



Research article

Colistin resistance and resistance determinants are mobile among *Salmonella enterica* isolates from diseased and healthy pigs in Thailand

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Abstract

Salmonella is an important enteric pathogen that poses a threat to human and livestock animal health, with emerging multidrug resistance (MDR) a major public health issue globally. A total of 150 fecal samples were collected from pigs housed from 4 separate pig farms in southern Thailand and tested for the presence of *Salmonella*. Confirmed *Salmonella* isolates were tested for their susceptibility to 11 antimicrobials, and PCR used to detect antimicrobial resistance genes (ARGs). *Salmonella* isolates were cultured from 69% (103/150) of all fecal samples, with higher prevalence in diseased pigs (12/15; 80%), compared with healthy pigs (91/135; 67%). Serotype Rissen was the most frequently identified serotype among the *Salmonella* isolates. Resistance to ampicillin (AMP) (97%), sulfonamide-trimethoprim (SXT) (97%), and tetracycline (TET) (94%) were the most common phenotypes observed. The most common ARGs identified were *bla*_{TEM} (99%), *tetA* (87%), *sul1* (77%), and *dfraA1* (74%), and more than 95% of the *Salmonella* isolates tested were MDR - based on resistance to three or more antimicrobial classes. The most common antimicrobial resistance pattern exhibited was AMP-TET-SXT (76%), and resistance to colistin (via the *mcr-1* gene) was observed in both healthy and diseased pigs. The clonal groups of PFGE analysis in each serotype revealed the genetic relationship among *Salmonella* isolated from healthy and diseased pigs from different pig farms. The pulsotype of *Salmonella* is clonal in both healthy and diseased pigs, providing crucial genetic evidence for the circulation of *Salmonella* in the area and its potential spread of MDR through the pig production chain.

Keywords: Antimicrobial resistance, Colistin, Multidrug resistant, Pig farms, *Salmonella*

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INTRODUCTION

Non-typhoidal *Salmonella* (NTS) are a common cause of human gastroenteritis (Phongaran et al., 2019; Ahmad et al., 2021). Infection is usually the result of consumption raw or undercooked food of livestock origin (Sévellec et al., 2020). The global expansion of intensive swine production since the 1960s has elevated stocking densities and environmental stressors, thereby accelerating pathogen transmission and herd infection rates (VanderWaal and Deen, 2018; Kim et al., 2023; Wang and Li, 2024). The detection levels of *Salmonella* in European swine livestock production were 28.7% breeding herds, rising to 33.3% in fattening herds (EFSA, 2009; Mainar-Jaime et al., 2025). In Southeast Asia, the prevalence of *Salmonella* infection across swine farms varies considerably, driven by differences in farming systems, biosecurity protocols, climatic conditions, and hygienic practices (Alarcón et al., 2021; Holohan et al., 2022). The reported *Salmonella* prevalence range from 13.93% in Cambodia (Chea et al., 2025), 19.5%–44.5% in Myanmar (Trung et al., 2024), 21.5%–32.7% in Thailand (Anuchatkitcharoen et al., 2020; Tadee et al., 2021), and 84.2%–91.9% in Vietnam (Tu et al., 2015). In Thailand, the most common serotypes identified from pigs are S. Rissen, S. Typhimurium and S. 1,4,[5],12:i:- (Phongaran et al., 2019; Prasertsee et al., 2019; Anuchatkitcharoen et al., 2020; Patchanee et al., 2020; Eiamsam-Ang et al., 2022). While these serovars commonly induce gastroenteritis in warm-blooded animals, they typically do not cause severe illness in pigs. The symptomatic and asymptomatic animals act as a shedding carriers, spreading the pathogen to other animals and environments (Tanner and Kingsley, 2018).

Since the 1950s, antibiotics have been widely used in livestock production for increased yields within intensive farming systems globally (Caniça et al., 2019; Wojnarowski et al., 2025). Global antimicrobial usage in food producing animals was at 63,151 tons in 2010, rising to nearly 99,502 tons by 2022 (Van Boeckel et al., 2015; Mulchandani et al., 2023). The excessive use of antimicrobial agents for non-therapeutic purposes, such as prophylaxis and growth promotion, presents a critical public health concern by driving a dramatic increase in antimicrobial resistance (AMR) (Ikhimiukor and Okeke, 2023; Patra et al., 2025). Although the antibiotics used as growth promoters has been prohibited in the livestock sectors of many countries, prophylactic, metaphylactic, and therapeutic applications remain widespread globally. Consequently, intensive industrial farming practices have made swine production as a primary reservoir for the development and dissemination of multidrug-resistant (MDR) pathogens (Peng et al., 2022; Wojnarowski et al., 2025).

