Original Article

Prevalence, antimicrobial resistance characteristics and virulence genes of *Streptococcus suis* in pigs in upper northern Thailand

Wanida Mala[1] Rangsiya Prathan[2] Si Thu Hein[2] Sunpetch Angkittitrakul[3] Asinamai Athliamai Bitrus[4] Rungtip Chuanchuen[2*]

Abstract

This study aimed to investigate the prevalence, virulence genes and antimicrobial resistance of *Streptococcus suis* from pigs in Upper-Northern Thailand. A total of 768 nasal swabs were obtained from pigs at the municipal slaughterhouses in five provinces including Phayao (n=202), Nan (n=190), Chiang Rai (180), Chiang Mai (n=130), and Mae Hong Son (n=66) in 2018-2019. The prevalence of *S. suis* varied from 3.0%-9.4%, of which the highest prevalence was in Chiang Rai (9.4%) and Mae Hong Son (9.1%). Of all 59 isolates confirmed to be *S. suis*, serotype 8 (50.8%) was most commonly identified, followed by serotype 10 (3.4%), 2 (1.7%) and 9 (1.7%). The only one *S. suis* serotype 2 originated from Phayao. Twenty-five isolates (42.4%) could not be typed. The *sly* gene was the most frequent virulence genes, while *mrp-sly-arcA-hyl* (33.9%) was the most common virulence gene profile. All the isolates carried *arcA*, but none were positive to *epf*. The *S. suis* isolates exhibited high resistance rates to all antimicrobial agents tested (more than 50%) and all were multidrug resistant (100%). The most common resistance pattern was CLI-CTC-ERY-GEN-NEO-OTC-TET-TIL-TYL (5.1%). The isolates were positive to *tet*(M) (32.3%), *tet*(O) (30.5%) and *mefA* (8.47%). In conclusion, the results confirm the important role of subclinical carrier pigs of *S. suis* and emphasize the need for routine detection of *S. suis* in clinically healthy pigs.

Keywords: antimicrobial resistance, pigs, slaughterhouse, *Streptococcus suis*, virulence genes

[1]*Medical Technology Program, Faculty of Science, Nakhon Phanom University, Nakhon Phanom, Thailand*

[2]*Research Unit in Microbial Food Safety and Antimicrobial Resistance, Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*

[3]*Research Group for the Prevention Technology in Livestock, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand*

[4]*Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, University of Jos, PMB 2084 Jos Pateau, Nigeria)*

****Correspondence**: chuanchuen.r@gmail.com* (R. Chuanchuen)

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Introduction

Streptococcus suis is a zoonotic agent that causes severe infections in both humans and pigs and has become an important pathogen affecting pig industry worldwide (Goyette-Desjardins *et al.*, 2014; Segura *et al.*, 2020). The pathogen naturally colonizes the upper respiratory tract of pigs, particularly in tonsils and nasal cavities (Petrocchi-Rilo *et al.*, 2021), and can cause meningitis, septicemia, endocarditis, arthritis, pneumonia and sudden death (Gottschalk & Segura, 2000). Healthy pigs serve as a carrier for the spread of *S. suis* in herds that may be transmitted to humans mainly via either direct contact with infected pigs or consumption of contaminated raw pork (Goyette-Desjardins *et al.*, 2014; Segura, 2020). People at risk of *S. suis* infection include pig farmers, workers in pig slaughterhouses, and meat consumers (Goyette-Desjardins *et al.*, 2014). Currently, 29 *S. suis* serotypes are recognized according to the distinct capsular polysaccharides, of which serotype 2 is the most prevalent (Goyette-Desjardins *et al.*, 2014; Oh *et al.*, 2017; Segura *et al.*, 2020). *S. suis* serotypes 20, 22, 26, 32, 33, and 34 were removed due to being reclassified as other bacterial species (Kerdsin *et al.*, 2014; Segura *et al.*, 2020). The distribution of *S. suis* serotypes vary depending on geographical regions (Lunha *et al.*, 2022).

The pathogenesis and disease severity of *S. suis* depend on the presence of virulence factors e.g., muraminidase released protein (MRP), extracellular factor (EF), suilysin (SLY) and hyaluronidase (Rajkhowa & Rajesh, 2021). One of the major virulence factors is enzyme arginine deiminase (ArcA), which plays an important role in survival of the bacterium under stress conditions (Gruening *et al.*, 2006; Rajkhowa & Rajesh, 2021). Even though *S. suis* carrying virulence factors are highly virulence and potentially zoonotic agent, their roles in the pathogenesis of pig and human infections remain unclear. Different virulence profiles of *S. suis* are identified in different serotypes and different strains of the same serotype (Petrocchi-Rilo *et al.*, 2021).

Antimicrobial therapy is an important choice of treatment and control of *S. suis* infection in pigs and humans. The most frequently used antimicrobials for the treatment of *S. suis* infections are β-lactams, tetracyclines, sulfonamides, and macrolides (Lunha *et al.*, 2022). However, antimicrobial resistance (AMR) in *S. suis* has increasingly emerged at high rates worldwide (Varela *et al.*, 2013). Recently, a progressively increasing trend of resistance to tetracyclines, sulfonamides, macrolides and lincosamides was reported among *S. suis* strains (Segura *et al.*, 2020). However, these antibiotics seem to remain drugs of choice with low levels of resistance (Lunha *et al.*, 2022).

In Thailand, *S. suis* has been isolated from pigs in all regions (Lunha *et al.*, 2022) with the highest incidence rate (730 cases/year) in Northern provinces in particular Phayao, Lamphun and Phrae (Kerdsin *et al.*, 2022). Clinically healthy pigs were previously reported to be an important reservoir of *S. suis* (Kerdsin *et al.*, 2018). The contamination of traditional raw pork and pigs' blood products mainly occurs at the slaughterhouse (Kerdsin *et al.*, 2018; Takeuchi *et al.*, 2012) these contained pork serves as a carrier for transmission to consumers. *S. suis* infection is frequently identified in Hill tribe, who usually slaughter pigs for family consumption and traditional celebration. To date, the number of illnesses and deaths due to *S. suis* infection has been increasingly reported, and poor hygienic handling of raw pork at slaughterhouses represents a predominant cause (Kerdsin *et al.*, 2020).

