ST9 MRSA strains carrying a variant of type IX SCC\textit{mec} identified in the Thai community

Aroonlug Lulitanond, Teruyo Ito, Shanshuang Li, Xiao Han, Xiao Xue Ma, Chulapan Enghani, Aroonwadee Chanawong, Chotechana Wilailuckana, Netchanok Jiwakanon and Keiichi Hiramatsu

Abstract

Background: Infections caused by methicillin-resistant \textit{Staphylococcus aureus} (MRSA) in Thailand occur most frequently in healthcare facilities. However, reports of community-associated MRSA are limited.

Methods: We characterized 14 MRSA isolates from outpatients (O-1 to O-14) by phenotypic and genotypic methods and compared them with 5 isolates from inpatients (I-1 to I-5). Thai MRSA isolates from a healthcare worker (N-1) and a pig (P-1) were also included as ST9 MRSA strains from other sources.

Results: All MRSA isolates from the outpatients and inpatients were multidrug-resistant (resistant to $\geq$ 3 classes of antimicrobials). All of them except strains O-2 and I-3 carried type III SCC\textit{mec} and belonged to \textit{agr} I, coagulase IV, \textit{spa} type t037 or t233, which related to ST239. The strain O-2 (JCSC6690) carried type IX SCC\textit{mec} and belonged to \textit{agr} II, coagulaseXIc, \textit{spa} type t337 and ST9, whereas the strain I-3 carried a type III SCC\textit{mec} and belonged to ST1429. Nucleotide sequence determination revealed that the type IX SCC\textit{mec} element in strain O-2 was distinct from that in a Thai ST398 strain (JCSC6943) previously identified in 2011; nucleotide identities of \textit{ccrA} and \textit{ccrB} were 93 and 91%, respectively and several open reading frames (ORFs) at the joining regions were different. PCR experiments suggested that strain O-2 and N-1 carried similar SCC\textit{mec} element, whereas that of strain P-1 was different, suggesting that distinct ST9-MRSA–IX clones might be spreading in this province.

Conclusions: The SCC\textit{mec}IX-ST9 MRSA clones of distinct SCC\textit{mec} subtypes might have emerged in the Thai community and might also have disseminated into the hospital.

Keywords: \textit{S. aureus}, CA-MRSA, SCC\textit{mec}, ST9, Livestock

Background

\textit{Staphylococcus aureus} is an important human pathogen that causes a broad spectrum of infections, from mild to life-threatening. The organism becomes methicillin-resistant \textit{Staphylococcus aureus} (MRSA) by acquiring the \textit{mecA} gene. Expression of the \textit{mecA} results in production of a special penicillin-binding protein, PBP2a, which has reduced affinity to $\beta$-lactam antibiotics [1] and confers resistance to all practically used $\beta$-lactam antimicrobials. The \textit{mecA} gene is usually carried on a mobile genetic element called the staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}), which contains two main parts: the \textit{ccr} gene complex (\textit{ccr}) and the \textit{mec} gene complex (\textit{mec}). The \textit{ccr} gene complex comprises \textit{ccr} gene (8), \textit{ccrA} and \textit{ccrB}, or \textit{ccrC}, which encode site-specific recombinases responsible for the mobility of SCC\textit{mec}, and surrounding open reading frames (ORFs). The \textit{mec} gene complex comprises the \textit{mecA} gene, its regulatory genes, and insertion sequences upstream or downstream of the \textit{mecA} gene [1]. SCC\textit{mec} elements identified in \textit{S. aureus} have been classified according to the combination of \textit{ccr} allotypes with the \textit{mec} gene complex: 11 types (I-XI) have been reported to date [2,3].

