Original Article

Multidrug-resistant ESBL-producing *Escherichia coli* coexisting with colistin-resistance genes in pig farms, Central Thailand

Wipawee Songsaeng[1] Nutthee Am-in[2] Nuvee Prapasarakul[3] Wandee Sirichokchatchawan[1*]

Abstract

The presence of extended-spectrum beta-lactamase and colistin resistance *E. coli* in food-producing animals is a public health concern. This study was conducted using a rigorous methodology to survey the prevalence of ESBL-producing and colistin-resistant *E. coli* from pig farms in the central part of Thailand. A total of 519 samples were collected from fecal, feed, and waste in 53 farms located in central Thailand. All samples were inoculated onto selective agar, and species identification was performed using biochemical assays and MAlDI-TOF MS. Antimicrobial susceptibility was determined by broth microdilution, and ESBL production was confirmed by the combination disc diffusion method. Resistance genes were detected by PCR and confirmed by sequencing. Thirty-four isolates from fecal and waste samples were identified as ESBL-producing *E. coli*. All were considered multidrug-resistant, resisting to beta-lactams, aminoglycosides, quinolones, and colistin. The $bla_{\text{CTX-M}}$ Group 1 was detected in all isolates, with additional $bla_{\text{CTX-M}}$ Group 2 (74%) in some isolates. Furthermore, 94% of these ESBL-producing E.coli were found to coexist with *mcr-1* and/or *mcr-3*. In conclusion, this surveillance study found that although the prevalence of ESBL-producing *E. coli* is low in pig farms, the co-occurrence of $bla_{\text{CTX-M}}$ and *mcr-* genes is high among positive isolates. Therefore, the results indicated a chance that ESBL and colistin resistance genes may exist together in one bacterial isolate, raising threats in treatment for bacterial infections. Further analysis is needed to indicate the location and transferability of these genes in order to understand how to prevent the dissemination of these resistance pathogens in the future.

Keywords: multidrug resistance, pig farms, ESBL, colistin resistance, *E. coli*

[1]*College of Public Health Science, Chulalongkorn University, Bangkok, 10330, Thailand*
[2]*Department of Obstetrics Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand*
[3]*Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand*
****Correspondence****: wandee.s@chula.ac.th* (W. Sirichokchatchawan)
Received June 16, 2024
Accepted July 10, 2024

Introduction

Antimicrobial resistance (AMR), especially among bacterial pathogens with multidrug resistance (MDR), has emerged as one of the major urgent public health threats that result in high morbidity and mortality rates worldwide (Dadgostar, 2019). Resistance to last-resort antibiotics is of particular concern as it limits the treatment options available for serious infections in both humans and animals (Lay *et al.,* 2021). Therefore, bacteria resistant to beta-lactams (especially the new generation of cephalosporins), colistin, and carbapenems are considered high-risk pathogens as they may result in treatment complications or even make the treatments become impossible (Ben Sallem *et al.,* 2012).

E. coli are commensal bacteria in humans and animals. However, *E. coli* is a frequently implicated bacteria that can cause a wide range of diseases, including diarrhea, septicemia, blood infection, and urinary tract infection (Chotinantakul *et al.,* 2022). Since *E. coli* can acquire antimicrobial resistance genes through horizontal gene transfer, multidrug-resistant *E. coli* has been widely discovered. For example, ESBL-producing *E. coli* are capable of hydrolyzing numerous antibiotics, which leads to high impacts on patient outcomes as these multidrug ESBL-producing *E. coli* are associated with high mortality, longer length of hospital stay, and high cost (Runcharoen *et al.,* 2017).

The obstacles to effective treatment have further escalated following the emergence of multidrug-resistant *E. coli* with acquired resistance to carbapenems and/or colistin, the last resort antibiotics for treating multidrug-resistant infections (Nordmann *et al.,* 2011; Schwarz and Johnson, 2016). The coexistence of multidrug ESBL with colistin resistance (*MCR*-) or carbapenem resistance genes is of particular concern as this combination of resistance genes in *E. coli* makes infections harder to treat and limits the effectiveness of these antibiotics. The appearance of these resistance genes is largely due to the irrational use of antibiotics for preventive prophylactic and growth-promoting purposes, which also leads to the threats in the One Health approach that seek the well-being of humans, animals, and environments (Bastidas-Caldes *et al.,* 2023; Plata *et al.,* 2022).

This leads to another worrying phenomenon, which is the distribution of multidrug-resistant ESBL outside of hospital settings, such as the distribution in livestock and environments. The extensive and inappropriate use of beta-lactams and colistin in animal production has triggered a natural selection of ESBL, colistin- and carbapenem-resistance (Gupta *et al.,* 2021). The resistant pathogens may remain in food animals or environments and potentially transmit to humans via direct or indirect contact. However, the transmission of bacteria with antimicrobial resistance is difficult to demonstrate since its potential reservoirs and transmission routes are so diverse (Almansour *et al.,* 2023).

