ESCHERICHIA COLI CARRYING CEPHALOSPORIN (*BLA*) AND COLISTIN (*MCR*) RESISTANCE GENES ISOLATED FROM BROILERS AND PIGS IN THAILAND

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Abstract. Spread of transferable mobile genetic elements (MGEs)-mediated antimicrobial resistance in human and veterinary medicine, especially of important antimicrobials in human medicine, is of global concern. Cephalosporin- and colistin-resistant *Escherichia coli* isolates and their MGEs-mediated resistance genes (*bla* and *mcr*) in broilers and pigs in Thailand were investigated using fecal samples (n = 45) from 4 broiler farms and 5 pig farms (5 fecal samples per farm) during 2014 - 2015. Broiler and pig farm samples were 60 and 90% resistant to cephalosporin respectively. Among cefotaxime-resistant *E. coli* isolates (n = 99), bla_{TEM} was the most predominant (74%), followed by $bla_{\text{CMY-2}}$ (45%), $bla_{\text{CTX-M-55}}$ (32%), $bla_{\text{CTX-M-14}}$ (29%), and bla_{SHV} (2%); 73% of isolates harbored multiple gene types. Among *mcr*-positive *E. coli* isolates (n = 15) from broiler and pig farms, *mcr-1*, *mcr-2*, *mcr-3*, and *mcr-2* + *mcr-3* were present in 33, 7, 53, and 7% of the samples; except for one isolate, the remainings were also resistant to cefotaxime. Five *bla*- and *mcr*-positive isolates exhibited co-transfer of the genes in conjugation experiments. To the best of our knowledge, this is the first study to report *mcr-2*-positive isolates in a non-European country.

Keywords: AmpC, cephalosporin resistance, colistin, ESBL, *mcr-1*, *mcr-2*, *mcr-3*

INTRODUCTION

Emergence and spread of antimicrobial resistance are of great concern to public health. The World Health Organization has provided a list of critically important antimicrobials for human medicine (WHO, 2017), and in order to preserve the effectiveness of currently available antimicrobials, risk management is required for preventing the spread of antimicrobial resistance, especial antimicrobials classified as of highest priority, such as cephalosporin, polymyxin, and quinolone. Antimicrobials are used in both human and veterinary medicine, and there is a threat of transmission of antimicrobial resistance from livestock to humans (Koch *et al*, 2017). To address this issue, it is important to ensure appropriate use of antimicrobials and control of antimicrobial resistance in livestock by monitoring antimicrobial resistance of zoonotic bacteria, animal pathogens

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and indicator bacteria from livestock (Sharma *et al*, 2018)one among the most common priority areas identified by both national and international agencies, is mushrooming as a silent pandemic. The advancement in public health care through introduction of antibiotics against infectious agents is now being threatened by global development of multidrug-resistant strains. These strains are product of both continuous evolution and unchecked antimicrobial usage (AMU.

Resistance to cephalosporin is spreading among Enterobacteriaceae throughout the world and is mainly associated with transferable cephalosporin resistance genes mediated by mobile genetic elements (MGEs), such as plasmids, integrons and transposons. (van Hoek *et al*, 2011). MGEs-encoded extended-spectrum β-lactamase ($bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV}) and AmpC β-lactamase ($bla_{\text{CMY-2}}$) are the predominant genes detected in cephalosporin-resistant bacteria (Pfeifer *et al*, 2010). Of even greater concern is most cephalosporin-resistant bacteria exhibit resistance to multiple antimicrobials (Seiffert *et al*, 2013). In Thailand, β-lactam, and in some cases colistin (CL) and tetracycline, are used as therapeutic and routine prophylactic antimicrobials for livestock (Lugsomya *et al*, 2018; Wongsuvan *et al*, 2018). Cephalosporin-resistant bacteria have been detected from livestock and their products in the country; however, only limited studies have been conducted on prevalence of MGEs-mediated cephalosporin resistance genes and susceptibility to other classes of antimicrobials, especially in broilers (Trongjit *et al*, 2016; Tansawai *et al*, 2019).

