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Genetic characterization of methicillin-resistant *Staphylococcus aureus* isolated from pigs and pork meat in Thailand

(タイにおいてブタ及び食肉から分離されたメチシリン耐性黄色ブドウ球菌の遺伝学的特徴)

Wimonrat Tanomsridachchai

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ABBREVIATIONS

Abbreviation or symbol	Term
AMP	Ampicillin
BHI	Brian Heart Infusion
bp	Base pair
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLI	Clindamycin
CC	Clonal complex
CLSI	Clinical and Laboratory Standards Institute
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanine triphosphate
dNTP	Deoxyribonucleotide triphosphate
dTTP	Deoxythymine triphosphate
ENR	Enrofloxacin
ERY	Erythromycin
FOX	Cefoxitin
gDNA	Genomic DNA
GEN	Gentamicin
h	Hour
MHA	Mueller Hinton agar
min	Minute
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NSS	Normal saline solution
ORSAB	Oxacillin resistance screening agar base
OXA	Oxacillin
PCR	Polymerase Chain Reaction

ABBREVIATIONS (cont.)

Abbreviation or symbol	Term
SCC*mec*	staphylococcal cassette chromosome *mec*
SPSS	Statistical Package for the Social Sciences
ST	Sequence typing
SXT	Sulfamethoxazole/trimethoprim
TE	Tetracycline
U	Unit
VAN	Vancomycin
w/v	Weight per volume
WGS	Whole-genome sequencing

PREFACE

Staphylococcus aureus is a commensal bacterium and an opportunistic pathogen that colonizes the nares, gut, skin surfaces of humans, and several animal species [1]. *S. aureus* is the major cause of hospital and community-acquired infections that have serious consequences. It can cause bloodstream (BSI), skin and soft tissues (SSTI), lower respiratory tract infections (LRTI), etc. *S. aureus* carried of virulence factors and toxins. It is often responsible for many toxin-mediated diseases such as toxic shock syndrome, scalded skin syndrome, and staphylococcal foodborne diseases (SFD) [1]. *S. aureus* infections have been complicated by the acquisition of antimicrobial resistance, including methicillin resistance. Methicillin-resistant *S. aureus* (MRSA) is a significant cause of infection in the health care industry.

Many countries have experienced an increasing burden of MRSA that has notable geographical variation. The highest prevalence of MRSA was in parts of America and Asia (Figure 1) [2]. In recent years, public health concern has risen from isolates harbored in the community and livestock species [3]. Hospital-associated MRSA (HA-MRSA) is currently endemic in many hospitals. Community-associated MRSA (CA-MRSA) clones have been spreading rapidly in the communities and infiltrating healthcare in many regions worldwide [1]. To date, livestock-associated MRSA (LA-MRSA) is found in various animals and certain high-risk groups of workers that are in direct contact with live animals [4]. The evolutionary changes of MRSA have contributed to its continued threat to public health [4]. Figure 2 shows the MRSA populations geographically. It is evident that some HA-MRSA and CA-MRSA clones overlap each other and showing a very close genetic association. Conversely, the LA-MRSA has little correlation with either CA-MRSA or HA-MRSA clones (Figure 2) [1].

Moreover, many bacteria can initially cause a silent carrier state and may later give rise to infections as being of foodborne origin. MRSA is one of the bacteria that are of concern regarding the transmission of this strain to human populations in relation to high-density swine production [5,6]. The bacteria can be transmitted to humans in close contact with the LA-MRSA colonized animals and their products. Recently, MRSA strains from livestock (e.g., swine, cattle, and poultry) or their products have emerged throughout Europe, America, and Asia. Most of the LA-MRSA strains

belonged to clonal complex (CC) 398 as defined by multilocus sequence typing (MLST), while ST9 was mostly found in Asian countries such as Taiwan [7], Hong Kong [8,] and Thailand [9]. Human infections of LA-MRSA ST398 and ST9 have been reported [10]. In Thailand, CC9 MRSA was isolated from 10%-40% of swine [10,11] and 50% of retail pork [10]. The previous studies showed the potential risk of spread from livestock reservoirs to the communities and hospitals. In addition, CC9-SCC*mec* IX MRSA isolated from humans (a patient and a healthy healthcare worker) attracted attention as it was unique and new community clone in Thailand [9].

The origin and molecular evolution of LA-MRSA especially ST398, seem to be associated primarily with pigs [1]. LA-MRSA ST9 represents the most common sequence type in Thailand [9,10]. LA-MRSA ST398 isolated from pigs in Thailand was first detected between 2015 to 2017 as a major lineage in the previous study in the central of Thailand [12]. The information about the importation of live pigs in Thailand was shown in Figure 3. In this Figure, nearly 100% of the imported live pigs were from the American and European countries which have experienced a remarkable increase in LA-MRSA ST398 prevalence in pigs and other animal species [6]. However, the origin or the transmission routes of LA-MRSA ST398 in Thailand are unclear and have not been investigated so far.

The high-throughput whole-genome sequencing (WGS) is the comprehensive method for analyzing entire genomes and has provided the enhanced resolution required to accurately track the spread of LA-MRSA. It facilitates the formulation of more effective infection prevention and control strategies. This method has also revolutionized investigations of the evolution of established and emerging clones. This thesis also used WGS to investigate the transmission dynamics of LA-MRSA in slaughtered pig and pork samples in Thailand.

In Chapter I, an investigation about the prevalence, phenotype, and genotype of MRSA in slaughtered pigs and retailed pork samples in the 2-year study (2017-2018) in the central region of Thailand was covered. The slaughtered pigs were collected from three slaughterhouses. The retailed pork samples were collected from butcher shops in four fresh markets. The goal of this study was to determine prevalence of MRSA in livestock animals especially swine and pork in Thailand. In Chapter II, LA-MRSA ST398 are characterized. A genomic screen was performed to identify important genes for LA-MRSA ST398 in slaughtered pig and pork samples. This Chapter presented the possible associations among Thai samples of LA-MRSA ST398 that were established

with WGS. The phylogenetic analysis of these samples based on single nucleotide polymorphism (SNP) was also done. Further, we investigated the molecular characteristics of MRSA to evaluate the potential relationships between livestock animals, animal food products, and humans.

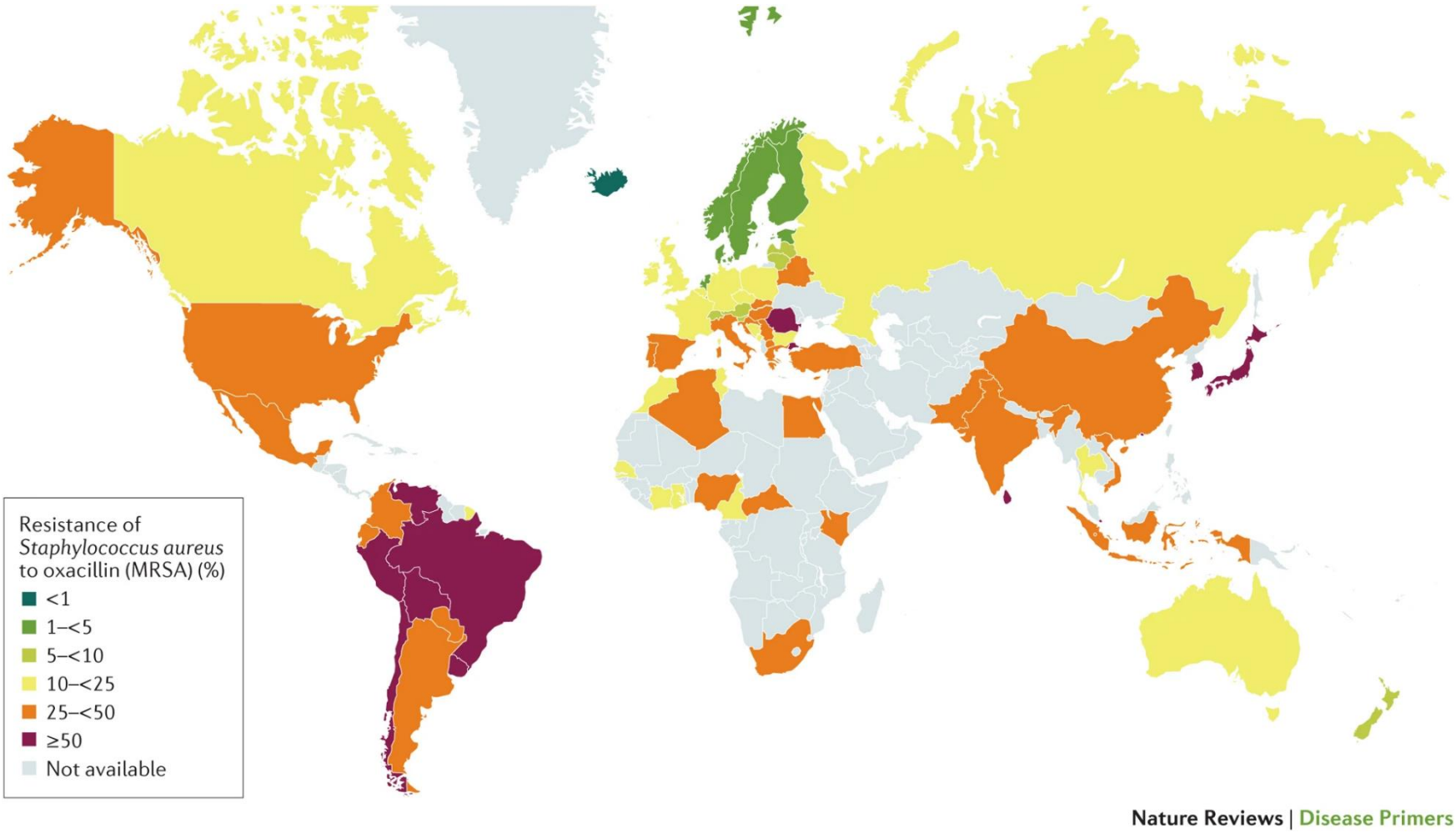


Figure 1. Worldwide prevalence of MRSA. The percentage of Staphylococcus aureus isolates that are resistant to oxacillin (that is, methicillin-resistant *S. aureus* (MRSA) isolates) is shown [2].

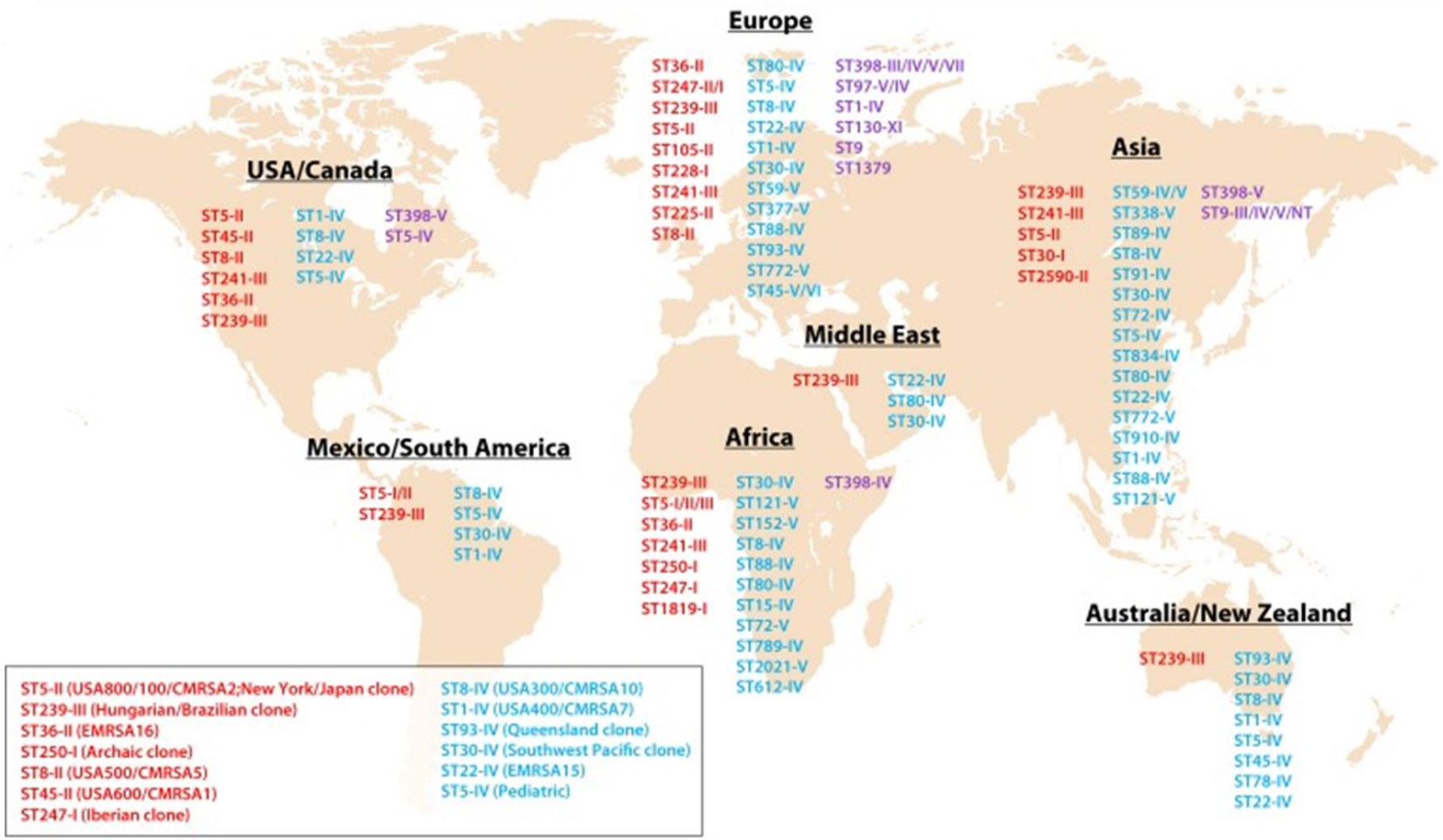
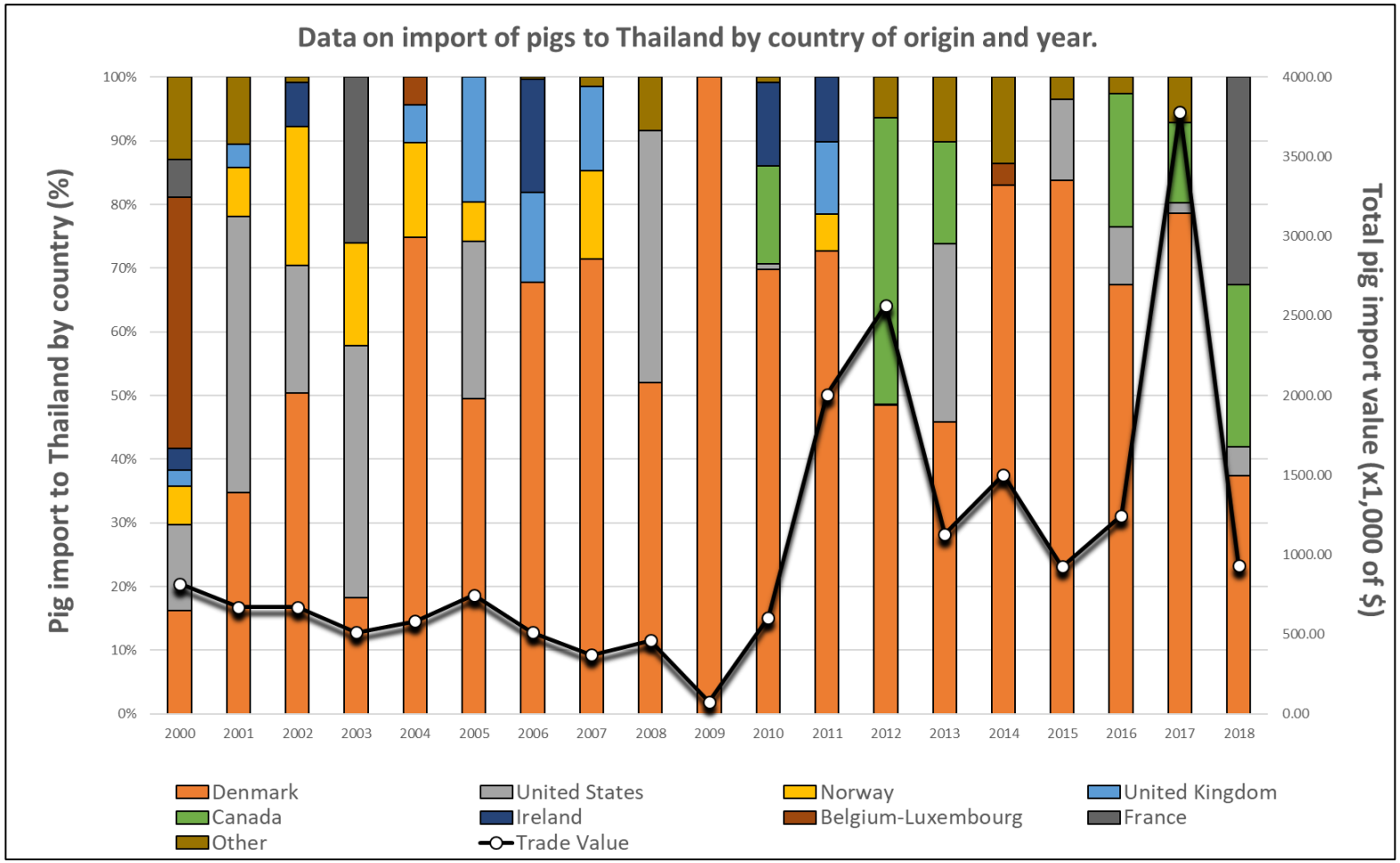


Figure 2. MRSA population structure, showing the major clones reported in each continent or region along with the commonly associated SCC*mec* types. There are many STs that show marked region specificity. Red represents STs belonging to HA-MRSA, blue represents belonging to CA-MRSA, and purple represents belonging to LA-MRSA. Traditional names for the mainly predominant epidemic strains are at the bottom [1].



