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Molecular characterization of methicillin-resistant *Staphylococcus aureus* genotype ST764-SCCmec type II in Thailand

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant causative agent of hospital-acquired infections. We characterized MRSA isolated from August 2012 to July 2015 from Thammasat University Hospital. Genotypic characterization of MRSA SCCmec type II and III isolates were scrutinized by whole genome sequencing (WGS). The WGS data revealed that the MRSA SCCmec type II isolates belonged to ST764 previously reported mainly in Japan. All of tested isolates contained ACME Type II', SaPI_n2, SaPI_n3, *seb*, interrupted SA1320, and had a virulence gene profile similar to Japan MRSA ST764. Rigorous surveillance of MRSA strains is imperative in Thailand to arrest its potential spread.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infection (HA-MRSA) worldwide. There have been increasing reports of community-acquired MRSA (CA-MRSA)¹ and livestock-associated MRSA (LA-MRSA)². The resistance is generally conferred by the acquisition of a SCCmec element, which carries *mecA*, a gene encoding for PBP-2', a penicillin-binding protein that is not inhibited by most beta-lactam antibiotics. The SCCmec element may also carry resistance genes to other classes of antibiotics such as aminoglycosides and glycopeptide antibiotics³. Many MRSA strains have become global multidrug resistant. For instance, MRSA strains isolated from a tertiary hospital in Malaysia showed co-resistance of tetracycline, ciprofloxacin and erythromycin⁴. MRSA-infected patients have a higher mortality rate than those infected with susceptible strains. Treatment of MRSA infection relies primarily on vancomycin. Although *S. aureus* isolates with complete resistance to vancomycin (VRSA) are still not common, milder vancomycin resistance phenotypes may cause problems in treatment. These include vancomycin-intermediate resistant *S. aureus* (VISA), heterogeneous vancomycin-intermediate resistant *S. aureus* (hVISA), and even the strains with reduced susceptibility to vancomycin at the MIC level of 1.5–2.0 µg/ml (vancomycin MIC creep)^{5–7}. Strains with decreased susceptibility to vancomycin were reported to be a cause of treatment failure⁸. We selected the MRSA isolates by various methods. Despite the MRSA ST239 SCCmec type III genotype being commonly reported in Thailand, 41% of MRSA in Thammasat University Hospital (TUH)⁹ was SCCmec type II MRSA (unpublished data), which differed from other tertiary hospitals in Thailand^{10,11}.

In this study, five isolates of HA-MRSA SCCmec type II and two isolates of SCCmec type III were whole-genome sequenced. All of the MRSA SCCmec type II isolates belonged to an unexpected MLST type, ST764 which had been reported mostly in Japan. The MRSA ST764 strains frequently contained ACME type II¹². This strain was first discovered in a community setting in Japan in 2009 as a variant of ST5 genotype with SCCmec type II/*spa2/seb2/PVL*-¹³. The ST764 genotype was later reported as a common genotype in many settings in Japan, including tertiary hospitals^{14,15}, outpatient departments¹⁶, long-term care facilities¹⁷ and communities¹⁸, and this genotype has been increasing¹⁶.

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Strain code	ST	Date of isolation	Age	Sex	Diagnosis	Sample type	Days to negative culture	Underlying diseases		Treatment outcomes
								DM	CKD	
SATU130	ST764	10/10/2013	84	M	Septicemia	Blood	8	Yes	Yes	Death
SATU131	ST764	5/11/2013	78	M	Pneumonia	Urine	8	No	No	Death
SATU132	ST764	9/4/2013	61	M	Pneumonia	Sputum	19	Yes	Yes	Success
SATU133	ST239	1/2/2013	18	M	Osteomyelitis of left femur	Bone	17	No	No	Success
SATU134	ST764	15/1/2013	80	F	Pneumonia	Sputum	13	Yes	Yes	Death
SATU135	ST22	21/2/2015	78	M	Pneumonia	Sputum	NA	No	No	Success
SATU136	ST764	29/1/2014	80	F	Endocarditis and septicemia	Blood	8	Yes	Yes	Death

Table 1. Profiles of patients whose MRSA isolates were studied by WGS. WGS whole genome sequencing, ST sequence type, NA not available, DM diabetes mellitus, CKD chronic kidney disease.