Colistin, or polymyxin E, is a bactericidal antibiotic which used as a last-resort drug against infections caused by MDR gram-negative bacteria (El-Sayed Ahmed et al., 2020; Mondal et al., 2024; Zeb et al., 2026). Colistin resistance can arise from mutations in chromosomal genes or acquisition of transposable genetic elements carrying mobilized colistin resistance (*mcr*) genes, which mediated phenotypic resistance (Gharaibeh and Shatnawi, 2019; Patchanee et al., 2021). Currently, ten *mcr* gene, *mcr-1* to *mcr-10*, have been identified globally, occurring most prevalently within the *Enterobacteriaceae* family, particularly impacting major foodborne pathogens such as *E. coli* and *Salmonella* spp. (Mmatli et al., 2022; Xu et al., 2022; Liu et al., 2025).

The objective of this study was to investigate the prevalence of *Salmonella* on healthy and diseased pigs from pig farms in Southern Thailand and characterise their phenotypic antimicrobial resistance profiles and associated carriage of specific antimicrobial resistance genes. We examined the genetic relatedness among the different *Salmonella* serotypes isolated from healthy and diseased pigs using the PFGE fingerprint typing method. Our work contributes to surveillance effort in the region and help inform public health interventions for improved food security.

MATERIALS AND METHODS

Sample collection and *Salmonella* spp. isolation

All isolates from pig farms in this study were collected in the previous studies (Prasertsee et al., 2024). The sample population was categorized into two groups of pigs based on clinical status: healthy and diseased pigs. Diseased pigs were defined as pigs displaying symptoms of gastrointestinal infection (e.g., watery diarrhea with/without blood or mucus, and loss of appetite) and were separated into quarantine cages. The samples were collected from pig feces on the floor of each group of pig. Prince of Songkla University (PSU) Animal Standard did not require the study to be reviewed or approved by an ethics committee because the fecal samples were collected off the ground which non-invasive sampling procedure for animals. Oral informed consent was obtained from all farm owners and informed about the purpose of this study and the study procedures at the time of sample collection.

Salmonella was isolated using ISO 6579-1:2017 as the standard protocol. Twenty-five-gram samples of fresh fecal samples were placed in sterile bags using aseptic techniques. To each of the samples was added 225 ml of buffered peptone water (BPW; Oxoid, England) and the mixtures were homogenized in a stomacher for 2 min, then incubated for 18 ± 2 h at $37 \pm 1^\circ\text{C}$. Aliquot 3 drops of incubated BPW were inoculated into a modified semisolid Rappaport Vassiliadis (MSRV; Oxoid, England) medium with novobiocin supplement, and incubated for 24 ± 3 h at $41.5 \pm 1^\circ\text{C}$ (negative samples were re-incubated for an additional 24 ± 3 h). Transferred 1 μl sterile loop of the presumptive *Salmonella*, white/grey turbid zones radiating from the point of inoculation on MSRV, streak onto xylosine lysine deoxycholate (XLD; Oxoid, England) and on brilliant green phenol red lactose sucrose (BPLS; Oxoid, England) incubated for 24 ± 3 h at $37 \pm 1^\circ\text{C}$. Suspected colonies, a red color with a black center on XLD and red and surrounded by a bright red zone on BPLS, were selected to biochemical testing for *Salmonella* spp. confirmation. The serotyping of *Salmonella* was identified by Kauffmann-White scheme.

Antimicrobial susceptibility testing and minimum inhibitory concentrations

Susceptibility to different antimicrobial agents of all *Salmonella* isolates were performed by disc diffusion method. A total of ten antimicrobials as follows: ampicillin (AMP, 10 μg), amoxycillin/clavulanate (AMC, 20/10 μg), chloramphenicol (CHL, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NAL, 30 μg), norfloxacin (NOR, 10 μg), streptomycin (STR 10 μg), sulfisoxazole (SX, 250 μg), tetracycline (TET, 30 μg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg) were performed using Mueller-Hinton agar (Oxoid, England). Inhibition zones were measured according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018). *Escherichia coli* ATCC 25922 was used as an internal quality control.

Gradient diffusion method was used to determine resistance to colistin (COL) according to European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2021). Interpretation of colistin resistance were compared to the Annex 1: EUCAST clinical breakpoints and epidemiological cut-off values of *Salmonella* spp. (MIC dilution ≤ 2 mg/L was recorded as sensitive, while MICs > 2 mg/L were considered resistant).

