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ABSTRACT

This study investigated the prevalence of antibiotic-resistant *Escherichia coli* using extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase as exemplars of multidrug-resistant phenotypes in poultry meat samples taken from open-air and supermarkets in Phitsanulok province, Northern Thailand. Two hundred and fifty poultry meat samples from open-air (n = 147) and supermarkets (n = 103) were analyzed. In total, 143 cefotaxime-resistant *E. coli* isolates comprising 78 isolates (53.1%) from open-air markets and 65 isolates (63.1%) from supermarkets were obtained. No significant difference could be observed in the prevalence of ESBL-positive *E. coli* between samples taken from open-air (70.5%) and supermarkets (69.2%). ESBL genotypes comprised of $bla_{\text{CTX-M-group 1}}$ (69%), $bla_{\text{CTX-M-group 9}}$ (13%), $bla_{\text{TEM-116}}$ (1%), $bla_{\text{SHV-2a}}$ (1%) and $bla_{\text{SHV-12}}$ (1%) were detected. 39.5% of the ESBL-negative *E. coli* possessed $bla_{\text{CMY-2}}$. The $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 9}}$ and $bla_{\text{CMY-2}}$ were successfully transferred into *E. coli* by conjugation at high frequencies. Repetitive palindromic-PCR of some $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$-positive *E. coli* isolates revealed identical DNA patterns suggesting clonal spread. Phylogenetic grouping and MLST analysis revealed that 3 isolates were *E. coli* ST131. Of these, 2 isolates were ESBL-negative and carried $bla_{\text{CMY-2}}$. The other isolate was ESBL-positive and carried $bla_{\text{TEM-116}}$. This is the first study to demonstrate ESBL and AmpC genotypes in *E. coli* and the first discovery of human pathogen ST131 from Thai poultry meat. Our data raises serious concerns for food safety and biosecurity in the Thai food industry.

Keywords: poultry; meat; ESBL; CTX-M; AmpC; CMY-2; *E. coli*; ST131

1. Introduction

Resistance to broad spectrum β-lactams such as third-generation cephalosporins, monobactam and carbapenems in Enterobacteriaceae, especially *Escherichia coli*, is rapidly increasing (Pitout, 2013). Reports on extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase-producing Enterobacteriaceae from clinical and environmental samples are continuously published from the majority of countries indicating a worldwide dissemination. Several types of ESBLs have been reported such as SHV, TEM and CTX-M. CTX-M is the most prevalent ESBL while CMY-2 is frequently encountered AmpC in human infections. ESBL- and AmpC-encoding genes are usually associated with mobile genetic elements which strongly facilitate their spread within a bacterial population (Jacoby 2009; Poirel, Bonnin, & Nordmann, 2012).

Contamination of meat with antibiotic-resistant bacteria has the potential to transfer to humans and is a clear public health concern. Several studies have shown that meat, especially poultry meat, is an important reservoir of antibiotic-resistant *E. coli* (Nguyen et al., 2016; Schwaiger et al., 2012). The prevalence of ESBL- and AmpC-positive *E. coli* as well as their respective resistant genes in different types of meat even in organic meat has been reported from several countries (Cohen Stuart et al., 2012; Egea et al., 2012; Ghodousi, Bonura, Di Noto, & Mammina, 2015; Kawamura, Goto, Nakane, & Arakawa, 2014). Furthermore, transmission of ESBL and AmpC-positive *E. coli* from meat to human has been previously reported outside Thailand (Overdevest et al., 2011; Vincent et al., 2010).

In Thailand, consumption of poultry meat is popular. Meat is often sold in open-air markets, traditional Thai markets which are seen extensively throughout the country, and western-style supermarkets which are becoming increasingly popular throughout Thailand.

