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Research article

Occurrence of ciprofloxacin resistance, plasmid-mediated quinolone resistance genes and virulence factors in *Salmonella enterica* serovar Enteritidis isolated from broiler farms in the central and northeastern parts of Thailand

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Abstract

Salmonella Enteritidis is one of the most common serovars associated with food-prisoning and gastroenteritis in humans. Fluoroquinolone resistance in non-typhoidal *Salmonella* including *S.* Enteritidis has been increasing globally and is considered as an urgent threat to public health. In this study, we aimed to investigate the occurrence of ciprofloxacin resistance and plasmid-mediated quinolone resistance (PMQR) genes, and to examine the virulence gene profiles of 69 *S.* Enteritidis isolates. The isolates were obtained from 47 boot swab and 22 intestinal content samples, collected from 69 Good Agricultural Practice (GAP)- certified broiler farms located in the central and northeastern regions of Thailand. One isolate was randomly selected from each farm for analysis. Ciprofloxacin susceptibility of these isolates was determined using microbroth dilution method. PCR was used to detect 5 common PMQR genes *(qnrA, qnrB, qnrS, aac(6')-Ib-cr* and *qepA)* and 12 important virulence genes *(agfA, invA, spaN, prgH, sitC, ssaQ, mgtC, sopB, sifA, tolC, cdtB* and *spvC)*. All *S.* Enteritidis showed reduced susceptibility to ciprofloxacin, with the MIC values of 0.125-0.50 µg/mL. However, these isolates did not carry PMQR genes investigated. All 69 *S.* Enteritidis isolates exhibited an identical virulence profile, characterized by the presence of 11 virulence genes, except for *cdtB*. The presence of virulence genes identified in invasive salmonellosis among *S.* Enteritidis isolates with reduced susceptibility to ciprofloxacin could pose public health concerns. Our findings underline the need for constant monitoring of ciprofloxacin-resistant *S.* Enteritidis in the poultry production chain to reduce public health risk

Keywords: *Salmonella* Enteritidis, broiler farms, virulence genes, Plasmid-Mediated Quinolone Resistance Genes, Thailand

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INTRODUCTION

Salmonella spp. is an important zoonotic pathogen affecting human health and well-being by causing foodborne gastroenteritis worldwide. More than 2,500 serovars of *Salmonella* have been discovered (Jajere, 2019). *Salmonella* Enteritidis is one of the common serovars most frequently isolated from food-prisoning and gastroenteritis in humans (Campioni et al., 2018; Yue et al., 2022). *S.* Enteritidis is also considered a pathogen of poultry (Wang et al., 2020). Although it generally causes no symptom or mild illness in chickens, *S.* Enteritidis colonizes gastrointestinal and reproductive tracts leading to contaminated chicken meats and eggs which are the main sources of foodborne salmonellosis in humans (Campioni et al., 2014; Gast et al., 2022). An increasing trend of *S.* Enteritidis infection in humans has been observed globally including Thailand (Whistler et al., 2018; Balasubramanian et al., 2019) and *S.* Enteritidis in poultry farms has been closely and legally monitored (DLD, 2010).

Fluoroquinolones, such as ciprofloxacin, are the recommended drugs for treatment of severe *Salmonella* infections in humans (Li et al., 2018). However, the prevalence of ciprofloxacin resistance in non-typhoidal *Salmonella* isolates has increased in several countries, including Thailand (Utrarachkij et al., 2017; Sriyapai et al., 2021; Hengkrawit et al., 2022; Piekarska et al., 2023). With an increasing global trend of ciprofloxacin resistant *Salmonella*, WHO has announced this resistant bacterium as an urgent threat to public health (WHO, 2019). Treatment failures caused by *Salmonella* strains with resistance or reduced susceptibility to ciprofloxacin have been documented (Dimitrov et al., 2007; Pham Thanh et al., 2016). Several mechanisms of fluoroquinolone resistance have been described (Li et al., 2018). Mutations in the quinolone resistance-determining region (QRDR) of the topoisomerase and efflux pump encoding genes located on bacterial chromosome are the main mechanisms of resistance. Furthermore, plasmid-mediated quinolone resistance (PMQR) mechanisms were first discovered in 1998 (Martínez-Martínez et al., 1998). Since then, various PMQR genes, including *qnr* variants, *aac(6')-Ib-cr*, *qepA*, and *oqxAB*, have been identified (Li et al., 2018). PMQR genes encode for Qnr proteins that function to protect DNA gyrase and topoisomerase IV from quinolone inhibition, for aminoglycoside acethytransferase AAC(6′)-Ib that involves in acethylation of quinolone, and for pumps QepAB and OqxAB that enhance efflux of quinolone (Li et al., 2018). Although the quinolone resistance mechanisms mediated by PMQR provide only low-level of resistance, PMQR genes can facilitate the selection of higher-level resistance when the bacteria harboring these genes are exposed to quinolone (de Toro et al., 2010). Due to plasmid mobility, the dissemination of PMQR in pathogenic bacteria such as *Salmonella* is of great concern. PMQR genes appear to be spreading across the globe and have been found in *Salmonella* isolates from both humans and animals (Karp et al., 2018; Kuang et al., 2018), potentially causing infection by *Salmonella* containing PMQR genes more difficult to treat.

The pathogenicity of *Salmonella* is mediated by many virulence factors located on both chromosomal and plasmid (Wang et al., 2020). The expression of genes involved in adhesion and invasion of host cells, and extra-intestinal spreading has been demonstrated in severe cases of salmonellosis in both

humans and broiler chickens (Suez et al., 2013; Mezal et al., 2014). The main virulence factors of *Salmonella* are located in the *Salmonella* pathogenicity islands (SPIs) on the chromosome (Wang et al., 2020). The virulence genes located on SPIs plays several importance roles in *Salmonella*'s pathogenicity, such as host cell recognition and adaptation, cell adhesion and invasion, toxin production, and regulation of iron and magnesium uptake (Wang et al., 2020). Some virulence genes of *Salmonella* are located on the *Salmonella* plasmid virulence (spv) operon, which is a highly conserved region on the *Salmonella* virulence plasmids. The *spv* genes play essential role in intracellular survival and systemic infection (Wang et al., 2020). Information on the virulence genes of *S.* Enteritidis isolated from broiler farms in Thailand is still very limited.

Given the concern regarding the resistance of *Salmonella* to the drugs of choice for treatment of severe *Salmonella* infections, we aimed to investigate the occurrence of ciprofloxacin resistance and PMQR genes in *S.* Enteritidis isolated from broiler farms in the central and northeast Thailand. Additionally, we examined the virulence gene profiles of the *S.* Enteritidis isolates. Understanding the virulence gene profiles of locally isolated field *S.* Enteritidis strains could potentially help predict the clinical outcomes of infected cases.