Currently, no monitoring or surveillance program targeting *S. suis* exist in Thailand. While knowledge of *S. suis* prevalence is limited, it is expected that the real *S. suis* prevalence is much higher than reported in research studies. Data on AMR at both phenotype and genotype of the *S. suis* strains is even less. This study aimed to determine the prevalence, AMR characteristics and virulence genes of *S. suis* in pigs in Upper Northern Thailand.

Materials and Methods

Sample collection: A total of 768 nasal swab samples were obtained from pigs at slaughterhouses during April to October 2018. The cross-sectional sampling was performed 4 times at the municipal slaughterhouses in five provinces in Upper Northern Thailand including Phayao (n=202), Chiang Rai (n=180), Chiang Mai (n=130), Nan (n=190) and Mae Hong Son (n=66) (Fig. 1). The sampling areas were chosen based on the previous reports of *S. suis* cases, slaughterhouse owner agreement to participate in this project and distance not farther than 15-20 km from the public delivery service to ensure sample delivery within 24 hours after collection. The slaughterhouses were of large production facilities with daily throughput of 80 or more pigs or small-scale plant with daily throughput of 50 or less. One large production facility with daily throughput of 200 or more heads was included. The nasal swab samples were collected from pigs after stunning and bleeding and stored in Stuart transport medium (OXOID, Thermo Fisher Scientific, Hampshire, England).

Bacterial isolation and identification: Isolation of *S. suis* was performed as previously described (Nutravong *et al.*, 2014). Briefly, nasal swab was streaked on Columbia agar supplemented with 5% sheep blood containing 20 µg/ml colistin (Sigma-

Aldrich, St. Louis, MO, USA) and 15 µg/ml nalidixic acid (Sigma-Aldrich, Louis) and incubated at 37°C under 5% CO_2 for 24 hours. For primary identification, four alpha hemolytic colonies were chosen using Gram staining and conventional biochemical tests. The isolates with typical characteristics were confirmed by PCR targeting 16S rRNA and glutamate dehydrogenase (*gdh*) genes.

PCR amplification and nucleotide sequencing: The total DNA prepared by whole-cell boiling method was used as a PCR template for all reactions (Okwumabua *et al.*, 2003). The lysate originated from a 500 µl of overnight bacterial culture in Todd Hewitt broth (OXOID, Thermo Fisher Scientific, Hampshire, England) at 37°C. All PCR amplifications were performed using a PCR mastermix (GeNeiTM MasterMix; Merck, Munich, Germany) according to the manufacturer's instructions. All primers and PCR conditions used in this study are listed in Table 1.

Serotyping of S. suis: The PCR-confirmed *S. suis* isolates (n=59) were subjected to identification of serotypes by multiplex PCR amplification of cps genes for the serotype 1, 2, 3, 4, 5, 7, 8, 9, 10, 14, 16, 19, 23 and 25 (Kerdsin *et al.*, 2012). The PCR amplicons were gel purified using Nucleospin® Gel (Mccherey-Nagel, Düren, Germany) and submitted for sequencing at First BASE Laboratories (Selangor Darul Ehsan, Malaysia). The obtained nucleotide sequence was compared to available sequences at GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) using BLAST.

Detection of virulence genes: Five virulence genes of *S. suis* were detected using PCR assay, including *epf* encoding extracellular protein, *mrp* encoding muramidase-released protein, *sly* encoding suilysin, *arcA* encoding arginine deiminase and *hyl* encoding hyaluronidase.

Detection of AMR genes: All *S. suis* isolates werescreened for the presence of 22 AMR genes of 6 antimicrobial agents using PCR assay. The AMR genes included *catA, catB* and *cmlA* for chloramphenicol resistance, *aadA1, aadA2*, and *aadB* for gentamicin resistance, *mefA, ermA*, and *ermB* for macrolide resistance, *sul1* and *sul2* for sulfamethoxazoleresistance, *tet*(A), *tet*(B), *tet* (K), *tet*(L), *tet*(M), *tet*(O), *tet*(W) and *tet*(32) for tetracyclines resistance, and *dfrA1, dfrA10* and *dfrA12* for trimethoprim resistance. The isolates positive to *tet*(M) were further detected for the presence of the Tn*916*-like transposon.

Antimicrobial susceptibility testing: Antimicrobial susceptibility test was performed using agar dilution technique to determine the Minimum Inhibitory Concentrations (MICs) according to Clinical and Laboratory Standards Institute (CLSI, 2018). Seventeen antimicrobial agents (Sigma-Aldrich®, Steinheim, Germany) and clinical breakpoints (µg/ml) tested including ampicillin (AMP, 2), ceftiofur (CEF, 8), chloramphenicol (CHL, 16), chlortetracycline (CTC, 8), clindamycin (CLI, 1), erythromycin (ERY, 1), florfenicol (FLO, 8), gentamicin (GEN, 16), neomycin (NEO, 32), oxytetracycline (OTC, 8), tetracycline (TET, 2), tiamulin (TIA, 32), tilmicosin (TIL, 32), tylosin (TYL, 8). enrofloxacin (ENR, 2), sulfamethoxazole (SUL, 512), and trimethoprim (TRI, 4). *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 served as quality control strains.

Table 1 Primer sequences used in this study.