Source: https://oec.world/en/profile/hs92/pigs

Figure 3 Data on imported pigs to Thailand by country of origin each year. White dots denote the total import value of pigs per year, expressed in thousands of U.S. dollars, with the solid black line showing the yearly trend. Data were retrieved from the Observatory of Economic Complexity [14].

CHAPTER I

Antimicrobial Resistance and Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolated from Slaughtered Pigs and Pork in Central Region, Thailand

Introduction

MRSA has been a major public health concern as it causes nosocomial infections leading to high mortality and morbidity in humans [15]. MRSA strains with resistance to a wide range of antibiotics have been found in various sources globally [3,4,16]. LA-MRSA strains have always been associated with exposure to livestock or their products and have emerged in different countries in Europe, America, and Asia [17,18]. MRSA types have divergent genetic backgrounds, hence different MRSA strains carry different types of staphylococcal cassette chromosome *mec* (SCC*mec*) [17]. LA-MRSA belonging to ST398 has been reported to colonize livestock and people with close contact to them such as farmers and veterinarians [19–21].

However, infections by LA-MRSA were also found in people without livestock exposure [22,23]. ST398 and several others (ST9, ST97, ST5) have been isolated from pork, chicken, beef, and milk in many countries [24]. These finding demonstrate that handling and/or consumption of food-producing animals contaminated by MRSA is a potential zoonotic transmission source for humans [25,26]. When MRSA-carrying animals are slaughtered, MRSA may spread to carcasses, to the environment, and to abattoir workers. Moreover, if animal products are contaminated, MRSA can enter the human food chain [27]. Therefore, LA-MRSA has become an important public health issue that warrants intensive monitoring.

Thailand has a positive trend for the production and export of pork and live pigs especially to ASEAN countries and domestic pork consumption increased 2–3% from 2011 to 2016 [28]. As the central region of Thailand is the main pig production area [29], the risk of zoonotic transmission of LA-MRSA through pigs and/or pig products is high [12]. Although some studies have identified LA-MRSA from healthy pigs [30,31], and pork [11] in Thailand, the prevalence of them in slaughtered pigs is still unknown. Moreover, there is only one report on the description of the epidemiology and

molecular characteristics of LA-MRSA from slaughtered pigs and pork in Thailand [17]. Therefore, the purpose of this study was to investigate the prevalence, molecular characteristics, and antimicrobial resistance pattern of MRSA isolated from slaughtered pigs and retail pork in the central region of Thailand.

Materials and Methods

Study design and sample collection

The cross-sectional study was performed in two settings of the food chain—slaughterhouses and markets in the central region of Thailand in 2017 and 2018—to determine the prevalence of MRSA.

A total of 204 nasal swab samples were collected from three slaughterhouses (A, B, and C) during 2017–2018 (Figure 4). In each year, 34 nasal swab samples were collected from each of the three slaughterhouses. All slaughterhouses were under the control of Department of Livestock Development, Ministry of Agriculture and Cooperatives, but under different ownerships. Slaughterhouse A belonged to the town-municipal while slaughterhouses B and C belonged to private companies. Approximately 100–200 pigs were slaughtered per day. Slaughtering of animals was done according to common slaughtering practice; nasal swab samples were collected immediately after the scalding and dehairing and prior to washing the head with water. A cotton swab was inserted 2–7 cm (according to pig size) into both nostrils and gently rotated against the mucosal epithelium. Then, the cotton swab was inserted in the tube containing medium (Seed Swab γ No. 2 “Eiken”; Eiken Chemical, Tokyo, Japan) and the cap was tightly closed. All the swab samples were immediately stored in an ice box.

A total of 116 retailed pork samples were collected from 64 butcher shops in four fresh markets (D, E, F, and G in Figure 4) in the 2-year study. In 2017, a total of 57 pork samples were collected from 32 butcher shops, including market D (n=6), market E (n=37), market F (n=6), and market G (n=8). In 2018, a total of 59 pork samples were collected from 32 butcher shops, including market D (n=7), market E (n=38), market F (n=6), and market G (n=8). The unequal number of butcher shops for sample collection in each market was dependent on the capacity of the market. Approximately 50 g raw pork samples were purposely purchased from each butcher shop and collected in individual plastic bags.

Slaughterhouses and fresh markets were selected for convenience, based on the willingness of the producers to participate. All samples were kept individually in sterile bags, stored in an icebox, and transported to the laboratory within 6 h for further processing.

This study used meat and carcass from pigs in markets and slaughterhouses that had been legally registered. The Institutional Animal Care and Use Committee, Thammasat University (IACUC-TU) has confirmed that no ethical approval is required.

Isolation and identification of MRSA

All samples were inoculated into trypticase soy broth (TSB; Oxoid, Basingstoke, United Kingdom) containing ceftizoxime (5 µg/mL), aztreonam (75 mg/mL), and 6.5% NaCl, and incubated at 37 °C for 24 h. Subsequently, enrichment cultures from individual samples were streaked on oxacillin-resistance screening agar (ORSA) supplemented with 2 µg/mL oxacillin (Oxoid) and incubated at 37 °C for 24–48 h. Up to three suspected staphylococcal colonies (mannitol-positive) were selected per sample from ORSA and sub-cultured on trypticase soy agar (TSA) (Oxoid). Colonies on TSA were primarily identified by Gram stain, catalase test, coagulase test, DNase test, and growth on mannitol salt egg-yolk agar (Figure 5).

Presumptive MRSA isolates were further confirmed to species level by sequencing of 16S rRNA gene using primers Bact-*rrs*-F (5′-AGAGTTTGATCCTGGC TCAG-3′) and Bact-*rrs*-R (5′- TACGGCTACCTTGTTACGAC-3′) [32]. The PCR reaction mixture (total 20 µL) consisted of 1× Ex *Taq* buffer, 1 mM $MgCl_2$, 0.25 mM of each dNTP, 0.25 µM of each primer, 0.5 U *Taq* polymerase (Takara Bio Inc., Kyoto, Japan), and 1 µL of DNA template. Thermal cycling was performed in a Thermal Cycler (Applied Biosystems Veriti™ Thermal Cycler, Foster City, CA, USA). Amplification conditions entailed the following: initial denaturation at 96 °C for 1 min, 35 cycles of denaturation at 96 °C for 10 s, annealing at 55 °C for 10 s, DNA extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. This protocol was adapted from Neilan et al. (1997) [32]. Sequencing PCR was performed using a BigDye ver. 3.1 Terminator Cycle Sequencing Kit (Thermo Fisher Scientific), followed by Sanger sequencing using ABI 3500xL Genetic Analyzer (Thermo Fisher Scientific). After sequencing of the 16S rRNA gene, contiguous sequences were analyzed by the BLAST search engine (http://www.ncbi.nih.gov accessed on 19 February 2021) and compared with those registered in the GenBank database.

Detection of the *mecA* gene was done by PCR using specific primers *mecA*-F (5′-AAAATCGATGGTAAAGGTTGGC-3′) and *mecA*-R (5′- AGTTCTGCAGTACC GGATTTGC-3′) for methicillin-resistance confirmation [33]. The PCR mixture was

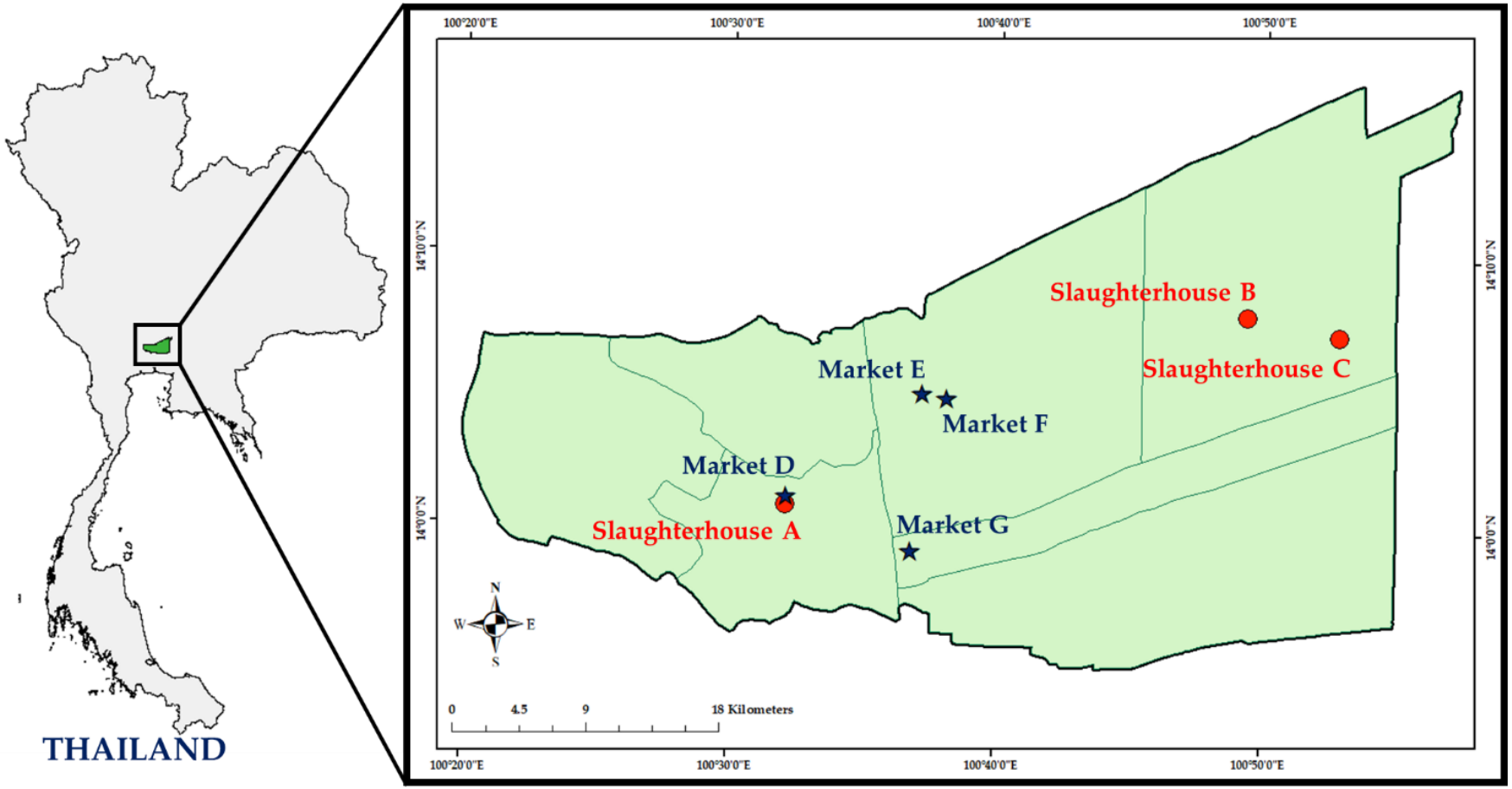


Figure 4. Geographic distribution of the three selected slaughterhouses and the four fresh markets in the central region of Thailand. Red and blue texts represent the name of slaughterhouses and markets, respectively.

Swab inner wall of pig nose using Culture swab™/ 10 g of pork

↓ Kept in ice box and transport to laboratory within 24 h

Inoculate a swab and pork into 3 ml and 90 ml, respectively, of Trypticase soy broth (contain ceftizoxime, aztreonam, 6.5% NaCl)

↓ Incubate at 37°C, 24 h

Streak the enrich culture on ORSAB agar (oxacillin resistance screening agar base)

↓ Incubate at 37°C, 24 - 48 h

Subculture 3 of representative single colony colored blue on Trypticase soy agar

↓ Incubate at 37°C, 24 h

Confirmed identify on mannitol salt egg-yolk agar, DNAase agar, Coagulase test, antimicrobial susceptibility test

↓

Molecular confirmation tests: 16s rRNA Gene, *mec*A gene

↓

Streak positive MRSA isolates (pure culture) onto a small section of TSA agar plate

↓ Incubate at 37°C, 24 h

Suspend all bacterial grown on TSA into 1ml of 10% skim milk

↓

Store at -80°C

Figure 5. Flow of isolation and identification for methicillin-resistance *Staphylococcus aureus* (MRSA)

prepared in a total volume of 20 µl per reaction. The mixture contained 1x Green Go*Taq* reaction buffer, 1 mM $MgCl_2$, 0.25 mM each of dNTP, 0.25 µM of each primer, 0.5 U GoTaq DNA polymerase (Promega, Madison, WI, USA), and 1 µl of DNA template. The final volume was adjusted to 20 µl with sterile deionized water. The PCR conditions were the same as explained in the previous study [33]. Isolates with *mecA* were kept frozen at −80 °C until further examination.

Antimicrobial susceptibility testing (AST)

Isolates identified as MRSA were examined for susceptibility to antimicrobial agents using the disk diffusion method on Mueller–Hinton agar (MHA; Oxoid) following the Clinical and Laboratory Standards Institute (CLSI) guidelines CLSI VET01 S5, 2018 for enrofloxacin [34]; and CLSI M100 S30, 2020 for all other antibiotics [35]. A total of 12 antimicrobial disks were used comprised of ampicillin (AMP, 10 µg), oxacillin (OXA, 1 µg), cefoxitin (FOX, 30 µg), chloramphenicol (CHL, 30 µg), clindamycin (CLI, 2 µg), erythromycin (ERY, 15 µg), ciprofloxacin (CIP, 5 µg), enrofloxacin (ENR, 5 µg), gentamicin (GEN, 10 µg), tetracycline (TET, 30 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), and vancomycin (VAN, 30 µg).

Molecular typing of MRSA

SCC*mec* typing of MRSA was performed by PCR amplification of the *mec* (classes A–C) and *ccr* (types 1, 2, 3, and 5) regions as previously described [36]. The combinations of *ccr* types and classes of *mec* gene complexes were used to determine the SCC*mec* types of each isolate.

Multilocus sequence typing (MLST) was performed following the protocol described elsewhere [37]. The seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqi*) were amplified by PCR. After agarose gel electrophoretic separation, PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The concentration and quality of the purified PCR products were measured by Qubit 3 using Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific). The purified products were sequenced by ABI 3500xL Genetic Analyzer (Thermo Fisher Scientific) using a BigDye ver. 3.1 Terminator Cycle Sequencing Kit (Thermo Fisher Scientific). The sequencing data were analyzed using BioEdit version 7.0.9.1 [38]. The allele numbers and sequence type (ST) of each *S. aureus* isolate were obtained using MLST Databases

(http://saureus.mlst.net accessed on 19 February 2021). Phylogenetic trees were constructed by Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (www.megasoftware.net accessed on 19 February 2021). Isolates showing identical antimicrobial resistance phenotype and genotype obtained from same sample were considered as clonal.

Data analysis

The SPSS software version 19.0 was used for statistical analysis. The chi-square tests or Fisher's exact tests were carried out to examine the differences in the prevalence of MRSA and antimicrobial resistance profiles among the MRSA isolates. The *p*-value less than 0.05 was considered statistically significant.

Results

Prevalence of MRSA

Among 204 nasal swab samples of pigs from three slaughterhouses and 116 pork samples from four markets, 63 (19.7%) were positive for MRSA based on the presence of the *mecA* (Table 1). The prevalence was significantly higher in pork samples (44.8%; 52/116) than in nasal swab samples (5.4%; 11/204) (p-value < 0.05) (Table 1 and Table 2). No MRSA was found in nasal swab samples from slaughterhouse C in both year (2017 and 2018) or in pork samples from market D in the first year (2017). Among nasal swab samples, the highest prevalence of MRSA was found at slaughterhouse A (11.8%; 8/68). For pork samples, the highest prevalence of MRSA was found at market F (58.3%; 7/12) followed by market G (50.0%; 8/16), market E (48.0%; 36/75), and market D (7.7%; 1/13). There were no significant differences between the sampling years (Table 1). In total, 67 MRSA isolates, 11 from nasal swab and 56 from pork samples, were used for further analyses (Table 1 and Table 3).

Table 1. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in slaughterhouses and markets located in the central region of Thailand in 2017 and 2018

Sample/place	No. of MRSA positive samples / total No. (%)		
	2017	2018	Total
Nasal swab/			
Slaughterhouse A	2/34 (5.9)	6/34 (17.6)	8/68 (11.8)
Slaughterhouse B	2/34 (5.9)	1/34 (2.9)	3/68 (4.4)
Slaughterhouse C	0/34	0/34	0/68
Total (n)	4/102 (3.9)	7/102 (6.9)	11/204 (5.4)
Pork/			
Market D	0/6	1/7 (14.3)	1/13 (7.7)
Market E	22/37 (59.5) [a]	14/38 (36.8)	36/75 (48.0)
Market F	3/6 (50.0)	4/6 (66.7) [a]	7/12 (58.3)
Market G	3/8 (37.5)	5/8 (62.5)	8/16 (50.0)
Total (n)	28/57 (49.1)	24/59 (40.7)	52/116 (44.8)

[a] Two MRSA isolates were derived from one sample (there were 2 samples).