Strain code	VAN	CPR	CLI	TMP	SXT	RIF	MUP	FUS	TEC	LZD
SATU130	1.5	>256	>256	0.5	0.012	0.012	0.094	0.125	0.75	2
SATU131	2	>256	>256	0.38	0.012	0.004	0.25	0.094	1	1
SATU132	0.75	>256	>256	0.25	0.008	0.008	0.094	0.25	1	1.5
SATU133	2	>256	>256	>32	1	>32	<0.064	0.094	1	0.75
SATU134	1.5	>256	>256	0.75	0.016	0.008	<0.064	0.19	0.38	1
SATU135	0.38	>256	0.125	0.38	0.032	0.008	0.19	0.19	0.5	1.5
SATU136	2	>256	>256	0.38	0.012	0.008	0.19	0.125	0.5	1.5

Table 2. Minimal inhibitory concentrations of antibiotics against the MRSA isolates. VAN vancomycin, CPR ceftiofime, CLI clindamycin, TMP trimethoprim, SXT trimethoprim-sulfamethoxazole, RIF rifampicin, MUP mupirocin, FUS fusidic acid, TEC teicoplanin, LZD linezolid.

This study reports, for the first time, WGS data of MRSA ST764 SCCmec type II in Thailand, using Illumina NextSeq 500 platform. All isolates here were from severe hospital-acquired infection patients with high mortality. Although these MRSA strains were still susceptible to vancomycin, their MICs of vancomycin were mostly at the high end of the susceptible range. The discovery in Thailand of the ST764 genotype, a well-known Japan CA-MRSA, indicates the increase in the geographic range of the bacteria beyond East Asia. Since the ST764 genotype can be community-acquired, this emerging clone poses a significant threat not only to TUH but also to the other hospitals in the region. The presence of this MRSA strain warrants vigorous surveillance in hospitals and immediate investigation of community transmissions of the strains.

Results

Five isolates of HA-MRSA SCCmec type II [SATU130 (SRA: SRR16971216), SATU131 (SRA: SRR16971215), SATU132 (SRA: SRR16971214), SATU134 (SRA: SRR16971213) and SATU136 (SRA: SRR16971212)] and two isolates of HA-MRSA SCCmec type III [SATU133 (SRA: SRR17033804) and SATU135 (SRA: SRR17033804)] were whole-genome sequenced. All isolates were from patients with invasive diseases, including pneumonia, septicemia, osteomyelitis and endocarditis as described in Table 1. The majority of the patients were over 60 years old. All five SCCmec type II isolates belonged to the ST764 genotype while the SCCmec type III isolates belonged to ST22 and ST239. None of the patients was HIV seropositive, but four patients with ST764 infection had diabetes with chronic kidney diseases. Only one patient with MRSA ST764 infection survived.

All isolates were resistant to ceftiofime and clindamycin yet were susceptible to vancomycin, trimethoprim-sulfamethoxazole, linezolid, mupirocin and teicoplanin. However, MICs of vancomycin against most ST764 isolates were relatively high at 1.5–2.0 µg/ml. The MICs of the drugs against each isolate were shown in Table 2.

The isolates were characterized genetically by SCCmec typing, spa typing, MLST, and WGS with the results shown in Supplementary Table S1. Isolates belonging to ST239 and ST22 harbored SCCmec type III while all ST764 isolates harbored SCCmec type II, similar to previous reports^{14,19,20}.

Characterization of single nucleotide variants (SNVs). More than 90% of reads were uniquely mapped to *S. aureus* N315 (ASM964v1, GenBank accession: BA000018.3), an ST5 isolate. The mean coverage of the sequence was 93% with 72X average depth. A total of 50,021 SNVs were identified based on alignment to the reference genome of *S. aureus* N315. The number of SNVs identified among ST764 isolates ranged from 237 to 261. The average pairwise SNV distance among ST764 isolates was 49.8.

