



Research article

Multidrug-resistance and mobile colistin resistance (*mcr*) genes of *Salmonella* isolates from pork in Thailand during 2014-2017: comparison between two different types of slaughterhouses and retails

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Abstract

Food-producing animals are the major reservoir for *Salmonella* infections in humans. *Salmonella* contamination and spread of antimicrobial resistance genes can occur during the production chain of animal products. The aims of this study were to investigate antimicrobial resistance patterns and compare the proportions of multidrug resistance and the presence of mobile colistin resistance (*mcr*) genes, *mcr-1*, *mcr-2* and *mcr-3*, among *Salmonella* isolates which were recovered from pork at two different standard practice slaughterhouses and retails during 2014-2017 in Thailand. *Salmonella* isolates recovered from good standard practice slaughterhouses (GSH, n=75), below standard practice slaughterhouses (BSH, n=75), good standard practice retails (GRT, n=75) and below standard practice retails (BRT, n=75) were examined for their antimicrobial resistance patterns and the existence of *mcr-1* to *mcr-3* genes. *Salmonella* strains of the 4 origins showed similar resistance rates to almost all antimicrobial agents tested. BRT origin (33/75, 44%) had slightly higher proportion of MDR *Salmonella* than the others group with no statistical difference. Five MDR *Salmonella* isolates carrying the *mcr-3* gene were detected among isolates of all origins, while only 4 isolates (1.33%) displayed colistin resistance phenotype (MIC 4-8 ug/mL). This study revealed that MDR *Salmonella* isolates have widely spread in both standard and low hygiene practice slaughterhouses and retails. This is the first report of *mcr-3* positive MDR *Salmonella* isolates from pork in Thailand. Effective monitoring program in slaughterhouses and retails should be continually implemented to reduce the contamination of MDR *Salmonella* carrying the *mcr* gene to consumers.

Keywords: Multidrug resistance, *mcr* genes, Pork, Retails, *Salmonella*, Slaughterhouses, Thailand

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INTRODUCTION

Salmonella spp. has been recognized over more than a century as a major and serious foodborne pathogen causing global morbidity and mortality in humans (Scallan et al., 2011). To date, more than 2,600 *Salmonella* serovars have been reported worldwide, and nearly all *Salmonella* can cause human and animal diseases (Majowicz et al., 2010; Mezal et al., 2014). Animal products, particularly chicken and pork meats, play major roles in the spread of *Salmonella* and are the main sources of *Salmonella* infections in humans (Evangelopoulou et al., 2014; Cavallo et al., 2015). Like other countries (Majowicz et al., 2010; Evangelopoulou et al., 2014), most foodborne disease outbreaks in Thailand have been caused by *Salmonella* spp. and most *Salmonella* outbreaks are associated with ingestion of contaminated livestock products (Chanachai et al., 2008). Although most *Salmonella* infections are uncomplicated and self-limited without the use of antibiotics, antibiotic treatments are essential in complicated cases and high-risk patients such as in immunocompromised cases (Rule, et al., 2019; Fukushima et al., 2020).

The emergence of multidrug-resistant (MDR) *Salmonella* has been continuously reported during the past few years which included the isolates from chicken and pork meats (Phongaran, et al., 2019; Vidayanti et al. 2021). In addition, resistance to colistin, the last-line treatment of multidrug-resistant Gram-negative bacterial infections, in *Salmonella* isolates from animal-based products has been frequently reported in several countries worldwide, including Germany, Spain (EFSA and ECDC, 2019), Portugal (Campos et al., 2016), China (Zhang et al., 2018) and Thailand (Sinwat et al., 2016). The occurrence of MDR and colistin resistant *Salmonella* in animal products poses a potential threat to humans since it may lead to increased severity of foodborne illness, and higher rates of hospitalization and death (Crump et al., 2015).

The use of antimicrobials during animal productions is one of the factors contributing to increased antimicrobial resistance. Previously, colistin was one of the most commonly used drugs for growth enhancement and prevention of *Enterobacteriaceae* infections in swine farms (Rhouma et al., 2016). However, colistin has been prohibited for its use as a preventive medication in food-producing animals in Thailand since 2017. Nevertheless, colistin is still permitted for a short-term therapeutic treatment under the approval of a veterinarian. Interestingly, colistin administration even in a short period of time was found to be related with the occurrence of colistin-resistant *Enterobacteriaceae* in swine (Poolperm et al., 2020).

Several measures have been implemented to reduce the numbers and spread of antimicrobial resistant bacteria in the food chain such as restriction of use of antibiotics in food-producing animals, and application of hazard analysis and critical control points (HACCP)-based procedures in food production. It has been demonstrated that good slaughtering process at HACCP slaughterhouses could reduce the number of *Salmonella* from pigs to pork (Gomes-Neves et al., 2012; Wilhelm et al., 2011; Wu et al., 2019). However, the frequencies of bacteria recovered during the meat production and distribution processes, from slaughterhouse to retail, were found to be not highly related (Choi et al., 2013), indicating that contamination or cross-contamination of meat products can occur along the pork supply chain. The environment of food production system which include processing and marketing of food products may contribute to the spread of antimicrobial resistant bacteria and antimicrobial resistance genes.