Table 2 Prevalence of MRSA among different sources

	No. of samples		*p*-value
	MRSA Positive	**MRSA Negative**	
Slaughterhouse in 2017	4	98	0.352
Slaughterhouse in 2018	7	95	
Market in 2017	28	29	0.361
Market in 2018	24	35	
Total 2017	32	127	0.845
Total 2018	31	130	
Slaughterhouse	11	193	<0.001*
Market	52	64	
Market D	1	12	0.035*
Market E	36	39	
Market F	7	5	
Market G	8	8	
Slaughterhouse A	8	60	0.008*
Slaughterhouse B	3	65	
Slaughterhouse C	0	68	

* The *p*-value less than 0.05 was considered statistically significant.

Table 3. Characteristics of staphylococcal cassette chromosome *mec* (SCC*mec)* type and ST type of MRSA isolated in slaughterhouses and markets located in the central region of Thailand in 2017 and 2018

Typing profiles		Slaughterhouse						Market								Total
		2017			2018			2017				2018				
SCC*mec* typing	ST	A	B	C	A	B	C	D	E	F	G	D	E	F	G	
IX	9	2	2	0	5	1	0	0	9	1	3	1	13	3	5	45
V	398	0	0	0	1	0	0	0	12	2	0	0	0	3	0	18
NT	9	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
IV	779	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
IX	5639	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1

Antimicrobial susceptibility

Drug susceptibility tests utilizing 12 antimicrobial agents of 10 drug classes revealed that all examined isolates were resistant to ampicillin and cefoxitin, and various degrees of resistance were observed in other 10 antimicrobial agents with all isolates susceptible to vancomycin as shown in Tables 4 and Table 5. There was no statistically significant difference between nasal swab and pork samples in the prevalence of each antimicrobial resistance (Fisher's test; p-value > 0.05). All MRSA isolates were multi-drug resistant (MDR) and classified into 18 different patterns of resistance (Table 4). Six and 16 different patterns of drug resistance were observed in isolates from nasal swabs and pork samples, respectively. Nevertheless, it was found that all of isolates showed resistance to at least two of the non-β-lactams antimicrobial classes. All isolates from nasal swab samples were MDR, resulting in resistance to at least three non-β-lactams antimicrobial classes, whereas only 39 (69.6%) MRSA isolates from pork samples were MDR. The antimicrobial resistance profile of AMP-OXA-FOX-CLI-TET, was the highest in frequency (23.9%; 16/67) and found only in pork samples from market E (in 2017) and market F (both 2017 and 2018), followed by AMP-OXA-FOX-CHL-CLI-ERY-CIP-ENR-GEN-TET (16.4%; 11/67) and AMP-OXA-FOX-CHL-CLI-CIP-ENR-GEN-TET (16.4%; 11/67) found in both nasal and pork samples. The other antimicrobial resistance patterns, which were mainly found in pork samples for both years, were diverse and low in number.

Table 4. Antimicrobial resistance patterns of MRSA isolates from pig and pork

Antimicrobial resistance pattern												Total of isolates	Sources (No. of isolates)		Genotype (No. of isolates)				
AMP	OXA	FOX	CHL	CLI	ERY	CIP	ENR	GEN	TET	SXT	VAN		Slaughterhouse	Market	ST9 -SCC*mec* IX	ST398 -SCC*mec* V	ST9 -SCC*mec* NT	ST779 -SCC*mec* IV	ST5639 -SCC*mec* IX
												5	B (2)	E (3)	5	-	-	-	-
												3	A (1)	E (2)	3	-	-	-	-
												8	A (3)	E (5)	8	-	-	-	-
												2	0	E (2)	2	-	-	-	-
												2	0	E (2)	2	-	-	-	-
												3	0	E (3)	3	-	-	-	-
												1	0	E (1)	1	-	-	-	-
												1	0	E (1)	-	-	1	-	-
												1	0	E (1)	-	-	1	-	-
												3	0	E (1), G (2)	3	-	-	-	-
												1	A (1)	0	1	-	-	-	-
												7	B (1)	E (3), G (3)	6	-	-	-	1
												1	0	F (1)	1	-	-	-	-
												1	0	D (1)	1	-	-	-	-
												2	0	G (1), E (1)	2	-	-	-	-
												1	0	F (1)	1	-	-	-	-
												1	0	E (1)	-	1	-	-	-
												2	A (1)	F (1)	2	-	-	-	-
												1	0	G (1)	1	-	-	-	-
												1	0	G (1)	1	-	-	-	-
												1	0	F (1)	1	-	-	-	-
												1	A (1)	0	1	-	-	-	-
												1	A (1)	0	-	1	-	-	-
												3	0	E (2), F (1)	-	3	-	-	-
												1	0	E (1)	-	1	-	-	-
												1	0	F (1)	-	1	-	-	-
												1	0	F (1)	-	1	-	-	-
												3	0	E (2), F (1)	-	3	-	-	-
												7	0	E (6), F (1)	-	7	-	-	-
												1	0	E (1)	-	-	-	1	-
Total												**67**	**11**	**56**	**45**	**18**	**2**	**1**	**1**

Abbreviation: AMP, ampicillin; OXA, oxacillin; FOX, cefoxitin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; ENR, enrofloxacin; GEN, gentamicin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim; VAN, vancomycin
Red, resistant; Yellow, intermediate; Green, susceptible; NT, nontypeable; ST5639, novel ST found from Market E in 2018

Table 5. Prevalence of antimicrobial resistance of MRSA isolated in slaughterhouse and market located in the central region of Thailand in 2017 and 2018.

Class/ Antimicrobial agents	No. (%) of antimicrobial resistance of MRSA isolates						
	Slaughterhouse (n=30)			Market (n=102)			Total (n=67)
	2017 (n=4)	2018 (n=7)	Total (n=11)	2017 (n=30)	2018 (n=26)	Total (n=56)	
Penicillin							
AMP	4 (100)	7 (100)	11 (100)	30 (100)	26 (100)	56 (100)	67 (100)
OXA	4 (100)	7 (100)	11 (100)	30 (100)	25 (96.2)	55 (98.2)	66 (98.5)
Cephem							
FOX	4 (100)	7 (100)	11 (100)	30 (100)	26 (100)	56 (100)	67 (100)
Phenicol							
CHL	3 (75.0)	5 (71.4)	8 (72.7)	15 (50.0)	19 (73.1)	34 (60.7)	42 (62.7)
Lincosamide							
CLI	3 (75.0)	7 (100)	10 (90.9)	27 (90.0)	23 (88.5)	50 (89.3)	60 (89.6)
Macrolide							
ERY	3 (75.0)	3 (42.9)	6 (54.5)	10 (33.3)	9 (34.6)	19 (33.9)	25 (37.3)
Fluoroquinolone							
CIP	4 (100)	7 (100)	11 (100)	16 (53.3)	23 (88.5)	39 (69.6)	50 (74.6)
ENR	4 (100)	6 (85.7)	10 (90.0)	16 (53.3)	23 (88.5)	39 (69.6)	49 (73.1)
Aminoglycoside							
GEN	4 (100)	6 (85.7)	10 (90.9)	10 (33.3)	23 (88.5)	33 (58.9)	43 (64.2)
Tetracycline							
TE	4 (100)	7 (100)	11 (100)	30 (100)	25 (96.2)	55 (98.2)	66 (98.5)
Folate partway -inhibitor							
SXT	2 (50.0)	0	2 (18.2)	6 (20.0)	8 (30.8)	14 (25.0)	16 (23.9)
Glycopeptide							
VAN	0	0	0	0	0	0	0

Abbreviation: AMP, ampicillin; OXA, oxacillin; FOX, cefoxitin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; ENR, enrofloxacin; GEN, gentamicin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim; VAN, vancomycin

Molecular characteristics (by Multilocus sequence typing (MLST) and SCC*mec* typing) of MRSA isolates

MRSA isolates were differentiated into four SCC*mec* types and four STs. SCC*mec* type IX was the most prevalent (68.7%; 46/67), followed by SCC*mec* type V (26.9%; 18/67) and SCC*mec* type IV (1.5%; 1/67), while two isolates (3.0%), consisting of class C2 *mec* complex but negative amplification of *ccr* complex were nontypeable (NT). The most frequently found ST was ST9 (70.1%; 47/67) followed by ST398 (26.9%; 18/67), ST779 (1.5%; 1/67), and ST5639 (1.5%; 1/67) (Table 6). ST5639 was a new single-locus variant of ST9 with a substitution mutation (G52T) of *glpF*, resulting in allelic profile 3-3-111-1-1-1-10, which belonged to CC 9. The five different genotype profiles were identified where ST9-SCC*mec* IX was predominant in both nasal swabs and pork samples. ST398-SCC*mec* V was identified at market F (in both years), market E (only in the first year), and at slaughterhouse A (only in the first year). MRSA at market E in the first year (2017) showed the most diverse molecular characteristic profiles (Table 3). Four samples were found to carry two strains with different genotype profiles in each. The characteristic genotype profile of ST9-SCC*mec* IX and ST398-SCC*mec* V were found in a pork sample from market E and two pork samples from market F. Moreover, ST398-SCC*mec* V and ST9-SCC*mec* NT were found in a pork sample from market E.

Association between antimicrobial resistance and molecular typing

Antimicrobial resistance rates obtained for five different genotype profiles are shown in Table 6 and Figure 6. ST9-SCC*mec* IX isolates showed significantly higher rates of resistance (p-value < 0.05) than isolates with other genotype profiles, exhibiting high prevalence of resistance to chloramphenicol, erythromycin, ciprofloxacin, enrofloxacin, gentamicin, and sulfamethoxazole/trimethoprim. Among ST398-SCC*mec* V isolates (n = 18), the antimicrobial resistance pattern AMP-OXA-FOX-CLI-TE was found with the highest frequency in pork samples from markets (88.9%; 16/18) (Figure 6 and Table 4). All MRSA ST398-SCC*mec* V from market F in both years exhibited the same antimicrobial resistance profile, whereas one MRSA isolate from market E in the first year was different in antimicrobial resistance pattern.

Table 6. Association between antimicrobial resistance and genotype profile

Genotype profiles		Antimicrobial agents (No. of isolates)											
		AMP	**OXA**	**FOX**	**CHL**	**CLI**	**ERY**	**CIP**	**ENR**	**GEN**	**TET**	**SXT**	**VAN**
ST9-SCC*mec* IX	**(n=45)**	45	44	45	38*	39	22*	45*	45*	42*	44	16*	0
ST398-SCC*mec* V	**(n=18)**	18	18	18	0	18	1	2	1	0	18	0	0
ST9-SCC*mec* NT	**(n = 2)**	2	2	2	2	2	2	2	2	0	2	0	0
ST779-SCC*mec* IV	**(n = 1)**	1	1	1	1	0	0	0	0	0	1	0	0
ST5639-SCC*mec* IX	**(n = 1)**	1	1	1	1	1	0	1	1	1	1	0	0

Abbreviation: AMP, ampicillin; OXA, oxacillin; FOX, cefoxitin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; ENR, enrofloxacin; GEN, gentamicin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim; VAN, vancomycin; Resistant: only resistant isolates, Non-resistant: including susceptible and intermediate isolates; NT, nontypeable; Significant differences between ST9-SCC*mec* IX and all other genotype profiles; * The *p*-value less than 0.05 was considered statistically significant.

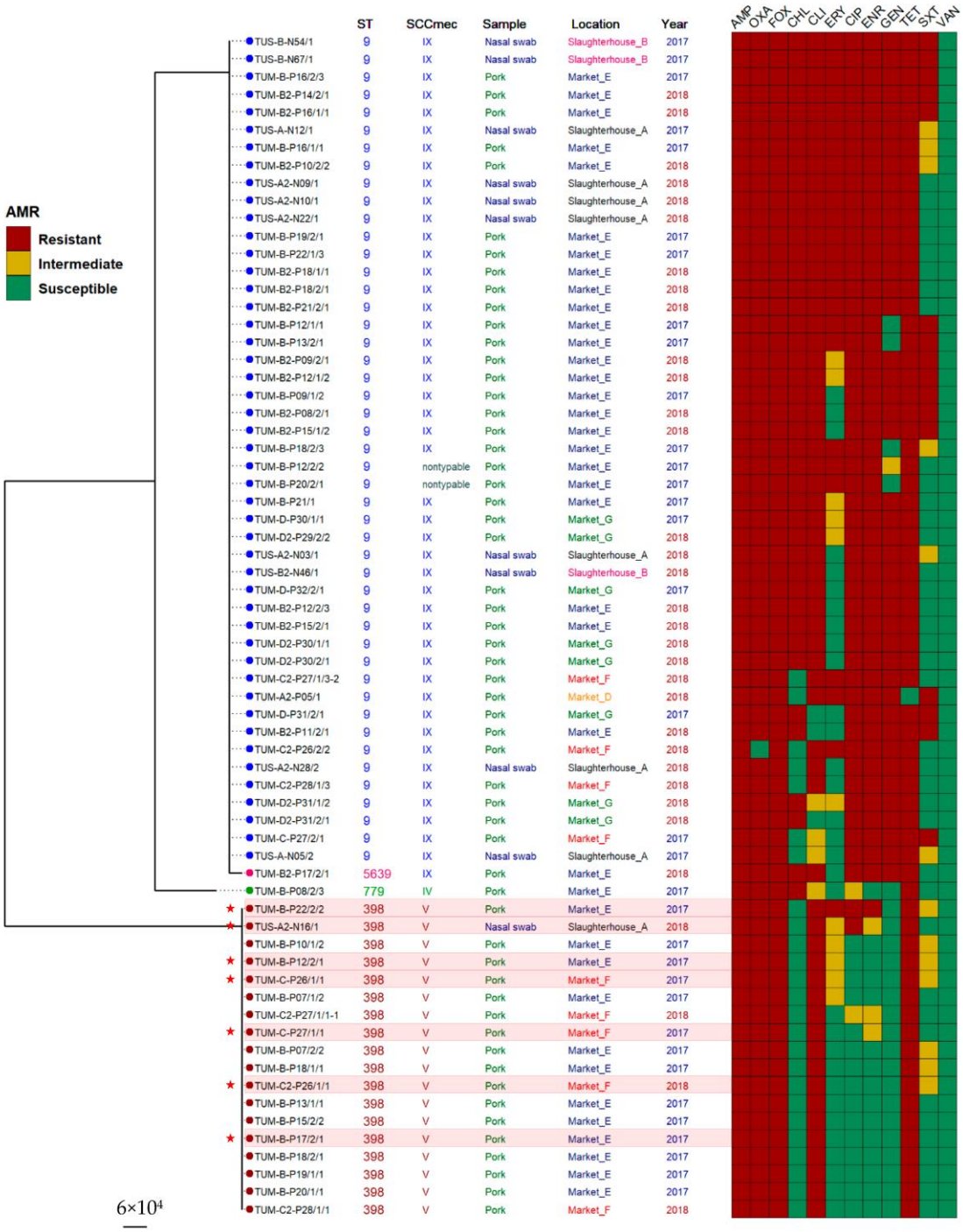


Figure 6. Phylogenetic tree showing the relationship between 67 MRSA strains isolated from nasal swab and pork samples based on the concatenated sequences of seven housekeeping enzyme genes' loci (3186 bp). Boxes showing resistant, dark red; intermediate, ocher; and susceptible, green. *S. aureus* selected for WGS analysis were marked in red asterisk.

Discussion

This study investigated the distribution of MRSA in individual slaughtered pigs and pork in markets at central region of Thailand. This is the first report investigating the epidemiology and molecular characteristics of MRSA in individual slaughtered pigs and pork in Thailand.

The prevalence of MRSA in nasal swab samples observed in this study (11/204; 5.4%) was lower than that in European countries such as Latvia (17/100; 17%) [39] and other Asian countries such as China (38/590; 6.4%) [40]. We estimated the prevalence of MRSA isolated from pork as 44.8% (52/116), which is slightly lower than that in the earlier study in the central region of Thailand (50%; 5/10) [11]. In contrast, these results were higher than 1.8–15.8% among pork in European countries [41], 3.6–9.6% in North American countries [42,43], and 7.1–21.5% in some Asian countries [44,45]. The prevalence may vary depending on several factors, such as geographical area, sampling methods, sample size, collection period, and laboratory methodologies.

In this study, the frequently observed STs were ST9 and ST398 which are known to be associated with animals. These are major endemic MRSA clones circulating in pigs in the central region of Thailand [11,12]. ST9 represents the most common sequence type in Asian countries [17] while ST398 is the dominant clone disseminating worldwide, especially in Europe and North America [3], and has been rarely identified in some Asian countries [46]. However, these strains are an infection-associated strain among pigs and humans in other Asian countries [17,46]. Although ST398 strains have been found from veterinarian [47] and swine farms (pigs and swine workers) [12] in Thailand, this report is the first to detect this strain from pork samples. ST9 and ST398 might be endemic in animal food production in the central region of Thailand.