MATERIALS AND METHODS

Salmonella Enteritidis isolates

A total of 69 *S.* Enteritidis isolates in this study were recovered from 47 boot swabs and 22 intestinal content samples of apparently healthy chicks aged 2-3 days. The samples were submitted as part of the surveillance program for *Salmonella* in broiler farms, conducted by the National Institute of Animal Health (NIAH) during 2015-2018. These samples were collected from 69 broiler farms that have obtained certification for complying with the Good Agricultural Practices (GAP) in broiler farm management, according to Thai agricultural standards (TAS 6901-2017, ACSF). These standards aim to ensure effective and hygienic operations for the production of safe broilers for further processing and consumption. Additionally, the administration of drugs on the GAP-certified broiler farms is closely monitored and regulated by specialized farm veterinarians. These 69 GAP-certified broiler farms were located across 11 provinces in the central and northeastern parts of Thailand. The details of isolates are shown in Table 3.

Boot swab samples were collected from the submitted farms by covering the boots with plastic shoe covers and immersing them in a solution of 0.8-0.85% sodium chloride in sterile water. The covered boots were then used to walk over the area within the house, and two pairs of shoe covers were used to cover the entire area (DLD, 2010). Following collection, the samples were placed in a tightly sealed sample collection bag and labeled with clear details before submission to the NIAH.

Salmonella spp. was isolated from the submitted samples using the ISO-6579:2002 standardized method. Briefly, the samples were pre-enriched in buffered peptone water (Difco, France) at 37°C for 18 h. Next, 100 microliters of the pre-enriched sample were transferred onto Modified Semi-

solid Rappaport-Vassiliadis agar (MSRV, Difco, France) and incubated at 42°C for 24 h. A loopful of inoculum from each MSRV agar culture was streaked onto Xylose Lysine Deoxycholate (XLD, Difco, France) and Brilliant Green Agar (BGA, Difco, France) plates and incubated at 37°C for 24 h. Typical colonies of *Salmonella* were selected from the culture plates for further analysis. *Salmonella* isolates were classified into serogroup D according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). One isolate of *Salmonella* serogroup D was randomly selected from each of the 69 farms. These *Salmonella* group D isolates were further identified as *S.* Enteritidis by the detection of serovar specific gene, *SdfI*, following the protocol described by Alvarez et al. (2004). *S.* Enteritidis DMST 15676 was used as a positive control for PCR identification.

Ciprofloxacin susceptibility testing

All isolates were determined for their susceptibility to ciprofloxacin using the microbroth dilution method following the guidelines of the Clinical and Laboratory Standards Institute M100 27th standard (CLSI, 2017). *Escherichia coli* ATCC 25922 was used as a quality control strain. Isolates were classified as susceptible to ciprofloxacin with a minimum inhibitory concentration (MIC) of ≤ 0.06 µg/mL, intermediate (reduced susceptibility) with an MIC of < 1 µg/mL and > 0.06 µg/mL, and resistant with an MIC of ≥ 1 µg/mL, based on the Clinical and Laboratory Standards Institute guideline (CLSI, 2020, Table 2.1A).

Bacterial DNA extraction

Bacterial DNA was extracted from each isolate using the rapid boiling method, as described by Dashti et al. (2009). Briefly, *S.* Enteritidis colonies were collected and resuspended in 100 µL of TE buffer. The suspensions were boiled at 100°C for 10 minutes and then centrifuged at 12,000*xg* for 5 minutes to pellet the cellular debris. The supernatant lysates were then transferred to 1.5 mL tubes and preserved at -80°C until further use.

PCR detection of plasmid-mediated quinolone resistance (PMQR) genes

Simplex PCRs for the detection of five common PMQR genes, including *qnrA*, *qnrB*, *qnrS*, *aac(6')-Ib-cr* and *qepA* were performed as previously described (Park et al., 2006; Robicsek et al., 2006; Shams et al., 2015). The PCR primers and cycle conditions for the detection of each gene are presented in Table 1. The final volume of each reaction mixture was at 25 µL containing 12.5 µL of 2× GoTaq® Green Master Mix (Promega, USA), 0.2 µM of each primer for each gene, and 2 µL of extracted DNA. No template DNA was included as a negative control and DNA of *Escherichia* coli containing PMQR genes was included as a positive control (Murase et al., 2022). PCR products were separated by electrophoresis with 1.5% agarose gel in 1×TBE buffer and were visualized under UV light using a gel documentation system (Bio-Rad, USA).

Table 1 List of primers and expected amplicon sizes for the detection of plasmid-mediated quinolone resistance (PMQR) genes

Target gene	Primer name	DNA sequence (5' to 3')	Size (bp)	Reference
qnrA	qnrA F	ATTTCTCACGCCAGGATTTG	516	Robicsek et al., 2006
	qnrA R	GATCGGCAAAGGTTAGGTCA		
qnrB	qnrB F	GATCGTGAAAGCCAGAAAGG	469	Robicsek et al., 2006
	qnrB R	ACGATGCCTGGTAGTTGTCC		
qnrS	qnrS F	ACGACATTCGTCAACTGCAA	417	Robicsek et al., 2006
	qnrS R	TAAATTGGCACCCTGTAGGC		
aac(6')-*Ib-cr*	aac(6')-Ib-cr F	TTGCGATGCTCTATGAGTGGCTA	482	Park et al., 2006
	aac(6')-Ib-cr R	CTCGAATGCCTGGCGTGTTT		
qepA	qepA F	GGACATCTACGGCTTCTTCG	720	Shams et al., 2015
	qepA R	AGCTGCAGGTACTGCGTCAT		

PCR Detection of virulence genes

The presence of 12 *Salmonella* virulence genes, including *agfA*, *invA*, *spaN*, *prgH*, *sitC*, *ssaQ, mgtC*, *sopB, sifA*, *tolC*, *cdtB* and *spvC* was determined by simplex PCR assays. These virulence genes are located in the *Salmonella* pathogenicity islands (SPI) 1-3, 5, 11, or outside of SPI of chromosomal DNA, or in the virulence plasmid. These virulence factors play important roles in the pathogenicity of *Salmonella*, as described in Table 2. The PCR primers for detection of each virulence gene are presented in Table 2. Each PCR reaction contained 12.5 µL of 2x Gotaq® Green Master Mix (Promega™, USA), 0.2 µM of each primer, 2 µL of extracted DNA and nuclease-free water adjusted to a final volume of 25 µL. PCR was run following the cycles described for each referenced primer pair (Table 2). The DNA of *S.* Weltevreden that harbours *prgH*, *spaN*, *sitC*, *sopB*, *tolC*, *sifA*, and *cdtB* genes was used as a PCR positive control. No template DNA was included as a negative control. PCR products were separated by electrophoresis with 1.5% agarose gel in 1×TBE buffer and the gel was visualized under UV light using a gel documentation system (Bio-Rad, USA). The amplified PCR products of each gene were confirmed by Sanger sequencing with specific primers. The obtained sequence data were compared to the GenBank database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 2 List of virulence genes, their genomic locations and functional roles, and primers for the detection of each virulence gene

