High prevalence and molecular characterization of methicillin-resistant 
Staphylococcus aureus isolated from retailed meats, south Thailand

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Article history
Received: 17 September 2013
Received in revised form: 12 November 2013
Accepted: 21 November 2013

Abstract
Methicillin-resistant Staphylococcus aureus is an important pathogenic bacterium playing
crucial roles in healthcare institutions including the communities. Moreover, food-borne
illnesses caused by MRSA were also reported. In this study, 50 meat samples were investigated
for the presence of MRSA. Suspected S. aureus colonies were selected and confirmed as S.
aureus by 16S rRNA gene amplification using S. aureus specific primers. Investigation of mecA
and other virulence genes, were carried out by PCR approach. Of 474 suspected isolates, 185
(39.02%) were confirmed to be mecA+ S. aureus. All mecA+ S. aureus were isolated from 34
meat samples (68%). Of these 185 isolates, 103, 42 and 40, were obtained from beef, pork
and chicken, respectively. Two (1.08%) mecA+ S. aureus from beef, PSU123 and PSU124,
and two (1.08%) from chicken, PSU125 and PSU126, possessed van Willebrand factor binding
protein gene. One (0.54%) of mecA+ S. aureus obtained from chicken, PSU127, revealed the
possession of spa gene. MRSA typing by SCCmec type revealed that PSU127 belonged to type
IV while PSU123 to PSU126, were thought to be classified as SCCmec type other than type I
to VI. Pulsed-field gel electrophoresis exhibited the distantly relationship among five mecA+
S. aureus strains. The majority of pathogenic strains exhibited the resistance to penicillin G,
erthyromycin, ceftazidime, clindamycin and fusidic acid while PSU125 revealed the resistance
to extra antimicrobial agent, tetracycline. This is the first report of MRSA contamination in
retailed meats sold in south of Thailand.

Keywords
MRSA
Retailed meats
Thailand
mecA

Introduction
Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most frequent pathogens
causing nosocomial infections worldwide (de Sousa et al., 2003; Johnson et al., 2005; Witte
et al., 2007). The reported cases of MRSA infections have been gradually increased in several countries
along a past decade. In Songklanagarind hospital, Hat-Yai city, Thailand, the nosocomial infection
caused by MRSA reached epidemic circumstances in late December, 1986 (Jamulitrat et al., 1988). At
that moment, 6 of 90 patients died within 14 months and the outbreak could not be attenuated although
the infected people were immediately quarantined. The outbreak and sporadic cases by MRSA in this area
were continually reported to date. Despite the fact that MRSA is frequently transmitted from
person-to-person in hospitals or infections may be occurred in the populations which are related to
the healthcare institutions, food-borne illnesses by MRSA have also been reported (Jones et al., 2002).

Resistance to methicillin is mediated through the mec operon which is a part of the staphylococcal cassette
chromosome mec (SCCmec) (El Karamany et al., 2013). The mecA gene codes for an altered penicillin-
binding protein, PBP2a, which has a lower affinity for binding β-lactam antibiotics (Mostafa, 2013). S.
aureus possesses several virulence factors responsible for its pathogenicity to the host. It produces many
virulence factors such as coagulase, γ-hemolysin, Panton-Valentine leukocidin (PVL), staphylococcal
protein A. These virulence factors are responsible for the host attachment, host invasion and destruction
of host defense system. Moreover, it may possess the staphylococcal enterotoxins (SEs) and toxic
shock syndrome toxin-1 (TSST-1), the superantigens (Hwang et al., 2007). Both the SEs and TSST-1 belong
to the pyrogenic toxin superantigen (PTSAg) family (Hwang et al., 2007). These superantigens (SAg) are
able to bind to the major histocompatibility complex (MHC) class II and form a complex with Vβ chain of
a T-cell receptor, resulting in a nonspecific manner stimulation of T-cell proliferation. Many of SAg genes

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are located in the mobile genetic elements, plasmids, prophages and staphylococcal pathogenicity islands (Baba et al., 2002). Although the additional types of SEs have newly been described, the classical SEs, staphylococcal enterotoxin A (SEA) to staphylococcal enterotoxin E (SEE), are the common causes of staphylococcal food poisoning.

