

Research article

Low Prevalence of *Streptococcus suis* and Bacterial Contamination in Raw Pork Products from Fresh Markets in Nakhon Ratchasima, Thailand

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Abstract

Streptococcus suis is a zoonotic pathogen associated with severe human infections, particularly in regions where consumption of raw pork is common. This study aimed to investigate the prevalence of *S. suis* in raw pork products sold in fresh markets in Mueang District, Nakhon Ratchasima Province, Thailand. A total of 113 raw pork samples were collected from four fresh markets and analyzed microbiologically. *S. suis* was detected in one liver sample, corresponding to a low overall prevalence of 0.88%. Nonetheless, microbial growth was observed in 55.75% (63/113) of samples, yielding 68 isolates. Liver and loin samples demonstrated the greatest microbial diversity and frequency of contamination. Biochemical tests of the *S. suis* isolate revealed characteristic features including alpha-hemolytic activity, catalase negativity, and selective sugar fermentation, which were consistent with known profiles of the species. Identification was confirmed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and detected species included *Enterococcus faecalis*, *Enterococcus casseliflavus*, *Staphylococcus hominis*, and *Staphylococcus saprophyticus*. Antimicrobial susceptibility testing showed that the isolate was susceptible to beta-lactams, quinolones, chloramphenicol, tigecycline, linezolid, trimethoprim/sulfamethoxazole, and vancomycin,

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while resistance was observed to macrolides, lincosamides, and tetracycline. Multiplex PCR confirmed the isolate as *S. suis* serotype 2, and multilocus sequence typing (MLST) further identified it as sequence type 104 (ST104). These findings indicate the microbial contamination risk associated with raw pork sold at fresh markets and emphasize the importance of routine surveillance and improved hygiene practices.

Keywords: food safety; raw pork; serotype; *Streptococcus suis*; zoonotic pathogen

1. Introduction

Streptococcus suis is a zoonotic bacterial pathogen responsible for a range of severe diseases in humans, including meningitis, septicemia, arthritis, and endophthalmitis. Complications following infection often leads to permanent hearing loss, commonly referred to as "deafness fever", vestibular dysfunction, or other sequelae (Özsürekcı et al., 2023). This organism is predominantly found in the respiratory tract and blood of diseased pigs and can be transmitted to humans through wounds, abrasions, mucous membranes, or by the consumption of raw or undercooked pork products, including blood and organs. Due to these modes of transmission, *S. suis* infections are classified as zoonotic infectious diseases (Papatsiros et al., 2011).

Streptococcus suis belongs to the genus *Streptococcus* and the family Streptococcaceae. It is a gram-positive, coccoid bacterium classified into Lancefield groups D, R, or S. This bacterial species is capable of producing a capsule and exhibits hemolytic activity. Based on capsular antigens, *S. suis* is divided into 35 serotypes, each with varying levels of virulence (Okura et al., 2016). The virulence of individual serotypes is influenced by the production of factors such as muramidase-released protein (MRP) and extracellular protein (EP). Among these, serotype 2 and serotype 1 are identified as highly virulent strains in humans. The distribution of serotypes varies geographically, with specific serotypes being more prevalent in certain regions (Li et al., 2017). *Streptococcus suis* was first identified as a human pathogen in 1968 in Denmark, where two cases of meningitis and one case of septicemia were reported (Perch et al., 1968). Since then, *S. suis* infections have been documented globally (Arends & Zanen, 1988).

In Thailand, the first reported human case occurred in 1987, and subsequent reports have indicated widespread infections across the country, particularly in the northern and northeastern regions, where the incidence is highest (Kerdsin, 2022). Zoonotic transmission in Thailand primarily occurs through the consumption of raw or undercooked pork and pork-derived products, often in association with close contact with infected pigs or contaminated pork. Among the diverse *S. suis* serotypes identified, serotype 2 is the most prevalent and is associated with severe invasive diseases such as meningitis and septicemia, accounting for 92% of human cases in Phayao Province (Takeuchi et al., 2012). A case fatality rate of 9.5% has been reported in Nakhon Ratchasima Province (Karavanonth et al., 2019). While serotype 2 is the primary strain implicated in human infections, other serotypes, including 1, 14, and 9, have been isolated from pigs, though their contribution to zoonotic transmission appears limited (Kerdsin et al., 2020).

