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Genetic Relationship between SCC*mec* Types and Virulence Factors of Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates in Korea

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The molecular epidemiological characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates have demonstrated their genetic diversity and evolution. A total of 137 strains of MRSA clinical isolates was collected from Korean healthcare facility in 2007. The MRSA clinical isolates were analyzed by molecular typings (SCC*mec* element and *agr* locus typing), virule nce factor gene detections {(Panton-Valentine leukocidin (PVL), enterotoxin, exfoliative toxin and toxic shock syndrome toxin-1), and amplified fragment length polymorphism (AFLP)}. The MRSA clinical isolates were classified as SCC*mec* type II-*agr* type 1 (2 strains), type II-*agr* type 2 (79 strains), type III-*agr* type 1 (24 strains), type III-*agr* type 2 (2 strains), type IV-*agr* type 1 (27 strains), type IV-*agr* type 2 (2 strains), and non-typable (1 strain, *agr* type 3). Based on SCC*mec* types, SCC*mec* type II (95.1%) and III (88.5%) indicated higher multidrug resistance rate than SCC*mec* type IV (10.3%) (P<0.001). The most common enterotoxin genes were *seg* (83.8%), *sei* (83.1%), and *sec* (80.2%). The *tst* gene was present in 86 out of 137 (62.8%) MRSA isolates. All MRSA isolates were negative for PVL and exfoliative toxin genes. The combinations of toxin genes were observed in particular SCC*mec* types; 97.6% of SCC*mec* type II strains carried *sec*, *seg*, *sei* and *tst* genes, 73.0% of SCC*me*c type III strains carried *sea* gene, and 89.7% of SCC*mec* type IV strains carried *sec*, *seg* and *sei* genes. Each of the SCC*mec* types of MRSA isolates had distinct AFLP profile. In conclusion, SCC*mec* type II, *agr* type 1 and 2 have demonstrated to be the most common types in Korea, and the results indicated that the virulence factors are closely associated with their molecular types (SCC*mec* and *agr* types).

Key Words: MRSA, SCC*mec*, *agr* locus, Staphylococcal toxin gene, AFLP, PCR

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* is one of the major human pathogens both in nosocomial and community-associated infections, such as skin and soft tissue infections (SSTIs), pneumonia, and bacteremia. The emergence of MRSA was due mainly to the acquisition of *mec*A gene, which is located on a mobile genomic island, named as staphylococcal cassette chromosome *mec* (SCC*mec*) (Ito et al., 2003). Seven main types of SCC*mec* (type I to VII) are recognized as of current. While SCC*mec* type II and III cause resistance to multiple classes of antibiotics due to the additional drug resistance genes integrated into SCC*mec*, other SCC*mec* types (I, IV, V, VI and VII) are known to cause only β-lactam antibiotic resistance (Ito et al., 2001; Oliveira et al., 2006).

The pathogenicity of *S. aureus* infections is linked to the expression of several extracellular protein toxins, including enterotoxins (SE), exfoliative toxin (ET), toxic shock syndrome toxin-1 (TSST-1), and the leukocidins (Dinges et al., 2000). These toxins are known to be responsible for specific clinical syndromes, staphylococcal enterotoxins for food poisoning, and Panton-Valentine leukocidins (PVL)

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for severe skin and soft tissue infections (Lina et al., 1999).

Virulence gene expression in *S. aureus* is controlled by RNA III, a central pleiotropic regulator transcribed from the accessory gene regulator (*agr*) locus, including those involved in exoprotein, exotoxin and adhesin expression (Arvidson et al., 2001). The central segment of the *agr* locus, which consists of C-terminal two-thirds of AgrB, AgrD, and N-terminal half of AgrC, shows striking interstrain variation. This finding has led to the division of *Staphylococcus aureus* isolates into four different *agr* specificity groups (Gilot et al., 2002).

Accurate molecular epidemiological typing has become of primary importance for the classification of MRSA isolates and the control of their spread. In many laboratories, conventional methods of typing such as serotyping and bacteriophage typing have been replaced by PCR-based methods (van Belkum A. 1994). To investigate the virulence factors of MRSA clinical isolates, SCC*mec* typing, *agr* locus typing and amplified fragment length polymorphism (AFLP) were used to analyze the relationship among the three methodologies in this study.

