ANTIBIOGRAM, ANTIBIOTIC AND DISINFECTANT RESISTANCE GENES, BIOFILM-PRODUCING AND -ASSOCIATED GENES, AND GENOTYPE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES FROM NORTHERN THAILAND

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Abstract. Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a variety of infectious diseases in both hospital and community. The study determined prevalence of antibiotic resistance and associated genes, biofilm-producing phenotype and associated genes, SCC*mec* types, and clonal subtype ST239 of MRSA clinical isolates obtained from three hospitals in northern Thailand during January 2013 to October 2015. Some 95% of MRSA isolates were multidrug resistant, with 82%, 60% and 47% harboring *erm*A, *erm*B and *qac*AB, respectively. Although all MRSA isolates were positive for slime (biofilm) production on Congo red agar, quantitative measurement of biofilm generation using microtiter plate assay (MTP) indicated 60% were low biofilm producers, with prevalence of biofilm-associated genes, *bab*, *cna*, *fnb*A, and *ica*AD, ranging from 50% to 100%. MRSA SCC*mec* type III was predominant, but the presence of SCC*mec* type IV and type V (albeit at low frequency) indicated acquisition of community-acquired infection. Clonal subtype ST239 was detected in 29% of MRSA isolates in hospitals located in the lower and upper northern regions. The information provided by this study should be useful for future active surveillance of MRSA and in the development of the strategies to lower prevalence and to control the spread of this virulent staphylococcal infection in hospitals and the community at large.

Keywords: *Staphylococcus aureus*, antibiogram, biofilm, SCC*mec* type, MRSA, Thailand

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium causing infection in both community and hospital. Most strains of MRSA have developed resistance to most of the other beta-lactam antibiotics (Stefani *et al*, 2012). Resistance to methicillin and other beta-lactam antibiotics is caused by an acquisition of *mec*A located within staphylococcal cassette chromosome *mec* element (SCC*mec*), with SCC*mec* types I, II and III carried by hospital-acquired (HA)-MRSA, rendering resistance to non-beta-lactam antibiotic, while SCC*mec* types IV, V and VI (encoding beta-lactam resistance factors) are carried by community-acquired (CA)-MRSA (encoding beta-lactam resistance (Tulinski *et al*, 2012; Eed *et al*, 2015). Multilocus sequence typing (MLST) has allowed characterization of approximately 90% of HA-MRSA in nine Southeast Asian countries into a single clonal subgroup ST239 (Chongtrakool *et al*, 2006). However, there is little information of MRSA SCC*mec* types as well as their clonal distribution in Thailand.

The majority of MRSA possess an ability to generate biofilm, a crucial property for intracellular persistence and virulence involved in pathogenesis (Oyama *et al*, 2016). Four major genes play key roles in biofilm development and adhesion, namely, bap (encoding biofilm-associated protein; BAP), *cna* (encoding collagen adhesin), *fnb*A (encoding fibronectin-binding protein FnbA), and *ica*AD (located within the intracellular adhesion (*ica*) operon and encoding polysaccharide intracellular adhesin PIA). Bap is involved in the initial attachment and intracellular adhesion (Cucarella *et al*, 2004); PIA mediates cell-to-cell adhesion (Becker *et al*, 2014); FnbA mediates biofilm formation accumulation phase through forming homophilic interactions or through binding of proteins to surface receptors on adjacent cells (Herman-Bausier *et al*, 2015); and collagen adhesin is a virulence factor associated with *S. aureus* adhesion (Saei, 2012). Limited research has been conducted on *S. aureus* biofilm formation in Thailand. From 73% to 95% of Thai *S. aureus* isolates form biofilm (Indrawattana *et al*, 2013; Tangchaisuriya *et al*, 2014; Daoda *et al*, 2015), but 44.4% of clinical MRSA isolates only demonstrated weak biofilm production and there is no association between biofilm formation and MRSA genotypes (Boontha *et al*, 2015). Study of biofilm generation in clinical MRSA isolates from different hospitals in Thailand is necessary to broaden our understanding of biofilm formation.

