Extended spectrum ß-lactamase-producing *Escherichia coli* among backyard poultry farms, farmers, and environments in Thailand

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ABSTRACT

Food-producing animals, including poultry, have been considered as potential sources of extended spectrum ß-lactamase (**ESBL**)-producing *Escherichia coli*. This study investigates the occurrence and dissemination of ESBL-producing *E. coli* among backyard poultry farms, farmers, and environments in Northern Thailand. Antimicrobial-resistant phenotypes, resistant determinants, genotypic characterizations, and spread of these isolates were studied. Fecal samples from poultry, farmers, and environments were captured from 27 farms. In total, 587 samples were collected and the overall 27.1% (159/587) of ESBL-producing *E. coli* isolates were obtained. Among these, ESBL-producing *E. coli* was isolated from 50% (farmers), 25.9% poultry (24.9% chicken and 36.6% duck) of the fecal samples, and 25.0% of the environmental samples. All isolates demonstrated multidrug resistance, most frequently to ≥ 10 different antimicrobial agents. Molecular analysis of ESBL-encoding genes showed that the predominant gene was $bla_{\text{CTX-M-55}}$ (54.1%), followed by $bla_{\text{CTX-M-14}}$ (28.3%), and $bla_{\text{CTX-M-15}}$ (8.8%). $bla_{\text{CTX-M-27}}$ (3.8%) and $bla_{\text{CTX-M-65}}$ (0.6%) were also detected at low frequencies. Conjugation assays demonstrated that $bla_{\text{CTX-M}}$ could be transferred to *E. coli* J53 with the transfer frequencies ranging from 10^{-7} to 10^{-2}. Pulsed field gel electrophoresis (**PFGE**) revealed diverse genotypes, however, identical and closely related PFGE profiles were detected among isolates within and between farms, suggesting the clonal transmission. In addition, our study identified 4 $bla_{\text{CTX-M-27}}$-positive *E. coli* B2-ST131 isolates. Interestingly, two ST131 isolates, obtained from a farmer and chicken in the same area, showed closely related PFGE profiles. Our results suggest the presence and spread of ESBL-producing *E. coli* between backyard poultry farms, farmers, and environments in Thailand.

INTRODUCTION

The increasing reports on resistance to extended-spectrum cephalosporins in Enterobacteriaceae, especially *Escherichia coli*, is a matter of great public health concern since these drugs are frequently used to treat human and animal diseases. Mechanism of extended-spectrum cephalosporin resistance is mainly caused by the production of extended spectrum ß-lactamase (**ESBL**). Several types of ESBL have continuously been documented, however the CTX-M is the most common ESBL found in Enterobacteriaceae (Ruppé et al., 2015). Genes encoding for ESBL are usually associated with mobile genetic elements which can be readily transferred to other bacteria (Ruppé et al., 2015).

It is generally accepted that ESBL-producing *E. coli* is not only found in humans but also in food-producing animals including poultry. This is considered as a serious threat to human health since *E. coli* can be transmitted to humans, spread within communities, and cause common infections such as diarrhea and cystitis (Seiffert et al., 2013). Studies suggest that multidrug-resistant (**MDR**) *E. coli*, especially those producing ESBLs, have increasingly been isolated from poultry worldwide (Seiffert et al., 2013; Saliu et al., 2017). For the European countries where the use of antibiotics in poultry farming is controlled, such as Germany and the

Netherlands, high proportions of ESBL-producing *E. coli* in poultry farms were still noted (Reich et al., 2013; Huijbers et al., 2014) whereas lower isolation rate was found in Finland (Päivärinta et al., 2016). For Asian countries, such as Japan, Vietnam, and China, ESBL-producing *E. coli* in poultry farms have been previously reported, however, the prevalence of these isolates varied depending on the study catchment area and sampling strategy (Kameyama et al., 2013; Ueda et al., 2015; Li et al., 2016). Furthermore, clonal disseminations of ESBL-producing *E. coli* isolates among poultry and environments within and between farms have been reported (Ma et al., 2012; Kameyama et al., 2013).

Backyard poultry farming is a household-farming system that can be seen extensively throughout Thailand and provides local meat sources as well as cash income. It has been reported that among the 2.9 million poultry flocks in Thailand, approximately 73% are backyard flocks (Otte et al., 2006). Backyard poultry are usually raised under free-range systems and have open access to external environments, this system therefore provides low biosecurity. Backyard poultry have very close contact with humans, thus people working closely with animals will have higher exposure and a high risk for backyard farming-mediated bacterial colonization and subsequent infections (El-Tras et al., 2015; Bi et al., 2018).

In Thailand a number of studies have demonstrated the occurrence and dissemination of MDR *E. coli* isolates as well as their resistant genes among food-producing animals. The high prevalence of ESBL-producing *E. coli* isolates in healthy pigs and broilers, farm workers and water collected from animal farms have been reported (Boonyasiri et al., 2014; Changkaew et al., 2015). However, most studies on the ESBL-producing *E. coli* have been performed on large commercial poultry farms and less is known about the occurrence of these organisms in backyard farms, which are very common for Thai residents. Given the close proximity of families to their animals and their higher risk of sharing bacteria through contaminated food matter and suboptimal hygiene conditions, there is a need to understand the dynamics of transmission of resistance in backyard farms. It is also worth noting that, in India, the prevalence of ESBL-producing *E. coli* in pigs was significantly higher in backyard farms than those in organized farms (Samanta et al., 2015a). We therefore investigate the occurrence and dissemination of ESBL-producing *E. coli* in poultry, farmers, and environments in backyard poultry farms in Northern Thailand.

MATERIALS AND METHODS

Ethics

Ethical approval was obtained from Naresuan University Institutional Review Board (COA No. 83/2013). Written informed consent was obtained from farmers and their family members prior to participating in this study.