Although, recently there have been more reports indicating the coexistence of ESBL and *mcr*- genes among livestock in Thailand, the cause of resistance dissemination, including the antimicrobials used on farms, has not been clearly defined (Khine *et al.,* 2020; Sudatip *et al.,* 2023). In fact, the knowledge of resistance to new generation beta-lactams and colistin in bacteria from livestock and products remains largely unexplored in most regions of Thailand, especially the central regions with the highest number of pig productions (Lay *et al.,* 2021). The objective of this study was to find the prevalence of ESBL-producing *E. coli* among pig farms in the central part of Thailand and examine the genetic characteristics and coexistence with other resistant genes of these isolates.

Materials and Methods

Before the sample collection process, the ethical approval was done by the Faculty of Veterinary Science, Chulalongkorn University (approval No. 2031041). Samples were collected between May and September 2021 from 53 medium-sized farms located in Ratchaburi province, central Thailand. Ratchaburi province was selected as a representative of the central part of Thailand as it is among one of the provinces with the highest numbers of pig production in Thailand. In each farm, up to 5 pooled feces, 5 feed tray samples, and one or two waste samples were collected. For feces samples, approximately 3-5 grams of pig feces were collected from the pen floor of different pig housings by using a transport media swab. Approximately 5-10 grams of pig feed samples were collected directly from the tray in different housings and put into a Ziplock bag. Finally, the waste samples from the septic tank were collected in a sterile tube and put in a plastic bag. After the sample collection processes, all samples were put into an icebox and taken to the laboratory for analysis within 24 hours.

All samples, including fecal swabs, feed samples, and farms' waste, were enriched in trypticase soy broth (TSB) in the ratio of 1:9 (sample to enrichment broth) and incubated overnight at 37°C. After the enrichment process, one loopful of each enrichment culture was inoculated onto chromogenic Brilliance ESBL agar and Brilliance CRE agar (Oxoid Hampshire, United Kingdom) to select ESBL and carbapenemase producers. Following that, the agar plates were incubated at 37°C for 24 hours under aerobic conditions (Zurfluh *et al.,* 2013). Once the incubation time was over, the growth on agar plates with different colors and morphologies was chosen for bacteria species identification. The confirmation of *E.coli* was accomplished by using biochemical assays, including methyl red, Voges-Proskauer, indole, Simmons' citrate, and motility assay. The ambiguous isolates were further identified using matrix-assisted laser desorption ionization-time of flight of mass spectrometry (MALDI-TOF-MS) (Mollenkopf *et al.,* 2017).

The minimal inhibitory concentration (MIC) of antimicrobial agents against the *E. coli* isolates was determined using the broth microdilution method, and ATCC 25922 was used as a control strain. The antibiotic tested were included doripenem (DOR), imipenem (IPM), meropenem (MEM), ertapenem (ETP), amikacin (AMK), amoxicillin-clavulanic acid (AMC), ampicillin (AMP), cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), cefotaxime (CTX), ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), colistin (CST),

ciprofloxacin (CIP), ceftriaxone (CRO), levofloxacin (LVX), gentamicin (GEN), netilmicin (NET), and trimethoprim-sulfamethoxazole (SXT). The results were interpreted according to the cutoff value from CLSI guideline 2015 (CLSI, 2015). Isolates displaying resistance to three or more classes of antibiotics were defined as multidrug resistance.

In addition, isolates resistant to at least one of the indicators of cephalosporins were subjected to confirm ESBL production with ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (10 µg) (Oxoid). The difference of ≥5 mm between the inhibition zone of the cephalosporin/ clavulanic acid combination and the corresponding cephalosporin disks alone was interpreted as positive ESBL (CLSI, 2022).

The isolates with positive ESBL were assessed for genotype by using previously reported polymerase chain reaction (PCR) assays and Sanger sequencing. The PCR primers for ESBL, carbapenem- and colistin-resistance, along with the amplification cycles of each set of primers, were indicated in Table 1 (Poirel *et al.*, 2011; Rebelo *et al.*, 2018; Xu *et al.*, 2005). Once the cycles were completed, the PCR products were analyzed by electrophoresis with 2% agarose gels at 100 V for 1 hour in 1x TAE. The gel was stained with SYRB Safe DNA gel stain, and the PCR product was visualized with UV light (Doyle *et al.*, 2012; Subramanya *et al.*, 2021). In addition, the selected PCR products from isolates carrying different ESBL and *mcr*- genes were extracted from the gel and purified by using NucleoSpin Gel and PCR Clean-up, Mini Kit (Macherey-Nagel, Germany) and sent for Sanger sequencing to confirm the presence of the genes.

Result

In this study, 519 samples were systematically collected to assess the incidence of ESBL, carbapenem-, and colistin-resistant bacteria. The samples included 264 fecal samples, 159 feed samples from feeding trays, and 96 waste samples from septic tanks. After the screening processes, 34 isolates were identified as ESBL-producing *E. coli* (6.6%). The majority of these ESBL-producing *E. coli* isolates were retrieved from fecal samples (26/34), followed by waste samples (8/34) (Fig.1).