In this study, phenotypic properties of biofilm and biofilm-associated genes of clinical MRSA isolates from three hospitals in Thailand were characterized, including prevalence of SCC*mec* type, clonal subgroup ST239, antibiogram profile, resistance to disinfectants and carriage of antibiotic and disinfectant resistance genes. Understanding virulence properties, such as biofilm formation, in such pathogenic microbes can provide insightful information towards development of new strategies to ameliorate severity and prevalence of infectious diseases. Molecular characteristics, such as clonal distribution, should be useful for future active surveillance aimed at controlling the spread of existing antimicrobial resistant bacteria such as MRSA.

MATERIALS AND METHODS

Isolation and identification of MRSA isolates

A total of 38 MRSA isolates were col-

lected from three hospitals in Thailand, namely, Chiangrai Prachanukroh Hospital (HA), a large tertiary hospital located in the northern region, Naresuan University Hospital (HB) and Sawanpracharak Hospital (HC) located in the lower northern region (see Fig 2). Samples from HA (n = 23) and HB (n = 9) were collected from November 2014 to October 2015 and from HC (n = 6) in January 2013. Isolates were collected from multiple sites, such as blood, pus, sputum, urine and other body fluids, and identified as *S. aureus* using a coagulase test, detection of 16S rDNA and *nuc* by PCR amplification (Sasaki *et al*, 2010) and as MRSA using a cefoxitin disk (30 μg) (Oxoid, Hampshire, UK) diffusion test on Mueller-Hinton agar (Hi-Media, Mumbai, India) and PCR-based detection of *mec*A (Kitti *et al*, 2011).

Antibiogram determination

Antibiotic susceptibilities to chloramphenicol (C; 30 μg), ciprofloxacin (CIP; 5 μg), clindamycin (DA; 2 μg), erythromycin (E; 15 μg), fusidic acid (FD; 10 μg), gentamicin (CN; 10 μg), linezolid (LZD; 30 μg), mupirocin (MUP; 5 μg), novobiocin (NV; 5 μg), oxacillin (OX, 1 μg), penicillin (P; 10 units), rifampicin (RD; 5 μg), sulfamethoxazole/trimethoprim (SXT; 1.25/23.75 μg), and vancomycin (VA; 30 μg) (Oxoid, Hampshire, UK) were determined using a disk diffusion method (CLSI, 2014). An isolate is categorized as multidrug resistant (MDR) if resistant to at least three classes of antibiotics.

Detection of SCC*mec* types and clonal subgroup ST239

SCC*mec* types were determined by multi- and uniplex PCR according to a method modified from a previous study (Zhang *et al*, 2005). In brief, amplification was performed in a total volume of 25 μl containing 3 μl of 10X buffer (RBC Bioscience corp, Taipei, Taiwan) containing 15 mM Mg^{2+}, 2.5 μl of 2.5 mM dNTPs, 0.2 μl of 5 U *Taq* polymerase (RBC Bioscience), various concentrations of each primer (Zhang *et al*, 2005) and 3 μl of DNA template. Thermocycling (GeneMate thermal cycler, Hangzhou, China) was conducted as follows: 94ºC for 4 minutes; followed by 30 cycles of 94ºC for 20 seconds, 55ºC for 30 seconds and 72ºC for 30 minutes; and a final step of 72ºC for 5 minutes.

Clonal subgroup ST239 was identified by a PCR method using primer sets and conditions as described previously (Feil *et al*, 2008). In short, a total volume of 25 μl containing 1X PCR buffer (RBC Bioscience), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.7 μM each of the four primers, 1.25 U *Taq* polymerase (RBC Bioscience), and 2.5 μl of DNA was amplified with the following thermocycling (GeneMate thermal cycler, Hangzhou, China) conditions: 95°C for 15 minutes; followed by 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; and a final step of 72°C for 7 minutes. Amplicons were analyzed by 1% agarose gel-electrophoresis and staining with ethidium bromide.

