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RESEARCH ARTICLE

Antimicrobial susceptibility profiles of *Salmonella* Enteritidis isolated from broiler chicken farms in the Northeast of Thailand

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Abstract

Objectives: This study was performed to investigate antimicrobial susceptibility profiles of *Salmonella enterica* serovar Enteritidis isolated from broiler farms located in the northeastern region of Thailand during 2015 and 2018. Additionally, we examine the presence of mobile colistin resistance (*mcr*) genes in these isolates.

Materials and Methods: This study included 30 *Salmonella* group D isolates, which were obtained from boot swab samples collected from 30 Good Agricultural Practice (GAP)- certified broiler farms. A single isolate was chosen at random from each farm for analysis. The isolates were identified as *S.* Enteritidis using PCR specific to the *SdfI* gene. Antimicrobial susceptibility testing was carried out on the isolates using an automated microbroth dilution method for 27 antimicrobial agents from 12 different classes. The presence of mobile colistin resistance (*mcr*) genes was detected using PCR analysis.

Results: All *Salmonella* group D isolates were genetically identified as *S.* Enteritidis by PCR. The *S.* Enteritidis isolates displayed resistance only to ampicillin (100%), cephalexin (90%), and nitrofurantoin (3.33%). Intermediate resistance to ticarcillin/clavulanate (100%), ciprofloxacin (100%), nitrofurantoin (96.7%), and amoxicillin/clavulanate (36.7%) was also detected. The majority of isolates (90%) exhibited an ampicillin-cephalexin resistance pattern, and only one isolate (3.33%) was multidrug resistant, displaying resistance to ampicillin, cephalexin, and nitrofurantoin. The multiple antibiotic resistance (MAR) index was 0.07 for most isolates, with the highest MAR index of 0.11 found for the multidrug resistance (MDR) strain. Three isolates (10%) harbored the *mcr*-1 gene, but none of these isolates were multidrug resistant.

Conclusions: A low prevalence of multidrug resistance was observed among *S.* Enteritidis isolates. This may be attributed to the implementation of antimicrobial stewardship practices in broiler farms that follow the GAP standard. Nonetheless, the facts that all isolates exhibited reduced susceptibility to ciprofloxacin and the presence of *mcr*-1 gene highlight the importance of ongoing monitoring of antimicrobial susceptibility profiles and *mcr* genes in *S.* Enteritidis from poultry sources.

Keywords: *Salmonella* Enteritidis, broiler, antimicrobial susceptibility, mobile colistin resistance (*mcr*) genes, northeast, Thailand

Introduction

Salmonella remains a significant cause of foodborne illness in humans worldwide, with contaminated eggs and poultry meat being major sources of foodborne salmonellosis (Batz et al., 2012). In Thailand, *Salmonella* is the second leading cause of food poisoning, with *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) being continuously reported as the predominant serovar isolated from human clinical samples and the increasing trend of *S.* Enteritidis infected cases being observed (Bangtrakulnonth et al., 2004; Utrarachkij et al., 2016). *S.* Enteritidis is also the major cause of foodborne salmonellosis outbreaks in humans globally and is considered a bacterial pathogen of poultry (Campioni et al., 2018; Cui et al., 2021; Punchihewage-Don et al., 2022; Yue et al., 2022). Infected chickens generally have no clinical signs, but *S.* Enteritidis colonization of the gastrointestinal and reproductive tracts can lead to prolonged fecal shedding, contaminated environments and contaminated meats and eggs (Campioni et al., 2014; Suzuki, 1994). The shedding of *Salmonella* via feces can facilitate dissemination and colonization, allowing the bacteria to persist and circulate within the poultry farm, and boot swab sampling is an effective method for monitoring and surveying the spread of *Salmonella* (Soria et al., 2017).

The emergence of antimicrobial resistant bacteria (AMR) caused by the improper use of antibiotics poses a global health threat to both humans and animals. According to the World Health Organization (WHO, 2014), AMR has been estimated to cause approximately 23,000 deaths in the United States (US) and 25,000 deaths in the European Union (EU) annually. In Thailand, the significant impact of AMR on health and the economy has been well-documented (Pumart et al., 2012). Healthcare costs have increased due to longer hospital stays, treatment failures, increased antimicrobial usage, and laboratory confirmation test expenses (Founou et al., 2017; Gulen et al., 2015; Hengkrawit and Tangjade, 2022). Antimicrobial-resistant bacteria from animal sources, such as *S.* Enteritidis, can be transmitted to humans through the food production chain. Therefore, continuous monitoring of drug-resistant *S.* Enteritidis is essential.

