX\text{-}M\text{-}14}$	1	$bla_{CTX\text{-}M\text{-}14}$, *qnr*S1	1
ST 1112	$bla_{CTX\text{-}M\text{-}55}$, *qnr*S1	1	-	-
ST 1114	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1B}$, *sul*3, *qnr*S1, *mef*B	1	-	-
ST 1146	$bla_{CTX\text{-}M\text{-}55}$, *sul*2, *sul*3, *qnr*S1	1	-	-
ST 2345	$bla_{CTX\text{-}M\text{-}14}$, *qnr*S1 $bla_{CTX\text{-}M\text{-}55}$, *qnr*S1	1 1	-	-
ST 4646	$bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1B}$, *str*A, *str*B, *tet*B	1	-	-

Table 6 Distribution of the clonal complexes and their sequence type members detected in ESBL-*E.coli* isolates from humans and swine

Clonal complex	Number of isolates				
	Sequence types	Healthy humans (n=59)	Sequence types	Healthy swine (n=73)	Total (n=132)
CC 10	ST 10, 34, 48, 617	18	ST 10, 34, 48, 218	30	48
CC 14	ST 1193	2	ST 1193	1	3
CC 23	ST 23, 410	5	ST 23, 410	9	14
CC 38	-	-	ST 38	4	4
CC 46	-	-	ST 46	2	2
CC 86	ST 86	1	ST 86	1	2
CC 95	ST 142	1	-	-	1
CC 101	ST 101	8	ST 101	1	9
CC 131	ST 131	17	ST 131	7	24
CC 155	ST 55, 56, 58	5	ST 58, 155	9	14
CC 156	-	-	ST 156	3	3
CC 165	-	-	ST 165	2	2
CC 168	ST 93	1	ST 168	2	3
CC 206	-	-	ST 206	1	1
CC 405	-	-	ST 405	1	1
CC 648	ST648	1	-	-	1

(n=1), had only bla_{TEM}. Among the three most common shared STs found in both human and swine isolates, ST131 (n=24), ST10 (n=18), and ST48 (n=17), there were noteworthy patterns of ESBL encoding genes. Among the ST131 isolates, 15 isolates harboured $bla_{CTX\text{-}M}$+bla_{TEM} (11 human and 4 swine isolates), whereas the remaining 9 isolates harboured $bla_{CTX\text{-}M}$ (6 human and 3 swine isolates).

Among ST10 isolates, there were 8 isolates harbouring b$la_{CTX\text{-}M}$+bla_{TEM} (3 human and 5 swine isolates), whereas the other 8 isolates harboured $bla_{CTX\text{-}M}$ (2 human and 6 swine isolates). The other two swine isolates harboured only bla_{TEM}. A variety of ESBL encoding genes was found among the ST48 isolates. There were 10 isolates harbouring $bla_{CTX\text{-}M}$+bla_{TEM} (2 human and 8 swine isolates), 3 isolates harbouring $bla_{CTX\text{-}M}$ (2 human and 1 swine isolates), 2 isolates harbouring $bla_{CTX\text{-}M}$+bla_{TEM}+bla_{SHV} (1 human and 1 swine isolate), 1 human isolate harbouring $bla_{CTX\text{-}M}$+bla_{SHV}, and one swine isolate harbouring only bla_{TEM}.

Discussion

ESBL is one of the beta-lactamase enzymes and is considered the essential mechanism of resistance to b-lactam antibiotics among gram-negative bacilli. The ESBL enzymes are usually acquired by horizontal gene transfer, which rapidly supports the spreading of resistant bacterial strains.[18] Several publications have revealed data of spreading of ESBL-*E. coli* in communities worldwide.[7–9] In Thailand, *ESBL-E*. coli was previously isolated from the food of healthy adults and animal farm workers, as well as farm animals and environmental samples.[19] In our study, we assessed the spreading of ESBL-*E. coli* among humans and swine from a community by MLST. ESBL-*E. coli* was isolated from healthy humans living in Amphor Mueang, Lamphun Province and from healthy swine living in livestock in the same community as the sampled humans.