Beyond serotyping, molecular epidemiological tools such as multilocus sequence typing (MLST) have been widely used to characterize *S. suis* populations and to elucidate genetic diversity, evolutionary relationships, and transmission pathways. MLST classifies isolates into sequence types (STs) based on allelic profiles of conserved housekeeping genes, providing a high-resolution method for comparing strains across geographic regions and host species. Several STs have been strongly associated with virulence and zoonotic

potential, particularly ST1, ST7, and ST104, which are frequently linked to human infections and invasive disease (Kerdsin et al., 2022; King et al., 2002). MLST has also been instrumental in tracing outbreaks, identifying clonal expansion, and correlating specific STs with antimicrobial resistance patterns and clinical outcomes.

Recent studies have indicated the emergence of zoonotic *S. suis* strains in Thailand with increased antimicrobial resistance, posing a growing public health concern (Brizuela et al., 2023). Resistance to penicillin, the first-line treatment for *S. suis* infections, has increased from approximately 5% to 12% in recent years (Singpoltan, 2019; Kiatkangwanchon et al., 2023), alongside resistance to macrolides and tetracyclines, further complicating treatment options. MLST-based analyses have revealed that certain sequence types associated with human infections also harbor multidrug resistance traits, emphasizing the importance of integrating molecular typing with phenotypic surveillance. These trends likely reflect the widespread misuse and overuse of antibiotics in both human and veterinary medicine. Regional variations in serotype and sequence type distribution are influenced by farming practices, pork consumption behaviors, and environmental factors, emphasizing the need for continuous molecular surveillance, antimicrobial stewardship, and public health education to mitigate the burden of *S. suis* in Thailand.

Mueang District, Nakhon Ratchasima Province was selected for the study because it is a major hub of livestock production, pork distribution, and traditional fresh markets in Nakhon Ratchasima Province. Its high population, high pork consumption, and prevalent cultural practices of eating raw pork dishes make it a key area for zoonotic pathogen surveillance, particularly for *S. suis*.

Given that the culture methodology in this study employed NNCC selective agar - which primarily targets gram-positive cocci - the present investigation was designed to focus on the detection of *S. suis* and other gram-positive bacteria in raw pork products, rather than a comprehensive survey of all microbial groups.

Accordingly, this study aimed to determine the prevalence and to characterize *S. suis* and other gram-positive bacteria present in raw pork products sold in fresh markets in Mueang District, Nakhon Ratchasima Province. By incorporating serotyping, antimicrobial susceptibility testing, and MLST analysis, the findings contribute to baseline molecular surveillance data and support improved risk assessment and public health strategies for food safety and zoonotic disease prevention in northeastern Thailand.

2. Materials and Methods

2.1 Study design

A cross-sectional study was conducted to detect *S. suis* in raw pork products, determine its antimicrobial susceptibility profiles, and identify its serotypes. The study was performed in August 2024 in Mueang District, Nakhon Ratchasima Province, Thailand. The study population consisted of raw pork products sold in four fresh markets (FM1, FM2, FM3, and FM4) within the Mueang District Municipality. Samples included various pork tissues and organs such as belly, blood, bone, collar, ham, heart, liver, loin, lung, minced pork, shank, shoulder, solid fat, spleen, and tenderloin, as summarized in Table 1.

