SCCmec IX in meticillin-resistant *Staphylococcus aureus* and meticillin-resistant coagulase-negative staphylococci from pigs and workers at pig farms in Khon Kaen, Thailand

Siwaporn Sinlapasorn,1 Aroonlug Lulitanond,2 Sunpetch Angkititrakul,3 Aroonwadee Chanawong,2 Chotechana Wilailuckana,2 Ratree Tavichakorntrakool,2 Kanoksak Chindawong,3 Charinya Seelaget,4 Mana Krasaesom,4 Sarawut Chartchai,4 Lumyai Wonglakorn5 and Pipat Sribenjalux2

Correspondence
Aroonlug Lulitanond
arolul@kku.ac.th

1Graduate School, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
2Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
3Research Group for Preventive Technology in Livestock, Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
4Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
5Clinical Microbiology Laboratory, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Livestock-associated meticillin-resistant *Staphylococcus aureus*, clonal complex (CC) 398, has been reported in Europe, whereas CC9 MRSA has mostly been found in Asia. Therefore, we aimed to detect MRSA on pig farms in north-eastern Thailand. A total of 257 nasal swabs (159 samples from pigs and 98 from pig-farm workers) were collected from three pig farms in north-eastern Thailand from 2010 to 2011. MRSA isolates were confirmed for *femA* and *mecA* genes by PCR. The MICs of eight antimicrobials, namely vancomycin (VA), cefazolin (CZ), ofloxacin (OF), tetracycline (TET), erythromycin (ER), oxacillin (OX), cefoxitin (FOX) and gentamicin (GN), were tested by agar dilution method. The virulence genes for Panton–Valentine leukocidin toxin (*lukSF-PV*), toxic shock syndrome toxin-1 (*tst*) and α-haemolysin (*hla*) were detected by PCR. Strain typing was performed by staphylococcal cassette chromosome (SCC) *mec, agr, spa* and multilocus sequence typing. Four MRSA were isolated: three from workers and one from a pig. All the MRSA isolates were resistant to OX, GN, ER, TET and CZ, and they all carried *hla* only. Two MRSA from humans carried SCCmec II-sequence type (ST)764-**agr**II, whereas the two remaining MRSA (one each from a human and a pig) contained SCCmec IX-ST9-**agr**II. Interestingly, meticillin-resistant coagulase-negative *Staphylococcus* isolates carrying SCCmec IX were also obtained from five workers and three pigs. This study suggests that the SCCmec IX element is distributed among the *Staphylococcus* found in pigs and pig-farm workers, and pigs may be a reservoir for MRSA in the community.

Abbreviations: CC, clonal complex; CNS, coagulase-negative *Staphylococcus*; CPS, coagulase-positive *Staphylococcus*; CZ, cefazolin; ER, erythromycin; FOX, cefoxitin; GN, gentamicin; HA-MRSA, healthcare-associated MRSA; LA-MRSA, livestock-associated MRSA; MLST, multilocus sequence typing; MRCNS, meticillin-resistant coagulase-negative *Staphylococcus*; MRCFS, meticillin-resistant coagulase-positive *Staphylococcus*; MRS, meticillin-resistant *Staphylococcus* spp.; MRSA, meticillin-resistant *Staphylococcus aureus*; OF, ofloxacin; OX, oxacillin; SCC, staphylococcal cassette chromosome; ST, sequence type; TET, tetracycline; VA, vancomycin.
INTRODUCTION