Target gene	Virulence loci	Functional role	Primer name	DNA sequences (5'-3')	Product size (bp)	References
invA	SPI-1	Invasion into host cells	invA-F	TCATCGCACCGTCAAAGGAACC	284	Zishiri et al., 2016
			invA-R	GTGAAATTATCGCCACGTTCGGGCAA		
spaN	SPI-1	Invasion into host cells	spaN-F	AAAAGCCGTGGAATCCGTTAGTGAAGT	504	Skyberg et al., 2006
			spaN-R	CAGCGCTGGGGATTACCGTTTTG		
prgH	SPI-1	Invasion and survival within host cells	prgH-F	GCCCGAGCAGCCTGAGAAGTTAGAAA	756	Skyberg et al., 2006
			prgH-R	TGAAATGAGCGCCCCTTGAGCCAGTC		
sitC	SPI-1	Iron transport	sitC-F	CAGTATATGCTCAACGCGATGTGGGTCTCC	768	Skyberg et al., 2006
			sitC-R	CGGGGCGAAAATAAAGGCTGTGATGAAC		
ssaQ	SPI-2	Replication in macrophages and systemic infection	ssaQ-F	GAATAGCGAATGAAGAGCGTCC	678	Soto et al., 2006
			ssaQ-R	CATCGTGTTATCCTCTGTCAGC		
mgtC	SPI-3	Magnesium and Phosphate transport and survival within macrophages	mgtC-F	TGACTATCAATGCTCCAGTGAAT	655	Soto et al., 2006
			mgtC-R	ATTACTGGCCGCTATGCTGTTG		
sopB	SPI-5	Survival within B cells and chloride secretory	sopB -F	CGGACCGGCCAAGCAACAAAACAAGAAGAAG	220	Skyberg et al., 2006
			sopB-R	TAGTGATGCCCGTTATGCGTGAGTGTATT		
cdtB	SPI-11	Enterotoxin production	cdtB-F	ACAACTGTCGCATCTCGCCCCGTCATT	268	Skyberg et al., 2006
			cdtB-R	CAATTTGCGTGGGTTCTGTAGGTGCGAGT		
agfA	*a*gfA operon	Host cell adhesion and biofilm formation	agfA-F	TCCACAATGGGGCGGCG	350	Borges et al., 2013
			agfA-R	GCGCCTGACGCACCATTACGCTG		
sifA	Outside SPIs	Replication in host cells	sifA-F	TTTGCCGAACGCGCCCCCACACG	450	Skyberg et al., 2006
			sifA-R	GTTGCCTTTTCTTGCGCTTTCCACCCATCT		
tolC	Outside SPIs	Efflux pump, host cell invasion, and maintenance of cell membrane integrity	tolC-F	TACCCAGGCGCAAAAAGAGGCTATC	161	Skyberg et al., 2006
			tolC-R	CCGCGTTATCCAGGTTGTTGC		
spvC	Virulence plasmid	Systemic infection	spvC-F	CGGAAATACCATCTACAAATA	699	Swamy et al. 1996
			spvC-R	CCCAAACCCATACTTACTCTG		

SPI: Salmonella Pathogenicity Island

Data analysis

The data on the presence of ciprofloxacin resistance, plasmid-mediated quinolone resistance genes, and virulence factors were subject to both descriptive and analytical statistical analysis. The 95% confidence interval was calculated using the exact binomial confidence intervals for proportions (Kohn et al., 2021). The Mann-Whitney test was used to compare the ciprofloxacin MIC values of *S.* Enteritidis between the two regions, and the Kruskal-Wallis test was used to compare the ciprofloxacin MIC values over a four-year period. The tests were conducted using GraphPad Prism version 9.5.1 for Windows (www.graphpad.com). The p-value ≤ 0.05 was considered statistically significant.

RESULTS

Occurrence of ciprofloxacin-resistant *Salmonella* Enteritidis

All 69 *S.* Enteritidis recovered from 69 GAP-certified broiler farms in this study showed no resistance to ciprofloxacin. However, all of the isolates (100%, 95% CI: 94.79-100%) exhibited an intermediate susceptibility phenotype to ciprofloxacin, with the MIC values ranging from 0.125 to 0.5 µg/mL (Figure 1 and Table 3). Most isolates (92.8%, 95% CI: 83.89-97.61%) had an MIC value of 0.25 µg/mL, and only 3 (4.35%, 95% CI: 0.91-12.18%) isolates showed an MIC value of 0.50 µg/mL. Thus, the MIC_{50} and MIC_{90} of ciprofloxacin that inhibited 50% and 90% of the isolates were equal at 0.25 µg/mL. In addition, we found no significant differences in the ciprofloxacin MIC values of *S.* Enteritidis strains isolated from farms in two different regions (p value = 0.29) and over a four-year period (p value = 0.99), as shown in Figure 1.

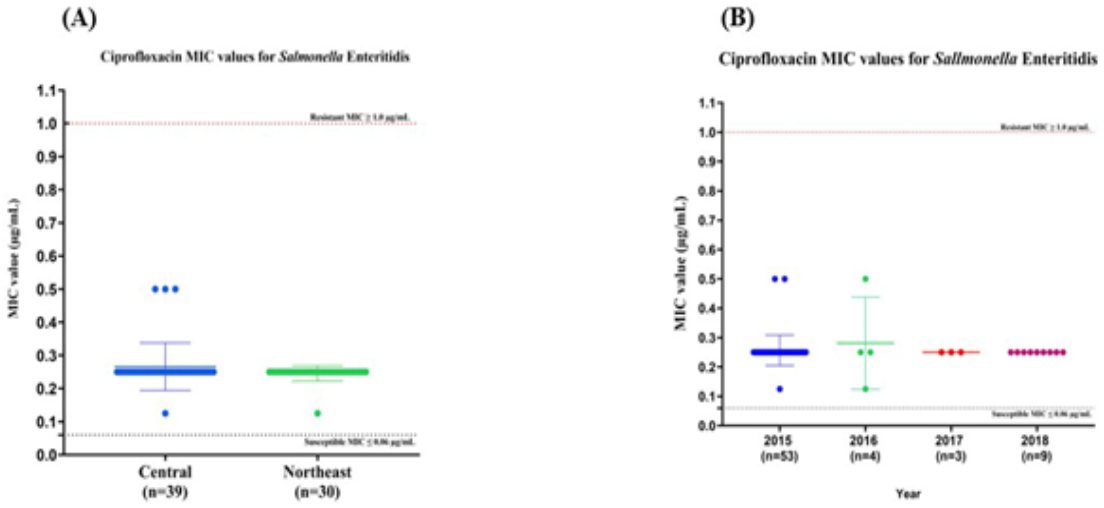

Figure 1 The minimum inhibitory concentrations (MIC) of ciprofloxacin in *Salmonella* Enteritidis isolates, grouped by region (A) and by year (B). Each dot on the graph represents a single isolate of *S.* Enteritidis.