CL is a last-resort option for treatment of multidrug-resistant Gram-negative bacterial infection in humans (WHO, 2017). Since 2015, several types of MGEs-mediated CL resistance (*mcr*) genes have been reported in succession among Enterobacteriaceae isolated from humans, livestock and environment globally (Kluytmans, 2017; Yang *et al*, 2018). In some cases, CL-resistant bacteria and carriage of *mcr* were detected in high numbers from livestock, especially in pigs, but not in such high numbers in humans (Kluytmans, 2017; Fukuda *et al*, 2018a). These findings were linked to the use of CL in livestock and humans, making CL resistance in the former a risk hazard to humans (Rhouma *et al*, 2016). In Thailand, *mcr-1* and/or *mcr-3* were detected in human urine, canal water samples and flies (Paveenkittiporn *et al*, 2017; Runcharoen *et al*, 2017; Fukuda *et al*, 2018b); however, the prevalence of *mcr* and CL resistance in livestock in the country remains unknown.

In developing countries, especially in Southeast Asia, several studies showed high rates of antimicrobial resistance (Sheng *et al*, 2013; Nhung *et al*, 2016). In Thailand, one of the most agriculturally productive countries, meat, especially chicken and pig meat, is produced and exported (Costales, 2004). Livestock in Thailand is frequently administered antimicrobials to treat and prevent infections, and high prevalence of bacteria resistant to critically important antimicrobials for human medicine was reported in livestock and livestock-associated foods (Padungtod *et al*, 2008; Koch *et al*, 2017). Previous research in Thailand showed bacteria resistant to these antimicrobials harboring transferable antimicrobial resistance genes (ARGs) are present in environmental samples (flies and water), and isolates obtained from surrounding environment samples in farm areas have higher resistance prevalence to cephalosporin than those in urban areas (Usui *et al*, 2016; Fukuda *et al*, 2018b). These results indicate a great risk of exposure to livestock-associated antimicrobial-resistant bacteria (ARB) through the food chain.

Prevalence of resistance to highest-priority antimicrobials in human medicine by molecular characterization of ARGs in *Escherichia coli*, as an indicator bacteria, obtained from livestock farms where samples in surrounding areas had been investigated (Usui *et al*, 2016; Fukuda *et al*, 2018b). Focused was placed on cephalosporin- and CL-resistant *E. coli* isolates obtained from broiler and pig fecal samples and their transferable resistance genes.

MATERIALS AND METHODS

Fecal sampling

Forty-five fecal samples were obtained using cotton swabs (Seedswab gamma 1; Eiken Chemical, Tokyo, Japan) from 4 broiler farms and 5 pig farms (five fecal samples per farm), including two broiler farms and two pig farms in Ratchaburi Province, western Thailand in September 2014, and two broiler farms and three pig farms in Nakhon Si Thammarat Province, southern Thailand in September 2015 (Fukuda *et al*, 2018b).

Bacteria isolation

Swabs were used to inoculate deoxycholate-hydrogen sulfate-lactose (DHL) agar (Nissui Pharmaceutical, Tokyo, Japan) and DHL agar supplemented with 4 μg/ml cefotaxime (CTX) (DHL-C; Sigma-Aldrich, St Louis, MO) plates, which were incubated overnight at 37°C. For each agar plate, up to three suspected *E. coli* colonies were subcultured on nutrient agar (Nissui Pharmaceutical, Tokyo, Japan) and each *E. coli* isolate was confirmed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI–TOF MS) with a Bruker MALDI Biotyper system (Bruker Daltonics, Bremen, Germany) (Dierig *et al*, 2015). Two or three isolates from any agar plate exhibiting the same antibiogram profile and possessing ARGs were considered a single isolate.

Antibiogram profiling

Minimum inhibitory concentrations (MICs) of amikacin (AMK), cefazolin (CEZ), ceftazidime (CAZ), ciprofloxacin (CPFX), chloramphenicol (CP), CTX, gentamicin (GM), kanamycin (KM), nalidixic acid (NA), and tetracycline (TET) (all from Sigma-Aldrich) for each isolate were determined using an agar dilution method and that of CL using a microbroth dilution method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2019). Resistance breakpoint is defined according to CLSI guidelines (CLSI, 2019). *E. coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as controls. The breakpoint of CL, which is not defined by the CLSI guidelines, is based on the European Committee on Antimicrobial Susceptibility Testing Breakpoints for Enterobacteriaceae (EUCAST, 2019).