All 34 *E. coli* were classified as ESBL producers according to the disk-diffusion test. The antimicrobial susceptibility test also indicated that these *E. coli* isolates were resistant to at least one beta-lactam, especially 3rd generation cephalosporin such as cefotaxime. All isolates were also resistant to colistin. None of the isolates were resistant to carbapenems. In addition, all *E. coli* isolates were found to be multidrug-resistant, giving resistance to three or more groups of antibiotics, including beta-lactams, aminoglycosides, quinolones, and colistin (Fig. 2).

Furthermore, the PCR results indicated that all 34 isolates carried $bla_{\text{CTX-M}}$ Group 1, with the majority also carrying additional $bla_{\text{CTX-M}}$ Group 2 (74%). In addition, 32 isolates (94%) revealed the coexistence of ESBL with colistin-resistance (*mcr*- genes), including 16 isolates with *mcr-1*, 9 isolates with *mcr-3*, and 7 isolates with both *mcr-1* and *mcr-3*. None of the *E. coli* samples were found to carry carbapenemase genes. In this study, the Sanger sequence was used to validate the resistance genes previously detected by the PCR primers; therefore, a few isolates carrying different sets of genes were submitted for sequencing. The results from the Sanger sequence were matched with the PCR results of ESBL and *mcr*- genes. The details of resistance genes detected in each isolate are indicated in Supplementary Table 1.

Discussion

The detection of ESBL and colistin-resistant bacteria in pig farms and the environment is worrisome due to its implications for One Health. Both ESBL- and colistin-resistant *E. coli* represent a public health hazard as they reduce and limit the therapeutic options for the treatment of infections, making the treatment impossible. We detected that ESBL resistance genes in this study were relatively low (6.6%) when compared to the other studies in Phayao province (58.8%), northern Thailand provinces (61.0%), and central Thailand (36.7%) (Nuangmek *et al.*, 2018; Nuanmuang *et al.*, 2018; Sudatip *et al.*, 2023). However, even the low detection of resistance genes in food-producing animal production and the surrounding farm environments can raise significant concerns as these resistant bacteria could infect healthy populations in the community.

For example, several studies indicated that direct contact with pigs or contaminated environments is considered a risk factor for human ESBL-producing *E. coli* carriage through occupational and environmental exposure or consumption of contaminated meat (Dohmen *et al.*, 2017a; Nuanmuang *et al.*, 2018). With this, many studies also specified the potential transmission events between these reservoirs based on genetic relatedness and epidemiological associations by suggesting the possibility of clonal and horizontal dissemination of ESBL-producing *E. coli* between pigs, pig farmers, the farm environments, and humans in the community (Dohmen *et al.*, 2023; Dohmen *et al.*, 2017b; Hammerum *et al.*, 2014). To better understand the transmission patterns, further studies are needed to identify genetic characteristics, the location of resistance genes, and the genetic relatedness of isolates retrieved from this study compared with isolates from humans, animals, and environments previously detected in Thailand.

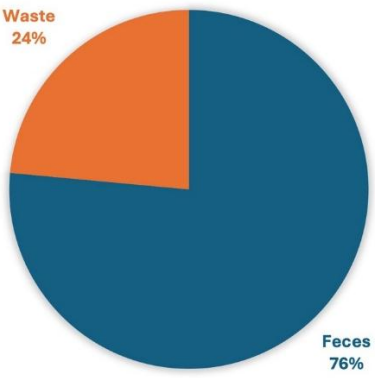

Figure 1 ESBL positive isolates retrieved from different sources in the pig farms.

Table 1 Primer sequence and PCR amplification cycles of each antimicrobial resistance gene.