Meticillin-resistant Staphylococcus aureus (MRSA) has been a major cause of various infections in hospitals for over 50 years. During the last 15 years, the prevalence of MRSA infections in the community has been increasing. Recently, MRSA has been emerging in livestock. Since 2005, a novel MRSA isolate called clonal complex (CC) 398 has been isolated from pigs and people associated with pig farming in Europe (Voss et al., 2005). Consequently, pigs were assumed to be a possible reservoir of community-associated MRSA. At present, MRSA strains that are isolated from animals are called livestock-associated MRSA (LA-MRSA). However, CC398 LA-MRSA strains have been found frequently in Europe, and non-CC398 MRSA isolates are predominant in Asia. In particular, the sequence type (ST) MRSA (CC9) strains were mostly reported in association with pig farming (Cui et al., 2009; Guardabassi et al., 2009; Neela et al., 2009). Staphylococcal cassette chromosome (SCC) mec IX-ST9-t337 MRSA isolates from pork and pigs were also reported in Thailand (Vestergaard et al., 2012). In addition, ST239 made up the majority of healthcare-associated MRSA (HA-MRSA) clones in Thailand, whereas ST5 was a minor clone. Interestingly, a CC9 (SCCmec IX-ST9-1337) MRSA strain was first isolated from a patient in north-eastern Thailand whose PFGE pattern was closely related to an SCCmec IX-ST9-t044 strain of MRSA that was isolated from a diseased pig in the same area (Lulitanond et al., 2013). The occurrence of CC9 MRSA in this geographical area is indicative that the clone may have originated from pigs and spread to humans. Hence, the aim of this study was to investigate MRSA isolates from pigs and pig-farm workers in north-eastern Thailand.

METHODS

Volunteer recruitment. The study plan was clarified, and all volunteers signed a consent form and filled out a questionnaire to collect their personal demographics, health status and previous antimicrobial usage.

Bacterial isolation. A total of 257 skin and nasal swab samples, including 159 samples from pigs and 98 from humans working on pig farms, were collected from 2010 to 2011 from three pig farms in Khon Kaen, Thailand. The samples were pre-enriched for 24 h in tryptic soy broth supplemented with 5 % NaCl, subcultured on mannitol salt agar and then incubated at 37 °C for 24 h. The suspected Staphylococcus colonies were restreaked on blood agar and primarily identified by Gram stain, catalase, coagulase and phenol red mannitol tests. Meticillin resistance was detected by using oxacillin (OX) and cefoxitin (FOX) disc diffusion tests (CLSI, 2012). The meticillin-resistant coagulase-positive Staphylococcus (MRCPs) isolates were further confirmed by PCR identification of the femA gene, whereas the meticillin-resistant coagulase-negative Staphylococcus (MRCNS) isolates were further identified by using the Vitek II automated microbiology system (bioMérieux). The meticillin-resistant Staphylococcus spp. (MRS) isolates were kept frozen at −70 °C in skimmed milk supplemented with 20 % glycerol until further examination.

Ethics approval. This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Ethics Committee of Khon Kaen University (project number HE532170).

Antimicrobial susceptibility testing. The MRS isolates were tested for the MICs for eight antimicrobial agents (from Sigma-Aldrich), that is, vancomycin (VA), cefazolin (CZ), ofloxacin (OF), tetracycline (TET), erythromycin (ER), OX, FOX and gentamicin (GN), by using an agar dilution method (CLSI, 2012). S. aureus ATCC 29213 was used as an antimicrobial-susceptible reference strain.

DNA preparation. The genomic DNA of each MRS isolate was extracted by using achromopeptidase enzyme (Sigma-Aldrich) according to the method of Shittu et al. (2004). The crude extract solution was kept at −20 °C and used as a DNA template in the PCR.

Detection of virulence-associated genes. The genes encoding the Panton–Valentine leukocidin toxin ( lukSF-PV ) (Lina et al., 1999), toxic shock syndrome toxin-1 ( tst ) (Monday & Bohach, 1999) and α-haemolysin ( hla ) (Cafiso et al., 2007) of the MRS isolates were investigated using a simple PCR technique according to published studies. The DNA of S. aureus strains MR108 and N315 were used as positive controls for lukSF-PV, tst and hla.

Detection of mecA and mecC genes. The mecA and femA genes of the MRCPs isolates were detected by using multiplex PCR assays as described by Kondo et al. (2007) and Berger-Bachi et al. (1989), respectively. The mecC gene was detected by using the PCR technique described by Paterson et al. (2012). The internal positive control for the PCR was performed simultaneously by using primer sets against the staphylococcal 16S rRNA (Monday & Bohach, 1999).