The maximum likelihood phylogenetic tree of five ST764 isolates, with N315 as the outgroup, was shown in Fig. 1. There were two pairs, SATU130–SATU131 and SATU132–SATU136, with the same SNV distances of 11 (Table 3), suggesting epidemiological linkages between each pair²¹. SATU130 and SATU131 were isolated from the same male surgical ward, 26 days apart, and therefore probably belonged to the same clones. Isolates SATU132

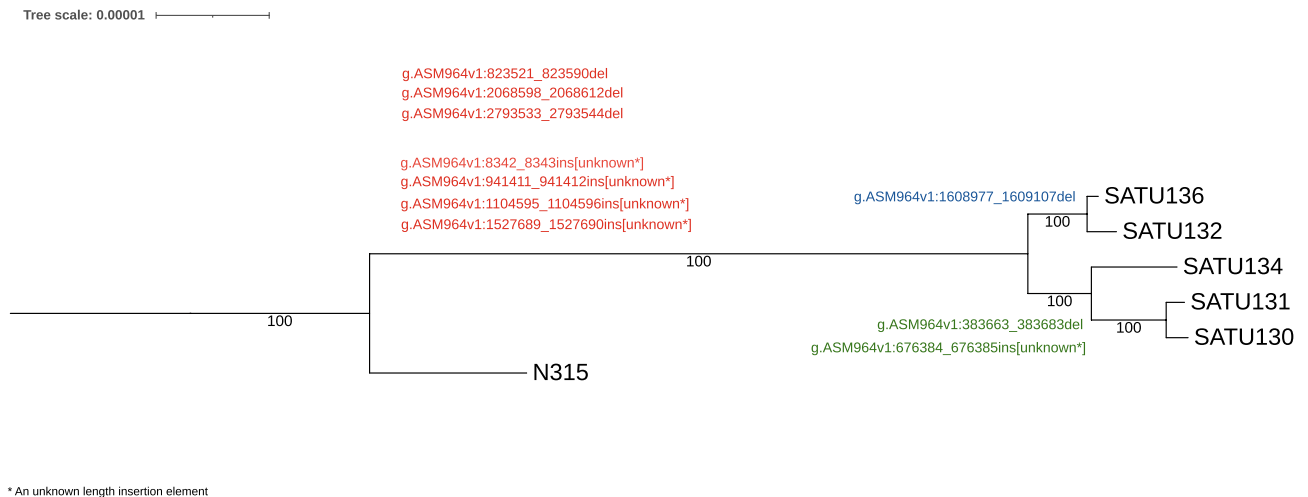


Figure 1. An ML phylogenetic tree of five ST764 isolates with N315 (ST5) as the outgroup. The deletions and insertions were labeled alongside with the branches shared by all isolates that shared the deletions/insertions as indicated with red (common to all isolates), blue and green. The HGVS nomenclature (available from <http://varnomen.hgvs.org/>) were used to describe the deletion and insertion events. The “unknown” indicated in the nomenclature means unknown inserted sequences.

	N315	SATU130	SATU131	SATU132	SATU134	SATU136	SATU133	SATU135
N315								
SATU130	261							
SATU131	258	11						
SATU132	238	67	66					
SATU134	258	49	48	64				
SATU136	237	62	61	11	59			
SATU133	22,720	22,893	22,890	22,880	22,897	22,877		
SATU135	31,919	32,087	32,084	32,074	32,093	32,071	35,871	

Table 3. Number of SNV differences between each pair of isolates.

and SATU136 were from male and female medicine wards, 9 months apart. Moreover, isolate SATU136 did not contain *seb*, while SATU132 did. The epidemiological link between the two isolates was therefore uncertain.

All five ST764 isolates shared three deletions (g.ASM964v1:823521_823580del, g.ASM964v1:2068598_2068612del, g.ASM964v1:2793533_2793544del) and four insertions (g.ASM964v1:8342_8343ins [unknown], g.ASM964v1:941411_941412ins [unknown], g.ASM964v1:1104595_1104596ins [unknown], g.ASM964v1:1527689_1527690ins [unknown]), which were not present in the ST22 and ST239 isolates. There were some indels found in some but not all ST764 isolates. A deletion (g.ASM964v1:1608977_1609107del) was shared by SATU132 and SATU136 while a deletion (g.ASM964v1:383663_383683del) and an insertion (g.ASM964v1:676384_676385ins [unknown]) could be found only among isolates SATU130, SATU131, and SATU134, as shown in Fig. 1 and Supplementary Tables S2 and S3.

Characterization of SCCmec-ACME II' composite island of ST764. We investigated the presence of various genes and genetic elements previously described in ACME-SCCmec composite island (ACME-SCCmec CI)¹². All ST764 isolates here contained *arcA–D*, but not *opp3*; thus the ACME element was classified as ACME II'. They did not harbor any SNV in *arcA–D* as compared with N315, which was similar to those Japan ST764 isolates. It should be noted that C4913T mutation in *arcB* was found in an ST764 isolate in Japan²². ACME-SCCmec CI was previously classified into five types. The five ST764 isolates harbored cJR1 DNA segment but carried neither pUB110 nor *ccrC*. Thus, these isolates belonged to type A3²³. The ACME-SCCmec CI type A3 is the shortest form of the complex and probably puts less burden on the fitness cost for the bacteria.