Template	Size (bp)	Sequence (5′-3′)	Reference	PCR amplification cycles
bla_{KPC}	798	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	(Poirel *et al.,* 2011)	Initial denaturation at 95°C for 5 min; 30 cycles of DNA denaturation at 95°C for 30 s; annealing at 55°C for 40 s; elongation at 72°C for 3 min; and final elongation at 72°C for 10 min (Poirel *et al.,* 2011).
bla_{IMP}	232	GGAATAGAGTGGCTTAAYTCTC GGTTTAAYAAAACAACCACC	(Poirel *et al.,* 2011)	
bla_{VIM}	390	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	(Poirel *et al.,* 2011)	
bla_{NDM}	621	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	(Poirel *et al.,* 2011)	
bla_{Oxa-48}	438	GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	(Poirel *et al.,* 2011)	
bla_{SPM}	271	AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG	(Poirel *et al.,* 2011)	
bla_{AIM}	322	CTGAAGGTGTACGGAAACAC GTTCGGCCACCTCGAATTG	(Poirel *et al.,* 2011)	
bla_{BIG}	537	TATGCAGCTCCTTTAAGGGC ATCATTGGCGGTGCCGTACAC	(Poirel *et al.,* 2011)	
bla_{GIM}	477	TCGACACACCTTGGTCTGAA AACTCCAACTTTGCCATGC	(Poirel *et al.,* 2011)	
bla_{SIM}	570	TACAAGGGATTCGGCATCG TAATGGCCTGTTCCCATGTG	(Poirel *et al.,* 2011)	
$bla_{CTX\text{-}group\ 1}$	260	GCGTGATACCACTTCACCTC TGAAGTAAGTGACCAGAATC	(Xu *et al.,* 2005)	Initial denaturation at 95 °C for 2 min; 30 cycles of DNA denaturation at 95 °C for 1 min; annealing at 55 °C for 1 min; elongation at 72 °C for 1 min, and final elongation at 72 °C for 10 mins (Xu *et al.,* 2005)
$bla_{CTX\text{-}group\ 2}$	341	TGATACCACCACGCCGCTC TATTGCATCAGAAACCGTGGG	(Xu *et al.,* 2005)	
$bla_{CTX\text{-}group\ 8}$	207	CAATCTGACGTTGGGCAATG ATAACCGTCGGTGACAATT	(Xu *et al.,* 2005)	
$bla_{CTX\text{-}group\ 9}$	293	ATCAAGCCTGCCGATCTGGTTA GTAAGCTGACGCAACGTCTGC	(Xu *et al.,* 2005)	
mcr-1	320	AGTCCGTTTGTTCTTGTGGC AGATCCTTGGTCTGGCTTG	(Robelo *et al.,* 2018)	Initial denaturation at 94 °C for 15 min; 25 cycles of DNA denaturation at 94 °C for 30 s; annealing at 58 °C for 90 s; elongation at 72 °C for 60 s; and final elongation at 72 °C for 10 min (Rebelo *et al.,* 2018).
mcr-2	715	CAAGTGTGTTGGTCGCAGTT TCTAGCCCGACAAGCATACC	(Robelo *et al.,* 2018)	
mcr-3	929	AAATAAAAATTGTTCCGCTTATG AATGGAGATCCCCGTTTTT	(Robelo *et al.,* 2018)	
mcr-4	1,116	TCACTTTCATCACTGCGTTG TTGGTCCATGACTACCAATG	(Robelo *et al.,* 2018)	
mcr-5	1,644	ATGCGGTTGTCTGCATTTATC TCATTGTGGTTGTCCTTTTCTG	(Borowiak *et al.,* 2017)	

The majority of isolates in this study were multidrug resistant. Most ESBL productions in bacteria are associated with MDR and pose significant challenges in the treatment of infections. Similar to this study, the previous studies have identified multidrug-resistant ESBL, which confer resistance to a wide range of beta-lactam antibiotics, including penicillin and cephalosporins, as well as resistance to other classes of antibiotics beyond beta-lactam such as colistin, fluoroquinolones, aminoglycosides and sulfonamides (Bandy and Tantry, 2021; Shrestha *et al.,* 2017; Yadav *et al.,* 2015). Evidently, the inappropriate use of antibiotics both in humans and animals can contribute to elevated rates of AMR. Therefore, the resistance to beta-lactam antibiotics implies the challenge in managing diseases triggered by ESBL-producing *E. coli*. Furthermore, this resistance poses significant public health concerns, being linked to increased rates of illness and death as well as a reduced array of treatment options available (Srichumporn *et al.,* 2022).

In this study, the $bla_{CTX\text{-}M}$ group was predominantly detected. The worldwide studies, including the previous ones from Thailand, also reported the particular group of genes as predominant in humans, animal farms, and foods (Dohmen *et al.,* 2015; Dohmen *et al.,* 2017a; Hammerum *et al.,* 2014; Srichumporn *et al.,* 2022; Wu *et al.,* 2008). However, the other genes, such as bla_{SHV} or bla_{OXA}, which have also been previously detected in pig farms and environments (Kaleva *et al.,* 2023; Ye *et al.,* 2018), were not detected in this study. Evidently, the genotypic diversity of ESBL-producing *E. coli* varies across countries and regions, likely due to differences in antibiotic usage policies. Besides ESBL resistance genes, colistin-resistance genes (*mcr-1* and *mcr-3*) were also detected. However, no carbapenem-resistance gene was detected in this study. This may be due to the prohibition of carbapenem use in pig farms and the effective management to control the dissemination of this particular resistance gene.