Occurrence of plasmid-mediated quinolone resistance (PMQR) genes in *Salmonella* Enteritidis

All 69 *S.* Enteritidis isolates exhibiting reduced susceptibility to ciprofloxacin were subject to screening for five PMQR genes, including *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*, in order to investigate whether the intermediate susceptibility phenotypes observed in these isolates were mediated by PMQR.These selected genes have been commonly reported in non-typhoidal *Salmonella* and *E. coli* strains in Thailand (Murase et al., 2021; Sriyapai et al., 2021). None of the 69 isolates (0%, 95% CI: 0.00-5.21%) were found to harbor any of the five PMQR genes using the PCR assay (Table 3).

Virulence gene patterns of *Salmonella* Enteritidis

To investigate the potential pathogenicity of *S.* Enteritis isolates, 12 virulence genes known to be associated with *Salmonella*-induced gastroenteritis and systemic infections were examined by PCR. The virulence gene profiles of all 69 *S.* Enteritis isolates (100%, 95% CI: 94.79-100%) obtained from either boot swab or intestinal content samples were found to be identical (Table 3). Specifically, all isolates harbored 11 virulence genes, including *agfA*, *invA*, *spaN*, *prgH*, *sitC*, *ssaQ, mgtC*, *sopB, sifA*, *tolC*, and *spvC*. The *cdtB* gene was not detected in any of the isolates.

Table 3 Isolation and results of ciprofloxacin susceptibility test, plasmid-mediated quinolone resistance (PMQR) gene analysis and virulence gene profiles of *Salmonella* Enteritidis in this study.

No.	Sample type	Province	Region	Year	CIP susceptibility phenotype	MIC µg/mL	PMQR	Virulence gene profile
1	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
2	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
3	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
4	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
5	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
6	Intestinal content	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
7	Intestinal content	LRI	CEN	2015	I	0.50	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
8	Intestinal content	LRI	CEN	2015	I	0.50	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
9	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
10	boot swab	NYK	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
11	boot swab	SRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
12	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
13	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
14	boot swab	PNB	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
15	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
16	boot swab	SRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
17	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
18	boot swab	NSN	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
19	boot swab	NSN	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
20	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
21	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
22	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
23	boot swab	PNB	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
24	boot swab	LRI	CEN	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
25	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*

Table 3 Isolation and results of ciprofloxacin susceptibility test, plasmid-mediated quinolone resistance (PMQR) gene analysis and virulence gene profiles of *Salmonella* Enteritidis in this study. (Cont.)

No.	Sample type	Province	Region	Year	CIP susceptibility phenotype	MIC µg/mL	PMQR	Virulence gene profile
26	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
27	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
28	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
29	boot swab	NMA	NE	2015	I	0.125	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
30	boot swab	SRN	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
31	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
32	boot swab	MKM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
33	boot swab	KKN	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
34	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
35	boot swab	SRN	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
36	boot swab	KKN	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
37	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
38	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
39	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
40	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
41	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
42	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
43	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
44	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
45	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
46	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
47	boot swab	NMA	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
48	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
49	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
50	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*

Table 3 Isolation and results of ciprofloxacin susceptibility test, plasmid-mediated quinolone resistance (PMQR) gene analysis and virulence gene profiles of *Salmonella* Enteritidis in this study. (Cont.)

No.	Sample type	Province	Region	Year	CIP susceptibility phenotype	MIC µg/mL	PMQR	Virulence gene profile
51	boot swab	CPM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
52	boot swab	KKN	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
53	boot swab	BRM	NE	2015	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
54	boot swab	LRI	CEN	2016	I	0.125	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
58	Intestinal content	LRI	CEN	2016	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
55	Intestinal content	ND	CEN	2016	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
56	Intestinal content	ND	CEN	2016	I	0.50	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
57	Intestinal content	LRI	CEN	2017	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
59	Intestinal content	LRI	CEN	2017	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
60	Intestinal content	LRI	CEN	2017	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
61	Intestinal content	LRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
62	Intestinal content	LRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
63	Intestinal content	LRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
64	Intestinal content	LRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
65	Intestinal content	LRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
66	Intestinal content	PNB	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
67	Intestinal content	SRI	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
68	Intestinal content	PNB	CEN	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*
69	boot swab	CPM	NE	2018	I	0.25	-	*agfA, invA, spaN, sifA, prgH, sopB, sdfI, tolC, mgtC, ssaQ, spvC*

NE: Northeastern part, BRM: Buriram, CPM: Chaiyaphum, KKN: Khon Kaen, NMA: Nakhon Ratchasima, MKM: Mahasarakham, SRN: Surin, CEN: Central part, NSN: Nakhon Sawan, LRI: Lopburi, SRI: Saraburi, NYK: Nakhon Nayok, PNB: Phetchabun, ND: Not determine, CIP: Ciprofloxacin, I: Intermediate (reduced susceptibility), PMQR: plasmid-mediated quinolone resistance genes: *qnrA, qnrB, qnrS, aac(6')-Ib-cr, qepA*

DISCUSSION

Resistance to ciprofloxaxin in *Salmonella* has been increasing worldwide. Emergence and dissemination of several PMQR genes play important roles for a rising of ciprofloxacin-resistant *Salmonella* (Chang et al., 2021). Fluoroquinolones have long been common antibiotic drugs used in broiler farms in several countries, including Thailand (Na Lampang et al., 2007; Roth et al., 2019; Tenhagen et al., 2021). In this study, we found that *S.* Enteritidis isolates recovered from GAP-certified broiler farms in central and northeast Thailand showed no resistance to ciprofloxacin. However, reduced susceptibility to ciprofloxacin was detected in all isolates, regardless of geographical area and year of isolation. Similarly, previous studies found very low or no resistance to ciprofloxacin in *Salmonella* recovered from broiler supply chain in Thailand (Chotinan and Tadee, 2015; Perestrelo et al., 2016). The study by Pelyuntha et al. (2022) found 29.4% ciprofloxacin-resistant *Salmonella* in the broiler production chain including a free-range farm, slaughterhouses, and wet markets, but not in commercial broiler farms. Higher rates of ciprofloxacin-resistant *Salmonella* were reported in isolates recovered from broilers at local slaughterhouses in nine provinces of Thailand at 23.85% (Phongaran et al., 2019) and from integrated broiler chicken supply chain in China at 37% (Cui et al., 2019). Reduction and appropriate use of antimicrobials in the GAP- certified broiler farms in this study may have contributed to low or no resistance to ciprofloxacin in *Salmonella* isolates. However, the finding of reduced susceptibility phenotype indicates that ciprofloxacin-resistant *Salmonella* in poultry production chain should be constantly and closely monitored.