MRSA strains were regarded to be pathogens that cause hospital-acquired (HA) infections; however, MRSA infections in the community have been increasingly reported [4,5]. MRSA strains that colonized or caused infections in persons who meet the following criteria are considered to be CA-MRSA: healthy persons; person with no prior...
history of a healthcare-associated risk factors such as a recently (within the past year) hospitalized or had a medical procedure (such as dialysis, surgery, catheters) [5,6]. Recently, MRSA strains from livestock (e.g., pigs) or their products have emerged throughout Europe, America and Asia. Most of livestock-associated MRSA (LA-MRSA) strains belonged to clonal complex (CC) 398 as defined by multilocus sequence typing [7], although the other clones, e.g., CC5, CC9, CC30, and CC97, have been occurred [8].

Most of MRSA infection in Thailand was hospital-associated whereas CA-MRSA infection was very rare [9]. The hospital-associated MRSA strains usually carried types III or II SCCmec elements [10,11], while characteristics of CA-MRSA strains were not fully investigated. We aimed to characterize CA-MRSA strains disseminating in the Thai community compared with HA-MRSA strains from a teaching hospital in Thailand. Thai MRSA strains from other sources such as hospital staff, pig were also included and compared to a previously identified type IX SCCmec MRSA strain from a Thai veterinarian [12].

Methods

Bacterial strains

MRSA isolates (O-1 to O-14) from 14 outpatients at Srinagarind Hospital, Khon Kaen University, Thailand, were collected between September 2005 and March 2006: 11 isolates from skin or tissue infection samples and 3 from respiratory tracts. These strains met the category of CA-MRSA since they were isolated from samples collected from the outpatient, who did not admit to hospitals within a year. Five MRSA strains (I-1 to I-5) isolated from inpatients >72 hours after admission were also used as HA-MRSA control strains. In addition, 2 MRSA strains isolated during personnel surveillance in early 2006: the P-1 strain (from a pig with pneumonia) was chosen from 19 S. aureus strains isolated during personnel surveillance in early 2006: the P-1 strain (from a pig with pneumonia) was chosen from 4 S. aureus strains collected at the Veterinary Research and Development Center of Upper Northeastern Thailand in 2006.

All isolates were identified by conventional coagulase and DNase tests. All of the MRSA strains from people were taken as part of standard care.

This study was conducted in accordance with the declaration of Helsinki and good clinical practice. It was approved by the Ethics Committee of Khon Kaen University (project number HE551393).

Antimicrobial susceptibility test

The minimum inhibitory concentrations of 7 antimicrobials (vancomycin, cefazolin, oxacillin, cefoxitin, tetracycline, erythromycin and ofloxacin) (Sigma Chemical, St. Louis, USA) were determined using the agar dilution method described by the Clinical and Laboratory Standards Institute [13]. S. aureus ATCC29213 was used as a drug-susceptible standard strain.

Multiplex PCR for SCCmec type

Chromosomal DNA was extracted using the DNeasy tissue Kit (Qiagen, Valencia, USA). Multiplex PCR for detection of the mecA, ccr gene complex type and mec gene complex class were performed using primer sets and conditions as per Kondo et al. [14]. MLEP (mec left extremity polymorphism) typing was performed to estimate the insertion of SCCmec as per Ito et al. [15]. The S. aureus strains used as reference strains for SCCmec typing were: NCTC10442 (type I), N315 (type II), 85/2082 (type III), MR108 (type IV), JCSC3624 (type V), and HDE288 (type VI).

PCR identification for heavy metal resistance genes and potential virulence genes

The mer operon and copA genes and genes encoding for virulent factors such as exfoliative toxin type a, b, d (eta, etb, etd), PVL (lukS-PV, lukF-PV) and TSST (tst) were detected by PCR methods [15-17].

Molecular typing

Coagulase and agr typing was performed as per Sakai et al. [18] and Shopsin et al. [19], respectively. Typing of spa was performed as per Shopsin et al. [20]. The PCR products of the spa genes were then purified using High Pure PCR Product Purification kits (Roche Diagnostics, Indianapolis, USA) and used as DNA templates for sequencing by ABI PRISM 3100 (Applied Biosystems, Foster City, USA). The designation of the spa type was conducted using the RidomStaph Type program (www.ridom.de).