Our results suggested that the majority of ESBL *E. coli* isolates were found to coexist with colistin resistance, *mcr-1,* and *mcr-3* genes. This coexistence of ESBL, specifically $bla_{CTX\text{-}M}$ group, and *mcr-* genes in *E. coli* have been reported globally, especially among sick patients in clinical settings (Chen *et al.,* 2019; Lü *et al.,* 2020; Wu *et al.,* 2018; Zhang *et al.,* 2022). However, recently, the co-occurrence of $bla_{CTX\text{-}M}$ and *mcr* was observed in various studies in food-producing animals and environments in Thailand. These studies included the detection of these pathogens in pig and poultry farms, pork meat, human workers in farms, and natural water sources (Chotinantakul *et al.,* 2022; Lay *et al.,* 2021; Sudatip *et al.,* 2023). Our findings also suggested that the majority of our isolates harbored both ESBL and colistin resistance genes, which indicated the high risk of disseminating the extensively

drug-resistant *E. coli*, posing a threat to public health. Additionally, the ESBL and colistin co-resistant strains may arise from selective pressure resulting from the use of antibiotics, including bata-lactams and polymyxins (Feng *et al*., 2023).

The increasing number of *E. coli* with the coexistence of ESBL and *mcr* genes in Thailand, especially in food-producing animals and environments, raises local public health concerns. The significant points regarding the coexistence of these resistance genes in *E. coli* include the treatment challenges, increased severity of infections, risk of transmission, and public health implications (concerns about potential outbreaks of MDR infections) (Larsson and Flach, 2022). Therefore, there is an urgent need for comprehensive strategies to combat these pathogens.

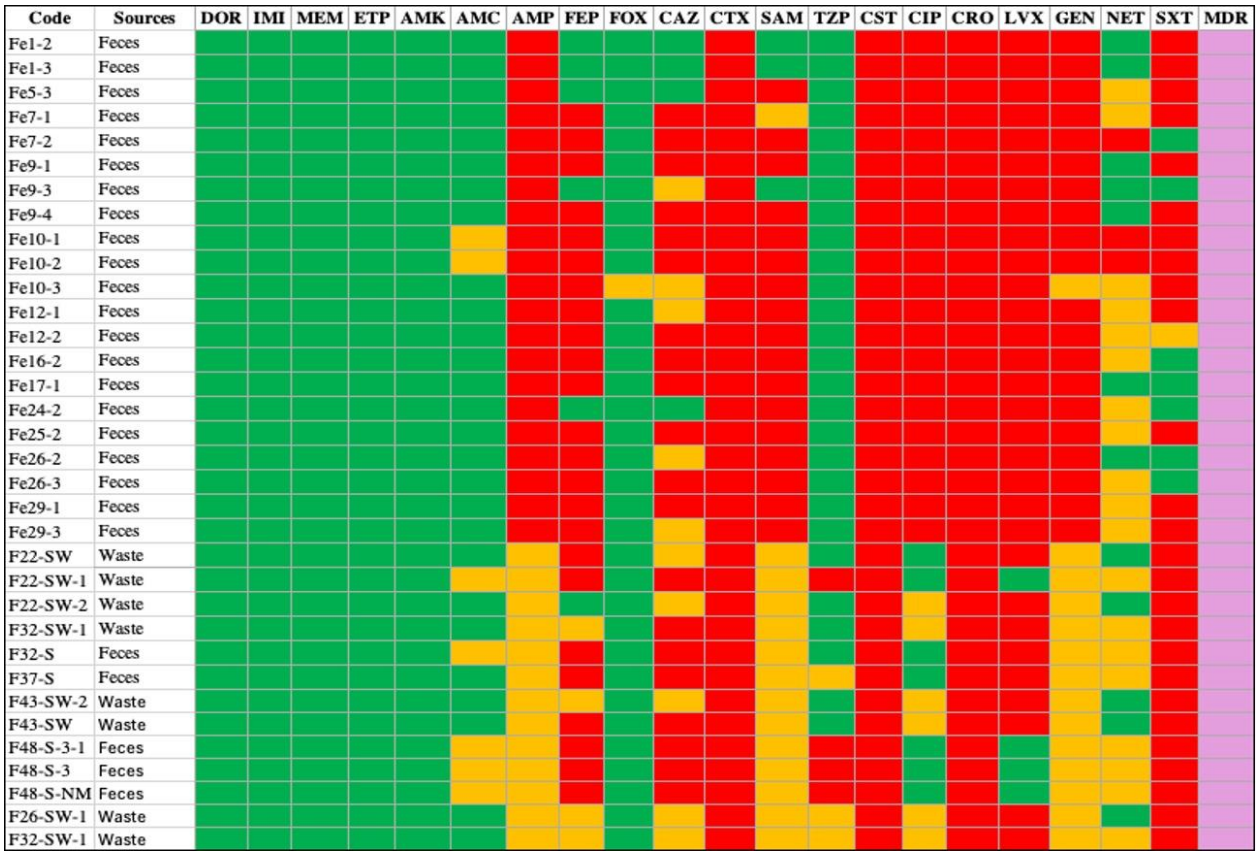

Figure 2 Antibiotic susceptibility profiles of ESBL-producing *E.coli* isolated from pig farms. Abbreviations: DOR- doripenem, IPM-imipenem, MEM-meropenem, ETP-ertapenem, AMK-amikacin, AMC-amoxicillin-clavulanic acid, AMP-ampicillin, FEP-cefepime, FOX-cefoxitin, CAZ-ceftazidime, CTX-cefotaxime, SAM-ampicillin-sulbactam, TZP-piperacillin-tazobactam, CST-colistin, CIP- ciprofloxacin, CRO-ceftriaxone, LVX-levofloxacin, GEN-gentamicin, NET-netilmicin, SXT-trimethoprim-sulfamethoxazole, MDR-multidrug-resistant. The color of squares categorizing antibiotic resistance profile: red is resistant; yellow is intermediate; green is susceptible; purple is multidrug resistance.