Plasmid-mediated quinolone resistance (PMQR) is a common mechanism that provides low-level resistance to fluoroquinolones in gram-negative bacteria (Li et al., 2018). Importantly, *Salmonella* isolates with reduced susceptibility to ciprofloxacin can adversely affect the effectiveness of treatment for salmonellosis (Dimitrov et al., 2007; Pham Thanh et al., 2016). The spread of PMQR in *Salmonella* is of great concern due to plasmid mobility. In our study, the common PMQR genes were not detected in *S.* Enteritidis isolates. These findings differ from a study conducted in China, which reported a high prevalence of *qnrS* (41.1%) and *qnrB* (30%) genes in *Salmonella* isolated from broiler chicken supplies (Cui et al., 2019). Similarly, in a study conducted in Egypt by Ammar et al. (2019), *Salmonella* with reduced susceptibility to ciprofloxacin isolated from broilers were found to carry *qnrS* and *qnrA* at 100% and 20%, respectively. In this study, the reduced susceptibility of *S.* Enteritidis to ciprofloxacin may be due to mutations in QRDR genes. However, it is also possible that other PMQR genes, such as *qnrC*, *qnrD*, *qnrE*, and *oqxAB*, could contribute to this reduced susceptibility phenotype. Therefore, additional investigations are required to determine the role of these genes.

Salmonella possesses various virulence factors that contribute to its pathogenicity and ability to cause serious infections in humans and animals. The virulence genes are located on the chromosome or virulence plasmid, and the presence of specific virulence genes can be serovar-specific (Suez et al., 2013; Wang et al., 2020). For example, the virulence genes *sefA* and *spvC* were detected in *S.* Enteritidis but not in *S.* Typhimurium (Siddiky et al., 2021). Variations in virulence genes among strains within specific serovar have also

been observed (Mezal et al., 2014; Kim and Lee, 2017). In this study, the same virulence profile was detected among all 69 *S.* Enteritidis isolates from both types of samples collected from farms across two geographical areas over a four-year period. These isolates contained 11 out of 12 virulence genes tested, indicating similar pathogenicity and the potential to infect and cause disease in humans. None of the 69 *S.* Enteritidis isolates carried the *cdtB* gene. This enterotoxin-encoding gene was highly detected in *S.* Typhi (Haghjoo and Galán, 2004). The nonexistence of the *cdtB* gene observed in *S.* Enteritidis in this study is consistent with previous studies (Elemfareji and Thong, 2013; Wang, et al., 2020). Interestingly, the presence of *cdtB* was found in 7.7% of *S.* Enteritidis isolated from poultry samples in Iran (Bahramianfard et al., 2021)

Salmonella infections primarily require cell adhesion and invasion into host epithelial cells, and the ability to survive and propagate within macrophages is critical for the systemic infection (Wang et al., 2020). Fimbriae are the key virulence factors for adhesion and biofilm formation of *Salmonella*. The *agfA* gene encoding for thin, aggregative fimbriae was detected in all *S.* Enteritidis in this study. Similarly, *S.* Enteritidis of poultry origin in Brazil and Malaysia were found to carry *agfA* at high percentages of 96% and 100%, respectively (Borges et al., 2013; Elemfareji and Thong, 2013). The invasion ability of *Salmonella* requires effector proteins of the type III secretion system (T3SS) encoded by genes on the SPI-1 (Wang et al., 2020). The *invA*, *spaN* and *prgH* genes of the SPI-1 are among the common core virulence genes of T3SS. Similar to other studies (Elemfareji and Thong, 2013; Campioni et. al. 2014; Kim and Lee, 2017), all of our *S.* Enteritidis isolates harbored *invA*. However, the presence of *prgH* and *spaN* genes can vary among *S.* Enteritidis isolates and *Salmonella* serovars (Mezal et al., 2014; Kim and Lee, 2017; Tarabees et al., 2017; Bahramianfard et al., 2021). The *tolC* virulence gene, located outside SPIs, is involved in host cell invasion, efflux pump, and maintenance of cell membrane integrity (Horiyama et al., 2012). We also detected *tolC* in all of our *S.* Enteritidis isolates. The *invA*, *spaN*, *prgH* and *tolC* genes were found in *S.* Enteritidis isolated from both poultry environment and clinical samples, as well as in non-typhoidal *Salmonella* from invasive *Salmonella* disease in humans (Suez et al., 2013; Mezal et al., 2014).

Several virulence genes are involved in the ability of *Salmonella* to survive and replicate within host cells. For example, the protein product of the *sifA* gene mediates the maturation of *Salmonella*-containing vacuole (SCV) during replication of *Salmonella* within cells (Wang et al., 2020). The *ssaQ* gene located on the SPI-2 encodes a T3SS apparatus protein that is required for replication within macrophage. Additionally, the functional protein of the SPI-3 gene, *mgt*C, mediates magnesium and phosphate transports for intramacrophage survival. The presence of the *sifA* gene ranged from 90% to 100% in *S.* Enteritidis isolated from healthy or diseased chickens in previous studies (Mezal et al., 2014; Wang et al., 2020). The occurrence of the *ssaQ* and *mgt*C genes in *S.* Enteritidis was also high (100%) in previous studies conducted in broiler supply chain (Ren et al., 2016; Andesfha et al., 2019). In our study, all *S.* Enteritidis isolates harbored the *sifA*, *ssaQ*, and *mgtC* genes, indicating their potential to cause systemic infection.

The *sopB* gene, located on SPI-5, contributes to *Salmonella* survival in B cells and mediates chloride secretory involving enteritis (Galyov et al.,

1997; Garcia-Gil et al., 2018). The *sopB* gene was detected in 50% of *S.* Enteritidis isolates from diseased chickens in Egypt (Ammar et al., 2016). The occurrence of the *sopB* gene was much higher in our *S.* Enteritidis, with 100% of the isolates carrying this gene, which is comparable to isolates from poultry sources in other countries (Elemfareji et al., 2013; Mezel et al., 2014; Kim and Lee, 2017). The *sitC* effector protein, which regulates iron transportation, was also detected in all our isolates, similar to the findings in Brazil by Borges et al. (2017). The occurrence of *sitC* in *Salmonella* of poultry origins can vary depending on serovar and geographical region (Mezel et al., 2014; Sripaurya et al., 2018).