This study aimed to investigate antimicrobial resistance profiles and compare the proportions of multidrug resistance and the presence of mobile colistin resistance (*mcr*) genes, *mcr-1*, *mcr-2* and *mcr-3* genes, between *Salmonella* isolates recovered from pork at two different standard practice slaughterhouses and retails during 2014-2017 in Thailand. Such information is imperative for planning effective prevention and control measures to reduce public health risks from MDR and colistin-resistant *Salmonella*.

MATERIALS and METHODS

Ethical approval

No ethical approval was obtained as this study did not involve animal subjects.

Study area, study period and sample collections

A total of 300 *Salmonella* isolates used in this study were recovered from 300 pork samples collected as part of the activities of national surveillance system for microbiological contamination in livestock products conducted by the Bureau of Quality Control of Livestock Products Laboratory (BQCLP), Thailand. Of 300 isolates, 83 (27.7%), 65 (21.7%), 75 (25%) and 77 (25.7%) isolates were obtained each year for a period of 4 years, 2014 to 2017, respectively. Pork samples were collected from two different types of slaughterhouses or retails located in 18 provinces of Thailand, including 10, 2, 3, 1, 1 and 1 provinces in the central, north, northeast, east, west and south, respectively (Figure 1). Good standard practice slaughterhouses (GSH) are the slaughterhouses with an appropriate handling and processing following the Thai Agricultural Standard (TAS) practical guidelines for abattoir TAS 9004 (2004) and certified as good manufacturing practice (GMP) by the Department of Livestock Development (DLD), Thailand. Below standard practice slaughterhouses (BSH) are the local slaughterhouses with slightly poorer processing technique and hygiene without certified GMP. Good standard practice retails (GRT) are mostly located in supermarkets where meat products are stored at 0 to 4 °C without adding any preservative. Below standard practice retails (BRT) are the local fresh markets where meats are stored without temperature control and under slightly poor hygiene conditions. Of 300 isolates, equal number of isolates were derived from pork samples collected from the good standard practice slaughterhouses (GSH = 75) or retails (GRT=75), and the below standard practice slaughterhouses (BSH = 75) or retails (BRT = 75).

