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ESBL-producing *Enterobacterales* in food and clinical samples: antimicrobial resistance organisms and genes in Chiang Mai, Thailand

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Globally, food safety and security are negatively impacted by antimicrobial resistance (AMR). The emergence of resistance caused by extended-spectrum β-lactamases (ESBLs) during the AMR era has resulted in increased morbidity, longer hospital stays, and more costly treatment alternatives. This study aimed to detect the contamination of ESBL-producing *Enterobacterales* antibiotic-resistant bacteria and ESBL genes in food materials and clinical samples in Chiang Mai, Thailand. Microbial culture-based, molecular and nucleotide sequencing methods were used in this study. Of 279 food samples, chicken meat was the most contaminated with ESBL-producing organisms (50%), followed by pork meat (48%), and tomato (2%). The prevalence rate of ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca* were 15.41%, 2.15%, and 0.36%, respectively. Moreover, we detected 96.15% of bla_{CTX-M} gene and 43.59% of bla_{TEM} gene from these organisms. While of 39 clinical samples, 22 samples were ESBL-producing *E. coli* and 17 samples were ESBL-producing *K. pneumoniae*. The prevalence of bla_{CTX-M} and bla_{TEM} genes in these samples were 94.87% and 66.67%, respectively. These study findings raise concerns about the consumption of AMR bacteria. All parties involved in the supply chain for food production and consumption must engage in behavioral communication, excellent agricultural and industrial practices, and awareness-raising initiatives.

Keywords Antimicrobial resistance, ESBL, Bla_{CTX-M}, Bla_{TEM}, *Enterobacterales*, Food safety

Antibiotics are a class of antimicrobial agents that can combat bacteria and typically used in medicine and agriculture. Globally, antibiotics are the most popular and efficient drugs for treating bacterial infections, the incapacity of these pathogens to be treated is known as antibiotic resistance[1].

Antibiotic or antimicrobial resistance is defined as the ability to withstand the medications intended to treat them. Thus, treatment-resistant illnesses are the result of these germs. Nowadays, antibiotic resistance is a significant health concern worldwide that contributes to increased mortality and morbidity, longer hospital stays, and higher medical expenses[2].

Bacterial antibiotic resistance poses a serious threat to human, animal, and environmental health[3]. The treatment choices and effectiveness are restricted when these bacteria are more prevalent. Consequently, harmful bacteria that develop resistance to antibiotics are a serious threat to both human and animal health[4].

Extended spectrum β-lactamases (ESBLs) are gram-negative bacteria belonging to the *Enterobacterales* family. ESBLs are enzymes produced by certain bacteria, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), which render them resistant to many commonly used antibiotics[5].

These bacteria can cause serious infections such as urinary tract infections (UTIs), bloodstream infections, and pneumonia. The World Health Organization (WHO) correctly considers ESBLs to be among the most

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difficult diseases[6]. Bacteria with β-lactam-resistant ESBL are one of the primary causes of concern. Since ESBLs are enzymes that confer resistance to several beta-lactam antibiotics, inactivation of multiple antimicrobial types, such as monobactams, cephalosporins, penicillin, and β-lactamases, complicates the treatment of infections[5].

Most ESBL-encoding genes are found in transposon or plasmid insertion sequences. As a result, they have the potential to spread swiftly and cause resistance to other antimicrobials such as tetracyclines, aminoglycosides, trimethoprim, sulfonamides, fluoroquinolones, and chloramphenicol[7,8]. In both human and animal populations, the misuse or overuse of antibiotics results in the formation of drug-resistant bacteria through gene changes or horizontal spread of resistance genes by plasmids[9].

According to reports from around the world, ESBL-producing *Enterobacterales* organisms or ESBL-E is found in animal and human commensals, environmental contaminants, and various biological niches. Therefore, animal production has been a major concern in recent decades because of its tight linkages to the food chain and its ability to serve as reservoirs, vehicles, or routes of transmission and dissemination of ESBL-E[10].

Many factors contribute to antibiotic resistance, such as the use of antibiotics in agriculture, the way people ingest antibiotics, and environmental contamination by antimicrobial chemicals. The increase in the demand for antibiotics and their widespread and negligent use are major contributors to the development of resistant strains. One of the main causes of antibiotic-resistant bacteria in food animals is the ongoing use of antibiotics in their production[11]. Monitoring the spread of resistant bacteria from animals to humans at each step of the transmission pathway is crucial, because certain antibiotic classes are used in both humans and animals[12].

As a result, ESBL-producing bacteria can be directly transferred to humans through food products or the oral fecal route from infested animals that eat raw foods[13]. However, the presence of enteropathogenic bacteria leads to an increase in consumer health problems, and fruits consumed by the skin, such as pears, apples, blackberries, grapes, and strawberries, are more likely to have these bacteria on their surfaces[14].

From overall, monitoring antibiotic-resistant bacteria and genes in human and food materials is necessary in settings where humans have routine contact with agriculture and livestock products because of the increased risk of spread of resistance genes in these interactions.

For Southeast Asian (SEA) review study has documented a rise in multidrug resistance in Gram-negative bacteria, particularly ESBL and carbapenem resistance in *Enterobacterales*[15]. In addition, in order to address the growing antimicrobial resistance (AMR) in the setting, it is necessary to obtain a comprehensive assessment of the available evidence in order to guide future policies and initiatives, particularly with regard to monitoring and surveillance.

According to previous studies reported, the genes that encode these enzymes are commonly located on mobile genetic elements such plasmids and transposons, which can promote horizontal transmission of resistance among *Enterobacterales*[16,17]. And due to the outbreak of the *E. coli* ST131 lineage in SEA, ESBLs type $bla_{\text{CTX-M}}$ have become more and more isolated[18]. AMR gene levels were also found to be high in SEA *K. pneumoniae* isolates, underscoring the significance of genomics-based surveillance, which can help standardize data for site comparison and detect regional and national variations[19].