S. Enteritidis and some *Salmonella* serovars carry virulence plasmids containing the *spv* operon, a highly conserved region that houses virulence genes (Wang et al., 2020). The virulence genes in the *spv* operon play important roles in survival and growth of *Salmonella* within the cells of the reticuloendothelial system, and in systemic infections (Wang et al., 2020). We found the virulence plasmid gene, *spvC*, in all of the *S.* Enteritidis isolates (100%) which was similar to a study in China by Wang et al (2020). However, other studies in Brazil and Iran reported lower percentages of this gene in *S.* Enteritidis of poultry origins, ranging from 51% to 93% (Castilla et al., 2006; Amini et al., 2010; Borges et al., 2013; Bahramianfard et al., 2021). Meanwhile, Kanaan et al. (2022) did not detect *spvC* gene in *S.* Enteritidis recovered from chicken meat and eggs in Iraq. The prevalence of the *spvC* gene in *Salmonella* has been shown to be related to the host origin and serovar of the isolates (Suez et al., 2013; Amini et al., 2010). The differences in the frequencies of the *spvC* in *S.* Enteritidis of poultry origins across various studies indicate differences in the genetic profiles of the strains from different geographical areas.

One of the limitations of this study is the relatively small number of *S.* Enteritidis isolates detected in the GAP broiler farms under investigation, and the majority of these isolates were found in 2015, which limits our ability to comprehensively investigate annual trends in the ciprofloxacin-resistant phenotype. Moreover, the absence of information regarding antimicrobial usage history in the study farms precludes us from analyzing any potential correlation between drug usage and the observed pattern of reduced susceptibility to ciprofloxacin. Furthermore, the samples for this study were collected from boot swabs and intestinal contents of healthy chicks, not from diseased broilers. This indicates that broilers on the farms are colonized with and shedding *S.* Enteritidis. It is not possible to determine their pathogenicity based solely on the detected virulence genes. Further studies including gene expression analysis and *in vivo* experiments are needed to provide a more comprehensive understanding of the pathogenic phenotype of these isolates in chickens.

In conclusion, our study found that all *S.* Enteritidis strains isolated from certified GAP broiler farms in central and northeast Thailand exhibited reduced susceptibility to ciprofloxacin, irrespective of their geographical origin or isolation time. Furthermore, we observed an identical virulence profile in all isolates, with almost all virulence genes detected, indicating that the *S.* Enteritidis strains in these regions are genetically related and have the potential to cause systemic infections. Therefore, continuous monitoring of ciprofloxacin-resistant *S.* Enteritidis in the poultry production chain is crucial in mitigating public health risks.

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AUTHOR CONTRIBUTIONS

The contributions of each author for this paper are as follows; Suwannachot N- design, literature search, experimental studies, manuscript preparation, Phuektes P- concept, design, experimental studies, manuscript editing, manuscript review, Jittimanee S-design, manuscript editing and manuscript review, Ketphan W-design, experimental studies, and manuscript review.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

ACFS, 2017. Guidance on the application of Thai Agricultural Standard TAS 6901(G)-2017. Available online: https://www.acfs.go.th/standard/download/GAP_BROILER_FARM.pdf (In Thai)

Alvarez, J., Sota, M., Vivanco, A.B., Perales, I., Cisterna, R., Rementeria, A., Garaizar, J., 2004. Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. J. Clin. Microbiol. 42, 1734–1738.

Amini, K., Salehi, T.Z., Nikbakht, G., Ranjbar, R., Amini, J., Ashrafganjooei, S.B., 2010. Molecular detection of *inv*A and *spv* virulence genes in *Salmonella* Enteritidis isolated from human and animals in Iran. Afr. J. Microbiol. Res. 4, 2202-2210.

Ammar, A.M., Mohamed, A.A., Abd El-Hamid, M.I., El-Azzouny, M.M., 2016. Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. J. Infect. Dev. Ctries. 10, 337-346.

Ammar, A.M., Abdeen, E.E., Abo-Shama, U.H., Fekry, E., Kotb Elmahallawy, E., 2019. Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. Lett. Appl. Microbiol. 68, 188–195.

Andesfha, E., Indrawati, A., Mayasari, N.L.P.I., Rahayuningtyas, I., Jusa, I., 2019. Detection of *Salmonella* pathogenicity island and *Salmonella* plasmid virulence genes in *Salmonella* Enteritidis originated from layer and broiler farms in Java Island. J. Adv. Vet. Anim. Res. 6, 384.

Bahramianfard, H., Derakhshandeh, A., Naziri, Z., Khaltabadi Farahani, R., 2021. Prevalence, virulence factor and antimicrobial resistance analysis of *Salmonella* Enteritidis from poultry and egg samples in Iran. BMC vet. Res. 17, 196.

Balasubramanian, R., Im, J., Lee, J.S., Jeon, H.J., Mogeni, O.D., Kim, J.H., Rakotozandrindrainy, R., Baker, S., Marks, F., 2019. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. Hum. Vaccines. Immunother. 15, 1421–1426.

Borges, K.A., Furian, T.Q., Borsoi, A., Moraes, H.L.S., Salle, C.T.P., Nascimento, V.P., 2013. Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in South of Brazil. Pesq. Vet. Bras. 33, 1416-1422.

Borges, K.A., Furian, T.Q., de Souza, S.N., Menezes, R., Salle, C.T.P., de Souza Moraes, H.L., Tondo, E.C., do Nascimento, V.P., 2017. Phenotypic and molecular characterization of *Salmonella* Enteritidis SE86 isolated from poultry and salmonellosis outbreaks. Foodborne. Pathog. Dis. 14, 742-754

Campioni, F., Zoldan, M.M., Falcão, J.P., 2014. Characterization of *Salmonella* Enteritidis strains isolated from poultry and farm environments in Brazil. Epidemiol. Infect. 142, 1403-1410.

Campioni, F., Cao, G., Kastanis, G., Janies, D.A., Bergamini, A.M.M., Rodrigues, D.D.P., Stones, R., Brown, E., Allard, M.W., Falcão, J.P., 2018. Changing of the genomic pattern of *Salmonella* Enteritidis Strains Isolated in Brazil over a 48 year-period revealed by Whole Genome SNP analyses. Sci. Rep. 8, 10478.

Castilla, K.S., Ferreira, C.S.A., Moreno, A.M., Nunes, I.A., Ferreira, A.J.P., 2006. Distribution of virulence genes *sefC*, *pefA* and *spvC* in *Salmonella* Enteritidis phage type 4 strains isolated in Brazil. Braz. J. Microbiol. 37, 135-139.

Chang, M.X., Zhang, J.F., Sun, Y.H., Li, R.S., Lin, X.L., Yang, L., Webber, M.A., Jiang, H.X., 2021. Contribution of different mechanisms to ciprofloxacin resistance in *Salmonella* spp. Front. Microbiol. 12, 663731.

Chotinan, S., Tadee, P., 2015. Epidemiological survey of *S. Enteritidis* pulsotypes from salmonellosis outbreak in Chiang Mai and Samut Songkhram provinces, Thailand. Vet. Integr. Sci. 13, 73-80.