DNA extraction

All *Salmonella* isolates were grown on nutrient broth (Oxoid, England) at $37 \pm 1^\circ\text{C}$ for 24 ± 3 h. Transferred 1 ml of sample to a 1.5 ml microcentrifuge tube for DNA extraction by boiling method. The suspension was centrifuged for 10 min at

14,000 ×g and discarded the supernatant. The pellet was resuspended in 300 µl of DNase-RNase-free distilled water and mixed by vortex. The tube was centrifuged for 5 min at 14,000 ×g, and the supernatant was discarded carefully. The pellet was resuspended in 200 µl of DNase-RNase-free distilled water by vortexing. Then, boiling at 100°C for 15 min and immediately chilled on ice. The suspension was centrifuged for 5 min at 14,000 ×g at 4°C. Transferred the supernatant to a new microcentrifuge tube and boiling at 100°C for 10 min and chilled immediately on ice. The supernatant was used as the DNA template for PCR (Xu et al., 2020).

Detection of antimicrobial resistance genes

All *Salmonella* DNA samples were screened for the presence of antimicrobial resistance genes by PCR using gene-specific primer in Table 1. The PCR final reaction volume was 25 µl, consisting of 12.5 µl of OmniPCR Supermix (BIO-HELIX, Taiwan), 0.5 µl each of forward and reverse primers (Macrogen, Korea), 6.5 µl of sterile distilled water, and 5 µl of DNA sample. The PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 amplification cycles of denaturation at 94°C for 30 sec, annealing temperature (Table 1) for 30 sec, and extension at 72°C for 30 sec and final extension at 72°C for 7 min and kept on hold at 4°C. Gel electrophoresis was done using 1.5% w/v of agarose gel (HydraGene, Xiamen China), and 0.5X Tris-acetate-EDTA (TAE) buffer at 100 volts for 25 min.

Table 1 Primers used for detection of genes encoding resistance to different antimicrobials

Antimicrobial groups	Genes	Primer sequences (5' to 3')	Annealing temperature (°C)	References
Aminoglycosides	<i>aadA</i>	F: GTGGATGGCGGCCTGAAGCC R: AATGCCAGTCGGCAGCG	60	(Fazel et al., 2019)
	<i>strA</i>	F: TCAATCCCGACTTCTTACCG R: CACCATGGCAAACAACCATA	54	(Salvador-Membreve and Rivera, 2021)
Beta-lactam group	<i>bla_{CTX-M}</i>	F: ATGTGCAGYACCAAGTAARGTKATGGC R: TGGGTRAARTARGTSACCAGAAAYCAGC	60	(Salvador-Membreve and Rivera, 2021)
	<i>bla_{SHV}</i>	F: ATGCGTTATATTCGCCTGTG R: TGCTTTGTTATTCGGGCCAA	60	
	<i>bla_{TEM}</i>	F: TCGCCGCATACACTATTCTCAGAATGA R: ACGCTCACC GGCTCCAGATTTAT	60	
Chloramphenicol	<i>cmlA</i>	F: GCGGGCTATCTTTGCGTTTT R: AAGTAGACTGCCGTGACCGTTCC	59	(Cao et al., 2020)
Sulfonamide	<i>sul1</i>	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	56	(Cao et al., 2020)
Trimethoprim	<i>dhfrA1</i>	F: CAATGGCTGTTGGTTGGAC R: CCGGCTCGATGTCTATTGT	55	(Herrera-Sánchez et al., 2020)
Tetracyclines	<i>tetA</i>	F: GCTACATCCTGCTTGCCCTC R: CATAGATCGCCGTGAAGAGG	57	(Salvador-Membreve and Rivera, 2021)
Quinolones	<i>qnrS1</i>	F: ACGACATTCGTCACCTGCAA R: TAAATTGGCACCCTGTAGGC	53	(Mahmud et al., 2018)
	<i>gyrA</i>	F: TGCTTATCACACCGGACAAG R: CCAAAGCCGTTTTCAGTCAT	55	(Madhupriya et al., 2019)
Polymyxins	<i>mcr-1</i>	F: CTTGGTCGGTCTGTAGGG R: CGGTCAGTCCGTTTGTC	54	(Cao et al., 2020)

Pulsed-Field Gel Electrophoresis (PFGE)

Ten *Salmonella* samples resistant to colistin were selected to perform PFGE according to the PulseNet protocol from the Centers for Disease Control and Prevention procedures. In brief, a single colony of each isolate was streaked onto Nutrient agar (Oxoid, England) at 37°C overnight. *Salmonella* colonies were suspended in Cell Suspension Buffer (CBS; 100 mM Tris:100 mM EDTA, pH 8.0) at concentration of optical density (OD) 600 nm. Lysis of bacterial cells used Cell Lysis Buffer (CLB; 50 mM Tris:50 mM EDTA, pH 8.0; 1% Sarcosyl) and Proteinase K (Thermo Fisher Scientific, Korea). Restriction enzyme *XbaI* (Thermo Fisher Scientific, Korea) was used for digestion of genomic DNA samples. The standard

strain *S. Braenderup* H9812 (ATCC® BAA-664™) was chosen as the universal size standard. Staining gel after electrophoresis used ethidium bromide and sterile ultrapure water was used to destain (Kang et al., 2020).