Due to its high association with human gastroenteritis worldwide, a surveillance program for *S.* Enteritidis in poultry farms has been implemented in Thailand to prevent dissemination and contamination of this important zoonotic pathogen (DLD, 2010). Multidrug-resistant *S.* Enteritidis has been globally reported in isolates from severe human foodborne illness and broiler chicken sources (Liang et al., 2015; Lu et al., 2014; Kipper et al., 2022). Colistin is a critical "last-line" treatment for multidrug-resistant gram-negative infections, including *Salmonella*. The emergence and spread of mobile colistin resistance (*mcr*) genes are a significant concern in public health, as they could limit treatment options. Since the discovery of the *mcr-1* gene in *Escherichia coli* in China by Liu et al. (2016), several other *mcr* genes have been found in *Enterobacteriaceae*, including *Salmonella* spp., in animals and humans worldwide (Carroll et al., 2019; Wang et al., 2018; Wang C et al., 2020; Skove and Monnet, 2016). In Thailand, Sakdinun et al. (2018) detected the *mcr-1* gene in *Salmonella* isolates recovered from poultry farm located in the western region. Recently, Wongsrichai et al. (2021) reported the recovery of the *mcr-3* gene from colistin-resistant MDR *Salmonella* isolated from pork.

Therefore, this study was conducted to monitor multidrug resistance and mobile colistin resistance (*mcr*) genes in *S.* Enteritidis in good agricultural practice (GAP) broiler farms in northeastern Thailand.

Material and Methods

This study did not involve direct contact with animals. Thus, ethical approval for animal experimentation was not required.

Source of samples

A total of 30 *S.* Enteritidis isolates were obtained from 30 boot swab samples collected through the passive surveillance program conducted by the National Institute of Animal Health (NIAH) over a 4-year period (2015-2018). The samples were submitted by broiler farms adhering to Thai agricultural standards (TAS 6901-2017) for good agricultural practices (GAP) in broiler farm management, which are designed to ensure effective and hygienic operation and the production of safe broilers for further processing and consumption. Drug use on these GAP broiler farms is closely monitored and controlled by farm veterinarians.

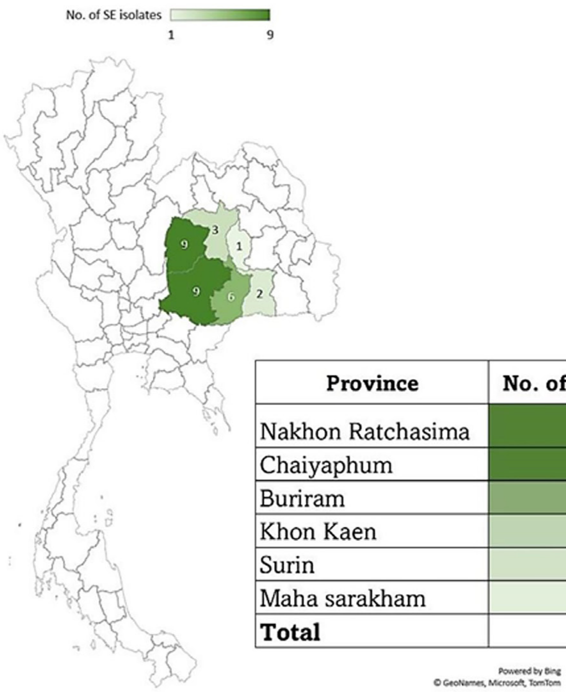
Salmonella spp. was isolated from the submitted samples using the standardized ISO-6579:2002 method, and the isolates were serotyped according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). Between 2015 and 2018, 30 GAP broiler farms located in the northeastern region of Thailand were identified as positive for *Salmonella* serogroup D. These farms were distributed across six provinces in the region (Figure 1), including Nakhon Ratchasima (9 farms), Chaiyaphum (9 farms), Buriram (6 farms), Khon Kaen (3 farms), Surin (2 farms), and Mahasarakham (1 farm). One isolate of *Salmonella* serogroup D was randomly selected from each positive farm for further analysis. Of the 30 isolates, 29 were recovered in 2015 and one in 2018.