Previous studies in Thailand have revealed the presence of antibiotic-resistant Enterobacteriaceae isolates in chicken meat and chicken rectal swab including those producing ESBL (Boonyasiri et al., 2014; Chaisatit, Tribuddharat, Pulsrikarn, & Dejsirilert, 2012; Trongjit, Angkittitrakul, & Chuanchuen, 2016). However, the prevalence of ESBL- and/or AmpC-producing *E. coli* from meat samples in Thailand remains poorly understood. This study investigated the prevalence of ESBL- and AmpC-encoding genes from poultry meat samples obtained from both open-air and supermarkets as well as determined *E. coli* pathogenicity groups.

2. Materials and Methods

2.1 Samplings

Samplings of fresh poultry meat were performed in 29 open-air markets and 22 supermarkets in Phitsanulok province, Northern Thailand. A total of 250 poultry meat samples (chicken = 218, duck = 14, bird = 18) from open-air markets (n = 147) and supermarkets (n = 103) were sampled. Frozen poultry meat was excluded from the study. All samples were originated from Thailand. Samples were maintained at 4 °C and processed immediately.

2.2 Isolation and identification of third generation cephalosporin-resistant E. coli

Twenty-five grams of each sample were homogenized with a Stomacher in 225 mL buffered peptone water (Oxoid, Basingstroke, UK). Then, 10 mL of this homogenate were enriched in 90 ml EE broth (Becton, Dickinson and Company, MD, USA) for 24 h at 37 °C. The enrichment was plated onto EMB agar (Oxoid) supplemented with 2 µg/mL cefotaxime (as an exemplar of broad-spectrum cephalosporins) (Sigma Aldrich, MO, USA) and incubated under aerobic condition for 24 h at 37 °C. Presumptive *E. coli*

colonies isolated from each sample were subcultured on Tryptic Soy Agar and incubated as described above for further characterizations. Species identification was performed by using RapID™ ONE System (REMEL Inc., KS, USA) according to the manufacturers' instructions and confirmed by sequencing of 16S rRNA gene (Lane, 1991).

2.3 Antimicrobial susceptibility and ESBL detection

All isolates were tested for susceptibility to 18 antimicrobial agents by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) protocols and the results were evaluated according to CLSI criteria (CLSI, 2013). The antibiotics tested were ampicillin, cefoxitin, ceftazidime, cefotaxime, cefpodoxime, cefepime, aztreonam, imipenem, amoxicillin/clavulanic acid, ampicillin/sulbactam, amikacin, gentamicin, doxycycline, tetracycline, ciprofloxacin, levofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole. Isolates showing intermediate results were considered as resistant. Minimum Inhibitory Concentration (MICs) were determined by broth microdilution method according to CLSI guidelines (CLSI, 2013). The MIC of each antimicrobial agent was defined as the lowest concentration, which inhibited visible growth of the organism.

Isolates were tested for ESBL production by combination disk method with ceftazidime and cefotxime in the presence or absence of clavulanic acid, according to CLSI guidelines (CLSI, 2013).

2.4 Screening for ESBL- and AmpC-encoding genes by PCR and sequencing

All ESBL-producing isolates were investigated for the presence of ESBL-encoding genes; bla_{TEM}, bla_{SHV} and $bla_{\mathrm{CTX\text{-}M}}$, by PCR as previously described (Dallenne, Da Costa, Decré, Favier, & Arlet, 2010; Woodford, Fagan, & Ellington, 2006). Isolates showing

resistance to cefoxitin were examined for the presence of AmpC-encoding genes by multiplex PCR (Pérez-Pérez, & Hanson, 2002). PCR products were analyzed by agarose gel electrophoresis.

Selected PCR products were analyzed by DNA sequencing. Amplicons were purified using a DNA purification kit (RBC Bioscience, New Taipei City, Taiwan) and sequenced by First BASE Laboratories (Selangor, Malaysia). The obtained sequences were compared with those available in the GenBank database using the BLAST algorithm available on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

2.5 Conjugation experiments

To investigate the transfer of antibiotic resistance, conjugation experiments were carried out by broth mating method using rifampin-resistant *E. coli* DH5α as the recipient. Cultures of donor and recipient cells were mixed and incubated overnight at 37 °C without shaking. Transconjugants were selected on Tryptic Soy Agar supplemented with rifampin (16 µg/mL) and cefotaxime (1 µg/mL). Conjugation frequency was expressed as the number of transconjugants divided by the number of recipient cells. Transferrable of antibiotic-resistant gene was confirmed by PCR. MICs of transconjugants were determined by broth microdilution method.