Backyard Poultry Farms and Sample Collections

This study was conducted in Phitsanulok province, Northern Thailand (Figure 1). Twenty-seven backyard chicken farms willingly participated in this study. Among these, 10 farms additionally raised duck. Samplings were performed during August 2013 to April 2014. Fresh fecal samples from chicken and duck were randomly collected using a sterile spoon and container. Only fecal materials that had not contacted with soil surface were taken. Fecal swabs from consenting farmers and family members were obtained by subjects using sterile Amies swab (Deltalab, Barcelona, Spain). Environmental samples from farms; such as soil, sewage, and water samples, were randomly collected from different locations in each farm. Soil (30 g/site) and water (100 mL/site) were collected in sterile containers approximately 10 cm and 50 cm below the surface, respectively.

Overall, 587 samples were included in this study. Four hundred and sixty-seven fecal samples from poultry (426 chicken and 41 duck), 88 samples from environments within farms (54 soil and 34 water) were collected. In addition, 32 farmers and their family members from 21 farms agreed to provide fecal swabs for this study. All samples were transported to the laboratory and processed within 24 h.

Microbiological Analysis

Fecal swabs from farmers and their family members were inoculated on Eosin Methylene Blue (**EMB**) agar (Oxoid, Basingstoke, UK) supplemented with 2 μg/mL cefotaxime (**EMB-CTX**). For poultry feces, 1 g of fecal material was suspended in 9 mL Tryptic Soy Broth (**TSB**) (Oxoid) and incubated overnight at 37°C. Subsequently, the resulting growth was streaked onto EMB-CTX agar. For environmental samples, 1 gram of soil sample and 10 mL of water sample was added to 9 mL and 90 mL TSB, respectively, incubated overnight at 37°C and streaked on EMB-CTX agar as described above. All plates were incubated at 37°C under aerobic condition for 24 h.

One presumptive *E. coli* colony isolated from each sample was subcultured on Tryptic Soy Agar (Oxoid) and incubated at 37°C for 24 h for further characterizations. Species identification was performed by standard biochemical testing (Gram stain, growth on EMB agar, oxidase test, citrate test). Suspected colonies were identified by using RapID™ ONE System (REMEL Inc., KS, USA) according to the manufacturer's instruction. Phenotypic detection of ESBL production was performed using combination disk method with CTX and CTX/clavulanate or ceftazidime and ceftazidime/clavulanic acid disks according to the Clinical and Laboratory Standards Institute (**CLSI**) guidelines (CLSI, 2013).

Antimicrobial Susceptibility Test

Antimicrobial susceptibilities were performed against 16 antimicrobial agents by the disk diffusion method according to CLSI protocols and the results were interpreted according to CLSI criteria (CLSI, 2013). Nine ß-lactam antibiotics (ampicillin, cefazolin, cefuroxime, CTX, ceftazidime, cefpodoxime cefepime, imipenem, aztreonam), and 7 non-ß-lactam antibiotics (streptomycin, gentamicin, amikacin, doxycycline, ciprofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole) (Oxoid) were used.

Determination of blaCTX-M by PCR and Sequencing

Detection of $bla_{\text{CTX-M}}$ was performed by multiplex PCR using previously published primers and conditions (Woodford et al., 2006). PCR products were analyzed by agarose gel electrophoresis. Selected PCR products were purified using a DNA purification kit (RBC Bioscience, New Taipei City, Taiwan) and sent to a commercial facility for sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). Nucleotide 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).

Identification of $bla_{\text{CTX-M}}$ alleles in *E. coli* was performed by PCR using primers: $bla_{\text{CTX-M group 1}}$, 5′-ATGGTTAAAAAATCACTGCG-3′ and 5′-TTACAAACCGTCGGTGAC-3′ (876 bp); $bla_{\text{CTX-M group 9}}$, 5′-CAAAGAGAGTGCAACGGATG-3′ and 5′-TTACAGCCCTTCGGCGATGA-3′(866 bp). The PCR conditions were 5 min of initial denaturation at 94°C, followed by 30 cycles at 94°C for 1 min, 48°C for 45 s, and 72°C for 1 min s and a final extension at 72 at 94°C for 5 min. PCR products were purified, sequenced, and analyzed as described above.

Conjugation Experiments

The transferability of *bla*$_{CTX-M}$ was investigated by broth mating method using sodium azide-resistant *E. coli* J53 as a recipient. Briefly, 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 sodium azide (150 μg/mL) and CTX (2 μg/mL). Conjugation frequency was expressed as the number of transconjugants divided by the number of donor cells. Transfer of *bla*$_{CTX-M}$ was confirmed by PCR. Minimum Inhibitory Concentrations (**MICs**) of transconjugants were determined by broth microdilution method according to CLSI guidelines (CLSI, 2013). Plasmid incompatibility groups were determined by PCR-based replicon typing (**PBRT**) as previously described (Carattoli et al., 2005).

Pulsed Field Gel Electrophoresis (PFGE)

To study the genetic relationship of *bla*$_{CTX-M}$-positive ESBL-producing *E. coli*, PFGE was performed. Chromosomal DNA of *E. coli* in agarose plugs was prepared and digested with the restriction enzyme *Xba*I (Thermo Fisher Scientific, Waltham, MA, USA) as previously described (Kiddee et al., 2013). Plugs were then subjected to PFGE analysis in 1% agarose gel (Pulsed Field Certified™ agarose; Bio-Rad Laboratories, CA, USA) and 0.5X Tris-borate-EDTA buffer using a CHEF Mapper® XA System (Bio-Rad Laboratories, Hercules, CA, USA). The gels were run at 6.0 V/cm with an angle of 120 at 14°C for 20 h. *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad Laboratories) was used as a molecular size standard. Pulsed field gel electrophoresis profiles were analyzed by GelCompar II software Version 6.6 (Applied Maths, Kortrijk, Belgium). A dendrogram was constructed from the Dice coefficient and the unweighted pair-group method using arithmetic mean (**UPGMA**). Both the position tolerance and the optimization were set at 1%. Isolates were considered to be related and to belong to the same PFGE profile if their Dice similarity index was ≥ 85% (Carriço et al., 2005).