Genotypic study: SCCmec types, agr types, spa types and multilocus STs. The SCCmec types of the MRS isolates were determined by using a multiplex PCR technique as described by Kondo et al. (2007) with S. aureus strains NCTC 10442, N315, 85/2082, HDE288 and JCS6690 as positive controls for SCCmec types I, II, III, IV and IX, respectively. The SCCmec IX isolates were further confirmed for mec class C2 according to the method of Lawung et al. (2014). The MRSA were isolated into agr and spa types according to published reports (Lina et al., 2003; Shopsin et al., 1999). The DNA of S. aureus strains MS37, MS3, MS16 and MS38 were used as positive controls for agr types I, II, III and IV, respectively. The designation of the spa type was conducted by using the Ridom StaphType program (www.ridom.de).

Multilocus sequence typing (MLST) of the representative isolates from each group was performed as indicated by Enright et al. (2000). The alleles of seven loci were examined by comparing the nucleotide sequences to those of the corresponding loci in the S. aureus MLST database (www.mlst.net). The STs were determined according to the combination of the seven alleles, and the CC types were defined by using the eBURST program (based on related STs) available from the MLST website.

PFGE. The Smal-digested chromosomal DNA of the staphylococci isolates was used for PFGE analysis in a CHEF system according to the manufacturer’s instructions (Bio-Rad). The gel was stained with ethidium bromide and photographed under UV light. The band patterns were compared visually and were classified as indistinguishable (clonal), closely related (representative of clonal variants with a difference in three or fewer bands), possibly related (exhibiting a four- to six-band difference), and unrelated, according to previously described criteria (Tenover et al., 1995).

Statistical analysis. SPSS 17.0 statistical software (SPSS) was used for statistical analysis. A P value of <0.05 was considered statistically significant.
RESULTS AND DISCUSSION

Bacterial isolates

Of the 257 samples from pigs and humans, 4 MRCPS and 40 MRCNS were isolated. A total of 3 MRSA (3.1%) were isolated from the 98 pig-farm workers, whereas only 1 MRSA was isolated from the 159 live pigs (0.6%). The 40 MRCNS identified by automation were 17 Staphylococcus sciuri isolates (11 isolates from pigs and 6 isolates from humans), 6 Staphylococcus haemolyticus (2 from pigs and 4 from humans), 3 Staphylococcus arlettae (from humans), 3 Staphylococcus cohnii subspecies urealyticus (1 from a pig and 2 from humans), 2 Staphylococcus hominis subspecies hominis (from humans), 2 Staphylococcus chromogenes (1 from a pig and 1 from a human), 2 Staphylococcus saprophyticus (from pigs), 1 Staphylococcus xylosus (from a pig), 1 Staphylococcus caprae (from a pig), 1 Staphylococcus epidermidis (from a human), 1 Staphylococcus vitulinus (from a pig) and 1 Staphylococcus hyicus (from a pig). Only 68 complete questionnaires from pig-farm workers were available for data analysis. Most of the volunteers were males (43/68). The mean age of the volunteers was 39.4 years (with a range from 17 to 64 years). The demographic information and health characteristics are shown in Table 1.

Staphylococci are major bacteria in human and veterinary medicine. Coagulase-positive Staphylococcus (CPS), particularly S. aureus, can colonize both humans and animals (Sung et al., 2008). The present study showed that the rate of MRSA colonization among pig farmers (3.1%) and pigs (0.6%) was similar to the rates found in a recent study from northern Thailand, which reported that the prevalence of nasal MRSA carriers in pig-farm workers and pigs was 1.28% (4/312) and 0.68% (2/292), respectively (Patchanee et al., 2014). These rates are lower than those reported in Europe and North America, but are somewhat comparable to those of Asian countries such as China [11.39% from pigs (58/509) and 1.19% (2/167) from humans] (Cui et al., 2009), Malaysia [1.4% from pigs (5/360) and 5.5% (5/90) from humans] (Neela et al., 2009), and Taiwan [13.88% from pigs (89/641) and 13% (13/100) from humans] (Fang et al., 2014). However, coagulase-negative Staphylococcus (CNS) consisted of a variety of species and had a trend of increasing resistance to meticillin (Diekema et al., 2001). Interestingly, the rates of MRCNS among pig-farm workers and pigs in the present study were 16.3% (16/98) and 15.09% (24/159), respectively. This finding confirmed that both MRSA and various species of MRCNS can be found in pigs and humans (Tulinski et al., 2012).