MATERIALS AND METHODS

Specimen collection

A total of 137 MRSA clinical isolates was collected from Kangwon-do healthcare facility from July to November in 2007. These were collected from wound, urine, tissue, sputum, pus, eye, abscess, blood, urine/catheter tip and other sites.

Antimicrobial susceptibility test

Minimal inhibitory concentrations of clinical antimicrobials for all isolates were determined by MicroScan Walk/Away 96 AI system (Dade Behring, Sacramento, CA, USA) and were interpreted according to CLSI M100-S16 (formerly NCCLS, 2006). Twenty-four antimicrobial agents were tested: penicillin, oxacillin, amoxicillin/K clavulanate, ampicillin, imipenem, meropenem, ticarcillin/K clavulanate, cefazolin, cephalothin, cefuroxime, cefotaxime, cefepime, ciprofloxacin, ofloxacin, chloramphenicol, clindamycin, gentamicin, erythromycin, tetracycline, synercid, rifampin, vancomycin, teicoplanin, and trimethoprim-sulfamethoxazole. For this study, multidrug resistance was defined as resistance to penicillin and oxacillin plus three or more antibiotics.

Bacterial DNA extraction

To prepare DNA templates, brain heart infusion (Difco, Detroit, USA) broth was inoculated with one colony of MRSA strain. After 24 hours incubation at 37℃, 1 ml of the culture were centrifuged at 12,000 × g for 10 minutes. The pellets were resuspended in 1 ml of saline, harvested by centrifugation. Bacterial genomic DNA was extracted using the *AccuPrep*® Genomic DNA Extraction Kit (Bioneer Co., Daejeon, Korea), and kept at -20℃ until use.

SCC*mec*, *agr* locus typing, and *mec*A gene detection

Presence of *mec*A gene, SCC*mec*, and *agr* locus typing were investigated by PCR using primers and previously described conditions (Ito et al., 2001; Gilot et al., 2002; Zhang et al., 2005).

Detection of staphylococcal toxin genes

Sequences specific for staphylococcal enterotoxin (*sea* to *sej*), exfoliative toxin (*eta* and *etb*) and toxic shock syndrome toxin-1 (*tst*) genes were detected by PCR as previously described (Mehrotra et al., 2000; Sharma et al., 2000; Rosec and Giguad, 2002). The PVL genes (*lukS-PV* and *lukF-PV*) were detected by duplex PCR. The 20 μl of PCR mixture contained 2 μl template DNA, PCR buffer (40 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$), 2.5 mM (each) dNTP, 20 pmol of lukS-PV-F (5'ATCACTCCTA-TTGCTACTTC3'), lukS-PV-R (5'ATCTACATTGGGGTC-ATTTG3'), 40 pmol of lukF-PV-F (5'GTAAGTGAGAA-AAAGGTTGA3'), lukF-PV-R (5'TGGATAACACTGGC-ATTTTG3'), and 0.5 U of G-*Taq* DNA polymerase (Cosmo Genetech Co., Seoul, Korea). The amplification was performed in a thermal cycler (GeneAmp® PCR System 2700, Perkin-Elmer Cetus, Boston, USA) beginning with an initial denaturation step at 94℃ for 5 minutes followed by 25 cycles of 94℃ for 30 seconds, 48℃ for 30 seconds, and 72℃ for 30 seconds, ending with a final extension step at 72℃ for 10 minutes followed by a hold at 4℃. The PCR products were analyzed using 1.5% agarose gel

electrophoresis with 0.5 μg/ml of ethidium bromide and visualized under UV light.