Detection of biofilm-associated, antibiotic and disinfectant resistance genes in MRSA isolates

Primers employed in detection of MRSA biofilm-associated genes bab, *fnb*A and *ica*AD, *cna* and antibiotic and disinfectant resistance genes *erm*A, *erm*B, *erm*C, and *qac*AB are listed in Table 1. Bacteria were grown on tryptone soya agar (Hi-Media, Mumbai, India) and DNA was extracted using a boiling method. For bab, *fnb*A, *ica*AD and *cna*, PCR mixture (20 μl) contained 2 μl of 10X PCR buffer, 0.2 μl of 25 mM $MgCl_2$, 2 μl of 2.5 mM dNTPs, 0.2 μl of 10 μM each primer, 0.15 μl of 5 U Taq polymerase (RBC Bioscience), and 2 μl of DNA. Thermocycling of each gene

Table 1
Primer used in the study.

Target gene	Primer	Amplicon size (bp)	Tm (°C)	Reference
*erm*A	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190	56.7	Strommenger *et al* (2003)
*erm*B	F: AATCGTCAATTCCTGCATGT R: TAATCGTGG AATACGGGTTTG	142	55.9	Chaieb *et al* (2007)
*erm*C	F: AATCGTCAATTCCTGCATGT R: TAATCGTGG AATACGGGTTTG	299	62	Strommenger *et al* (2003)
*qac*AB	F: GCAGAAAGTGCAGAGTTCG R: CCAGTCCAATCATGCCTG	361	57.3	Zhang *et al* (2011)
ST239/ST 30	F: TCGCACTCTCGTTGAACA R: AAATCCGCTTCGACAAACATT	484	57	Feil *et al* (2008)
ST239/ST 8	F: CACTTTAAATACTGACGAAAAT R: TTGAAAATTGATCATTCAGCAA	220	53	Feil *et al* (2008)
bap (MRSA)	F: CCCTATATCGAAGGTGTAGAATTGCAC R: GCTGTTGAAGTTAATACTGTACCTGC	971	58	Cucarella *et al* (2001)
*fnb*A (MRSA)	F: GATACA AACCCAGGTGGTGG R: TGTGCTTGACCATGCTCTTC	191	55	Arciola *et al* (2005)
*ica*AD (MRSA)	F: AATGTGCTTGGATGCAGATACTAT C R: GAATCGTCATCTGCATTTGCA	1,216	61	This study
cna (MRSA)	F: AAAGCGTTGCCTAGTGGAGA R: AGTGCCTTCCCAAACCTTTT	192	55	Smith *et al* (2010)
bap (MR-CoNS)	F: GGCGCAAGCAGCAGAATTA R: CATAGTTCTTTGTGGTGTTGC	901	63	Seng *et al* (2017)
*fnb*A (MR-CoNS)	F: CCCTCTTCGTTATTCAGCC R: CAGGAGGCAAGTCACCTTG	422	58	Seng *et al* (2017)
*ica*AD (MR-CoNS)	F: GACAGTCGCTACGAAAAG R: AATAAGCTCCTAACTA	211	55	Seng *et al* (2017)

MR-CoNS, methicillin-resistant coagulase negative *Staphylococcus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

was conducted in DNA thermal cycler (GeneMate thermal cycler) individually as previously described (Table 1) and amplicons were analyzed as described above.

The other antibiotic and disinfectant resistance genes, *erm*A, *erm*B, *erm*C, and *qac*AB were detected by PCR modified as previously described (Ardic *et al*, 2005; Zhang *et al*, 2011; Youn *et al*, 2014). In brief, PCR mixture (20 µl) consisted of 2.4 µl of 10X PCR buffer containing 15 mM $MgCl_2$, (RBC Biosience) 2 µl of 2.5 mM dNTPs, 0.2 µl of 10 µM each primer, 0.2 µl of 5 U HotStart Taq DNA polymerase (RBC Biosience), and 2 µl of DNA. Thermocycling was performed in a GeneMate thermal cycler as follows: 95ºC for 5 minutes; followed by 30 cycles of 94ºC for 25 seconds, 57ºC for 30 seconds and 72ºC for 30 seconds; with a final step of 72ºC for 5 minutes. Amplicons from representative isolates were purified from gels using QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and directly sequenced by Macrogen (Seoul, Korea).