Phylogenetic Grouping and Multilocus Sequence Typing Analysis (MLST) Analysis

All ESBL-producing *E. coli* isolates were assigned to phylogenetic groups A, B1, B2, and D by a multiplex PCR assay (Clermont et al., 2000). MLST of the isolates belonging to the B2 group was performed by amplification and sequencing of 7 house-keeping 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)

RESULTS

Isolation of ESBL-producing E. coli

Twenty-seven backyard poultry farms participated in this study (Figure 1). A total of 587 samples were collected (12 to 46 samples/farm). Overall, 172 CTX-resistant *E. coli* isolates were recovered and 159 isolates (27.1%) were presumptive ESBL-producers. Almost all farms yielded ESBL-producing *E. coli* with the exception of farms 21, 22, and 26 (Table 1 and Table S1). Among these, ESBL-producing *E. coli* was isolated from 50% (human), 25.9% poultry (24.9% chicken and 36.6% duck) of the fecal samples, and 25.0% of the environmental samples.

Antimicrobial Susceptibility Test

All ESBL-producing *E. coli* isolates were tested for susceptibilities to a wide range of antibiotics (Table 2). Of the 121 isolates from chicken and duck, all isolates showed resistance to ampicillin and 1st, 2nd, and 3rd generation cephalosporins such as cefazolin, cefuroxime, CTX, and cefpodoxime. Resistance to ceftazidime, cefepime, aztreonam, doxycycline, chloramphenicol, trimethoprim/sulfamethoxazole, and streptomycin was

common (49.6 to 75.2%). Resistance to ciprofloxacin and gentamicin were found at low frequencies (28.9 to 43.0%). A large number of isolates (>90%) remained susceptible to amikacin and none of isolates were resistant to imipenem. The resistant rates of ESBL-producing *E. coli* isolates recovered from environmental samples and farmers demonstrated similar results. Furthermore, as many as 61.6% of isolates showed resistant to at least 10 antimicrobial agents tested (Table 2).

Detection of blaCTX-M and Their Transferability

Among 159 ESBL-producing *E. coli*, 152 isolates were positive for $bla_{\text{CTX-M}}$. Of these, $bla_{\text{CTX-M-group 1}}$ and $bla_{\text{CTX-M-group 9}}$ were found in 62.9% (100/159) and 32.7% (52/159) of isolates, respectively (Table 1). Sequence analysis revealed that the most common $bla_{\text{CTX-M-group 1}}$ were $bla_{\text{CTX-M-55}}$ (54.1%) followed by $bla_{\text{CTX-M-15}}$ (8.8%). $bla_{\text{CTX-M-14}}$ was predominant (28.3%) among $bla_{\text{CTX-M-group 9}}$-carrying isolates.. In addition, $bla_{\text{CTX-M-27}}$ (3.8%) and $bla_{\text{CTX-M-65}}$ (0.6%) were detected at low frequencies.

Conjugation experiments were performed with randomly selected *E. coli* containing $bla_{\text{CTX-M-55}}$ (n = 12) and $bla_{\text{CTX-M-14}}$ (n = 8) as donors. Nineteen of the 20 isolates carrying $bla_{\text{CTX-M}}$ genes were able to transfer their genes to the recipient, *E. coli* J53, with the conjugation frequencies of 8.33×10^{-7} to 7.0×10^{-2} and 4.50×10^{-7} to 8.15×10^{-2} for $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-14}}$, respectively (Table S2). A single isolate was not able to transfer $bla_{\text{CTX-M-55}}$. The presence of $bla_{\text{CTX-M}}$ in transconjugants was confirmed by PCR. The CTX MICs were > 8-fold and > 16-fold increase in transconjugants carrying $bla_{\text{CTX-M-55}}$ (MICs = 1 to > 8 μg/mL) and $bla_{\text{CTX-M-14}}$ (MICs = 2 to > 8 μg/mL), respectively, compared with the recipient *E. coli* J53 (MICs < 0.125 μg/mL). Some transconjugants showed more than 128-fold increase in CTX MICs (MICs > 8 μg/mL) compared with the recipient strain (Table S2).

Sixteen transconjugants were successfully typed by PBRT. Various replicon types, including IncF (n = 3), IncY (n = 1), IncX (n = 2), IncHI1 (n = 1), IncFIA (n = 2), IncI1 (n = 1), were found in the plasmids carrying the $bla_{\text{CTX-M-55}}$. The plasmid incompatibility groups IncF (n = 1), IncFIA (n = 2), IncFIB (n = 2), and IncI1(n = 1) were found in the plasmids carrying the $bla_{\text{CTX-M-14}}$ (Table S2).