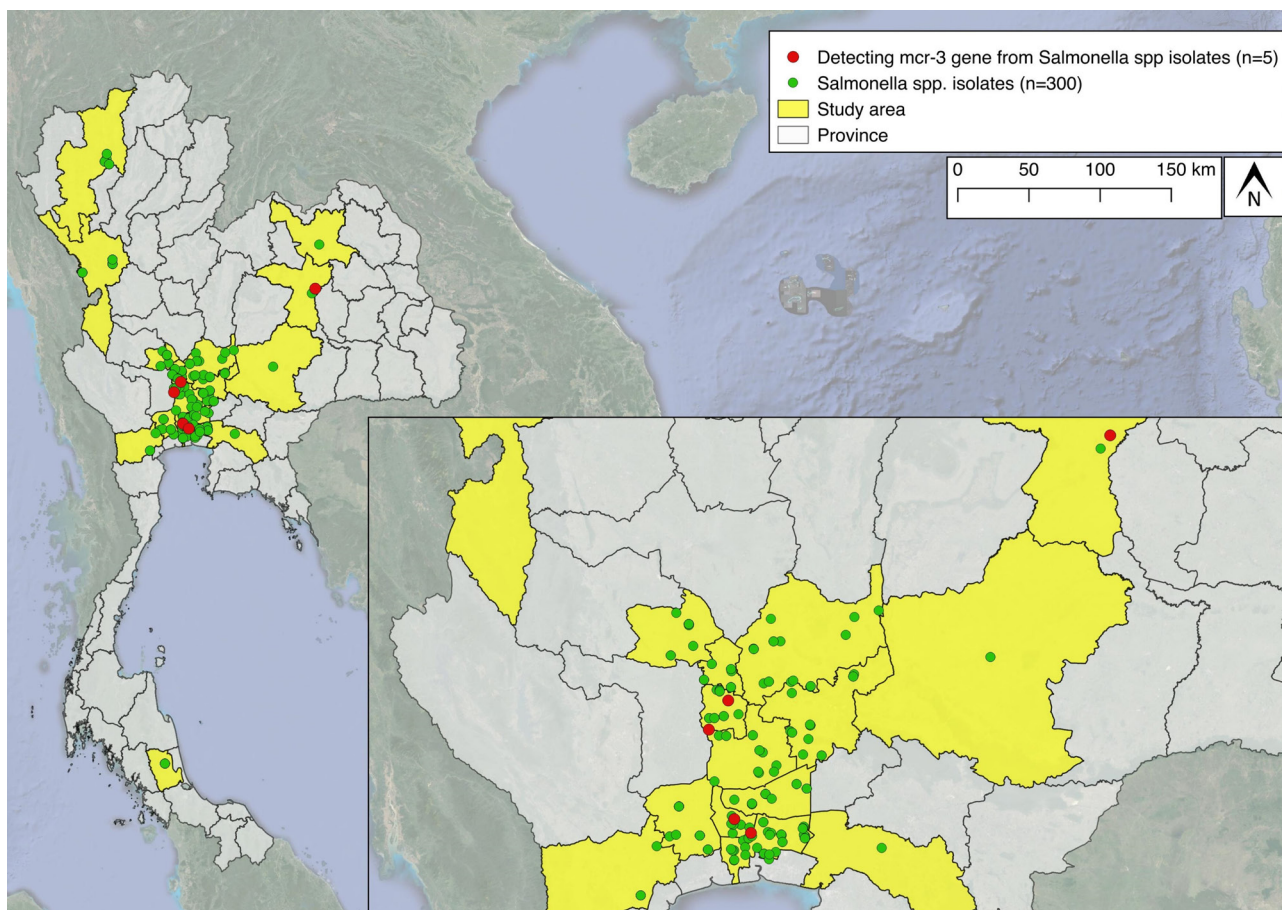


Figure 1 Map of the study area, 18 provinces included in this study are highlighted in yellow color; green spot refers to the locations where 300 *Salmonella* isolates were obtained from pork in Thailand, 2014-2017; red spot designates the locations where five *mcr-3*-positive *Salmonella* isolates were recovered from pork which are in the Central (4 isolates) and North-East part (1 isolate) of Thailand, 2014-2017.

***Salmonella* serotyping**

Salmonella isolates were recovered on tryptic soy agar (TSA) media (Oxoid, England). The isolates were classified into serogroup according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). Serotyping was performed by slide agglutination with poly-specific anti-*Salmonella* sera followed by serogroup-specific, O-specific and H-specific sera (S & A Laboratory Ltd., Thailand).

Antimicrobial susceptibility testing

All *Salmonella* isolates were examined for their susceptibilities to 20 antimicrobial drugs belonging to 10 clinically relevant antimicrobial classes, including 1, beta-lactams and subclass monobactam: amoxicillin/clavulanate (AMC), ampicillin (AMP), piperacillin/tazobactam (TZP), aztreonam (ATM); 2, cephalosporins: cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO); 3, chloramphenicol (CHL); 4, fluoroquinolones: norfloxacin (NOR), ciprofloxacin (CIP); 5, polypeptides: colistin (COL); 6, carbapenems: ertapenem (ETP), imipenem (IPM), meropenem (MEM); 7, aminoglycosides: gentamicin (GEN); 8, nitrofurans: nitrofurantoin (FM); 9, tetracyclines:

tetracycline (TET); 10, trimethoprim and sulfonamides: trimethoprim (TRI) and trimethoprim/sulfamethoxazole (SXT). The susceptibility of *Salmonella* isolates to antimicrobials were tested using BD Phoenix M50 (Becton Dickinson Phoenix™ ID & AST System, USA). Briefly, *Salmonella* colonies were suspended in the Pheonix ID/Inoculum broth and the optical density of the prepared inoculum was adjusted to 0.5 McFarland. Twenty-five microliters of the suspension were then added into the AST broth tube containing one drop of the AST indicator, followed by transferring the whole suspension into the BD Pheonix panel, NMIC/ID-95 lot 448783. The panel card was then loaded into the instrument BD Phoenix M50 and incubated for 24 h at 37°C. *Escherichia coli* ATCC 25922 was used as the quality control strain. Susceptibility or resistance to antimicrobial agents was identified based on the antimicrobial resistance breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016). *Salmonella* isolate demonstrating intermediate susceptibility or resistance to three or more classes of antimicrobial agents was defined as a multidrug-resistant strain (MDR).

Detection of *mcr* genes

Salmonella DNA was extracted from all 300 isolates by boiling method as previously described (Jimenez et al., 1999). Briefly, two colonies of overnight growth culture were suspended in 1 ml of Tris-EDTA (TE) buffer in a 1.5 ml tube. The suspension was then boiled at 100 °C for 10 minutes, followed by centrifugation at 10,000 rpm for 3 minutes. The DNA supernatant was collected for detection of 3 *mcr* genes, *mcr*-1, *mcr*-2 and *mcr*-3, using real-time PCR. Primers specific to each *mcr* gene used in this study were as described previously (Li et al., 2017) as shown in Table 1. The real-time PCR reactions of all extracted samples, and positive and negative controls were performed using the SensiFAST™ SYBR No-ROX Kit (Bioline USA Inc., MA, USA) following the manufacturing procedures. *E. coli* ATCC 25922 was used as a negative control strain, and *E. coli* harboring *mcr* genes were used as positive control strains. The real-time PCR was run on ECO Real-Time PCR System (Illumina, USA) using conditions as follows: a cycle of 50°C for 2min, 95°C for 3min, then 40 cycles of 95°C for 30s, 60°C for 30s, and 72°C for 30s, followed by a ramp from 72 to 95°C for melting curve stage. All positive *mcr*-3 genes have been confirmed by DNA sequencing and blast with the NCBI database *mcr*-3 genes obtained by PCR were sequenced and blasted against the NCBI nucleotide database (<https://blast.ncbi.nlm.nih.gov>).

Table 1 Primers for detection of the *mcr-1*, *mcr-2*, and *mcr-3* gene (Li et al., 2017).