Pulsed-field gel electrophoresis (PFGE) was conducted as a method for genotyping. The MSSA strain NCTC8325 and MRSA strain N315 were used as reference strains for PFGE. The analysis of Smal-digested chromosomal DNA was performed using a contour-clamped homogeneous electric field mapper system as per the manufacturer’s instructions (Bio-Rad, Hercules, USA), with a 48.5-kb ladder (Bio-Rad) as DNA-sized markers. The PFGE patterns for all of the isolates were analyzed using BioNumerics software (version 6.5; Applied Maths, Kortrijk, Belgium) and interpreted as per Tenover et al. [21].

Multilocus sequence typing (MLST) was conducted as per Enright et al. [22]. The alleles of the 7 loci were evaluated by comparing the sequences to those of the corresponding loci in the S. aureus MLST database (www.mlst.net). The sequence types (STs) were determined according to the combined patterns of the 7 alleles,
while the clonal complexes were defined using the BURST program, based on related sequence types available on the MLST website.

**Determination of nucleotide sequences in and around the SCCmec element and coa gene**

We determined the entire nucleotide sequences of type IX SCCmec carried by strain O-2 (JCSC6943) and nucleotide sequences of coa genes that could not be identified by PCRs using DNAs of three strains, O-2, N-1, and P-1. The DNA fragments of the SCCmec element from the strain O-2 and its genetics surrounding were amplified using long-range PCRs (list of primers sets in Additional file 1: Table S1). The region from Tn916 to copA gene was amplified by using a constructed DNA template from the Fosmid Library Production Kit (Epicentre Biotechnologies, Madison, WI). The nucleotide sequences were determined by primer-walking. The sequences were analyzed for ORFs with the In Silico Molecular Cloning Program (IMC Load command) and compared with the sequence databases from the National Center for Biotechnology Information using the basic local alignment search tool, BLAST (National Library of Medicine, Bethesda, MD) for annotation and prediction of functions. The nucleotide sequences of the coa gene from strains O-2, N-1 and P-1 were determined as per Watanabe et al. [23].

**PCR identification of ORFs at J3 regions of type IX SCCmec elements**

The carriage of ORFs at the J3 region of SCCmecIX elements was examined using chromosomal DNAs of strains N-1 and P-1 with three sets of primers (sets a and b as used for the strain O-2 and set c as used for the strain JCSC6943) [12]. The locations and nucleotide sequences of the primers used are indicated in Figure 1 and Additional file 1: Table S1, respectively.

**Results**

**Characteristics of MRSA isolates**

All outpatient and inpatient MRSA strains were multidrug-resistant with high MIC values to cefazolin, oxacillin, cefoxitin, tetracycline, erythromycin and ofloxacin (32 - >64 μg/ml). However, all of them were still susceptible to vancomycin.

None of the MRSA isolates carried the virulence-related genes tested. Thirteen out of 14 outpatient isolates and all 5 inpatient isolates carried a type 3 ccr gene and class A mec gene complex, although 12 out of 13 outpatient isolates and 4 out of 5 inpatient isolates also carried the ccrC. Further PCR experiments showed that these 16 strains contained mercury resistance gene (mer) and the extremity of SCCmec, therefore, they were identified as type III SCCmec strains carrying SCCHg [24]. In conclusion, 18 out of 19 strains carried type III SCCmec. The remaining strain, O-2, carried ccrA1B1 genes and class C2 mec gene complex, which was categorized to type IX SCCmec.

All isolates from both sources were of the agr group I and coagulase type IV except for strain O-2 that belonged to agr group II. Since the coagulase type of strain O-2 could not be determined neither by a genetic method using multiplex PCRs nor by serological typing based on a coagulation inhibition, its coagulase nucleotide sequences