Nevertheless, reports of these issues are limited in Thailand. Therefore, the purpose of this study was to assess the potential contamination of ESBL-producing *Enterobacterales* antibiotic-resistant bacteria and ESBL genes in food materials collected from fresh markets in Chiang Mai province, Northern Thailand, to determine risk factors for contamination and prevent the transfer of resistant microbes from farms to people. Furthermore, ESBL genes in clinical specimens were determined and evaluated to understand the genetic diversity of antibiotic-resistant organisms that affect human health in this area.

Material and methods

Sample collection

Food material samples collection

This cross-sectional study collected food material samples from fresh markets in Chiang Mai Province, Northern Thailand. All raw food material samples, including chicken and pork meat, fruit, and vegetables, were randomly purchased and collected between September 2023 and February 2024. All sample types were random sampling for 20–50 samples. These samples were kept in clean plastic and zip lock bags, and then transported to the laboratory in a cold container with an ice pack as soon as possible or within eight hours to detect extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales* antibiotic-resistant bacteria. The total number of food samples collected is listed in Table 1.

Residual of clinical cultured specimens collection

Ethics approval and consent to participate This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Research ID. 0537 Study: NONE-256-0537) for the collection of clinical cultured specimen residuals which were categorized as exempted specimens. All study methods were carried out in accordance with relevant guidelines and regulations. In addition, due to the study did not directly involve patients, human material, or personal data identifiers therefore the patient informed consent was not obtained in this study. The need to obtain informed consent was waived by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Specimen collection All bacterial isolates included in this study were collected from the microbiology laboratory in Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, at the same time of food material samples collected. A total of 39 residual isolated colonies of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* which cultured from different kinds of clinical specimens include blood, urine, pus, sputum and body fluids between September 2023–February 2024 (Table 2) was collected.

Type of food materials	Number of food material collected
Total	**279**
Pork meat	43
Chicken meat	33
Basil	31
Cabbage	31
Chinese cabbage	28
Lettuce	30
Peppermint	28
Tomato	28
Grape	27

Table 1. Total numbers of food material samples collected from difference fresh markets in Chiang Mai, Thailand between September 2023–February 2024, $n = 279$. The bold indicates the importance of each number.

Detail of sample collected	No. of samples	
Type of ESBL-producing organisms	Total	**39**
	E. coli	22
	K. pneumoniae	17
Source of cultured specimens	Total	**39**
	Blood	3
	Body fluid	3
	Pus	5
	Sputum	6
	Urine	22

Table 2. The details of residual isolated colonies of ESBL-organisms collected from clinical cultured specimens between September 2023–February 2024. The bold indicates the importance of each number.

The isolated colonies were subjected to antimicrobial susceptibility tests using fully automated VITEK2, bioMérieux, and Sensititre Aris®2X, Thermo Scientific™ machines. The standardized antimicrobial sensitivity test for ESBL detection was confirmed, a double disk synergy test was performed using Muller Hinton Agar (MHA) media agar, and the results were interpreted according to the Standards for Antimicrobial Susceptibility of the Clinical Laboratory Standards Institute (CLSI) protocol[20].

Briefly, presumptive ESBL-positive isolated colonies were suspended in broth culture at a McFarland standard of 0.5. A sterile swab was dipped into the standardized suspension of bacteria and streaked on the entire surface of the MHA plate. After Subsequently, ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg), Ceftazidime/Clavulanic acid (CAC 30/10 µg), and cefotaxime/clavulanic acid (CEC 30/10 µg) were applied to the inoculated plate and incubated at 37 ± 2 °C for 18–24 h. The zones of inhibition were measured to the nearest millimeter at the back of the inverted culture plate to examine the antimicrobial drug susceptibility test, which was considered ESBL-positive when an increase of ≥ 5 mm in the zone diameter of any of the antibiotic disks was used towards the center[21]. While an increase < 5 mm in the zone diameter, the result was considered ESBL-negative. For quality control, *E. coli* ATCC® 25,922 and ATCC® 35,218 were used.

Microbial culture, identification and drug susceptibility test (DST) for ESBL-producing bacteria

All food material samples were tested for ESBL-producing organisms by microbial culture on selective media and confirmed using the DST test.

Bacterial culture test (culture-based method)

A sterile swab was used to swab the surface of each 100 g of raw food sample (one by one), which was then brought to the detection of ESBL-producing bacteria by streaking back and forth in a zigzag pattern on the surface of the CHROMID® ESBL selective media plate (bioMerieux SA, Marcy l'Etoile, France)[22]. Subsequently, these selective media plates were incubated at 35 ± 2 °C for 18–24 h. The processing procedures for *Enterobacterales* isolation and selective enrichment were based on previous studies[23,24].

Bacterial isolation and identification test

The plates were visually inspected to determine whether the colony growth colors corresponded to the presumed *Enterobacterales* bacteria (pink or blue/turquoise colonies on CHROMID® ESBL agar). Suspected colonies were isolated on MacConkey agar plates. After incubation at 35 ± 2 °C for 18–24 h, the isolated single colony of suspected bacteria was identified as the organism strain using the automated matrix-assisted laser desorption-

ionization time of flight mass spectrometry (MALDI-TOF MS), VITEK® MS (bioMerieux, Marcy l'Etoile, France)[22].

Antimicrobial resistant drug susceptibility test (DST)
Isolated colonies were identified as members of the order *Enterobacterales* that grew on the CHROMID® ESBL selective media plate (bioMerieux SA, Marcy l'Etoile, France) and were then confirmed to produce ESBL by antimicrobial-resistant DST. The standardized antimicrobial sensitivity test for ESBL detection was performed by a double-disk synergy test using Muller Hinton Agar (MHA) media agar, and the results were interpreted according to the Standards for Antimicrobial Susceptibility of the Clinical Laboratory Standards Institute (CLSI) protocol[20]. For quality control, *E. coli* ATCC® 25,922 and ATCC® 35,218 were used.