Table 1. Specimens from raw pork product samples and sampling fresh markets

Specimens	Fresh Markets				Total
	FM1	FM2	FM3	FM4	
Belly	5	2	-	5	12
Blood	-	-	1	1	2
Bone	-	-	-	1	1
Collar	5	2	-	2	9
Ham	5	2	10	9	26
Heart	-	1	-	1	2
Liver	10	1	-	6	17
Loin	15	1	-	2	18
Lung	-	1	-	-	1
Minced pork	-	-	-	2	2
Shank	-	-	-	1	1
Shoulder	-	2	-	-	2
Solid fat	-	1	-	-	1
Spleen	-	1	-	-	1
Tenderloin	10	4	-	4	18
Total	50	18	11	34	113

2.2 Ethical considerations

This study was reviewed and approved by the Human Research Ethics Committee of Nakhon Ratchasima College, Thailand, under approval number NMCEC-0053/2567. The research involved the collection and microbiological analysis of raw pork and edible pig organ samples obtained from fresh markets and did not involve human participants or the use of identifiable personal data. All procedures were conducted in accordance with relevant ethical guidelines and regulations to ensure responsible research practice.

2.3 Sample collection and handling

A total of 113 raw pork samples were collected from seven vendors across four fresh markets in Mueang District Municipality, Nakhon Ratchasima Province, Thailand. Each sample (minimum 25 g) was aseptically placed into sterile plastic bags, labeled with collection details, and transported in iceboxes to the laboratory for same-day processing. The collection method followed Guntala et al. (2024).

2.4 Screening of *S. suis*

Each 25 g sample was homogenized in 225 mL of sterile normal saline (0.85% NaCl; Merck, Germany). A 0.1 mL aliquot of the suspension was plated onto NNCC agar prepared from Todd-Hewitt broth (BD Difco, USA) containing 1.5% Bacto agar (BD Difco, USA) and 5% defibrinated sheep blood (Thermo Fisher Scientific, USA), supplemented with sodium azide (50 µg/mL; Sigma-Aldrich, USA), nalidixic acid (25 µg/mL; Sigma-Aldrich, USA), colistin (12.5 µg/mL; Sigma-Aldrich, USA), and crystal violet (2 µg/mL; Sigma-Aldrich, USA), following the formulation adapted from Kataoka et al. (1991). Plates were incubated at 37°C in a 5% CO₂ atmosphere for 18-24 h.

From each presumptive plate, all morphologically distinct colonies (typically 1-3 colonies per plate) displaying typical *S. suis* characteristics (small, translucent,

occasionally mucoid, with alpha-hemolysis) were selected for further screening. Colonies were sub-cultured on sheep blood agar (Thermo Fisher Scientific, USA) for purification and stored in Todd-Hewitt broth containing 20% glycerol (Merck, Germany) at -80°C.

2.5 Identification of isolated colonies

All isolates were sub-cultured on Dorset egg slants (HiMedia Laboratories, India) and sent to Phramongkutklao College of Medicine. Species confirmation was performed using Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany) following the manufacturer's instructions. When multiple colonies of the same species were obtained from a single sample, isolates with identical phenotypic characteristics were considered duplicates, and only one representative isolate was retained for further analysis.

2.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the broth microdilution method with the Sensititre ARIS 2X ID/AST System (Thermo Fisher Scientific, USA). Minimum inhibitory concentrations (MICs) were determined for a panel of antibiotics supplied in the Sensititre plates (Thermo Fisher Scientific, USA), including beta-lactams, macrolides, carbapenems, fluoroquinolones, chloramphenicol, daptomycin, linezolid, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin. Interpretations followed Clinical and Laboratory Standards Institute guidelines (CLSI, 2014).

2.7 Serotyping by Multiplex PCR

Multiplex PCR for serotyping was performed following Kerdsin et al. (2014) and Thu et al. (2021). Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany) and amplified with serotype-specific primer sets synthesized by Integrated DNA Technologies (IDT, Singapore). PCR reactions were performed using Taq DNA Polymerase and dNTPs (Thermo Fisher Scientific, USA). Amplicons were analyzed by gel electrophoresis using agarose (Promega, USA) and compared with reference serotype profiles.

2.8 Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) was performed on the confirmed *S. suis* isolate to determine its sequence type (ST). Genomic DNA extracted as described above was used as the template for PCR amplification of seven housekeeping genes (*aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA*, and *thrA*) according to the standardized *S. suis* MLST scheme described by King et al. (2002). PCR amplification was conducted using gene-specific primers, and reaction conditions followed the published protocol.