Bacterial DNA extraction

Bacterial DNA was extracted from each *Salmonella* isolate by using the rapid boiling method as described by Dashti et al. (2009). Briefly, colonies of were resuspended in 100 µL of TE buffer. The suspensions were then boiled at 100°C for 10 minutes followed by centrifugation at 12,000*xg* for 5 minutes. The supernatant lysates were transferred to 1.5 mL tube and stored at -80°C until used for PCR assays.

Identification of *Salmonella enterica* serovar Enteritidis by PCR

The *Salmonella* isolates were genetically identified as *S.* Enteritidis by the detection of *S.* Enteritidis serovar specific gene, *SdfI*, using specific PCR primers, ENTF: 5'- TGTGTTTTATCTGATGCAAGAGG-3', and ENTR: 5'-TGAACTACGTTCG TTCTTCTGG-3' (Alvarez et al., 2004). The PCR reaction mixture comprised 12.5 µL of 2x Gotaq® Green Master Mix (Promega™, USA), 0.2 µM of each primer, 1 µL of extracted DNA, and nuclease-free water adjusted to a final volume of 25 µL. No template DNA was included as a negative control and DNA of *S.* Enteritidis DMST 15676 was used as a positive control. The PCR cycles were carried out using a Thermal cycler (Bio-Rad™, USA). The cycling conditions consisted of an initial denaturation step of 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds, and a final elongation step at 72°C for 5 min-



Province	No. of SE isolates
Nakhon Ratchasima	9
Chaiyaphum	9
Buriram	6
Khon Kaen	3
Surin	2
Maha sarakham	1
Total	**30**

Figure 1. Geographic map of the provinces in the northeastern region where the broiler chicken farms were found positive for *Salmonella* Enteritidis. One isolate was randomly selected from each of the positive farms.

utes. A 6 µL of PCR product was separated by electrophoresis with 1.5% agarose gel in 1xTBE buffer, and the gel was visualized under UV light using a gel documentation system (Bio-Rad™, USA).

Antimicrobial susceptibility profile of *S.* Enteritidis isolates

Antimicrobial susceptibility of each *S.* Enteritidis isolate was determined using BD Phoenix Automated Microbiology System (Becton Dickinson Phoenix™ ID and AST System, USA). The gram-negative susceptibility panel, NMIC203 which contains 27 antimicrobials belonging to 12 classes including 1) beta-lactams, subclass monobactam and beta-lactamase inhibitors: amoxicillin/clavulanate (AMC), ampicillin (AMP), aztreonam (ATM), piperacillin/tazobactam (TZP), ticarcillin/clavulanate (TIM), 2) cephalosporins: cefazolin (CZ), cephalexin (CN), cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), 3) amphenicols: chloramphenicol (C), 4) fluoroquinolones: norfloxacin (NOR), ciprofloxacin (CIP), 5) polymyxins: colistin (CL), 6) carbapenems: ertapenem (ETP), imipenem (IPM), meropenem (MEM), 7) aminoglycosides: gentamicin (GM), amikacin (AN), Tobramycin (NN), 8) nitrofurantoin: nitrofurantoin (FM), 9) tetracyclines: tetracycline (TE), 10) trimethoprim and sulfonamides: trimethoprim (TMP), trimethoprim/sulfamethoxazole (SXT), 11) glycylcyclines: tigecycline (TGC), and 12) phosphonic acid derivatives: fosfomycin (FF) (Table 1). The test was performed according to manufacturer's instructions. Briefly, *S.* Enteritidis isolates were grown on blood agar plate at 37°C for 18 hours. Each bacterial culture was suspended in 3 mL of AST buffer solution and the optical density of bacterial suspension was adjusted to 0.5 McFarland. The suspension was transferred into the BD Phoenix panel, NMIC203, and then the panel card was placed on antimicrobial drug tray of the instrument BD Phoenix M50 before incubation at 37°C for 24 hours. *Escherichia coli* ATCC 25922 was used as a quality control strain. Results were analyzed with BD Epicenter version 7.22A and V6.61A. The susceptible, intermediate, and resistant properties were determined based on the guideline of Clinical and Laboratory Standard Institute (CLSI, 2020, Table 2.1A), or through breakpoint interpretation by BD Epicenter version 7.22A and V6.61A.

The multiple antibiotic resistance (MAR) phenotypes were assessed for all isolates. The MAR index was calculated as the ratio of the number of antibiotics resistant to the total number of antibiotics tested, as described by Akinola et al. in 2019.