Table 1. Demographic information and health characteristics of pig-farm workers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Category</th>
<th>Mean (range)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (n=68)</td>
<td></td>
<td>39.4 (17–64)</td>
<td></td>
<td></td>
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<td>No. of family members</td>
<td></td>
<td>5 (1–9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>43</td>
<td>63.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>Educational background</td>
<td>University</td>
<td>7</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vocational training</td>
<td>11</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>18</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primary</td>
<td>32</td>
<td>47.0</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Pig-farm worker</td>
<td>60</td>
<td>88.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Butcher</td>
<td>8</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Working period</td>
<td>Less than 5 years</td>
<td>18</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5–15 years</td>
<td>37</td>
<td>54.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>More than 15 years</td>
<td>13</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Direct contact with pigs</td>
<td>Yes</td>
<td>67</td>
<td>98.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Exercise per week</td>
<td>3 times</td>
<td>25</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less than 1 time</td>
<td>18</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>25</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>Problem with skin abscesses</td>
<td>Yes</td>
<td>13</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>55</td>
<td>80.9</td>
<td></td>
</tr>
<tr>
<td>Previous antimicrobial usage</td>
<td>Yes</td>
<td>6</td>
<td>8.8</td>
<td></td>
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<tr>
<td></td>
<td>No</td>
<td>62</td>
<td>91.2</td>
<td></td>
</tr>
<tr>
<td>Place to clean body after work</td>
<td>Home</td>
<td>37</td>
<td>54.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Workplace</td>
<td>31</td>
<td>45.6</td>
<td></td>
</tr>
<tr>
<td>Underlying conditions</td>
<td>Diabetes mellitus</td>
<td>5</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver and biliary disease</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Respiratory syndrome and psychosis</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>61</td>
<td>89.7</td>
<td></td>
</tr>
</tbody>
</table>
ANTIMICROBIAL SUSCEPTIBILITIES

All 44 MRS isolates were resistant to OX and susceptible to VA, whereas most of them were resistant to TET, GN, ER and OF (Table 2). The MRS isolates from humans and pigs showed different resistance rates to FOX (31.8 % compared with 22.7 %, respectively, \( P = 0.026 \)), and all four MRSAs were resistant to FOX, GN (13.6 % compared with 6.8 %, respectively, \( P = 0.012 \)) and TET (36.4 % compared with 6.8 %, respectively, \( P = 0.026 \)). The antimicrobial resistance rates of most of the MRS isolates from humans were higher than the rates in pigs. These findings are different from those of a study in Taiwan that reported the antimicrobial resistance rates of MRSA isolates from pigs were significantly higher than those from humans (Fang et al., 2014). This difference may be related to the different drug usage in each country. The antimicrobials tested in this study were drugs that are commonly used in both humans and livestock. An inappropriate antimicrobial usage would lead to an increase in drug-resistant bacteria. Increasing antimicrobial usage in animals and the emergence of antimicrobial resistance among food-borne bacteria are currently associated with human diseases (Marshall & Levy, 2011).

GENOTYPES OF MRS

Of the 44 MRS isolates, 39 were positive for the \( \text{mecA} \) gene (17 from humans and 22 from pigs). Of the four MRSAs, two isolates from pig-farm workers carried \( \text{SCCmec II-ST764-agrII-spa} \) 17-20-17-12-17 and one isolate carried \( \text{SCCmec IX-ST9-agrII-spa} \) 23-02, whereas one isolate from a pig also carried \( \text{SCCmec IX-ST9-agrII-spa} \) 16-23-06-02-12-23-02-34. Interestingly, of the 35 MRCNS isolates, 8 isolates carrying \( \text{SCCmec IX} \) were obtained from 5 workers and 3 pigs (Table 3), and 17 isolates carried non-typable \( \text{SCCmec} \) (Table 4). The remaining 10 isolates carried \( \text{mec} \) classes and \( \text{ccr} \) types that were undetermined. All the 44 isolates were negative for \( \text{mecC} \) and \( \text{lukSF-PV} \) genes. Remarkably, only four MRSA isolates were positive for the \( \text{hla} \) gene and one MRCNS isolate (\( \text{S. hominis} \) subspecies \( \text{hominis} \)) from a human was positive for the \( \text{tst} \) gene (Tables 3 and 4).