Detection of ESBL-antibiotic resistance genes

PCR amplification and nucleotide sequencing of ESBL genes were performed in these steps. The genomic DNA of all ESBL isolate colonies collected from food material and clinical specimens was extracted and amplified for ESBL-genes, including $bla_{\text{CTX-M}}$ and bla_{TEM}, using specific primers followed these procedures.

Extraction of bacterial DNA
Plasmid DNA was extracted using the boiling method[25]. Briefly, the isolated bacterial colonies were resuspended in a 500 µL of sterile deionized water tube and then boiled at 100 °C for 10 min. Lysates were centrifuged at 12,000 × g for 10 min. The supernatant was collected and used as a DNA template. DNA quantity and purity were determined using NanoDrop One (Thermo Fisher Scientific™, Madison, WI, USA) at wavelengths of 260 and 280 nm.

Molecular identification of ESBL-genes by polymerase chain reaction (PCR)
Extracted bacterial DNA was amplified by PCR to detect antibiotic resistance genes using gene-specific primers listed in Table 3.

Briefly, the amplification reaction (25 µL) contained 13 µl of 2 × Taq PCR StarMix (Dye) (GenStar, China), 1 µL of 10 µM each forward and reverse primer, 10 µL of sterile deionized water, and 2 µL of extracted DNA (> 50 ng/µL). The thermal cycling conditions were 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Finally, PCR products were analyzed by gel electrophoresis to visualize the quality and expected size of plasmid DNA, using 1.5% (w/v) agarose gel and Super GelBlue™ (US EVERBRIGHT® INC., China) staining.

For quality control, known negative and positive samples for $bla_{\text{CTX-M}}$ and bla_{TEM} amplification were used to perform along the procedure.

Interpretation of nucleotide sequences
The amplified DNA with representative of antibiotic drug resistance genes were sent to the company of Hunan Branch, Beijing Tsingke Biotech Co., Ltd. (China) to perform DNA sequencing tests. The nucleotide sequences were then examined using the Basic Local Alignments Search Tool (BLAST) software available at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) sequence databases[28], which determines the statistical significance by comparing protein or nucleotide sequences to sequence databases and finds regions of similarity between biological sequences.

Results

Prevalence of ESBL-producing bacteria in food materials

In this study, raw food material samples were used to detect ESBL-producing organisms by culturing on CHROMID® ESBL selective media (bioMerieux SA, France). The presumptive ESBL-positive isolated colonies were visually inspected to determine whether the colony growth colors corresponded with the presumed *Enterobacterales* bacteria. The expected isolated colonies with pink or blue/turquoise colonies on CHROMID® ESBL agar (Fig. 1a and b) were identified as the microorganism strain using the automated MALDI-TOF MS, VITEK® MS (bioMerieux, Inc., Germany) machine. Finally, ESBL-positive isolated colonies were confirmed to be ESBL-producing organisms by a standardized antimicrobial sensitivity test for ESBL detection following the CLSI protocol [20,21].

From overall 279 raw food material samples; 43 pork and 33 chicken meats, 28 tomatoes, 33 cabbages, 30 lettuces, 27 grapes, 28 Chinese cabbage, 31 basils and 28 peppermints were collected during September 2023–February 2024 from fresh markets in Chiang Mai province, Thailand.

Antibiotic resitance genes	5' to 3' sequence	Product size (bp)	References
$bla_{\text{CTX-M}}$	F-ATGTGCAGYACCAGTAARGTKATGGC	593	26
	R-TGGGTRAARTARGTSACCAGAAYCAGCGG		
bla_{TEM}	F-CGCCGCATACACTATTCTCAGAATGA	445	27
	R-ACGCTCACCGGCTCCAGATTTAT		

Table 3. The primers used for PCR amplification of ESBL genes. F: Forward; R: Reverse.

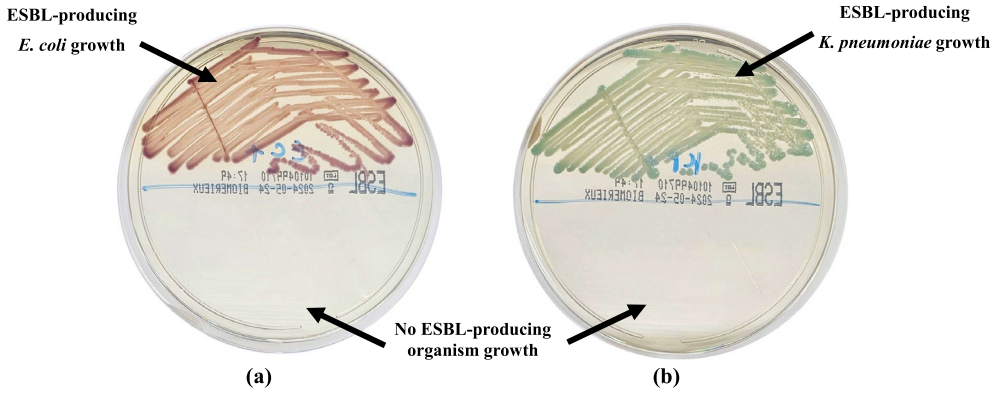

Fig. 1. The visually inspected colony growth colors corresponded with presumed *Enterobacterales* bacteria on selective CHROMID® ESBL agar plate (bioMerieux SA, France). (**a**) The result of pink color colonies on the upper part of CHROMID® ESBL agar (bioMerieux SA, France) demonstrated the ESBL-producing *E. coli* growth; (**b**) the result of blue/turquoise color colonies on the upper part of CHROMID® ESBL agar demonstrated the ESBL-producing *K. pneumoniae* growth. The lower part of both CHROMID® ESBL agar plates with no colony found demonstrated no ESBL-producing organism growth.