Primer name	Sequence (5' → 3')	Gene	Product length (bp)
<i>mcr1</i> -qf	AAAGACGCGGTACAAGCAAC	<i>MCR-1</i>	213
<i>mcr1</i> -qr	GCTGAACATACACGGCACAG		
<i>mcr2</i> -qf	CGACCAAGCCGAGTCTAAGG	<i>MCR-2</i>	92
<i>mcr2</i> -qr	CAACTGCGACCAACACACTT		
<i>mcr3</i> -qf	ACCTCCAGCGTGAGATTGTTCCA	<i>MCR-3</i>	169
<i>mcr3</i> -qr	GCGGTTTCACCAACGACCAGAA		

Statistical analysis

Descriptive statistics were used to determine the pattern of AMR and MDR, and the proportion of multidrug resistance and the presence of *mcr-1* to *mcr-3* genes among *Salmonella* isolates of the 4 origins (GSH, BSH, GRT and BRT). Differences in the frequencies of MDR *Salmonella* between sources of sample or years, and the odds ratios were calculated using Fisher's exact test by R version 3.6.2. The p-values of ≤ 0.05 are considered to be statistically significant.

RESULTS

Serogroup and antimicrobial susceptibility patterns of *Salmonella* isolates

Identification of *Salmonella* serogroup by serotyping revealed that *Salmonella* isolates from pork samples belonged to 4 serogroups, B, C, D and E. Forty two percent (126/300) of the isolates belonged to serogroup B, and 30.7% (92/300) belonged to serogroup C. Serogroup E was found at 20% (60/300) of the isolates, and only 3.3% (10/300) of them belonged to serogroup D. Twelve *Salmonella* isolates could not be serotyped.

The resistance profiles of 300 *Salmonella* isolates are summarized in Table 2 and Figure 2. *Salmonella* isolates were most commonly resistant to ampicillin (75%, 225/300) and tetracycline (62.7%, 188/300). Over one-fourth of the isolates showed resistant to trimethoprim (30%, 90/300), sulfamethoxazole/trimethoprim (29.3%, 88/300) and chloramphenicol (25.3%, 76/300). All strains were susceptible to meropenem and imipenem. Interestingly, an isolate of GRT origin was resistant to ertapenem. In almost all antimicrobial agents tested, similar rates of resistance of each drug were observed in *Salmonella* strains of different origins (Table 2). Although resistance to nitrofurantoin was not found in the group of *Salmonella* of BRT origin, the resistance rates of this drug among the isolates of the other origins were also very low, at 1.3%.

Analysis of the MIC values of each drug revealed 36 antimicrobial resistance patterns (Figure 2). Sixty-seven isolates (67/300, 22.3%) were sensitive to all drugs tested. Similar proportions of these isolates were obtained from each source of samples (GSH, BSH, GRT and BRT). Seven predominant drug-resistance profiles were 1) AMP-TET (78/300, 26%) followed by 2) AMP-TET-TRI (31/300, 10.3%), 3,4) AMP and AMP-CHL-

TET-TRI (24/300, 8%), 5) AMP-CHL-TRI (10/300, 3.3%), 6) AMP-CHL-TET (9/300, 3%) and 7) TET (6/300, 2%). GRT source was observed to have the highest percentage of *Salmonella* isolates in the predominate patterns 1 and 2. Resistance to 6 drug classes were observed in *Salmonella* isolates of all origins, except the BSH origin.

Table 2 Antimicrobial susceptibility profiles of *Salmonella* isolates from two different types of slaughterhouses and retails in Thailand during 2014 - 2017.

Antimicrobial agents	Number of resistance isolates									
	GSH (n=75)		BSH (n=75)		GRT (n=75)		BRT (n=75)		Total (n=300)	
	n	%	n	%	n	%	n	%	n	%
Ciprofloxacin	5	6.7	3	4	6	8	4	5.3	18	6
Norfloxacin	1	1.3	0	0	1	1.3	0	0	2	0.7
Colistin	1	1.3	2	2.7	0	0	1	1.3	4	1.3
Amoxicillin-clavulanate	3	4	0	0	2	2.7	2	2.7	7	2.3
Ampicillin	52	69.3	57	76	59	78.7	57	76	225	75
Piperacillin-tazobactam	0	0	1	1.3	0	0	0	0	1	0.3
Aztreonam	1	1.3	4	5.3	3	4	7	9.3	15	5
Chloramphenicol	20	26.7	17	22.7	19	25.3	20	26.7	76	25.3
Gentamicin	5	6.7	5	6.7	10	13.3	8	10.7	28	9.3
Cefepime	0	0	4	5.3	3	4	3	4	10	3.3
Cefoxitin	2	2.7	0	0	3	4	1	1.3	6	2
Ceftazidime	2	2.7	2	2.7	4	5.3	5	6.7	13	4.3
Ceftriazone	2	2.7	5	6.7	6	8	10	13.3	23	7.7
Ertapenem	0	0	0	0	1	1.3	0	0	1	0.3
Tetracycline	40	53.3	42	56	55	73.3	51	68	188	62.7
Nitrofurantoin	1	1.3	1	1.3	1	1.3	0	0	3	1
Trimethoprim	23	30.7	19	25.3	27	36	21	28	90	30
Sulfamethoxazole-Trimethoprim	23	30.7	18	24	27	36	20	26.7	88	29.3