Statistical and clonal analysis

Association between antimicrobial resistance characteristics and their related resistance genes were analysed by Pearson Chi-square or Fisher's exact tests (where appropriated) in Epi Info™ statistical software using StatCalc modules. The results were considered as statistically significant when p -value < 0.05.

The clonal analysis of ten *Salmonella* DNA fingerprint were analysed by using BioNumerics software version 7.6.3 (Applied Maths, Belgium). The relatedness dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with Dice coefficient at an optimization setting of 1% and a position tolerance setting of 1%. Interpretation of pulsotype based on the percentage of similarity.

RESULTS

Prevalence of *Salmonella* positive and serotype distribution

Table 2 presented the prevalence of *Salmonella* positive from healthy and diseased pigs. The overall prevalence of healthy and diseased pigs was 67.41% and 80.00%, respectively. *Salmonella* was just as likely to be sampled from healthy pigs, with successful identification of isolates from 82.61%, 80.00%, 60.00% and 51.06% of pigs from farms B, C, D and A, respectively. *Salmonella* isolates were identified from all diseased pigs (100.00%) from farms A and B, and 80.00% and 60.00% of pigs from farms D and farm C, respectively.

Table 2 Prevalence of *Salmonella* positive from healthy and diseased pigs

Farm	Healthy pig		Diseased pigs	
	No. of positive samples (No. of samples)	Prevalence (95% confidence interval)	No. of positive samples (No. of samples)	Prevalence (95% confidence interval)
A	24 (47)	51.06 (36.77-65.36)	3 (3)	100.00
B	19 (23)	82.61 (67.12-98.10)	2 (2)	100.00
C	36 (45)	80.00 (68.31-91.69)	3 (5)	60.00 (22.91-88.40)
D	12 (20)	60.00 (38.60-78.17)	4 (5)	80.00 (35.96-97.97)
Total	91 (135)	67.41 (59.11-74.74)	12 (15)	80.00 (54.81-92.95)

The serotype distribution of *Salmonella* isolates is shown in Table 3, with eighteen different serotypes observed in this study. *S. Rissen* (n=21) was the most common serotype found within pig farms, followed by serotypes Typhimurium (n=17), Enteritidis (n=11) and Stanley (n=10). Fourteen serotypes were observed in only healthy pigs while four serotypes; *S. Typhimurium*, *S. Enteritidis*, *S. Brunei*, and *S. 4,5,12:i:-*, were isolated from both healthy and diseased pigs. The dominant serotypes from healthy and diseased pigs were *S. Rissen* (n=21) and *S. 4,5,12:i:-* (n=5), respectively.

Table 3 Serotypes distribution of *Salmonella* isolated from healthy and diseased pigs

Serotype	Healthy pigs	Diseased pigs	Total
S. Rissen	21	0	21
S. Typhimurium	13	4	17
S. Enteritidis	9	2	11
S. Stanley	10	0	10
S. Lexington	8	0	8
S. Brunei	6	1	7
S. 4,5,12:i:-	1	5	6
S. Panama	5	0	5
S. Weltevreden	5	0	5
S. Anatum	3	0	3
S. Krefeld	2	0	2
S. Newport	2	0	2
S. Albany	1	0	1
S. Derby	1	0	1
S. Emek	1	0	1
S. Kedougou	1	0	1
S. London	1	0	1
S. 1,4,5,12:i:-	1	0	1

Prevalence of *Salmonella* positive and serotype distribution

Summary results of antimicrobial susceptibility testing among *Salmonella* isolates are shown in Table 4. All 103 *Salmonella* isolates from healthy and diseased pigs were susceptible to AMC, CHL and CIP. High rates of resistance were observed for AMP and SXT (97.09%; 100/103), followed by TET (94.17%; 97/103), COL (9.71%; 10/103), STR (7.77%; 8/103), SX (5.83%; 6/103), NAL (3.88%; 4/103), and NOR (1.94%; 2/103). 88.44% of healthy pig isolates was resistant to AMP and SXT while 11.65% of diseased pig isolates was resistant to AMP, TET and SXT. Polymyxins resistance was low in both healthy pigs (3.88%) and diseased pigs (5.83%).