In this study, the majority of the isolated ESBL-*E. coli* comprised multidrug-resistant strains; these bacteria may serve as an unintentional source of difficult-to-treat endogenous infections due to their multidrug-resistant phenotypes that limit the options for antibiotic therapy. The risk factors involved with the faecal carriage of AMR include the use of antibiotics without prescriptions such as beta-lactams and fluoroquinolones use for lower urinary tract infections.[20] The use of antibiotics in food animals contributes to selective pressures that promote AMR in livestock.[11] Our data show that, apart from beta-lactam antibiotics, ESBL-*E. coli* was further resistant to fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole. WGS revealed several transferable antimicrobial resistance genes other than bla_{ESBL} co-existing in ESBL-*E. coli*. Many isolates carried three or more resistance gene categories, reflecting the multidrug-resistant phenotype of the isolates. Although, not all isolates that were antimicrobial susceptibility tested were whole genome sequenced in this study. A mechanism of plasmid-mediated

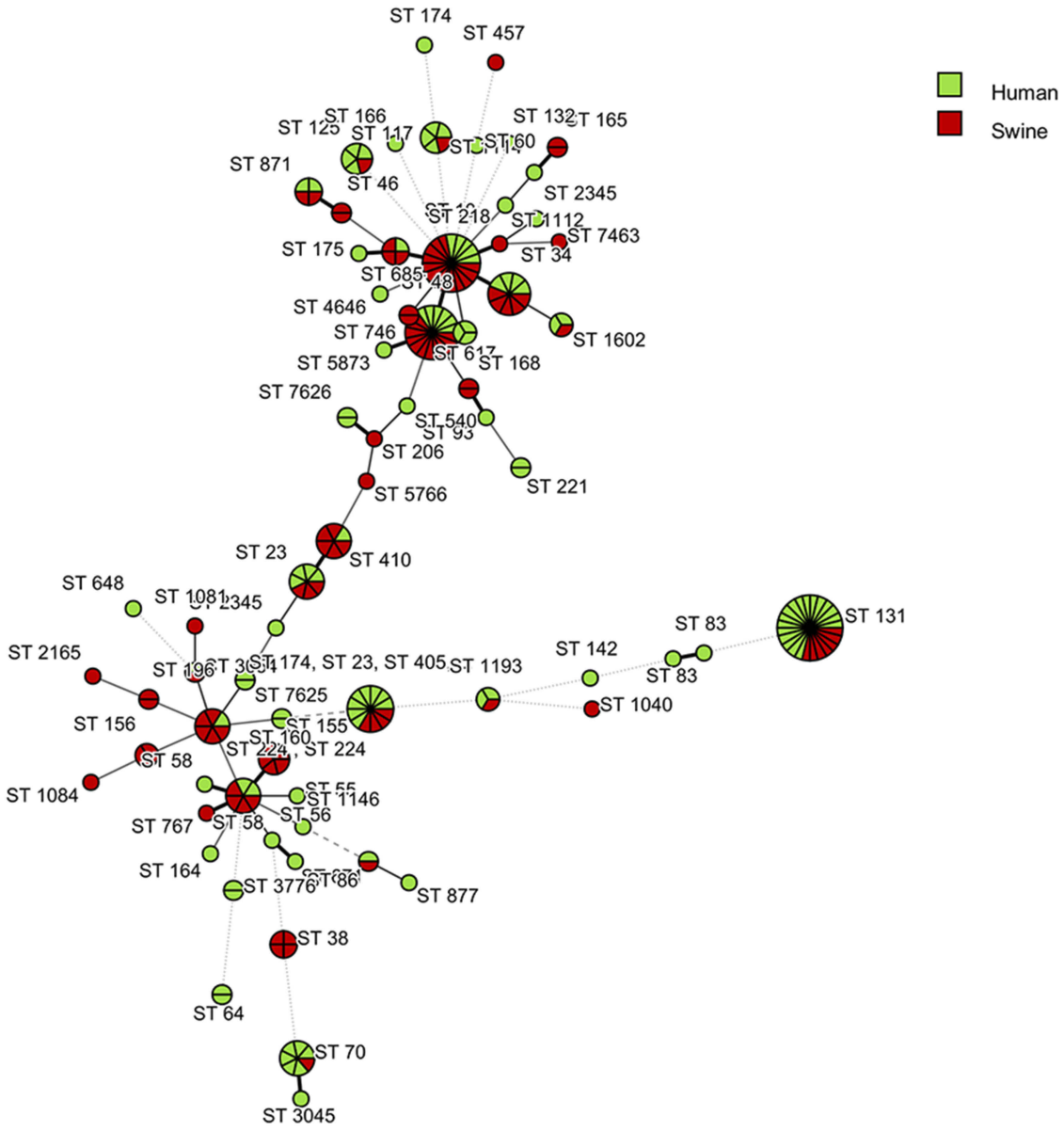

Figure 2 MLST dendrogram created based on STs of the 212 ESBL-*E.coli* isolates from human and swine coloured according to the strain's origin. Each circle represents a given allelic profile and is named according to the MLST sequence type. Each circle corresponds to an ST, and the size of the circle is related to the number of isolates in this study. (BioNumerics version 7.6 software, Applied Maths, Sint-Martens-Latem, Belgium).