Abbreviations: GSH, good standard practice slaughterhouses; BSH, below standard practice slaughterhouses; GRT, good standard practice retails; BRT, below standard practice retails

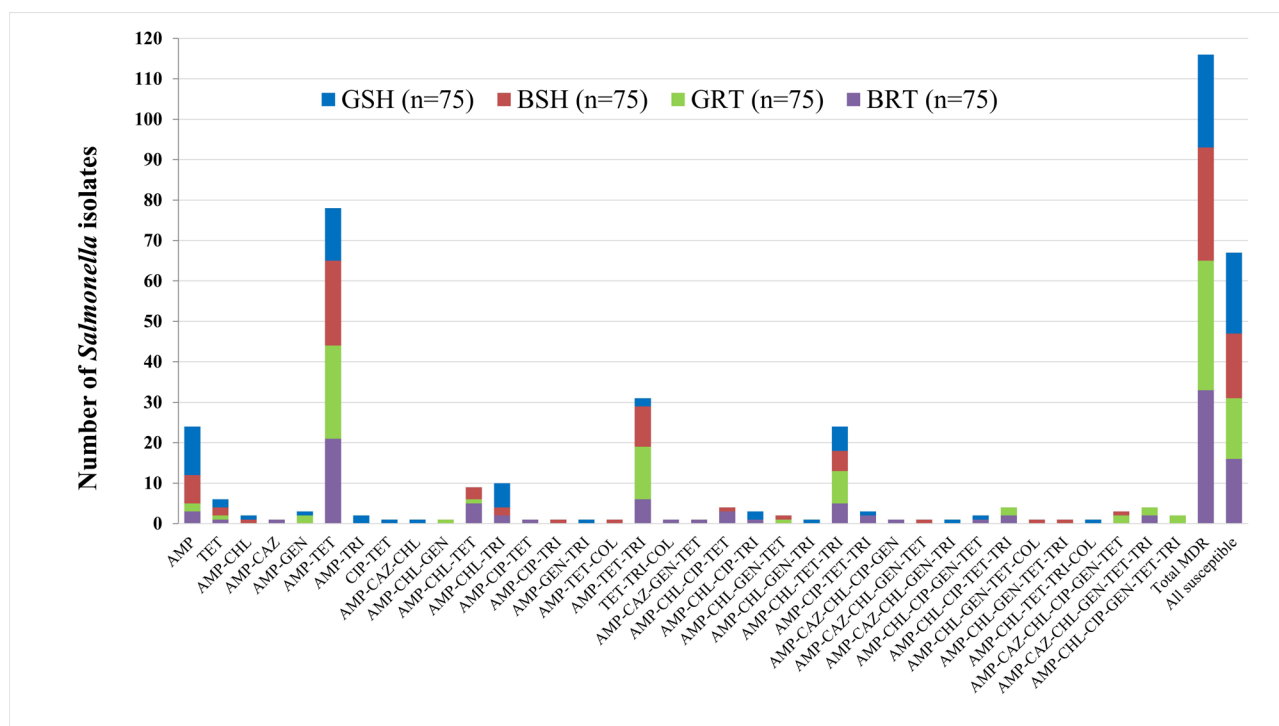


Figure 2 Antimicrobial resistance patterns of *Salmonella* isolates in Thailand during 2014-2017. Total 36 resistance patterns were observed in 300 *Salmonella* isolates of the 4 groups including BRT (n=75), BSH (n=75), GRT(n=75) and GSH (n=75). Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; MEM, meropenem TET, tetracycline; TRI, trimethoprim.

Multidrug resistance of *Salmonella* isolates from two different types of slaughterhouses and retails

Salmonella strains were defined as multidrug resistance (MDR) when the isolates were resistant to at least 3 different classes of antimicrobials. Of 300 *Salmonella* isolates, 116 isolates (38.7%) exhibited MDR phenotype. The highest percentage of MDR *Salmonella* was observed in the group of BRT origin at 44% (33/75, 95% CI: 32.54-55.94), followed by GRT, BSH, and GSH at 42.7% (32/75, 95% CI: 31.30-54.62), 37.3% (28/75, 95% CI: 26.43-49.26) and 29.3% (23/75, 95% CI: 19.38-40.97), respectively (Table 3). However, there was no statistical difference between the 4 different sources. Over 4 years comparison, BRT was revealed as the leading source of MDR-*Salmonella* strains in the year 2015, 2016 and 2017, with no statistical difference. Only in 2014, the frequency of MDR *Salmonella* strains was found to be the highest in the GRT group at 65% (13/20, 95% CI: 40.78-84.60) which was significantly higher than the other groups ($P \leq 0.05$ and odds ratio = 3.71).

Table 3 Comparison of the percentage and odds ratio of MDR-*Salmonella* isolates from two different types of slaughterhouses and retails in Thailand during 2014-2017.