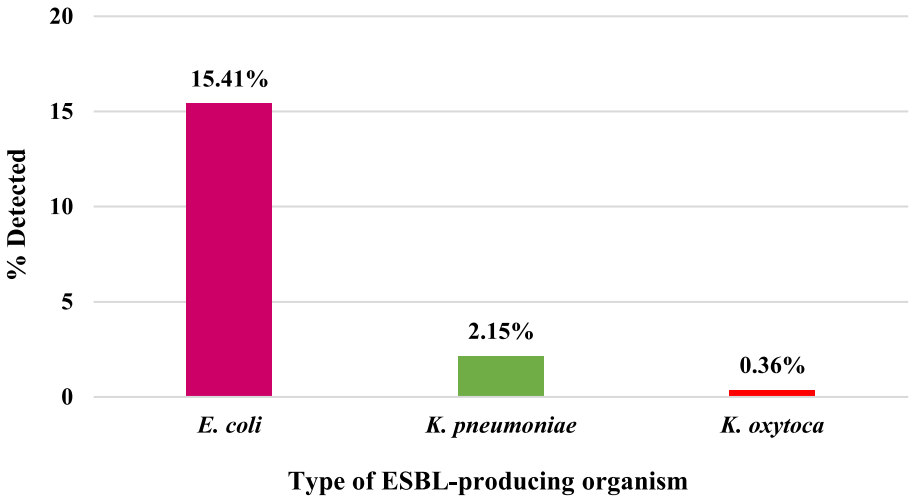

Fig. 2. The prevalence of ESBL-producing *Enterobacterales* bacteria detected on raw food material samples collected from fresh markets in Chiang Mai Province, Thailand ($n = 279$).

Of these raw food material samples, this study found that the prevalence rate of ESBL-producing organisms was 17.92% (50/279), including 15.41% (43/279), 2.15% (6/279), and 0.36% (1/279) for ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* and ESBL-producing *K. oxytoca*, respectively (Fig. 2).

Among the 50 ESBL-producing *Enterobacterales* bacteria contaminating the food samples, 86.00% (43/50), 12.00% (6/50), and 2.00% (1/50) were ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* and ESBL-producing *K. oxytoca*, respectively (Fig. 3a). Raw chicken meat samples had the highest detection of these organisms at 50% (25/50), followed by raw pork meat samples at 48% (24/50), and raw tomato at 2% (1/50) (Fig. 3b).

ESBL-producing *Enterobacterales* organisms were detected in 23 of 33 (69.70%) ESBL-producing *E. coli* and 2 of 33 (6.06%) ESBL-producing *K. pneumoniae* isolates obtained from chicken meat samples. While 20 of 43 (46.51%) were ESBL-producing *E. coli*, 4 of 43 (9.30%) were ESBL-producing *K. pneumoniae* obtained from pork meat samples, and 1 of 28 (3.57%) were ESBL-producing *K. oxytoca* obtained from tomato. No ESBL-producing organisms were detected in the other raw food material samples. The number of ESBL-producing organisms isolated from different raw food material samples is shown in Table 4.

Detection results of ESBL-antibiotic resistance genes

ESBL-antibiotic resistance genes in food material samples

From the 50 food material samples with ESBL-producing bacteria, 78 isolated colonies were obtained. Sixty-eight isolated colonies were obtained from ESBL-producing *E. coli,* 9 colonies were obtained from ESBL-producing *K. pneumoniae* and 1 colony was obtained from ESBL-producing *K. oxytoca*. All colonies were detected and

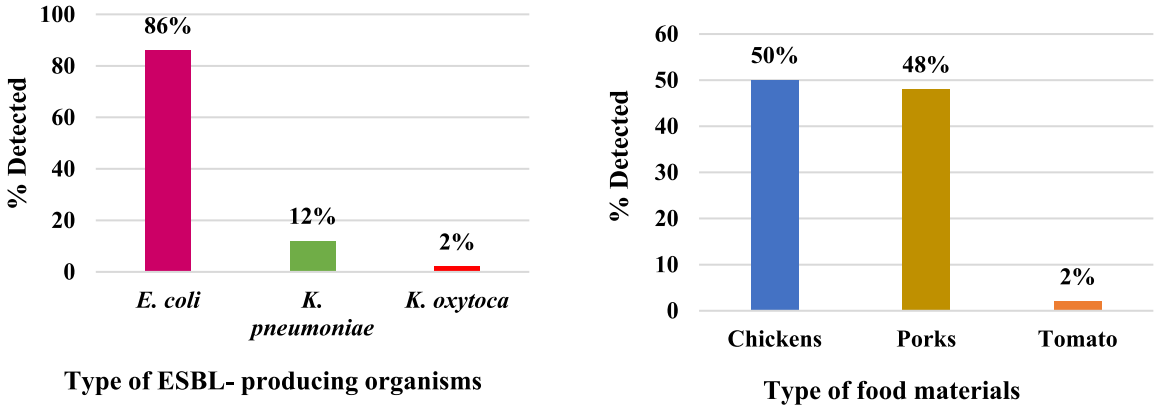

Fig. 3. (**a**) The prevalence of ESBL-producing *Enterobacterales* bacteria detected in raw food material samples ($n = 50$). (**b**) The percentage of raw food material samples were ESBL-producing *Enterobacterales* bacteria detected. For basil, cabbage, Chinese cabbage, lettuce, peppermint and grape samples, ESBL-producing organisms were not detected.

