PRELIMINARY REPORT OF SCC*MEC*-TYPES AND ANTIMICROBIAL SUSCEPTIBILITIES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM A UNIVERSITY HOSPITAL IN THAILAND

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Abstract. Methicillin-resistant *Staphylococcus aureus* (MRSA) has spread worldwide. It is a major cause of hospital-acquired infections in most hospitals for nearly half century. The present study was conducted to examine the antimicrobial susceptibilities and staphylococcal cassette chromosome *mec* (SCC*mec)*-type for MRSA isolates from 237 patients treated at Srinagarind Hospital between September 2002 and August 2003. Antimicrobial susceptibility testing for all isolates was performed using an agar dilution method and SCC*mec*-types of 81 representatives from 237 isolates were determined using multiplex PCR. The minimum inhibitory concentration (MIC) ranges for the MRSA isolates were as follows: cefazolin 8 to ≥ 64; erythromycin ≤ 0.5 to ≥ 64; gentamicin ≤ 0.5 to ≥ 64; imipenem ≤ 0.5 to > 16; ofloxacin ≤ 0.5 to ≥ 64; oxacillin 16 to ≥ 64; tetracycline 2 to ≥ 64 and vancomycin ≤ 0.5 to 2 μg/ml. All MRSA isolates were susceptible to vancomycin, but only 0.4% to 8.9% was susceptible to the remaining antimicrobial agents. Of the 81 isolates tested, 2 types of SCC*mec* were found (76 with type III and 2 with type II) and no *mecA* gene was detected in 3 isolates. Sixty-seven of the 78 isolates carried the mercury-resistant operon. The multilocus sequence type in isolates with type III SCC*mec* was ST239 and in isolates with type II SCC*mec* was ST5.

Keywords: antimicrobial susceptibility, MRSA, SCC*mec,* Thailand

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first recognized in 1961, one year after methicillin was introduced (Jevons *et al*, 1961). Aminoglycosides were used to control early MRSA. Gentamicin-resistant MRSA emerged in the late 1970s and had led to a new wave of hospital outbreaks (Speller *et al*, 1976). Subsequently, MRSA has spread throughout the world (Townsend *et al*, 1987) causing in-

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fections in hospitals (Klimek *et al*, 1976; Doebbeling *et al*, 1995) and communities (Frenay *et al*, 1994; Kefala-Agoropoulou, *et al*, 2009). Glycopeptides are the principal agent for treating infections caused by MRSA. MRSA infections have been increasingly more common in various parts of the world, *eg*, the USA-National Nosocomial Infection Survey System reported a marked increase in the rate of MRSA infection among hospitals from 2.4% in 1975 to 29% in 1991 (Panlilio *et al*, 1992). MRSA infections in Europe have also increased significantly in several countries: Belgium (22% in 1999, 27% in 2002); Ireland (39 to 45%); Germany (9 to 19%); the Netherlands (0.4 to 1%); and the United Kingdom (31 to 45%) (Tiemersma *et al*, 2004). In Thailand, MRSA has been an important pathogen in hospitalized patients for several decades. The infection rates with MRSA among Thai university hospitals vary between 20% and 40% (Mekviwattanawong *et al*, 2006).

S. aureus becomes MRSA by receiving a *mecA* gene which encodes for penicillin binding protein (PBP) 2a, leading to the expression of high-level methicillin resistance. The *mecA* gene is located on a genomic island called the staphylococcal cassette chromosome *mec* (SCC*mec*) (Ito *et al*, 1999; Katayama *et al*, 2000) and may be transferred horizontally (Musser and Kapur, 1992; Archer and Niemayer, 1994). SCC*mec* is composed of two essential genetic elements: 1) the *mec* gene complex, which contains the *mecA* gene, its regulatory genes, *mecR1* and *mecI*, and an insertion element, IS431; and 2) the cassette chromosome recombinase (*ccr*) gene complex, containing two site-specific recombinase genes, *ccrA* combined with *ccrB* or *ccrC* alone (Ito *et al*, 2003). The SCC*mec* types are classified as types I to VI, based on differences in the *mec* gene complex (class A, B or C), the *ccr* gene complex (types I, II, III, IV or V) and other flanking genetic elements, such as insertion sequences and non-essential parts designated as junkyard (J) regions (Ito *et al*, 2001, 2004; Ma *et al*, 2002). Type I SCC*mec* contains only *mecA* as the antimicrobial-resistance gene, whereas types II and III carry both *mecA* and other genes encoding for non-β-lactam antimicrobial agents and/or heavy metal resistance. These resistance genes may either derive from plasmids or transposons (Ito *et al*, 1999). MRSA with type IV or type V SCC*mec* is usually susceptible to the majority of other non-β-lactam antibiotics and is associated with severe community infections, whereas types I, II and III SCC*mec* mostly occur in the health-care setting (Ma *et al*, 2002; Centers for Disease Control and Prevention, 2007; Kefala-Agoropoulou *et al*, 2009).

The present study reports the antimicrobial susceptibilities and molecular characteristics of MRSA isolates from patients at a university hospital in Thailand. This study provides primary epidemiological data regarding MRSA in northeastern Thailand.