PCR products were purified and subjected to bidirectional Sanger sequencing. The obtained sequences were edited and assembled using standard sequence analysis software, and allele numbers were assigned by comparison with the *S. suis* MLST database. The allelic profile was used to determine the corresponding sequence type (ST). Novel alleles or allelic profiles, if identified, were submitted to the MLST database curator for assignment.

2.9 Statistical analysis

Descriptive statistics, including percentages, means, and standard deviations, were used to summarize the findings. The contamination rate of *S. suis* was expressed as the percentage of positive samples among the total number of samples analyzed.

3. Results and Discussion

3.1 Prevalence of *S. suis* and other organisms in raw pork products

Out of the 113 raw pork samples collected from four fresh markets, *S. suis* was isolated from 1 sample, resulting in an overall prevalence of 0.88%. In total, 63 samples (55.75%) showed organism growth on NNCC agar, yielding 68 isolates (Figure 1). Among these, the most predominant organism identified was *Enterococcus faecalis* (42 isolates, 61.76%), followed by coagulase-negative staphylococci (11 isolates, 16.18%) and other enterococci (9 isolates, 13.24%). The remaining isolates consisted of a mixture of other gram-positive cocci and yeast as shown in Figure 1.

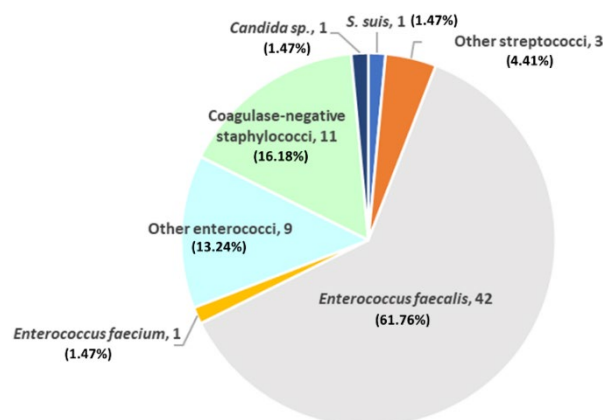


Figure 1. Proportional distribution of organism isolates on NNCC Agar

3.2 Distribution of organism isolates by sample type and market

Table 2 presents the distribution of organism isolates obtained from various raw pork products collected across four fresh markets. A total of 68 isolates were recovered, with the highest number of isolates obtained from FM1 (30 isolates), followed by FM4 (20 isolates), FM2 (12 isolates), and FM3 (6 isolates).

Among the identified organisms, *E. faecalis* was the most frequently isolated species, found in multiple sample types including liver, loin, ham, collar, and tenderloin, with a total of 42 isolates across all markets. *S. suis* was detected in one liver sample from Fresh Market 1. Other bacterial groups identified include coagulase-negative staphylococci (11 isolates), other streptococci (3 isolates), *E. faecium* (1 isolate), other enterococci (9 isolates), and a single *Candida sp.* isolate from a liver sample. The liver and loin samples yielded the highest diversity and frequency of bacterial isolates, emphasizing these products as important targets for microbial monitoring in raw pork sold at fresh markets.

Table 2. Distribution of organism isolates from raw pork products collected at four fresh markets

Organisms	Specimens	Number of Isolates				Total
		FM1	FM2	FM3	FM4	
<i>S. suis</i>	Liver	1	-	-	-	1
Other streptococci	Belly	1	-	-	-	1
	Collar	2	-	-	-	2
<i>E. faecalis</i>	Belly	-	-	-	2	2
	Collar	3	-	-	1	4
	Ham	2	1	3	2	8
	Heart	-	-	-	1	1
	Liver	4	1	-	4	9
	Loin	5	2	-	2	9
	Lung	-	1	-	-	1
	Minced pork	-	-	-	1	1
	Shank	-	-	-	1	1
	Shoulder	-	1	-	-	1
	Spleen	-	1	-	-	1
	Tenderloin	1	1	-	2	4
<i>E. faecium</i>	Ham	-	-	1	-	1
Other enterococci	Belly	1	-	-	-	1
	Collar	-	2	-	-	2
	Ham	-	-	-	2	2
	Liver	-	-	-	1	1
	Loin	-	1	-	1	1
	Tenderloin	1	-	-	-	1
Coagulase-negative staphylococci	Belly	1	-	-	-	1
	Collar	1	-	-	-	1
	Ham	1	-	2	-	3
	Liver	1	-	-	-	1
	Loin	1	-	-	-	1
	Tenderloin	3	1	-	-	4
<i>Candida</i> sp.	Liver	1	-	-	-	1
Total		30	12	6	20	68