Type of food materials* ($n = 279$)	ESBL-producing organisms detected			
	Total organism	*E. coli*	*K. pneumoniae*	*K. oxytoca*
Chicken meat ($n = 33$)	**25/33 (75.76%)**	**23/33 (69.70%)**	**2/33 (6.06%)**	0/33 (0%)
Pork meat ($n = 43$)	**24/43 (55.81%)**	**20/43 (46.51%)**	**4/43 (9.30%)**	0/43 (0%)
Tomato ($n = 28$)	**1/28 (3.57%)**	0/28 (0%)	0/28 (0%)	**1/28 (3.57%)**

Table 4. The prevalence of ESBL-producing *Enterobacterales* bacteria detected on each raw food material samples. *For basil, cabbage, Chinese cabbage, lettuce, peppermint and grape samples, ESBL-producing organisms were not detected in these samples. The bold indicates the importance of each number.

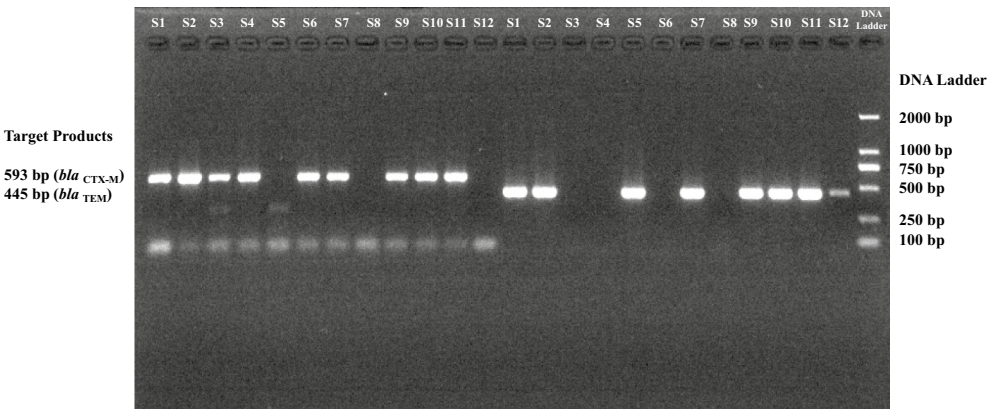

Fig. 4. The gel electrophoresis results of ESBL-producing genes detected by PCR amplification. The PCR products size 593 bp are represented for $bla_{CTX\text{-}M}$ gene positive samples whereas PCR products size 445 bp are represented for bla_{TEM} gene positive samples. For $bla_{CTX\text{-}M}$ gene, sample no. S1-S4, S6-S7, S9-S11 were provided positive results whereas sample no. S5, S8 and S12 were provided negative results. For bla_{TEM} gene, sample no. S1-S2, S5, S7, S9-S12 were provided positive results whereas sample no. S3-S4, S6 and S8 were provided negative results.

confirmed for ESBL-antibiotic resistance genes, $bla_{CTX\text{-}M}$ and bla_{TEM}, using molecular identification techniques with PCR and nucleotide sequencing.

The PCR products of ESBL genes detected by gel electrophoresis for the PCR amplification results of this study are shown in Fig. 4. Among the 78 extracted DNA samples of ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca;* 75 samples (75/78, 96.15%) provided positive PCR amplification results for $bla_{CTX\text{-}M}$ gene with

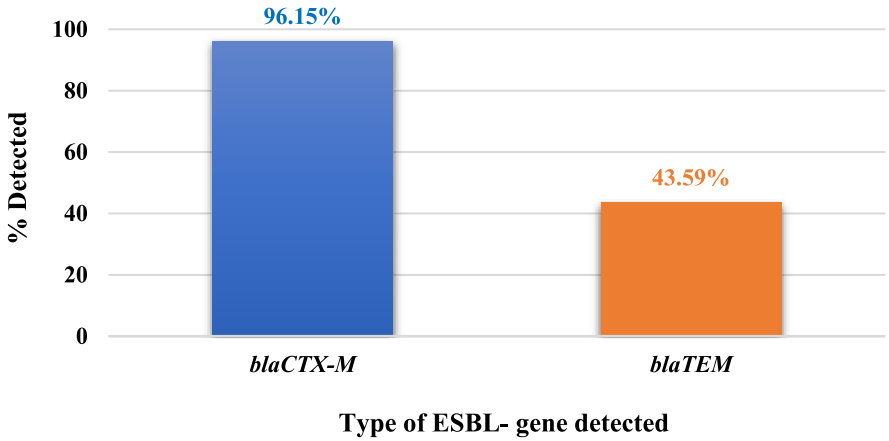

Fig. 5. The prevalence of ESBL-genes detected from 78 extracted DNA samples of ESBL-producing organism obtained from food materials samples.

ESBL-producing organisms	Results of ESBL-gene detected	
	bla_{CTX-M}	bla_{TEM}
Total (*n* = 78)	**75/78 (96.15%)**	**34/78 (43.59%)**
E. coli (*n* = 68)	68/68 (100%)	28/68 (41.18%)
K. pneumoniae (*n* = 9)	6/9 (66.67%)	6/9 (66.67%)
K. oxytoca (*n* = 1)	1/1 (100%)	0 (0%)

Table 5. The prevalence of ESBL-genes detected in ESBL-producing organisms from food material samples by using PCR amplification and confirmed with nucleotide sequencing methods. The bold indicates the importance of each number.

593 bp of PCR product on gel electrophoresis, and 34 samples (34/78, 43.59%) provided positive results for bla_{TEM} gene with 445 bp of PCR product (Fig. 5).

Moreover, for these 78 positive PCR amplification samples, the amplified DNA or PCR products were further subjected to DNA sequencing tests and nucleotide sequences were examined using the BLAST program to confirm the representative ESBL genes. Among these samples, for bla_{CTX-M} gene; 100% (68/68), 66.67% (6/9) and 100% (1/1) were detected from ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca*, respectively. For bla_{TEM} gene, 41.18% (28/68) were detected in ESBL-producing *E. coli*, 66.67% (6/9) were detected in ESBL-producing *K. pneumoniae* and no bla_{TEM} gene was detected in ESBL-producing *K. oxytoca*. The overall results of the ESBL-producing genes detected by PCR Amplification and DNA sequencing are shown in Table 5.