2.6 Repetitive-palindromic polymerase chain reaction (Rep-PCR)

E. coli isolates carrying either ESBL- or AmpC genes were typed by rep-PCR as described previously by Versalovic, Koeuth, and Lupski (1991).

2.7 Phylogenetic grouping and multilocus sequence typing (MLST) analysis

Phylogenetic group (A, B1, B2 and D) of ESBL- and pAmpC-producing *E. coli* was performed by a multiplex PCR assay for *chuA*, *yjaA* and DNA fragment TspE4C2 as previously described (Clermont, Bonacorsi, & Bingen, 2000). Isolate belonging to group B2 was investigated for the presence of *pabB* gene by PCR (Clermont et al., 2009). MLST was performed by amplification and sequencing of 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) according to the protocols from *E. coli* MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

2.8 Statistical analysis

Fisher's exact test was used to compare proportions using Minitab software version 15. The differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Isolation of cefotaxime-resistant (Ctx-R) E. coli from poultry meat samples and ESBL production

In this study, 250 poultry meat samples from open-air markets and supermarkets in Phitsanulok province, Northern Thailand were analyzed (Table 1). In total, 143 Ctx-R *E. coli* isolates comprising 78 isolates (53.1%, 95% CI = 45.0–61.1%) from open-air markets and 65 isolates (63.1%, 95% CI = 53.8–72.4%) from supermarkets were obtained. The percentages of Ctx-R *E. coli* isolates were not statistically different between samples obtained from open-air and supermarkets ($p = 0.121$). Of the 143 isolates, 70.5% (55/78, 95% CI = 60.4–80.6%) and 69.2% (45/65, 95% CI = 58.0–80.5%) from open-air and supermarkets, respectively, were ESBL-positive.

3.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* isolates recovered from poultry meat samples was determined (Table 2). β-Lactam susceptibility test revealed that all 143 *E. coli* isolates were resistant to ampicillin, cefotaxime and cefpodoxime and >90% were resistant to aztreonam and ceftazidime. Resistance to amoxicillin/clavulanate and ampicillin/sulbactam was 60.1% and 82.5%, respectively. A small number of isolates were resistant to imipenem (16.1%). High resistant rates (>60%) were also observed for cefepime, amikacin, doxycycline, tetracycline and ciprofloxacin. Furthermore, we observed that ESBL-positive isolates showed significantly higher resistant rates to cefepime, gentamicin, doxycycline, tetracycline, levofloxacin and trimethoprim/sulfamethoxaole ($p < 0.05$) than the ESBL-negative isolates (Table 2). In contrast, ESBL-negative isolates showed significantly higher resistant rates to cefoxitin, amoxicillin/clavulanate and ampicillin/sulbactam ($p < 0.05$).

3.3 Detection of antibiotic-resistant genes

Of the 143 Ctx-R isolates, 100 were shown to be ESBL-positive (Table 2). Detection of ESBL- and AmpC-encoding genes in Ctx-R *E. coli* isolates was performed and results were shown in Table 3. Of the 100 ESBL-positive isolates, 82 were found to carry genes encoding for $bla_{\text{CTX-M}}$. $bla_{\text{CTX-M-group 1}}$ was the most prevalent (n = 69, 69%), followed by $bla_{\text{CTX-M-group 9}}$ (n = 13, 13%). bla_{TEM} and bla_{SHV}-related ESBL-genes were also found i.e. $bla_{\text{TEM-116}}$ (n = 1, 1%), $bla_{\text{SHV-2a}}$ (n = 1, 1%) and $bla_{\text{SHV-12}}$ (n = 1, 1%). Additionally, from the 43 ESBL-negative isolates, 17 (39.5%) were shown to be $bla_{\text{CMY-2}}$-positive.