PCR amplification of mobile-colistin resistance (*mcr*) genes

All *S.* Enteritidis isolates were screened for the presence of *mcr-1* to *mcr-9* genes using simplex PCR on a Thermal cycler (BioRad), and the resulting amplicon products were visualized by agarose gel electrophoresis in TBE buffer system. PCR primers are listed in Table 1. Each PCR reaction contained 12.5 µL of 2x Gotaq® Green Master Mix (Promega™, USA), 0.2 µM of each forward and reverse primer, 1 µL of extracted DNA, and nuclease-free water adjusted to a final volume of 25 µL. PCR was performed following the PCR cycle conditions described for each referenced primer pair. Negative controls with no template DNA were included, and DNA of *Escherichia coli* containing the *mcr* genes was used as a positive control. The amplified PCR products were confirmed by Sanger sequencing (ATGC Co; Ltd. Thailand).

The obtained DNA sequences were compared to GenBank database using BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis

Descriptive and numerical statistics were used to analyze the data of antimicrobial susceptibility. The Multiple antibiotic resistance (MAR) index was calculated for each isolate using the formular MAR= a/b, where a corresponds to the number of antibiotics that each isolate was resistant to, and b represents the total number of antibiotics that each isolate was tested for susceptibility phenotype.

Results

Genetic identification of *Salmonella* Enteritidis

The *Salmonella* isolates, which belonged to serogroup D, were identified genetically as serovar *S.* Enteritidis using a conventional PCR that targeted the *Sdfl* gene. The PCR amplicon specific to *S.* Enteritidis at 304 bp was produced by all 30 *Salmonella* isolates examined in this study (Figure 2), indicating that all isolates were *S.* Enteritidis.

Table 1. List of primers and expected product size of mobile colistin resistance (*mcr*) genes, *mcr-1* to *mcr-9*

Target	Primer name	DNA sequence (5' to 3')	Product size (bp)	References
mcr-1	CLR F	CGGTCAGTCCGTTTGTTC	309	Liu et al., 2016
	CLR R	CTTGGTCGGTCTGTAGGG		
mcr-2	MCR2-IF	TGTTGCTTGTGCCGATTGGA	715	Xavier et al., 2016
	MCR2-IR	AGATGGTATTGTTGGTTGCTG		
mcr-3	MCR3-qf	ACCTCCAGCGTGAGATTGTTCCAC	169	Li et al., 2017
	MCR3-qr	GCGGTTTCACCAACGACCAGAA		
mcr-4	MCR-4F	TCACTTTCATCACTGCGTCGTTG	1116	Rebelo et al., 2018
	MCR-4R	TTGGTCCATGACTACCAATG		
mcr-5	MCR-5F	ATGCGGTTGTCTGCATTTATC	1644	Rebelo et al., 2018
	MCR-5R	TCATTGTGGTTGTCCTTTTCTG		
mcr-6	mcr-6_mp_fw	AGCTATGTCAATCCCGTGAT	252	Borowiak et al., 2020
	mcr-6_mp_rev	ATTGGCTAGGTTGTCAATC		
mcr-7	mcr-7_mp_fw	GCCCTTCTTTTCGTTGTT	551	Borowiak et al., 2020
	mcr-7_mp_rev	GGTTGGTCTCTTTCTCGT		
mcr-8	mcr-8_mp_fw	TCAACAATTCTACAAAGCGTG	856	Borowiak et al., 2020
	mcr-8_mp_rev	AATGCTGCGCGAATGAAG		
mcr-9	mcr-9_mp_fw	TTCCCTTTGTTCTGGTTG	1011	Borowiak et al., 2020
	mcr-9_mp_rev	GCAGGTAATAAGTCGGTC		