Gene	Primer sequences (5′-3′)	PCR condition	Amplicon (bp)	Reference
16S rRNA	F: CAGTATTTACCGCATGGTAGATAT R: GTAAGATACCGTCAAGTGAGAA	94 °C, 15 s; 60 °C, 30 s; 72 °C, 2 min (30 cycles)	294	(Marois *et al.*, 2004)
gdh	F: TTCTGCAGCGTATTCTGTCAAACG R: TGTTCCATGGACAGATAAAGATGG	95 °C, 20 s; 58 °C, 30 s; 72 °C, 40 s (30 cycles)	695	(Kerdsin *et al.*, 2012)
Serotypes				
1 and 14	F: AATCATGGAATAAAGCGGAGTACAG R: ACAATTGATACGTCAAAATCCTCACC	95 °C, 20 s; 58 °C, 30 s; 72 °C, 40 s (30 cycles)	550	(Kerdsin *et al.*, 2012)
2 and ½	F: GATTTGTCGGGAGGGTTACTTG R: TAAATAATATGCCACTGTAGCGTCTC		450	(Kerdsin *et al.*, 2012)
3	F: TGGGAGAAGGCAGAAAGTACGAGA R: ACCCCCAGAAGAGCCGAAGGA		1273	(Kerdsin *et al.*, 2012)
4	F: ACTTGGAGTTGTCGGAGTAGTGCT R: ACCGCGATGGATAGGCCGAC		783	(Kerdsin *et al.*, 2012)
5	F: TGATGGCGGAGTTTGGGTCGC R: CGTAACAACCGCCCCAGCCG		166	(Kerdsin *et al.*, 2012)
7	F: GATGATTTATGGCACCCGAGTAAGC R: AGTCACAATTGCTGGTCCTGACACC		150	(Kerdsin *et al.*, 2012)
8	F: ATGGGCGTTGGCGGGAGTTT R: TTACGGCCCCCATCACGCTG		320	(Kerdsin *et al.*, 2012)
9	F: GGCTACATATAATGGAAGCCC R: CCGAAGTATCTGGGCTACTG		300	(Kerdsin *et al.*, 2012)
10	F: TCGCTCTGCGTTCGTCGAGT R: GCCCACCCGCCACGAGAAAG		1756	(Kerdsin *et al.*, 2012)
16	F: TGGAGGAGCATCTACAGCTCGGAAT R: TTTGTTTGCTGGAATCTCAGGCACC		202	(Kerdsin *et al.*, 2012)
19	F: AGCAGGGTTGCGTATGGCGG R: ACAAGCACCAGCAAAGACCGCA		1024	(Kerdsin *et al.*, 2012)
23	F: GCGGGCATATGCAGTGGGCA R: ACCGAATGCCACATCGGGTG		825	(Kerdsin *et al.*, 2012)
25	F: GGAGGAGCTGCGGGCTCATA R: GGAGGAGCTGCGGGCTCATA		1211	(Kerdsin *et al.*, 2012)

Table 1 Primer sequences used in this study (continued).

Gene	Primer sequences (5′-3′)	PCR condition	Amplicon (bp)	Reference
Virulence genes				
mrp	F: ATTGCTCCACAAGAGGATGG R: TGAGCTTTACCTGAAGCGGT	94 °C, 1 min; 58 °C, 1 min; 72 °C, 90 s (30 cycles)	188	(Silva *et al.*, 2006)
epf	F: ATCTACTGGGTATCCTTCTGC R: CTATCTGGATCTGTGATTGGA		626	(Maneerat *et al.*, 2013)
sly	F: GCTTGACTTACGAGCCACAA R: CCGCGCAATACTGATAAGC		248	(Takamatsu *et al.*, 2008)
arcA	F: TGATATGGTTGCTGCTGGTC R: GGACTCGAGGATAGCATTGG	94 °C, 30 s; 54 °C, 1 min; 72 °C, 1 min (30 cycles)	118	(Takamatsu *et al.*, 2008)
hyl	F: CTCAGATGAAAGCCTTTCTA R: TTTGTCCTTGGTCGTTGTC		1290	(King *et al.*, 2004)
AMR genes				
aadA1	F: CTCCGCAGTGGATGGCGG R: GATCTGCGCGCGAGGCCA	94 °C, 45 s; 56 °C, 45 s; 72 °C, 1 min (30 cycles)	631	(Chuanchuen, *et al.*, 2008b)
aadA2	F: CATTGAGCGCCATCTGGAAT R: ACATTTCGCTCATCGCCGGC		500	(Chuanchuen, *et al.*, 2008b)
aadB	F: CTAGCTGCGGCAGATGAGC R: CTCAGCCGCCTCTGGGCA		300	(Chuanchuen & Padungtod, 2009)
catA	F: CCAGACCGTTCAGCTGGATA R: CATCAGCACCTTGTCGCCT		452	(Chuanchuen, *et al.*, 2008b)
catB	F: CGGATTCAGCCTGACCACC R: ATACGCGGTCACCTTCCTG		416	(Chuanchuen, *et al.*, 2008b)
cmlA	F: TGGACCGCTATCGGACCG R: CGCAAGACACTTGGGCTGC	94 °C, 45 s; 57 °C, 45 s; 72 °C, 1 min (30 cycles)	641	(Chuanchuen, *et al.*, 2008a)
dfrA1	F: TGGACCGCTATCGGACCG R: CGCAAGACACTTGGGCTGC		254	(Chuanchuen, *et al.*, 2008b)
dfrA10	F: CAATGGCTGTTGGTTGGAC R: CCGGCTCGATGTCTATTGT		432	(Chuanchuen & Padungtod, 2009)
dfrA12	F: TCAAGGCAAATTACCTTGGC R: ATCTATTGGATCACCTACCC		330	(Chuanchuen, *et al.*, 2008b)

Table 1 Primer sequences used in this study (continued).