Table 4 Antimicrobial resistance characteristics of *Salmonella* isolates from healthy and diseased pigs

Antimicrobial agents	No. of resistance (%)	
	Healthy pigs	Diseased pigs
AMP	88 (88.44)	12 (11.65)
AMC	0 (0.00)	0 (0.00)
CHL	0 (0.00)	0 (0.00)
CIP	0 (0.00)	0 (0.00)
NAL	2 (1.94)	2 (1.94)
NOR	1 (0.97)	1 (0.97)
STR	5 (4.85)	3 (2.91)
SX	4 (3.88)	2 (1.94)
TET	85 (82.52)	12 (11.65)
SXT	88 (85.44)	12 (11.65)
COL	4 (3.88)	6 (5.83)

Table 5 showed the results of antimicrobial resistance genes among the *Salmonella* isolates from healthy and diseased pigs. The presence of twelve known resistance genes were assessed, from eight different categories of antimicrobials including aminoglycosides (*aadA*, *strA*), beta-lactams (*bla_{CTX-M}*, *bla_{SHV}*, *bla_{TEM}*), chloramphenicol (*cmlA*), sulfonamide (*sul1*), trimethoprim (*dfrA1*), tetracyclines (*tetA*), quinolones (*qnrS1*, *gyrA*), and polymyxins (*mcr-1*). The beta-lactam

resistance gene, *bla*_{TEM}, was the most prevalent resistance gene, found in 99.03% (102/103) of isolates - both healthy and diseased pigs. The *bla*_{SHV} gene, from the same group, was not found in any of the isolates. High rates of prevalence were observed for the antimicrobial resistance genes: *tetA* (87.38%; 90/103), *sul1* (76.70%; 79/103), and *dfrA1* (73.79%; 76/103), and were observed in both healthy and diseased pigs. Carriage of the *mcr-1* gene was identified in 15.53% (16/103) of isolates, of which most were healthy pigs (9/16) (Table 5).

The antimicrobial resistance patterns of all *Salmonella* isolates were presented in Table 6, and the S. 1,4,5,12:i:- (ID: D_Healthy_SL4) isolated from healthy pigs in farm D was sensitive to all eleven antimicrobial agents tested.

Table 5 Antimicrobial resistance genes of *Salmonella* isolates from healthy and diseased pigs

Resistance genes	No. of positive resistance gene (%)	
	Healthy pigs	Diseased pigs
<i>aadA</i>	3 (2.91)	1 (0.97)
<i>strA</i>	8 (7.77)	3 (2.91)
<i>bla</i> _{CTX-M}	2 (1.94)	0 (0.00)
<i>bla</i> _{SHV}	0 (0.00)	0 (0.00)
<i>bla</i> _{TEM}	90 (87.38)	12 (11.65)
<i>cmlA</i>	5 (4.85)	0 (0.00)
<i>sul1</i>	69 (66.99)	10 (9.71)
<i>dfrA1</i>	67 (65.05)	9 (8.74)
<i>tetA</i>	79 (76.70)	11 (10.68)
<i>qnrS1</i>	3 (2.91)	3 (2.91)
<i>gyrA</i>	4 (2.88)	1 (0.97)
<i>mcr-1</i>	9 (8.74)	7 (6.80)

Table 6 Antimicrobial resistance patterns of *Salmonella* isolates from healthy and diseased pigs

Antimicrobial resistance patterns	No. of isolates (%)	
	Healthy pigs	Diseased pigs
Pan-susceptible	1 (0.97)	0 (0.00)
Non-MDR		
SXT	1 (0.97)	0 (0.00)
AMP-TET	1 (0.97)	0 (0.00)
AMP-SX	2 (1.94)	0 (0.00)
MDR		
NOR-SX-SXT	1 (0.97)	0 (0.00)
AMP-STR-TET	1 (0.97)	0 (0.00)
AMP-STR-SXT	1 (0.97)	0 (0.00)
AMP-TET-SXT	72 (69.90)	6 (5.83)
AMP-SX-TET-SXT	2 (1.94)	0 (0.00)
AMP-NAL-TET-SXT	2 (1.94)	0 (0.00)
AMP-STR-TET-SXT	3 (2.91)	0 (0.00)
AMP-TET-SXT-COL	4 (3.88)	2 (1.94)
AMP-NAL-TET-SXT-COL	0 (0.00)	1 (0.97)
AMP-STR-TET-SXT-COL	0 (0.00)	1 (0.97)
AMP-STR-SX-TET-SXT-COL	0 (0.00)	1 (0.97)
AMP-NAL-NOR-STR-TET-SXT-COL	0 (0.00)	1 (0.97)