3.4 Conjugation experiments

Conjugation experiments were performed with randomly selected *E. coli* containing $bla_{\text{CTX-M-group 1}}$ (7 isolates), $bla_{\text{CTX-M-group 9}}$ (2 isolates) and $bla_{\text{CMY-2}}$ (3 isolates) as donors. The $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 9}}$ and $bla_{\text{CMY-2}}$ genes from donor isolates could be transferred to the recipient strain *E. coli* DH5α with conjugation frequencies of 10^{-5}-10^{-2}, 10^{-4} and 10^{-7}-10^{-5}, respectively (Table 4). The presence of $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 9}}$ and $bla_{\text{CMY-2}}$ in transconjugants was confirmed by PCR. The cefotaxime MICs were markedly increased in transconjugants carrying $bla_{\text{CTX-M}}$ (> 128 fold) and $bla_{\text{CMY-2}}$ (> 16-32 fold) compared with the recipient strain *E. coli* DH5α (Table 4).

3.5 Typing of ESBL- and AmpC-positive E. coli by rep-PCR

The DNA profiles of $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$-positive *E. coli* generated with rep-PCR primers showed diverse banding patterns. However, the identical rep-PCR profiles in some *E. coli* isolates were detected. The representative rep-PCR patterns of the isolates were shown in Fig. 1. Rep-PCR analysis differentiated 69 and 13 isolates of $bla_{\text{CTX-M group 1}}$- and $bla_{\text{CTX-M group 9}}$-positive *E. coli* into 34 and 9 distinct patterns, respectively. However, the identical patterns among isolates carrying $bla_{\text{CTX-M group 1}}$ (Fig. 1A, Lanes 1 & 5, 2 & 3 and 4 & 6) and $bla_{\text{CTX-M group 9}}$ (Fig. 1B, Lanes 1 & 2 and 3 & 4) were found. In addition, a total of 11 distinct DNA profiles were found among 17 $bla_{\text{CMY-2}}$-positive *E. coli* and identical DNA patterns were detected (Fig. 1C, Lanes 1-3). Importantly, $bla_{\text{CTX-M group 1}}$-positive *E. coli* isolates recovered from different open-air and supermarkets shared the same rep-PCR pattern (Fig. 1A). In addition, we found that 22 isolates carried an identical rep-PCR type but different CTX-M genes i.e. $bla_{\text{CTX-M group 1}}$ (n = 19) and $bla_{\text{CTX-M group 9}}$ (n = 3).

3.6 Phylogenetic grouping and analysis of sequence type (ST)

Phylogenetic grouping of 85 ESBL- and 17 AmpC-positive *E. coli* isolates were determined. The results showed that 51% belonged to group A, 27.5% to group B1 and 17.6% to group D. Only 4 isolates (3.9%) belonged to group B2 (Table 5). The prevalence of $bla_{\text{CTX-M}}$ was significantly higher in the commensal phylogroups A and B1 than pathogenic groups B2 and D ($p < 0.001$). In contrast, the prevalence of $bla_{\text{CMY-2}}$ was significantly higher in the pathogenic phylogroups D and B2 than commensal groups A and B1 ($p = 0.002$). Of the 4 isolates, belonging to group B2, 3 isolates contained the *pabB* gene, which is specific for subgroup I/ O25b. These 3 isolates were identified as ST131, by MLST analysis. One carried $bla_{\text{TEM-116}}$ and 2 carried $bla_{\text{CMY-2}}$.

4. Discussion

In this study, poultry meat samples from open-air and supermarkets in Phitsanulok province, Northern Thailand were analyzed and a total of 143 Ctx-R *E. coli* isolates were recovered from all types of poultry meat (chicken, duck and bird, Table 1).