**Figure 1 Structural comparison of type IX SCCmec elements carried by strains O-2 and JCSC6943.** Structures of SCCmec are illustrated based on the nucleotide sequences deposited in databases DDBJ/EMBL/GenBank, under accession nos. AB505628 for JCSC6943, AB705452 (region 1, indicated by black bar) and AB705453 (region 2, indicated by black bar) for O-2. The red arrowhead indicates the location of ISS, and the nucleotide sequences are given below. ORFs are colored as follows: yellow, insertion sequences; red, cad operons; lawn green, genes for arsenical resistance operons; orange, copB; salmon, ORFs for ccr gene complexes. The three joining regions (J1, J2, and J3 regions) are indicated in green. The locations of DNA fragments amplified with three pairs of primers (a, b, and c) are indicated in red bars. Nucleotide sequences of primers used for amplification for three loci are listed in Additional file 1: Table S1.
were determined, and classified into type X1c, a novel coagulase subtype. The majority of type III SCCmec strains belonged to spa type t037 except for strains O-3 and I-3, which belonged to spa type t233 and nontypeable, respectively. No amplification product of spa-typing was seen when used chromosomal DNA of strain I-3 as DNA templates. MLST genotypes of 5 spa t037 isolates, which were chosen based on their unique PFGE banding patterns, were identified as the ST239, whereas those of strain O-3 and I-3 were ST239 and ST1429 respectively. The strain O-2 belonged to spa type t337 and ST9. The characteristics of strain O-2 were distinct from other 18 strains.

The PFGE profiles revealed that 12 out of 14 outpatient isolates showed similar banding patterns with 1–2 different bands, while strains O-2 and O-3 had a distinct DNA profile (Figure 2). Three of the five inpatient isolates showed closely related PFGE patterns and strain I-3 belonging to ST1429 presented a distinct pattern, whereas the band pattern of strain I-5 was related to the outpatient strains (Figure 2).

Structure of type IX SCCmec in strain O-2 (SCCmecO-2)
The nucleotide sequences of the two long regions containing SCCmec element in the strain O-2 were determined. The first 25 kb region contained a class C2 mec gene complex, a type 1 ccr gene complex and the downstream end of a conjugative transposon, Tn916. The second 32 kb region started from the upstream region of Tn916 and ended at the extremity of an SCC. The overall structure of the approximately 70-kb region from the strain O-2 containing the SCCmec element and an SCC without ccr gene (γSCC) is illustrated in Figure 1 and the ORFs locating in these regions are listed in Table 1.

Three integration site sequences (ISS) comprising directly repeated (DR) sequences were identified: the first ISS, at the 3’ end of orfX; the second ISS, at the downstream of the copB gene on the 32-kb fragment; and the third ISS, at the end of the 32 kb fragment (Figure 1). The SCCmecO-2 was estimated to be 43 kb in size, in which the Tn916 was integrated. At the downstream of the SCCmec, an SCC encoding truncated ccrA1 had 83% nucleotide identity with that of type I SCCmec in strain NCTC10442.

We then compared the structure of SCCmecO-2 to a previously reported type IX SCCmec of strain JCSC6943 [12]. The class C2 mec gene complexes of both strains were highly identical, four ORFs around the mecA gene of the strain O-2 had 100% identity with SCCmecJCSC6943. The ccr gene complexes of both strains were located upstream to the mec gene complex. The nucleotide sequences of the ccrA1B1 in SCCmecO-2 showed 91–93% identities with those of SCCmecJCSC6943 and 91–92% identities with those of the type I SCCmec in strain NCTC10442.

The ORFs at J3 region of SCCmecO-2 which encoded for hypothetical proteins related to modification methylase and restriction enzymes were less similar to those of the JCSC6943 (Table 1). At the J1 region of both SCCmec elements, the ORFs encoding resistance genes for heavy metals (e.g., cadmium and copper) were present. But a truncated copy of Tn916 encoding tetracycline resistance gene (tetM) was identified only in the SCCmecO-2 (Table 1).

Comparisons of type IX SCCmec MRSA isolates
The characteristic of type IX SCCmec MRSA strains, N-1 and P-1, were compared with strain O-2. All 3 strains belonged to the same agr type and ST type (Table 2). Nucleotide sequences of coa genes of these three strains were exactly identical, which were classified into type X1c. However, their spa types were not totally identical. Strain P-1 belonged to spa type t044, whereas strains N-1 and O-2 belonged to the same spa type t337. These three isolates showed closely related PFGE patterns with 2–4 different bands (Figure 2).