The nucleotide sequences of the positive PCR amplification samples for bla_{CTX-M} and bla_{TEM} are shown in Figs. 6 and 7, respectively.

ESBL-antibiotic resistance genes in clinical specimen samples

From a total of 39 isolated colonies of ESBL-producing organisms collected, 22 were obtained from ESBL-producing *E. coli* and 17 colonies were obtained from ESBL-producing *K. pneumoniae*. The determination and confirmation of ESBL-antibiotic resistance genes included bla_{CTX-M} and bla_{TEM} genes using molecular identification techniques with PCR and nucleotide sequencing by BLAST (blastn) software to search a nucleotide database, and the results are shown in Table 6.

In this study, 37 of 39 samples (94.87%) were bla_{CTX-M} genes, whereas 20 and 17 positive samples were from ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, respectively. While bla_{TEM} was detected in 26 of 39 samples (66.67%), 12 and 14 positive samples were from ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*, respectively.

ESBL-antibiotic resistance genes in food material samples and clinical specimen samples

In this study, the nucleotide sequences of ESBL genes detected in food material samples were further analyzed to evaluate the protein database using BLAST (blastx) software. Among the 75 bla_{CTX-M} genes in raw food material samples, the results found $bla_{CTX-M15}$ as 62.67% (47/75) and $bla_{CTX-M14}$ in 37.33% (28/75). Among the 37 bla_{CTX-M} genes in the clinical cultured specimens, $bla_{CTX-M15}$ was 83.78% (31/37), $bla_{CTX-M14}$ was 10.81% (4/37), and $bla_{CTX-M27}$ was 5.41% (2/37). For overall bla_{TEM} genes, the study results found 100% bla_{TEM-1} in both raw food material samples and clinical specimens (Fig. 8).

Origin

1	GCGGGGCAGA	TCACCGCATA	TCATTGGTGG	TGCCGTAGTC	GCCGCTGCCG
51	GTCTTATCAC	CCACAGTCCA	CGACGTCGGT	AAGCCGGCCC	GAATGCTGGC
101	TGCGCCGGTC	GTATTGCCTT	TGAGCCACGT	CACCAACTGC	GCCCGCTGGG
151	TTTCGCCCAG	CGCATGACCC	AGCGTAAGCT	GACGCAACGT	CTGCGCCATC
201	GCCCGCGGCG	TGGTGGTGTC	TCTCGGGTCG	CCGGGAATGG	CGGTATTCAG
251	CGTAGGTTCA	GTGCGATCCA	GACGAAACGT	CTCATCGCCG	ATCGCGCGGG
301	CAAAAGCCGT	CACGCCTCCC	GGGCCACCGA	GCTGGGCAAT	CAATTTGTTC
351	ATGGCGGTAT	TGTCGCTGTA	CTGCAACGCG	GCCGCGCTCA	GTTCTGCCAG
401	CGTCATTGTG	CCGTTGACGT	GTTTTTCGGC	AATCGGATTG	TAGTTAACCA
451	GATCGGCAG	GCTTGATCTC	GACAGGCTGA	TTAAGCAGCT	GCTTTTGCGT
501	TTCACTCTGC	TTAAGCACCG	CCGCGGCCGC	CATCACCTTA	CTGGGGCTGC
551	ACATAAAAAA	ACGGG			

Fig. 6. The nucleotide sequences of Sample no. S1 which positive for ESBL $bla_{\text{CTX-M}}$ gene by PCR amplification method. The sample results examined from BLAST software available at the National Center for Biotechnology Information (NCBI) website represented 99.27% identities with the *E. coli* strain CTX-M family extended-spectrum class A beta-lactamase ($bla_{\text{CTX-M}}$) gene, partial cds from GenBank Sequence accession ID. OP491380.1 data.

Origin

1	GCGTGAGTCT	CCAGTCACAG	AAAAGCATCT	TACGGATGGC	ATGACAGTAA
51	GAGAATTATG	CAGTGCTGCC	ATAACCATGA	GTGATAACAC	TGCTGCCAAC
101	TTACTTCTGA	CAACGATCGG	AGGACCGAAG	GAGCTAACCG	CTTTTTTGCA
151	CAACATGGGG	GATCATGTAA	CTCGCCTTGA	TCGTTGGGAA	CCGGAGCTGA
201	ATGAAGCCAT	ACCAAACGAC	GAGCGTGACA	CCACGATGCC	TGCAGCAATG
251	GCAACAACGT	TGCGCAAACT	ATTAACTGGC	GAACTACTTA	CTCTAGCTTC
301	CCGGCAACAA	TTAATAGACT	GGATGGAGGC	GGATAAAGTT	GCAGGACCAC
351	TTCTGCGCTC	GGCCCTTCCG	GCTGGCTGGT	TTATTGCTGA	TAAATCTGGA
401	GCCGGTGAGC	GTAAAGAAA			

Fig. 7. The nucleotide sequences of Sample no. S1 which positive for ESBL bla_{TEM} gene by PCR amplification method. The sample results examined from BLAST software available at the National Center for Biotechnology Information (NCBI) website represented 100% identities with the *E. coli* strain TEM family beta-lactamase (bla_{TEM}) gene, partial cds from GenBank Sequence accession ID. MN158355.1 data.

ESBL-producing organisms	Results of ESBL-gene detected	
	$bla_{\text{CTX-M}}$	bla_{TEM}
Total (*n* = 39)	**37/39 (94.87%)**	**26/39 (66.67%)**
E. coli (*n* = 22)	20/22 (90.91%)	12/22 (54.55%)
K. pneumoniae (*n* = 17)	17/17 (100%)	14/17 (82.35%)

Table 6. The prevalence of ESBL-genes detected in ESBL-producing organisms from clinical cultured specimens by using PCR amplification and confirmed with nucleotide sequencing methods. The bold indicates the importance of each number.