A previous study in Thailand revealed the presence of ESBL-positive *E. coli* from chicken meat however a limited number of meat samples were investigated (Boonyasiri et al., 2014). In contrast, we found a comparatively high prevalence of ESBL-positive *E. coli* isolates from open-air (70.5%) and supermarkets (69.2%).

Antimicrobial susceptibility test of 143 Ctx-R *E. coli* isolates revealed the high resistant rates for broad-spectrum cephalosporins, aztreonam and ciprofloxacin (Table 2). These results were different from those reported previously from Thailand where *E. coli* obtained from chicken meat was mainly susceptible to third generation cephalosporins and ciprofloxacin (Chaisatit, Tribuddharat, Pulsrikarn, & Dejsirilert, 2012). Interestingly, ESBL-positive *E. coli* isolates showed significantly higher resistance rates to many

antibiotics than ESBL-negative isolates, consistent with the common feature of ESBL-positive bacteria (Woerther, Burdet, Chachaty, & Andremont, 2013). In addition, we found that all *E. coli* isolates, regardless of the ESBL production, exhibited multidrug-resistant (MDR) phenotype as defined by Magiorakos et al. (2012).

bla$_{\text{CTX-M}}$ was commonly found among ESBL-positive isolates, although genes encoding for TEM-, and SHV-type ESBLs such as *bla*$_{\text{TEM-116}}$, *bla*$_{\text{SHV-2a}}$ and *bla*$_{\text{SHV-12}}$ were also detected (Table 3). Similar results were found in other studies which reported the predominance of *bla*$_{\text{CTX-M}}$ in poultry meat samples (Cohen Stuart et al., 2012; Kawamura, Goto, Nakane, & Arakawa, 2014; Nguyen et al., 2016). The high prevalence of *bla*$_{\text{CTX-M}}$ seen in this study coincided with the report on community-acquired CTX-M-positive infections in Thailand (Apisarnthanarak et al., 2007). For almost all ESBL-negative *E. coli* isolates, resistance to cefoxitin was noted (Table 2) suggesting that AmpC may be responsible for cefotaxime-resistant phenotypes. Accordingly, *bla*$_{\text{CMY-2}}$ was found in 39.5% of ESBL-negative isolates.

For the rest of *E. coli* isolates, neither ESBL- nor AmpC-encoding genes were detected and were not further investigated. It is possible that overexpression of chromosomally-encoded AmpC β-lactamases, efflux pump overexpression or outer membrane alteration may contribute to reduced susceptibility to antibiotics (Pitout, 2013). It is also interesting to note that distribution of ESBL-genes was similar between open-air and supermarkets. In contrast, *bla*$_{\text{CMY-2}}$ was found more frequently in poultry meat taken from open-air markets (Table 3).

Conjugation experiments revealed that *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{CMY-2}}$ were successfully transferred to *E. coli* DH5α at high frequencies. The increased cefotaxime MICs in transconjugants suggested that *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{CMY-2}}$ are responsible for cefotaxime resistance. These results are consistent with the fact that horizontal gene transfer plays a

major role in the spread of *bla*$_{CTX-M}$ and *bla*$_{CMY-2}$ in *E. coli* (Woerther, Burdet, Chachaty, & Andremont, 2013; Sidjabat et al., 2014). Genotypic analysis of *bla*$_{CTX-M}$ and *bla*$_{CMY-2}$-positive *E. coli* by rep-PCR revealed the genetic diversity among these isolates. However, identical genotypes were found among isolates carrying the same genes suggesting the clonal spread of MDR *E. coli* in poultry meat samples. This observation is further supported by the same genotypes being found in isolates obtained from open-air and supermarkets. Moreover, we found 22 identical isolates carrying different *bla*$_{CTX-M}$ inferring the recent transfer of *bla*$_{CTX-M}$ among dominant Thai *E. coli* clades.