The carriage of genes in strains N-1 and P-1 examined by using primer’s pairs (a, b and c) showed that strains...
O-2 and N-1 were positive in PCRs using primer pairs a and b, while strain P-1 was negative with all three primer's pairs. Although strains N-1 and P-1 were positive in PCRs identifying \textit{tetM} and \textit{copB} genes, the locations of these genes were not examined yet.

**Discussion**

**Characteristics of type IX SCC\textit{mec} in strain O-2**

The SCC\textit{mec}O-2, which was located on the downstream region of the \textit{orfX}, was classified into type IX. It had type I \textit{ccr} gene complex carrying \textit{ccrA} and \textit{ccrB} genes that were classified into \textit{ccrA1} and \textit{ccrB1} based on the criteria suggested by IWG-SCC [2]. The class C2 \textit{mec} gene complex of SCC\textit{mec}O-2 was very similar to that of SCC\textit{mec}JCSC6943 [12], the \textit{ccr} gene complex was located between \textit{orfX} and \textit{mec} gene complex. Furthermore, a number of ORFs encoding genes associated with heavy metal resistance (i.e., \textit{cadD}, \textit{cadX}, \textit{arsC}, \textit{arsB}, \textit{arsR} and \textit{copB}) were also found and showed 92-100% nucleotide identities to those of the SCC\textit{mec}JCSC6943.

**Table 1 ORFs of SCC\textit{mec} in the \textit{S. aureus} strain O-2 compared to \textit{S. aureus} JCSC6943 and \textit{S. haemolyticus} JCSC1435**

| ORFs | Genes | Description of products | % identity to type IX SCC\textit{mec} in \textit{S. aureus} JCSC6943 | % identity to \textit{S. haemolyticus} JCSC1435 |
|------|-------|-------------------------|-------------------------------------------------|----------------------------------|
| KK01 | \textit{orfX} | hypothetical protein | 97 | 80 |
| KK02 | hypothetical protein, similar to modification methylase LlaFl | <50 | 51.8 |
| KK03 | hypothetical protein, similar to modification methylase LlaFl | <50 | 50.3 |
| KK04 | hypothetical protein, similar to restriction enzyme LlaFl | <50 | <50 |
| KK05 | hypothetical protein | 52 | 83 |
| KK06 | hypothetical protein | 65 | 86.3 |
| KK07 | hypothetical protein | 78 | 61.9 |
| KK08 | hypothetical protein | 85 | 83 |
| KK09 | hypothetical protein | <50 | 51.6 |
| KK10 | \textit{ccrB1} | cassette chromosome recombinase B1 | 91 | 50 |
| KK11 | \textit{ccrA1} | cassette chromosome recombinase A1 | 93 | <50 |
| KK12 | hypothetical protein | 81 | 51.1 |
| KK13 | hypothetical protein | 61 | <50 |
| KK14 | hypothetical protein | 95 | <50 |
| KK15 | \textit{tnp} | I5431 transposase | 97.5 | 99.1 |
| KK16 | [truncated]putative HMG-CoAsynthase | 100 | 83 |
| KK17 | Glycerophosphoryl diester phosphodiesterase | 100 | 100 |
| KK18 | hypothetical protein | 100 | 100 |
| KK19 | \textit{mecA} | penicillin-binding protein 2' | 100 | 100 |
| KK20 | \textit{tnp} | transposase for I5431 | 100 | 98 |
| KK21 | \textit{tnp} | Tn916, transposase | <50 | <50 |
| KK22 | Tn916, hypothetical protein | <50 | <50 |
| KK23 | Tn916, transcriptional regulator | <50 | 50 |
| KK24 | \textit{tetM} | tetracycline resistance protein TetM | <50 | <50 |
| KK25 | hypothetical protein | <50 | 52.5 |
| KK26 | \textit{cadD} | cadmium binding protein | 96 | 94 |
| KK27 | \textit{cadX} | cadmium resistant accessory protein | 96 | 77 |
| KK28 | \textit{arsC} | arsenate reductase | 93 | 82 |
| KK29 | \textit{arsB} | arsenic efflux pump protein | 88 | 73 |
| KK30 | \textit{arsR} | arsenical resistance operon repressor | 100 | 97 |
| KK31 | \textit{copA} | copper-exporting ATPase | 98 | 67 |
| KK32 | putative lipoprotein | 92 | 98 |