Abbreviations: MLST, Multilocus sequence typing; ST, Sequence type; ESBL, Extended-spectrum beta-lactamase.

quinolone resistance (PMQR) includes fluoroquinolone target protection by the Qnr protein (encoded by *qnr*) and aminoglycoside-modifying enzyme production (encoded by *aac-6'-Ib-cr*) that induce resistance against fluoroquinolones and aminoglycosides simultaneously. Interestingly, the PMQRs have been previously shown to co-exist on plasmids carrying $bla_{\text{CTX-M}}$.[21] This may explain the association of fluoroquinolone and/or aminoglycoside resistance that was found among the ESBL-*E. coli* strains in our study. *sul*1, *sul*2, and *sul*3 are known as plasmid-encoded sulfonamide resistance genes that induce resistance against sulfonamides. A coexistence of ESBL genes with *sul* genes in ESBL-*E. coli* has previously been reported.[22]

In the last two decades, the majority of the ESBL strains identified in human clinical isolates were SHV or TEM types, until 10 years ago when CTX-M became the most widespread type of ESBL.[11] Our study showed that $bla_{\text{CTX-M}}$ was the most frequently detected (95.75%) ESBL encoding gene either alone or in combination with other *bla* genes, followed by bla_{TEM} (60.85%) and bla_{SHV} (2.40%). Both groups of human and swine isolates had no significant differences in the distribution of these *bla* genes. Similar to our

