PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG UNIVERSITY STUDENTS IN THAILAND

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Abstract. We studied the prevalence of methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) nasal colonization among healthy young Thai adults. MSSA nasal colonization was found in 30 of 200 subjects (15%). The prevalence of MRSA nasal carriage was 1% (2 of 200) detected by cefoxitin/oxacillin disk diffusion and oxacillin salt screening methods. These carriers were associated with health care risk factors. The two MRSA isolates were *mecA* positive, SCC*mec* type II. All *S. aureus* isolates were tested for antibiotic resistance. Their resistance rates to penicillin, erythromycin, clindamycin, oxacillin and cefoxitin were 96.7, 26.7, 26.7, 6.7 and 6.7%, respectively. All MSSA and MRSA isolates were susceptible to gentamicin, chloramphenicol, trimethoprim/sulfamethoxazole, rifampicin, linezolid, fusidic acid, mupirocin, ciprofloxacin and vancomycin. The results of this first study of MRSA nasal colonization among healthy young Thai adults suggests MRSA is present in the Thai community.

Keywords: *Staphylococcus aureus,* antimicrobial resistance, methicillin resistance nasal colonization, *mecA*

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* (SCC*mec*) (Malachowa and Deleo, 2010). To date, eight different SCC*mec* 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-

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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 35ºC for 24 hours. Cultures with yellow colonies were selected and evaluated using Gram's stain, catalase, DNase, mannitol 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; Siripornmongcolchai *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 g), penicillin (10 UI), erythromycin (15 g), vancomycin (30 g), trimethoprim/sulfamethoxazole (1.5 g/23.75 g), chloramphenicol (30 g), gentamycin (10 g), rifampicin (5 g), clindamicin (2 g), linezolid (30 g), cefoxitin (30 g), mupirocin (5 g), ciprofloxacin (5 g) and fusidic acid (10 g) (Oxoid, UK). The plates were incubated at 35ºC for 24 hours. The zones of inhibition determined whether the microorganism was susceptible, intermediately resistant, or resistant to each antibiotic.

Detection of *S. aureus mec*A, *femA* and SCC*mec* types by PCR

The methicillin resistance gene (*mecA*) was detected according to the method of Ryffel *et al* (1990). Two oligonucleotide primers (5'TGGCTATCGTGTCACAATCG 3'and 5'CTGGAACTTGTTGAGCAGAG 3') were used and the amplification reaction was performed: 1 cycle for 2 minutes

Table 1
Drug resistance patterns of *S. aureus* isolated from nasal carriers.

Antibiotics	MRSA (*n*=2) (%)	MSSA (*n*=28) (%)	All *S. aureus* isolates (*n*=30) (%)
Cefoxitin (30 g)	2 (100)	0	2 (6.7)
Oxacillin (1 g)	2 (100)	0	2 (6.7)
Penicillin (10 UI)	2 (100)	27 (96.4)	29 (96.7)
Erythromycin (15 g)	1 (50)	7 (25)	8 (26.7)
Clindamycin (2 g)	1 (50)	7 (25)	8 (26.7)

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

at 94ºC, 30 cycles for 20 seconds at 94ºC, 30 seconds at 56ºC, and 30 seconds at 72ºC, and 1 cycle for 5 minutes at 72ºC 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 94ºC), 30 cycles for 20 seconds at 94ºC, 30 seconds at 59ºC, and 45 seconds at 72ºC, and 1 cycle for 5 minutes at 72ºC using *S. aureus* strain COL as a positive control.

SCC*mec* typing was determined using specific primers for SCC*mec* 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 SCC*mec* 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 SCC*mec* type was performed, both isolates carried the type II SCC*mec* element (Fig 3).