3.3 Identification of isolated colonies

MALDI-TOF MS was employed to confirm the species-level identity of 68 isolates obtained from raw pork products as shown in Table 3. The majority of the isolates were bacteria, with a predominance of catalase-negative enterococci, particularly *E. faecalis*, which accounted for 42 isolates (61.76%). Other enterococcal species identified included *E. casseliflavus*, *E. faecium*, and *E. gallinarum*, each comprising 1.47% of the total.

Among catalase-positive organisms, coagulase-negative staphylococci were commonly detected, including *S. hominis* (5.88%), *S. saprophyticus* (2.94%), and other species such as *S. arlettae*, *S. capitis*, *S. epidermidis*, *S. haemolyticus*, and *S. warneri* (each 1.47%). The genus *Streptococcus* was represented by *S. suis*, *S. canis*, *S. dysgalactiae*, and *S. porcinus*, each also contributing 1.47% of isolates. A single isolate was confirmed as *S. suis* by MALDI-TOF MS with acceptable score value, indicating high-confidence species-level identification. Additionally, *Vagococcus* species were detected, with *V. fluvialis* and *V. lutrae* representing 8.82% and 1.47% of isolates, respectively. A single fungal isolate, *C. parapsilosis*, was also identified (1.47%).

Table 3. Microorganisms identified in raw pork products by MALDI-TOF MS

Organisms		Genus	Species	Number of Isolates	Proportion (%)
Bacteria	Catalase positive	<i>Staphylococcus</i> (all species are coagulase-negative staphylococci)	<i>S. arlettae</i>	1	1.47
			<i>S. capitis</i>	1	1.47
			<i>S. epidermidis</i>	1	1.47
			<i>S. haemolyticus</i>	1	1.47
			<i>S. hominis</i>	4	5.88
			<i>S. saprophyticus</i>	2	2.94
			<i>S. warneri</i>	1	1.47
	Catalase negative	<i>Streptococcus</i>	<i>S. canis</i>	1	1.47
			<i>S. dysgalactiae</i>	1	1.47
			<i>S. porcinus</i>	1	1.47
			<i>S. suis</i>	1	1.47
		<i>Enterococcus</i>	<i>E. casseliflavus</i>	1	1.47
			<i>E. faecalis</i>	42	61.76
			<i>E. faecium</i>	1	1.47
			<i>E. gallinarum</i>	1	1.47
		<i>Vagococcus</i>	<i>V. fluvialis</i>	6	8.82
			<i>V. lutrae</i>	1	1.47
Fungi	<i>Candida</i>	<i>C. parapsilosis</i>	1	1.47	
Total				68	

3.4 Antimicrobial susceptibility profiles of *S. suis*

The *S. suis* isolate was susceptible to most tested antimicrobials, including beta-lactams, carbapenems, fluoroquinolones, chloramphenicol, tigecycline, linezolid, trimethoprim/sulfamethoxazole, and vancomycin. Resistance was observed to azithromycin, erythromycin, clindamycin, and tetracycline. Overall, the susceptibility profile indicates broad sensitivity to first-line and critically important antimicrobials, with resistance mainly confined to macrolides and tetracyclines as shown in Table 4.