CLSI, 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA: Pennsylvania.

CLSI, 2020. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA: Pennsylvania.

Cui, M., Zhang, P., Li, J., Sun, C., Song, L., Zhang, C., Zhao, Q., Wu, C., 2019. Prevalence and characterization of fluoroquinolone resistant *Salmonella* Isolated from an integrated broiler chicken supply chain. Front. Microbiol. 10, 1865.

Dashti, A.A., Jadaon, M.M., Abdulsamad, A.M., Dashti, H.M., 2009. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. Kuwait. Med. J. 41(2), 117-122.

de Toro, M., Rojo-Bezares, B., Vinué, L., Undabeitia, E., Torres, C., Sáenz, Y., 2010. In vivo selection of *aac(6')-Ib-cr* and mutations in the *gyr*A gene in a clinical *qnrS*1-positive *Salmonella enterica* serovar Typhimurium DT104B strain recovered after fluoroquinolone treatment. J. Antimicrob. Chemother. 65, 1945–1949.

Dimitrov, T., Udo, E.E., Albaksami, O., Kilani, A.A., Shehab, E.M.R., 2007. Ciprofloxacin treatment failure in a case of typhoid fever caused by *Salmonella enterica* serotype Paratyphi A with reduced susceptibility to ciprofloxacin. J. Med. Microbiol. 56, 277–279.

Department of Livestock Development (DLD), 2010. Control *Salmonella* in Poultry. Available online: https://ratchakitcha.soc.go.th/documents/1859886.pdf (In Thai)

Elemfareji, O.I., Thong, K.L., 2013. Comparative Virulotyping of *Salmonella typhi* and *Salmonella enteritidis*. Indian J. Microbiol. 53, 410–417.

Galyov, E.E., Wood, M.W., Rosqvist, R., Mullan, P.B., Watson, P.R., Hedges, S., Wallis, T.S., 1997. A secreted effector protein of *Salmonella dublin* is translocated into eukaryotic cells and mediates inflammation and fluid secretion in infected ileal mucosa. Mol. Microbiol. 25, 903-912.

García-Gil, A., Galán-Enríquez, C.S., Pérez-López, A., Nava, P., Alpuche-Aranda, C., Ortiz-Navarrete, V., 2018. *Sop*B activates the Akt-YAP pathway to promote *Salmonella* survival within B cells. Virulence. 9, 1390–1402.

Gast, R.K., Jones, D.R., Guraya, R., Garcia, J.S., Karcher, D.M., 2022. Research note: internal organ colonization by *Salmonella* Enteritidis in experimentally infected layer pullets reared at different stocking densities in indoor cage-free housing. Poult sci, 101, 102104.

Grimont, P.A., Weill, F.X., 2007. Antigenic Formulae of the *Salmonella* Serovars, 9th edition. WHO, France.

Haghjoo, E., Galán, J.E., 2004. *Salmonella typhi* encodes a functional cytolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway. Proc. Natl. Acad. Sci. U.S.A. 101, 4614–4619.

Hengkrawit, K., Tangjade, C., 2022. Prevalence and Trends in Antimicrobial Susceptibility Patterns of Multi-Drug-Resistance Non-Typhoidal *Salmonella* in Central Thailand, 2012-2019. Infect. drug resist. 15, 1305–1315.

Horiyama, T., Yamaguchi, A., Nishino, K., 2010. *TolC* dependency of multidrug efflux systems in *Salmonella enterica* serovar Typhimurium. J. Antimicrob. Chemother. 65, 1372–1376.

Jajere S.M., 2019. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet. World. 12, 504–521.

Kanaan, M.H.G., Khalil, Z.K., Khashan, H.T., Ghasemian, A., 2022. Occurrence of virulence factors and carbapenemase genes in *Salmonella enterica* serovar Enteritidis isolated from chicken meat and egg samples in Iraq. BMC Microbiol. 22, 1-8.

Karp, B.E., Campbell, D., Chen, J.C., Folster, J.P., Friedman, C.R., 2018. Plasmid-mediated quinolone resistance in human non-typhoidal *Salmonella* infections: An emerging public health problem in the United States. Zoonoses. Public. Health. 65, 838–849.

Kohn, M.A., Senyak J. Sample Size Calculators for designing clinical research. Available online: https://www.sample-size.net/ (Accessed on April 6, 2023)

Kim, J.E., Lee, Y.J., 2017. Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea. Ir. Vet. J. 70, 20.

Kuang, D., Zhang, J., Xu, X., Shi, W., Chen, S., Yang, X., Su, X., Shi, X., Meng, J., 2018. Emerging high-level ciprofloxacin resistance and molecular basis of resistance in *Salmonella enterica* from humans, food and animals. Int. J. Food Microbiol. 280, 1–9.

Lee, S., Park, N., Yun, S., Hur, E., Song, J., Lee, H., Kim, Y., Ryu, S., 2021. Presence of plasmid-mediated quinolone resistance (PMQR) genes in non-typhoidal *Salmonella* strains with reduced susceptibility to fluoroquinolones isolated from human salmonellosis in Gyeonggi-do, South Korea from 2016 to 2019. Gut. Pathog. 13, 35.

Li, J., Hao, H., Sajid, A., Zhang, H., Yuan, Z., 2018. Fluoroquinolone Resistance in *Salmonella*: Mechanisms, Fitness, and Virulence. In: Mascellino, M.T. (Ed.), *Salmonella* - A Re-Emerging Pathogen. Available online: https://www.intechopen.com/chapters/60197

Martínez-Martínez, L., Pascual, A., Jacoby, G. A., 1998. Quinolone resistance from a transferable plasmid. Lancet. 351, 797–799.

Mezal, E.H., Sabol, A., Khan, M.A., Ali, N., Stefanova, R., Khan, A.A., 2014. Isolation and molecular characterization of *Salmonella enterica* serovar Enteritidis from poultry house and clinical samples during 2010. Food. Microbiol. 38, 67–74.

Murase, T., Phuektes, P., Ozaki, H., Angkititrakul, S., 2022. Prevalence of *qnrS*-positive *Escherichia coli* from chicken in Thailand and possible co-selection of isolates with plasmids carrying *qnrS* and trimethoprim-resistance genes under farm use of trimethoprim. Poult. Sci. 101, 101538.

Na lampang, K., Chongsuvivatwong, V., Kitikoon, V., 2007. Pattern and determinant of antibiotics used on broiler farms in Songkhla province, southern Thailand. Trop. Anim. Health. Prod.39, 355–361.

Park, C.H., Robicsek, A., Jacoby, G.A., Sahm, D., Hooper, D.C., 2006. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents. Chemother. 50(11), 3953–3955.