Gene	Primer sequences (5′-3′)	PCR condition	Amplicon (bp)	Reference
AMR genes				
ermA	F: CCCGAAAAATACGCAAAATTTCAT R: CCCTGTTTACCCATTTATAAACG	94 °C, 1 min; 58 °C 1 min; 72 °C, 1 min (30 cycles)	590	(Malhotra-Kumar *et al.*, 2003)
ermB	F: TGGTATTCCAAATGCGTAATG R: CTGTGGTATGGCGGGTAAGT	94 °C, 45 s; 60 °C 1 min; 72 °C, 1 min (30 cycles)	745	(Malhotra-Kumar *et al.*, 2005)
mefA	F: CAATATGGGCAGGGCAAG R: AAGCTGTTCCAATGCTACGG		317	(Malhotra-Kumar *et al.*, 2005)
sul1	F: CGGACGCGAGGCCTGTATC R: GGGTGCGGACGTAGTCAGC		591	(Chuanchuen *et al.*, 2007)
sul2	F: GCGCAGGCGCGTAAGCTGAT R: CGAAGCGCAGCCGCAATTC		514	(Chuanchuen & Padungtod, 2009)
tet(A)	F: GCTGTCGGATCGTTTCGG R: CATTCCGAGCATGAGTGCC	94 °C, 1 min; 58 °C 1 min; 72 °C, 1 min (30 cycles)	658	(Chuanchuen, *et al.*, 2008b)
tet(B)	F: CTGTCGCGGCATCGGTCAT R: CAGGTAAAGCGATCCCACC		615	(Chuanchuen, *et al.*, 2008b)
tet(K)	F: GATCAATTGTAGCTTTAGGTGAAGG R: TTTTGTTGATTTACCAGGTACCATT		155	(Malhotra-Kumar *et al.*, 2005)
tet(L)	F: TGGTGGAATGATAGCCCATT R: CAGGAATGACAGCACGCTAA		229	(Malhotra-Kumar *et al.*, 2005)
tet(M)	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC		406	(Malhotra-Kumar *et al.*, 2005)
tet(O)	F: AACTTAGGCATTCTGGCTCAC R: TCCCACTGTTCCATATCGTCA		515	(Malhotra-Kumar *et al.*, 2005)
tet(32)	F: GAACCAGATGCTGCTCTT R: CATAGCCACGCCCACATGAT		620	(Melville *et al.*, 2001)
Tn*916*	F: GCCATGACCTATCTTATA R: CTAGATTGCGTCCAA	94 °C, 1 min; 45 °C 1 min; 72 °C, 1 min (35 cycles)	476	(Agersø *et al.*, 2006)

Results

Prevalence of S. suis in pigs: Based on PCR detection of 16S rRNA and *gdh*, the prevalence of *S. suis* in four provinces varied from 3.0-9.4%. None of the isolates collected in Chiang Mai were confirmed to be *S. suis* by PCR. The highest prevalence was observed in Chiang Rai 9.4% (17/180), followed by Mae Hong Son 9.1% (6/66) Nan 6.3% (12/190) and Phayao 3.0% (6/202). Among these samples, 59 isolates (7.7%) were confirmed to be *S. suis* (Table 2).

Serotypes of S. suis: Of all 59 *S. suis* isolates, 4 serotypes including 2, 8, 9 and 10 were identified and the others were designated as "Unidentified" (Table 2, Fig. 1). Serotype 8 (50.8%) was most commonly identified in all provinces, followed serotype 10 (3.4%), serotype 2 (1.7%) and 9 (1.7%). All the isolates from Mae Hong Son 18.6% (11/11) and most isolates from Nan 18.6% (11/12) were serotype 8. Only one *S. suis* serotype 2 isolate was obtained, and it originated from a sample collected from Phayao. Most positive samples carried only one *S. suis* serotype, with the exception for 3 samples from Chiang Rai (n=2, serotype 10 and unidentified serotype) and Phayao (n=1, serotype 2 and 8) carried 2 *S. suis* serotypes.

Presence of virulence genes of S. suis: All *S. suis* isolates carried *arcA* whereas all isolates were negative for *epf*. The *sly* gene was very common (91.5%), followed by *mrp* (69.5%) and *hyl* (37.3%). Six different virulence gene patterns (VGP1-6) were identified in different serotypes (Table 3). The most identified pattern was *mrp-sly-arcA-hyl* (VGP1, 33.9%), followed by *mrp-sly-arcA* (VGP2, 28.8%) and *sly-arcA* (VGP5, 25.4%).

Antimicrobial resistance profiles: All *S. suis* isolates obtained from slaughtered pigs were resistant to tilmicosin (100%) and expressed a multidrug-resistant phenotype (MDR, resistance to at least 3 different antimicrobial classes). Almost all were resistant to chlortetracycline (98.3%), clindamycin (98.3%), tetracycline (96.6%) (Fig. 2). The distributions of MIC values of all *S. suis* isolates are presented in Table 4. AMR phenotypes of all *S. suis* isolates were classified into 48 profiles (Table 5) of which CLI-CTC-ERY-GEN-NEO-OTC-TET-TIL-TYL was the most common (n=3, 5.1%).

Presence of antimicrobial resistance genes: Of all AMR genes tested, *tet*(M) (19/59, 32.3%), *tet*(O) gene (18/59, 30.5%), and *mefA* (5/59, 8.5%) were detected (Table 6). When considering specific resistance phenotype, *tet*(M) was the most commonly found in tetracycline-resistant strains (32.3%) and followed by *tet*(O) (30.5%). The *mefA* was detected in macrolide-resistant strains (8.5%). For resistance gene patterns, the *tet*(M), *tet*(O) (8.5%) was most common followed by *tet*(M), *mefA* (3.4%) and *tet*(M), *tet*(O), *mefA* (1.7%). Five *tet*(M) positive *S. suis* isolates additionally carried Tn*916*- like transposons.