ORFs, KK1-KK24 and KK25-32 are from nucleotide sequences deposited at the DDBJ/EMBL/GenBank databases under accession nos. AB705452 and AB705453, respectively.

\* % identities of less than 50% were expressed as <50%.

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Table 2 Characteristics of MRSA strains tested in this study

| Isolate no.* | Origin of specimen | Disease | Minimum inhibitory concentrationb (μg/mL) | ccr gene type | mec gene complex class | mer operon | copA type | Coagulase type | spa type | MLST (ST) |
|--------------|-------------------|---------|------------------------------------------|---------------|-----------------------|-----------|---------|---------------|---------|-----------|
|              |                   |         | Van Cefa Oxa Cefox Tetra Erythro Ofx     |               |                       |           |         |               |         |           |
| Isolates from outpatients (n = 14) |                   |         |                                           |               |                       |           |         |               |         |           |
| O-1          | Pus Left thigh (Human) | Accident, infected wound | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3 | A - - I IV t037       | ST239     |         |               |         |           |
| O-2          | Pus Left foot (Human) | Atopic dermatitis | 1 32 32 32 > 64 > 64 > 64 > 64 > 64 | ccrA1B1 | C2 - + II Xlc t337   | ST9       |         |               |         |           |
| O-3          | Wound (Human)      | Electrical burn | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t233     | ST239     |         |               |         |           |
| O-4          | Pus (Human)        | NA      | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-5          | Pus (Human)        | Infected wound | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + + I IV t037   | NT        |         |               |         |           |
| O-6          | Tracheal suction (Human) | NA         | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-7          | Sputum (Human)     | Pulmonary TB | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-8          | Wound (Human)      | Accident, head injury | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-9          | Wound (Human)      | Accident, infected wound | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-10         | Pus Left cheek (Human) | NA         | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-11         | Pus scalp (Human)  | Chronic scalp impetigo | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-12         | Tissue (Human)     | Gas gangrene | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| O-13         | Sputum (Human)     | NA       | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + + I IV t037   | NT        |         |               |         |           |
| O-14         | Pus Lt thigh (Human) | Accident, infected wound | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | NT        |         |               |         |           |
| Isolates from inpatients (n = 5) |                   |         |                                           |               |                       |           |         |               |         |           |
| I-1          | Blood (Human)      | NA      | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3 | A + - I IV t037   | NT        |         |               |         |           |
| I-2          | Blood (Human)      | NA      | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + + I IV UD² ST1429 |         |         |               |         |           |
| I-3          | Blood (Human)      | NA      | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | ST239     |         |               |         |           |
| I-4          | Sputum (Human)     | NA      | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A + - I IV t037   | ST239     |         |               |         |           |
| I-5          | Sputum (Human)     | NA      | 2 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA3B3ccrC  | A - - I IV t037   | ST239     |         |               |         |           |
| An isolate from healthy worker (n = 1) |                   |         |                                           |               |                       |           |         |               |         |           |
| N-1          | Nasal swab (Human) | colonize | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA1B1 | C2 - + II Xlc t337   | ST9       |         |               |         |           |
| An isolate from pig (n = 1) |                   |         |                                           |               |                       |           |         |               |         |           |
| P-1          | Lung (pig)         | pneumonia | 1 > 64 > 64 > 64 > 64 > 64 > 64 > 64 | ccrA1B1 | C2 - + II Xlc t044 | ST9       |         |               |         |           |