ARGs Characterization

CTX-resistant isolates (MIC ≥4 μg/ml) were screened for presence of bla_{ACC}, bla_{CIT}, $bla_{\mathrm{CMY\text{-}2}}$, $bla_{\mathrm{CTX\text{-}M}}$, bla_{DHA}, bla_{EBC}, bla_{FOX}, bla_{MOX}, bla_{SHV}, and bla_{T} by PCR as previously described (Pérez-Pérez and Hanson, 2002; Kojima *et al*, 2005; Xu *et al*, 2005; Kozak *et al*, 2009), and for identification of $bla_{\mathrm{CTX\text{-}M}}$ groups, an additional PCR was performed using external primers (Saladin *et al*, 2002) CTX-M-2, CTX-M-9, CTX-M-14 and two novel plasmid-mediated CTX-M beta-lactamases (CTX-M-20, and CTX-M-21.

Amplicons were subsequently sequenced (Fasmac Co., Ltd, Kanagawa, Japan). Isolates with CL MIC ≥ 2 μg/ml were analyzed for presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, and *mcr-7* by PCR as previously described (Fukuda *et al*, 2018a; Yang *et al*, 2018).

Conjugation assay

Transfer of cephalosporin and CL resistance was determined using previously described broth- and/or filter-mating methods with slight modifications (Stout and Iandolo, 1990; Fukuda *et al*, 2018b). In brief, rifampicin-resistant *E. coli* K12 DH5α strain carrying bla_{EBC} was used as recipient and mating was conducted at 37°C. Transconjugants were selected on Mueller–Hinton agar (OXOID, Cambridge, UK) supplemented with 50 μg/ml rifampicin (Sigma-Aldrich) and 32 μg/ml CEZ for selection of CEZ-resistant transconjugants or on MacConkey agar (Becton, Dickinson and Co, Franklin Lakes, NJ) supplemented with 50 μg/m rifampicin and 2 μg/ml CL for selection of CL-resistant transconjugants, which then were subjected to antibiogram profiling and presence of ARGs as described above.

Statistical analysis

Chi-square test was used to compare antimicrobial resistance and *bla* prevalence between isolates from broiler and pig farms. Significance is accepted at *p*-value <0.01.

RESULTS

***E. coli* from fecal samples**

Using DHL agar, 103 *E. coli* isolates were obtained from the fecal samples (47 and 56 isolates from broiler farm and pig farms samples respectively) and using DHL-C agar, 69 *E. coli* isolates were obtained from fecal samples (18 and 51 isolates respectively). Prevalence of CTX-resistant *E. coli* in pig fecal samples (96%) is significantly higher than that of broiler samples (60%) (*p*-value = 0.009).

Antibiogram profiles

Sixty-seven to one hundred percent isolates were resistant to TET, 39-61% to CP and 6-14% to CL; all isolates were susceptible to AMK (Fig 1). On DHL agar, prevalence of resistance to cephalosporins (CAZ, CEZ and CTX) are significantly higher in pig compared to broiler farm samples (*p*-value <0.0001). On DHL-C agar, prevalence of resistance to quinolones (CPFX and NA) are significantly higher in broiler compared to pig farm samples (*p*-value <0.0001).

Carriage of cephalosporin and CL resistance genes

A total of 99 CTX-resistant *E. coli* isolates (22 from broiler and 77 from pig farm) were obtained using DHL (30 isolates) and DHL-C (69 isolates) agar. *bla* alleles were detected from all CTX-resistant isolates, with *bla* bla_{TEM} the most prevalent (73 isolates), followed by $bla_{\mathrm{CTX\text{-}M}}$ (61 isolates), $bla_{\mathrm{CMY\text{-}2}}$ (45 isolates) and bla_{SHV} (2 isolates); other *bla* alleles were not detected (Table 1). Among $bla_{\mathrm{CTX\text{-}M}}$ group, $bla_{\mathrm{CTX\text{-}M\text{-}55}}$ (32 isolates) and $bla_{\mathrm{CTX\text{-}M\text{-}14}}$ (29 isolates) variants were also identified, with prevalence of latter in CTX-resistant isolates from pig farms (36%) significantly higher compared to that in CTX-resistant isolates from broiler farms (4%) (*p*-value = 0.003). Seventy-two isolates harbored multiple types of *bla* alleles.

Fifteen isolates from broiler and pig farm samples were *mcr*-positive and exhibited resistance to CL (MIC = 4 or 8 μg/ml). With the exception of a single isolate from a broiler farm, isolates resistant to CTX co-harbored $bla_{\mathrm{CTX\text{-}M\text{-}14}}$,

Table 1
Prevalence of *bla* alleles in cefotaxime-resistant *Escherichia coli* isolates from broiler and pig farms in Thailand.

bla allele	Number (%) (n = 99)	Broiler farm Number (%) (n = 22)	Pig farm Number (%) (n = 77)
CTX-M-55	32 (32)	11 (50)	21 (27)
CTX-M-14	29 (29)	1 (4)	28 (36)*
TEM	73 (74)	12 (54)	61 (79)
SHV	2 (2)	1 (4)	1 (1)
CMY-2	45 (45)	7 (32)	38 (49)

*p-value <0.01 compared to broiler farm.