Among the *Salmonella* isolates, 4.0% were non-MDR (resistant to <3 antimicrobial classes). Resistance to SXT was detected from *S. Typhimurium*, which isolated from healthy pig. Additionally, two-class resistance patterns were identified in *S. Rissen* from healthy pigs, specifically exhibiting the resistance

profiles AMP-TET (n=1) and AMP-SXT (n=2). MDR was defined as resistance to at least one agent from three or more different antimicrobial classes, and twelve different MDR patterns of resistance were observed (Table 6). In total, 95.15% (98/103) of *Salmonella* isolates were found to be MDR. Eighty-six from ninety-one of *Salmonella* isolates from healthy pigs were MDR. All twelve *Salmonella* isolates from diseased pigs were identified as MDR, and half of them demonstrated the same MDR pattern (AMP-TET-SXT) (Table 6). The highest prevalence MDR pattern was AMP-TET-SXT (75.73%; 78/103), followed by AMP-TET-SXT-COL (5.82%; 6/103), and AMP-STR-TET-SXT (2.91%; 3/103). Two MDR patterns including AMP-TET-SXT and AMP-TET-SXT-COL were demonstrated in *Salmonella* isolates sampled from both healthy and diseased pigs. Additionally, The MDR patterns presented only in *Salmonella* isolates from diseased pigs comprised of AMP-NAL-TET-SXT-COL (n=1), AMP-STR-TET-SXT-COL (n=1), AMP-STR-SX-TET-SXT-COL (n=1), and AMP-NAL-NOR-STR-TET-SXT-COL (n=1). However, resistance to six different antimicrobial classes and seven antimicrobial agents (AMP-NAL-NOR-STR-TET-SXT-COL) was observed in *S.* 4,5,12:i:- from diseased pigs (ID: C_Diseased_N1).

Association between antimicrobial resistance phenotype and corresponding genes

Statistical analysis of the antimicrobial resistance phenotypes and the corresponding genes revealed that AMP and *bla*_{TEM} gene, SXT and *sul1* gene, TET and *tetA* gene, COL and *mcr-1* gene, STR and *aadA* gene, STR and *strA* gene, NAL and *qnrS1* gene, NAL and *gyrA* gene, NOR and *qnrS1* gene, and NOR and *gyrA* gene were significantly related to the observed resistance (p-value<0.05) (Figure 1).

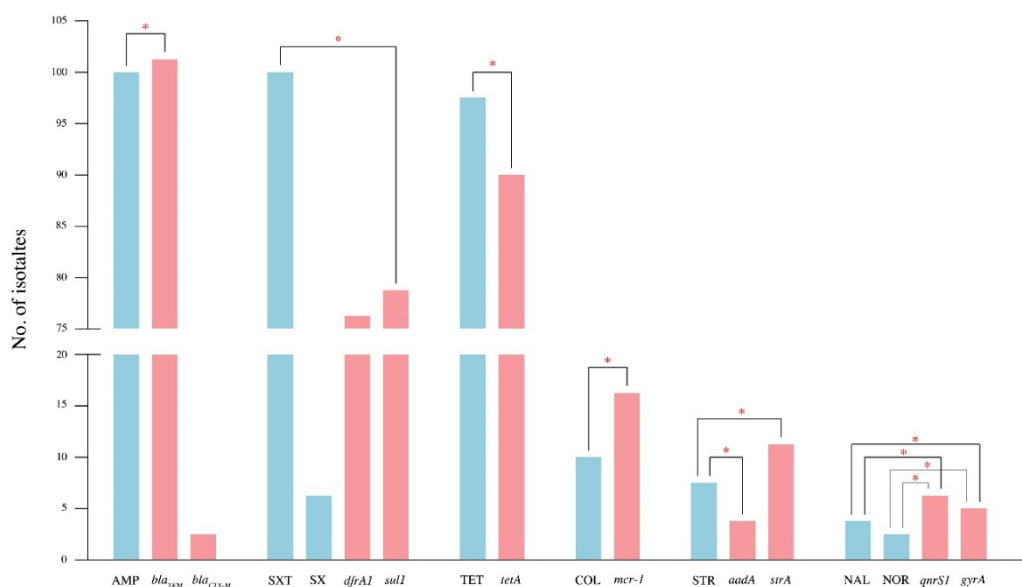


Figure 1 The relationship between AMR phenotype (blue bars) and related genes (pink bars) of all 103 *Salmonella* isolates. * p value < 0.05

Clonal relatedness

All ten *Salmonella* isolates that demonstrated colistin resistance were selected for PFGE, including isolates from *S.* Typhimurium (n=4), *S.* 4,5,12:i:- (n=3), *S.* Rissen (n=2), and *S.* Weltevreden (n=1) serovars. Within each serovar, isolates grouped into clonal clusters of pulsotypes (Figure 2). Cluster 1 belonged to *S.*

4,5,12:i:- isolated from diseased pigs in farm C and D. The antimicrobial resistance profile of three isolates was different within cluster 1. Four *S. Typhimurium* were grouped into cluster 2. The isolates in this cluster were from both healthy pig and diseased pigs and carried the same resistance genes profile (Figure 2). The identical clone of two *S. Rissen* from healthy pigs was in cluster 3. Although *S. Rissen* in this group isolated from different farms (B and D), these two isolates had the same antimicrobial resistance characteristics (Figure 2). *S. Weltevreden* from healthy pig in farm D was classified into cluster 4.