In conclusion, the detection of ESBL and colistin-resistant bacteria in pig farms underscores the importance of surveillance and monitoring in environmental and agricultural settings. The prevalence of these resistant bacteria raises concerns regarding the potential transmission of antibiotic resistance in humans, animals, or even the environment. More efforts should be focused on implementing strict regulations on antibiotic usage in livestock and developing better hygiene for farm workers. In addition, continued research surveillance is essential to better understand the dynamics of multidrug-resistant ESBL dissemination in animals' farms or surrounding environments. In our study, we only focused on the surveillance of initial screening for resistance genes using the PCR method; future studies should emphasize the analysis of detail in the genomic characteristics of the isolates as well as the transmission pattern of these genes.

Supplement Table 1 ESBL and *mcr- genes* detection for each isolate and the confirmation by Sanger sequencing for selected isolates.

No	Code	Species	β-lactam resistance genes (CTX-M)		Additional *mcr-* genes	Sources	Sanger Sequencing
1	Fe1-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
2	Fe1-3	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
3	Fe5-3	*Escherichia coli*	Group 1		*mcr-1*	Feces	Confirmed CTX-M Group 1 and *mcr-1*
4	Fe7-1	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
5	Fe7-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
6	Fe9-1	*Escherichia coli*	Group 1	Group 2	*mcr-1, mcr-3*	Feces	Confirmed CTX-M Group 1, Group 2, *mcr-1* and *mcr-3*
7	Fe9-3	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
8	Fe9-4	*Escherichia coli*	Group 1	Group 2	*mcr-3*	Feces	
9	Fe10-1	*Escherichia coli*	Group 1	Group 2	*mcr-3*	Feces	
10	Fe10-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
11	Fe10-3	*Escherichia coli*	Group 1	Group 2	*mcr-1, mcr-3*	Feces	
12	Fe12-1	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
13	Fe12-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
14	Fe16-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
15	Fe17-1	*Escherichia coli*	Group 1	Group 2	*mcr-1, mcr-3*	Feces	
16	Fe24-2	*Escherichia coli*	Group 1	Group 2	*mcr-1, mcr-3*	Feces	
17	Fe25-2	*Escherichia coli*	Group 1		*mcr-3*	Feces	Confirmed CTX-M Group 1 and *mcr-3*
18	Fe26-2	*Escherichia coli*	Group 1	Group 2	-	Feces	Confirmed CTX-M Group 1 and Group 2
19	Fe26-3	*Escherichia coli*	Group 1		*mcr-1, mcr-3*	Feces	
20	Fe29-1	*Escherichia coli*	Group 1		*mcr-1, mcr-3*	Feces	
21	Fe29-3	*Escherichia coli*	Group 1		*mcr-1, mcr-3*	Feces	
22	F22-SW	*Escherichia coli*	Group 1		*mcr-3*	Waste	
23	F22-SW-1	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Waste	
24	F22-SW-2	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Waste	
25	F32-SW-1	*Escherichia coli*	Group 1	Group 2	*mcr-3*	Waste	
26	F32-S	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Feces	
27	F37-S	*Escherichia coli*	Group 1		*mcr-3*	Feces	
28	F43-SW-2	*Escherichia coli*	Group 1	Group 2	*mcr-3*	Waste	
29	F43-SW	*Escherichia coli*	Group 1	Group 2	*mcr-3*	Waste	
30	F48-S-3-1	*Escherichia coli*	Group 1	Group 2	-	Feces	
31	F48-S-3	*Escherichia coli*	Group 1		*mcr-3*	Feces	
32	F48-S-NM	*Escherichia coli*	Group 1		*mcr-1*	Feces	
33	F26-SW-1	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Waste	
34	F32-SW-1	*Escherichia coli*	Group 1	Group 2	*mcr-1*	Waste	

Acknowledgments

This study was funded by "The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship" and "The 90th Anniversary Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund)."

References

Almansour AM, Alhadlaq MA, Alzahrani KO, Mukhtar LE, Alharbi AL and Alajel SM 2023. The Silent Threat: Antimicrobial-Resistant Pathogens in Food-Producing Animals and Their Impact on Public Health. Microorganisms. 11.

Bandy A and Tantry B 2021. ESBL Activity, MDR, and Carbapenem Resistance among Predominant Enterobacterales Isolated in 2019. Antibiotics (Basel). 10.