MATERIALS AND METHODS

Bacterial isolates

Two hundred thirty-seven non-repetitive clinical isolates of MRSA were taken from patients at Srinagarind Hospital, Khon Kaen University, Thailand between September 2002 and August 2003 (Table 1). The reference strains of *S. aureus* included in this study were: ATCC 29213 (methicillin susceptible *S. aureus)*, NCTC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), MR108 (SCC*mec* type IV), JCSC 3624 (SCC*mec* type V) and HDE288 (SCC*mec* type VI). All isolates were identified by

Table 1
Sources of MRSA isolate specimens.

Specimens	No. of isolates
Respiratory tract	114
Pus	82
Body fluid	18
Urine	8
Eye	6
Blood	5
Soft tissue	2
Lymph node	1
Appendix	1
Total	237

conventional biochemical tests and stored in skim milk with 20% glycerol at -70ºC.

Susceptibility testing

The antimicrobial agents used in this study were supplied by Sigma Chemical (Switzerland): cefazolin, erythromycin, gentamicin, ofloxacin, oxacillin, tetracycline and vancomycin. Imipenem was supplied by Bristol-Myers Squibb (Hounslow, UK). The minimum inhibitory concentrations (MICs) of these antimicrobial agents were determined using the agar dilution method described by the National Committee for Clinical Laboratory Standard Institute (CLSI, 2005).

Chromosomal DNA isolation

Chromosomal DNA from each isolate was prepared using a phenol-chloroform extraction method (Sjostrom *et al*, 1975). Briefly, bacterial cells were harvested from an overnight culture in tryptic soy broth and lysed with achromopeptidase enzyme. Protein from the solution was digested with proteinase-K. The cell debris was then removed with a phenol buffer and chloroform-isoamyl alcohol; carried out twice for each reagent. The DNA was then precipitated with absolute ethanol and drying with a centrifugal concentration machine (VC-36, Taiyo). The DNA pellet was then dissolved in 20 µl of distilled water and used as a DNA template for the PCR reaction.

Molecular characterization

SCC*mec* elements were determined for 81 of the 237 isolates by amplifying the *ccr* and *mec* gene complexes using multiplex PCR as described by Okuma *et al* (2002). The multilocus sequence type (MLST) for 4 representative isolates with different SCC*mec* types was carried out as described previously (Enright *et al*, 2000). The presence of a mercury resistance (*mer*) operon was also investigated. The primers used in this study are shown in Table 2.

RESULTS

Antimicrobial susceptibility

MIC determination revealed most MRSA isolates were resistant to at least 5 of the 8 tested-antimicrobial agents. Of the 237 isolates, the sensitivities were: 0.8% for cefazolin (2 isolates), 0.4% for erythromycin (1 isolate), 1.3% for gentamicin (3 isolates), 8.9% for imipenem (21 isolates), 0.8% for ofloxacin (2 isolates) and 0.8% for tetracycline (2 isolates). All isolates were susceptible to vancomycin but resistant to oxacillin with MICs for oxacillin of 16 µg/ml is 4 isolates, 32 µg/ml in 3 isolates and ≥ 64 µg/ml in 230 isolates (Table 3). The MIC range, MIC_{50}, MIC_{90} and percentages of susceptibility are shown in Table 4.

Molecular determination

Using multiplex PCR, 78 of 81 MRSA isolates contained a *mecA* gene. SCC*mec* types were determined in 78 isolates: all isolates had *mecI-mecR1* genes, indicating they had class A *mec* gene complex.

Table 2
Primers used for PCR.

Genes	Primer name	Nucleotide sequence (5'- 3')	PCR product(s)
mecA	mA1	TGCTATCCACCCTCAAACAGG	280 bp
	mA2	AACGTTGTAACCACCCCAAGA	
ccrB	βc	ATTGCCTTGATAATAGCCITCT	
ccrA1	α1	AACCTATATCATCAATCAGTACGT	type1; 700 bp
ccrA2	α2	TAAAGGCATCAATGCACAAACACT	type2; 1 Kb
ccrA3	α3	AGCTCAAAAGCAAGCAATAGAAT	type3; 1.6 Kb
mecI	mI4	CAAGTGAATTGAAACCGCCT	1.9 Kb
mecR1	mcR3	ATCTCCACGTTAATTCCATT	
mer	merA2	TCT TCA CAG CCT GTG CAT GTC ATG CCT	1,545 bp
	merG	TGA TAC CGC GAA TGA ATC AAA GGT-	

Table 3
Minimum inhibitory concentrations (MIC) for MRSA with various antimicrobial agents.