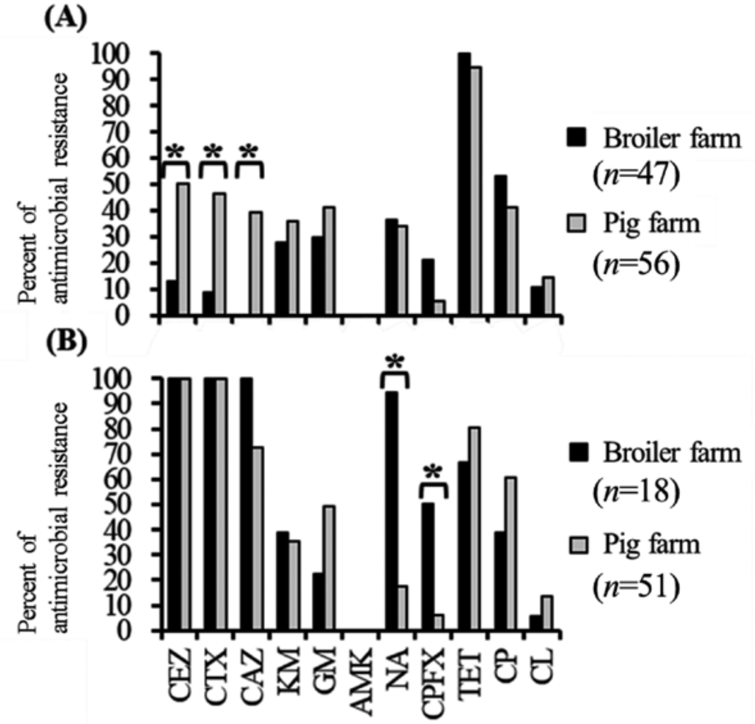

Fig 1-Antimicrobial-resistance prevalence of *Esherichia coli* isolates from broiler and pig farms in Thailand.

Isolates were obtained using DHL agar (A) and DHL agar supplemented with 4 μg/ml cefotaxime (B). Resistance is defined according to the CLSI guideline breakpoints, but resistance to CL according to the European Committee on Antimicrobial Susceptibility Testing breakpoints. *p-value <0.01. AMK: amikacin; CAZ: ceftazidime; CEZ: cefazolin; CL: colistin; CP: chloramphenicol; CPFX: ciprofloxacin; CTX: cefotaxime; KM: kanamycin; GM: gentamicin; NA: nalidixic acid; TET: tetracycline.

bla$_{CTX-M-55}$, *bla*$_{TEM}$, and/or *bla*$_{CMY-2}$ (Table 1). Two *mcr-3*-positive isolates were detected from broiler farm samples, and one *mcr-2*-, one *mcr-2* + *mcr-3*-, five *mcr-1*-, and six *mcr-3*-positive isolates from pig farm samples.

Co-transfer of cephalosporin and CL resistance genes

From the *bla*-positive isolates, 30 (30%) CEZ-resistant transconjugants were obtained using the broth-mating method (Table 2). Two CEZ-resistant transconjugants derived from isolates D226 and D227 carrying *bla* and *mcr-3* presented decreased susceptibility to CL (MIC = 2-4 μg/ml) compared to original recipient strain (MIC for CL = 0.5 μg/ml).

From the *mcr*-positive isolates, CL-resistant transconjugants could not be established using the broth-mating method, but with the filter-mating method, seven (47%) CL-resistant transconjugants were established. Four CL-resistant transconjugants derived from B73, B80, D226, and D228 strains acquired *mcr* along with *bla* alleles.

DISCUSSION

The study shows cephalosporin-resistant *E. coli* was isolated from a high proportion of broiler and pig fecal samples when DHL-C agar was used. In Thailand, cephalosporin-resistant bacteria are frequently isolated from livestock especially pigs; in some cases, the prevalence in pig and pork meat are higher than of other countries (Seiffert *et al*; 2013; Sheng *et al*, 2013; Boonyasiri *et al*, 2014; Trongjit *et al*, 2016). These findings suggest, in Thailand, pig and their products pose relatively high risk as sources for transmission of ARB from livestock to humans through the food chain.