Pulsed Field Gel Electrophoresis (PFGE)

Genetic relationship among ESBL-producing *E. coli* isolates carrying *bla*CTX-M was investigated by PFGE. Eighty-five and 47 isolates of *bla*CTX-M group 1- and *bla*CTX-M group 9-positive ESBL-producing *E. coli*, respectively, were available for analysis. The majority of isolates showed unique and unrelated PFGE profiles (Figure 2 and Figure 3). However, identical or closely related PFGE profiles were common among isolates, obtaining from poultry and environments, within the same farm, i.e., *bla*CTX-M-55-positive isolates in farms 1, 2, 3, 6, 7, 8, 9, 10, 11, 13, 14, and *bla*CTX-M-14-positive isolates in farms 1 and 12. In contrast, only 3 cases of genetic similarity of isolates obtained from different farms was noted, i.e *bla*CTX-M-55-positive isolates from farm 1 (farmer isolate) and farm 5 (soil isolate) (Figure 2) and *bla*CTX-M-14-positive chicken isolates from farms 10 and 24 (Figure 3). Interestingly, the similar PFGE profiles of the *bla*CTX-M-27-positive isolates from farm 15 (chicken isolate) and farm 16 (farmer isolate) were observed.

Phylogenetic Grouping and MLST Analysis

Phylogenetic analysis of the 159 ESBL-producing *E. coli* isolates revealed that most isolates belonged to group A (59.8%), followed by group B1 (23.9%), D (11.3%), and B2 (5.0%). MLST analysis of the 8 isolates belonging to B2 group showed the presence of ST131 (n = 4), ST95 (n = 1), ST219 (n = 1), ST127 (n = 1). One isolate was untypeable by MLST

scheme. Identification of *bla*$_{CTX-M}$ alleles in these isolates revealed that all 4 ST131 isolates possessed *bla*$_{CTX-M-27}$. ST95 and ST219 carried *bla*$_{CTX-M-14}$ and ST127 carried *bla*$_{CTX-M-15}$.

DISCUSSION

Whilst there are many studies on large poultry farms, few studies have been conducted regarding the occurrence of ESBL-producing *E. coli* from local backyard poultry farms which are common not only in Thailand but throughout Southeast Asia. A recent report from Vietnam showed that the prevalence of ESBL-producing *E. coli* among backyard chicken farms was 42.6% (Ueda et al., 2015). In contrast, similar studies from India and Finland showed no ESBL-positive *E. coli* (Samanta et al., 2015b; Pohjola et al., 2016). Our study also found similar findings to those of Vietnam; however, the overall ESBL-producing *E. coli* isolates from poultry detected was lower (25.9%). Our results on the prevalence of ESBL-producing *E. coli* in backyard poultry is less than that previously reported from commercial broiler farm in Thailand (ca. 40%) (Boonyasiri et al., 2014). This may be due to the use of antibiotics for prophylaxis in commercial farm (Wongsuvan et al., 2017). We noted that the prevalence of ESBL-producing *E. coli* from duck (36.6%) is higher than that from chicken (24.9%) although this is not statistically significant ($P > 0.05$, two proportion Z-test). These results were similar to those reported previously from China where MDR *E. coli* isolates were more common in duck than those in chicken (Yassin et al., 2017). The reason for this is not known. However, as a waterfowl, ducks usually excrete their feces directly into water reservoir, thereby enhancing the rapid spread of ESBL-producing *E. coli* within the duck populations. Prevalence of ESBL-producing *E. coli* from feces of farmers and their family members was 50%, which is consistent with those reported from healthy volunteers in this area and other parts of Thailand (Luvsansharav et al., 2011; Niumsup et al., 2018). In addition, we obtained 13 CTX-resistant *E. coli* isolates, which all tested negative for ESBL production (Table S1). Mechanism of CTX resistance in these isolates may be due to the presence of other enzymes, i.e., AmpC ß-lactamase or inhibitor-resistant ß-lactamase, or porin alteration (Ruppé et al., 2015). With regard to the antimicrobial susceptibility testing, whilst about half or more than half of all isolates were resistant to extended-spectrum ß-lactams (ceftazidime, cefepime, aztreonam), resistance to ciprofloxacin and amikacin was not common (Table 2). However, all isolates were classified as MDR (Falagas and Karageorgopoulos, 2008), consistent with another study in the neighboring country, Vietnam, where the high prevalence of MDR *E. coli* (> 80%) in backyard chicken was observed (Nguyen et al., 2015).

Molecular analysis of the genetic determinants associated with ESBL production was performed focusing on the presence of *bla*$_{CTX-M}$ since *bla*$_{CTX-M}$ types are highly endemic in Thailand (Kiratisin et al., 2008; Runcharoen et al., 2017). Our data showed that *bla*$_{CTX-M-55}$ (54.1%) is the most prevalent followed by *bla*$_{CTX-M-14}$ (28.3%) and *bla*$_{CTX-M-15}$ (8.8%). These results were in agreement with the fact that *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ were commonly found in clinical specimens and environments in Thailand (Kiratisin et al., 2008; Assawatheptawee et al., 2017; Runcharoen et al., 2017). *bla*$_{CTX-M}$-positive *E. coli* was also common in backyard chickens and pigs in other Asian countries such as Vietnam, China, and India (Ueda et al., 2015; Samanta et al., 2015a; Li et al., 2016). No *bla*$_{CTX-M}$ was found in 7 isolates of ESBL-producing *E. coli*. It is possible that other ESBL-genes that were not investigated in this study may be responsible for the ESBL production in these isolates (Ruppé et al., 2015).