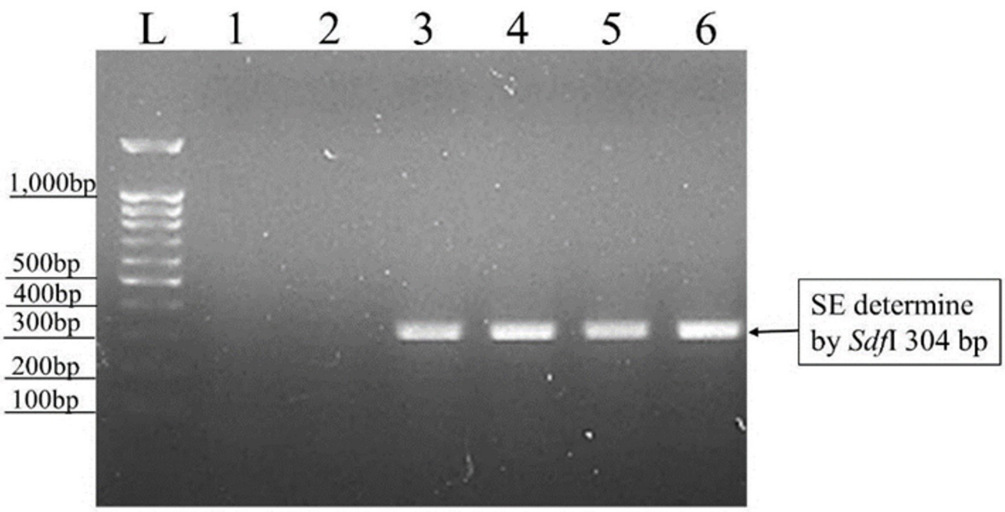


Figure 2. PCR amplification of *SdfI* gene of *Salmonella* Enteritidis (SE) isolates. L: 100 bp DNA ladder (Vivantis), Lane 1: negative control (nuclease free water), Lane 2: *S.*Weltevreden, Lane 3: *S.* Enteritidis DMST 15676, Lane 4-6: *S.* Enteritidis isolates

Antimicrobial susceptibility profile

Antimicrobial susceptibility testing conducted in accordance with the CLSI breakpoints (CLSI, 2020) revealed that all *S*. Enteritidis isolates were susceptible to the tested antimicrobials belonging to 9 classes including carbapenems, aminoglycosides, tetracyclines, amphenicols, polymyxins, fluoroquinolones, trimethoprim and sulfonamides, glycylcyclines, and phosphonic acid derivatives as shown in Table 2. Resistance to at least one drug was observed in the beta-lactams, cephalosporins, and nitrofurantoin classes. We found that all *S.* Enteritidis isolates (100%, 95% CI:88.43-100.00) were phenotypically resistant to ampicillin, and 27 (90%, 95% CI:73.47-97.89) and 1 (3.33%, 95% CI:0.08-17.22) isolates were resistant to cephalexin and nitrofurantoin, respectively. All isolates displayed an intermediate resistance phenotype to ticarcillin/clavulanate (100%) and ciprofloxacin (100%). Additionally, intermediate resistance to nitrofurantoin (96.7%, 95% CI:82.78-99.92) and amoxicillin/clavulanate (36.7%, 95% CI:19.93-56.14) was also detected among these isolates.

Antimicrobial resistance pattern

Most of *S*. Enteritidis isolates in our study had an MAR index of 0.07, as shown in Table 3. The highest MAR index at 0.11 was observed in only one isolate from a boot swab sample collected from a farm in Nakhon Ratchasima. This isolate was identified as a multidrug-resistant strain, displaying resistance to AMP-CN-FM. Most isolates (90%, 95% CI:73.47-97.89) exhibited the same resistant pattern of AMP-CN (Table 3). Only two *S*. Enteritidis isolates (6.67%, 95% CI: 0.82-22.07) were found to be resistant to ampicillin alone.

Presence of mobile colistin resistance (*mcr*) genes

To investigate the spread of mobile colistin resistance genes (*mcr*) among the study isolates, we performed PCR analysis for the detection of *mcr-1* to *mcr-9* genes. Of all 30 *S.* Enteritidis isolates, 3 isolates (10%, 95% CI: 2.11-22.53) collected from three farms located in Chaiyaphum, Khon Kaen, and Nakhon Ratchasima in 2015 were found to harbor *mcr*-1 genes (Table 3, supplementary 1). No isolates were found to carry *mcr-2* to *mcr-9* genes.

Discussion

In this study, all *S.* Enteritidis isolates exhibited a resistant phenotype to ampicillin. The ampicillin resistant rate of 100% is consistent with those observed in *S.* Enteritidis from poultry sources in a previous study conducted in northern Thailand (Chotinan and Tadee, 2015). A high prevalence of resistance to ampicillin at 73% was also reported in *S.* Enteritidis isolated from broiler farms in China (Lu et al., 2014). Reduced susceptibilities to amoxicillin-clavulanate (36.7%) and ticarcillin/clavulanate (100%) were detected in *S.* Enteritidis isolates in this study. These findings are different from those found in *Salmonella* isolated from broilers in Thailand in which resistance to amoxicillin-clavulanate was found at 26.6% (Pongaran, 2019).