Year	Source of samples	no. of isolates	no. of MDR isolates	Percentage (95% CI)	Odds ratio (95% CI)	P-value
2014	GSH	27	8	29.6 (13.75-50.18)	Reference	
	BSH	18	8	44.4 (21.53-69.24)	1.60 (0.41-6.40)	0.49
	GRT	20	13	65 (40.78-84.60)	3.71 (1.00-15.14)	0.05 ^a
	BRT	18	6	33.3 (13.34-59)	0.84 (0.23-3.12)	0.79
2015	GSH	6	2	33.3 (4.32-77.72)	Reference	
	BSH	21	10	47.6 (25.71-70.21)	1.81 (0.28-15.25)	0.53
	GRT	20	8	40.2 (19.11-63.94)	1.33 (0.20-11.30)	0.76
	BRT	18	12	66.7 (40.99-86.65)	4 (0.60-35.47)	0.16
2016	GSH	15	4	26.7 (7.78-55.10)	Reference	
	BSH	14	4	28.6 (8.38-58.10)	1.10 (0.20-5.83)	0.90
	GRT	17	5	29.4 (10.31-55.95)	1.14 (0.24-5.69)	0.86
	BRT	29	10	34.5 (17.93-54.33)	1.44 (0.37-1.06)	0.59
2017	GSH	27	8	29.6 (13.75-50.18)	0.90 (0.17-4.80)	0.90
	BSH	22	6	27.3 (10.72-50.22)	Reference	
	GRT	18	6	33.3 (13.34-59)	1.04 (0.21-5.22)	0.95
	BRT	10	5	50 (18.70-81.29)	1.31 (0.33-5.77)	0.69
Total	GSH	75	22	29.3 (19.38-40.97)	Reference	
	BSH	75	28	37.3 (26.43-49.26)	1.10 (0.20-5.83)	0.90
	GRT	75	32	42.7 (31.30-54.62)	1.14 (0.24-5.69)	0.86
	BRT	75	33	44 (32.54-55.94)	1.44 (0.37-6.28)	0.59

Abbreviations: GSH, good standard practice slaughterhouses; BSH, below standard practice slaughterhouses; GRT, good standard practice retails; BRT, below standard practice retails

^a statistically significant difference ($P \leq 0.05$)

Occurrence of colistin-resistance and *mcr*-positive *Salmonella* isolates

Very low occurrence of colistin-resistance as determined by the MIC values was demonstrated. The colistin-resistant strains had MIC values of 4 or 8 µg/mL as shown in Table 4. Of 300 *Salmonella* isolates, only 4 isolates (1.3%, 4/300) were resistant to colistin, comprising 3 isolates (2%, 3/150) of 150 slaughterhouse-derived origin, and 1 (0.7%, 1/150) of retail-derived isolates. Three out of four colistin-resistant *Salmonella* isolates were recovered from the below standard practice slaughterhouses or retails located in the central area (Table 5 and Figure 1).

The presence of mobile colistin resistant genes, *mcr*-1, *mcr*-2, and *mcr*-3 in all 300 *Salmonella* isolates was investigated using the real-time PCR. None of the *Salmonella* isolates with the MIC value of <1 µg/mL were found positive for *mcr* genes. The *mcr*-1 and *mcr*-2 genes were not detected from all isolates. However, all phenotypic colistin-resistant *Salmonella* isolates were found to

carry the *mcr-3* gene (Table 4). In addition, the *mcr-3* gene was detected in another isolate with decreased susceptibility to colistin, with the MIC value of 2 µg/mL. This isolate was recovered from the GRT source as shown in Table 3. The *mcr*-positive *Salmonella* isolates were almost equally distributed among *Salmonella* isolates of all 4 sources.

Table 4 Presence of colistin resistant and *mcr*-positive *Salmonella* isolates from pork of 4 different sources in Thailand, 2014 to 2017

Sample sources	Number of <i>Salmonella</i> isolates (n)	Resistance to colistin			Presence of <i>mcr-3</i> gene		
		Number of resistant isolates	MIC value (µg/mL)	Percentage (%)	Number of <i>mcr-3</i> positive isolates	MIC value (µg/mL)	Percentage (%)
GSH	75	1	8	0.3	1	8	0.3
BSH	75	2	4-8	0.7	2	4-8	0.7
GRT	75	0	0	0	1	2	0.3
BRT	75	1	8	0.3	1	8	0.3
Total	300	4	4-8	1.3	5	2-8	1.7

MIC breakpoint for colistin ≥ 4 µg/mL

Serogroup and antimicrobial resistance phenotypes of colistin-resistant and *mcr*-positive *Salmonella* isolates

Four out of five *mcr-3*-positive *Salmonella* isolates belonged to serogroup B, and another isolate belonged to serogroup C (Table 5). These isolates were recovered from three slaughterhouses and two retail supermarkets in Thailand in 2014 (2 isolates), 2015 (2 isolates) and 2016 (1 isolate), respectively (Table 5). All *mcr*-positive *Salmonella* isolates were multi-drug resistant in which each isolate had distinct antimicrobial resistant pattern. Eighty percent of these isolates (4/5) were resistant to ampicillin and tetracycline, and only 1 isolate (20%) was resistant to gentamicin. All *mcr*-positive *Salmonella* isolates were non-extended spectrum beta-lactamase (ESBL) and were still susceptible to carbapenems.