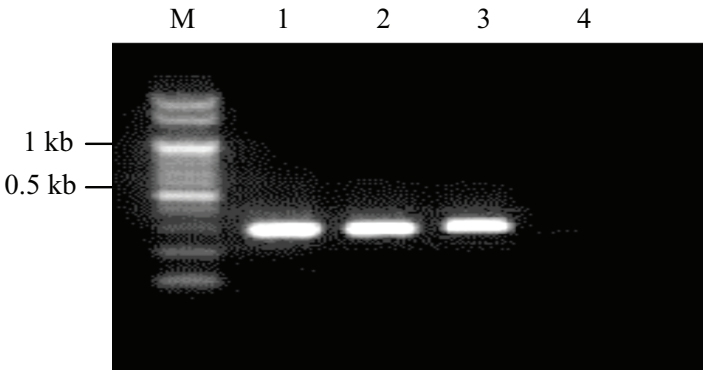

Fig 1–Agarose gel electrophoresis of PCR product of *mecA* gene. A 310 bp band of *mecA* gene from *S. aureus* isolates detected by 0.7% agarose gel electrophoresis. Lane M, DNA marker; lane 1, positive control *S. aureus* strain COL; lane 2, MRSA isolate NU007; lane 3, MRSA isolate NU042; lane 4, negative control.

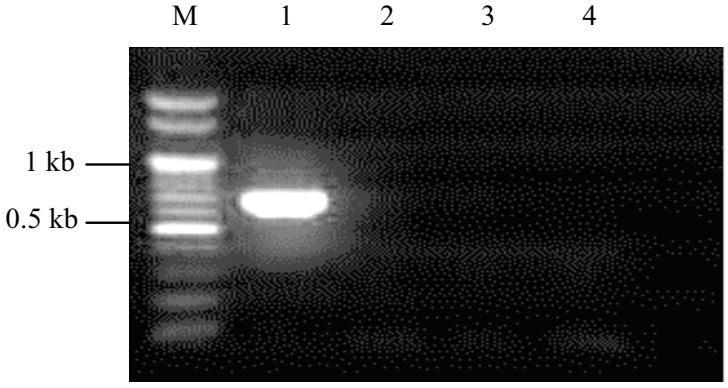

Fig 2–Agarose gel electrophoresis of PCR product of *femA* gene. A 686 bp band of *femA* gene from *S. aureus* isolates detected by 0.7% agarose gel electrophoresis. Lane M, DNA marker; lane 1, positive control *S.aureus* strain COL; lane 2, MRSA isolate NU007; lane 3, MRSA isolate NU042; lane 4, negative control.

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 habor 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 SCC*mec* type revealed both MRSA isolates were type II SCC*mec* element. A previous

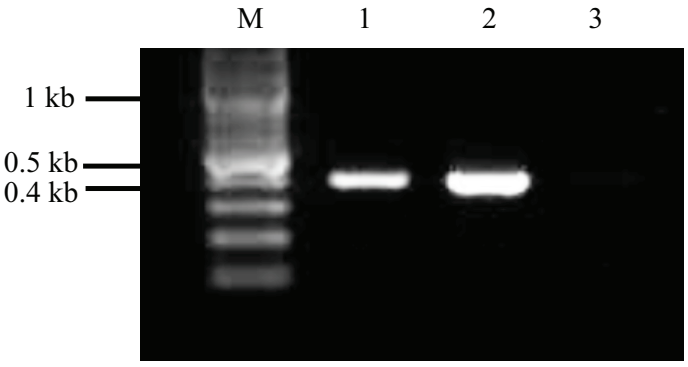

Fig 3–Agarose gel electrophoresis of PCR product to identify SCC*mec* types. A 398 bp band of SCC*mec* type II from *S. aureus* isolates detected by 0.7 % agarose gel electrophoresis. Lane M, DNA marker; lane 1, MRSA isolate NU007; lane 2, MRSA isolate NU042; lane 3, negative control.

study in Thailand (Lulitanond *et al*, 2010) showed patients with HA-MRSA at Srinagarind Hospital in Thailand carried type II and type III SCC*mec* elements. The main molecular marker of HA-MRSA is SCC*mec* 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 betalactamases; this gene spreads efficiently by horizontal transmission to other strains of *S. aureus*. Previous studies of antibiotic susceptibilities among *S. aureus* strains harboring SCC*mec* 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 SCC*mec* 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.

ACKNOWLEDGEMENTS

This work was partially supported by a grant-in-aid from the Faculty of Medical Sciences, Naresuan University. The authors thank Dr Norman Scholfield for his critical reading of this manuscript. We are also grateful to all the volunteers who participated in this study.

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