The presence of genetic elements specific to ST764. In order to further evaluate similarity between the ST764 isolates in this study and the isolates reported from Japan, we identified other genetic elements previously described for ST764 in Japan apart from the ACME-SCCmec CI.

The five ST764 isolates contained SaPin2 and SaPin3 pathogenic islands. The former exotoxin island, SaPin2, contained *set6* to *set15*. The latter enterotoxin island contained *seg*, *sei*, *sem*, *sen*, and *seo*. These isolates did not have SaPin1, evidenced by the absence of *tst*, *sea*, *sec*, *sel* or *sek*. Our ST764 isolates did not contain *sed*, *see*, *seq*,

or *sep* either. They did not contain exfoliative toxin genes (*eta* and *etb*). These ST764 isolates were characterized as having *cna* with *pvl*-negative but also having *lukE-lukD*. These isolates contained *hla*, *hld* and *hlgA-C*. Such genetic characteristics matched the genetic profile of the ST764 isolates in Japan. The Thai isolates contained adhesin genes, *icaA*, *sdrC-E*, *ebpS*, *clfA*, *clfB*, *bbp*, *fnbA* and *fnbB* (partial sequence).

The ST764 isolates in Japan were described as containing three more insertions, SaPI_{inn54}-13.5 kb fragment containing *seb*, prophage carrying *SA1320* gene, and *tet(M)*-carrying Tn5251. All except one ST764 here harbored *seb*, which was reported missing in some ST764 isolates in Japan²². All ST764 isolates here harbored the insertion in *SA1320*, *tet(M)* and Tn5251. The similarity between genetic profiles of the isolates studied here and the MRSA ST764 SCC_{mec} type II in Japan suggests that the isolates in Thailand were from the outbreak clone in Japan.

Discussion

HA-MRSA is a global problem, especially in large tertiary hospitals. Previous WGS studies in Thailand focused mainly on ST239 SCC_{mec} type III, which was the most common MLST type^{24–26}. It is, therefore, intriguing to find that SCC_{mec} type II MRSA was common in TUH, unlike in other tertiary hospitals in Thailand^{10,11}. Moreover, all belonged to ST764-MRSA-II and were from patients with severe hospital-acquired invasive diseases. The apparently high incidence remains to be confirmed.

ST764-MRSA-II, is a recently recognized genotype, reported as a common MRSA genotype in many settings in Japan and might be spreading¹⁶. Such hospital settings encompass outpatient departments and healthcare professionals²⁷. This spreading may be attributed to the presence of ACME type II, which may provide the bacteria a better ability to colonize on skin and mucous membrane which could consequently lead to better transmission. It has recently been reported as a common genotype in Shanghai²⁸ and Eastern China²⁹ but not yet in Southern China³⁰.

ST764 is a hybrid variant of the globally disseminated ST5 lineage of HA-MRSA, carrying a few virulence traits similar to community-acquired MRSA (CA-MRSA). ST764 has evolved via multiple steps, including the acquisition of ACME *arcA* and the staphylococcal enterotoxin B gene (*seb*) in SaPI_{inn54}¹².

Here we reported, for the first time, the WGS of the five MRSA SCC_{mec} type II isolates identified from 2013 to 2015 and demonstrated that all ST764 isolates in this study carried ACME-SCC_{mec} CI type A3 similar to some ST764 isolates in Japan²³. The ST764 strains from Thailand and those from Japan also shared other genetic characteristics, such as the lack of *pvl*, *tst*, *sec*, *sel* and *sep* with the presence of SaPI_{inn2}, SaPI_{inn3}, *seb*, *tet(M)*¹². This implies that the ST764-MRSA-II clone in Thailand was similar to and probably originated from the clone in Japan.

The genetic similarity of ST764-MRSA-II in Thailand to ST764-MRSA-II in Japan suggested a possibility that the ST764-MRSA-II strain was brought to Thailand by a healthy carrier, and later was transmitted into the hospital settings. There is also a possibility that the ST764-MRSA-II strain may continue to spread wider via colonization of healthy carriers.