ST9 has been rarely associated with human diseases [3]; however, a report from Thailand identified ST9 in 2.5% (7/276) of pig farm workers' isolates [48]. In this study, ST5639, a novel single-locus variant of ST9, with a single base substitution in *glpF* was detected in pork from the market. This finding supports the notion that pigs or food animals are reservoirs for the emergence of new MRSA lineages or the evolution of existing clones [49]. Of note, one ST779 isolate from pork in the market (Table 3) was closely related to CA-MRSA or HA-MRSA observed among the population in Australia,

UK, Ireland, and France [50,51]. We detected MRSA ST779 clone carrying SCC*mec* type IV (Table 3) distinct from a previous report by Kinnevey et al. [52,53] and Roberts et al. [54]. The former and the latter found ST779 carrying pseudo-element ΨSCC*mec*-SCC-SCC_{CRISPR} and SCC*mec* type V, respectively. The emergence of human-related STs indicates that slaughter pigs and pork could become important reservoirs for MRSA and increase the potential risk of human infections. Thus, the MRSA lineage described in this study should be considered as a possible public health threat. These data suggest the need to investigate production practices in farms supplying pork products to markets.

A high prevalence of SCC*mec* IX and V among MRSA isolates from markets and slaughterhouses (Table 3 and Figure 6) indicates that this MRSA genotype is rapidly spreading among swine processing chain. ST9 isolates carry different types of SCC*mec* depending on the country [8,30,40,55]. Moreover, a large variety of SCC*mec* types have been found in CC9 strains; much more so than CC398 stains [18]. Therefore, the structures of the non-typeable SCC*mec* found in ST9 in this study need to be characterized by whole-genome sequencing in future study.

We discovered a high diversity of MRSAs genotypes in markets. The major genotype profiles of MRSA isolates were different in each year and each source (slaughterhouses and markets). This analysis suggests that it may be linked to multiple sources of pork in each market and to a temporal shift in the epidemiology of genotype (STs and SCC*mec* type) in Thailand. Hence, further study is needed to monitor the evolution of these pathogens among livestock especially in pig farms and food production stages. Moreover, investigations of LA-MRSA compared to HA-MRSA and CA-MRSA in the same area should be conducted to elucidate the source of cross-contamination of MRSA among the human population, since certain clones may spread in this population.

Notably, oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) found in one pork-sample isolate belonged to ST9-SCC*mec* IX (Table 6 and Figure 6). All of LA-MRSA ST398 (Table 6 and Figure 6) displayed resistance to tetracycline similar to the previous reports [56]. This demonstrates that the LA-MRSA ST398 strain originated as methicillin-susceptible *S. aureus* in humans, then acquired methicillin and tetracycline resistance by antimicrobial selective pressure within the pig farms [57]. Thus, human exposure to LA-MRSA ST 398 might lead to the re-adaptation of this clone by re-acquisition of human pathogenicity genes [57,58]. The MRSA ST9 strain

showed more diverse antimicrobial resistance profiles than ST398 clones. Similar profiles to ST9 have been reported in central Thailand [12]. Previous reports have shown that LA-MRSA isolates were resistant to at least one agent of the fluoroquinolone class in Thailand [9,11,12,48,59,60]. Only LA-MRSA ST9 in this study was associated with fluoroquinolone resistance. It is possible that several fluoroquinolones are available for treatment of animals in farms, and thus, their use may increase resistance among LA-MRSA. These results indicated that appropriate use of antimicrobials in farms is necessary to avoid emergence of high antimicrobial resistance rates of MRSA which can be sources of transmission to humans via food and other routes.

Summary

This is the first report investigating the distribution of MRSA in individual slaughter pigs and pork in Thailand. A high prevalence of SCC*mec* IX and V with high-level antimicrobial resistance among MRSA isolates from markets and slaughterhouses indicated that MRSA with this genotype was rapidly spreading in Thai swine-processing chains. For planning countermeasures, further research is required to understand the nationwide epidemiology of LA-MRSA among livestock, especially in pig farms and food production. In accordance with the information obtained from this study, reduced usage of antimicrobials in farms, prevention of MRSA contamination in animals along the entire pig production chain, and improved hygiene in food practices can be recommended to control the spread of MRSA and reduce the risk of MRSA to a minimum.

CHAPTER II

Whole-genome sequencing of livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 in Thailand

Introduction

LA-MRSA isolated from pigs was the first reported in France in 2005 that belonged to clonal complex (CC) 398 [61]. The MRSA ST398 clone was discovered widespread in pigs in the Netherlands [62]. This MRSA ST398 has been widely identified for having a broader host-spectrum compared to other MRSA strains. LA-MRSA ST398 is the most widely disseminated in European countries [3,6], while ST9 is more predominant in Asian countries [17]. Although LA-MRSA ST398 is the most dominant clone in EU, this strain has been identified outside EU such as North America [42,43], and some Asian countries [10,12,13,44–46]. LA-MRSA ST398 was not only found to colonize pigs, but also in other species of animals such as mink [64], horse [65], cattle [66], poultry [67], and dogs [68]. LA-MRSA ST398 is also found in animal-derived foods such as pork meat, turkey, and milk [69]. Thus, Animal food products might serve as potential vehicles for the transmission of antimicrobial resistance of LA-MRSA ST398 due to manual handing of contaminated raw material [70]. This is an increasing concern regarding the presence of foodborne MRSA encoding antimicrobial resistance (AMR) and virulence genes through mobile genetic elements (MGEs). This increases its adaptability to the host representing a serious public health threat [6]. MRSA ST398 in humans is associated with skin and soft tissue infections (SSTIs) and has also caused bloodstream infections (BSIs) [5,22].

Swine LA-MRSA ST398 in Thailand was first detected between 2015 to 2017 as a major lineage in the previous study in the central of Thailand [12]. While the same strain in retail pork, has been reported in Chapter I [13]. However, the transmission routes of LA-MRSA ST398 in Thailand are unclear and have not been investigated so far. The possible relations among Thai samples of LA-MRSA ST398 isolated from the slaughtered pig and pork can be established with whole-genome sequencing (WGS) and phylogenetic analysis based on single nucleotide polymorphism (SNP).

The objectives of this study were: (i) to investigate the genotypes of LA-MRSA ST398 isolated from slaughtered pigs and retail pork, and (ii) to investigate the possible sources of LA-MRSA ST398 in Thailand.

Materials and Methods

Selection criteria of LA-MRSA 398 isolates from Thailand

A total of seven LA-MRSA ST398 isolates from a cross-sectional study conducted in central region of Thailand during 2017-2018 were selected for WGS analysis [13]. Detailed data on the characteristics of MRSA isolates from each nasal swab or pork samples were showed in Figure 6. Isolates were selected according to the following criteria: (i) showing distinct phenotypic traits of antimicrobial susceptibility pattern (resistant, intermediate, or susceptible following CLSI guidelines) compared with other isolates (8 patterns), (ii) belonging to distinct years in each pattern, (iii) originating from sources in which LA-MRSA ST398 was isolated from distinct markets or slaughterhouses. Therefore, isolates displaying identical antimicrobial susceptibility profiles were considered duplicates in each profile, and only one representative isolate (from each profile) was selected for WGS analysis (Figure 6).

According to the described criteria, six strains originated from markets and one strain from slaughterhouse. The strain from slaughterhouse A were isolated in 2018. For six strains from markets, one strain was isolated in 2018 from market F, and five strains in 2017 were isolated in 2018 from market E (3 strains) and market F (2 strains).

DNA extraction and WGS

Genomic DNA of seven LA-MRSA ST398 was extracted using bead-beating method. The DNA extraction protocol was modified to include an initial bead-beating step whereby the 500 µL of heat-killed MRSA was poured into a bead-beater tube (containing beads). Then, 500 µL of chloroform was added to the tubes, shaken by a bead beater for 1 min at 3,000 rpm, followed by centrifuging the tube for 5 min at 10,000 rpm (25°C) and take the upper layer (aqueous layer, about 400 µL) to a new tube. Next, the supernatant in each tube was mixed with 40 µL (1/10 volume of the supernatant) of 3 M sodium acetate and 1 mL (2.5 times vol.) of ethanol, upside-down mixing, and then incubated at 4°C for 30 min in a refrigerator (or on ice). Samples were centrifuged for 10 min (4°C) at 10,000 rpm. The supernatant from each tube was discarded by pouring. Then 1 mL of 70% ethanol was added to the supernatant in each tube and mixed by light tapping. Samples were centrifuged for 5 min (4°C) at 10,000 rpm and removed the

supernatant with a pipet. Pellets in tubes were dried by opening the lid and laying it inside of a safety cabinet at room temperature for 10 min to evaporate ethanol. Once dried, the pellet was dissolved in 20-100 µL of sterilized TB buffer. The Qubit 3.0 Fluorometer (Invitrogen) and the Nanodrop were used to determine the DNA concentration and indicate the purity of samples produced. The DNA samples were stored in the freezer (-20°C). All samples were diluted to a concentration of ~0.2 ng/µL before performed WGS.

DNA sequence library preparation was performed using an Illumina Nextera XT Kit (Illumina) in accordance with the manufacturer's instruction. Libraries were sequenced on an Illumina MiSeq (Illumina) platform with paired-end operating mode. Following each sequencing reaction, the forward and reverse fastq files for each isolate were exported from the MiSeq computer.

Single nucleotide polymorphism (SNP) calling and phylogenetic analysis

SNPs were identified by mapping reads against the LA-MRSA ST398 reference genome (strain S0385; GenBank accession no. AM9900992) through CFSAN SNP pipeline [71]. SNPs falling into regions of putative recombination [57] were removed from SNP alignment using Gubbins version 2.4.1 [72]. The maximum-likelihood phylogenetic tree was established in IQ-TREE version 2.1.2 [73].The tree was rooted according to Sieber et al. (2018) [6] by using R version 3.6.3 with the package ggtree [74] and ggplot2 [75]. The genetic distance between isolates was calculated as the number of sites that differ between each pair of sequences in the detected core genome.

For comparison, 88 *S. aureus* ST398 isolates from the international reference collection (48 MRSA and 44 MSSA) [57], 283 LA-MRSA ST398 isolates from pigs and humans in Denmark [6], 143 *S. aureus* ST398 isolates from samples in China (65 MRSA and 78 MSSA) [76–79] were included in the phylogenetic analysis. Metadata for all isolates is provided in Data set (Table 7). The fastq file of *S. aureus* or MRSA ST398 belonged to Danish lineage from the previous studies [5,6,57,64–66,69,80] were also downloaded from GenBank and used to study the relationship. The variants were also called using the preceding strategy.

Genotypic characterization of isolates

The virulence genes and antimicrobial resistance genes were identified with the online tools (http://www.genomicepidemiology.org/) VirulenceFinder v2.0 [81,82] and ResFinder v4.1 [83,84], respectively, with a minimum query coverage of 60% and similarity threshold value of 90%. The webserver MyDbFinder v2.0 (https://cge.cbs.dtu.dk/services/MyDbFinder/) was used to determine the *czrC* gene encoding resistance to cadmium and zinc (GenBank accession no. KF593809) with a minimum query coverage of 60% and similarity threshold value of 98%.

The online tool (https://cge.cbs.dtu.dk/services/spatyper/) spaTyper v1.0 [85] was applied to identify *spa* typing in *S. aureus* isolates.

Table 7. Description of 521 *S. aureus* ST398 isolates analyzed in this study.

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
ERR1992226	SSI_80629	2004	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992378	SSI_81109	2004	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992379	SSI_81699	2004	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992380	SSI_87885	2004	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992381	SSI_89393	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992382	SSI_89475	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992384	SSI_92855	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992385	SSI_94863	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992386	SSI_95389	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992388	SSI_95543	2005	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992389	SSI_104579	2006	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992227	SSI_105035	2006	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992228	SSI_105887	2006	Human	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992229	SSI_106337	2006	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992232	SSI_113509	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992233	SSI_113843	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992234	SSI_114345	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992235	SSI_114473	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992236	SSI_114657	2007	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992238	SSI_114673	2007	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992240	SSI_114695	2007	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992241	SSI_114697	2007	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992246	SSI_115637	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992248	SSI_115915	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992249	SSI_116001	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992250	SSI_116561	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992251	SSI_116897	2007	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992139	55-114-001	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992140	55-114-002	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992141	55-114-003	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992142	55-114-004	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992143	55-114-005	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992144	55-114-006	2008	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992145	55-114-007	2008	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992253	SSI_120535	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992254	SSI_120551	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992255	SSI_120561	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992256	SSI_121215	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992257	SSI_121217	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992259	SSI_121939	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992260	SSI_122129	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992261	SSI_122625	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992262	SSI_122661	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992263	SSI_122935	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992264	SSI_122983	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992265	SSI_122987	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992266	SSI_123193	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992267	SSI_123197	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992269	SSI_123725	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992271	SSI_123817	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992272	SSI_124381	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992273	SSI_124457	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992276	SSI_125007	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992277	SSI_125087	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992278	SSI_125089	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992279	SSI_125091	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992280	SSI_125527	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992281	SSI_125835	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992282	SSI_125843	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR2437214	SSI_125845	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992284	SSI_125847	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992285	SSI_125849	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992286	SSI_125851	2008	Human	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992287	SSI_125853	2008	Human	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992288	SSI_125855	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992289	SSI_125857	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992290	SSI_125859	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992291	SSI_125861	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992292	SSI_125865	2008	Human	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992293	SSI_125871	2008	Human	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992294	SSI_125873	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992295	SSI_125875	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992296	SSI_125877	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR2437215	SSI_125879	2008	Human	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992298	SSI_125881	2008	Human	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992299	SSI_125883	2008	Human	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992300	SSI_125887	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992302	SSI_126031	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992303	SSI_126159	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992304	SSI_126267	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992305	SSI_126523	2008	Human	Denmark	MRSA		Sieber et al., 2018