Detection of biofilm formation

Biofilm formation of MRSA isolates was detected on Congo red agar (CRA) plates as previously described (Seng *et al*, 2017). In brief, MRSA isolate was streaked on CRA plate (Hi-Media) and incubated at 35°C under aerobic conditions for 24-48 hours. Slime (biofilm)-producing MRSA formed black (indicated by a darkening of colony with an absence of dry crystalline colonial morphology) or very black (indicated by a darkening of colony with presence of dry crystalline colonial morphology) colonies while non-slime (biofilm) producer strain formed red colonies. Qauntification of biofilm formation was performed by a microtiter plate assay (MTP) (Bekir *et al*, 2011). In short, MRSA isolates were cultured in trypticase soy broth (Hi-Media) supplemented with 0.25% glucose in 96-well polystyrene tissue culture microtiter plates (Nunc, Roskilde, Denmark) overnight at 37°C, each experiment being conducted in triplicate. Culture medium then was removed and adherent cells were treated with 95% ethanol and stained with 1% crystal violet. $A_{570\,nm}$ was measured and data interpreted as follows: highly positive ($A_{570\,nm} \geq 1$), low grade positive ($0.1 \leq A_{570\,nm} < 1$) and negative ($A_{570\,nm} < 0.1$).

RESULTS

Antibiogram profile

Based on a disc diffusion assay, 76-96% of the MRSA isolates were resistant to ciprofloxacin, clindamycin, erythromycin, gentamicin, and penicillin; <10% resistant to chloramphenicol, mupirocin and rifampicin; and none resistant to fusidic acid, linezolid, novobiocin, and vancomycin (Fig 1). MDR-MRSA constituted 95% of the isolates.

Prevalence of antibiotic and disinfectant resistance genes

Employing PCR to identify the target genes, prevalence of *erm*A, *erm*B, *erm*C, and *qac*AB among the MRSA isolates was 82, 60, 8 and 47%, respectively (Table 2).

SCC*mec* types and prevalence of clonal subgroup ST239

Using multi- and uniplex PCR, MRSA isolates were characterized as SCC*mec* type I (n = 10; 26%), type II (n = 10; 26%), type III (n = 15; 39%), type IV (n = 2; 5%), and type V (n = 1; 3%) (Fig 2). Clonal subgroup ST239 was detected in 11 (29%) isolates, 10 from HA and 1 from HB.

Biofilm formation and biofilm-associated genes

Observation of colony phenotypes on CRA plates, 7 (18%) isolates formed very black colonies, 31 (82%) black colonies and none red colonies, indicating that all

Table 2
Prevalence of antibiotic and disinfectant resistance genes of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from three hospitals (HA, HB and HC), Thailand, January 2013 - October 2015.

Antibiotic/disinfectant resistance gene	MRSA from HA Number (%) (n = 23)	MRSA from HB Number (%) (n = 9)	MRSA from HC Number (%) (n = 6)	Total (%) (n = 38)
*erm*A	18 (78)	8 (89)	5 (83)	31 (82)
*erm*B	17 (74)	1 (11)	5 (83)	23 (60)
*erm*C	1 (4)	1 (11)	1 (17)	3 (8)
*qac*AB	6 (26)	7 (78)	5 (83)	18 (47)
SCC*mec* I	6 (26)	2 (22)	2 (33)	10 (26)
SCC*mec* II	1 (4)	5 (56)	4 (67)	10 (26)
SCC*mec* III	13 (56)	2 (22)	0 (0)	15 (39)
SCC*mec* IV	2 (9)	0 (0)	0 (0)	2 (5)
SCC*mec* V	1 (4)	0 (0)	0 (0)	1 (3)
ST239	10 (43)	1 (11)	0 (0)	11 (29)

isolates were slime (biofilm) producers. However, quantification of biofilm formation using a MTP assay showed 23 isolates (60%) were low producers and the remaining non-biofilm producers (Table 3). Only 1 isolate (11%) of MRSA isolated from HB was a non-biofilm producer, while almost 50% of isolates from HA and HC were negative for biofilm production.