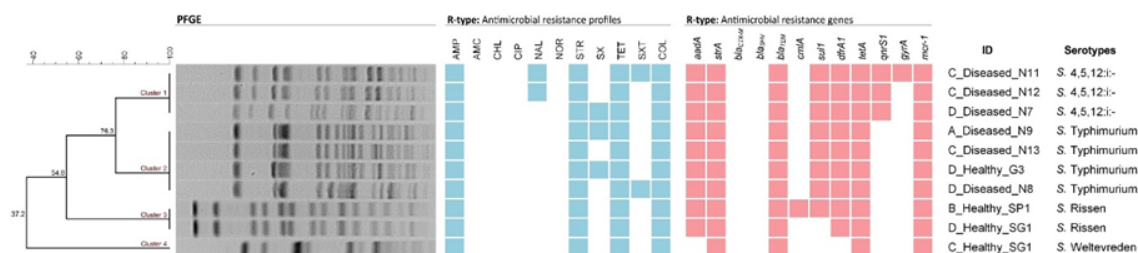


Figure 2 Phylogenetic tree of DNA fingerprint of ten colistin-resistant *Salmonella* isolates with R-type of antimicrobial resistance phenotype and genotype. Pink box = resistance phenotype, blue box = resistance genotype

DISCUSSION

In this study, the prevalence of *Salmonella* isolated from healthy pig was 67.41% and 80.00% from diseased pigs. This prevalence is higher than that reported in many recent studies, including those conducted in Thailand. Recent Thai estimated reported *Salmonella* prevalence of 6% (Padungtod and Kaneene, 2006), 34.98% (Tadee et al., 2014) and 21.51% (Anuchatkitcharoen et al., 2020). The differences in prevalence could be a result of differences in farm management strategies, sanitation, hygiene practices, and the individual environmental conditions of each farm (Cao et al., 2020). Furthermore, fifteen isolates in this study collected from feces of pigs that had gastrointestinal problems such as diarrhea in quarantine housing. Specific sampling from diseased pigs may have contributed to the higher prevalence observed in this study.

In this study, Rissen was the dominant serotype on healthy pigs, consistent with several other studies reporting serotype Rissen as the most common serotype found in pig production in Thailand (Tadee et al., 2014; Trongjit et al., 2017; Yang et al., 2018; Phongaran et al., 2019). Despite this, there have been few reports of this serotype from pig farms in Southern Thailand. In 2013, Rissen was found as the predominant serotype isolated from pork in Phatthalung province (Southern Thailand) (Lertworapreecha et al., 2013). Taken together, our findings suggest that serotype Rissen is a common serovar infecting pigs from the region, it has likely spread around the world through global pork markets (Elbediwi et al., 2021). Furthermore, this serotype was reported as the top twenty *Salmonella* serovars linked with human salmonellosis, underscoring its public health significance (Xu et al., 2020; Ali et al., 2026).

Although NTS rarely cause severe illness in healthy pigs, presenting as gastroenteritis and diarrhea, which effects pig growth. Consequently, antimicrobials are often used prophylactically to prevent disease in asymptomatic animals, with ampicillin and tetracycline the preferred antimicrobials to treat diarrheal symptoms (Rosager et al., 2017). This was reflected in our results, with extensive resistance to AMP (97.09%), SXT (97.09%), and TET (94.17%) reported.

Previous studies in the north-east of Thailand and Laos have also observed extremely high levels of resistance to AMP (100.00%), SXT (100.00%), and TET (91.40%) (Sinwat et al., 2016). This may be attributed to the common usage of the antimicrobial agents in livestock production.

Resistance to more than one antimicrobial agent was observed in 98.06% of *Salmonella* isolates, and approximately 95% were MDR. This is consistent with the current crisis in southeast Asia. MDR *Salmonella* have been isolated from livestock production animals in extremely high numbers, including 98.00% in Thailand, 98.40% Laos, 59.40% in Vietnam and 52.00% in Cambodia (Tu et al., 2015; Sinwat et al., 2016; Trongjit et al., 2017; Holohan et al., 2022). The ubiquitous prevalence of MDR isolates from pigs in this region is alarming and poses a current public health risk.