3.5 Serotyping of *S. suis*

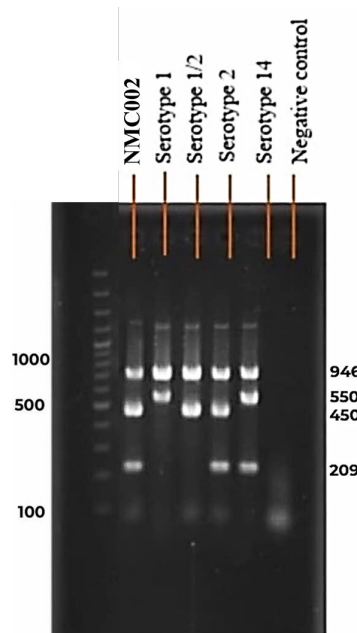
Multiplex PCR was employed to determine the serotype of the *S. suis* isolate. Amplified DNA fragments of 946 bp, 450 bp, and 209 bp were detected, which were consistent with the genetic profile of *S. suis* serotype 2, as shown in Figure 2. The serotyping was conducted following established protocols, confirming the isolate as serotype 2. The isolate has been documented and assigned the code NMC002.

3.6 Multilocus sequence typing (MLST) analysis

MLST analysis of the confirmed *S. suis* isolate was performed based on the allelic profiles of seven housekeeping genes (*aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA*, and *thrA*). Sequence analysis and comparison with the *S. suis* MLST database assigned the isolate to sequence type 104 (ST104). This sequence type has previously been reported among zoonotic *S. suis* strains associated with human infections in Thailand.

Table 4. Antimicrobial susceptibility profile of *S. suis* isolate

Antimicrobial Agent	MIC ($\mu\text{g/mL}$)	Interpretation
Amoxicillin/Clavulanic acid	≤ 2	Susceptible
Azithromycin	>2	Resistant
Cefepime	≤ 0.5	Susceptible
Cefotaxime	≤ 0.12	Susceptible
Ceftriaxone	≤ 0.12	Susceptible
Cefuroxime (sodium)	≤ 0.5	Susceptible
Chloramphenicol	4	Susceptible
Clindamycin	>1	Resistant
Daptomycin	≤ 0.06	Susceptible
Ertapenem	≤ 0.5	Susceptible
Erythromycin	>2	Resistant
Levofloxacin	≤ 0.5	Susceptible
Linezolid	0.5	Susceptible
Meropenem	≤ 0.25	Susceptible
Moxifloxacin	≤ 1	Susceptible
Penicillin	≤ 0.03	Susceptible
Tetracycline	>8	Resistant
Tigecycline	0.03	Susceptible
Trimethoprim/Sulfamethoxazole	≤ 0.5	Susceptible
Vancomycin	≤ 0.5	Susceptible

**Figure 2.** Multiplex PCR results for *S. suis* serotyping. The gel electrophoresis image shows amplified DNA bands at 946 bp, 450 bp, and 209 bp, corresponding to serotype 2.

Lane 1: DNA ladder; Lane 2: *S. suis* isolate NMC002;
 Lanes 3-6: *S. suis* serotypes; Lane 7: Negative controls