As far as we know, this is the first report of ST764 MRSA isolates from humans in Thailand. The ST764 MRSA is a single-locus variant of ST5 MRSA. This variant was proposed to have evolved from ST5-\( \text{SCCmec II HA-MRSA} \) (Takano et al., 2013). It was reported as a community-associated MRSA according to the criteria of the Center for Disease Control and Prevention (Takano et al., 2013; Klevens et al., 2007). The PFGE patterns of the two \( \text{SCCmec II-ST764 MRSA} \) isolates from pig-farm workers were identical to that of an \( \text{SCCmec II-ST5} \) strain from a hospital in the same province (Fig. 1) (Lulitanond et al., 2015). This finding was indicative that ST5 HA-MRSA may disseminate and evolve in the community and livestock. Further investigations of their genome sequences will elucidate this hypothesis.

### Table 2. MIC values for MRS isolates from pig-farm workers and pigs

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Organism</th>
<th>No. of isolates yielding the indicated MICs (CPS, ( n = 4 ); CNS, ( n = 40 ))</th>
<th>% Resistance</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;&lt;0.125&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OX</td>
<td>CPS</td>
<td>1 1 2</td>
<td>100</td>
<td>128</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>24 10 2 2</td>
<td>100</td>
<td>1 4</td>
<td></td>
</tr>
<tr>
<td>FOX</td>
<td>CPS</td>
<td>1 1 2</td>
<td>100</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>1 1 2</td>
<td>47.5</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>GN</td>
<td>CPS</td>
<td>1 4 7 3 1</td>
<td>75</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>20 1 4 7 3 1</td>
<td>17.5</td>
<td>&lt;0.125</td>
<td>16</td>
</tr>
<tr>
<td>CZ</td>
<td>CPS</td>
<td>1 1 2</td>
<td>75</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>2 18 7 9 2 2</td>
<td>5</td>
<td>1 4</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>CPS</td>
<td>1 3</td>
<td>75</td>
<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
<td></td>
<td>CNS</td>
<td>2 15 5 1 1</td>
<td>42.5</td>
<td>0.5</td>
<td>&gt;128</td>
</tr>
<tr>
<td>TET</td>
<td>CPS</td>
<td>1 1 2</td>
<td>100</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>11 3 1 2 4 5 14</td>
<td>62.5</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td>OF</td>
<td>CPS</td>
<td>1 1 2</td>
<td>100</td>
<td>32</td>
<td>&gt;128</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>3 3 18 10 2 1 2 1</td>
<td>15</td>
<td>1 4</td>
<td></td>
</tr>
<tr>
<td>VA</td>
<td>CPS</td>
<td>2 2</td>
<td>0</td>
<td>1 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>1 20 19</td>
<td>0</td>
<td>1 2</td>
<td></td>
</tr>
</tbody>
</table>

*The shading indicates the resistant concentrations for each antimicrobial.*
Table 3. Characteristics of SCCmec-typable MRS isolates from humans and pigs