Antimicrobial agents	Number of isolates yielding indicated MICs (μg/ml)								
	≤ 0.5	1	2	3	4	8	16	32	≥ 64
Cefazolin	-	-	-	-	-	2	1	-	234
Erythromycin	1	-	1	-	1	13	21	9	191
Gentamicin	3	-	-	-	-	1	-	2	231
Imipenem	7	1	7	-	6	4	34	178	-
Ofloxacin	1	1	-	-	-	10	80	70	75
Oxacillin	-	-	-	-	-	-	4	3	230
Tetracycline	-	-	1	-	1	-	1	27	207
Vancomycin	19	156	62	-	-	-	-	-	-

Seventy-six isolates (97.4%) had *ccr* complex type III, consistent with SCC*mec* type III, and 2 isolates (2.6%) had type II *ccr* complex corresponding with SCC*mec* type II. The *mec* operon was detected in 2 and 65 isolates with type II and type III SCC*mec*, respectively (85.9% total). The MLST for all 3 representatives with type III SCC*mec* were ST239, clonal complex 9, and the one representative strain with type II SCC*mec* was ST5, clonal complex 5.

DISCUSSION

MRSA is a worldwide important cause of nosocomial and community-acquired infections. The antimicrobial resistance of this organism is a growing problem with serious clinical implications. Only a few antimicrobial agents have good efficacy against multidrug-resistant MRSA. Vancomycin, the drug of choice for treating MRSA, has failed in some cases

Table 4
Antimicrobial susceptibilities of MRSA.

Antimicrobial agents	Minimum inhibitory concentration (μg/ml)			% Susceptibility
	Range	MIC_{50}	MIC_{90}	
Cefazolin	8 to ≥ 64	≥ 64	≥ 64	0.84
Erythromycin	≤ 0.5 to ≥ 64	≥ 64	≥ 64	0.42
Gentamicin	≤ 0.5 to ≥ 64	≥ 64	≥ 64	1.26
Imipenem	≤ 0.5 to 32	32	32	8.86
Ofloxacin	≤ 0.5 to ≥ 64	32	≥ 64	0.84
Oxacillin	16 to ≥ 64	≥ 64	≥ 64	0
Tetracycline	2 to ≥ 64	≥ 64	≥ 64	0.84
Vancomycin	≤ 0.5 to 2	1	2	100

(Hiramatsu *et al,* 1998; Rotun *et al,* 1999; Smith *et al,* 1999; Trakulsomboon *et al,* 2001; Lulitanond *et al,* 2009).

In the present study, 85.6% of MRSA isolates were resistant to at least 5 antimicrobial agents: cefazolin, erythromycin, gentamicin, ofloxacin and tetracycline. Resistance rates to the antimicrobial agents were slightly higher than previously reported (Marshall *et al,* 1998; Pfaller *et al,* 1999; Arias *et al,* 2003), except for tetracycline. Imipenem was effective in only 21 of the 237 MRSA isolates (8.9%), while vancomycin was effective against 100% of isolates. Recent studies have found MRSA isolates with reduced susceptibility to vancomycin (Lulitanond *et al,* 2005, 2009). Since heterogeneously vancomycin-resistant *S. aureus* cannot be detected by routine disk diffusion or agar dilution (Tenover *et al,* 2001), the use of vancomycin to treat MRSA infection should be carefully monitored. Eighty-five point nine percent of the MRSA isolates in our study had the *mer* operon. The *mer* operon is usually associated with type III SCC*mec* elements (Ito *et al,* 2003; Chongtrakool *et al,* 2006; Arakere *et al,* 2009).

Investigation of SCC*mec* type revealed 97.4% of MRSA isolates in our study had type III SCC*mec* with a MLST of ST239; a minority of isolates had type II SCC*mec* with a MLST of ST5. The clinical information in 51 patients suggests the infections were hospital acquired. It could not be determined of the infections was hospital or community acquired in the remaining 27 patients. Type III SCC*mec* MRSA is found in several countries in Asia: China, India, Vietnam, Indonesia, Malaysia, the Philippines, Singapore, Saudi Arabia, Sri Lanka, Taiwan and Hong Kong (Chongtrakool *et al,* 2006). In Japan and Korea the MRSA isolates are mainly type II SCC*mec* (Ito *et al,* 2001; Ko *et al,* 2005). MRSA in healthcare facilities is usually one of three SCC*mec* types (*ie,* I, II or III) and usually multidrug-resistant, while MRSA from the community is usually type IV or V SCC*mec,* and is usually susceptible to antimicrobial agents other than β-lactams (Merlino *et al,* 2002; Berglund *et al,* 2008; 2009). In the present study, 3 of the 81 MRSA isolates were negative for the *mecA* gene; all were resistant to all antimicrobial agents tested except

vancomycin. The MICs for oxacillin in these isolates were all > 32 μg/ml, suggesting hyper-production of β-lactamase enzyme in these isolates (Malouin *et al*, 2003).

ST239-SCC*mec* type III was the major clone in this hospital; however, ST5-SCC*mec* type II was also detected in a minority of cases.

ACKNOWLEDGEMENTS

This work was partially supported by Khon Kaen University. We thank: 1) the Japan Society for the Promotion of Science for their support and expertise; 2) the staff of the Clinical Microbiology Laboratory at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, for collecting the clinical isolates; 3) the Faculty of Associated Medical Sciences and Center for Research and Development in the Medical Diagnostic Laboratory (CMDL), Khon Kaen University, for their support; and, 4) Mr Bryan Hamman for assistance with English language editing.

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