Bastidas-Caldes C, Cisneros-Vásquez E, Zambrano A, Mosquera-Maza A, Calero-Cáceres W, Rey J, Yamamoto Y, Yamamoto M, Calvopiña M and de Waard JH 2023. Co-Harboring of Beta-Lactamases and *mcr*-1 Genes in *Escherichia coli* and *Klebsiella pneumoniae* from Healthy Carriers and Backyard Animals in Rural Communities in Ecuador. Antibiotics (Basel). 12.

Ben Sallem R, Ben Slama K, Sáenz Y, Rojo-Bezares B, Estepa V, Jouini A, Gharsa H, Klibi N, Boudabous A and Torres C 2012. Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)- and CMY-2-producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia. Foodborne Pathog Dis. 9: 1137-1142.

Chen Y, Liu Z, Zhang Y, Zhang Z, Lei L and Xia Z 2019. Increasing Prevalence of ESBL-Producing Multidrug Resistance *Escherichia coli* From Diseased Pets in Beijing, China From 2012 to 2017. Front Microbiol. 10: 2852.

Chotinantakul K, Chusri P and Okada S 2022. Detection and characterization of ESBL-producing *Escherichia coli* and additional co-existence with *mcr* genes from river water in northern Thailand. PeerJ. 10: e14408.

CLSI 2015. Performance standards for antimicrobial susceptibility testing; 20th informational supplement M100-S25. Wayne, PA, Clinical Lab Standards Institute.

CLSI 2022. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA, Clinical Lab Standards Institute.

Dadgostar P 2019. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 12: 3903-3910.

Dohmen W, Bonten MJ, Bos ME, van Marm S, Scharringa J, Wagenaar JA and Heederik DJ 2015. Carriage of extended-spectrum β-lactamases in pig farmers is associated with occurrence in pigs. Clinical Microbiology and Infection. 21: 917-923.

Dohmen W, Dorado-García A, Bonten MJ, Wagenaar JA, Mevius D and Heederik DJ 2017a. Risk factors for ESBL-producing *Escherichia coli* on pig farms: A longitudinal study in the context of reduced use of antimicrobials. PLoS One. 12: e0174094.

Dohmen W, Liakopoulos A, Bonten MJ, Mevius DJ and Heederik DJ 2023. Longitudinal Study of Dynamic Epidemiology of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Pigs and Humans Living and/or Working on Pig Farms. Microbiol Spectr. 11: e0294722.

Dohmen W, Schmitt H, Bonten M and Heederik D 2017b. Air exposure as a possible route for ESBL in pig farmers. Environmental Research. 155: 359-364.

Doyle D, Peirano G, Lascols C, Lloyd T, Church DL and Pitout JD 2012. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. J Clin Microbiol. 50: 3877-3880.

Feng J, Wu J, Zhuang Y, Luo J, Chen Y, Wu Y, Fei J, Shen Q, Yuan Z and Chen M 2023. Stability and genetic insights of the co-existence of $bla_{\text{CTX-M-65}}$, $bla_{\text{OXA-1}}$, and *mcr-1.1* harboring conjugative IncI2 plasmid isolated from a clinical extensively-drug resistant *Escherichia coli* ST744 in Shanghai. Front Public Health. 11: 1216704.

Gupta CL, Blum SE, Kattusamy K, Daniel T, Druyan S, Shapira R, Krifucks O, Zhu YG, Zhou XY, Su JQ and Cytryn E 2021. Longitudinal study on the effects of growth-promoting and therapeutic antibiotics on the dynamics of chicken cloacal and litter microbiomes and resistomes. Microbiome. 9: 178.

Hammerum AM, Larsen J, Andersen VD, Lester CH, Skovgaard Skytte TS, Hansen F, Olsen SS, Mordhorst H, Skov RL, Aarestrup FM and Agersø Y 2014. Characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. Journal of Antimicrobial Chemotherapy. 69: 2650-2657.

Khine NO, Lugsomya K, Kaewgun B, Honhanrob L, Pairojrit P, Jermprasert S and Prapasarakul N 2020. Multidrug Resistance and Virulence Factors of *Escherichia coli* Harboring Plasmid-Mediated Colistin Resistance: *mcr-1* and *mcr-3* Genes in Contracted Pig Farms in Thailand. Front Vet Sci. 7: 582899.

Larsson DG and Flach CF 2022. Antibiotic resistance in the environment. Nat Rev Microbiol. 20: 257-269.

Lay KK, Jeamsripong S, Sunn KP, Angkititrakul S, Prathan R, Srisanga S and Chuanchuen R 2021. Colistin Resistance and ESBL Production in *Salmonella* and *Escherichia coli* from Pigs and Pork in the Thailand, Cambodia, Lao PDR, and Myanmar Border Area. Antibiotics (Basel). 10.