Two sets of primers including the primers designed from MRSA and methicillin-resistant coagulase negative *Staphylococcus* (MR-CoNS) were used to amplify bab, *fnb*A and *ica*AD. Only a pair of primers was used to detect *cna*. PCR amplification of target genes using MRSA-based primers showed prevalence of bab, *fnb*A, *ica*AD, and *cna* was 100%, 63%, 53% and 50%, respectively; and employing MR-CoNS-based primers, prevalence of bab, *fnb*A and *ica*AD was 100%, 81% and 100%, respectively (Table 4). It is worth noting that the frequencies of *fnb*A and *ica*AD detected using MR-CoNS-primers were higher than those of MRSA-based primers.

DISCUSSION

MRSA is a pervasive pathogen recognized as a major health threat worldwide that possesses a variety of virulence factors involved in pathogenesis such as biofilm formation (Oyama *et al*, 2016). In order to strengthen an understanding of MRSA antibiotic resistance, dissemination and virulence, presence, phenotypic and genotypic antibiotic resistance, and biofilm formation were examined in 38 MRSA isolates from three different hospitals in northern Thailand.

High rates of MRSA resistance to ciprofloxacin, clindamycin, erythromycin, and gentamicin in this study were similar to other reports in middle region of Thailand (Phokhaphan *et al*, 2017) and Asia (Song *et al*, 2011), but the rate was approximately 40-50% higher than clinical MRSA isolated in India (Pai *et al*, 2010). A recent study found only 48% and 29% of MRSA isolated from Oman were resistant to erythromycin and clindamycin, respec-

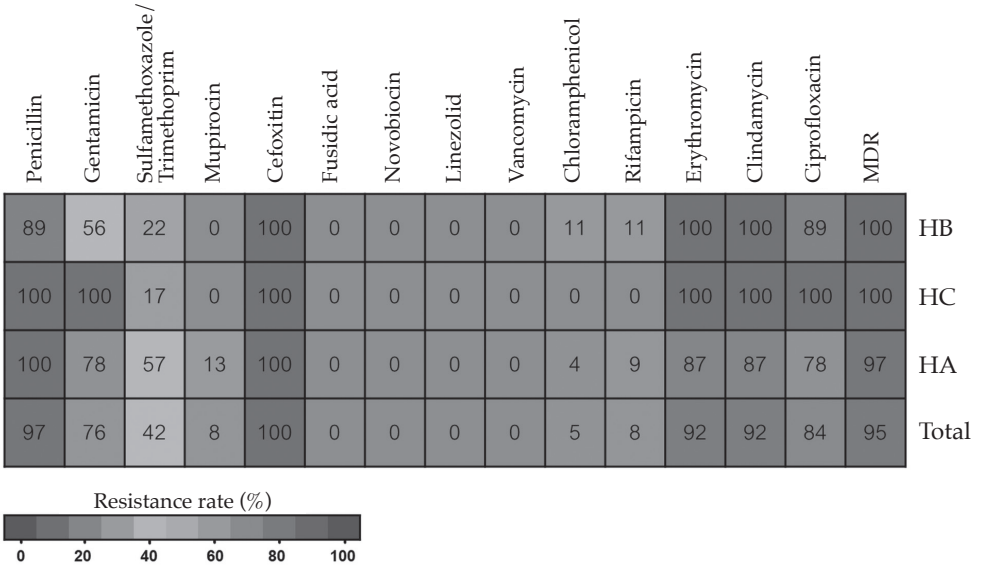

Fig 1 - Antibiogram profiles of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from three hospitals (HA, HB and HC), Thailand, January 2013 - October 2015. Antibiotic susceptibility was determined using a disc diffusion assay. Number of isolates from HA, HB and HC was 23, 9 and 6, respectively. MDR, multidrug resistant.