Table 5 Serogroup and antimicrobial susceptibility profile of *mcr-3*-positive *Salmonella* isolates from pork, by source of sample, location and year of isolation

Sample source	Year	Location	<i>Salmonella</i> serogroup	Colistin MIC (mg/L)	Antimicrobial resistance profile
GSH	2014	North-East	B	8	AMP-CHL-TMP-COL
BSH	2014	Central	B	4	AMP-TET-COL
BSH	2015	Central	B	8	AMP-CHL-GEN-TET-COL
BRT	2015	Central	C	8	TET-TRI-COL
GRT	2016	Central	B	2	AMP-COL-TET

Abbreviations: AMP, Ampicillin; CHL, Chloramphenicol; COL, Colistin; GEN, Gentamicin; TET, Tetracycline; TRI, Trimethoprim.

DISCUSSION

Multidrug antimicrobial resistance has been recognized as a serious and rising threat to both human and animal health worldwide (EFSA and ECDC, 2019). One of the major concerns is the presence of MDR *Salmonella* isolates that are resistance to colistin, the last-line treatment of serious human infections, in animal-based products (Mendelson et al., 2018). The increasing occurrence of MDR *Salmonella* in food-producing animals and their products has been continuously reported during the past few years in Thailand (Sinwat et al., 2016). Contamination or cross-contamination of *Salmonella* in animal products can occur at any point along the supply chain, which may lead to dissemination of antimicrobial resistance bacteria and resistant genes. Several previous studies examined the level of *Salmonella* contamination in pork collected at the slaughter stage or at the retail stage (Wu et al., 2019; Patchanee et al., 2016). Common findings of these studies were that pork from the good hygiene slaughterhouses or retails had significantly lower levels of *Salmonella* contamination than those of the poor hygiene type. However, the levels of bacterial contamination in pork at the slaughterhouses and at the retail markets were observed to be highly unrelated in the previous study (Choi et al., 2013). Our study focused on investigating the presence of MDR and *mcr* genes among the *Salmonella* isolates recovered from different hygienic practice slaughterhouses and retails with the aim of providing valuable insight into the potential impact of both pork production and distribution systems on the occurrence of MDR *Salmonella* and the *mcr* genes in pork in Thailand.

The most prominent *Salmonella* serogroups in our study are group B (42%) and C (30.7%). Recent studies also found groups B and C be the two most common serogroups in *Salmonella* isolates from pork at slaughterhouses and retails shops (Nuanmuang and Kummasook, 2018; Phongaran et al., 2019). Nuanmuang and Kummasook (2018) reported the prevalence of serogroup B and C of *Salmonella* isolated from mince pork samples at retail shops around the University of Phayou, Thailand be at 16.7% and 54.8%, respectively. Phongaran (2019) found that *Salmonella* isolates from pigs at slaughterhouses belonged to serogroup B and C at 33.6% and 38.9%, respectively. Our study found that group B was the most frequent serogroup, followed by group C, while the opposite results were found in those studies. Discrepancies between our results and the previous studies may be due to different study areas. *Salmonella* isolates in our study were recovered from slaughterhouses and retails in the provinces located in the Central (10), North (2), Northeast (3), East (1), West (1) and South (1) of Thailand whereas *Salmonella* isolates in those studies were obtained from nine provinces located in North (6), North-East (2) and East (1) (Nuanmuang and Kummasook, 2018; Phongaran et al., 2019).

Results of our research showed that large number of *Salmonella* isolates displayed resistance to ampicillin and tetracycline, and some were resistance to trimethoprim and chloramphenicol. High rates of resistance to ampicillin and tetracycline were observed in *Salmonella* isolates obtained from pork at both slaughterhouses and retailers: ampicillin (72.7% slaughterhouse and 77.3% retailed market) and tetracycline (54.7% slaughterhouse and 70.7% retailed market). Other studies in Thailand also revealed that large proportion of *Salmonella* isolates were resistant to ampicillin and tetracycline (Phongaran

et al., 2019; Nuanmuang and Kummasook, 2018; Patchanee et al., 2016). The resistant rates of ampicillin and tetracycline in *Salmonella* isolates reported in each study were at 69.05% and 66.19% (Phongaran et al., 2019), 64.3% and 61.9% (Nuanmuang and Kummasook, 2018), and 52.9% and 52.9% (Patchanee et al., 2016). High proportions of resistance to tetracycline (71.4%) and ampicillin (64.3%) were also reported in Greece (Evangelopoulou et al., 2014). The high resistant rates to ampicillin and tetracycline observed in our study and these studies are most likely because penicillins and tetracyclines have been the two most commonly used antibiotics in swine farming in many European countries (EFSA and ECDC, 2019) and Thailand (Lekagul et al., 2020; Hallenberg et al., 2020). The main MDR-resistant pattern in *Salmonella* isolates of our study was AMP-TET-TRI (10.33%). This result was slightly different from the previous studies in Thailand, which discovered that the most prominent MDR resistant profile was AMP-TET-SXT at 19.1% in 2018 (Nuanmuang and Kummasook, 2018) and 28.91% in 2019 (Phongaran et al., 2019). Minor difference between TRI and SXT in the MDR resistant profiles observed in our study and the previous studies is because we analyzed antimicrobial profile using a representative drug of each class, not a combine drug, as recommended by CLSI (2016) and EFSA and ECDC, (2019). Thus, trimethoprim was used as a representative drug of the trimethoprim and sulfonamide class. Limitation of this study was that only *Escherichia coli* ATCC 25922 was used as the reference strain for susceptibility testing following the previous studies (Patchanee et al., 2016; Vidayanti et al. 2021).