Previous studies have shown the clonal transmission of ESBL-producing *E. coli* among poultry within the same farms (Ma et al., 2012; Kameyama et al., 2013). In addition, similar genotypes of ESBL-genes and plasmids in *E. coli* obtained from food-producing animals (chickens, pigs, and calves) and humans within and between farms have been documented (Huijbers et al., 2014;

Börjesson et al., 2016). In our study, PFGE analysis showed the high genetic diversity among ESBL-producing *E. coli* isolates, suggesting that the presence of these isolates in backyard poultry farms was not necessary due to the spread of a single clone (Figure 2 and Figure 3). These results suggest that horizontal gene transfer may play an important role in the transmission of the *bla*$_{CTX-M}$ among ESBL-producing isolates in backyard farms, which was supported by the finding that *bla*$_{CTX-M}$ were readily transferable to *E. coli* J53 (Table S2). Plasmid replicon typing demonstrated that *bla*$_{CTX-M}$ were associated with plasmids belonging to different replicon types, i.e., F, FIA, FIB, Y, X, HI1, I1. Among these, IncF replicons (including FIA and FIB) were the most frequently detected (n = 10), consistent with those of plasmid carrying *bla*$_{CTX-M}$ previously reported from Enterobacteriaceae (Carattoli, 2009). Nonetheless, *E. coli* isolates with similar PFGE profiles (≥85% similarity) were found within a single farm and between farms suggesting that clonal transmission of ESBL-producing *E. coli* isolates has also occurred, both among poultry and between poultry and environments (Figure 2 and Figure 3). However, the spread between farms is rare. For instance, we found the identical PFGE profiles of *bla*$_{CTX-M-14}$-positive *E. coli* from farms 10 and 24 (Figure 3), which are approximately 98 km away from each other. We observed only 1 instance of a commonality of a single clone from chicken in farm 15 and farmer in farm 16 (Figure 3), which are located within a 1-km radius of each other (Figure 1). The latter results are in agreement with previous study from Vietnam which suggested that clonal transmission of *bla*$_{CTX-M}$-positive *E. coli* between human and chicken in backyard farm was not common (Ueda et al., 2015).

E. coli isolates belonging to the phylogenetic group B2 are considered as highly virulent human pathogen. Of the B2 group, *E. coli* ST131 is an extraintestinal pathogenic *E. coli* (ExPEC) that commonly caused community-acquired infections in different countries (Banerjee et al., 2013; Ciesielczuk et al., 2016) and ST131 is now distributed in animals and environments worldwide (Nicolas-Chanoine et al., 2014). Recent studies in Thailand have identified ST131 in natural water resources, human clinical specimen, healthy population, and chicken meat (Netikul et al., 2014; Assawatheptawee et al., 2017; Niumsup et al., 2018; Tansawai et al., 2018). In the present study, 4 isolates, from different farms, were identified as *bla*$_{CTX-M-27}$-positive *E. coli* B2-ST131 (2, 1, and 1 isolate from chicken, human and soil, respectively) (Figure 3). It is worrying to note that *bla*$_{CTX-M-27}$-positive ST131 isolates from farmer and chicken, obtaining from the same area, showed closely related PFGE profile (91.9% similarity), suggesting that these isolates may originate from the same clone. These results suggest the dissemination of ExPEC between human and chicken.

In conclusion, our results showed the presence of ESBL-producing *E. coli* in backyard poultry farms in Thailand, emphasizing the importance of the household farming as potential sources of MDR *E. coli*. Clonal spread of these isolates within the same farm was demonstrated yet transmission of ESBL-producing *E. coli* from poultry to humans was rare. The presence of genetically-related *E. coli* ST131 between farmer and chicken is noteworthy, providing the evidence that transfer of MDR ExPEC between human and poultry may occur. Since ExPEC isolates belonging to pathogenic group B2 are of public health threat due to the fact that they contain many virulent traits, the occurrence of these isolates should be regularly monitored. Additionally, appropriate hygiene practices should be implemented to limit the dissemination of the MDR organisms within the community.

Table 1
Distribution of ESBL-producing *E coli* isolates, the presence of *bla*$_{CTX-M}$ and phylogenetic group from 27 backyard poultry farms

Samples	*la*$_{CTX-M\ group\ 1}$ (%)	*bla*$_{CTX-M\ group\ 9}$ (%)						Phylogenetic groups (n)			
	ESBL-producing *E. coli* (%)	*bla*$_{CTX-M-15}$	*bla*$_{CTX-M-55}$	*bla*$_{CTX-M-14}$	*bla*$_{CTX-M-27}$	*bla*$_{CTX-M-65}$	No *bla*$_{CTX-M}$	A	B1	B2	D
Farmers (n = 32)	16 (50.0)	4	4	5	2	0	1	9	2	3	2
Poultry samples											
– chicken feces (n = 426)	106 (24.9)	5	64	30	2	1	4	62	30	3	11
– duck feces (n = 41)	15 (36.6)	2	7	6	0	0	0	9	2	0	4
subtotal (n = 467)	121 (25.9)	7	71	36	2	1	4	71	32	3	15
Environmental samples											
– soil (n = 54)	9 (16.7)	1	5	1	1	0	1	6	1	2	0
– water (n = 34)	13 (38.2)	2	6	3	1	0	1	9	3	0	1
subtotal (n = 88)	22 (25.0)	3	11	4	2	0	2	15	4	2	1
Total (n = 587)	159 (27.1)	14 (8.8)	86 (54.1)	45 (28.3)	6 (3.8)	1 (0.6)	7 (4.4)	95	38	8	18

Table 2
Antimicrobial-resistant rates of ESBL-producing *E coli* recovered from 27 backyard poultry farms (n=159)