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
ERR1992306	SSI_126545	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992307	SSI_126547	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992308	SSI_126577	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992309	SSI_126837	2008	Human	Denmark	MRSA		Sieber et al., 2018
ERR1992146	55-114-047	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992147	55-114-048	2010	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992148	55-114-049	2010	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992149	55-114-050	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992150	55-114-052	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992151	55-114-053	2010	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992152	55-114-054	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992153	55-114-055	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992154	55-114-056	2010	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992155	55-114-057	2010	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992156	55-114-058	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992157	55-114-059	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992158	55-114-060	2010	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992159	55-114-061	2010	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992160	55-114-062	2010	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992377	55-100-001	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR2437216	55-100-002	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR2437206	55-100-003	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991975	55-100-004	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1991976	55-100-005	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1991977	55-100-006	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991978	55-100-007	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991979	55-100-008	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1823524	55-100-009	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991980	55-100-010	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1991981	55-100-011	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1991982	55-100-012	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991983	55-100-013	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991984	55-100-014	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991985	55-100-015	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823525	55-100-016	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1991986	55-100-017	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991987	55-100-018	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991988	55-100-019	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991989	55-100-020	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1991990	55-100-021	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991991	55-100-022	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1991992	55-100-023	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1991993	55-100-024	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1991994	55-100-025	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1823526	55-100-026	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1991995	55-100-027	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991996	55-100-028	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991997	55-100-029	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991998	55-100-030	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1991999	55-100-031	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992000	55-100-032	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992001	55-100-033	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992002	55-100-034	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992003	55-100-035	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992004	55-100-036	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992005	55-100-037	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992006	55-100-038	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992007	55-100-039	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992008	55-100-040	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992009	55-100-041	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823527	55-100-042	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992010	55-100-043	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992011	55-100-044	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992012	55-100-045	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992013	55-100-046	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992014	55-100-047	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992015	55-100-048	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992016	55-100-049	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992017	55-100-050	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992018	55-100-051	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992019	55-100-052	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992020	55-100-053	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992021	55-100-054	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992022	55-100-055	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823528	55-100-056	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992023	55-100-057	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992024	55-100-058	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992025	55-100-059	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992026	55-100-060	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992027	55-100-061	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992028	55-100-062	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992029	55-100-063	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992030	55-100-064	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992031	55-100-065	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992032	55-100-066	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
ERR1823529	55-100-067	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992033	55-100-068	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992034	55-100-069	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992035	55-100-070	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992036	55-100-071	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992037	55-100-072	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1823530	55-100-073	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992038	55-100-075	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992039	55-100-076	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992040	55-100-077	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992041	55-100-078	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1823531	55-100-079	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1823532	55-100-080	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823533	55-100-081	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR2437207	55-100-082	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992043	55-100-083	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR2437208	55-100-084	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992045	55-100-086	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR2437209	55-100-087	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992046	55-100-088	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR2437210	55-100-089	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992048	55-100-090	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992049	55-100-091	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR2437211	55-100-092	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR2437212	55-100-093	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR2437213	55-100-094	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992053	55-100-095	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1823535	55-100-096	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992054	55-100-097	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992055	55-100-098	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992056	55-100-099	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992057	55-100-100	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992058	55-100-101	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992059	55-100-102	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992060	55-100-103	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992061	55-100-104	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992062	55-100-105	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992063	55-100-106	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992064	55-100-107	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992065	55-100-108	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992066	55-100-109	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992067	55-100-110	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992068	55-100-111	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992069	55-100-112	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992070	55-100-113	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992071	55-100-114	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992072	55-100-115	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992073	55-100-116	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992074	55-100-117	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992075	55-100-118	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992076	55-100-119	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992077	55-100-120	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992078	55-100-121	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992079	55-100-122	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992080	55-100-123	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1823536	55-100-124	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992081	55-100-125	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992082	55-100-126	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992083	55-100-127	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992084	55-100-128	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992085	55-100-129	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992086	55-100-130	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992087	55-100-131	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1823537	55-100-132	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823538	55-100-133	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992088	55-100-134	2014	Pig	Denmark	MRSA		Sieber et al., 2018
ERR1992089	55-100-135	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992090	55-100-136	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1823539	55-100-137	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992091	55-100-138	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992092	55-100-139	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992093	55-103-001	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992094	55-103-002	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992095	55-103-003	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992096	55-103-004	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992097	55-103-005	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992098	55-103-006	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992099	55-103-007	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992100	55-103-008	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992101	55-103-009	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992102	55-103-010	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992103	55-103-011	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992104	55-103-012	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992105	55-103-013	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992106	55-103-014	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
ERR1992107	55-103-015	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992108	55-103-016	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992109	55-103-017	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992110	55-103-018	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992111	55-103-019	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992112	55-103-020	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992113	55-103-021	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992114	55-103-022	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992115	55-103-023	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992116	55-103-024	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992117	55-103-025	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992118	55-103-026	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992119	55-103-027	2014	Pig	Denmark	MRSA	L2	Sieber et al., 2018
ERR1992120	55-103-028	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992121	55-103-029	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992122	55-103-030	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992123	55-103-031	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992124	55-103-032	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992125	55-103-033	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992126	55-103-034	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992127	55-103-035	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992128	55-103-041	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992129	55-103-042	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992130	55-103-043	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992131	55-103-044	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992132	55-103-045	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992133	55-103-046	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992134	55-103-047	2014	Pig	Denmark	MRSA	L1	Sieber et al., 2018
ERR1992135	55-103-048	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992136	55-103-049	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992137	55-103-050	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
ERR1992138	55-103-051	2014	Pig	Denmark	MRSA	L3	Sieber et al., 2018
SRR445240	324	2009	Pig	Germany	MSSA		Price et al., 2012
SRR445234	1061	2009	Pig	Germany	MSSA		Price et al., 2012
SRR445235	1953	2007	Human	United States	MSSA		Price et al., 2012
SRR445238	2046	2007	Human	United States	MSSA		Price et al., 2012
SRR445278	23225	2008	Pig_dust	Austria	MRSA		Price et al., 2012
SRR445284	23824	2008	Pig_dust	Austria	MRSA		Price et al., 2012
SRR445239	29139	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445029	30116	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445045	47771	2005	Human	Denmark	MRSA		Price et al., 2012
SRR445053	50148	2006	Human	Denmark	MRSA		Price et al., 2012
SRR445280	51225	2006	Human	Denmark	MRSA		Price et al., 2012
SRR445057	51726	2006	Human	Denmark	MRSA		Price et al., 2012
SRR445289	55488	2007	Human	Denmark	MRSA		Price et al., 2012
SRR445073	60036	2008	Human	Denmark	MRSA		Price et al., 2012
SRR445074	61888	2008	Human	Denmark	MRSA		Price et al., 2012
SRR445075	62951	2008	Human	Denmark	MSSA		Price et al., 2012
SRR445071	089B_2	2009	Pig	Peru	MRSA		Price et al., 2012
SRR445233	09/01691/3	2009	Pig	Poland	MSSA		Price et al., 2012
SRR445027	12152-5	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445232	13349_2	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445283	13349_6	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445025	199/08	2008	Pig_dust	Slovenia	MRSA		Price et al., 2012
SRR445275	2007-70-91-4-SPA	2007	Pig	Denmark	MRSA		Price et al., 2012
SRR445274	2007-70-92-6-SPA	2007	Pig	Denmark	MSSA		Price et al., 2012
SRR445273	2007-70-95-9-SPA	2007	Pig	Denmark	MSSA		Price et al., 2012
SRR445276	2008-60-1662-5	2008	Pig_dust	The Netherlands	MRSA		Price et al., 2012
SRR445236	2008-60-3254	2008	Pig_dust	Denmark	MRSA		Price et al., 2012
SRR445237	2008-60-970	2008	Pig_dust	Denmark	MRSA		Price et al., 2012
SRR445290	2296-MRSA	2008	Pig_dust	Germany	MRSA		Price et al., 2012
SRR445028	25207-25126/08	2008	Pig_dust	Poland	MRSA		Price et al., 2012
SRR445072	34-M-B-1_11	2008	Pig	Denmark	MSSA	L1	Price et al., 2012
SRR445286	374/08	2008	Pig_dust	Slovenia	MRSA		Price et al., 2012
SRR445229	3-S-1	2007	Pig	Denmark	MRSA		Price et al., 2012
SRR445030	44523-1	2008	Pig_dust	Italy	MRSA		Price et al., 2012
SRR445031	6919/08_8	2008	Pig_dust	Poland	MRSA		Price et al., 2012
SRR445288	7_2007-70-77-4	2007	Pig	Denmark	MRSA		Price et al., 2012
SRR445032	7-1	2007	Pig	Canada	MRSA		Price et al., 2012
SRR445033	7-109	2007	Pig	Canada	MRSA		Price et al., 2012
SRR445034	7-12	2007	Pig	Canada	MRSA		Price et al., 2012
SRR445035	7-14	2007	Pig	Canada	MRSA		Price et al., 2012
SRR445036	3-Jul	2007	Pig	Canada	MRSA		Price et al., 2012
SRR445282	7413532-2	2002	Pig	Denmark	MSSA	L3	Price et al., 2012
SRR445281	7SPA_72-13850-1	2000	Pig	Denmark	MSSA		Price et al., 2012
SRR445277	9B	2007	Pig	Denmark	MRSA		Price et al., 2012
SRR445292	Aureus_56	2008	Cattle	Belgium	MRSA		Price et al., 2012
SRR445279	AV4	2008	Horse	Belgium	MRSA		Price et al., 2012
SRR445287	AV6	2008	Horse	Belgium	MRSA		Price et al., 2012
SRR445293	DC38_BP_TET_F_7	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445026	DC57_BP_GEN_I_7	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445241	F10	2008	Pork	United States	MSSA		Price et al., 2012
SRR445242	F20	2008	Pork	United States	MSSA		Price et al., 2012
SRR445243	F38	2008	Pig	United States	MSSA		Price et al., 2012
SRR445086	LY19990171	1999	Human	France	MSSA		Price et al., 2012

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
SRR445037	M2009_10003479	2009	Pig_dust	Hungary	MRSA		Price et al., 2012
SRR445038	M2009_10004208	2009	Pig_dust	Hungary	MRSA		Price et al., 2012
SRR445291	M-5	2008	Pig_dust	Belgium	MRSA		Price et al., 2012
SRR445263	P23-01_SW31.1	2008	Pig	United States	MRSA		Price et al., 2012
SRR445265	P23-02_SW62.1	2008	Pig	United States	MRSA		Price et al., 2012
SRR445262	P23-03_SW181.1	2008	Pig	United States	MRSA		Price et al., 2012
SRR445039	P23-10_WZ-103	2003	Human	China	MSSA		Price et al., 2012
SRR445040	P23-11_HF-446	2007	Human	China	MSSA		Price et al., 2012
SRR445041	P23-12_HF-80520	2007	Human	China	MSSA		Price et al., 2012
SRR445042	P23-13_HF-724402	2007	Human	China	MSSA		Price et al., 2012
SRR445076	P23-14_SD4.1	2008	Pig_dust	China	MSSA		Price et al., 2012
SRR445043	P23-9_WZ-1	2002	Human	China	MSSA		Price et al., 2012
SRR445231	PR7/08	2007	Pig_dust	Portugal	MRSA		Price et al., 2012
SRR445077	ST20071083	2007	Human	French Guiana	MSSA		Price et al., 2012
SRR445078	ST20082015	2008	Human	France	MSSA		Price et al., 2012
SRR445079	ST20090121	2008	Human	France	MSSA		Price et al., 2012
SRR445080	ST20091155	2009	Human	France	MSSA		Price et al., 2012
SRR445081	ST20091526	2009	Human	France	MSSA		Price et al., 2012
SRR445082	ST20091826	2009	Human	France	MSSA		Price et al., 2012
SRR445083	ST20100011	2009	Human	France	MSSA		Price et al., 2012
SRR445084	ST20100537	2010	Human	France	MSSA		Price et al., 2012
SRR445085	ST20101526	2003	Human	France	MSSA		Price et al., 2012
SRR445264	SW356	1993	Cattle	Swizerland	MRSA		Price et al., 2012
SRR445266	SWK35	2009	Pig	United States	MSSA		Price et al., 2012
SRR445267	T2	2009	Turkey_meat	United States	MRSA		Price et al., 2012
SRR445268	T3	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445269	T4	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445270	T5	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445271	T6	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445272	T7	2009	Turkey_meat	United States	MSSA		Price et al., 2012
SRR445060	UB08116	2008	Pig_dust	France	MSSA		Price et al., 2012
SRR445066	UB08187	2008	Pig_dust	France	MRSA		Price et al., 2012
SRR445228	USA42	1993	Cattle	United States	MRSA		Price et al., 2012
SRR445285	Veo08/01292-1	2008	Pig_dust	Spain	MSSA		Price et al., 2012
SRR445230	Veo8/003845st	2008	Pig_dust	Spain	MRSA		Price et al., 2012
SRR11526821	18-398-15	2018	Human	China	MRSA		Lu et al., 2021
SRR11526822	18-398-14	2018	Human	China	MRSA		Lu et al., 2021
SRR11526823	18-398-13	2018	Human	China	MRSA		Lu et al., 2021
SRR11526824	18-398-11	2018	Human	China	MRSA		Lu et al., 2021
SRR11526825	18-398-05	2018	Human	China	MRSA		Lu et al., 2021
SRR11526826	15-398-9	2015	Human	China	MRSA		Lu et al., 2021
SRR11526827	18-398-04	2018	Human	China	MRSA		Lu et al., 2021
SRR11526828	18-398-01	2018	Human	China	MRSA		Lu et al., 2021
SRR11526829	17-398-43	2017	Human	China	MRSA		Lu et al., 2021
SRR11526830	17-398-42	2017	Human	China	MRSA		Lu et al., 2021
SRR11526831	17-398-40	2017	Human	China	MRSA		Lu et al., 2021
SRR11526832	17-398-38	2017	Human	China	MRSA		Lu et al., 2021
SRR11526833	17-398-37	2017	Human	China	MRSA		Lu et al., 2021
SRR11526834	17-398-36	2017	Human	China	MRSA		Lu et al., 2021
SRR11526835	17-398-35	2017	Human	China	MRSA		Lu et al., 2021
SRR11526836	17-398-30	2017	Human	China	MRSA		Lu et al., 2021
SRR11526837	15-398-7	2015	Human	China	MRSA		Lu et al., 2021
SRR11526838	17-398-29	2017	Human	China	MRSA		Lu et al., 2021
SRR11526839	17-398-26	2017	Human	China	MRSA		Lu et al., 2021
SRR11526840	17-398-25	2017	Human	China	MRSA		Lu et al., 2021
SRR11526841	17-398-21	2017	Human	China	MRSA		Lu et al., 2021
SRR11526842	17-398-20	2017	Human	China	MRSA		Lu et al., 2021
SRR11526843	17-398-18	2017	Human	China	MRSA		Lu et al., 2021
SRR11526844	17-398-12	2017	Human	China	MRSA		Lu et al., 2021
SRR11526845	17-398-11	2017	Human	China	MRSA		Lu et al., 2021
SRR11526846	17-398-10	2017	Human	China	MRSA		Lu et al., 2021
SRR11526847	17-398-09	2017	Human	China	MRSA		Lu et al., 2021
SRR11526848	15-398-6	2015	Human	China	MRSA		Lu et al., 2021
SRR11526849	16-398-39	2016	Human	China	MRSA		Lu et al., 2021
SRR11526850	16-398-36	2016	Human	China	MRSA		Lu et al., 2021
SRR11526851	16-398-35	2016	Human	China	MRSA		Lu et al., 2021
SRR11526852	16-398-31	2016	Human	China	MRSA		Lu et al., 2021
SRR11526853	16-398-30	2016	Human	China	MRSA		Lu et al., 2021
SRR11526854	16-398-22	2016	Human	China	MRSA		Lu et al., 2021
SRR11526855	16-398-15	2016	Human	China	MRSA		Lu et al., 2021
SRR11526856	16-398-8	2016	Human	China	MRSA		Lu et al., 2021
SRR11526857	16-398-14	2016	Human	China	MRSA		Lu et al., 2021
SRR11526858	16-398-7	2016	Human	China	MRSA		Lu et al., 2021
SRR11526859	16-398-1	2016	Human	China	MRSA		Lu et al., 2021
SRR11526860	15-398-19	2015	Human	China	MRSA		Lu et al., 2021
SRR11526861	18-398-33	2018	Human	China	MRSA		Lu et al., 2021
SRR11526862	18-398-32	2018	Human	China	MRSA		Lu et al., 2021
SRR11526863	15-398-10	2015	Human	China	MRSA		Lu et al., 2021
SRR11526864	18-398-31	2018	Human	China	MRSA		Lu et al., 2021
SRR11526865	18-398-29	2018	Human	China	MRSA		Lu et al., 2021
SRR11526866	18-398-26	2018	Human	China	MRSA		Lu et al., 2021
SRR11526867	18-398-21	2018	Human	China	MRSA		Lu et al., 2021
SRR11526868	18-398-16	2018	Human	China	MRSA		Lu et al., 2021
SRR11526869	16-398-12	2016	Human	China	MRSA		Lu et al., 2021

