INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that causes a variety of diseases ranging from mild skin and wound infections to life-threatening diseases. In humans, it commonly inhabits the nasal passages and skin surface. Most strains of S. aureus have developed antibiotic resistance, which is a serious problem for treating infections with this bacterium. Methicillin-resistant S. aureus (MRSA) has acquired the ability to survive in the presence of beta-lactam antibiotics, including penicillin, methicillin, and cephalosporins. The resistance mechanism is the production of a penicillin-binding protein PBP-2' (PBP-2a) encoded by the mecA gene (Malachowa and Deleo, 2010). The mecA gene is located on a mobile genetic element called Staphylococcal Cassette Chromosome mec (SCCmec) (Malachowa and Deleo, 2010). To date, eight different SCCmec types have been identified (Zhang et al, 2008; Nastaly et al, 2010). Expression of the mecA gene is controlled by the regulator proteins, MecI and MecR1. High level methicillin resistance related to femA, a gene in the femAB operon, is required for the formation of the pentaglycine interpep-
tide bridge that serves as the crosslink for peptidoglycan (Li et al., 2008).

Hospital acquired MRSA (HA-MRSA) is an important nosocomial pathogen and is usually associated with predisposing risk factors. Community acquired MRSA (CA-MRSA) has emerged worldwide, particularly among children (Shopsin et al., 2000; Mollaghan et al., 2010; Mollema et al., 2010; Simor et al., 2010). CA-MRSA causes skin and soft tissue infections and 40-90% of CA-MRSA strains are accompanied by an exotoxin called Panton-Valentine leukocidin toxin (Vandenesch et al., 2003).

*S. aureus* nasal colonization increases the risk of acquiring *S. aureus* infections as evidenced by the same *S. aureus* genotypes found in the nose and in the clinical infections (Reinoso et al., 2006). Carriers are asymptomatic and transmission of MRSA can occur from one individual to another. Since community-based studies are required to understand the epidemiology of *S. aureus* nasal colonization, the aim of this study was to identify the prevalence of nasal colonization with methicillin sensitive *S. aureus* (MSSA) and MRSA and to study drug resistance patterns in these isolates from healthy young adults.

**MATERIALS AND METHODS**

**Bacterial isolation and identification of *S. aureus***

Nasal swab samples were collected from 200 healthy volunteers (36 males and 164 females; age range 19-25 years) from October 2009 to September 2010. All subjects were third-year students (Bachelor of Science degree in Microbiology or Medical Sciences), and graduate students (degree in Microbiology), Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand.

Swab samples were streaked onto mannitol salt agar (HiMedia, Mumbia, India) plates and incubated at 35°C for 24 hours. Cultures with yellow colonies were selected and evaluated using Gram’s stain, catalase, DNase, mannositol fermentation and coagulase tests. Isolates were identified as *S. aureus* if they had positive test results for catalase, DNase, and coagulase tests.

**Detection of MRSA**

All *S. aureus* isolates were screened for MRSA, using the oxacillin salt screening method (Shopsin et al., 2000; Siripornmongcolchae et al., 2002), cefoxitin and oxacillin disk agar diffusion method (Broekema et al., 2009).

**Antimicrobial susceptibility test**

The antibiotic resistance of *S. aureus* isolates was determined using the disc diffusion method (CLSI, 2007). Mueller-Hinton agar plates were used to determine antibiotic susceptibility to oxacillin (1 µg), penicillin (10 UI), erythromycin (15 µg), vancomycin (30 µg), trimethoprim/sulfamethoxazole (1.5 µg/23.75 µg), chloramphenicol (30 µg), gentamycin (10 µg), rifampicin (5 µg), clindamycin (2 µg), linezolid (30 µg), cefoxitin (30 µg), mupirocin (5 µg), ciprofloxacin (5 µg) and fusidic acid (10 µg) (Oxoid, UK). The plates were incubated at 35°C for 24 hours. The zones of inhibition determined whether the microorganism was susceptible, intermediate resistant, or resistant to each antibiotic.

**Detection of *S. aureus* mecA, femA and SCCmec types by PCR**

The methicillin resistance gene (*mecA*) was detected according to the method of Ryffel et al. (1990). Two oligonucleotide primers (5’TGGCTATCGTGTCAACAATCG 3’ and 5’CTGGAACTTTGAGGCAGCAG 3’) were used and the amplification reaction was performed: 1 cycle for 2 minutes
at 94°C, 30 cycles for 20 seconds at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C, and 1 cycle for 5 minutes at 72°C using *S. aureus* strain COL as a positive control and *S. aureus* strain NTCT 8325 as a negative control.

**FemA** was detected according to the method of Vannuffel *et al* (1995) using oligonucleotide primers (5’ CTTACTTACTG GCTGTACCTG 3’ and 5’ ATGTCGCTTGT TATGTGC 3’). A PCR was carried out: (1 cycle for 2 minutes at 94°C), 30 cycles for 20 seconds at 94°C, 30 seconds at 59°C, and 45 seconds at 72°C, and 1 cycle for 5 minutes at 72°C using *S. aureus* strain COL as a positive control.

**SCC**mec typing was determined using specific primers for SCCmec types (I-V) as described by Zhang *et al* (2005). The PCR assay was performed using the protocol as described previously (Zhang *et al*, 2005).

### RESULTS

**Nasal colonization of methicillin-sensitive *S. aureus* (MSSA) and MRSA**

The incidences of nasal colonization with coagulase negative staphylococci (CNS) and *S. aureus* among 200 healthy young Thai adults were 11% (22 of 200), and 15% (30 of 200), respectively. Of the 30 *S. aureus* isolates 28 were MSSA and 2 were MRSA.