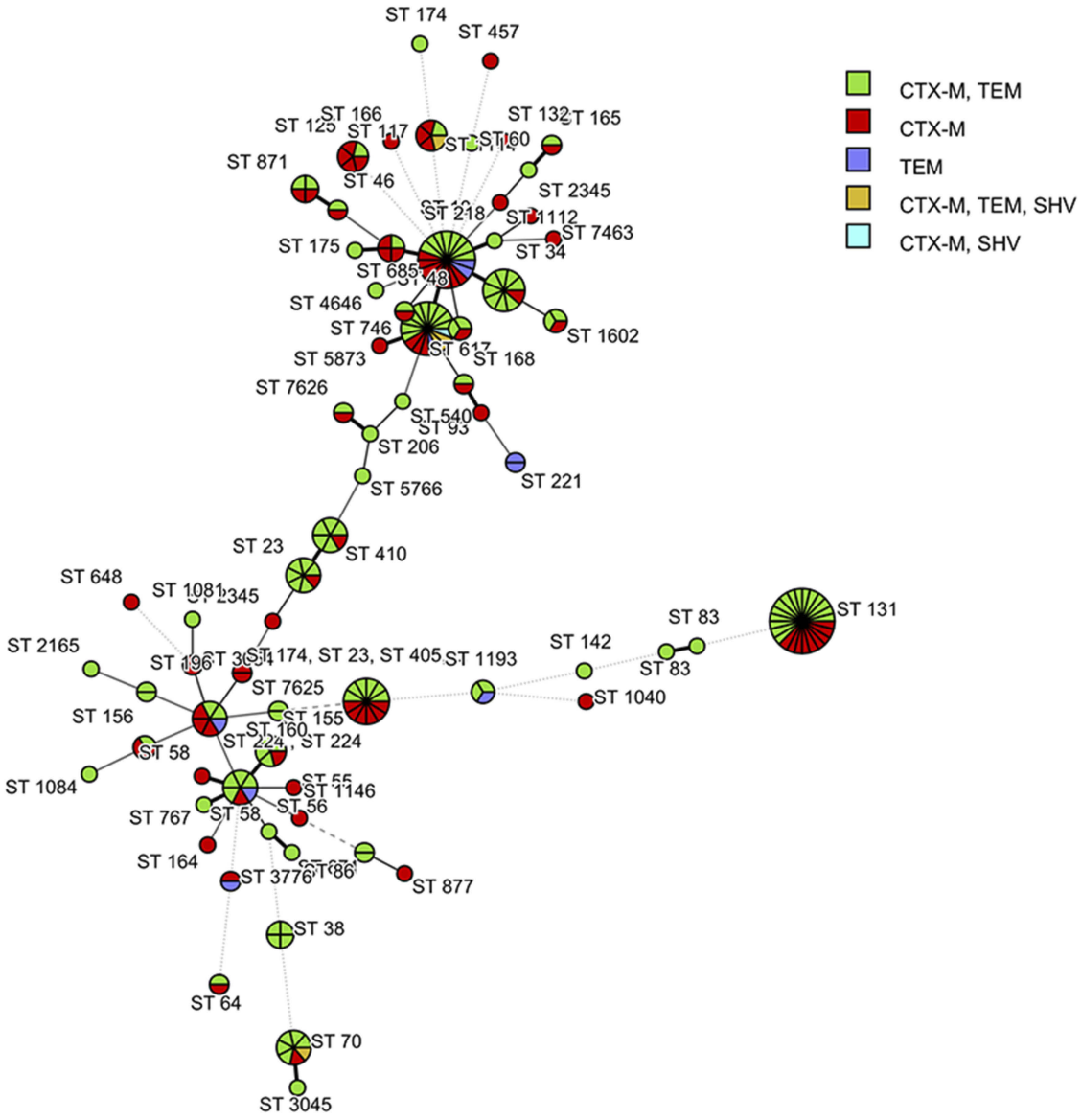

Figure 3 The relationship between the MLST-based dendrogram and the distribution of the ESBL encoding genes of the 212 *E. coli* strains isolated from human and swine coloured according to ESBL genes. (BioNumerics version 7.6 software, Applied Maths, Sint-Martens-Latem, Belgium).
Abbreviations: MLST, Multilocus sequence typing; ST, Sequence type; ESBL, Extended-spectrum beta-lactamase.

findings, previous studies showed that the $bla_{\text{CTX-M}}$ family had the highest prevalence among those typical *E. coli* ESBL encoding genes, followed by bla_{TEM} and bla_{SHV} either from clinical, animal, or environmental isolates.[23–25] The prevalence of $bla_{\text{CTX-M-15}}$ has risen over time worldwide and is dominant in most countries. Exceptions are China, Japan, Spain, and South-East Asia, where $bla_{\text{CTX-M-14}}$ genes are dominant.[11] Here, $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-14}}$ were the most prevalent identified $bla_{\text{CTX-M}}$ genes. However, only 42 randomly selected ESBL-*E. coli* samples out of 212 samples were investigated for a subgroup of $bla_{\text{CTX-M}}$ and may not be a representative of the $bla_{\text{CTX-M}}$ distribution in the country. In Thailand, $bla_{\text{CTX-M-55}}$ has been previously demonstrated to be the most common CTX-M in clinical isolates, farm waste and canals, followed by $bla_{\text{CTX-M-14}}$.[25]

MLST presents data on the genetic relationship between the STs of ESBL-*E. coli* strains and host origins. The dominant ST identified in our study was ST131, which was the first and third most common ST identified in healthy humans and swine, respectively. In general, ST131 *E. coli* is the most common ST obtained from human clinical isolates globally. Previous studies revealed that ST131 was predominant among ESBL-*E. coli*, ranging from 28 to 38%, isolated from human clinical samples in Sweden, Germany and Thailand.[25–27]

However, ST131 seems to be host restricted, and there have been very few reports on ST131 from either animals (healthy or sick), foods, or environments.[25,28] Our study provides another line of evidence showing a possibility that ST131 could disseminate to various animal species and ecosystems.