During slaughtering processes, MRSA can be contaminated on carcasses (Boost et al., 2013), contributing to the high contamination rates of MRSA on retailed meats in fresh markets. The superantigens can be found in those MRSA strains. Therefore, meats and meat products act as the vehicle in transmission of MRSA to the buchers and consumers (Boost et al., 2012). The surveillance of MRSA in retailed meats was thought to be important. Although several studies of MRSA in meats have been documented in various countries (Jones et al., 2002; Katai et al., 2005; van Loo et al., 2007; Weese et al., 2010), insufficient evidences of MRSA in meats were documented in Thailand. Thus, we aimed to investigate the prevalence of MRSA strains from retailed meats in Southern Thailand, which may be contaminated with pathogenic MRSA, capable of causing food poisoning, including the examination of their cumulative virulence factors which contribute to the severe human infections. Furthermore, the antimicrobial resistant patterns were elucidated, providing the informations about potential drug of choice useful for the therapy in either sporadic or outbreak cases by MRSA isolated from the environment.

Materials and Methods

Sample collection

A total of 50 meat samples (pork = 16, chicken = 14, beef = 20) were collected from various meat retailers in Hat-Yai city, Thailand between April and September, 2013. Five to ten different meat samples from different markets were randomly selected. The samples were collected once a week to ensure that the meats were from different batches. The meats were brought to the laboratory within two hours for processing. Ten grams of meats were mixed with 90 ml of tryptic soy broth (TSB) and homogenized for 1 min. The liquid portion was incubated at 37°C without shaking for 6 h. Subsequently, one ml of bacterial culture was diluted as dilution of 10⁻¹ and 10⁻² and plated on Mannitol Salt agar (MSA) (Difco, USA). The plates were incubated 37°C for 18 h. Ten yellow colonies per sample were picked up and grown in TSB for 6 h before kept stock at -80°C.

DNA template preparation

A single bacterial colony was inoculated into 3 ml of TSB and incubated at 37°C for 6 h with 150 rpm shaking. The culture was subjected to boiling for 10 min and immediately on ice for 5 min. After centrifugation at 11,000×g for 10 min, ten-fold dilution of the supernatant portion was performed to obtain the DNA template for PCR.

Virulence genes detection

Seven virulence genes were determined by uniplex PCR method, mecA, sea, coa, luk-PV, vWbp, femB and spa genes. The sequences of oligonucleotide primers and annealing temperature used in this study were shown in Table 1. vWbp oligonucleotide primers were designed based upon the consensus sequences of vWbp genes complete sequence obtained from National Center for Biotechnology Information (NCBI). The amplification was carried out using GoTaq® Flexi system (Promega, USA), consisting of 3.0 mM MgCl₂, 0.1 mM dNTPs, 0.4 μM each primer pair, 0.5 unit of GoTaq DNA polymerase, 1X GoTaq Flexi buffer and 2 μl of boiled DNA template. The total reaction volume was 25 μl. The amplicons were analyzed in 1.0% agarose gel electrophoresis, stained with ethidium bromide before image capture by Gel documentation system (SYNGENE, USA) (Figure 1).