Analysis of whole genome sequencing data suggested potential transmission between a pair of isolates. The isolates had a SNP-distance of 11 and were isolated from the same ward within a month. This finding of MRSA genotype ST764-SCC_{mec} type II is the first HA occurrence reported in Southeast Asia. Two isolates of CA ST764-SCC_{mec} type II from Thai pig farm workers were also reported³¹. The potential danger of spreading necessitates rigorous surveillance of emerging MRSA strains.

Methods

Bacterial strains. One-hundred and one clinical isolates of MRSA, confirmed by the detection of *mecA*, at TUH from August 2012 to July 2015⁹, were characterized for SCC_{mec} types, pulsed-field gel electrophoresis (PFGE) patterns, virulence genes patterns, and antibiotic resistance phenotypes. There were 41 samples belonging to SCC_{mec} type II that revealed either PFGE pattern A or P. Five isolates with both A- and P-PFGE patterns were randomly selected for sequencing, together with two isolates belonging to SCC_{mec} type III. Bacterial collection, demographic data, clinical and microbiological data retrieval from Microbiology laboratory, Thammasat University Hospital (TUH) were carried out in accordance with relevant guidelines and regulations after approval of the Human Research Ethics Committee of Thammasat University (approval no. MTU-EC-DS-1-193/63 and MTU-EC-DS-4-197/63) and TUH.

Microbiological and clinical data. Demographic data, clinical and microbiological data were retrieved from MRSA-infected patients' record files from the TUH database. Examples of patients' clinical data include lengths of stay, clinical outcomes and underlying diseases including diabetes mellitus (DM) and chronic kidney diseases (CKD). Successful treatment outcomes referred to events when the patients were cured or improved and MRSA was eradicated. Outcomes of persistent-MRSA, recurrent-MRSA or death due to MRSA were regarded as failure of treatments. The patients who were diagnosed with MRSA infection at an outpatient clinic or within 48 h of hospitalization without history of exposure to any healthcare facilities during the previous two months were defined as community-acquired MRSA infection (CA-MRSA). Hospital-acquired MRSA infection (HA-MRSA) referred to those patients who became infected after 48 h of hospitalization.

Antimicrobial susceptibility testing. Minimum inhibitory concentration (MIC) of vancomycin (VAN) was carried out by both broth microdilution method^{32,33} and E-test for determining the high end MICs. Other antibiotics including cefpirome (CPR), clindamycin (CLI), trimethoprim (TMP), trimethoprim-sulfamethoxazole (SXT), rifampicin (RIF), mupirocin (MUP), fusidic acid (FUS), teicoplanin (TEC) and linezolid (LZD) were tested by E-test (Liofilchem, Italy).

PCR detection of *S. aureus mecA* and *pvl* genes. Total DNA was purified from each overnight *S. aureus* culture using Genomic DNA Extraction Mini Kit (RBC Bioscience, New Taipei City, Taiwan). Amplification of *mecA* (5'-TCC AGA TTA CAA CTT CAC CAG G-3' and 5'-CCA CTT CAT ATC TTG TAA CG-3')³⁴ and *pvl* (5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3' and 5'-GCA TCA AST GTA TTG GAT AGC AAA AGC- 3')³⁵ (Integrated DNA Technologies, Singapore) was carried out using the following conditions. The PCR mixture consisted of 50 µl of 10X PCR buffer, 50 mM MgCl₂, 10 mM dNTPs, 100 µM specific primer pair and 1.25 U *Taq* polymerase (RBC Bioscience). Thermo-cycling was conducted in a MyCycler Thermal Cycler (Bio-Rad, Hercules, CA) as follows: 94 °C for 2 min; followed by 30 cycles of 94 °C for 30 s, 51 °C (for *mecA*) or 56 °C (for *pvl*) for 30 s, and 72 °C for 1 min; with a final elongation step at 72 °C for 5 min. Amplicons (162 bp and 433 bp of *mecA* and *pvl*, respectively) were analyzed by 1% agarose gel electrophoresis, stained with GelStar™ Nucleic Acid Gel Stain (Lonza Rockland, Rockland, ME) and visualized under UV light. *S. aureus* N315 and KCU-MS14 strains were used as *mecA*- and *pvl*-positive control, respectively.