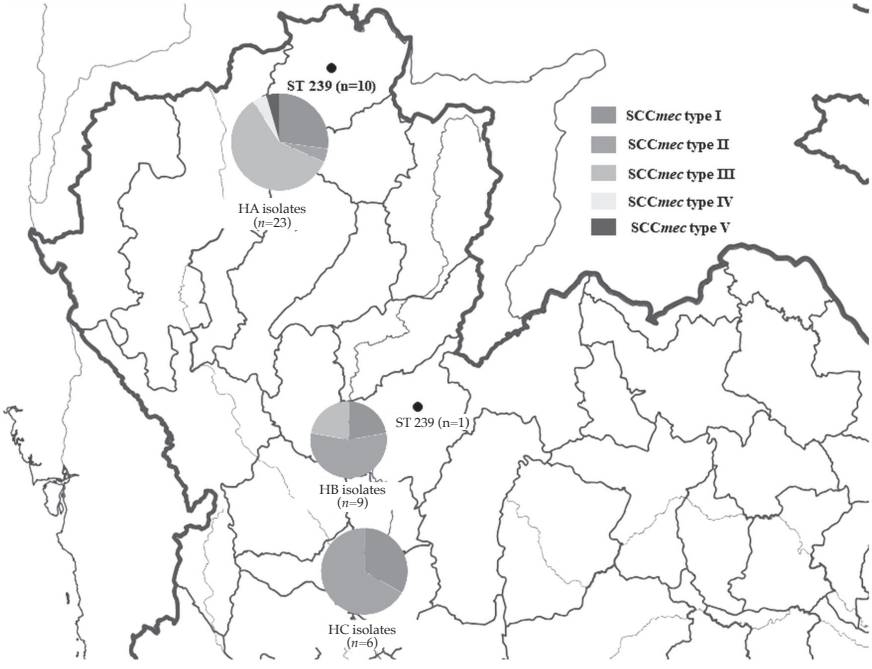

Fig 2 - Location of the three hospitals (HA, HB and HC) in northern Thailand, and frequency of SCC*mec* types and number of clonal subtype ST239 of clinical methicillin-resistant *Staphylococcus aureus* isolates at each hospital, January 2013 - October 2015. SCC*mec* types clonal subtype ST239 were identified using PCR-based methods.

Table 3
Biofilm formation using microtiter plate (MPT) and Congo red agar (CRA) assays of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from three hospitals (HA, HB and HC), Thailand, January 2013 - October 2015.

Assay	MRSA from HA Number (%) (n = 23)	MRSA from HB Number (%) (n = 9)	MRSA from HC Number (%) (n = 6)	Total (%) (n = 38)
CRA				
Very black	5 (22)	2 (22)	0 (0)	7 (18)
Black	18 (78)	7 (78)	6 (100)	31 (82)
Red	0 (0)	0 (0)	0 (0)	0 (0)
MTP				
Strong biofilm producer	0 (0.0)	0 (0)	0 (0)	0 (0)
Low biofilm producer	12 (52)	8 (81)	3 (50)	23 (60.5)
Non-biofilm producer	11 (48)	1 (11)	3 (50)	15 (39.5)

Table 4
Biofilm-associated genes of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from three hospitals (HA, HB and HC), Thailand, January 2013 - October 2015.

Biofilm-associated gene	MRSA from HA Number (%) (n = 23)	MRSA from HB Number (%) (n = 9)	MRSA from HC Number (%) (n = 6)	Total (%) (n = 38)
MRSA-based primers				
bap	23 (100)	9 (100)	6 (100)	38 (100)
*fnb*A	23 (100)	0 (0)	1 (17)	24 (63)
*ica*AD	14 (61)	3 (33)	3 (50)	20 (53)
cna	6 (26)	7 (78)	6 (100)	19 (50)
MR-CoNS-based primers				
bap	23 (100)	9 (100)	6 (100)	38 (100)
*fnb*A	20 (87)	7 (78)	4 (67)	31 (82)
*ica*AD	23 (100)	9 (100)	6 (100)	38 (100)

MR-CoNS, methicillin resistant coagulase negative *Staphylococcus*.

tively (Pathare *et al*, 2016). Therefore, resistance to erythromycin and clindamycin in MRSA are likely related to collection period, source and geographical region.