Identification of S. aureus by 16S rRNA gene

To confirm whether the isolates were S. aureus, amplification of 16S rRNA gene specific to S. aureus was carried out by PCR method using oligonucleotide primers described by Monday and Bohach (1999) (Table 1). The amplicons were analyzed by 1.0% agarose gel electrophoresis. The gel was stained with ethidium bromide and image captured.
Table 1. Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Target</th>
<th>Name</th>
<th>Sequences (5’ to 3’ )</th>
<th>Annealing temperature</th>
<th>Amplicon size (bp)</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>mecA</td>
<td>mecA1</td>
<td>GTAGAAAATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>310</td>
<td>Geha et al., 1994</td>
</tr>
<tr>
<td></td>
<td>mecA2</td>
<td>CCAATTCGATATGTTCTGCGCTTT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>luk-PV</td>
<td>luk-PV1</td>
<td>ATCGATGGATATGTTCTGCGCTTT</td>
<td>57ºC</td>
<td>433</td>
<td>Lin et al., 1999</td>
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<tr>
<td></td>
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<td>GACATCAATGATGGTCGAGCCCGATGAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coa</td>
<td>COA1</td>
<td>CAGAGCAAGATGATGGTCGAGCCCGATGAA</td>
<td>51ºC</td>
<td>730</td>
<td>Wichelhaus et al., 2001</td>
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<tr>
<td></td>
<td>COA2</td>
<td>AAAGAATTCTGCGCTTT</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>spa</td>
<td>SPA1</td>
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<td>1500</td>
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<tr>
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<td>SPA2</td>
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<tr>
<td>femB</td>
<td>FemB1</td>
<td>TTACAGGATGATGGTCGAGCCCGATGAA</td>
<td>48ºC</td>
<td>651</td>
<td>Kobayashi et al., 1994</td>
</tr>
<tr>
<td></td>
<td>FemB2</td>
<td>ATCAATCTGCGCTTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWbp</td>
<td>vWbp-F</td>
<td>GCCGATATGTTCTGCGCTTT</td>
<td>50ºC</td>
<td>320</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>vWbp-R</td>
<td>GACATCAATGATGGTCGAGCCCGATGAA</td>
<td></td>
<td></td>
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<tr>
<td>sea</td>
<td>SEA-F</td>
<td>GACGATATGTTCTGCGCTTT</td>
<td>52ºC</td>
<td>520</td>
<td>Monday and Bohach, 1999</td>
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<tr>
<td></td>
<td>SEA-R</td>
<td>GACATCAATGATGGTCGAGCCCGATGAA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SEA+</td>
<td>SEA+S</td>
<td>GTTCTAATGGATATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>228</td>
<td>Monday and Bohach, 1999</td>
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<tr>
<td></td>
<td>J1 region</td>
<td>CIF F2</td>
<td>TCGGATGGATATGTTCTGCGCTTT</td>
<td>53ºC</td>
<td>495</td>
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<tr>
<td>(type V)</td>
<td>ccr C2</td>
<td>GATACGATGATGGTCGAGCCCGATGAA</td>
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<td>femB</td>
<td>RIF5 F10</td>
<td>TTCATAGGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
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<td>Milheiroço et al., 2007</td>
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<td>(type III)</td>
<td>RIF5 R13</td>
<td>GTCACAGAATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>377</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>J1 region</td>
<td>SCCmecV1F</td>
<td>TTCATAGGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>342</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type V)</td>
<td>SCCmecV1R</td>
<td>AGACGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>342</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>femB</td>
<td>dcs F2</td>
<td>CATCAGGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>342</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type II, IV, VI)</td>
<td>dcs R1</td>
<td>CTCAATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>342</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>ccr complex</td>
<td>ccr B2 F2</td>
<td>AGTCTTTCGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>311</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type II, IV)</td>
<td>ccr B2 R2</td>
<td>CCGATATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>311</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>J1 region</td>
<td>kdp F1</td>
<td>ATCAATCTGCGCTTT</td>
<td>53ºC</td>
<td>284</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type II)</td>
<td>kdp R1</td>
<td>GATACGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>284</td>
<td>Milheiroço et al., 2007</td>
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<tr>
<td>J1 region</td>
<td>SCCmecHUIF</td>
<td>CTTAGTCAATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>234</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type III)</td>
<td>SCCmecHUIR</td>
<td>GTTCTATCAGGATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>234</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>mec complex</td>
<td>mec P2</td>
<td>ATCAAGAATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>210</td>
<td>Milheiroço et al., 2007</td>
</tr>
<tr>
<td>(type II and III)</td>
<td>mec P3</td>
<td>GCGGATATGATGGTCGAGCCCGATGAA</td>
<td>50ºC</td>
<td>210</td>
<td>Milheiroço et al., 2007</td>
</tr>
</tbody>
</table>