ST131 *E. coli* has a broad spectrum of infections both in community and hospital settings. ST131 *E. coli* also has a large number of virulence-associated genes, which make these bacteria truly pathogenic.[28] ST131 isolates are commonly reported to produce ESBL, mostly by CTX-M-15, and almost all isolates are resistant to fluoroquinolones.[28] Our findings showed that all ST131 harboured $bla_{\text{CTX-M}}$ either alone or in combination with other *bla* genes. However, only two strains of ST131 were randomly identified for a subgroup by WGS, and CTX-M-55 was found (data not shown). Thus, in our study, it is too early to conclude which CTX-M subgroup is mostly produced by the ST131 ESBL-*E. coli*. In addition, most of the ST131 isolates were resistant to ciprofloxacin and gentamicin. A global expansion of the clone ST131 carrying $bla_{\text{CTX-M}}$ is considered one major reason for the rise in ESBL-*E. coli*. Increased virulence of ST131 strains has been previously discussed, but the proof of this virulence is unclear.[26] Lavigne et al proposed a key explanation for the success in the spread of ST131 *E. coli* using a zebrafish model; CTX-M-producing ST131 and non-CTX-M-producing ST131 had decreased virulence but improved persistence during infection compared with non-ST131 *E. coli*.[29]

The next most common ST of ESBL-*E. coli* identified in this study was ST10, which was obtained from both human and swine isolates. In contrast to ST131, ST10 is commonly isolated from a variety of sources.[20] Our finding is supported by a previous study that showed that ST10 ESBL-*E. coli* is globally disseminated in humans and animals.[20,30] ST48 ESBL-*E. coli* was the third most common ST obtained in our study. This clone was previously isolated from both hospitalized and non-hospitalized patients as well as from poultry.[31,32] Nearly all ST10 and ST48 harboured $bla_{\text{CTX-M}}$ either alone or in combination with other *bla* genes; the two exceptions to the former scenario and the one exception to the latter scenario all carried only bla_{TEM}. Unlike ST131, in which $bla_{\text{CTX-M}}$ was strictly associated, both ST10 and ST48 seemed to be less restricted in their association with $bla_{\text{CTX-M}}$.

In a study from Denmark, highly similar clones of ESBL-*E. coli* were observed in swine livestock and farm workers, suggesting that swine may play a significant role as vectors in the transfer ESBL-*E. coli* via close contact.[33] Nonetheless, evidence showing a direct transfer of ESBL-*E. coli* from animals to humans is still inadequate. The presence of shared STs, particularly the human-restricted ST131 and the non-host-restricted ST10 and ST 48 isolates, between people in the community and the swine on farms in our study seemed to be transferred by means other than close contact. Commensal ESBL-*E. coli* in the gut of animals and humans may be an important source of bacteria causing opportunistic infections or act as a resistant gene reservoir, serving as a source of bacterial spread.[18] However, it would be interesting to further investigate the ESBL-*E. coli* isolates that shared identical STs by more discriminatory typing methods, such as WGS, to prove the relatedness among isolates and characterize the mechanisms of transfer between humans and animals.

Conclusion

Proof of a direct transfer of ESBL-*E. coli* from animals to human is required for further elucidation. However, this study showed several shared identical STs of ESBL-*E. coli* from the healthy humans in a community and the swine in the community livestock, suggesting epidemiological links or that transmission between these two hosts is possible. ESBL-*E. coli* isolates from both hosts indicate that healthy humans and livestock animals can serve as a reservoir for AMR. Minimizing the transmission between hosts is essential to control the spread of ESBL- *E. coli* in community settings. Additional monitoring of the epidemiology of ESBL-*E. coli* in humans and livestock, as well as the proof of possible transmission routes, are needed.

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Disclosure

The authors declare that they have no conflicts of interest related to the publication of this article.

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