CNSH, CNS from humans; CNSP, CNS from pigs; CPSH, CPS from humans; CPSP, CPS from pigs; ND, not done; pvl, Panton–Valentine leukocidin.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>SCCmec type</th>
<th>Virulence gene</th>
<th>Agr type</th>
<th>Spa type</th>
<th>ST</th>
<th>Antimicrobial resistance pattern</th>
<th>Species</th>
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<tbody>
<tr>
<td>Human CPS</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CPHS1</td>
<td>II</td>
<td>+ – –</td>
<td>II</td>
<td>New</td>
<td>764</td>
<td>OX, FOX, GN, CZ, ER, TET, OF</td>
<td>S. aureus</td>
</tr>
<tr>
<td>CPHS8</td>
<td>II</td>
<td>+ – –</td>
<td>II</td>
<td>New</td>
<td>764</td>
<td>OX, FOX, GN, CZ, ER, TET, OF</td>
<td>S. aureus</td>
</tr>
<tr>
<td>CPHS11</td>
<td>IX</td>
<td>+ – –</td>
<td>II</td>
<td>New</td>
<td>9</td>
<td>OX, FOX, GN, TET, OF, CZ*</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Pig CPS</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPSP4</td>
<td>IX</td>
<td>+ – –</td>
<td>II</td>
<td>New</td>
<td>9</td>
<td>OX, FOX, GN*, ER, TET, OF</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Human CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNSH6</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, GN*, ER, TET, OF*</td>
<td>S. hominis</td>
</tr>
<tr>
<td>CNSH5</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, FOX, ER, TET, OF</td>
<td>S. arlettae</td>
</tr>
<tr>
<td>CNSH7</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, ER, TET, OF*</td>
<td>S. arlettae</td>
</tr>
<tr>
<td>CNSH14</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, ER, TET</td>
<td>S. arlettae</td>
</tr>
<tr>
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<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, FOX, GN*, ER, TET, OF*</td>
<td>S. haemolyticus</td>
</tr>
<tr>
<td>Pig CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNSP15</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, FOX, GN, TET, OF*</td>
<td>S. haemolyticus</td>
</tr>
<tr>
<td>CNSP40</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, FOX, GN*, ER, TET, OF*</td>
<td>S. haemolyticus</td>
</tr>
<tr>
<td>CNSP47</td>
<td>IX</td>
<td>– – –</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>OX, GN, ER, TET</td>
<td>S. hyicus</td>
</tr>
</tbody>
</table>

*Intermediate resistance.

The ST9 MRSA is an endemic LA-MRSA in Asia, whereas ST398 is predominant in Europe and North America. However, the ST9 MRSA also has been detected from pigs and humans in Europe, e.g. in Italy in 2008 and in The Netherlands in 2010 (Battisti et al., 2010; Mulders et al., 2010). The first report of ST9 MRSA from pigs and farm workers in Asia was from China (Cui et al., 2009). Since then, many reports of ST9 LA-MRSA were made

Table 4. Characteristics of SCCmec-non-typable MRCNS isolates from pig-farm workers and pigs