Discussion

In this study, we assessed the rate of ESBL genes and antibiotic-resistant Enterobacterales bacterial contamination in food materials and further determined ESBL genes in clinical specimens to evaluate the relative abundance of antibiotic-resistant genes in these samples. The prevalence of ESBL-producing *Enterobacterales* contamination and ESBL-genes was investigated by gathering raw food, vegetables, fruits, and animal productsfrom fresh

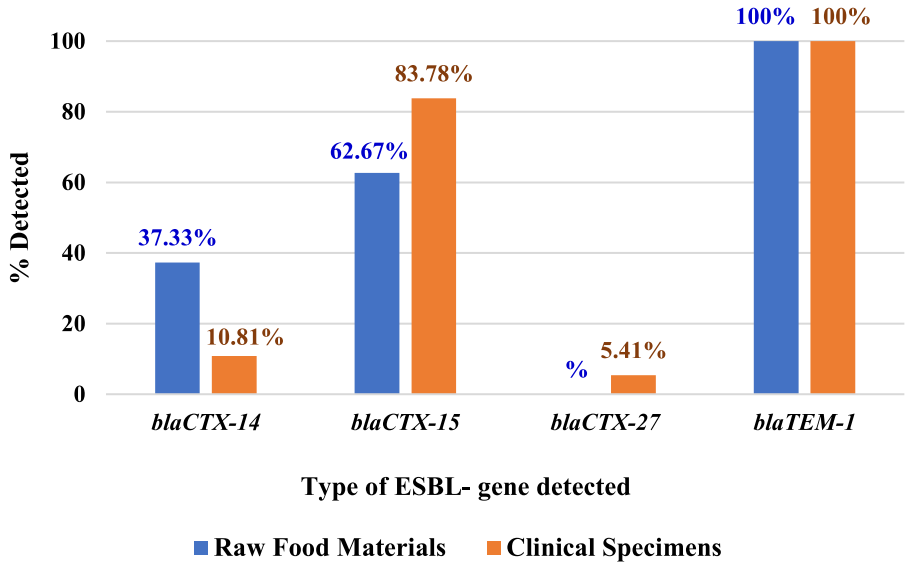

Fig. 8. The prevalence of ESBL-genes detected in food material samples and clinical specimen samples by using PCR amplification and confirmed with nucleotide sequencing methods.

marketplaces. The majority of fruits and vegetables gathered for this study were typically raw, flesh-and-leaf-eaten foods. These study results showed that raw chicken meat samples had the highest prevalence rate of ESBL-producing organisms at 50%, followed by raw pork meat samples at 48%, and raw tomato at 2%, while no ESBL-producing organisms were found in other raw food material samples. Nevertheless, other pathogenic organisms were detected in these samples (data not shown)[22], and almost all of them were opportunistic pathogens associated with various potential infections in immunocompromised and immunocompetent individuals.

Furthermore, we found that the total rate of ESBL-producing organisms was 17.92%, with high contamination of ESBL-producing *E. coli* (5.41%), ESBL-producing *K. pneumoniae* (2.15%), and ESBL-producing *K. oxytoca* (0.36%). Similar to other studies, antimicrobial organisms have also been detected in raw food materials, especially raw animal products, fruits, and vegetables[29,30]. Some studies have found that ESBL-producing bacteria, particularly *E. coli* and *K. pneumoniae*, can be found in various food products, especially those of animal origin (e.g., poultry, beef, pork), and contamination can occur during slaughtering, processing, or handling[31]. *E. coli* is the most prevalent host among gram-negative bacterial species from various families that carry ESBL genes, followed by *K. pneumoniae*[11].

From previous reported, a study of *Enterobacterales*-order bacteria that produce ESBLs indicated that up to 18% of human community-onset infections may be caused by food products, with household transmission accounting for the majority of cases[32]. ESBL-producing *Enterobacterales* or ESBL-E can pose serious risks to food security and safety in both humans and animals. Animal and human populations can co-transmit ESBL-E through a variety of direct and indirect pathways, which has led to significant increases in commensal ESBL reservoirs in the environment. Because pathogenic bacteria in the environment can acquire ESBL genes from commensal bacteria, both humans and animals may be at a significant risk for health issues[33].

Additionally, ESBL-producing bacteria have been detected in plants, soil, and water near cattle, markets, and agricultural settings[34,35]. Foodborne disease outbreaks have been associated with the consumption of fresh fruits and vegetables[36]. Several detrimental microorganisms have been linked to fruits and vegetables offered for sale in outdoor retail markets[37]. Owing to their collection from open fields and lack of washing prior to distribution for retail, food material samples are susceptible to microbial contamination. From farm harvest suppliers to selling locations, they may exhibit some mechanical or physiological damage, which could result in contamination by the time they are offered for sale in low-cost markets[38]. Therefore, the possible causes of fresh fruit contamination on farms, including biological soil amendments, human handlers, domestic animals, and irrigation water, must be considered.

In this study, to confirm the ESBL genes detected, we compared the nucleotide sequences to the sequence databases of the Basic Local Alignment Search Tool (BLAST) program. This tool was used to identify regions of similarity between the biological sequences[28]. In addition to identifying members of gene families, BLAST can be used to infer functional and evolutionary relationships between sequences. In our study, we found that the majority of the ESBL genes detected were those that generate enzymes of the CTX-M type, followed by the TEM type in both raw food material samples and clinical specimen samples. We detected 96.15% and 94.87% $bla_{\text{CTX-M}}$ genes and 43.49% and 66.67% bla_{TEM} in raw food and clinical samples, respectively. No significantly different between the prevalence of resistance genes detected in these two groups of sample ($p > 0.05$, by Fisher's Exact test). Moreover, in this study, we also found that the majority of ESBL genes detected in raw food and clinical samples were $bla_{\text{CTX-15}}$ gene, followed by $bla_{\text{CTX-14}}$ genes. The $bla_{\text{CTX-27}}$ gene was detected in clinical samples but not in raw food samples. In this study, for raw food samples, the prevalence of $bla_{\text{CTX-M}}$ gene detected in ESBL-producing *E. coli* and *K. oxytoca* was higher than in ESBL-producing *K. pneumoniae* as 100% and 66.67%,