The proportions of MDR were compared between pork-derived *Salmonella* isolates collected from 4 different sources: HACCP slaughterhouse (GSH), low standard practice slaughterhouse (BSH), supermarket (GRT) and wet market (BRT). Overall, there is no significant difference in the proportion of MDR *Salmonella* among the 4 groups (BRT=44%, GRT= 42.7%, BSH =37.3% and GSH=29.3%). This finding suggested that MDR *Salmonella* isolates have widely disseminated across the pork supply chain in both type of slaughterhouses and retails. Although, HACCP slaughterhouses have been demonstrated to be able to reduce the number of *Salmonella* isolates from rectal swab (61.11%) to carcass (12.78%) (Wu et al., 2019), and pork in supermarkets (9.8%) have also been revealed to have a lower prevalence of *Salmonella* isolates than in wet markets (73.2%) (Patchanee et al., 2016). However, there was no information to support that HACCP slaughterhouses or good hygiene retails could reduce the proportion of MDR *Salmonella* isolates. Interestingly, *Salmonella* isolates of GRT origin displayed MDR at a higher proportion than isolates in the other groups in the year 2014 with a statistically difference ($P \leq 0.05$). The likelihood of *Salmonella* presence in the retail shops with good hygiene (GRT) was 3.71 times higher than in the good hygiene slaughterhouses (OR = 3.71, $P = 0.05$). This information is in agreement with the previous study in Argentina (Colello et al., 2018) that revealed the higher prevalence of *Salmonella* in retail markets (8.0%) than slaughterhouses (2.0%). High prevalence of *Salmonella* in retails is mostly due to cross-contamination during the stages of dressing and distribution in the pork supply chain (Colello et al., 2018), since the frequency of bacteria recovered during the meat production and distribution processes, from slaughterhouse to retail, was found to be not highly related (Choi et al., 2013).

This study provides some insight into the existence of the *mcr-1*, *mcr-2* and *mcr-3* genes and colistin resistance in *Salmonella* isolates obtained from pork at slaughterhouses and retail markets in Thailand during 2014 to 2017. The *mcr-1* and *mcr-2* genes were not detected from all isolates in our study. Our findings are similar to the previous study in Czechia, which reported that *Enterobacteriaceae* carrying the *mcr-1* to *mcr-5* genes were not detected in pork, chicken and beef originating from the EU and non-EU countries (Gelbířová et al., 2019). The very low numbers of colistin resistance, and the absence of *mcr-1* and *mcr-2* genes in *Salmonella* isolates in pork from slaughterhouses and retail markets in our study might be the consequence of the restriction for the use of colistin in food animals in Thailand (DLD, 2018). In addition, a recent study in Thailand reported that *Enterobacteriaceae* isolates carrying *mcr-1* gene were only observed in *Escherichia coli* and *Klebsiella pneumoniae*, not *Salmonella*, after the use of colistin for short-term treatment in 2 pig farms (Poolperm et al., 2020). However, *Salmonella* isolates carrying *mcr-1* gene were reported in pork from Portugal (Campos et al., 2016), and China (Hu et al., 2019), and also found in a *S. Typhimurium* isolate from a healthy pig in South Korea (Moon et al., 2021). In this study, only *mcr-3* gene was discovered at the low percentages, ranging from 0.3% - 0.7% in all 4 groups of *Salmonella* isolates originating from both types of slaughterhouses and retails which are located within Central and North-East part of Thailand. All *mcr-3*-positive *Salmonella* isolates are multi-drug resistant. Our findings demonstrated that MDR *Salmonella* isolates are widely spread across all types of slaughterhouses and retails in the pig production chain. Notably, this is the first report of MDR *Salmonella* isolates harboring the *mcr-3* gene and displaying colistin resistance phenotype recovered from pork in Thailand. On the other hand, the *mcr-1*, *mcr-2* and *mcr-3* genes were widely distributed in pigs in china (Zhang et al., 2018), with the high prevalence rates of 79.2%, 56.3% and 18.7%, respectively, being reported.

CONCLUSION

This study demonstrated the presence of colistin resistance in *Salmonella* isolates in pork from the two different types of slaughterhouses and retails in Thailand, where colistin is used in pigs under veterinary supervision. Notably, this is the first report of *mcr-3* positive MDR *Salmonella* isolates from pork in Thailand despite at the low percentage. Our study revealed that MDR *Salmonella* isolates have widely spread in both standard or low hygiene practices of slaughterhouses and retails in the pork supply chain. Nevertheless, HACCP program in slaughterhouses and better hygienic practices in retails are suggested as the major keys to decrease the prevalence of *Salmonella* isolate in the pork production line. Furthermore, effective monitoring and control programs in slaughterhouses and retails should be continually implemented to reduce the contamination of MDR *Salmonella* isolates carrying *mcr* gene to consumers and improve the safety of final pork products.

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AUTHORS' CONTRIBUTION

W applied laboratory testing, analysed the data and wrote the manuscript. SJ and PP designed the study and approved the final manuscript.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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