3.7 Discussion

In this study, *S. suis* was isolated from one of 113 raw pork and edible pig organ samples collected in Nakhon Ratchasima Province in the northeastern region of Thailand, corresponding to a low prevalence of 0.88%, with the single isolate identified as serotype 2. This prevalence is substantially lower than that reported in other studies from the northeastern region, including Khon Kaen Province, where *S. suis* serotype 2 was detected in 12.8% of uncooked pork samples (Noppon et al., 2014), and 6.25% of raw pork and edible pig organ samples, including serotypes 2 (2.50%), 7 (1.88%), and 9 (1.88%) (Direksin et al., 2023). Comparable low-to-moderate prevalence levels have also been reported in a nationwide risk assessment study, in which *S. suis* was detected in pork samples from only 5 of 9 surveyed provinces, including Mukdahan (1.99%) and Khon Kaen (0.91%) in the northeastern region, as well as Nakhon Pathom (3.17%), Saraburi (1.72%), and Bangkok (0.69%) in the central region, while no positive samples were identified from the northern or southern regions of Thailand (Wongnak et al., 2020). In contrast, markedly higher prevalence rates have been reported in other studies, including 85.23% overall positivity (17.05% for serotype 2) in raw pork and edible pig organ samples from the central region of Thailand (Boonyong et al., 2019) and up to 83.50% overall positivity (34.00% for serotype 2) in raw pork and edible pig organ samples from Chiang Mai Province in the northern region (Guntala et al., 2024). The low detection rate of *S. suis* observed in this study likely reflects both biological and methodological factors, including variability in *S. suis* carriage among apparently healthy pigs (Nutravong et al., 2014; Kittiwon et al., 2022), effective hygiene and cross-contamination control during slaughtering and retail handling (Boonyong et al., 2019; Wongnak et al., 2020), and differences in sample types or detection approaches compared with previous studies (Guntala et al., 2024). Importantly, the recovery of only a single isolate indicates the limited sensitivity of culture-based detection without enrichment, as direct plating typically has a theoretical detection limit of approximately 100 CFU/g and may be further constrained by selective media performance, bacterial viability loss, and generally low bacterial loads in raw pork and edible organs, particularly when using NNCC selective agar (Kataoka et al., 1991; Lunha et al., 2024). In contrast, a study from central Thailand employing loop-mediated isothermal amplification (LAMP) reported substantially higher detection rates, with 85.23% of samples positive for *S. suis* and 17.05% positive for serotype 2 (Boonyong et al., 2019), emphasizing the markedly greater sensitivity of molecular assays compared with conventional culture methods. Together, these findings suggest that the true prevalence of *S. suis* in retail pork may be underestimated in culture-based studies.

Although only a single isolate was recovered - limiting statistical inference - the identification of serotype 2, the most prevalent serotype found in pork in Thailand, is epidemiologically significant due to its strong association with zoonotic transmission and severe invasive diseases such as meningitis and septicemia (Fittipaldi et al., 2012; Takeuchi et al., 2012;). This clinical relevance is supported by a retrospective study conducted in northern Thailand between 2006 and 2008, in which *S. suis* infection was confirmed in 179 patients with a history of close contact with infected pigs or contaminated pork products, with serotype 2 accounting for 92% of human infections and a reported case fatality rate of 9.5% (Karavanonth et al., 2019). The isolate in the present study was obtained from a liver sample, suggesting possible contamination during slaughtering or subsequent meat-handling processes, consistent with previous reports on pork organs (Boonyong et al., 2019).

Beyond Thailand, similar zoonotic risks associated with *S. suis* have been reported across Southeast Asia. In southern Vietnam, *S. suis* was detected in up to 41% of clinically healthy slaughter pigs, with serotype 2 predominating (Ngo et al., 2011). High levels of antimicrobial resistance have also been reported in the region, complicating therapeutic management (Hoa et al., 2011). Moreover, large-scale outbreaks linked to specific sequence types - such as the ST7 epidemic in China - illustrate the potential for rapid dissemination following single contamination events (Ye et al., 2006; Hernández-García et al., 2017). A recent meta-analysis estimated a global serotype 2 prevalence of 13.6% in pigs, emphasizing its persistent risk to both animal and human health (Keonam et al., 2024). In Thailand, pork supply chain assessments continue to confirm ongoing transmission risks (Wongnak et al., 2020).

Importantly, MLST analysis identified the isolate as sequence type ST104, a lineage that has been reported among human *S. suis* infections in Thailand and is closely linked to serotype 2 pathogenicity. Population-based molecular epidemiological data from northern Thailand demonstrated that human *S. suis* infections are dominated by serotype 2, with ST1 representing the major sequence type among invasive human isolates, followed by other sequence types such as ST105 associated with serotype 14 (Takeuchi et al., 2012). Although ST104 was not the predominant sequence type in that cohort, global sequence typing analyses indicate that ST104 belongs to a clonal lineage circulating in both pigs and humans and forms part of the broader group of zoonotic *S. suis* sequence types with invasive potential (Goyette-Desjardins et al., 2014). While ST104 has not been linked to large-scale outbreak events comparable to ST7, its recurrent detection in sporadic human infections indicates its clinical relevance. The identification of ST104 in a retail pork product is therefore epidemiologically significant, as it reflects the circulation of potentially pathogenic clones within the food supply and supports the role of asymptomatic carrier pigs and slaughterhouse cross-contamination in human exposure. These findings emphasize the importance of molecular surveillance, including MLST, for monitoring the persistence and dissemination of high-risk *S. suis* clones in pork production systems.