respectively. Among the clinical samples, the prevalence of bla_{CTX-M} detected in ESBL-producing *K. pneumoniae* was slightly higher than that in ESBL-producing *E. coli* at 100% and 90.91%, respectively. For bla_{TEM} gene detected in both raw food and clinical samples, we found most of them were bla_{TEM-1} gene and the prevalence of gene detected in ESBL-producing *K. pneumoniae* was higher than in ESBL-producing *E. coli*. This result's finding was similar to several studies that reported the frequency of bla_{TEM} were high among *Enterobacterales* isolates from hospitalized patients due to this resistance gene related to the highly antibiotic used in this population[39,40].

From previous study, the most common ESBL category has been found to be CTX-M-type enzymes, which have displaced TEM and SHV as the most common ESBL types[41]. Isolates containing CTX-M have been discovered in companion animals, food products, livestock, the environment, and nosocomial and community environments[42]. And most CTX-M gene isolates containing the $bla_{CTX-M-14}$, $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$ genes[43–45]. In addition to hospitals and communities, over 80 different types of CTX-M have been found in fresh vegetables, water, animals, and the environment[46]. CTX-M variations have been reported in *P. aeruginosa, Acinetobacter* spp., and various members of the order *Enterobacterales*, including *E. coli* and *K. pneumoniae*[47,48]. Furthermore, *Shigella* species and *S. enterica* serovar *Typhimurium* have a high prevalence of CTX-M-type ESBL[49].

It is clear that various antibiotic regimes cause genotypic ESBL-producing *E. coli* to frequently differ among nations and regions[50]. Different genes were seen to coexist within the same isolation; the most prevalent genotypic pattern was the combination of bla_{TEM} and bla_{CTX-M}. This may result in problems with enzyme combinations. Additionally, multi-bla_{CTX-M} isolates may result in more tenacious infections[50]. Antibiotic resistance genes can spread from one cell to another by conjugation, transformation, or transduction and are commonly found on plasmids or transposons. The resistance can be swiftly acquired by bacteria of different species through gene exchange. Horizontal transmission is the term for this procedure. For doctors treating infectious disorders, this kind of drug resistance poses serious challenges[51].

In Thailand, it has been shown that the antimicrobial stewardship program (ASP) improves clinical outcomes, decreases antimicrobial consumption, encourages appropriate antimicrobial use, and slows the emergence of AMR without affecting patient outcomes in terms of infection-related morbidity and mortality[52]. Therefore, from overall study's finding results focused on the detection of antibiotic-resistant ESBL organisms and genes in raw food materials and clinical samples. We found similar ESBL-producing organisms and genes in both the sample types. Inadequate agricultural and livestock sanitation and hygiene standards, bacteria with resistant genetic components can migrate from farms to the surrounding environment by contaminating the soil and water near the farm with livestock dung. Therefore, acknowledging ESBL antibiotic resistance in food materials requires a multifaceted approach involving monitoring, public education, regulatory oversight, and research.

Nevertheless, to improve the study quality in the future, to decrease any limitation of the study must be concerned. Regarding to DNA extraction and purification technique, using the commercial kit in experiment will provided high DNA quality and yield. For the limitation of the small number of sample collected in the study area, due to this study was a preliminary study to detect antibiotic resistance organisms and genes in our setting, the expanding sample types and study area should be more evaluated further. The continued research is needed to evaluate the prevalence of antibiotic-resistant microorganisms contaminating other food material samples. Finally, the laboratory and hospital settings, routine screening and prompt identification of ESBL-producing bacteria are essential for infection management and treatment strategies to prevent outbreaks.

Conclusion

In this study, ESBL-producing *Enterobacterales* resistance organisms and genes were detected in food material and clinical samples. The study's findings on antibiotic-resistant organisms can be used to acknowledge the people involved in the food chain as collecting, slaughtering, processing, transporting, and storing raw food materials. As a result, people who handle raw meats and vegetables should be awareness and concerned about food safety and hygiene. It is necessary to be concerned about the possibility of acquiring illnesses from antibiotic-resistant bacteria and opportunistic pathogens when handling or consuming these foods. In addition, they should be mindful of the dangers and risks of infections caused by opportunistic and antibiotic-resistant pathogens. Therefore, it is crucial to enhance food safety and protect public health from the threats posed by antibiotic-resistant bacteria. Educating consumers about safe food handling and cooking practices can help to reduce the risk of exposure. Awareness campaigns can inform people about the risk of antibiotic misuse in agriculture. Policymakers may need to consider stricter regulations on antibiotic use in livestock to mitigate the development of antibiotic resistance. Development plans for the control and surveillance of antibiotic resistance of pathogens and the use of antibiotic drugs for the future treatment of bacterial infections that support prevention, healthcare, and public health systems are needed.

Data availability

The datasets generated and/or analysed during the current study are available in the National Center for Biotechnology Information (NCBI) repository, [http://www.ncbi.nlm.nih.gov in accession no. PV699623 and PV699624].

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Author contributions

Conceptualization, S.H. and S.D.; methodology, S.D., P.J., X.Z., Z.K., S.Ho. and S.H.; validation, S.D., Z.K. and S.H.; formal analysis, S.D., P.K., Z.K. and S.H.; investigation, S.Ho. and B.C.; writing—original draft preparation, S.D. and S.H.; writing—review and editing, S.D. and S.H.; visualization, S.D. and S.H.; supervision, S.H.; project administration, S.Y.; funding acquisition, S.H. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Research ID. 0537 Study: NONE-256–0537).

Additional information

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