Pham Thanh, D., Karkey, A., Dongol, S., Ho Thi, N., Thompson, C.N., Rabaa, M.A., Arjyal, A., Holt, K.E., Wong, V., Tran Vu Thieu, N., Voong Vinh, P., Ha Thanh, T., Pradhan, A., Shrestha, S.K., Gajurel, D., Pickard, D., Parry, C.M., Dougan, G., Wolbers, M., Dolecek, C., Thwaites, G.E., Basnyat, B., Baker, S., 2016. A novel ciprofloxacin-resistant subclade of H58 *Salmonella* Typhi is associated with fluoroquinolone treatment failure. eLife, 5, e14003.

Pelyuntha, W., Sanguankiat, A., Kovitvadhi, A., Vongkamjan, K., 2022. Broad lytic spectrum of novel *Salmonella* phages on ciprofloxacin-resistant *Salmonella* contaminated in the broiler production chain. Vet. World. 15, 2039–2045.

Perestrelo S, Thongkamkoon P, Narongsak W, Amavisit P, 2016. Antimicrobial resistance profiles of *Salmonella* isolated from poultry farms in central Thailand. J. Kasetsart Vet. 26, 119-130.

Phongaran, D., Khang-Air, S., Angkititrakul, S., 2019. Molecular epidemiology and antimicrobial resistance of *Salmonella* isolates from broilers and pigs in Thailand. Vet. World. 12, 1311–1318.

Piekarska, K., Wołkowicz, T., Zacharczuk, K., Stepuch, A., Gierczyński, R., 2023. The Mechanisms Involved in the Fluoroquinolone Resistance of *Salmonella enterica* Strains Isolated from Humans in Poland, 2018-2019: The Prediction of Antimicrobial Genes by In Silico Whole-Genome Sequencing. Pathogens (Basel, Switzerland). 12, 193.

Ren, X., Li, M., Xu, C., Cui, K., Feng, Z., Fu, Y., Zhang, J., Liao, M., 2016. Prevalence and molecular characterization of *Salmonella enterica* isolates throughout an integrated broiler supply chain in China. Epidemiol. Infect. 144, 2989-2999.

Robicsek, A., Strahilevitz, J., Sahm, D.F., Jacoby, G.A., Hooper, D.C., 2006. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob. Agents Chemother. 50, 2872–2874.

Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., Domig, K.J., 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. Poult. Sci. 98, 1791–1804.

Shams, E., Firoozeh, F., Moniri, R., Zibaei, M., 2015. Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* Human Isolates in Iran. J. Pathog. 2015, 434391.

Siddiky, N.A., Sarker, M.S., Khan, M.S.R., Begum, R., Kabir, M.E., Karim, M.R., Rahman, M. T., Mahmud, A., Samad, M.A., 2021. Virulence and antimicrobial resistance profiles of *Salmonella enterica* serovars Isolated from Chicken at Wet Markets in Dhaka, Bangladesh. Microorganisms. 9, 952.

Skyberg, J.A., Logue, C.M., Nolan, L.K., 2006. Virulence genotyping of *Salmonella* spp. with multiplex PCR. Avian dis. 50, 77–81.

Soto, S.M., Rodríguez, I., Rodicio, M.R., Vila, J., Mendoza, M.C., 2006. Detection of virulence determinants in clinical strains of *Salmonella enterica* serovar Enteritidis and mapping on macrorestriction profiles. J. Med. Microbiol. 55, 365–373.

Sripaurya, B., Ngasaman, R., Benjakul, S., Vongkamjan, K., 2019. Virulence genes and antibiotic resistance of *Salmonella* recovered from a wet market in Thailand. J. Food Saf. 39, e12601.

Sriyapai, P., Pulsrikarn, C., Chansiri, K., Nyamniyom, A., Sriyapai, T., 2021. Molecular Characterization of Cephalosporin and Fluoroquinolone Resistant *Salmonella* Choleraesuis Isolated from Patients with Systemic Salmonellosis in Thailand. Antibiotics. 10(7), 844.

Suez, J., Porwollik, S., Dagan, A., Marzel, A., Schorr, Y.I., Desai, P.T., Agmon, V., McClelland, M., Rahav, G., Gal-Mor, O., 2013. Virulence gene profiling and pathogenicity characterization of non-typhoidal *Salmonella* accounted for invasive disease in humans. PloS one. 8, e58449.

Swamy, S.C., Barnhart, H.M., Lee, M.D., Dreesen, D.W., 1996. Virulence determinants *invA* and *spvC* in salmonellae isolated from poultry products, wastewater, and human sources. Appl. Environ. Microbiol. 62, 3768–3771.

Tarabees, R., Elsayed, M.S., Shawish, R., Basiouni, S., Shehata, A.A., 2017. Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. J. Infect. Dev. Ctries. 11, 314-319.

Tenhagen, B.A., Flor, M., Alt, K., Knüver, M.T., Buhler, C., Käsbohrer, A., Stingl, K., 2021. Association of antimicrobial resistance in *Campylobacter* spp. in broilers and turkeys with antimicrobial Use. Antibiotics (Basel). 10(6), 673.

Utrarachkij, F., Nakajima, C., Changkwanyeun, R., Siripanichgon, K., Kongsoi, S., Pornruangwong, S., Changkaew, K., Tsunoda, R., Tamura, Y., Suthienkul, O., Suzuki, Y., 2017. Quinolone resistance determinants of clinical *Salmonella* Enteritidis in Thailand. Microb. Drug. Resist. 23, 885–894.

Wang, M., Qazi, I.H., Wang, L., Zhou, G., Han, H., 2020. *Salmonella* virulence and immune escape. Microorganisms. 8, 407.

Wang, J., Li, J., Liu, F., Cheng, Y., Su, J., 2020. Characterization of *Salmonella enterica* Isolates from Diseased Poultry in Northern China between 2014 and 2018. Pathogens. 9(2), 95.

World Health Organization, 2019. Critically important antimicrobials for human medicine, 6th revision. Available online: https://www.who.int/publications/i/item/9789241515528

Whistler, T., Sapchookul, P., McCormick, D.W., Sangwichian, O., Jorakate, P., Makprasert, S., Jatapai, A., Naorat, S., Surin, U., Koosakunwat, S., Supcharassaeng, S., Piralam, B., Mikoleit, M., Baggett, H.C., Rhodes, J., Gregory, C.J., 2018. Epidemiology and antimicrobial resistance of invasive non-typhoidal Salmonellosis in rural Thailand from 2006-2014. PLoS. 12, e0006718.

Yue, M., Liu, D., Li, X., Jin, S., Hu, X., Zhao, X., Wu, Y., 2022. Epidemiology, serotype and resistance of *Salmonella* isolates from a children's hospital in Hangzhou, Zhejiang, China, 2006–2021. Infect. Drug. Resist. 4735-4748.

Zishiri, O. T., Mkhize, N., Mukaratirwa, S., 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort. J. Vet. Res. 83, a1067.

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