Lü Y, Kang H and Fan J 2020. A Novel $bla_{\text{CTX-M-65}}$-Harboring IncHI2 Plasmid pE648CTX-M-65 Isolated from a Clinical Extensively-Drug-Resistant *Escherichia coli* ST648. Infect Drug Resist. 13: 3383-3391.

Mollenkopf DF, Stull JW, Mathys DA, Bowman AS, Feicht SM, Grooters SV, Daniels JB and Wittum TE 2017. Carbapenemase-Producing *Enterobacteriaceae* Recovered from the Environment of a Swine Farrow-to-Finish Operation in the United States. Antimicrob Agents Chemother. 61.

Nordmann P, Naas T and Poirel L 2011. Global spread of Carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis. 17: 1791-1798.

Nuangmek A, Rojanasthien S, Chotinun S, Yamsakul P, Tadee P, Thamlikitkul V, Tansakul N and Patchanee P 2018. Antimicrobial Resistance in ESBL-Producing *Escherichia coli* Isolated from Layer and Pig Farms in Thailand. Acta Scientiae Veterinariae. 46.

Nuanmuang N, Baiubon P, Wannathong N, Nunta B, Suriya N, Chaingam P and Kummasook A 2018. Phenotypic and genotypic characterization of Extended-Spectrum Beta-Lactamase- producing *Escherichia coli* isolated from pig feces in farms, Muang Phayao. Naresuan University Journal: Science and Technology. 26: 9.

Plata G, Baxter NT, Susanti D, Volland-Munson A, Gangaiah D, Nagireddy A, Mane SP, Balakuntla J, Hawkins TB and Kumar Mahajan A 2022. Growth promotion and antibiotic induced metabolic shifts in the chicken gut microbiome. Commun Biol. 5: 293.

Poirel L, Walsh TR, Cuvillier V and Nordmann P 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 70: 119-123.

Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A and Hendriksen RS 2018. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* for surveillance purposes. Euro Surveill. 23.

Runcharoen C, Raven KE, Reuter S, Kallonen T, Paksanont S, Thammachote J, Anun S, Blane B, Parkhill J, Peacock SJ and Chantratita N 2017. Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. Genome Med. 9: 81.

Schwarz S and Johnson AP 2016. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 71: 2066-2070.

Shrestha A, Bajracharya AM, Subedi H, Turha RS, Kafle S, Sharma S, Neupane S and Chaudhary DK 2017. Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. BMC Res Notes. 10: 574.

Srichumporn W, Chaisowwong W, Intanon M and Na-Lampang K 2022. Extended-spectrum beta-lactamase-producing *Escherichia coli* from pork in Muang district, Chiang Mai Province, Thailand. Vet World. 15: 2903-2909.

Subramanya SH, Bairy I, Metok Y, Baral BP, Gautam D and Nayak N 2021. Detection and characterization of ESBL-producing *Enterobacteriaceae* from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. Sci Rep. 11: 2091.

Sudatip D, Mostacci N, Tiengrim S, Thamlikitkul V, Chasiri K, Kritiyakan A, Phanprasit W, Thinphovong C, Abdallah R, Baron SA, Rolain JM, Morand S, Oppliger A and Hilty M 2023. The risk of pig and chicken farming for carriage and transmission of *Escherichia coli* containing extended-spectrum beta-lactamase (ESBL) and mobile colistin resistance (*mcr*) genes in Thailand. Microbial Genomics. 9.

Wu C, Wang Y, Shi X, Wang S, Ren H, Shen Z, Wang Y, Lin J and Wang S 2018. Rapid rise of the ESBL and *mcr-1* genes in *Escherichia coli* of chicken origin in China, 2008-2014. Emerg Microbes Infect. 7: 30.

Wu S, Chouliara E, Hasman H, Dalsgaard A, Vieira A and Jensen LB 2008. Detection of a single isolate of CTX-M-1-producing *Escherichia coli* from healthy pigs in Denmark. Journal of Antimicrobial Chemotherapy. 61: 747-749.

Xu L, Ensor V, Gossain S, Nye K and Hawkey P 2005. Rapid and simple detection of $bla_{\text{CTX-M}}$ genes by multiplex PCR assay. J Med Microbiol. 54: 1183-1187.

Yadav KK, Adhikari N, Khadka R, Pant AD and Shah B 2015. Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. Antimicrobial Resistance and Infection Control. 4: 42.

Zhang X, Fang C, Zhang J, Hua W, He R and Zhou M 2022. Carbapenemase- and Colistin Resistant *Escherichia coli* Strains from Children in China: High Genetic Diversity and First Report of $bla_{\text{NDM-5}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{OXA-10}}$, $bla_{\text{TEM-1}}$, and $mcr_{\text{-1.1}}$ Genes Co-Occurrence in *E. coli* ST156. Infect Drug Resist. 15: 5315-5320.

Zurfluh K, Hächler H, Nüesch-Inderbinen M and Stephan R 2013. Characteristics of Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Enterobacteriaceae* Isolates from Rivers and Lakes in Switzerland. Applied and Environmental Microbiology. 79: 3021-3026.