Consistent with the high frequency of erythromycin resistance, high carriage rates of *erm*A and *erm*B (encoding erythromycin ribosomal methylase A and B) were detected, in agreement with studies of *S. aureus* from patients in Malaysia (Lim *et al*, 2012). Reduced susceptibility to disinfectant chlorhexidine is associated with the carriage of *qac*AB (encoding *Qac*A protein) (Horner *et al*, 2012). Although suscepti-

bility of MRSA isolates to chlorhexidine was not determined, the proportion of isolates harboring *qac*AB was similar to that in West African (Conceição *et al*, 2016). MRSA carrying *qac*AB is more often diagnosed in patients with nosocomial and catheter-related blood than bone and soft-tissue infections (Cho *et al*, 2017). MRSA with *qac*AB also is associated with resistance to ciprofloxacin, clindamycin and mupirocin resistance (Lee *et al*, 2013). Thus, the presence of *qac*AB may play a role in the virulence of MRSA.

MRSA isolates collected from Thai hospitals previously were demonstrated to carry only SCC*mec* type II and III (Lulitanond *et al*, 2010), while SCC*mec* type IV and V were also detected in this study, suggesting the spread of CA-MRSA infection into hospitals. The invasive MRSA clonal subtype ST239 causes a wide range of infections across the world, particularly in Asia (Feil *et al*, 2008). Nearly 50% of ST 239 isolates were biofilm producers. Biofilm producing- ST 239 has been reported to resistant to ciprofloxacin, erythromycin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole (Shahsavan *et al*, 2011; Aparecida *et al*, 2012). Some 30% of MRSA isolates in this study were ST239, almost all obtained from hospitals located in both the upper and lower northern regions of the country. It is imperative to determine how widespread this MRSA strain in hospitals is across the country.

Biofilm formation is one of the virulence factors of staphylococci that decrease the treatment effectiveness by reducing sensitivity to antibiotics and immune response (Stewart and Costerton, 2001). All MRSA isolates exhibited phenotypic slime (biofilm) production by the presence of black or very black colonies on CRA plates as has been reported in Iran where 97.5% of MRSA isolates are slime (biofilm) producers (Moghadam *et al*, 2014). However, quantitative biofilm assay indicated no MRSA isolates were strong biofilm producers and less than two thirds were low biofilm producer, consisted with that (44%) reported by Boontha *et al* (2015), but much lower than other findings (73-95%) in Thailand (Indrawattana *et al*, 2013; Tangchaisuriya *et al*, 2014; Daoda *et al*, 2015), suggesting a possible decline in biofilm-producing MRSA in the country. Although CRA method is easy and takes little time to perform, the poor agreement between CRA and MTP methods was found in the present study and this phenomenon also was reported by previous studies (Darwish and Asfour, 2013; Mathur *et al*, 2013). Therefore, CRA alone could not be used to detect the biofilm formation. It is worth pointing out the majority of biofilm producers were SCC*mec* types I and II, indicators of HA-MRSA.

S. aureus adherence to a biotic or an abiotic surface is mediated by a number of microbial surface components-recognizing adhesive matrix compounds and the expression of such matrix-attachment molecules has received increasing attention following the discovery of biofilm associated genes (Otto, 2008). We detected four major biofilm associated genes (bab, *fnb*A, *ica*AD and *cna*) using two sets of primers (MRSA and MR-CoNS primers). Using MRSA-based primers, high prevalence of biofilm associated genes bab, *cna*, *fnb*A, and *ica*AD has been observed, ranging from 50% to 100%. Daoda *et al* (2015) reported prevalence of *fnb*A and *ica*AD of MSSA was equal to those of MRSA. Tangchaisuriya *et al* (2014) previously observed 93%, 92% and 61% of *S. aureus* carry *fnb*A, *ica*AD and *cna*, higher than the current study. In addition, the presence of *ica*AD, amplified using MRSA-based primers, was approximately half of that detected using MR-CoNS primers while

the frequency of other genes detected was unchanged. This difference may be due to cross-hybridization of the MR-CoNS primers.

In conculsion, this study reveals 95% of MRSA isolates from three hospitals in northern Thailand were MDR and carried a variety of SCC*mec* types, including those associated with community-acquired infection, and virulence genes. MRSA clonal subtype ST239 presented in two hospitals located in the lower and higher northern regions suggesting dissemination of this clone in northern Thailand. High prevalence of phenotypic and genotypic biofilm formation also were detected. These data are essential in the development new strategies to lower the severity and prevalence of infectious diseases caused by pathogenic MRSA.

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