In cephalosporin-resistant *E. coli* isolates, *bla*$_{CMY-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-55}$, and *bla*$_{TEM}$ were frequently detected; these genes are the main types of cephalosporin-resistance genes detected in South Asian countries (Kojima *et al*, 2005; Pfeifer *et al*, 2010; Sheng *et al*, 2013; Nhung *et al*, 2016). The predominant *bla*$_{CTX-M}$ allele was *bla*$_{CTX-M-55}$ in broiler farms and *bla*$_{CMX-M-14}$ in pig farms. Pig farm *E. coli* isolates using DHL agar showed higher resistance prevalence to cephalosporin than those from broiler samples. These distribution patterns corresponded to previous reports of flies and water samples obtained from the same sites (Usui *et al*, 2016; Fukuda *et al*, 2018b). Contamination of the surrounding environment by ARBs and antimicrobial agents derived from farms is of concern because of transmission of ARBs from livestock to humans via the environment (Sharma *et al*, 2018; Tansawai *et al*, 2019). These findings suggest ARBs and their resistance genes were disseminated from farms to the surrounding environment and, thus, restricting antimicrobial usage is essential for controlling spread of ARBs and their resistance genes to minimize potential risks to public health.

TET- and CP-resistant *E. coli* isolates were frequently obtained from broiler and pig farms. TET is used intensively in livestock as a feed additive as well as antibiotic (Usui *et al*, 2016). However, although CP was banned globally before the 20th century, CP resistance prevalence has remained constant (Harada *et al*, 2006). CP resistance is mainly caused by MGEs-mediated ARGs carrying multiple classes of ARGs (van Hoek *et al*, 2011) and can involve co-selection and co-transfer of resistance genotypes to several types of antimicrobials (Harada *et al*, 2006; Tacão *et al*, 2014). Conjugation experiments showed TET and/or CP resistance were

Table 2
Transfer to transconjugants of antimicrobial-resistance genes and phenotypes from *Escherichia coli* isolates from broiler and pig farms in Thailand.

Farm type	Isolate ID	*bla*/*mcr* allele	Resistance phenotype
	CEZ-resistant transconjugant		
Pig	D222	CTX-M-55/TEM	CAZ/CEZ/CTX/GM/NA/TET
Pig	C139	CTX-M-55/TEM	CAZ/CEZ/CTX/GM
Pig	C159	CTX-M-55/TEM	CAZ/CEZ/CP/CTX/GM/TET
Pig	C165	CTX-M-55/TEM	CAZ/CEZ/CP/CTX/GM/KM/NA/TET
Pig	B51	CTX-M-14	CEZ/CP/CTX
Pig	D231	CMY-2/CTX-M-14/TEM	CAZ/CEZ/CTX/GM/KM/NA/TET
Pig	C153	CMY-2/CTX-M-14/ TEM	CAZ/CEZ/CP/CTX/GM/KM/NA/TET
Pig	C167	CMY-2/CTX-M-14/ TEM/*mcr-1*	CAZ/CEZ/CL/CP/CTX/GM/KM/TET
Pig	C163	CMY-2/CTX-M-14/ TEM/*mcr-2*/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/TET
Pig	B52	CTX-M-14/TEM	CEZ/CTX/TET
Pig	B67	CTX-M-14/TEM	CEZ/CTX
Pig	D216	CMY-2	CAZ/CEZ/CTX/NA/TET
Broiler	C123	CMY-2	CAZ/CEZ/CTX/NA
Broiler	C126	CMY-2	CAZ/CEZ/CTX/NA
Broiler	C128	CMY-2	CAZ/CEZ/CTX/GM/NA
Pig	D210	CMY-2/TEM	CAZ/CEZ/CTX/NA/TET
Pig	D217	CMY-2/TEM	CAZ/CEZ/CTX/GM/TET
Broiler	C130	CMY-2/TEM	CAZ/CEZ/CTX/KM/TET
Pig	C146	CMY-2/TEM	CAZ/CEZ/CP/CTX/KM/TET
Pig	C149	CMY-2/TEM	CAZ/CEZ/CP/CTX/GM/NA/TET
Pig	C151	CMY-2/TEM	CAZ/CEZ/CP/CTX/GM/TET
Pig	C152	CMY-2/TEM	CAZ/CEZ/CP/CTX/KM/TET
Pig	C161	CMY-2/TEM	CAZ/CEZ/CP/CTX/KM/TET
Broiler	B85	CMY-2/TEM	CAZ/CEZ/CP/CTX/CPFX/NA/TET
Broiler	B89	CMY-2/TEM	CAZ/CEZ/CP/CTX/CPFX/NA/TET
Pig	D237	CMY-2/TEM/*mcr-1*	CAZ/CEZ/CL/CP/CTX/GM/NA/TET
Pig	D223	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/NA/TET
Pig	D226	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	D227	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	D234	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/NA/TET
	CL-selected transconjugant		
Pig	B73	CTX-M-55/*mcr-1*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	B80	CTX-M-55/TEM/*mcr-1*	CAZ/CEZ/CL/CP/CTX/KM/GM
Pig	D225	CMY-2/CTX-M-14/ TEM/*mcr-2*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	D228	CMY-2/CTX-M-14/ TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	D226	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Pig	D227	CMY-2/TEM/*mcr-3*	CAZ/CEZ/CL/CP/CTX/KM/GM/TET
Broiler	A188	*mcr-3*	CL/TET