Typing divided the *E. coli* isolates into 4 phylogenetic groups. Groups A and B1 constitute mainly the commensal strains while extra-intestinal pathogenic strains belong to groups B2 and D (Clermont, Bonacorsi, & Bingen, 2000). In this study, the majority of *E. coli* isolates carrying ESBL-genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$ or *bla*$_{TEM}$) significantly belonged to the low virulent groups A and B1. Similar results had been reported from *E. coli* isolates recovered from poultry meat in Spain and the Netherlands (Egea et al., 2012; Kluytmans et al., 2013). Our results suggest that *E. coli* isolates of the commensal origin may contribute to the dissemination of MDR *E. coli* within the community. It is also interesting to note that most *bla*$_{CMY-2}$-positive *E. coli* belonged to phylogroup D, consistent with previous reports (Oteo et al., 2010; Tamang et al., 2012).

Among the extraintestinal *E. coli*, O25b-ST 131, belonging to the phylogenetic group B2, has emerged as a highly virulent human pathogen worldwide. *E. coli* O25b-ST131 usually exhibits multidrug resistance and produces various types of ESBLs and AmpC. ST131 has been reported from different origins such as animals, environments and humans. Although the widespread occurrence of *E. coli* ST131 has been documented, its presence in raw meat is rare (Nicolas-Chanoine, Bertrand, & Madec, 2014). Previous studies in the Netherlands and Spain which many ESBL-producing *E. coli* isolates from

poultry meat were investigated but no ST131 was identified (Cohen Stuart et al., 2012; Egea et al., 2012; Overdevest et al., 2011). However other studies in Italy, Japan and Canada have reported the contamination of *E. coli* ST131 in poultry meat samples (Ghodousi, Bonura, Di Noto, & Mammina, 2015; Kawamura, Goto, Nakane, & Arakawa, 2014; Vincent et al., 2010). In our study, 3 MDR *E. coli* isolates were identified as *E. coli* B2-O25b ST131. Of these, 2 isolates were ESBL-negative and carried $bla_{\text{CMY-2}}$. The other ST131 isolate was an ESBL-positive and carried $bla_{\text{TEM-116}}$, the infrequent ESBL-gene found among ST131 isolates. In Thailand, all *E. coli* ST131 reported, to date, were recovered from clinical specimen (Netikul et al., 2014). Given the fact that *E. coli* ST131 is associated predominantly with community-onset urinary tract infections, our finding of ST131 isolates suggest that poultry meat could contribute to the dissemination of MDR and virulent *E. coli* within Thai community.

Our data demonstrates that poultry meat in Thailand acts as a reservoir for MDR *E. coli* especially those producing ESBL and AmpC. This is the first analysis of ESBL and AmpC genes and the first discovery of human pathogen ST131 from Thai poultry meat. Our results indicate that poultry meat may be a source of transmission of the MDR ST131 in Thailand.

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475 **Figure Caption**

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477 **Fig. 1.** Representative rep-PCR profiles of the cefotaxime-resistant *E. coli* carrying $bla_{\text{CTX-M-group 1}}$ (A), $bla_{\text{CTX-M-group 9}}$(B) and $bla_{\text{CMY-2}}$ (C), respectively.

479 M1, 1 kb Sharp Ladder (RBC Bioscience, New Taipei City, Taiwan)

480 M2, 100 bp Ladder RTU (GeneDireX, Taipei City, Taiwan)

Table 1 Occurrence of cefotaxime-resistant *E. coli* from poultry meat samples

Types of Samples	No. of *E. coli*	No. of ESBL-positive *E.coli*
open air markets (*n* = 147)		
Chicken (*n* = 126)	69	46
Duck (*n* = 3)	0	0
Bird (*n* = 18)	9	9
Total	78 (53.1%)	55 (70.5%)
supermarkets (*n* = 103)		
Chicken (*n* = 92)	61	41
Duck (*n* = 11)	4	4
Total	65 (63.1%)	45 (69.2%)

Table 2 Antimicrobial resistance (%) among cefotaxime-resistant *E. coli* isolates