SCC*mec* typing. SCC*mec* typing was carried out using a multiplex PCR assay. Specific primers for SCC*mec* types I, II, III, and IVc were included (Supplementary Table S4)³⁶. Positive controls, SCC*mec* type I (613 bp); SCC*mec* type II (398 bp) and SCC*mec* type III (280 bp), were added.

Whole-genome sequencing. DNA purity was assessed using a spectrophotometer, NanoDrop™ 2000 (Thermo Fisher, USA) and DNA concentration was determined by measuring fluorescence in a fluorometer, Qubit™ 3 Fluorometer (Invitrogen™, USA). DNA integrity was visualized by running the isolated samples in agarose gel electrophoresis to assess extent of DNA shearing.

A DNA input of 1 ng was used to prepare a 150-base read library, using the Nextera XT DNA Library Preparation Kit (Illumina, USA). The library preparation protocol includes tagmentation of genomic DNA, library amplification, library clean up, library normalization and library pooling. Library denaturation and dilution were performed, and then diluted PhiX control V3 was spiked into the libraries to obtain 1% PhiX as an external control. The prepared library pool was loaded into the reagent cartridge and then sequenced by 150 bp paired-end NextSeq 500 sequencing (Illumina, USA). Sequences of the five isolates of HA-MRSA SCC*mec* type II were submitted to SRA. The accession numbers of each isolate were listed as follows. SATU130 (SRA: SRR16971216), SATU131 (SRA: SRR16971215), SATU132 (SRA: SRR16971214), SATU134 (SRA: SRR16971213) and SATU136 (SRA: SRR16971212)] and two isolates of HA-MRSA SCC*mec* type III [SATU133 (SRA: SRR17033804) and SATU135 (SRA: SRR17033804).

Bioinformatic analyses. The sequencing reads were quality filtered using Trimmomatic version 0.39³⁷ with the following parameters: LEADING: 3 TRAILING: 3 SLIDINGWINDOW: 4:20 MINLEN: 36. The quality-passed reads were aligned to the MRSA ST5 N315 reference genome (ASM964v1, GenBank accession: BA000018.3) using Burrows-Wheeler Aligner (BWA) version 0.7.17³⁸ with the BWA-MEM algorithm. Sequencing read duplication was handled by Picard Toolkit version 2.18.27 (available from <http://broadinstitute.github.io/picard/>). For each MRSA isolate, variant identification of single nucleotide variants (SNVs) and short insertion-deletions (Indels) was carried out using the HaplopytpeCaller module of Genome Analysis Toolkit (GATK) version 4.1.4.1³⁹. Low quality variants that have read depth lower than 10 were discarded. Variant annotation was performed using SnpEff version 4.3⁴⁰. Structural variants (SVs) were identified by running the program Manta⁴¹ on an input BAM file (sorted and mapped reads) that has aligned to the reference sequence. Resulting SV events flagged with “IMPRECISE” were discarded. The remaining SV events were confirmed by visualizing them using integrative genomics viewer (IGV)⁴². De novo Genome assembly was done by SPAdes3.9 (available from <http://cab.spbu.ru/software/spades/>) (Supplementary Fig. S1).

Multilocus sequencing typing (MLST). MLST was done by identifying the presence of variants located in seven housekeeping genes, carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*)⁴³. Parts of the assembled contigs from each isolate were aligned with *Staphylococcus aureus* MLST primers (available from <https://pubmlst.org/organisms/staphylococcus-aureus/primers>). The allelic profiles of our isolates were matched with allele sequences present in the PubMLST database (available from <https://pubmlst.org/>)⁴⁴ (Supplementary Fig. S1).

Spa typing. The *spa* sequence types were assigned using spaTyper1.0 (available from <https://cge.cbs.dtu.dk/services/spatyper/>)⁴⁵.

Phylogenetic tree construction. To prepare an input sequence for building a phylogenetic tree, SNVs present on Mobile Genetic Element (MGE) were discarded and only homozygous and biallelic SNVs were concatenated into the SNV input sequence (in-house script). Multiple sequence alignment of five ST764 isolates and N315, representing an outgroup, were tested for recombination signals by using Recombination Detection Program v4.10 (RDP4)⁴⁶ that includes seven well-known recombination checking routines, namely RDP, GENECONV, Chimera, MaxChi, Bootscan, SiScan, and 3Seq. If more than four utilities reported recombination regions, these regions would be removed. These RDP-corrected sequences were used as the input for phylogenetic tree construction by the maximum likelihood (ML) method. PhyML version 3.0 was used to construct an ML-based phylogeny which can be visualized by the SeaView program^{47,48}. That includes seven well-known recombination checking routines, namely RDP, GENECONV, Chimera, MaxChi, Bootscan, SiScan, and 3Seq. If

more than four utilities reported recombination regions, these regions would be removed. These RDP-corrected sequences were used as the input for phylogenetic tree construction by the maximum likelihood (ML) method. PhyML version 3.0 was used to construct an ML-based phylogeny which can be visualized by the SeaView program⁴⁹.