		No. of antimicrobial-resistant isolate (%)			
Antimicrobial agents		**Farmers (n = 16)**	**Poultry (n = 121)**	**Environments (n = 22)**	**Total (n = 159)**
β-lactams					
	ampicillin	16 (100)	121 (100)	22 (100)	159 (100)
	cefazolin	16 (100)	121 (100)	22 (100)	159 (100)
	cefuroxime	16 (100)	121 (100)	22 (100)	159 (100)
	cefotaxime	16 (100)	121 (100)	22 (100)	159 (100)
	cefpodoxime	16 (100)	121 (100)	22 (100)	159 (100)
	ceftazidime	9 (56.2)	60 (49.6)	14 (63.6)	83 (52.2)
	cefepime	11 (68.8)	67 (55.4)	12 (54.5)	90 (56.6)
	aztreonam	12 (75.0)	91 (75.2)	19 (86.4)	122 (76.7)
	imipenem	0	0	0	0
Non-β-lactams					
	streptomycin	12 (75.0)	91 (75.2)	14 (63.6)	117 (73.6)
	gentamicin	6 (37.5)	52 (43.0)	7 (31.8)	65 (40.9)
	amikacin	1 (6.3)	8 (6.6)	1 (4.5)	10 (6.3)
	doxycycline	7 (43.8)	74 (61.2)	12 (54.5)	93 (58.5)
	ciprofloxacin	5 (31.3)	35 (28.9)	7 (31.8)	47 (29.6)
	chloramphenicol	10 (62.5)	73 (60.3)	11 (50.0)	94 (59.1)
	trimethoprim–sulfamethoxazole	12 (75.0)	78 (64.5)	10 (45.5)	100 (62.9)
	No. of antibiotics to which isolates are resistant				
	≤ 8	4 (25.0)	23 (19.0)	6 (27.3)	33 (20.8)
	9	3 (18.7)	20 (16.5)	5 (22.7)	28 (17.6)
	≥ 10	9 (56.3)	78 (64.5)	11 (50)	98 (61.6)

Figure 1

Location of participating backyard poultry farms (n=27) in Phitsanulok province, Northern Thailand. The black area is Phitsanulok province and the grey areas is the sampling sites. *E coli* ST131-positive farms were marked with an asterisk(*)

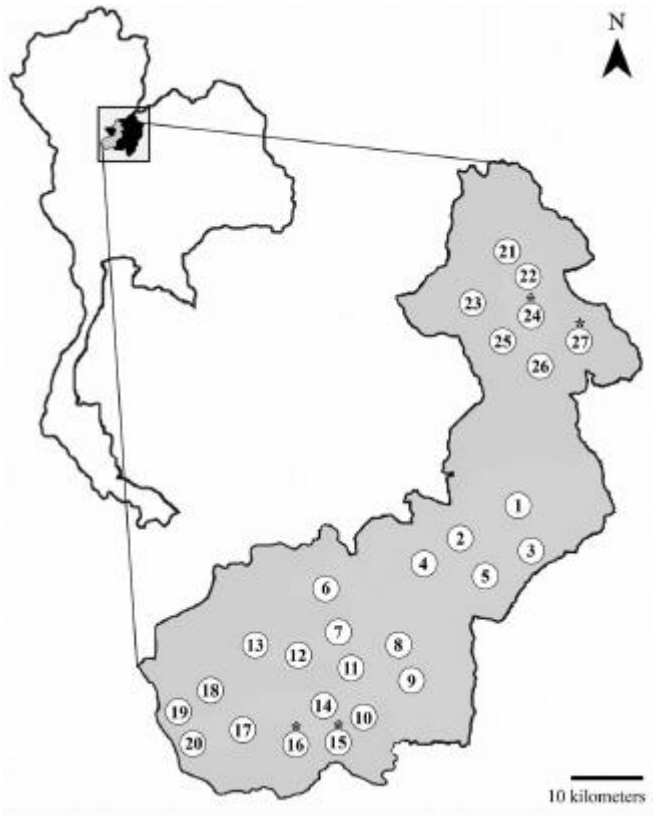


Figure 2

Dendrogram showing the genotypic relatedness of ESBL-producing *E. coli* carrying $bla_{\text{CTX-M-group 1}}$ from backyard poultry farms, farmers and environments based on the *Xba*I-PFGE. PFGE profiles were analyzed using the Dice coefficient and unweighted-pair group method using average linkages (UPGMA) (GelCompar II software Version 6.6). The scale bar represents the percentage of similarity and the vertical dotted lines indicates ≥85% similarity. Identical or closely related isolates are boxed.