Antimicrobial agents	Total (*n* = 143)	ESBL production		
		ESBL-positive (*n* = 100)	ESBL-negative (*n* = 43)	p values
ampicillin	100	100	100	NS
cefoxitin	41.3	17.0	97.7	< 0.001
ceftazidime	93	90	100	0.033
cefotaxime	100	100	100	NS
cefodoxime	100	100	100	NS
cefepime	60.1	83.0	7.0	< 0.001
aztreonam	95.8	96.0	95.3	NS
imipenem	16.1	16.0	16.3	NS
amoxicillin/clavulanate	60.1	43.0	100	< 0.001
ampicillin/sulbactam	82.5	75.0	100	< 0.001
amikacin	72	72.0	72.1	1
gentamicin	57.3	67.0	34.9	< 0.001
doxycycline	62.9	78.0	27.9	< 0.001
tetracycline	71.3	86.0	37.2	< 0.001
ciprofloxacin	62.9	64.0	60.5	NS
levofloxacin	26.6	34.0	9.3	0.002
chloramphenicol	34.3	39.0	23.3	NS
trimethoprim/sulfamethoxazole	45.5	59.0	14.0	< 0.001

NS, Not significance

Table 3 Prevalence of genes encoding for ESBL and AmpC among cefotaxime-resistant *E. coli* isolates.

β-lactamase genes	ESBL-positive *E. coli* (*n* = 100)			ESBL-negative *E. coli* (*n* = 43)		
	open-air markets (*n* = 55)	supermarkets (*n* = 45)	Total	open-air markets (*n* = 23)	supermarkets (*n* = 20)	Total
$bla_{\text{CTX-M group 1}}$	25	18	43	0	0	0
$bla_{\text{CTX-M group 1}}$ + $bla_{\text{TEM-1}}$	12	14	26	0	0	0
$bla_{\text{CTX-M group 9}}$	5	1	6	0	0	0
$bla_{\text{CTX-M group 9}}$ + $bla_{\text{TEM-1}}$	2	5	7	0	0	0
$bla_{\text{SHV-2a}}$	1	0	1	0	0	0
$bla_{\text{SHV-12}}$	0	1	1	0	0	0
$bla_{\text{TEM-116}}$	1	0	1	0	0	0
$bla_{\text{CMY-2}}$	0	0	0	14	2	16
$bla_{\text{CMY-2}}$ + $bla_{\text{TEM-1}}$	0	0	0	1	0	1
Total	46	39	85	15	2	17

Table 4 Conjugation frequency, transferred gene and cefotaxime MICs for *E. coli* DH5α and the respective transconjugants

Strain	Frequency of transfer	Transferred gene	Cefotaxime MIC (µg/mL)
E. coli DH5α[a]	-	-	< 0.0625
EC2_Tc	4.2×10^{-3}	$bla_{\text{CTX-group 1}}$	> 8
EC103_Tc	2.6×10^{-3}	$bla_{\text{CTX-group 1}}$	> 8
EC107_Tc	4.4×10^{-3}	$bla_{\text{CTX-group 1}}$	> 8
EC109_Tc	6.8×10^{-5}	$bla_{\text{CTX-group 1}}$	> 8
EC121_Tc	2.5×10^{-5}	$bla_{\text{CTX-group 1}}$	> 8
EC129_Tc	8.1×10^{-2}	$bla_{\text{CTX-group 1}}$	> 8
EC146_Tc	7.5×10^{-2}	$bla_{\text{CTX-group 1}}$	> 8
EC70_Tc	2.4×10^{-4}	$bla_{\text{CTX-group 9}}$	> 8
EC119_Tc	4.7×10^{-4}	$bla_{\text{CTX-group 9}}$	> 8
EC125_Tc	1.5×10^{-5}	$bla_{\text{CMY-2}}$	2
EC128_Tc	1.6×10^{-6}	$bla_{\text{CMY-2}}$	1