MRSA typing

Staphylococcal cassette chromosome mec (SCCmec) (type I to VI) was investigated by PCR method as described by Milheiroço et al. (2007) (Table 1). PCR was performed as a simplex PCR. After amplification, the amplicons were analyzed by agarose gel electrophoresis. The gel was stained with ethidium bromide and image captured. PCR amplification of mecA gene was also performed to be used as an internal control. In addition, pulsed-field gel electrophoresis was also carried out. Briefly, MRSA strains were treated with 50 μg/ml of lysostaphin (Sigma-Aldrich, USA) for 30 min to obtain spheroplast before plug preparation. The genomes of S. aureus were cleaved by FastDigest SmaI restriction enzyme (Thermo Scientific, USA) at 37ºC for 1 h. The digested DNA fragments were separated in 1.0% agarose gel (Invitrogen, USA) using 0.5× TBE buffer by CHEF DR III system (Bio-Rad, USA). Electrophoresis was performed at 6V/cm, field angle 120º at 14ºC. The initial and final switch times were 5.0 and 40.0 s, respectively. Overall run time was 21 h. After electrophoresis, the gel was stained with ethidium bromide and the image result was eventually captured. The 48.5 kb Lambda marker was used as a molecular size standard. Dendrogram was constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioNumerics software version 7.0, Applied maths, Belgium).

Antimicrobial susceptibility testing

All S. aureus samples were determined for the antimicrobial susceptibility by disk diffusion method (CLSI, 2013). Fifteen antimicrobial agents were used in this experiment: amikacin (30 μg), erythromycin (15 μg), gentamicin (10 μg), chloramphenicol (30 μg), cephalothin (30 μg), cefazidime (30 μg), penicillin G (10 μg), tetracycline (30 μg), norfloxacin (10 μg), clindamycin (2 μg), fosfomycin (50 μg), fusidic acid (10 μg), cefoxitin (30 μg), oxacillin (1 μg), and vancomycin. Clear zone was measured by Vernier caliper. Antimicrobial agents were purchased from Oxoid (UK). Vancomycin susceptibility was performed by E-test method. The susceptibility breakpoint for vancomycin was ≤4.

Results

Bacterial isolation and screening for mecA gene

In order to investigate MRSA in meats, three common meat types were purchased from fresh markets in Hat-Yai city, Thailand. A total of 474 isolates suspected to be S. aureus were collected from such 50 meat samples. mecA gene were first examined in all isolates by PCR. Of 474 isolates, the results revealed the possession of mecA gene in
Table 2. Contamination of mecA+ S. aureus in retailed meats sold in Hat-Yai city area

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of MRSA positive sample (%)</th>
<th>No. of isolated MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef (n = 20)</td>
<td>18 (90%)</td>
<td>103</td>
</tr>
<tr>
<td>Pork (n = 16)</td>
<td>8 (50%)</td>
<td>42</td>
</tr>
<tr>
<td>Chicken (n = 14)</td>
<td>8 (57.14%)</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 3. Virulence genes pattern of a total mecA+ S. aureus isolates from meats

<table>
<thead>
<tr>
<th>Isolate (n)</th>
<th>coa</th>
<th>vWbp</th>
<th>spa</th>
<th>luk-PV</th>
<th>sea</th>
<th>femB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef (103)</td>
<td>0 (0)</td>
<td>2 (1.94)</td>
<td>1 (0.97)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pork (42)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chicken (40)</td>
<td>0 (0)</td>
<td>2 (5)</td>
<td>1 (2.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (185)</td>
<td>0 (0)</td>
<td>4 (2.16)</td>
<td>1 (0.54)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Figure 2. PFGE-based dendrogram of mecA+ S. aureus isolated from meat samples

185 isolates (39.02%) (average number of detected mecA+ S. aureus was 3.9 isolates per sample). These mecA+ isolates belonged to 34 meat samples (68%) (Table 2). All 185 isolates were further confirmed to be S. aureus by amplification of 16S rRNA gene using primers specific to S. aureus. When focused on each group of meat, it was obvious that the highest mecA+ S. aureus contamination was found in beef, 18 of 20 samples (90%) (Table 2). Chicken exhibited the mecA+ S. aureus contamination as a second rank, 8 of 14 samples (57.14%).