**Antimicrobial susceptibility testing**

All *S. aureus* isolates were tested for antibiotic susceptibility patterns (Table 1). Resistance to penicillin, erythromycin, clindamycin, cefoxitin and oxacillin among all *S. aureus* isolates (both MSSA and MRSA) were found in 96.7, 26.7, 26.7, 6.7 and 6.7%, respectively. One MRSA isolate was resistant to penicillin, erythromycin, cefoxitin and oxacillin, the other isolates were resistant to penicillin, clindamycin, cefoxitin and oxacillin. None of the *S. aureus* isolates were resistant to gentamicin, chloramphenicol, trimethoprim/sulfamethoxazole, rifampicin, linezolid, fusidic acid, mupirocin, ciprofloxacin or vancomycin.

**Detection of *S. aureus* mecA, femA and SCCmec types by PCR**

The presence of the mecA gene in the 2 MRSA isolates was confirmed by PCR and an amplified 310 bp mecA DNA fragment was present in both MRSA isolates (Fig 1). Both MRSA isolates were also tested for the presence of femA and found to be negative (Fig 2). A PCR assay to determine SCCmec type was performed, both isolates carried the type II SCCmec element (Fig 3).

### Table 1

Drug resistance patterns of *S. aureus* isolated from nasal carriers.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MRSA (n=2) (%)</th>
<th>MSSA (n=28) (%)</th>
<th>All <em>S. aureus</em> isolates (n=30) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin (30 µg)</td>
<td>2 (100)</td>
<td>0</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Oxacillin (1 µg)</td>
<td>2 (100)</td>
<td>0</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Penicillin (10 UI)</td>
<td>2 (100)</td>
<td>27 (96.4)</td>
<td>29 (96.7)</td>
</tr>
<tr>
<td>Erythromycin (15 µg)</td>
<td>1 (50)</td>
<td>7 (25)</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Clindamycin (2 µg)</td>
<td>1 (50)</td>
<td>7 (25)</td>
<td>8 (26.7)</td>
</tr>
</tbody>
</table>

MRSA-methicillin resistant *S. aureus*  
MSSA-methicillin sensitive *S. aureus*
DISCUSSION

The discovery of MRSA among healthy subjects raises concerns about the changing epidemiology of MRSA. The prevalence of MRSA nasal colonization in the Thai community increased between previous studies in 1996 (Dhiraputra et al, 1996) and 2006 (Lertcanawanichakul et al, 2006) and our study. No MRSA carriers were detected in 1996 or 2006, but the prevalence of MRSA nasal colonization in our study was 1%. Other studies have shown a globally increasing prevalence of MRSA nasal colonization in the community (Grundmann et al, 2002; Beam and Buckley, 2006; Chatterjee et al, 2009; Mollaghan et al, 2010). Low prevalence rates (0.7-1%) were found in the USA and Europe, but much higher rates (2.5-5.3%) have been found in Asia. Risk factors that increase the prevalence of MRSA nasal colonization in Asia may be antibiotic misuse and lower socioeconomic status (Beam and Buckley, 2006; McMullen et al, 2009).

Patients who contract MRSA in the hospital may harbor the organisms for a long time. Both students with MRSA were retested and found to be colonized with MRSA for 6 months. Most MRSA isolated from the community is usually associated with people who have health associated risk factors or contact with high risk persons (Beam and Buckley 2006; Halablab et al, 2010). In our study, both MRSA carriers had been hospitalized, had outpatient visits and had been exposed to antibiotics during the past year. PCR characterization of SCCmec type revealed both MRSA isolates were type II SCCmec element. A previous
study in Thailand (Lulitanond et al, 2010) showed patients with HA-MRSA at Srinagarind Hospital in Thailand carried type II and type III SCCmec elements. The main molecular marker of HA-MRSA is SCCmec element types I, II and III (Nastaly et al, 2010). This data suggests both MRSA carriers in our study contracted HA-MRSA. The transmission of HA-MRSA to the community needs further investigation.

A PCR for mecA and femA genes is the gold standard for determining HA-MRSA. A previous study by Kobayashi et al (1994) found the mecA gene in 100% of MRSA cases and the femA gene in 89.4% of MRSA cases. In our study, the mecA gene was detected in both MRSA isolates, but the femA gene was not detected in either of the MRSA isolates. The failure to detect femA gene may have been from a mutation in the femA sequence preventing detection with PCR.

Similar to a study by Reinoso et al (2006), a high percentage of antibiotic resistance of S. aureus isolates was observed against penicillin and erythromycin; 96.7% of isolates were resistant to penicillin. Penicillin resistance is acquired with plasmid-encoded beta-lactamases; this gene spreads efficiently by horizontal transmission to other strains of S. aureus. Previous studies of antibiotic susceptibilities among S. aureus strains harboring SCCmec type II showed resistance to multiple antibiotics (Kilic et al, 2006). However, the MRSA isolates in this study were resistant to only 5 antimicrobial agents; penicillin, erythromycin, clindamycin, cefoxitin and oxacillin.

In this study, we assessed the nasal colonization of MSSA and MRSA in a community population. Two MRSA isolates were identified, both had SCCmec type II; both were in students who had a history of hospitalization. The data show prevention strategies for MRSA need to be developed for the community as well as in the clinical setting.

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REFERENCES

Antimicrobial Resistance of Methicillin-resistance S. aureus