A high proportion of resistance (90%) to cephalexin, a first-generation cephalosporin, was found in this study. Therefore, the most common resistance profile (90%) among the tested *S.* Enteritidis isolates was resistance to both ampicillin and cephalexin. Very low resistance rates to cephalexin in *Salmonella* from poultry sources were also observed in other countries (Plawinska-Czarnak, 2022; Shang K. et al., 2021). Nevertheless, all *S.* Enteritidis isolates in this study were still susceptible to third- and fourth-generation cephalosporins. A low rate of resistance to these generations of cephalosporins at 1.8% was also observed in *Salmonella* isolates from broilers in Thailand (Phongaran et al., 2019). However, a relatively high resistance rate to ceftazidime (36%) was found in *Salmonella* isolated from poultry farms in the central region of Thailand (Perestrelo et al., 2016). Resistance to ampicillin and cephalexin, as well as reduced susceptibilities to amoxicillin/clavulanate and ticarcillin/clavulanate found in our *S.* Enteritidis isolates could be due to the antimicrobial drugs used in broiler farms in the study area. It has been reported that amoxicillin, colistin, doxycycline, oxytetracycline, and tilmicosin are commonly used in broiler farms in Thailand (Wongsuwan et al., 2018). Differences in resistance rates observed in this study and previous studies suggested that the use of antimicrobial agents in broiler farms in Thailand may vary depending on farm type and study sites.

In this study, the prevalence of nitrofurantoin-resistant *S.* Enteritidis was low at 3.3%, which is much lower than the rates reported at 90% in Brazil (Cardoso et al., 2006) and at 96.1%-98.2% in China (Yu et al., 2021; Wang

Table 2. Antimicrobial susceptibility profiles of *Salmonella* Enteritidis isolated from the GAP broiler farms in the northeastern region of Thailand

Class	Antimicrobial drug lists	Drug concentration in PHOENIX NMIC 203 (µg/ml)	Resistance break point[a] (µg/ml)	MIC range of the study isolates (µg/ml)	Number of isolates		
					S	I	R
1.Beta-lactams (penicillins)	AMC	4/2-16/8	≥32/16	8/4-16/8	19	11	0
	AMP	4-16	> 16[b]	>16	0	0	30
	ATM	0.5-16	≥ 16	≤0.5	30	0	0
	TZP	4/4-64/4	≥ 128/4	≤4/4	30	0	0
	TIM	4/2-64/2	≥ 128/2	32/2-64/2	0	30	0
2.Beta-lactams (cephalosporins)	CZ	1-8	≥ 8	4	30	0	0
	CN	4-32	≥ 8[b]	≤4-8	3	0	27
	FEP	0.5-16	≥ 16	≤0.5	30	0	0
	FOX	4-32	≥ 32	≤4	30	0	0
	CAZ	0.5-32	≥ 16	≤0.5-1	30	0	0
	CRO	0.5-32	≥ 4	≤0.5	30	0	0
3. Amphenicols	CHL	4-32	≥ 32	≤4	30	0	0
4. Fluoroquinolone	NOR	0.25-8	≥ 16	1-2	30	0	0
	CIP	0.125-2	≥ 1	0.125-0.25	0	30	0
5. Polymyxin	CL	1-4	≥ 4	≤1	30	0	0
6. Carbapenems	ETP	0.25-2	≥ 2	≤0.25	30	0	0
	IPM	1-8	≥ 4	≤1	30	0	0
	MEM	0.25-16	≥ 4	≤0.25	30	0	0
7. Aminoglycosides	GM	1-8	≥ 16	≤1	30	0	0
	AN	4-32	≥ 64	≤4	30	0	0
	NN	1-8		≤1	30	0	0
8. Nitrofurantoin	FM	32-128	≥ 128	64-128	0	29	1
9. Tetracycline	TE	2-16	≥ 16	≤2	30	0	0
10.Sulfonamild Trimetoprim	TMP	1-8	≥ 16	≤1	30	0	0
	STX	1/19-8/152	≥ 4/76	≤1/19	30	0	0
11.Glycylcyclin	TGC	0.5-4	≥ 8[c]	≤0.5-1	30	0	0
12.Phosphonic acid derivative	FF	16- 64	≥ 256	≤16	30	0	0