Other virulence genes detection

In order to examine the presence of other six virulence genes, coa, vWbp, sea, spa, femB and luk-PV, in mecA+ S. aureus, PCR method using specific oligonucleotide primers to each gene, was performed. In this study, of 185 mecA+ S. aureus isolates, none of the strains exhibited the presence of coa gene. Thus, another protein responsible for the plasma clotting, von Willebrand factor binding protein, vWbp, was further investigated. Of 185 MRSA, 4 were shown to carry vWbp (2 from chicken and 2 from beef) (Table 3). spa gene was detected in one chicken sample (2.5%). The presence of mecA together with the absence of femB, are the indicator of coagulase negative MRSA (Jonas et al., 2002). In this study, all mecA+ isolates revealed the lack of femB, demonstrating that these S. aureus were coagulase negative MRSA. This result was corresponded to the lack of coa gene in these MRSA strains (Table 3). Staphylococcal enterotoxin A gene, one of the common superantigens responsible for food poisoning, was absence in all mecA+ S. aureus tested. Panton-Valentine leukocidin gene was also negative (Table 3).

MRSA typing

SCCmec typing was initially performed to distinguish mecA+ S. aureus strains. In this study, investigation of SCCmec type was carried out by PCR method using oligonucleotide primers specific to SCCmec type I to VI as previously described by Milheiro et al. (2007). The results revealed that PSU123 to PSU125 and PSU127 may be classified as SCCmec type other than type I to VI because no specific DNA band was observed. mecA gene that used as internal control could be normally observed. While PSU126 belonged to SCCmec type IV. PFGE clearly demonstrated the distantly relationship among mecA+ S. aureus strains. At 80% similarity, all mecA+ S. aureus were classified to be five distinguishable types, with PSU124 being located in the most distantly related (Figure 2).

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by disk diffusion method using 15 antimicrobial agents. Five mecA+ S. aureus strains that positive for vWbp and spa, were subjected to the test. One pathogenic strains, PSU127 resisted to penicillin G, erythromycin, cefazidime and fusidic acid while three pathogenic strains, PSU123, PSU124, and PSU126, revealed the same antimicrobial resistance pattern as PSU127 except they resisted to one extra antimicrobial agent, clindamycin. In addition, PSU125 exhibited the resistance to 6 antimicrobial agents, penicillin G, erythromycin, cefazidime, tetracycline, clindamycin, and fusidic acid. All mecA+ S. aureus were susceptible to vancomycin (Minimal Inhibitory Concentration ranged from 1.0 to 4.0).

Discussion

Various rates of MRSA contamination in meats were reported from many areas of the world. de Boer et al. (2009) investigated MRSA in various kinds of meat samples in retail trades, Netherlands. MRSA was found in beefs, porks, and chickens as 10.6%, 10.7%, and 16%, respectively. In addition, our study was in accordance with the work from Weese et al. (2010). They performed the MRSA investigation in four provinces in Canada. They also found the less contamination of MRSA in pork (9.6%), ground beef (5.6%), and chicken (1.2%). Particularly, no MRSA was detected in pork and beef from Saskatchewan province. Interesting report from Rhode Island, it was demonstrated that 0% of MRSA contamination was found in beef, pork, and chicken meat samples (Chan et al., 2008). Our results were contrasted to the
works from many countries. In this study, high rates of mec\textsuperscript{+} \textit{S. aureus} contamination were discovered in all meat types especially beef (90\%) (Table 2).

Despite the fact that selective and differential media such as CHROMagar MRSA supplemented with cefoxitin can be employed for isolation of MRSA, in our previous experiment, we demonstrated the high detection rate of mec\textsuperscript{+} \textit{S. aureus} in meat samples (unpublished data). Thus, based upon this information, general enrichment media such as tryptic soy broth followed by Mannitol salt agar, were thought to effectively amplify mec\textsuperscript{+} \textit{S. aureus} from the samples. Pu et al. (2009) used tryptcase soy broth supplemented with 10\% NaCl and 1\% sodium pyruvate in enrichment process for obtaining MRSA from retailed meats in Louisiana. Moreover, Weese et al. (2010) applied enrichment broth composed of 1\% tryptone, 7.5\% NaCl, 1\% mannitol, and 0.25\% yeast extract for MRSA isolation from meat marketed in four provinces in Canada. These works have been shown that the ordinary enrichment methods were efficiently capable of isolating MRSA from meat samples. This study emphasized the notion that in the samples obtained from low hygienic environments or where the high prevalence of MRSA has already been reported, the ordinary enrichment and selection methods followed by the indispensable screening, the detection of mec\textsuperscript{A}, can be employed for the isolation of MRSA.