* O, outpatient; I, inpatient; N, nurse; P, pig; NA, information was not available.

b Van, vancomycin; Cefa, cefazolin; Oxa, oxacillin; Cefox, cefoxitin; Tetra, tetracycline; Erythro, erythromycin; Ofx, ofloxacin.

c ND, Undetermined; because no PCR product was amplified.
number of ORFs were absent in the SCCmecCSC6943, e.g., hypothetical proteins related to restriction-modification system at J3 region and a conjugative transposon Tn916 encoding tetM at J1 region. The transposon Tn916 was located just downstream of the class C2 mec gene complex and the extremity of Tn916 including int gene was truncated. The transposition of Tn916 was not site-specific. We presume that Tn916 transposed firstly into the SCC element, then the class C2 mec gene complex was integrated to the SCC harboring Tn916 by disrupting its extremity.

The class C2 mec gene complex was formerly reported predominantly in S. haemolyticus [1]. Comparison of the ORFs of SCCmecO-2 and those of S. haemolyticus strain JCSC1435 revealed that their heavy metal-resistant genes (e.g., cadD, cadX, arsC, arsB, arsR, copB) had 67-97% identities. Three ORFs at the J3 region had more than 80% identities with those of the S. haemolyticus strain JCSC1435 [25]. These data suggested that although the SCCmecO-2 and SCCmecCSC6943 were classified into type IX, they might have evolved independently. Further study will clarify whether any staphylococcal strains such as the S. haemolyticus, might serve as reservoirs for SCC or SCCmec elements (Table 1).

MRSA clones in Thailand

MRSA infections in the hospitals have been a big concern in Thailand. So far the HA-MRSA strains have been analyzed, ST239-SCCmecIII clone was the major, although ST5-SCCmecII clone was identified as the minor one [10,11]. On the other hand, CA-MRSA in Thailand was reported to be very rare [9]. The incidence of CA-MRSA was undetermined because most outpatients with non severe infections were treated empirically without collecting samples for culture [26]. Most of the MRSA isolates from outpatients in the present study were ST239-SCCmecIII-spa type t037, which was the dominant clone reported in Asian countries except for Korea and Japan [10]. CA-MRSA has been reported to be susceptible to a wide range of β-lactam antibiotics, low oxacillin MIC, but our tested strains were multidrug-resistant to non-β-lactam antimicrobials similar to HA-MRSA strains [27].

Our results were consistent with the report from ANSORP that ST239-SCCmecIII and ST5-SCCmecII MRSA clones were found among CA-MRSA isolates from patients in Asian countries (e.g., Vietnam, Thailand, Sri Lanka, Taiwan, and Hong Kong), without any risk factors for HA-MRSA infection [5]. The results of our PFGE experiment suggested that ST239-SCCmecIII strains disseminated in the community were rather clonal and distinct from those in the hospital. Further detailed study will be needed to clarify this hypothesis.

A novel MRSA clone in the Thai community

It has been reported that CA-MRSA strains tend to carry types IV or V SCCmec element and belonged to particular genotypes. The distribution of CA-MRSA clones varied by geographical area: the ST1-SCCmecIV and ST8-SCCmecIV strains (called as USA400 and USA300 based on PFGE patterns) in the USA; the CCB80-SCCmecIV strains in Europe and Africa; ST22-SCCmecIV and SCCmecV- ST772 strains in India; ST30-SCCmecIV and ST93-SCCmecIV strains in Australia; and CCS9-SCCmecV strains in Asia [28,29]. Interestingly, most of these CA-MRSA strains carried the Panton-Valentine Leukocidin (PVL) gene that encodes a necrotizing cytotoxin, which may be responsible for the invasiveness and virulence of the organism [30]. Recently livestock associated MRSA (LA-MRSA) have emerged in the community and spread into hospitals [31-34]. The majority of LA-MRSA strains belonged to ST398, while strains belonged to other STs, e.g. ST9, has also been reported.