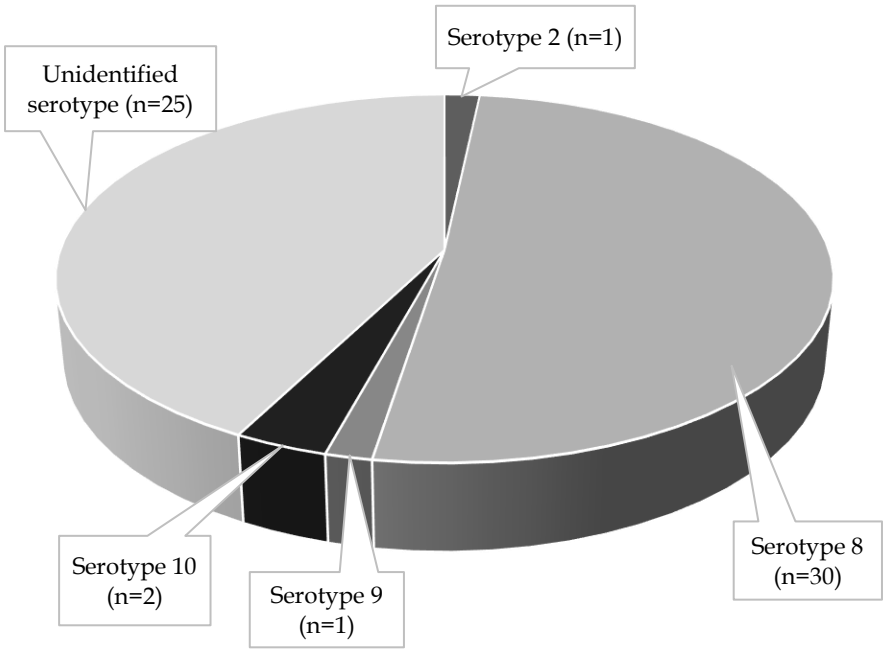

Figure 1 Serotypes of *S. suis isolated* from pigs in slaughterhouses in this study.

Table 2 Serotypes of *S. suis* isolated from pigs in slaughterhouses in each Upper Northern Province, Thailand (n=768)

Province (n)	No. of positive samples[a]	No. of *S. suis* isolates	Serotypes, No. of isolates (%)				
			2	8	9	10	Unidentified[b]
Phayao (202)	6	8	1 (1.7)	5 (8.5)	0	0	2 (3.4)
Nan (190)	12	13	0	11 (18.6)	0	0	2 (3.4)
Chiang Rai (180)	17	27	0	3 (5.1)	1 (1.7)	2 (3.4)	21 (35.6)
Mae Hong Son (66)	6	11	0	11 (18.6)	0	0	0
Total	**41**	**59**	**1 (1.7)**	**30 (50.8)**	**1 (1.7)**	**2 (3.4)**	**25 (42.4)**

[a]No positive samples in Chiangmai
[b]Not positive to 14 serotypes tested in this study.

Table 3 Distribution of serotypes and virulence genes in *S. suis* from different provinces (n=59)

Province (no. of isolate)	Serotypes	Number of isolates	Virulence gene profiles (%)[a]					
			VGP1	VGP2	VGP3	VGP4	VGP5	VGP6
Chiang Rai (27)	8	3	2 (3.4)	0	1 (1.7)	0	0	0
	9	1	0	1 (1.7)	0	0	0	0
	10	2	1 (1.7)	1 (1.7)	0	0	0	0
	Unidentified[b]	21	7 (11.9)	4 (6.8)	0	3 (5.1)	6 (10.2)	1 (1.7)
Nan (13)	8	11	3 (5.1)	6 (10.2)	0	0	2 (3.4)	0
	Unidentified[b]	2	1 (1.7)	0	0	0	1 (1.7)	0
Phayao (8)	2	1	1 (1.7)	0	0	0	0	0
	8	5	1 (1.7)	2 (3.4)	1 (1.7)	0	2 (3.4)	0
	Unidentified[b]	2	0	0	0	1 (1.7)	1 (1.7)	0
Mae Hong Son (11)	8	11	4 (6.8)	3 (5.1)	0	0	4 (6.8)	0
Total		**59**	**20 (33.9)**	**17 (28.8)**	**2 (3.4)**	**4 (6.8)**	**15 (25.4)**	**1 (1.7)**

[a]VGP1, *mrp-sly-arcA-hyl*; VGP2, *mrp-sly-arcA*; VGP3, *sly-arcA-hyl*; VGP4, *mrp-arcA*; VGP5, *sly-arcA*; VGP6, *arcA*
[b] Not positive to any serotypes tested in this study.