Letter abbreviation, AMC: Amoxicillin/Clavulanate, AMP: Ampicillin, ATM: Aztreonam, TZP: Piperacillin/tazobactam, TIM: Ticarcillin/Clavulanate, CZ: Cefazolin, CN: Cephalexin, FEP: Cefepime, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxone, CHL: Chloramphenicol, NOR: Norfloxacin, CIP: Ciprofloxacin, CL: Colistin, ETP: Ertapenem, IPM: Imipenem, MEM: Meropenem, GM: Gentamicin, AN: Amikacin, NN: Tobramycin, FM: Nitrofurantoin, TE: Tetracycline, TMP: Trimethoprim, STX: Trimethoprim/Sulfamethoxazole, TGC: Tigecycline, FF: Fosfomycin; S: susceptible; I: intermediate; R: resistant; MIC: minimal inhibitory concentration, a; breakpoints based on CLSI breakpoints 2020, b; breakpoint interpreted by BD Epicenter version 7.22A and V6.61A, c; breakpoints based on Yaghoubi et al., 2022

Table 3. Antimicrobial resistance pattern and presence of *mcr* genes in *Salmonella* Enteritidis isolated from the GAP-certified broiler farms in the northeastern region of Thailand

No.	Type of sample	Year	Province	No. of antimicrobial drug resistance	MAR index	Resistance pattern	Presence of *mcr* genes
1	boot swab	2015	BRM	1	0.04	AMP	-
2	boot swab	2015	BRM	2	0.07	AMP-CN	-
3	boot swab	2015	BRM	2	0.07	AMP-CN	-
4	boot swab	2015	BRM	2	0.07	AMP-CN	-
5	boot swab	2015	BRM	2	0.07	AMP-CN	-
6	boot swab	2015	BRM	2	0.07	AMP-CN	-
7	boot swab	2015	CPM	1	0.04	AMP	-
8	boot swab	2015	CPM	2	0.07	AMP-CN	-
9	boot swab	2015	CPM	2	0.07	AMP-CN	-
10	boot swab	2018	CPM	2	0.07	AMP-CN	-
11	boot swab	2015	CPM	2	0.07	AMP-CN	-
12	boot swab	2015	CPM	2	0.07	AMP-CN	*mcr-1*
13	boot swab	2015	CPM	2	0.07	AMP-CN	-
14	boot swab	2015	CPM	2	0.07	AMP-CN	-
15	boot swab	2015	CPM	2	0.07	AMP-CN	-
16	boot swab	2015	KKN	2	0.07	AMP-CN	-
17	boot swab	2015	KKN	2	0.07	AMP-CN	*mcr-1*
18	boot swab	2015	KKN	2	0.07	AMP-CN	-
19	boot swab	2015	MKM	2	0.07	AMP-CN	-
20	boot swab	2015	NMA	2	0.07	AMP-CN	-
21	boot swab	2015	NMA	2	0.07	AMP-CN	-
22	boot swab	2015	NMA	2	0.07	AMP-CN	-
23	boot swab	2015	NMA	2	0.07	AMP-CN	-
24	boot swab	2015	NMA	2	0.07	AMP-CN	-
25	boot swab	2015	NMA	2	0.07	AMP-CN	*mcr-1*
26	boot swab	2015	NMA	2	0.07	AMP-CN	-
27	boot swab	2015	NMA	2	0.07	AMP-CN	-
28	boot swab	2015	NMA	3	0.11	AMP-CN-FM	-
29	boot swab	2015	SRN	2	0.07	AMP-CN	-
30	boot swab	2015	SRN	2	0.07	AMP-CN	-

MAR: Multiple antibiotic resistance
BRM: Buriram, CPM: Chaiyaphum, KKN: Khon Kaen, NMA: Nakhon Ratchasima,
MKM: Mahasarakham, SRN: Surin, AMP: Ampicillin, CN: Cephalexin, FM: Nitrofurantoin

X et al., 2020). Resistance to nitrofurantoin is still present, although this drug has been withdrawn from use in animal production in Thailand since 2002 (Phongvivat, 2004). However, nitrofurantoin remains a drug of choice for treating urinary tract infections caused by *Enterobacteriaceae* in humans. Recent studies have shown an increasing prevalence of nitrofurans-resistant *Enterobacteriaceae* in humans (Osei Sekyere, 2018; Yue et al., 2020). The finding of nitrofurantoin-resistant *S.* Enteritidis in broiler farms could be due to the use of nitrofurans in humans and environmental contamination.