EC132_Tc	1.9×10^{-7}	*bla*$_{CMY-2}$	1

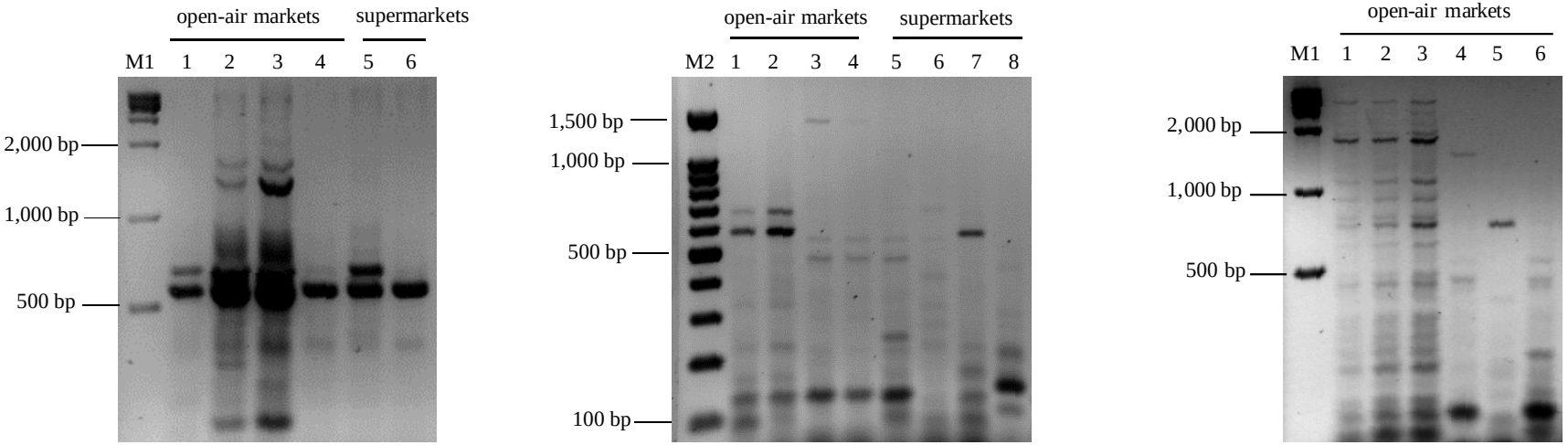
[a] rifampin-resistant *E. coli* DH5α was used a recipient strain.

Tc, transconjugant

Table 5 Distribution of ESBL- and AmpC-encoding genes among *E. coli* phylogroups (*n* = 102)

Phylogenetic groups	No. (%) of isolates producing:				Total (%)
	bla$_{\text{TEM-116}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{CTX-M}}$	*bla*$_{\text{CMY-2}}$	
A	0 (0)	1 (1.0)	50 (49.0)	1 (1.0)	52 (51.0)
B1	0 (0)	1 (1.0)	26 (25.5)	1 (1.0)	28 (27.5)
B2	1 (1.0)	0 (0)	0 (0)	3 (2.9)	4 (3.9)
D	0 (0)	0 (0)	6 (5.9)	12 (11.7)	18 (17.6)
Total	1 (1.0)	2 (2.0)	82 (80.4)	17 (16.6)	102 (100)

Fig. 1


open-air markets
supermarkets
M1 1 2 3 4 5 6
2,000 bp
1,000 bp
500 bp
(A)
open-air markets
supermarkets
M2 1 2 3 4 5 6 7 8
1,500 bp
1,000 bp
500 bp
100 bp
(B)
open-air markets
M1 1 2 3 4 5 6
2,000 bp
1,000 bp
500 bp
(C)

Highlights

High prevalence of ESBL-producing *E. coli* in poultry meat in Thailand was observed.

E. coli carrying $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ isolates were found in poultry meat samples.

Clonal spread of $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$-positive *E. coli* from poultry meat was detected.

E. coli ST131 isolates carrying $bla_{\text{TEM-116}}$ or $bla_{\text{CMY-2}}$ have emerged from poultry meat.