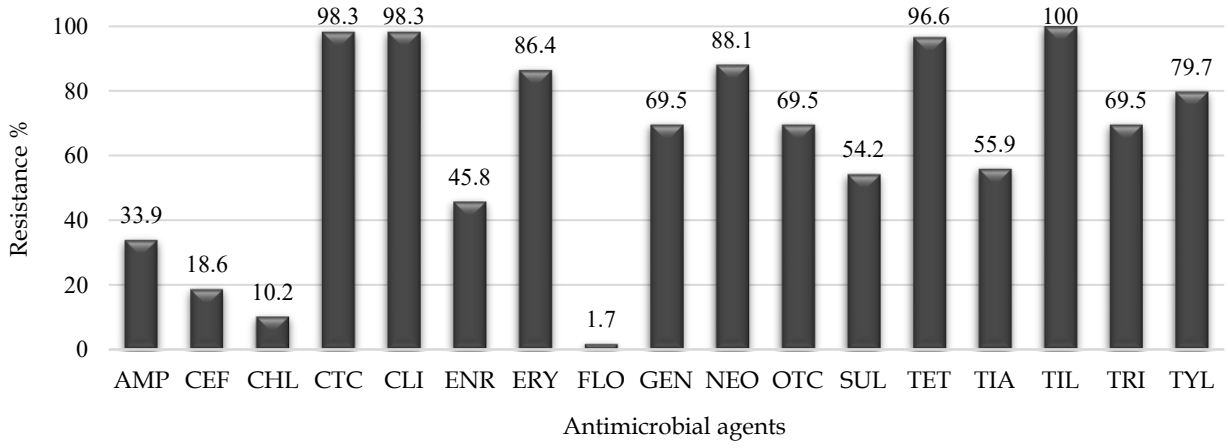

Figure 2 Distribution of antimicrobial resistance among *S. suis* (n=59)
Abbreviations: AMP, ampicillin; CEF, cetriofur; CHL, chloramphenicol; CTC, chlortetracycline; CLI, clindamycin; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; FLO, florfenicol; NEO, neomycin; OTC, Oxytetracycline; SUL, sulfamethoxazole; TET, tetracycline; TIA, tiamulin; TIL, tilmicosin; TRI, trimethroprim; TYL, tylosin.

Table 4 MIC distribution and resistance rates of *S. suis* (n=59)

Antimicrobial agents	MIC Range (µg/ml)[a]														Resistance
	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512	No. isolates (%)
Ampicillin	14	12	7	5	1	12	6	2	0	0	0	0	0	0	20 (3.9)
Cetiofur	0	24	3	4	2	2	13	9	0	2	0	0	0	0	11 (18.6)
Chloramphenicol	0	0	0	0	11	16	15	11	6	0	0	0	0	0	6 (10.2)
Chlortetracycline	0	0	0	0	0	0	1	1	6	21	30				58 (98.3)
Clindamycin	0	1	0	0	1	0	1	2	4	50					58 (98.3)
Erythromycin	0	7	1	0	0	0	0	0	51						51 (86.4)
Florfenicol	0	0	16	29	12	0	1	1	0		0	0	0	0	1 (1.7)
Gentamicin	0	1	0	0	1	1	1	14	14	20	0	7	0	0	41 (69.5)
Neomycin	0	0	0	0	0	1	4	0	2	5	7	26	14		52 (88.1)
Oxytetracycline	0	1	0	2	1	5	9	17	22	1	1				41 (69.5)
Tetracycline	0	0	1	0	1	0	1	3	18	13	17	5			57 (96.6)
Tiamulin	0	0	0	1	1	15	4	4	1	0	12	17	4		43 (55.9)
Tilmicosin	0	0	0	0	0	0	0	0	0	5	0	29	25		59 (100)
Tylosin	0	0	0	2	5	2	3	2	6	14	25				47 (79.7)
Enrofloxacin	0	6	14	10	2	2	0	2	0	23					27 (45.8)
Sulfamethoxazole	0	0	1	0	1	8	0	0	0	5	0	10	2	32	32 (54.2)
Trimethoprim	3		2	8	2	3	4	4	20	0	4	0	4	5	41 (69.5)

[a]black vertical lines indicated clinical breakpoint (µg/ml) used in this study.

Table 5 Antimicrobial resistance profiles of *S. suis* resistant isolates from pigs in Northern Thailand (n=59)

Profile	Antimicrobial resistance	No. of isolates (%)
1	CLI-CTC-NEO-TIL	1(1.7)
2	CTC-NEO-TET-TIL	1(1.7)
3	CLI-CTC-NEO-TET-TIL	2(3.4)
4	CLI-CTC-NEO-OTC-TET-TIL	1(1.7)
5	CLI-CTC-NEO-TET-TIL-TYL	1(1.7)
6	CLI-ERY-GEN-SUL-TIL-TRI	1(1.7)
7	CLI-CTC-ENR-ERY-OTC-TET-TIL	1(1.7)
8	CLI-CTC-ENR-SUL-TET-TIL-TRI	1(1.7)
9	CLI-CTC-ERY-GEN-NEO-OTC-TET-TIL	1(1.7)
10	AMP-CLI-CTC-ERY-NEO-OTC-SUL-TET-TIL	1(1.7)
11	AMP-CLI-CTC-GEN-NEO-SUL-TET-TIL-TRI	1(1.7)
12	CLI-CTC-ENR-ERY-NEO-OTC-TET-TIL-TYL	1(1.7)
13	CLI-CTC-ERY-GEN-NEO-OTC-TET-TIL-TYL	3(5.1)
14	CHL-CLI-CTC-ERY-GEN-NEO-OTC-TET-TIL-TYL	2(3.4)
15	CLI-CTC-ENR-ERY-GEN-NEO-OTC-TET-TIL-TYL	1(1.7)
16	CLI-CTC-ENR-ERY-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
17	CLI-CTC-ERY-GEN-NEO-SUL-TET-TIL-TRI-TYL	2(3.4)
18	CEF-CLI-CTC-ERY-GEN-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
19	CHL-CLI-CTC-ERY-GEN-NEO-OTC-SUL-TET-TIL-TYL	1(1.7)
20	CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TET-TIL-TYL	1(1.7)
21	CLI-CTC-ENR-ERY-NEO-OTC-TET-TIA-TIL-TRI-TYL	1(1.7)
22	CLI-CTC-ENR-GEN-NEO-OTC-TET-TIA-TIL-TRI-TYL	1(1.7)
23	CLI-CTC-ERY-GEN-NEO-OTC-SUL-TET-TIL-TRI-TYL	1(1.7)
24	CLI-CTC-ERY-GEN-NEO-OTC-SUL-TIA-TIL-TRI-TYL	1(1.7)
25	CLI-CTC-ERY-GEN-NEO-OTC-TET-TIA-TIL-TRI-TYL	1(1.7)
26	CLI-CTC-ERY-GEN-NEO-SUL-TET-TIA-TIL-TRI-TYL	2(3.4)
27	AMP-CHL-CLI-CTC-ERY-GEN-NEO-OTC-SUL-TET-TIL-TRI	1(1.7)
28	AMP-CLI-CTC-ENR-ERY-NEO-OTC-TET-TIA-TIL-TRI-TYL	2(3.4)
29	AMP-CLI-CTC-ERY-GEN-NEO-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
30	CEF-CLI-CTC-ENR-ERY-NEO-OTC-TET-TIA-TIL-TRI-TYL	2(3.4)
32	CEF-CLI-CTC-ERY-GEN-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
33	CHL-CLI-CTC-ERY-GEN-NEO-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
34	CLI-CTC-ENR-ERY-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
35	AMP-CEF-CLI-CTC-ENR-ERY-GEN-NEO-OTC-TET-TIA-TRI-TYL	2(3.4)
36	AMP-CEF-CLI-CTC-ERY-GEN-NEO-OTC-TET-TIA-TIL-TRI-TYL	1(1.7)
37	AMP-CHL-CLI-CTC-ERY-GEN-NEO-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
38	AMP-CLI-CTC-ENR-ERY-GEN-NEO-OTC-TET-TIA-TIL-TRI-TYL	2(3.4)
39	AMP-CLI-CTC-ENR-ERY-GEN-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
40	AMP-CLI-CTC-ENR-ERY-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
41	CLI-CTC-ENR-ERY-GEN-FLO-NEO-OTC-SUL-TET-TIL-TRI-TYL	1(1.7)
42	CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	2(3.4)
43	AMP-CEF-CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TIA-TIL-TRI-TYL	1(1.7)
44	AMP-CEF-CLI-CTC-ENR-ERY-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
45	AMP-CEF-CLI-CTC-ERY-GEN-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
46	AMP-CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	2(3.4)
47	CEF-CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
48	AMP-CEF-CLI-CTC-ENR-ERY-GEN-NEO-OTC-SUL-TET-TIA-TIL-TRI-TYL	1(1.7)
Total		**59**