Coagulase is the polypeptide that converts fibrinogen to fibrin, led to the plasma clot (McAdow et al., 2011). Coagulase is encoded by \textit{coa} gene. Approximately 670 amino acids of coagulase are varied among \textit{S. aureus} strains (McAdow et al., 2012). All mec\textsuperscript{+} \textit{S. aureus} in this study exhibited the lack of \textit{coa} gene. Therefore, it has been thought that another type of coagulase, von Willebrand factor binding protein, may be haboured by them. von Willebrand factor (vWF) is a glycoprotein synthesized by endothelial cells and megakaryocytes (Ruggeri, 1999). It is a large glycoprotein composed of 2,050 amino acids. vWF plays a pivotal role in the maintenance of haemostasis especially in the situation of rapid blood flow. Bjerketorp et al. (2002) described the novel virulence factor of \textit{S. aureus} called von Willebrand factor binding protein which functions as coagulase. In this study, vWbp gene was detected by the primers designed based upon the complete sequence of vWbp of seven \textit{S. aureus} strains. This primer pair was thought to effectively detect vWbp from various \textit{S. aureus} strains. We also applied this primer pair to the confirmed clinical MRSA strains containing vWbp (unpublished data) and it was found that the vWbp gene could also be detected clearly. Thus, this primer pair was suggested to be useful for the detection of pathogenic MRSA from various sources. Although vWbp was thought to play a role as coagulase in MRSA in this study, the subsequent investigation of vWbp gene revealed that only four strains, PSU123 to PSU126, possessed this gene. Even though these two main virulence factors responsible for plasma clotting were absence in most isolates, other virulence factors responsible for clotting phenomenon such as clumping factor may be exist and plays a role in pathogenesis.

Staphylococcal protein A is a 40 k Da antiphagocytic protein that covalently anchored to the peptidoglycan of bacterial cell wall. Approximately 90\% of protein A are present in the cell wall. However, the remaining 10\% are located in the bacterial cytoplasm (Shakeri et al., 2010). Protein A of \textit{S. aureus} is encoded by \textit{spa} gene. Shakeri et al. (2010) reported the prevalence of \textit{spa} gene in \textit{S. aureus}. Of 208 \textit{S. aureus}, 8 (3.8\%) lacked \textit{spa} gene detected by PCR method. However, \textit{spa} gene could be found in only 3.4\% of MRSA. Our investigation was corresponded to the work from Shakeri et al. (2010). It was found that only one MRSA strain, PSU127, possessed \textit{spa} gene.

Community-acquired MRSA (CA-MRSA) has been reported to cause an outbreak and gradually increase in playing the pivotal roles in human infections. The cases resulted from CA-MRSA infections were reported to be associated with necrotizing pneumonia or pulmonary abscesses and sepsis. The cases were considered severe and rapidly fatal. Gordon and Lowy (2008) showed that there was a strong epidemiological correlation between CA-MRSA infections and the presence of Panton-Valentine leukocidin. In addition, these CA-MRSA strains were found to harbour SCC\textit{mec} type IV. Therefore, the presence of SCC\textit{mec} type IV and Panton valentine leukocidin are the important makers of CA-MRSA. In this study, although PSU126 haboured SCC\textit{mec} type IV, this strain did not contain Panton Valentine leukocidin. Thus, we consider PSU126 as non-CA-MRSA strain.

Various MRSA strains either from clinical samples or raw meats, were reported to resist many antimicrobial agents (Lee et al., 2008; O’Brien et al., 2012; Boost et al., 2013). O’Brien et al. (2012) investigated MRSA from retail pork products. They found that 65.4\% of identified MRSA isolates were resistant to tetracycline, 38.5\% were resistant to erythromycin and 34.6\% were resistant to clindamycin. These resistant patterns were also found in our MRSA in this study. Recently, one study from Boost et al. (2013) which examined MRSA from...
retail meats in Hong Kong also demonstrated the multiple drug resistance.