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
SRR11526870	16-398-10	2016	Human	China	MRSA		Lu et al., 2021
SRR11526871	15-398-5	2015	Human	China	MRSA		Lu et al., 2021
SRR11526872	15-398-4	2015	Human	China	MRSA		Lu et al., 2021
SRR5062006	HO_MRSA_7	2011	Human	China	MRSA		He et al., 2018
SRR5054902	HO_MSSA_38	2010	Human	China	MSSA		He et al., 2018
SRR5054903	HO_MRSA_4	2011	Human	China	MRSA		He et al., 2018
SRR5054904	HO_MSSA_20	2014	Human	China	MSSA		He et al., 2018
SRR5054905	LA_MSSA_3	2014	Cattle	China	MSSA		He et al., 2018
SRR5054906	HO_MSSA_60	2011	Human	China	MSSA		He et al., 2018
SRR5054907	HO_MSSA_54	2011	Human	China	MSSA		He et al., 2018
SRR5054908	HO_MSSA_22	2012	Human	China	MSSA		He et al., 2018
SRR5054909	HO_MSSA_14	2014	Human	China	MSSA		He et al., 2018
SRR5054910	HO_MSSA_26	2012	Human	China	MSSA		He et al., 2018
SRR5054911	HO_MSSA_31	2012	Human	China	MSSA		He et al., 2018
SRR5054912	HO_MSSA_25	2010	Human	China	MSSA		He et al., 2018
SRR5054913	HO_MSSA_59	2012	Human	China	MSSA		He et al., 2018
SRR5054914	HO_MSSA_18	2014	Human	China	MSSA		He et al., 2018
SRR5054915	HO_MSSA_51	2012	Human	China	MSSA		He et al., 2018
SRR5054916	HO_MSSA_46	2012	Human	China	MSSA		He et al., 2018
SRR5054917	HO_MRSA_5	2012	Human	China	MRSA		He et al., 2018
SRR5054918	HO_MSSA_53	2014	Human	China	MSSA		He et al., 2018
SRR5054919	HO_MSSA_12	2014	Human	China	MSSA		He et al., 2018
SRR5054920	HO_MSSA_17	2012	Human	China	MSSA		He et al., 2018
SRR5054921	HO_MSSA_49	2014	Human	China	MSSA		He et al., 2018
SRR5054922	HO_MSSA_29	2012	Human	China	MSSA		He et al., 2018
SRR5054923	LA_MSSA_7	2014	Cattle	China	MSSA		He et al., 2018
SRR5054924	HO_MSSA_27	2012	Human	China	MSSA		He et al., 2018
SRR5054925	HO_MRSA_2	2014	Human	China	MRSA		He et al., 2018
SRR5054926	HO_MSSA_34	2011	Human	China	MSSA		He et al., 2018
SRR5054927	HO_MSSA_42	2014	Human	China	MSSA		He et al., 2018
SRR5054928	LA_MSSA_11	2015	Cattle	China	MSSA		He et al., 2018
SRR5054929	HO_MSSA_61	2010	Human	China	MSSA		He et al., 2018
SRR5054930	HO_MSSA_36	2012	Human	China	MSSA		He et al., 2018
SRR5054931	HO_MSSA_8	2010	Human	China	MSSA		He et al., 2018
SRR5054932	HO_MSSA_37	2011	Human	China	MSSA		He et al., 2018
SRR5054933	LA_MSSA_2	2014	Cattle	China	MSSA		He et al., 2018
SRR5054934	HO_MRSA_7	2011	Human	China	MRSA		He et al., 2018
SRR5054935	HO_MSSA_15	2010	Human	China	MSSA		He et al., 2018
SRR5054936	HO_MRSA_1	2014	Human	China	MRSA		He et al., 2018
SRR5054937	HO_MSSA_30	2010	Human	China	MSSA		He et al., 2018
SRR5054938	HO_MSSA_16	2011	Human	China	MSSA		He et al., 2018
SRR5054939	HO_MSSA_47	2012	Human	China	MSSA		He et al., 2018
SRR5054940	HO_MSSA_52	2014	Human	China	MSSA		He et al., 2018
SRR5054941	HO_MSSA_21	2014	Human	China	MSSA		He et al., 2018
SRR5054942	HO_MSSA_39	2012	Human	China	MSSA		He et al., 2018
SRR5054943	LA_MSSA_12	2015	Cattle	China	MSSA		He et al., 2018
SRR5054944	LA_MSSA_13	2015	Cattle	China	MSSA		He et al., 2018
SRR5054945	HO_MSSA_48	2012	Human	China	MSSA		He et al., 2018
SRR5054946	LA_MSSA_15	2015	Cattle	China	MSSA		He et al., 2018
SRR5054947	LA_MSSA_6	2014	Cattle	China	MSSA		He et al., 2018
SRR5054948	HO_MSSA_35	2014	Human	China	MSSA		He et al., 2018
SRR5054949	LA_MSSA_4	2014	Cattle	China	MSSA		He et al., 2018
SRR5054950	HO_MSSA_55	2010	Human	China	MSSA		He et al., 2018
SRR5054951	HO_MSSA_56	2011	Human	China	MSSA		He et al., 2018
SRR5054952	HO_MSSA_28	2012	Human	China	MSSA		He et al., 2018
SRR5054953	HO_MSSA_33	2012	Human	China	MSSA		He et al., 2018
SRR5054954	HO_MSSA_9	2014	Human	China	MSSA		He et al., 2018
SRR5054955	HO_MSSA_24	2012	Human	China	MSSA		He et al., 2018
SRR5054956	HO_MSSA_10	2011	Human	China	MSSA		He et al., 2018
SRR5054957	LA_MSSA_1	2014	Cattle	China	MSSA		He et al., 2018
SRR5054958	HO_MSSA_13	2014	Human	China	MSSA		He et al., 2018
SRR5054959	LA_MSSA_5	2014	Cattle	China	MSSA		He et al., 2018
SRR5054960	LA_MSSA_10	2015	Cattle	China	MSSA		He et al., 2018
SRR5054961	HO_MSSA_44	2011	Human	China	MSSA		He et al., 2018
SRR5054962	HO_MSSA_58	2014	Human	China	MSSA		He et al., 2018
SRR5054963	LA_MSSA_14	2015	Cattle	China	MSSA		He et al., 2018
SRR5054964	HO_MSSA_43	2011	Human	China	MSSA		He et al., 2018
SRR5054965	LA_MSSA_9	2014	Cattle	China	MSSA		He et al., 2018
SRR5054966	HO_MSSA_41	2012	Human	China	MSSA		He et al., 2018
SRR5054967	HO_MSSA_19	2010	Human	China	MSSA		He et al., 2018
SRR5054968	LA_MSSA_8	2014	Cattle	China	MSSA		He et al., 2018
SRR5054969	HO_MSSA_57	2014	Human	China	MSSA		He et al., 2018
SRR5054970	HO_MSSA_45	2011	Human	China	MSSA		He et al., 2018
SRR5054971	HO_MRSA_6	2012	Human	China	MRSA		He et al., 2018
SRR5054972	HO_MSSA_11	2014	Human	China	MSSA		He et al., 2018
SRR5054973	HO_MRSA_3	2012	Human	China	MSSA		He et al., 2018
SRR5054974	HO_MSSA_50	2014	Human	China	MSSA		He et al., 2018
SRR5054975	HO_MSSA_40	2012	Human	China	MSSA		He et al., 2018
SRR5054976	HO_MSSA_23	2012	Human	China	MSSA		He et al., 2018
SRR5054977	HO_MSSA_32	2012	Human	China	MSSA		He et al., 2018
ERR3792042	29MR_KINA	2019	Pork	China	MSSA		Li et al., 2021
ERR3792044	31MR_KINA	2019	Pork	China	MRSA		Li et al., 2021
ERR3792051	48_1_KINA	2019	Sushi	China	MSSA		Li et al., 2021
ERR3792052	48_2_KINA	2019	Sushi	China	MSSA		Li et al., 2021
ERR3792054	48_4_KINA	2019	Sushi	China	MSSA		Li et al., 2021

SRA	Sample_Name	Year	Sources	Country	MRSA /MSSA	Lineage	Ref.
SRR9046749	R09	2016	Human	China	MRSA		Chen et al., 2020
SRR9046750	X05	2016	Human	China	MRSA		Chen et al., 2020
SRR9046751	J01	2016	Human	China	MRSA		Chen et al., 2020
SRR9046752	J12	2016	Human	China	MRSA		Chen et al., 2020
SRR9046753	F18	2012	Human	China	MSSA		Chen et al., 2020
SRR9046754	07B04	2016	Human	China	MSSA		Chen et al., 2020
SRR9046755	X06	2016	Human	China	MRSA		Chen et al., 2020
SRR9046756	R3383	1999	Human	China	MSSA		Chen et al., 2020
SRR9046757	08B22	2016	Human	China	MSSA		Chen et al., 2020
sampleA107	TUM-B-P12/2/1	2017	Pork	Thailand	MRSA	L3	This study
sampleA131	TUM-B-P17/2/1	2017	Pork	Thailand	MRSA	L3	This study
sampleA154	TUM-B-P22/2/2	2017	Pork	Thailand	MRSA	L1	This study
sampleA170	TUM-C-P26/1/1	2017	Pork	Thailand	MRSA	L3	This study
sampleA172	TUM-C-P27/1/1	2017	Pork	Thailand	MRSA	L3	This study
sampleB10	TUS-A2-N16/1	2018	Slaughtered pig	Thailand	MRSA	L1	This study
sampleB53	TUM-C2-P26/1/1	2018	Pork	Thailand	MRSA	L3	This study

Results

Genotypic and phenotypic characteristic of LA-MRSA ST398 isolated from pork and slaughtered pig in Thailand

WGS was performed on seven recently isolated LA-MRSA ST398 strains representative of the major clones, as defined by phenotypic profiles from Chapter I [13]. All seven isolates, from pork (n=6) and slaughtered pig (n=1), were characterized based on genomic identification of virulence genes, antimicrobial resistance profiles, SCC*mec* type, and *spa* type as showed in Figure 7. None of the isolates were found to carry any of the human-related immune evasion gene cluster (IEC)-containing genes *sea, sep, sak, chp,* and *scn*.

All seven LA-MRSA ST398 strains were of SCC*mec* V type, six of which were *spa* t034 and one was *spa* t1255. (Figure 7). For the antimicrobial resistance genes, all isolates carried *blaZ, mecA, czrC, dfrG, lnu(B), lsa(E), tet(K),* and *tet(M)* gene. Moreover, all isolates were found to carry exoenzyme genes (*aur*) and toxin genes (*hlgA, hlgB,* and *hlgC*), whereas enterotoxins gene (*sem*) was detected only 2 (28.6%) isolates. There was one isolate carrying genes encoding staphylococcal enterotoxins (*seg, sei, sen,* and *seu*) that belonged to *spa* t1255.

Comparison of Thai LA-MRSA ST398 with Chinese *S. aureus* strains

The genomes of the seven LA-MRSA ST398 isolates from Thailand were compared with 143 Asian (Chinese) [76–79] and 88 international reference genomes of *S. aureus* ST398 [57]. In total, 238 genomes were considered for phylogenetic relationship analysis based on SNPs.

After removal of recombination regions, 13,932 core genomes SNPs from 238 isolates were used to construct a rooted maximum-likelihood tree (Figure 8). The analysis revealed the distribution of the isolates from Thailand into two groups (designated A and B) (Figure 8). These isolates also differed by source and year of collection. Group A composed of two isolates collected in 2018 from market E (1 isolate) and slaughterhouse A. Group B composed of other five isolates. These included (1 isolate) collected in 2018 from market F. While isolates from market E (2 isolates) and market F (2 isolates) were collected in 2017. The average SNP distance between

seven Thai LA-MRSA ST398 isolates and *S. aureus* ST398 of Danish pig (from international reference collection) was 109.9 (range, 91-118 SNPs). Whereas, the average shortest SNP distance to LA-MRSA ST398 isolates from Chinese samples was 273.7 SNPs (range, 271-279 SNPs) (Figure 8 and Table 8).

Comparison of Thai LA-MRSA ST398 with Danish and Chinese strains

Animal movement through trade is considered a driver for the spread of LA-MRSA ST398 among pigs. To trace the possible source and investigate any transboundary or local dissemination of recently identified Thai LA-MRSA ST398 isolates, the data on import of pigs to Thailand is shown in Figure 3 and Figure 9. The data reported that Denmark was the country with the highest number of pigs imported into Thailand especially in 2017. Moreover, Denmark experienced an increase in LA-MRSA ST398 prevalence in pig farms. To this purpose, additional genome data of 283 isolates from Danish pigs [6] were incorporated in this analysis. So, the genomes of the seven Thai LA-MRSA ST398 isolates were compared with 283 genomes from previously sequenced *S. aureus* ST398 isolates from the public database [6,57,76–79]. The total of 521 genomes were included in this analysis to reconstruct phylogenetic relationship based on SNPs.

The 17,395 core genomes SNPs after the removal of sites falling into recombination regions were used to construct a rooted maximum-likelihood tree shown in Figure 10. Thai LA-MRSA ST398 in group A and B in Figure 8 exhibited a close relatedness with the Danish L1 and L3 lineages (Figure 10), respectively. The seven LA-MRSA ST398 isolates had the shortest average SNP distances to LA-MRSA ST398 from Danish pig [6] that was 22.7 SNPs [range, 17-34 SNPs]). The average SNP distance to *S. aureus* ST398 from Danish pig in the international collection samples [57] was 110.1 SNPs (range, 91-119 SNPs), and LA-MRSA ST398 isolates from Chinese samples [78] was 272.1 SNPs (range, 268-280 SNPs) (Figure 10 and Table 9).

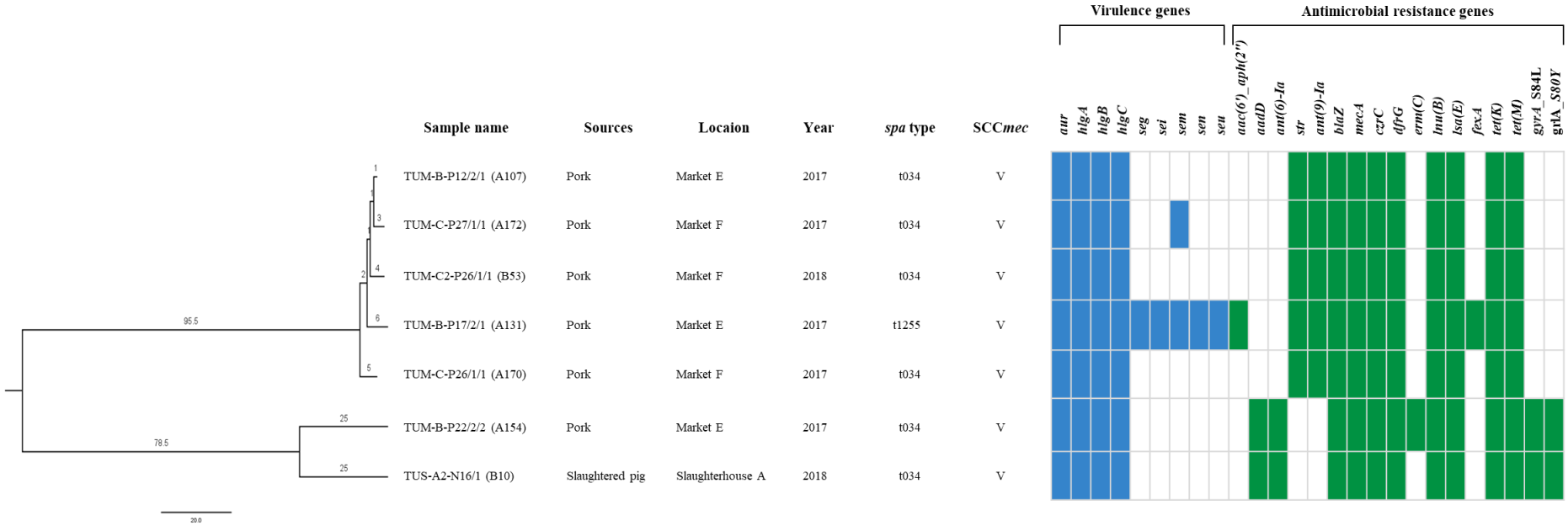


Figure 7. Maximum-parsimony phylogenetic analysis was established from 248 SNPs after filtering for recombination tracts (182 SNPs) and antimicrobial resistance profiles, SCC*mec* type, and spa type of seven LA-MRSA ST398

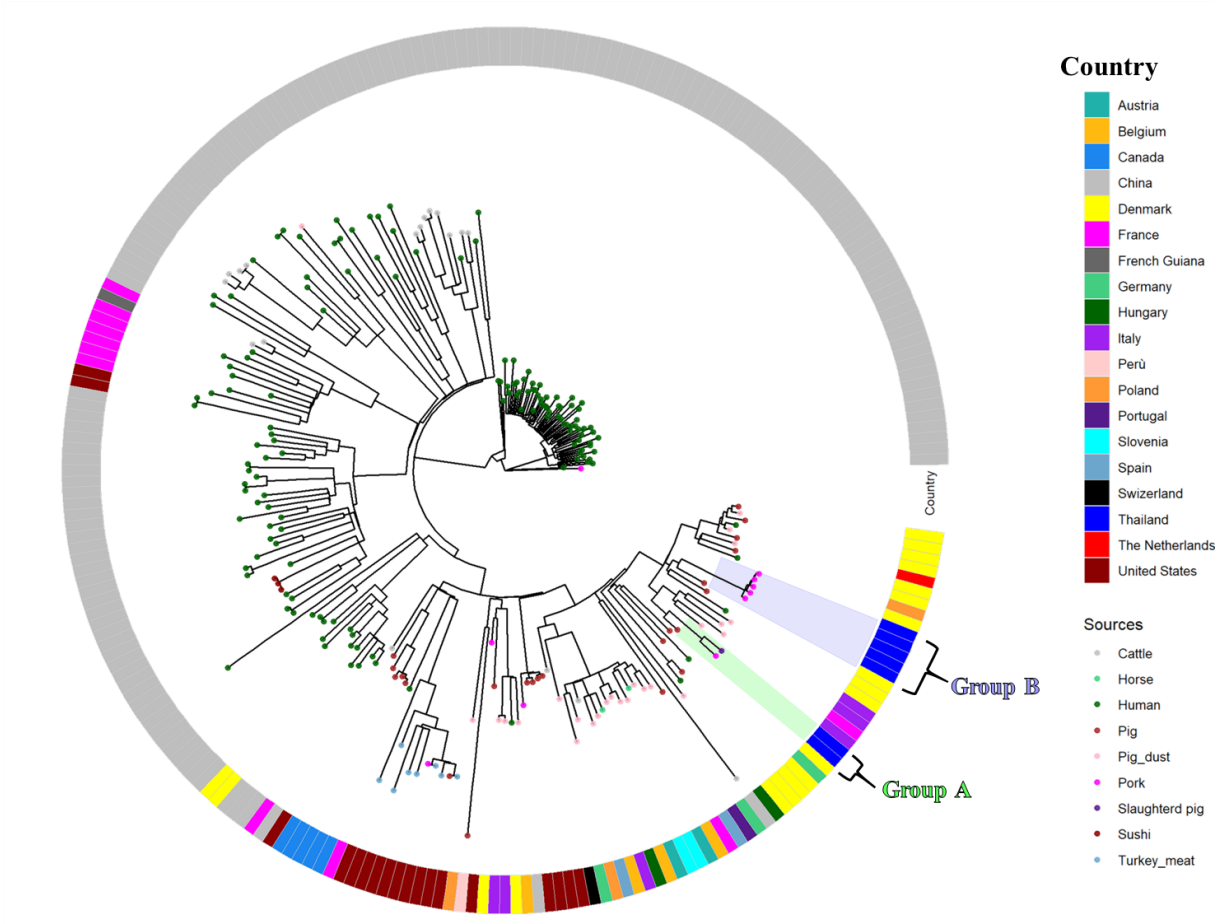
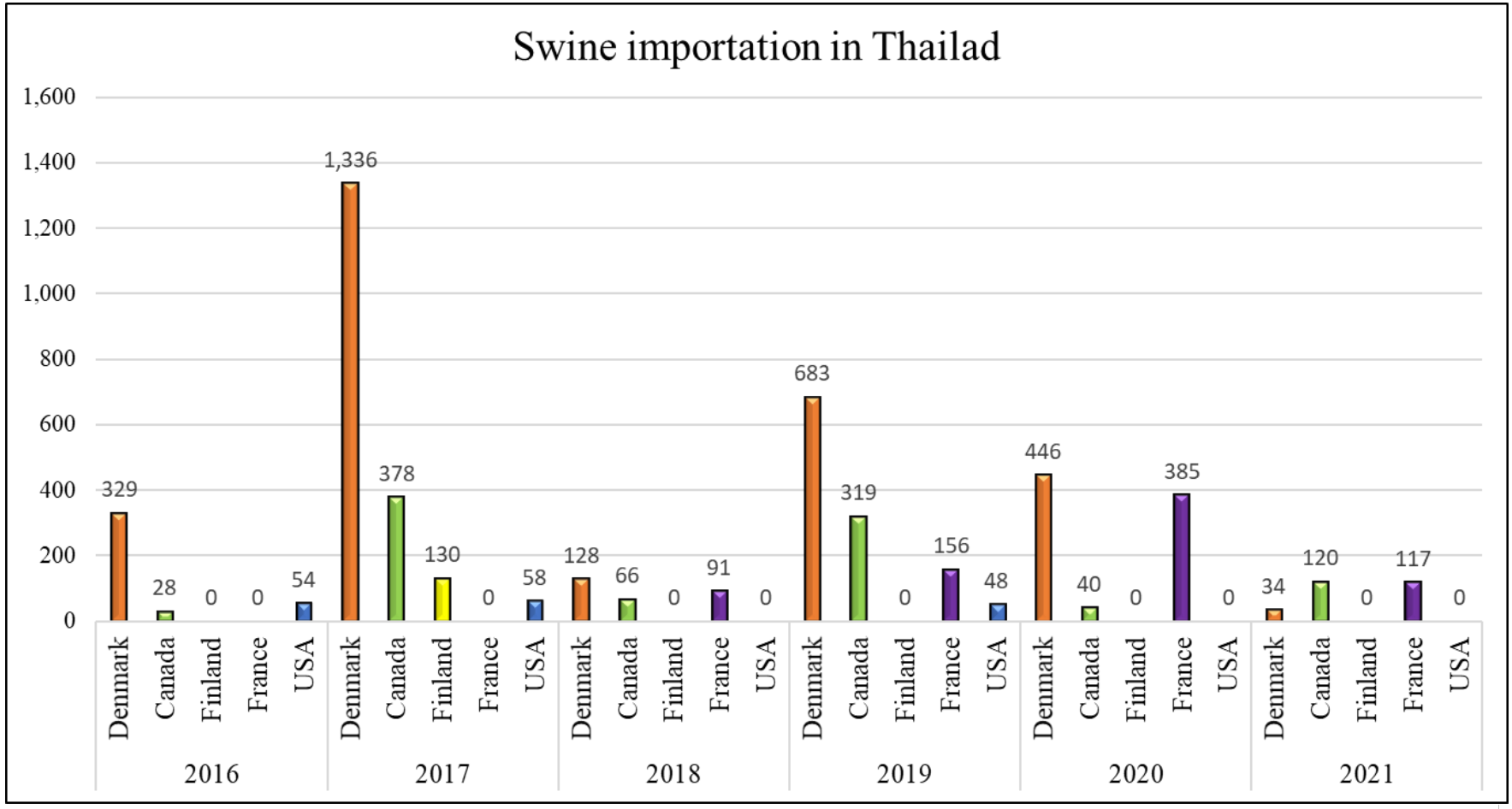