Table 6 Antimicrobial resistance genes of *S. suis* isolates from pigs in Upper Northern Thailand (n=59)

Antimicrobial resistance genes	Number of isolates (%)	Serotypes					Presence of Tn*916*
		2	8	9	10	Unidentified	
tet(M), *tet*(O), *mefA*	1 (1.7)	0	1 (1.7)	0	0	0	0
tet(M), *tet*(O)	5 (8.5)	0	5 (8.5)	0	0	0	+ (n=2)
tet(M), *mefA*	2 (3.4)	0	2 (3.4)	0	0	0	0
tet(M)	11 (18.6)	0	6 (10.2)	0	0	5 (8.5)	+ (n=3)
tet(O)	12 (20.3)	1 (1.7)	6 (10.2)	0	0	0	0
mefA	2 (3.4)	0	1 (1.7)	1 (1.7)	0	0	0
None	26 (44.1)	0	9 (15.3)	0	2	20 (33.9)	0
Total	**59**	**1**	**30**	**1**	**2**	**25**	**5**

Discussion

One of the major findings was the presence of *S. suis* in pigs at slaughterhouses in five provinces in Upper Northern Thailand, with prevalence ranging from 3.0 to 9.4%. The samples originated from clinically healthy pigs that were slaughtered for human consumption, in agreement with previous studies (Nutravong *et al.*, 2014; Thongkamkoon *et al.*, 2017). These *S. suis* can distribute and promote cross-contamination during slaughtering processes. It highlights the important role of pigs as carriers of *S. suis* that may enter food chain and potentially pose risk to farmers, butchers, slaughterhouse workers etc.

Previous studies revealed that serotype 2 was the main serotype for infections in patients, followed by serotypes 4, 5, 7, 9, 14, 16, 21, 24, and 31 (Goyette-Desjardins *et al.*, 2014; Segura *et al.*, 2020). These *S. suis* serotypes have been reported to increasingly emerge in Thailand and other countries in Southeast Asia (Kerdsin *et al.*, 2018; Nguyen Thi Hoang Mai *et al.*, 2008; Segura *et al.*, 2020). With the exception of serotypes 2 and 9, the other serotypes were not detected among isolates in the present study. Regardless, the results confirm the contribution of clinically healthy pigs as carriers for *S. suis* in the study. Multiple *S. suis* serotypes can be identified in tonsils of diseased pigs, while pigs can be infected with multiple serotypes of *S. suis* (Reams *et al.*, 1996). This agrees with the observations in this study, of which two samples from Chiang Rai (serotype 2 and unidentified) and a sample from Phayao (serotype 8 and 10) carried *S. suis* of two different serotypes. In addition, non-typable isolates were most prevalent in this study in agreement with previous studies conducted in clinically ill and healthy pigs in Thailand, Spain and other Asian countries (Arndt *et al.*, 2018; Prüfer *et al.*, 2019). These indicates the high genetic diversity and mutations of *S. suis* capsular biosynthesis genes (Werinder *et al.*, 2020). The phenomenon may contribute to the difficulty in disease control by vaccines (Lunha *et al.*, 2022). Currently, autogenous bacterins are used as vaccines for control of the disease. It has been hypothesized that a bacterin vaccine is serotype specific. It confers protection against the challenge with strains of a homologous serotype (Kebede *et al.*, 1990) and may not affect new outbreaks caused by *S. suis* strains belonging to other serotypes. This underlines the need for identification of specific *S. suis* strains, not only those involved in diseased animals but also those involved in the carrier state. It is to provide data essential for development of adequate control measures and vaccine.