Although regulations governing antimicrobial use vary globally, these agents continue to be deployed extensively in swine farming for therapeutic, prophylactic, and metaphylactic purposes. Furthermore, routine use as growth promoters is still widely reported in several countries, including Myanmar and Costa Rica (Manyi-Loh et al., 2018; Lekagul et al., 2019; Trinchera et al., 2025). Although Thailand prohibited the use of antibiotic growth promoters within the livestock sector in 2015, widespread use over decades has remained a key factor driving the emergence and persistence of AMR in bacteria (Ahmad et al., 2021; Lekagul et al., 2024; Alem et al., 2025; Nazir et al., 2025). However, several resistance phenotypes require multiple genes, as highlighted in our study where several genetic determinants showed little association with their predicted resistance phenotype (Figure 1). In this study, the most common antimicrobial resistant gene was *bla*_{TEM} gene (99.03%) and correlated well with AMP resistance. An additional two isolates carried the *bla*_{CTX-M} gene, but no isolate harboured the *bla*_{SHV} gene. The *bla*_{CTX-M} and *bla*_{SHV} genes are typically the most common extended-spectrum β -lactamase (ESBL) genes, and strains carrying these genes often demonstrate resistance to additional antimicrobial agents belonging to other classes (Pishtiwan and Khadija, 2019). In our collection, two *S. Typhimurium* isolates harboured the *bla*_{CTX-M} gene and were also found to be resistant to multiple antibiotic classes. Tetracycline resistance genes (*tetA*) and sulfonamide-trimethoprim (*sul1* and *dfrA1*) were also detected in high frequency. These genes were also common in other studies from the region, reflected in the high rates of resistance to β -lactam, tetracycline, and sulfonamide-trimethoprim (Hathai and Yamaguchi, 2012; Prasertsee et al., 2019; Karabasanavar et al., 2022).

Colistin resistance in *Enterobacteriaceae* family bacteria has been reported globally (Izdebski et al., 2016; Stefaniuk and Tyski, 2019; Gashaw et al., 2025). The *mcr-1* gene plays an important role in colistin resistance of *Salmonella* isolates from livestock animals, environmental and human (Lu et al., 2018; Cao et al., 2020). In this study, sixteen isolates contained the *mcr-1* gene, ten of which demonstrated correlating phenotypic colistin resistance. Six colistin resistant samples were obtained from symptomatic pigs (diarrhea) in farm A (n=1), C (n=3) and D (n=2) and other four isolates were collected from healthy pigs in farm B (n=1), C (n=1) and D (n=2). On farms C and D, symptomatic pigs were treated via colistin-medicated feed until clinical recovery. Additionally, after isolating symptomatic pigs to quarantine unit, prophylactic colistin-medicated feed was administered for 28 days to asymptomatic herdmates co-housed within the same pens as the symptomatic individuals. Conversely, individual colistin injections were administered to diarrheic pigs on farms A and B. Non-specific treatment durations and the prophylactic use of colistin in animal feed expose bacteria to continuous antimicrobial contact, intensifying selective pressure across the broader bacterial population and ultimately driving the development of AMR (Odey et al., 2024; Trinchera et al., 2025).

The DNA fingerprint of ten *Salmonella* isolates resistant to colistin was analysed and *Salmonella* isolates were grouped according to serotype in clonal

clusters 1-4 (Figure 2). The clonal groups of each serotype indicated that *Salmonella* isolates shared similar genetic backgrounds. Overall, diseased pigs were resistant to a broader spectrum of antibiotics, potentially due to the likely increased exposure to antimicrobial agents. It was therefore interesting to note that healthy pigs also represent a potential reservoir of MDR. This has been noted with other species, with healthy pigs harbouring diverse resistant genotypes of *Streptococcus suis* (Kittiwan et al., 2022).

CONCLUSIONS

In conclusion, this study confirmed that the *Salmonella* can be found and circulated within pig farm base on the evidence of high prevalence rate of *Salmonella* positive from both healthy and diseased pigs. High rates of MDR *Salmonella* were observed, especially in diseased pigs with more than half of the collected isolates resistant to three or more antibiotic classes. Taken with evidence from other studies, this poses a current public health risk in the region. Preventive control strategies must account for these extremely high levels of antibiotic resistance to stop the spread of MDR and reduce public exposure to MDR isolates via the pork production chain.

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AUTHOR CONTRIBUTIONS

Teerarat Prasertsee: Writing– original draft, Project administration, Methodology, Investigation, Conceptualization, Funding acquisition.

Ben Pascoe: Writing – review & editing, Validation, Supervision, Conceptualization.

Prapas Patchanee: Writing–review & editing, Software, Validation, Supervision, Conceptualization, Funding acquisition.

All authors read and approved the final.

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