Figure 8. Maximum-likelihood phylogeny was established from 13,932 SNPs after filtering for recombination tracts (21 SNPs). It include seven recent LA-MRSA ST398 isolates from Thailand, 88 *S. aureus* isolates from international reference collection [57], 52 Chinese isolates from hospital [77], 77 CA-MRSA ST398 and LA-MRSA ST398 of Chinese isolates [76], nine *S. aureus* ST398 isolates from patients in China [79], and five *S. aureus* ST398 isolates from retail foods in China [78]



Source: Department of Livestock Development (unpublished data, 2021)

Figure 9. Data on imported pigs to Thailand by country of origin each year between 2016-2021. (Sources: Department of Livestock Development (unpublished data, 2021)

Table 8. Shortest SNP distances from seven LA-MRSA ST398 to Chinese *S. aureus* ST 398 and international collection isolates

		TUM-B-P12/2/1 (A107)	**TUM-B-P17/2/1 (A131)**	**TUM-B-P22/2/2 (A154)**	**TUM-C-P26/1/1 (A170)**	**TUM-C-P27/1/1 (A172)**	**TUS-A2-N16/1 (B10)**	**TUM-C2-P26/1/1 (B53)**
	Group	**B**	**B**	**A**	**B**	**B**	**A**	**B**
Shortest distance to **another *S. aureus* ST398** isolate, SNPs	SNPs	**116**	**118**	**91**	**116**	**118**	**92**	**118**
	Closest isolate	7413532-2 (SRR445282)	7413532-2 (SRR445282)	34-M-B-1_11 (SRR445072)	7413532-2 (SRR445282)	7413532-2 (SRR445282)	34-M-B-1_11 (SRR445072)	7413532-2 (SRR445282)
	Sources	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig
	spa type	t011	t011	t t034	t011	t011	t034	t011
	Year	2002	2002	2008	2002	2002	2008	2002
Shortest distance to **Chinese** samples, SNPs	SNPs	**271**	**272**	**277**	**271**	**273**	**279**	**273**
	Closest isolate	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)
	Sources	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing
	spa type	t011	t011	t011	t011	t011	t011	t011
	Year	2019	2019	2019	2019	2019	2019	2019

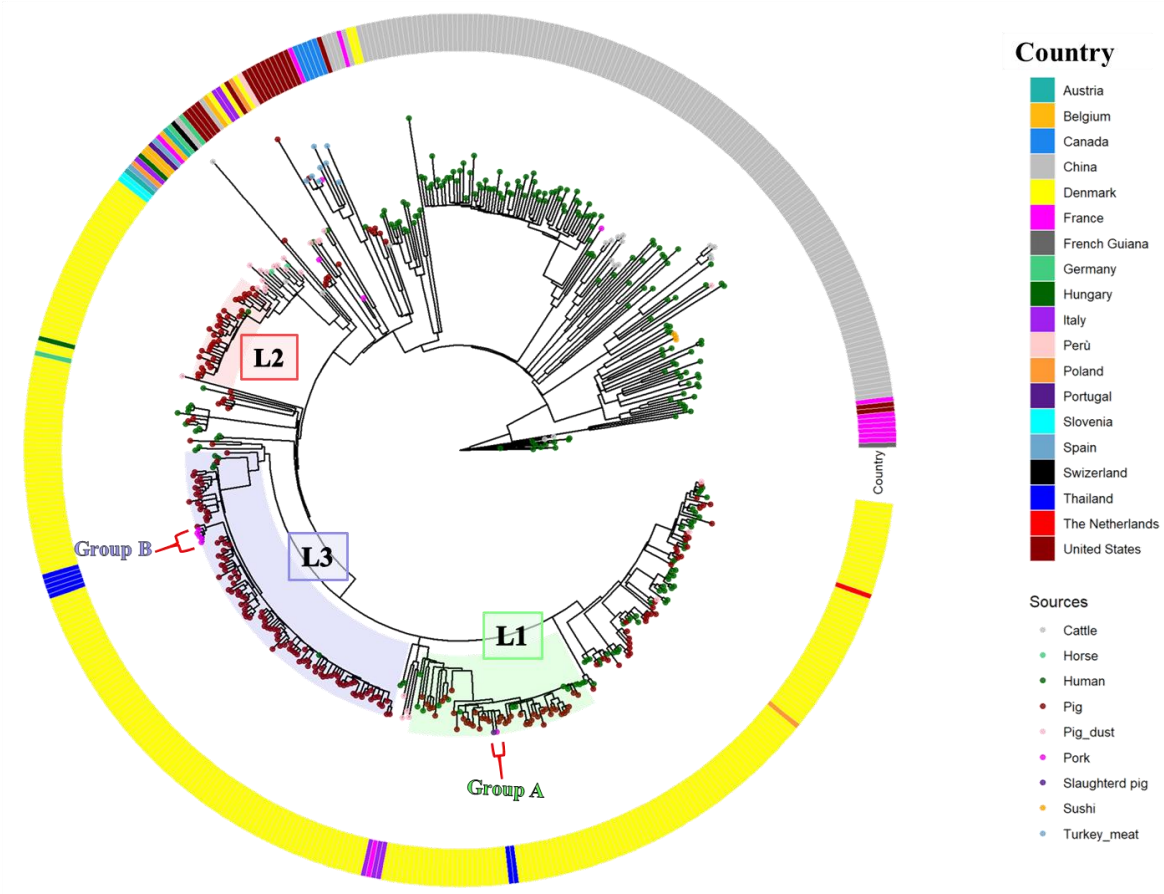


Figure 10. Maximum-likelihood phylogeny was established from 17,415 SNPs after filtering for recombination tracts (20 SNPs). It includes seven Thai LA-MRSA ST398 isolates from this study**,** 88 *S. aureus* isolates from international reference collection [57], 52 Chinese isolates from hospital [77], 77 CA-MRSA ST398 and LA-MRSA ST398 of Chinese isolates [76], nine *S. aureus* ST398 isolates from patients in China [79], five *S. aureus* ST398 isolates from retail foods in China [78], and 283 LA-MRSA ST398 of Danish isolates [6].

Table 9. Shortest SNP distances from seven LA-MRSA ST398 isolates to Danish pig LA-MRSA CC398 isolates, Chinese isolates, and other *S. aureus* CC398 isolates

		TUM-B-P12/2/1 (A107)	**TUM-B-P17/2/1 (A131)**	**TUM-B-P22/2/2 (A154)**	**TUM-C-P26/1/1 (A170)**	**TUM-C-P27/1/1 (A172)**	**TUM-A2-N16/1 (B10)**	**TUM-C2-P26/1/1 (B53)**
	Danish Lineage	**3**	**3**	**1**	**3**	**3**	**1**	**3**
Shortest distance to a **Danish pig LA-MRSA CC398** isolate, SNPs	SNPs	**17**	**19**	**34**	**17**	**19**	**34**	**19**
	Closest isolate	55-103-026 (ERR1992118)	55-103-026 (ERR1992118)	55-100-005 (ERR1991976)	55-103-026 (ERR1992118)	55-103-026 (ERR1992118)	55-100-005 (ERR1991976)	55-103-026 (ERR1992118)
	Sources	MRSA from Danish pig (Breeding farm)	MRSA from Danish pig (Breeding farm)	MRSA from Danish pig (Production farm)	MRSA from Danish pig (Breeding farm)	MRSA from Danish pig (Breeding farm)	MRSA from Danish pig (Production farm)	MRSA from Danish pig (Breeding farm)
	spa type	t034	t034	t034	t034	t034	t034	t034
	Year	2014	2014	2014	2014	2014	2014	2014
Shortest distance to **another *S. aureus* ST398** isolate, SNPs	SNPs	**116**	**117**	**91**	**117**	**119**	**92**	**119**
	Closest isolate	7413532-2 (SRR445282)	7413532-2 (SRR445282)	34-M-B-1_11 (SRR445072)	7413532-2 (SRR445282)	7413532-2 (SRR445282)	34-M-B-1_11 (SRR445072)	7413532-2 (SRR445282)
	Sources	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig	MSSA from Danish pig
	spa type	t011	t011	t034	t011	t011	t034	t011
	Year	2002	2002	2008	2002	2002	2008	2002
Shortest distance to **Chinese** samples, SNPs	SNPs	**268**	**269**	**277**	**269**	**271**	**280**	**271**
	Closest isolate	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)	31MR_KINA (ERR3792044)
	Sources	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing	Pork from Beijing
	spa type	t011	t011	t011	t011	t011	t011	t011
	Year	2019	2019	2019	2019	2019	2019	2019

Comparison of Thai LA-MRSA ST398 with Lineage 1 (L1) and 3 (L3) of *S. aureus* ST 398 from the database

Seven Thai LA-MRSA ST398 isolates clustered with 2 Danish Lineages (L1 and L3). Therefore, LA-MRSA ST398 was selected for further analysis to understand the genetic relationships between Thai LA-MRSA ST398 isolates and other *S. aureus* ST398 in L1 and L3.

To trace the other sources of *S. aureus* ST398 in L1, additional genome data of *S. aureus* in L1 from the previous studies were investigated. This was done through the construction of phylogenetic tree based on SNPs. After removing sites in recombination regions, 1,533 core genome SNPs in 103 isolates (including two Thai LA-MRSA ST398) were used to construct the rooted maximum-likelihood tree shown in Figure 11. The analysis revealed a non-uniform distribution of the isolates, which appeared dispersed throughout the phylogeny and did not cluster according to the source of isolation. The average SNP distance between two recently isolated LA-MRSA ST398 strains in the Danish L1 and other sources of *S. aureus* ST398 was 34 (Figure 11). Two Thai LAMRSA ST398 isolates in this study were more closely related to MRSA ST398 isolated from Danish pig production farm.

We further compared *S. aureus* in L3 with strains that have been published previously. Hence, additional genome data of 240 *S. aureus* ST398 isolates in the L3 from the previous studies were compared with five LA-MRSA ST398 isolates from my Thai samples. The total of 245 genomes (including five Thai LA-MRSA ST398) were considered for phylogenetic analysis based on SNPs. The 3,156 core genomes SNPs after the removal of sites falling into recombination regions were used to construct a rooted maximum-likelihood tree as shown in Figure 12. The average SNP distance between five recently isolated LA-MRSA ST398 strains in the Danish L3 and other sources of *S. aureus* ST398 from the previous study was 18.8 (range, 17-21 SNPs) (Figure 12). Five isolates in this study were closely related to MRSA ST398 isolated from Danish pig breeding farm.

Distribution of virulence genes and antimicrobial resistance genes

The genetic foundation for the spread of Danish Lineage in this study was investigated by comparing the prevalence of antimicrobial resistance determinants in L1 versus L3. Clustered Thai isolates within L1 and L3 showed virulence and AMR gene patterns very similar to those from the database belonging to the same lineages. The

analysis revealed that L1 was enriched for determinants conferring resistance to lincosamides, cadmium/zinc, quinolones compared to L3 (Figure 11, Figure 12, and Table 10). The *gyrA* and *grlA* mutations conferring resistance to quinolones were present only in L1. Aminoglycoside resistance was mainly encoded by *aadD* and *ant(6)-Ia* in L1 (Figure 11, Figure 12, and Table 10), whereas *str* and *ant(9)-Ia* was the most abundant aminoglycoside resistance gene in L3 (Figure 11, Figure 12, and Table 10). The tetracycline resistance genes, *tet(K)* and *tet(M)*, were ubiquitous in both L1 and L3.

One isolate in L3 from this study was found to carry *seg*, *sei*, *sem*, *sen*, *seu* (enterotoxin) (Table 11), *fexA* (phenicol resistance) and *aac(6')_aph(2'')* (aminoglycoside resistance) (Table 10).

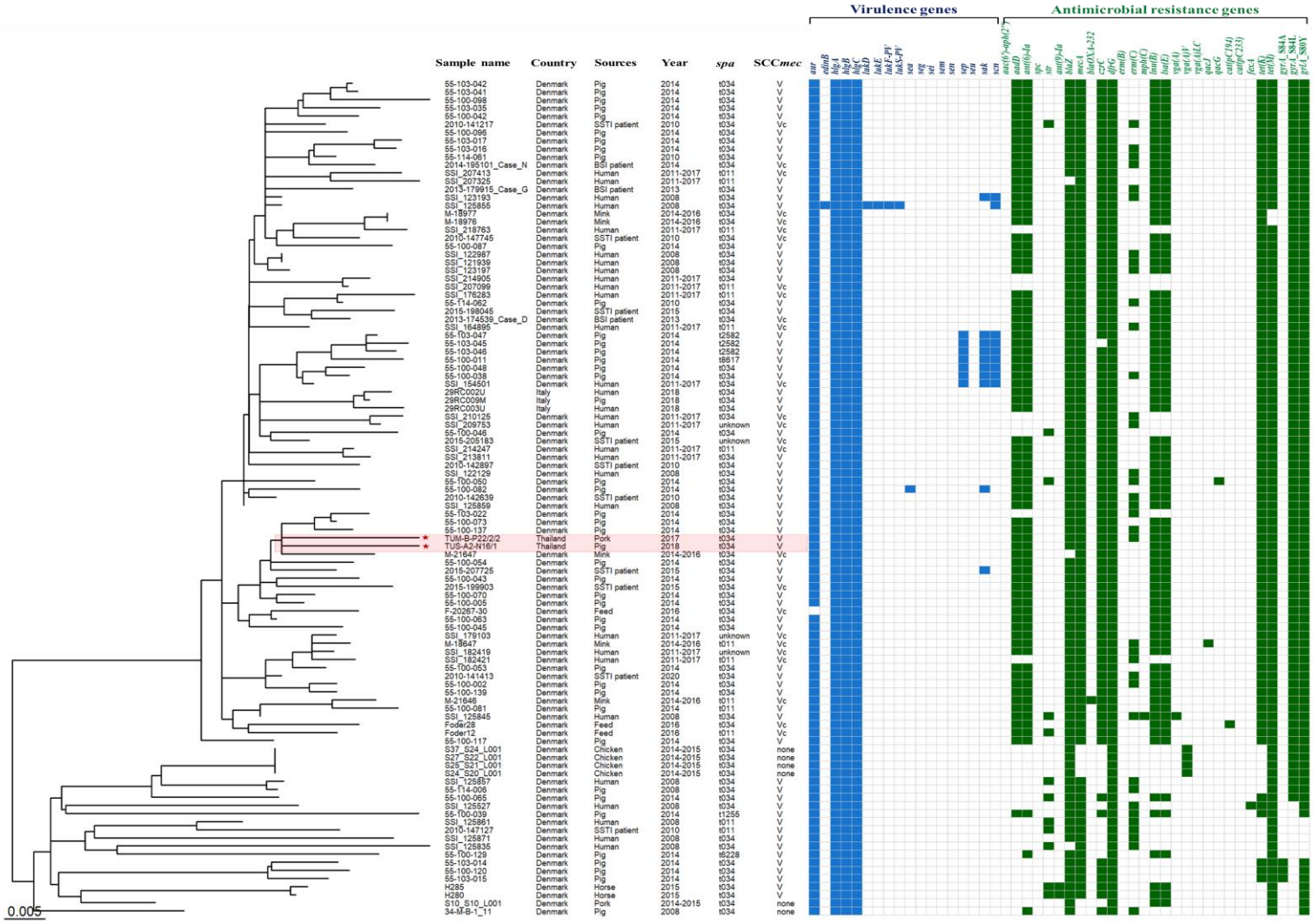


Figure 11. Maximum-likelihood phylogeny was established from 1,533 SNPs after filtering for recombination tracts (190 SNPs). It includes two LA-MRSA ST398 isolates in **Lineage1 (L1)** from this study and 101 isolates from previous studies [5,6,57,64–66,69,80]. The details of each isolate, presence of virulence genes (blue), as well as antimicrobial resistance genes (green), is indicated on the right. The filled squares indicate the presence of genes, while the empty squares indicate the absence of genes. The scale bar represents the number of nucleotide substitution per variable site. The isolates in this study were mark in red box.