In this study, we analyzed three ST9 MRSA strains carrying SCCmecIX, the same SCCmec type identified in an ST398-MRSA isolated from a Thai veterinarian (strain JCSC6943) [12].

The strain O-2 was isolated in March 2006, from a young boy with underlying atopic dermatitis and suffering from chronic impetigo of the left foot. He had not been admitted to hospital in the prior year. Therefore, we regarded the strains as CA-MRSA and as a novel MRSA clone emerged in the Thai community. We explored the clone and identified 2 strains, one (strain P-1) from a diseased pig with pneumonia in the same province and the other (strain N-1) from a nasal swab of a nurse isolated during hospital-personnel surveillance in the same year as the first case. The ST9-MSSA has occasionally been isolated from healthy human carriers [35] and has been able to colonize and be transmitted between humans and pigs as it has also been isolated from pigs and workers on pig farms [36,37]. Recently ST9-SCCmecIX strains have been isolated from swine in Thailand [38,39]. In the present study, 2 isolates of ST9-SCCmecIX were from humans. To our knowledge, this is the first report of ST9-SCCmecIX strain from humans (a patient and a healthy healthcare worker) and a diseased pig in Thailand. Unfortunately, information regarding any animal contact by these persons or their families was not available. The ST9-SCCmecIX strain would belong to a newly identified CA-MRSA clone disseminating in the Thai community, although no isolate harbored any potential virulence genes including the pvl gene, which is usually found in CA-MRSA strains.

The detailed comparison of 3 ST9-SCCmecIX strains: O-2, N-1, and P-1, suggested that strains isolated from human and pig were not exactly identical. Their PFGE
patterns were not totally identical but they were closely related, in contrast, that of ST398 strain JCSC6943 showed a non-typeable pattern (absence of DNA fragment). The spa type of strains O-2 and N-1 belonged to t337, whereas strain P-1 belonged to t044. Recently, MRSA isolates from pigs and pork in Thailand carrying the SCCmecIX-spa type t337 have been reported [38, 39]. The ST9-spa type t899 MRSA and MSSA were recently isolated from dust samples taken from Chinese pig farms [40]. Most of these isolates were resistant to various classes of antimicrobials such as β-lactams, macrolides and tetracycline, which is consistent with the strains O-2, N-1 and P-1. However, those strains belonged to the SCCmec type III.

PCR identification of J regions in strains O-2 and N-1 suggested that they carried the same subtype of type IX SCCmec, which was distinct from that of strain P-1 and JCSC 6943. On the other hand, strain P-1 was assumed to carry a novel subtype of type IX SCCmec, since it was distinct from that of strains O-2 and JCSC6943. We presume that livestock, e.g., pigs, might be the reservoir of ST9- S. aureus in Asian countries. The ST9-S. aureus strain might become an MRSA by acquiring SCCmec elements from different sources, since ST9-SCCmecIII strains were identified in China [40]. Identification of several subtypes of type IX SCCmec elements suggested that such elements might come from elsewhere and integrated into ST9 strains. In this study, it could not be concluded that SCCmecIX strains from human were derived from pig, since strain P-1 was not identical to strains O-2 and N-1. However, SCCmecIX strains with the same spa type (t337) have been identified from pigs (38, 39). Further study of these isolates will clarify whether ST9 MRSA strains isolated from human might be derived from livestock and disseminated in the Thai community.

Conclusions
ST9-SCCmecIX MRSA strains from humans (a patient and a healthy healthcare worker) and a diseased pig in Thailand were reported as a newly identified CA-MRSA clone disseminating in the Thai community.

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Author details
1Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. 2Department of Infection Control Science, Graduate School of Medicine, Juntendo University, Tokyo, Japan. 3Department of Bacteriology, Graduate School of Medicine, Juntendo University, 2-1-1 Hongo Bunkyo-ku, Tokyo 113-8421, Japan. 4Research and Diagnostic Center of Emerging Infectious Diseases and Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. 5Veterinary Research and Development (Upper Northeastern) Center, Khon Kaen, Thailand.

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