Underline indicates transferred *bla*, *mcr* or resistance phenotypes. CAZ: ceftazidime; CEZ: cefazolin; CL: colistin; CP: chloramphenicol; CPFX: ciprofloxacin; CTX: cefotaxime; KM: kanamycin; GM: gentamicin; NA: nalidixic acid; TET: tetracycline.

co-transferred with cephalosporin and/or CL resistance. In order to reduce co-selection of resistance to highest-priority antimicrobials together with resistance to multiple classes of other antibiotics, proper use of cephalosporin and CL, as well as of the other classes of antimicrobials, is required to ensure continuing efficacy of antibiotic treatment against bacterial infection (Roberts 2002; Harada *et al*, 2006; Tacão *et al*, 2014). Future steps in analyzing and controlling MGEs-mediated ARGs and multidrug-resistant bacteria, comprehensive analysis, such as next-generation genome sequencing, of resistance genes to these drugs is required (Sharma *et al*, 2018).

CTX-resistant isolates from broiler farms showed high prevalence of quinolone resistance. In Thailand, quinolones are used in broiler farms and high frequencies of resistance to both cephalosporin and quinolones in chickens have been reported (Nakayama *et al*, 2017; Wongsuvan *et al*, 2018). Quinolones are commonly used to treat a variety of bacterial infections because of their high efficacy against both Gram-negative and -positive bacteria (WHO, 2017). However, broiler farms in Thailand are at risk for emergence of resistance to multiple classes of antimicrobials, including those critically important in human medicine. If pathogenic bacteria resistant to both cephalosporin and quinolones are transmitted from livestock to humans, last-resort drugs such as CL would then be required for treatment (Kluytmans, 2017).

CL-resistant isolates harboring *mcr-1*, *mcr-2* and *mcr-3*, but not *mcr-4*, *mcr-5*, *mcr-6*, and *mcr-7*, were detected from broiler and pig farm samples. Some studies reported *mcr* alleles in various sources (livestock, humans, food, and the environment) and in several species of Enterobacteriaceae, with patterns of distribution of *mcr* alleles distinctive of study regions (Kluytmans 2017; Fukuda *et al*, 2018a). For instance, *mcr-2*-positive *E. coli* was previously been detected only in Belgium (Garcia-Graells *et al*, 2018; Zhang *et al*, 2018)1.90% (2/105. To the best of our knowledge, the present study is the first to report the detection of *mcr-2*-positive isolates in another country; interestingly, co-existence of *mcr-2* + *mcr-3* was observed. Several combinations of co-existing *mcr* alleles having an additive effect on CL resistance were reported (Fukuda *et al*. 2018a). These results suggest the prevalence of MGEs-mediated (*mcr*) CL resistance in bacteria should be monitored. However, data from the present study were limited, especially with regards to sample numbers, sampling sites and species. National surveillance and monitoring for antimicrobial resistance in humans and veterinary medicine will be required to obtain more reliable findings.

In summary, the study reveals a high prevalence of *E. coli* with MGEs-mediated cephalosporin resistance in livestock, especially in pig and broiler farms; some fifty percent of cephalosporin-resistant *E. coli* isolates were resistant to fluoroquinolone. Various types of *mcr* alleles were found to have been disseminated in Thailand, and to the best of our knowledge, this is the first report of the presence of *mcr-2*-carrying *E. coli* in Asia. These findings should assist in the monitoring of antimicrobial resistance in the food chain and help prevent their transfer to human pathogens.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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