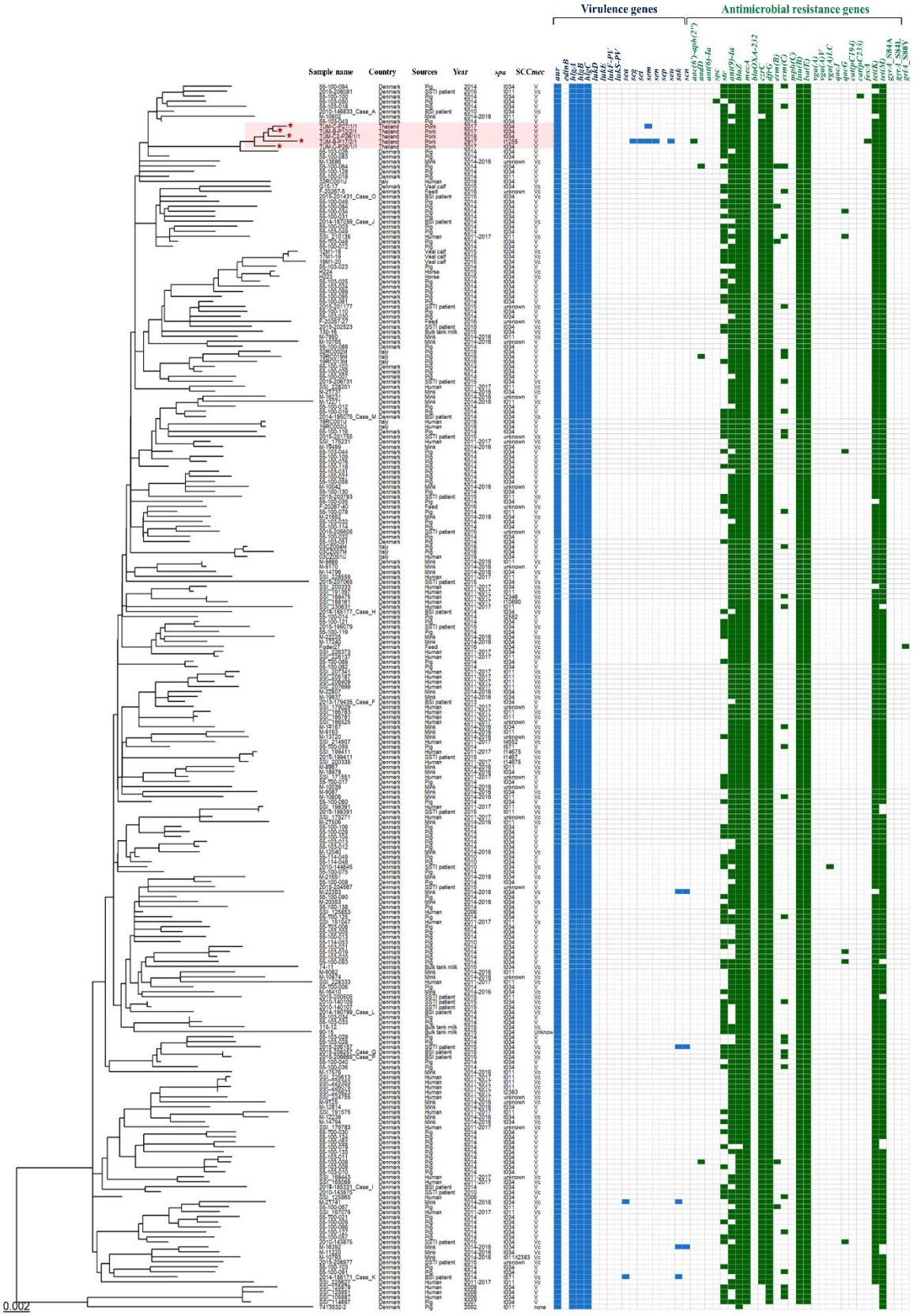


Figure 12. Maximum-likelihood phylogeny was established from 3,156 SNPs after filtering for recombination tracts (174 SNPs). It includes five LA-MRSA ST398 isolates in **Lineage3 (L3)** from this study and 240 isolates from previous studies [5,6,57,64–66,69,80]. The details of each isolate, presence of virulence genes (blue), as well as antimicrobial resistance genes (green), is indicated on the right. The filled squares indicate the presence of genes, while the empty squares indicate the absence of genes. The scale bar represents the number of nucleotide substitution per variable site. The isolates in this study were mark in red box.

Table 10. Prevalence of antimicrobial resistance determinants in the L1 and L3

Antimicrobial resistance determinants	No. (%) of isolates			
	L1 (n=103)		L3 (n=245)	
	Database (n=101)	Thai isolates (n=2)	Database (n=240)	Thai isolates (n=5)
Aminoglycoside				
aac(6')_aph(2'')	0	0	0	**1 (20.0)**
aadD	73 (72.3)	2 (100)	3 (1.3)	0
ant(6)-Ia	75 (74.3)	2 (100)	0	0
spc	0	0	1 (0.4)	0
str	12 (11.9)	0	96 (40.0)	5 (100)
ant(9)-Ia	2 (2.0)	0	193 (80.4)	5 (100)
β-lactam				
blaZ	98 (97.0)	2 (100)	239 (99.6)	5 (100)
mecA	95 (94.1)	2 (100)	239 (99.6)	5 (100)
blaOXA-232	1 (1.0)	0	0	0
Cadmium/zinc				
czrC	87 (86.1)	2 (100)	234 (97.5)	5 (100)
Trimethoprim				
dfrG	101 (100)	2 (100)	239 (99.6)	5 (100)
Macrolide				
erm(B)	0	0	5 (2.1)	0
erm(C)	35 (34.7)	1 (50.0)	32 (13.3)	0
mph(C)	1 (1.0)	0	0	0
Lincosamide				
lnu(B)	78 (77.2)	2 (100)	239 (99.6)	5 (100)
lsa(E)	78 (77.2)	2 (100)	239 (99.6)	5 (100)
Streptogramin B				
vga(A)	1 (1.0)	0	0	0
vga(A)V	4 (4.0)	0	0	0
vga(A)LC	0	0	1 (0.4)	0
Quaternary ammonium compounds				
qacJ	1 (1.0)	0	0	0
qacG	1 (1.0)	0	6 (2.5)	0
Phenicol				
cat(pC194)	1 (1.0)	0	0	0
cat(pC233)	0	0	1 (0.4)	0
fexA	1 (1.0)	0	0	**1 (20.0)**
Tetracycline				
tet(K)	86 (85.1)	2 (100)	230 (95.8)	5 (100)
tet(M)	99 (98.0)	2 (100)	231 (96.3)	5 (100)
Quinolone				
gyrA_S84A	3 (3.0)	0	0	0
gyrA_S84L	87 (86.1)	2 (100)	0	0
grlA_S80Y	96 (95.0)	2 (100)	1 (0.4)	0

Abbreviations: L1, lineage 1; L3, lineage 3

Table 11. Prevalence of virulence genes in the L1 and L3

Virulence genes	No. (%) of isolates			
	L1 (n=103)		L3 (n=245)	
	Database (n=101)	Thai isolates (n=2)	Database (n=240)	Thai isolates (n=5)
Exoenzyme genes				
aur	100 (99.0)	2 (100)	240 (100)	5 (100)
edinB	1 (1.0)	0	0	0
Toxin genes				
hlgA	101 (100)	2 (100)	240 (100)	5 (100)
hlgB	101 (100)	2 (100)	240 (100)	5 (100)
hlgC	101 (100)	2 (100)	240 (100)	5 (100)
lukD	1 (1.0)	0	0	0
lukE	1 (1.0)	0	0	0
lukF-PV	1 (1.0)	0	0	0
lukS-PV	1 (1.0)	0	0	0
sea	1 (1.0)	0	2 (0.8)	0
seg	0	0	0	**1 (20.0)**
sei	0	0	0	**1 (20.0)**
sem	0	0	0	**2 (40.0)**
sen	0	0	0	**1 (20.0)**
sep	7 (6.9)	0	0	0
seu	0	0	0	**1 (20.0)**
Hostimm genes				
sak	10 (9.9)	0	5 (2.1)	0
scn	9 (8.9)	0	3 (1.3)	0

Abbreviations: L1, lineage 1; L3, lineage 3

Discussion

The high-throughput WGS is the comprehensive method for gaining insights into the transmission dynamics and accurately track the spread of LA-MRSA. This tool was tested to determine whether LA-MRSA ST398 isolates from Thai animal-food products were closely related to each other, or with LA-MRSA ST398 isolates from Danish pigs [6] or other *S. aureus* ST398 isolates from international collection samples [57]. Pigs are the primary host of LA-MRSA ST398 in Denmark [5,26]. The prevalence of pig farms in Denmark that were positive for LA-MRSA STT398 increased from 16% in 2010 to more than 60% in 2019 [5]. Denmark is the leader country of pig exports to the European countries [26]. The data analysis revealed that Denmark has been the main provider of pigs to Thailand since 2004 (The data were retrieved from the Observatory of Economic Complexity [14]). These findings suggest the mode for LA-MRSA ST398 introduction and spread in the central region of Thailand. There is an expansion of LA-MRSA ST398 strain by trading of live pigs with other European countries. Denmark has experienced an increase in the prevalence of LA-MRSA ST398 in pig farms [6]. The increase of this Danish strain has been linked to the clonal expansion of three dominant lineages (L1, L2, and L3) [6]. All three lineages have spread beyond the pig farm level. They have been detected in the Danish food production chain and health care facilities [26,69].

The genomic comparison of seven Thai isolates to Danish isolates revealed that isolates from Thai pork samples were clustered within the dominant Danish lineages L1 and L3. This finding supports the data about the transmission of Danish LA-MRSA ST398 with imported pigs to Thailand. Previous studies revealed that some *S. aureus* ST398 strains isolated from other animal species were clustered in one of three dominant Danish lineages. LA-MRSA ST398 strains belonging to the Danish lineages (L1-L3) were also increasingly found in animal-derived foods such as pork meat, turkey, and milk [69]. It could be hypothesized that Danish lineages might have spread to other countries. However, only a few data about WGS of LA-MRSA in other Asian countries were available in the ST398 reference data set. Thus, other national collection samples of *S. aureus* ST398 isolates from other countries should be inspected to confirm this hypothesis.

All of MRSA ST398 isolates in this study from animal-food products belonged to the predominant SCC*mec* type V. However, the first finding in Thailand of MRSA ST398 from a human in 2006 carried SCCmec type IX. It suggests that epidemiological evolution may vary geographically. The most prevalent *spa* type was t034, which is recognized as the most common types in the Danish pig productions [6]. One isolate in this study belonged to spa type t1255. *spa* types t011, t034, and t1255 are widely distributed in most European countries [87].

Thai and Danish LA-MRSA isolates clustered within the predominant Danish lineages (L1, L2, and L3) and shared similarities in AMR genes which confer resistance to antimicrobials commonly used in pig farming in Denmark [88]. The recent Thai isolates were all positive for the zinc/cadmium resistance gene *czrC*. Zinc oxide is one of the most commonly used forms of zinc supplementation in animal feed [89]. Zinc oxide has widespread therapeutic use and prevents postweaning diarrhea in pigs [89]. Moreover, the therapeutic dose of zinc is effective in stimulating growth [89]. The *czrC* and *mecA* genes of LA-MRSA ST398 are located within the same MGEs of the SCC*mec* type V element [90]. It suggests that the increase of selection pressure to maintain the SCC*mec* element might be the potential contributor to the emergence and spread of MRSA in pigs via the use of zinc in feed as an antidiarrheal agent [90]. Danish lineage (L1 to L3) were enriched for AMR determinants [6]. The *czrC* and *tet(K)* gene are also integrated into the J region (the remaining parts of SC*Cmec*) of SCC*mec* type Vc [91]. Moreover, most animal-derived MRSA ST398 isolated in European countries exhibited multidrug resistance [92] that was in concordance with my study. MRSA characteristically carry two identical mutations: *gyrA* Ser84Leu and *grlA* Ser80Phe [93]. These double-serine mutations were reported to be the most frequent fluoroquinolone resistance mechanism of MRSA in multiple studies [93]. Although the double-serine mutations seem to associated with HA-MRSA strains [93], LA-MRSA ST398 in L1 of Danish pigs were also positive to double-serine mutations.

The virulence genes in this study were found in two of seven isolates. The isolates were positive for the enterotoxins gene and both were L3. This finding was in concordance with the fact that the majority of ST398-MRSA isolates are negative for major virulence factors such as enterotoxins, Panton-Valentine leucocidin, toxic shock syndrome toxins, and exfoliative toxins [69].

There are some limitations in this study. Firstly, the skewness of the Danish data set in this analysis may have caused the bias toward the origin of MRSA in Thai animal-

food products. From the previous studies, all LA-MRSA ST398 strains of other Asian countries analyzed by WGS were not clustered within Danish lineage [76–79]. Secondly, a small proportion of animal-food products collected only from the central region of Thailand was included in this study. Thus, the different genotypes in each Danish lineage of this strain may have been underestimated. Numerous previously studies clearly showed the persistence of *S. aureus* on environmental surfaces ranging from hours to weeks and even years [94]. It is possible that the origin and transmission of Thai LA-MRSA ST398 isolates in this study were not from imported live pigs, but they might be as a result of contamination from the environment [95], human carriers, contaminated fomites [96], or from humans or during transportation to slaughterhouses and markets. So, there is need to continue investigating the origin of LA-MRSA ST398 in pig farms in Thailand and compare the information with other local or international data sources.

Summary

This study provides genome-based evidence to investigate Thai LA-MRSA ST398 transmission through MESA colonized pigs between European countries and Thailand. Thai LA-MRSA ST398 in animal-food products were associated with lineage (L1, L2, and L3), also found in Danish pigs. L1 and L3 were the dominant lineage in this study. Two isolates in this study belonged to L1 and five to L3 of Danish lineage. Thai isolates in L1 and L3 were closely related to LA-MRSA ST398 isolates from Danish pigs than to isolates from other Asian countries. It suggested that there is a spillover of LA-MRSA ST398 from the Danish pig reservoir into pigs in Thailand and then to animal-food products. This finding showed that international trading of MRSA colonized pigs is an important factor contributing to the spread of LA-MRSA ST398 worldwide. It underscores the need for control measures on animal-food products to prevent transmission of LA-MRSA ST398 among pigs, humans, and animal-food products.

Conclusion

MRSA has been a major public health concern in humans and various animals. LA-MRSA strains have always been associated with livestock or their products. This strain has emerged in different countries globally. There are few reports on epidemiology of Thai LA-MRSA and their molecular characteristics. Moreover, prevalence of LA-MRSA in slaughtered pigs is still unknown.

In Chapter I, the objective was to investigate the prevalence, molecular characteristics, and antimicrobial resistance pattern of MRSA isolated from slaughtered pigs and retail pork in the central region of Thailand. A total of 204 nasal swab and 116 retailed pork samples were collected from three slaughterhouses and four fresh markets, respectively. Individual samples were used for screening for MRSA and obtained isolates were examined for drug-resistance profiling for 12 antimicrobial agents of 10 drug classes. In addition, SCC*mec* typing and MLST were conducted to obtain genotype profiles. MRSA were isolated from 11 and 52 nasal swab and pork samples, respectively. The prevalence was significantly higher in the pork than in the nasal swab samples (p-value < 0.05). A high prevalence of ST9-SCC*mec*IX and ST398-SCC*mec*V with high-level antimicrobial resistance from markets and slaughterhouses indicated the spreading of MRSA with these genotypes in the Thai swine processing chains.

In Chapter II, LA-MRSA ST398 isolates from Chapter I (one slaughtered pig and six retail pork samples) were compared by WGS with previous data found for China samples, international reference collection samples, and Danish pig samples. The results showed that Thai LA-MRSA ST398 from animal-food products were associated with lineages found in Danish pigs, especially L1 and L3 of Danish pigs.

This finding suggests that LA-MRSA can spread into the general population. Thus, it is important to identify MRSA among animal food production chain and implement effective control measures to prevent transmission of LA-MRSA among pigs, humans, and animal-food products.

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