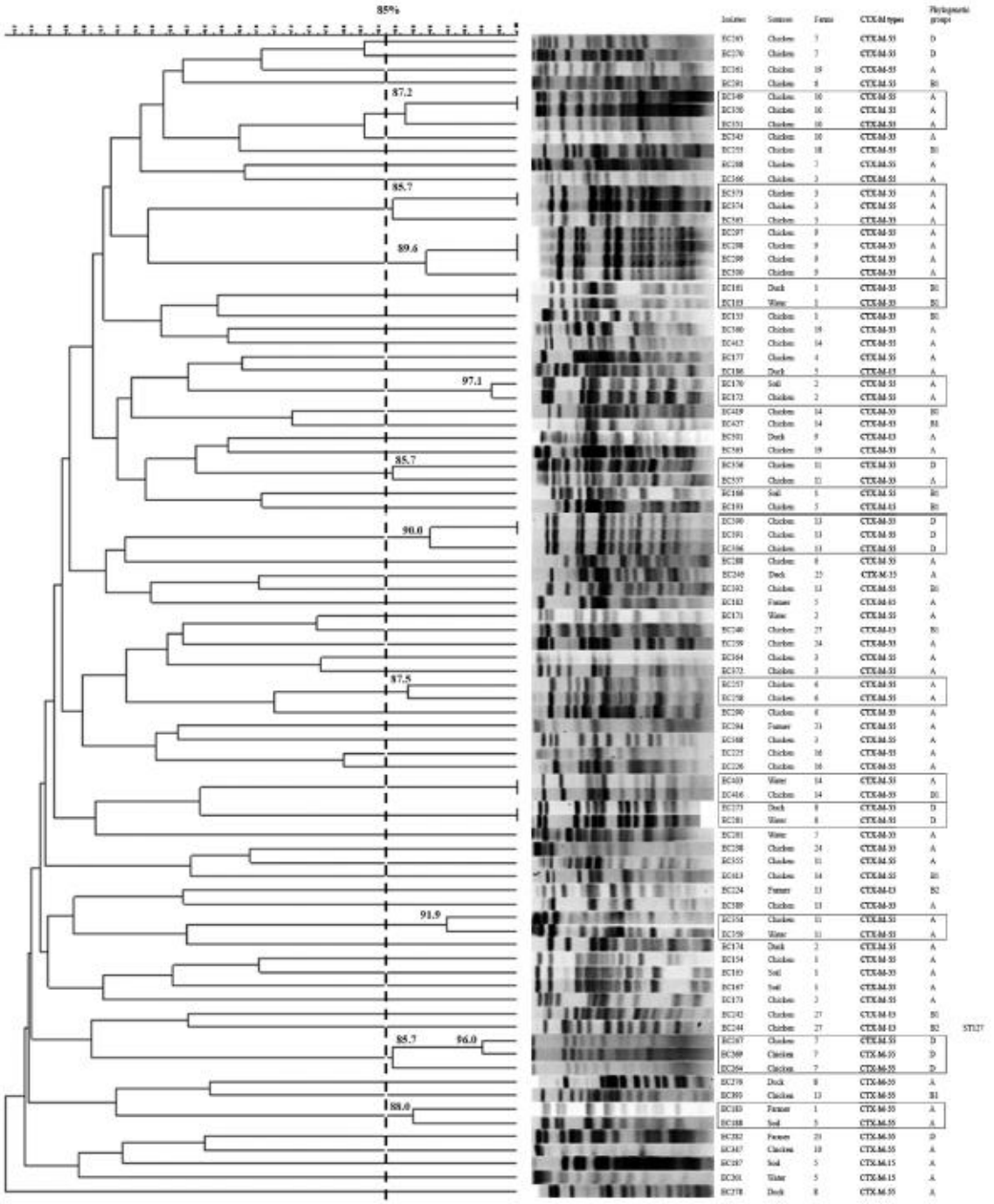
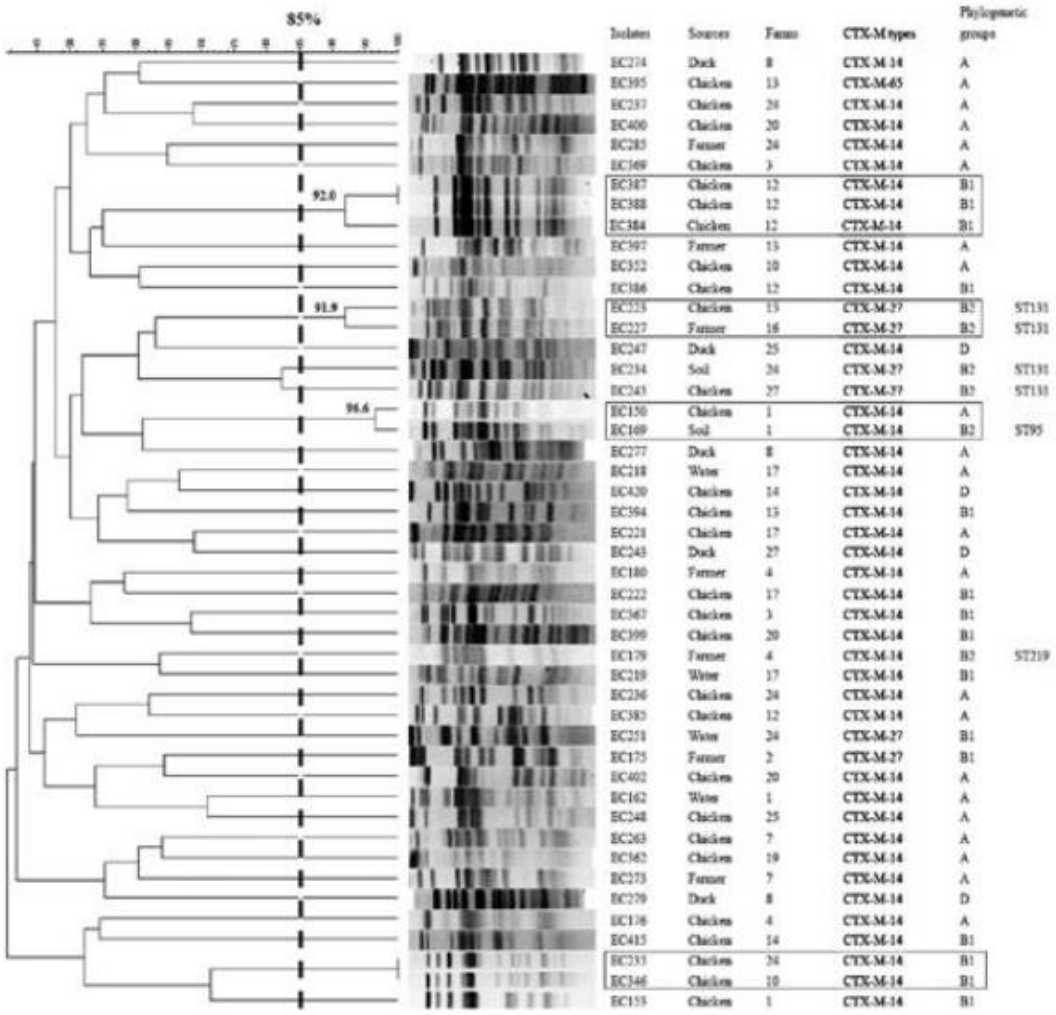


Figure 3

Dendrogram showing the genotypic relatedness of ESBL-producing *E. coli* carrying $bla_{\text{CTX-M-group 9}}$ from backyard poultry farms, farmers, and environments based on the *Xba*I-PFGE. PFGE profiles were analyzed using the Dice coefficient and unweighted-pair group method using average linkages (UPGMA) (GelCompar II software Version 6.6). The scale bar represents the percentage of similarity and the vertical dotted lines indicate ≥85% similarity. Identical or closely related isolates are boxed.