CNSH, CNS from humans; CNSP, CNS from pigs; pvl, Panton–Valentine leukocidin; UD, undetermined mec gene complex class and ccr complex type.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>SCCmec type</th>
<th>Virulence gene</th>
<th>Antimicrobial resistance pattern</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNSH9</td>
<td>Human</td>
<td>C A3B3</td>
<td>– – –</td>
<td>OX, FOX, GN, ER, TET, OF*</td>
<td>S. arlettae</td>
</tr>
<tr>
<td>CNSH16</td>
<td>Human</td>
<td>C A3B3</td>
<td>– – –</td>
<td>OX, FOX, TET</td>
<td>S. epidermidis</td>
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<tr>
<td>CNSH38</td>
<td>Human</td>
<td>C A3B3</td>
<td>– – –</td>
<td>OX, FOX, GN, ER, TET, OF</td>
<td>S. haemolyticus</td>
</tr>
<tr>
<td>CNSH39</td>
<td>Human</td>
<td>C A3B3</td>
<td>– – –</td>
<td>OX, FOX, TET</td>
<td>S. haemolyticus</td>
</tr>
<tr>
<td>CNSP27</td>
<td>Pig</td>
<td>C A3B3</td>
<td>– – –</td>
<td>OX, FOX, GN*, OF*</td>
<td>S. chromogenes</td>
</tr>
<tr>
<td>CNSP21</td>
<td>Pig</td>
<td>C UD</td>
<td>– – –</td>
<td>OX, FOX, ER, TET, OF</td>
<td>S. xylosus</td>
</tr>
<tr>
<td>CNSP75</td>
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<td>C UD</td>
<td>– – –</td>
<td>OX</td>
<td>S. sciuri</td>
</tr>
<tr>
<td>CNSP78</td>
<td>Pig</td>
<td>C UD</td>
<td>– – –</td>
<td>OX</td>
<td>S. sciuri</td>
</tr>
<tr>
<td>CNSP85</td>
<td>Pig</td>
<td>C UD</td>
<td>– – –</td>
<td>OX</td>
<td>S. sciuri</td>
</tr>
<tr>
<td>CNSP26</td>
<td>Pig</td>
<td>A A1B1</td>
<td>– – –</td>
<td>OX, FOX, ER, TET, OF*</td>
<td>S. caprae</td>
</tr>
<tr>
<td>CNSP27</td>
<td>Pig</td>
<td>A A1B1</td>
<td>– – –</td>
<td>OX, FOX, CZ, ER, TET</td>
<td>S. saprophyticus</td>
</tr>
<tr>
<td>CNSP56</td>
<td>Pig</td>
<td>A UD</td>
<td>– – –</td>
<td>OX, TET, OF*</td>
<td>S. sciuri</td>
</tr>
<tr>
<td>CNSP91</td>
<td>Pig</td>
<td>A UD</td>
<td>– – –</td>
<td>OX, FOX, CZ, ER, TET</td>
<td>S. saprophyticus</td>
</tr>
<tr>
<td>CNSH2</td>
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<td>– – +</td>
<td>OX, FOX, GN, ER*, TET</td>
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<tr>
<td>CNSH48</td>
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<td>UD A3B3</td>
<td>– – –</td>
<td>OX, GN, TET</td>
<td>S. sciuri</td>
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<tr>
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<td>Pig</td>
<td>UD A1B1, A3B3</td>
<td>– – –</td>
<td>OX, FOX, ER, TET</td>
<td>S. vitulinus</td>
</tr>
</tbody>
</table>

*Intermediate resistance.
from several countries, such as Malaysia, Hong Kong, Taiwan and Thailand (Guardabassi et al., 2009; Neela et al., 2012; Vestergaard et al., 2014; Fang et al., 2014). Our study confirmed that ST9 MRSA was an endemic LA-MRSA strain in pigs and pig-farm workers in the Khon Kaen province of Thailand. The PFGE of SCCmec IX-MRSA from workers and pigs showed possibly related patterns, which could have been related to that of the SCCmec IX-MRSA strains from patients and pigs in a previous report (Lulitanond et al., 2013). In addition, the strains carried different spa repeat sequences. This finding indicated that the SCCmec IX-MRSA strains in this area were not of a single clone. The SCCmec IX element may spread among different S. aureus strains.

Moreover, SCCmec IX-MRCNS isolates of different species (most of them were S. arlettae and S. haemolyticus) were also obtained from five workers and three pigs in the same geographical area. Of the three SCCmec IX-S. arlettae from pig-farm workers, two isolates showed closely related PFGE patterns, whereas one isolate showed a possibly related pattern. The PFGE patterns of one SCCmec IX-S. haemolyticus isolate from a human and two isolates from pigs also showed possibly related patterns. Taken together with the SCCmec IX-S. hominis from humans and the SCCmec IX-S. hyicus from pigs, these findings support the idea that the *Staphylococcus* species in the environment may be reservoirs of the SCCmec IX element and were disseminated to *S. aureus* in this area. This finding corresponded to previous reports on MRSA and CNS from pig farms in the same niche (Klevens et al., 2007), and many composite SCCmec elements have been found in MRCNS (IWG-SCC, 2009).

In addition, MRCNS was proposed as an origin of *mecA* genes that were transferred to *S. aureus* (Tsubakishita et al., 2010).

### CONCLUSION

This study suggests that there is a small number of MRSA strains distributed among pigs and humans on pig farms in Khon Kaen, Thailand, and pigs may be a reservoir of MRSA in the community. In addition, MRCNS may be a reservoir of the SCCmec IX element and transfer it to *S. aureus* strains in this area.

### ACKNOWLEDGEMENTS

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### REFERENCES