Fluoroquinolones are the recommended drugs for treatment of salmonellosis in humans (Chen et al., 2013; Crump et al., 2015). We found reduced susceptibility to ciprofloxacin in all *S.* Enteritidis isolates. Resistance to ciprofloxacin in *Salmonella* isolates from poultry sources has been reported in previous studies at rates ranging from 0% to 29.4% (Na Lampang et al., 2014; Phongaran et al., 2019; Chotinan & Tadee, 2015; Perestrelo et al., 2016; Pelyuntha et al., 2022). The reduced susceptibility or resistance to ciprofloxacin in *Salmonella* isolated from chickens or chicken products could be attributed to the use of fluoroquinolones at the farm level. A study by Na Lampang et al. (2007) reported that a combination of amoxicillin-enrofloxacin was the commonly used antimicrobial for disease prevention in broiler farms.

Multidrug-resistant *S.* Enteritidis is a global issue frequently reported among *Salmonella* isolates from severe human foodborne illness and broiler chicken sources. In this study, a relatively low prevalence of multidrug-resistant *S.* Enteritidis (3.3%) was observed, compared to the prevalence of MDR in *Salmonella* isolated from chicken farms in northern Thailand (6.45%) (Na Lampang, 2014). Interestingly, a considerably high prevalence of MDR was detected in *Salmonella* isolated from chicken farms in the western (84.7%) and central (64.4%) regions of Thailand (Sakdinun et al., 2016; Perestrelo et al., 2016), where intensive chicken production is clustered (Van Boeckel, 2012). In a previous study by Plawinska-Czarnak et al. in 2022, high MAR index values were observed in a significant proportion of multidrug-resistant (MDR) *Salmonella* isolates. In the present study, we investigated the MAR index values of *S. Enteritidis* isolates obtained from boot swab samples in the study farms. Our results indicate limited antibiotic resistance in the isolates, as evidenced by MAR indexes ranging from 0.04 to 0.11. These results are in contrast to the previous studies by Akinola et al. in 2019 and Plawinska-Czarnak et al. in 2022, which reported higher MAR index values for *S.* Enteritidis in chicken sources. (Akinola et al., 2019; Plawinska-Czarnak et al., 2022). The low prevalence of MDR and low MAR index observed in this study could be attributed to the judicious use of antimicrobials in GAP broiler farms in the study area.

In this study, all *S.* Enteritidis isolates exhibited susceptibility to colistin, although the *mcr-1* gene was detected in 10% of them. Sakdinun et al. (2018) reported the recovery of colistin-resistant *S.* Enteritidis strains from boot swab samples collected from broiler farms in western Thailand between 2013-2016, with a prevalence of 14.3% (1/8). However, the presence of the *mcr-1* gene was not detected in *S.* Enteritidis isolates, but in an isolate of *S.* Give with an MIC values of 8 µg/mL. The findings of *mcr* genes in colistin-susceptible *Salmonella* were also reported in previous studies (Bertelloni et al., 2022; Pungpian et al., 2021). This suggests that the presence of the *mcr* genes does not always correlate with phenotypic resistance to colistin. Therefore, screening for *mcr* genes should be performed in both colistin-susceptible and colistin-resistant *Salmonella* isolates.

One of the limitations of this study is the small number of *S.* Enteritidis isolates detected in the GAP broiler farms under investigation. Moreover, the majority of the isolates were found in 2015, and only one isolate was detected in 2018. As a result, it is impossible to examine the annual trends in antimicrobial resistance and the presence of mobile colistin resistance gene. Furthermore, the absence of information regarding the antimicrobial usage history in the study farms renders it impossible to analyze a correlation between drug usage and the observed pattern of antimicrobial resistance in the farms.

In conclusion, the information gathered from this study provides fundamental knowledge on the antimicrobial resistance pattern and occurrence of *mcr* gene in *S.* Enteritidis among GAP broiler farms located in the northeastern of Thailand. Despite the low prevalence of multidrug-resistant (MDR) *S.* Enteritidis observed in this study, the reduced susceptibility to ciprofloxacin detected in all isolates and the presence of the *mcr-1* gene underscore the need for continued monitoring of antimicrobial resistance in the *S.* Enteritidis strain collection of poultry origins. Such monitoring is essential to achieve the sustainable goal of controlling foodborne MDR *S.* Enteritidis infections in public health care.

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Conflict of Interest Statement

The authors declare that we have no conflict of interest in this work.

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