SUPPLEMENTARY DATA

Table S1. Prevalence of cefotaxime-resistant *Escherichia coli* (CTX-EC) and ESBL-producing *E. coli* (ESBL-EC) in backyard poultry farms in Phitsanulok Province, Northern Thailand.

	Samples (n)						No. of CTX-EC (%)	No. of ESBL-EC (%)
	Farmers	Poultry		Environments		Total		
		Chicken	Duck	Water	Soil			
1	3	17	3	5	7	35	14	13 (37.1)
2	1	10	3	2	5	21	6	6 (28.6)
3	1	24	0	0	0	25	12	12 (48.0)
4	3	6	0	2	3	14	5	5 (35.7)
5	3	3	3	3	3	15	11	11 (73.3)
6	2	27	0	2	1	32	8	6 (18.8)
7	1	11	0	2	3	14	12	10 (58.8)
8	0	0	14	0	1	15	8	8 (53.3)
9	1	19	4	0	1	25	6	6 (24.0)
10	0	24	0	0	1	25	8	7 (28.0)
11	0	20	0	1	1	22	6	5 (22.7)
12	1	30	0	1	2	34	5	5 (14.7)
13	1	41	0	2	2	46	9	9 (19.6)
14	1	31	0	3	0	35	17	15 (42.9)
15	1	13	0	1	1	16	2	2 (12.5)
16	1	15	0	2	3	21	3	3 (14.3)
17	0	9	0	2	1	12	4	4 (33.3)
18	1	23	0	1	2	27	7	7 (25.9)
19	2	19	0	0	0	21	4	4 (19.0)
20	1	17	0	2	1	21	5	3 (14.3)
21	1	8	0	1	4	14	0	0
22	1	13	0	0	2	16	0	0
23	2	8	2	1	2	15	2	2 (13.3)
24	2	8	0	1	2	13	8	8 (61.5)
25	0	6	5	0	2	13	3	3 (23.1)
26	2	12	4	0	2	20	1	0
27	0	12	3	0	2	17	6	5 (29.4)
Total	**32**	**426**	**41**	**34**	**54**	**587**	**172 (29.3)**	**159 (27.1)**

Table S2.
Conjugation frequency, transferred gene and cefotaxime MICs for *E. coli* J53 and the respective transconjugants.

Donors (Source)	Transconjugant	*bla*$_{CTX-M}$	Transfer frequency	cefotaxime MICs (μg/mL)	Plasmid replicon types
-	*E. coli* J53[a]	-	-	< 0.125	
E. coli 425 (wastewater)	Non-transferred	*bla*$_{CTX-M-55}$	-	-	-
E. coli 278 (duck)	EC278_Tc	*bla*$_{CTX-M-55}$	1.46×10^{-4}	2	F
E. coli 427 (chicken)	EC427_Tc	*bla*$_{CTX-M-55}$	4.8×10^{-5}	4	F
E. coli 393 (chicken)	EC393_Tc	*bla*$_{CTX-M-55}$	1.79×10^{-7}	4	Y
E. coli 252 (chicken)	EC252_Tc	*bla*$_{CTX-M-55}$	8.33×10^{-7}	4	X
E. coli 361 (chicken)	EC361_Tc	*bla*$_{CTX-M-55}$	6.67×10^{-7}	1	HI1
E. coli 379 (duck)	EC379_Tc	*bla*$_{CTX-M-55}$	1.13×10^{-3}	4	FIA
E. coli 401 (chicken)	EC401_Tc	*bla*$_{CTX-M-55}$	1.08×10^{-2}	8	I1
E. coli 291 (chicken)	EC291_Tc	*bla*$_{CTX-M-55}$	4.28×10^{-4}	8	FIA
E. coli 163 (wastewater	EC163_Tc	*bla*$_{CTX-M-55}$	6.4×10^{-2}	> 8	F
E. coli 173 (chicken)	EC173_Tc	*bla*$_{CTX-M-55}$	3.6×10^{-2}	> 8	untypeable
E. coli 174 (duck)	EC174_Tc	*bla*$_{CTX-M-55}$	2.5×10^{-4}	> 8	X
E. coli 397 (human)	EC397_Tc	*bla*$_{CTX-M-14}$	2.5×10^{-4}	> 8	FIB
E. coli 414 (chicken)	EC414_Tc	*bla*$_{CTX-M-14}$	4.5×10^{-7}	> 8	I1
E. coli 218 (wastewater)	EC218_Tc	*bla*$_{CTX-M-14}$	2.0×10^{-7}	2	FIA
E. coli 362 (chicken)	EC362_Tc	*bla*$_{CTX-M-14}$	1.63×10^{-4}	2	untypeable
E. coli 150 (chicken)	EC150_Tc (chicken)	*bla*$_{CTX-M-14}$	2.12×10^{-2}	2	F
E. coli 387 (chicken)	EC387_Tc (chicken)	*bla*$_{CTX-M-14}$	8.15×10^{-2}	4	FIB
E. coli 388 (chicken)	EC388_Tc (chicken)	*bla*$_{CTX-M-14}$	1.22×10^{-2}	2	FIA
E. coli 348 (chicken)	EC348_Tc (chicken)	*bla*$_{CTX-M-14}$	6.2×10^{-2}	8	untypeable

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