In the present study, the *S. suis* isolate recovered from retail pork exhibited broad susceptibility to beta-lactams, carbapenems, and fluoroquinolones, while resistance was limited to macrolides, lincosamides, and tetracycline. This resistance profile is consistent with previous genomic and phenotypic studies demonstrating that *S. suis* commonly harbors determinants conferring resistance to tetracyclines and macrolides (Palmieri et al., 2011). Importantly, the observed susceptibility to beta-lactam agents and glycopeptides corresponds with findings from Thailand showing that these antimicrobials remain effective for the treatment of *S. suis* infections in both pigs and humans, despite widespread resistance to macrolides and tetracyclines nationwide (Yongkiettrakul et al., 2019). The resistance pattern identified in this study aligns with regional and global trends, in which selective pressure associated with antimicrobial use in swine production has contributed to the persistence of macrolide and tetracycline resistance (Varela et al., 2013; Brizuela et al., 2023; Kiatkangwanchon et al., 2023). Although the isolate was not multidrug-resistant, its resistance phenotype emphasizes the ongoing circulation of antimicrobial-resistant zoonotic *S. suis* within the pork supply chain and indicates the need for continued surveillance.

Aside from *S. suis*, a total of 68 isolates from 63 samples (55.75%) were recovered, predominantly *Enterococcus faecalis*, coagulase-negative *Staphylococcus* spp., and other *Enterococcus* species. These organisms are commonly associated with fecal contamination, skin microbiota, and food-handling environments, reflecting cross-contamination during evisceration, carcass processing, or retail preparation. Such findings are consistent with previous studies demonstrating that slaughtering practices, carcass

washing, surface sanitation, and market hygiene strongly influence microbial contamination profiles in pork products (Kročko et al., 2011; Hammerum, 2012; Boonyong et al., 2019; Wongnak et al., 2020). Although the present study did not distinguish contamination sources along the supply chain, the observed bacterial distribution supports the need for targeted environmental and process-level investigations in future work. The incidental detection of *Candida parapsilosis* further indicates that non-bacterial contaminants may occasionally be introduced during processing, as opportunistic fungi have been reported in meat-associated environments (Shimada et al., 2021).

Although the generalizability of the present findings may be limited to the specific study area, market types, sampling period, and production systems examined, this study provides important baseline evidence that zoonotic *S. suis*, including serotype 2 ST104, can occur in retail pork. Moreover, the findings support the integration of selective enrichment with sensitive molecular detection approaches, such as PCR-based or LAMP methods, in future surveillance and risk assessment studies.

4. Conclusions

This study provides baseline evidence of *S. suis* contamination in retail pork products from fresh markets in Mueang District, Nakhon Ratchasima Province, Thailand, with a low prevalence of 0.88%. Despite the limited detection, the isolation of a zoonotic serotype 2 strain, identified as sequence type ST104 by MLST and previously associated with human infections, emphasizes the continued public health risk posed by pork products, particularly edible organs such as liver. The isolate showed broad susceptibility to clinically important antimicrobials, although resistance to macrolides and tetracycline was observed, which was consistent with regional resistance trends. Together with the frequent detection of other gram-positive contaminants, these findings indicate the importance of ongoing microbiological and molecular surveillance, including MLST-based monitoring of high-risk clones, as well as the implementation of improved hygiene practices along the pork supply chain, and strengthened food safety awareness to mitigate zoonotic transmission risks.

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6. Author's Contributions

Sudaluck Thunyaharn designed research; Thanaboon Chanwong, Chuleeporn Mutuwong, Marwah Abah and Natthakit Sathipchan performed research; Teerapat Phueakphong, Tanit Boonsiri and Anusak Kerdsin contributed analytic tools; Unchalee Visawapoka analyzed data; Phadungkiat Khamnoi coordinated research; and Nitchatorn Sungsirin wrote the paper.

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7. Conflicts of Interest

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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