ACME/SCCmec type II. The structure of ACME/SCCmec type II was determined by identification of *arcA*, *arcB*, *arcC*, *arcD*, *opp3* and *ccrC* using the sequence of MRSA USA300, (GenBank Accession: CP000255.1) as reference. Identification of pUB110 and cJR1 was done using N315 and NCTC10442 (SCCmec type I, GenBank Accession: UHCH0100003.1) as reference, respectively.

Virulence gene identification. Genes present in three pathogenicity islands (SaPI_n1, SaPI_n2 and SaPI_n3) were identified, which are SaPI_n1: [*tst*, *sea*, *sec*, *sel*, *sek*], SaPI_n2: [*set6* to *set15*] and SaPI_n3: [*seg*, *sei*, *sem*, *sen*, *seo*]. The presence of other enterotoxins (*seb* (AB630021.1), *sed*, *see*, *sep*, *seq*), exfoliative toxins (*eta*, *etb*), leukocidin (*lukE-lukD*), panton-valentine leukocidin (*pvl*), hemolysins (*hla*, *hld*, *hly*, *hlgA*, *hlgB* and *hlgC*), and adhesin genes (*icaA*, *sdrC-E*, *ebpS*, *clfA*, *clfB*, *cna*, *bbp*, *femA*, *fmbA* and *fmbB*), were also verified. The SA1320 gene was checked for the presence of an insertion in the middle of the genome by blasting the assembled contigs with SA1320 sequence of N315.

The following sequences were used as reference: MRSA N315/SCCmec II/ST5/ACMEII (GenBank accession: BA000018.3)⁵⁰, MRSA NN54/SCCmec II/ST764 (GenBank accession: BAFI01000000)¹², MRSA PT2/SCCmec II/ST5/*seb* (GenBank accession: AB630021.1)⁵¹, MRSA H0 5096_0412/SCCmec IV/ST22 (GenBank accession: HE681097)⁵², JKD6008/SCCmec III/ST239 (GenBank accession: CP002120), and TW20/SCCmec III/ST239 (GenBank accession: FN433596)⁵³.

The in-house script for converting SNV data in VCF file format to SNV concatenated sequences in fasta file format was deposited at https://github.com/voravich1/TB_platform/blob/5ef4ed3ddd93dd25df418a6fa632435663fc6f31/tb_convert_vcf_to_fasta_backbone.py.

Received: 9 September 2021; Accepted: 5 January 2022

Published online: 08 February 2022

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Acknowledgements

We owe thanks to Microbiology Laboratory, Thammasat University Hospital and Faculty of Medicine, Thammasat University, Pathum Thani, Thailand for strains collection and facility support, respectively. We also thank to Lalita Narachasima, Rujipat Wasitthanasem, Wanwisa Chareanchim, Nirinya Sudtachat and Maneerat Raksayot for technical support.

Author contributions

S.K. designed and directed the research project, P.Ph. performed experiments and clinical data analysis under supervision of S.K., A.A. and P.T., S.K., P.Ph., P.T., A.A. and P.Pa. interpreted results, C.N., W.P., W.R., P.Ph. and S.T. carried out bioinformatics analyses and prepared figures, A.D. and S.M. performed sequencing experiment,

A.L. provided bacterial strains for SCC mec typing, P.Pa., S.K. and P.Ph. wrote the main manuscript with input from all authors. All authors reviewed the manuscript.

Funding

This research was supported by the National Research Council of Thailand (94248/2557A10602093; 111854/2558A10602034), the Faculty of Medicine, Thammasat University, Thailand (2-07-2563), the National Science and Technology Development Agency (JRA-CO-2564-13515) and the Research Group in multidrug resistant bacteria and the antimicrobial herbal extracts, Faculty of Medicine, Thammasat University (RG2564).

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-05898-1>.

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