The mecA+ S. aureus in this study displayed the susceptibility to oxacillin and cefoxitin. This phenomenon was not surprising because there have been studies documented the oxacillin-sensitive MRSA. O’Brien et al. (2012) has demonstrated that 4 of 26 (15.38%) MRSA isolated from pork products, were susceptible to oxacillin by broth microdilution despite harbouring the mecA gene. Furthermore, the earlier report from Leedom Larson et al. (2011) supported O’Brien et al. (2012) and our works. They sought for MRSA in pork production shower facilities in Iowa and Illinois. Eight MRSA were subjected to antimicrobial susceptibility testing and it was found that two (25%) from swine’s nasal and one (12.5%) from shower floor did not resist to oxacillin. This suggested the presence of oxacillin-sensitive MRSA in the environment. To our comprehension, it was thought that these MRSA strains harboured mecA gene but not produce PBP2a, referred as cryptically methicillin resistant strains (Kobayashi et al., 1994). The primers detecting mecA gene in this study were thought to be reliable in mecA detection because this primer pair has been used for the screening of MRSA from 500 isolates obtained from Mayo Clinic patients, Minnesota, United States (Geha et al., 1994) and 439 patients swabs (nose, throat, groin, perineum, wound, and drainage) from German tertiary care hospital (Jonas et al., 2002). Furthermore, this primer pair was applied for confirmation of many oxacillin-resistant S. aureus from Songklanagarind and VachiraPhuket hospital (data not shown). In addition, mecA gene detection by PCR method was considered indispensable for MRSA screening. Despite the fact that these MRSA were resistant to multiple drugs such as tetracycline, clindamycin and fusidic acid, fortunately, they were still susceptible to vancomycin (Table 4).

MRSA is not generally considered as a food-borne pathogen. However, in this decade, several reports described the presence of enterotoxin-producing MRSA and toxic shock syndrome toxin, the superantigens in meats and other food samples (Jones et al., 2002; Hwang et al., 2007; Wang et al., 2008; de Boer et al., 2009; O’Brien et al., 2012). Thus, the presence of MRSA in meats and food samples are important and able to play a role in food poisoning. The presence of staphylococcal enterotoxins is important for food safety aspect. The classical SEs are known as the common causes of staphylococcal food poisoning. One report from the Republic of Korea, 143 S. aureus isolates obtained from pork and chicken meats were characterized for the presence of SE type by multiplex PCR (Hwang et al., 2007). They showed that 72 of 143 possessed at least one toxin gene. In their work, sea gene was found in only 10 (7%). Moreover, seb, sec, sek, sel and seq genes were not detected. In our study, we used sea gene, one of the common enterotoxins involved in staphylococcal food poisoning, as a sole representative of enterotoxin genes in the investigation of retailed meats. All mecA+ S. aureus isolates revealed the lack of sea gene (Table 3). Nevertheless, although sea was not observed in mecA+ S. aureus, another type of SE-encoding genes were probably exist and may capable of posing a health risk to the populations in this area.

Conclusion

The presence of MRSA in retailed meats is essential in the aspect of food safety. In this study, high numbers of mecA+ S. aureus were observed in all three common meat types sold in this Southern Thai area. Even though one of the common superantigen genes, sea, was not detected in all meat samples, other type of superantigens was probably existed. Moreover, MRSA virulence factors such as vWbp and spa were also detected in several mecA+ S. aureus strains and may contribute to the human pathogenesis. In addition, these strains revealed the resistance to several antimicrobial agents. These finding emphasizes the presence of pathogenic MRSA in Southern Thai environment. Frequent surveillance of MRSA in retailed meats sold in this area, including the attentive hygiene of buchers and consumers, were cautioned to be strictly performed to prevent the outbreaks of this organism.

Acknowledgement

This work was supported by the fund from Faculty of Science, Prince of Songkla University.

References