As a global important, the difference of virulence genes among strains of serotype 2 is well documented (Yongkiettrakul *et al.*, 2019). In this study, *mrp*, *epf*, and *sly* considered as the most relevant factors to the pathogenesis of *S. suis* were predominant (Prüfer *et al.*, 2019; Zhang *et al.*, 2015). The *arcA* encoding arginine deiminase was previously found in all pathogenic serotype 2 obtained from Thai isolates (Maneerat *et al.*, 2013), consistent with this study where all the *S. suis* isolates carried *arcA*. It was suggested that *arcA* has a presumptive role in the bacterial pathogenicity and could be one of the main virulence factors in the pathogen (Gruening *et al.*, 2006; Rajkhowa & Rajesh, 2021). It is important to observe that many *S. suis* isolates from clinically healthy pigs harbored *mrp*, *hyl* and *sly* virulence genes, even though none were positive for *epf* gene. Up-to-date knowledge on virulence factors in *S. suis* from clinically healthy pigs is still limited. It is suggested that genotyping based on virulence factors should be performed, and the comparison between the isolates from healthy and diseased individuals should be conducted.

Currently, most AMR studies are conducted in Enterobacterales, e.g. *Salmonella*, *Campylobacter* and *Escherichia coli* (Chuanchuen & Padungtod, 2009; Chuanchuen, Pathanasophon, *et al.*, 2008; Trongjit *et al.*, 2016). Despite a deadly pathogen, such study in *S. suis* is still limited. The *S. suis* isolates from pigs at slaughterhouse exhibited high resistance rates (>50%), to clinically important antimicrobials including those that have been extensively used in pig industry for a long time (Lunha *et al.*, 2022). Resistance to tetracycline and macrolide have been widely reported in *S. suis* strains isolated from pigs in Asia, Europe, and North America (Petrocchi-Rilo *et al.*, 2021; Segura *et al.*, 2020), in agreement with the result in this study, (tetracycline,

96.6% and erythromycin, 86.4%). Resistance to chloramphenicol (10.2 %) was still limited and lower than that of *Salmonella* and *E. coli* isolated from food animals and food of animal origins in the same region (Sinwat *et al.*, 2015). The similar phenomenon was observed for resistance rates to sulfamethoxazole, ampicillin and enrofloxacin. A study conducted in the pig isolates in Brazil reported that more than 80% of the strains were susceptible to ceftiofur and florfenicol (Takamatsu *et al.*, 2008) and suggested that both antimicrobials are drug of choice for empirical control of the infections caused by *S. suis* (Takamatsu *et al.*, 2008). Several studies demonstrated that the *S. suis* isolates from diseased and healthy pigs in different countries exhibited differences in the level of resistance (Matajira *et al.*, 2019; Petrocchi-Rilo *et al.*, 2021; Rajkhowa & Rajesh, 2021). The differences were observed between serotypes over time (Segura *et al.*, 2020; Tan *et al.*, 2021). However, the isolates from diseased pigs were not included in this study and the experiment design was for cross sectional study. Importantly, all *S. suis* isolates in this collection were MDR and most were resistant to more than 9 antibiotics simultaneously. This raises particular concern of limited antimicrobials of choice for the *S. suis* infection treatment.

Of all resistance genes tested, *tet*(M), *tet*(O) and *mefA* were detected. In this study, all the isolates carried *tet*(M) were resistant to either oxytetracycline or tetracycline and positive to Tn916-like transposon, consistent to a previous study (Liu *et al.*, 2020). Tn916 is a conjugative transposon that typically carries the transferable *tet*(M) gene. However, *tet*(M) gene can also be found on other genetic elements, such as plasmids and integrons (Agersø *et al.*, 2006). The resistance genes detected were previously reported among Salmonella and *E. coli* isolated from livestock in Thailand (Chuanchuen & Padungtod, 2009; Chuanchuen, Pathanasophon, *et al.*, 2008; Trongjit *et al.*, 2016). The presence of the same genes in different bacterial species suggested horizontal transfer of resistance genes that plays an important role for AMR distribution (Segura *et al.*, 2020). However, transferability of tetracycline resistance was not pursued in this study and should be further tested in both Gram positive and Gram-negative recipients.

Overall, the presence of AMR genes was well corresponded to resistance phenotypes. The *S. suis* isolates carrying *tet*(M), which encodes efflux protein and *tet*(O), which encodes ribosomal protection proteins exhibited high tetracycline MIC values (128 µg/mL), consistent with findings from a previous study (Hoa *et al.*, 2011). A high resistance rate to erythromycin (86.4%) was also observed, aligning with a study from China that reported a similar trend in *S. suis* isolated from healthy pigs (Zhang *et al.*, 2020). Isolates carrying *mefA*, which encodes a macrolide efflux pump, demonstrated high erythromycin MIC values (16 µg/mL), supporting earlier reports of the frequent presence of *mefA* in erythromycin-resistant *S. suis* isolates (D'Ercole *et al.*, 2005; Song *et al.*, 2004). However, the statistical association between the AMR phenotype and genotype was not determined due to limited number of isolates.

It is important to acknowledge that this study has certain limitations. First, it focused on *S. suis* in a specific region of Northern Thailand, which may not capture the full range of strain diversity that could exist in other areas with varying environmental conditions and farming practices. Including additional regions in future research could offer a more comprehensive picture of the pathogen's distribution and resistance patterns, potentially enhancing the effectiveness of control strategies and disease management efforts on a broader scale. Second, the study did not encompass all known *S. suis* serotypes, of which there are over 30. As a result, some aspects of the pathogen's diversity may not be fully represented. Incorporating a wider range of serotypes in future studies could support more robust surveillance and control measures for *S. suis* infections.

In conclusion, clinically healthy pigs at slaughterhouses serve as carriers for MDR *S. suis* carrying AMR genes. This highlights the importance of awareness raising on food safety and hygiene, in particular the consumption of well-cooked pork and other types of meat. Therefore, AMR monitoring in *S. suis* should be routinely conducted. Identification of *S. suis* serotypes is recommended for the benefit of future vaccine development.

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