Fingerprinting by AFLP

AFLP analysis was performed using the established procedures with minor modification (Janssen et al., 1996; Gibson et al., 1998). Briefly, the genomic DNA (1 μg) was digested with 10 U *Hin*dIII (Fermentas, Burlington, Canada) and *Apa*I (Fermentas, Burlington, Canada). For the ligation of the adapters, 5 pmol of *Hin*dIII adapter (ADH), *Apa*I adapter (Apa) and 10 Weiss unit of T4 DNA ligase (Fermentas, Burlington, Canada) were added. The primer used in the PCR step included the respective restriction enzyme and the adapter sequence plus one extension base for primer (G for *Hin*dIII primer). The DNA bands were analyzed by Quantity One Version 4.5.0 program (Bio-Rad, Hercules, CA, USA) and similarity index by dendrogram for genetical similarity to band pattern of each clinical isolates were examined.

Statistical methods

Statistical analysis was applied by using Microsoft® Office Excel® 2007 (Microsoft Co., Redmond, WA, USA). Comparisons of proportions were carried out using the χ^2 test.

RESULTS

Specimen collection

A total of 137 different MRSA isolates was examined. They were isolated from abscess (n=8), culture of blood (n=16), bronchial washing (n=3), catheter (n=1), drain (n=2), ear discharge (n=3), gastric juice (n=1), joint fluid (n=1), pus (n=2), sore (n=1), sputum (n=47), tip (n=17), tissue (n=3), urine (n=7), and wound (n=25).

Antimicrobial susceptibility test

Table 1 shows the antimicrobial susceptibility pattern based on SCC*mec* types (relationship between the antimicrobial susceptibility pattern and the SCC*mec* types). Our results indicate the resistance of MRSA isolates to ciprofloxacin (77.9%), ofloxacin (80.2%), chloramphenicol (0%), clindamycin (77.9%), gentamicin (72.8%), erythromycin (92.7%), tetracycline (57.4%), synercid (3.7%), rifampin (1.5%), vancomycin (0%), teicoplanin (0.7%), and trimethoprim/sulfamethoxazole (16.2%). All MRSA isolates were found to be resistant to β-lactam antibiotics (penicillin, oxacillin, amoxicillin/clavulanic acid, ampicillin, imipenem, meropenem, ticarcillin/clavulanic acid, cefazolin, cephalothin, cefuroxime, cefotaxime and cefepime). Antimicribial resistance rates differed significantly between SCC*mec* types; SCC*mec* type II and III were more resistant than SCC*mec* type IV against ciprofloxacin (P<0.001), ofloxacin (P<0.001), clindamycin (P<0.001), gentamicin (P<0.001), erythromycin (P<0.001) and tetracycline (P< 0.001), SCC*mec* type III was more resistant than SCC*mec* type II and IV against trimethoprim/sulfamethoxazole (P<

Table 1. Antimicrobial resistance of MRSA isolates based on SCC*mec* type

Antimicrobial agent	SCC*mec* type [n (%)]			
	II (n=81)	III (n=26)	IV (n=29)	Total (n=136)
Penicillin	81 (100)	26 (100)	29 (100)	136 (100)
Oxacillin	81 (100)	26 (100)	29 (100)	
Amox/k Clav	81 (100)	26 (100)	29 (100)	
Ampicillin	81 (100)	26 (100)	29 (100)	
Imipenem	81 (100)	26 (100)	29 (100)	
Meropenem	81 (100)	26 (100)	29 (100)	
Ticar/k Clav	81 (100)	26 (100)	29 (100)	
Cefazolin	81 (100)	26 (100)	29 (100)	
Cephalothin	81 (100)	26 (100)	29 (100)	
Cefuroxime	81 (100)	26 (100)	29 (100)	
Cefotaxime	81 (100)	26 (100)	29 (100)	
Cefepime	81 (100)	26 (100)	29 (100)	
Ciprofloxacin	80 (98.8)	23 (88.5)	3 (10.3)	106 (77.9)
Ofloxacin	79 (97.5)	23 (88.5)	7 (24.1)	109 (80.2)
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)
Clindamycin	78 (96.3)	22 (84.6)	6 (20.7)	106 (77.9)
Gentamicin	73 (90.1)	23 (88.5)	3 (10.3)	99 (72.8)
Erythromycin	80 (98.8)	26 (100)	20 (69.0)	126 (92.7)
Tetracycline	55 (67.9)	20 (76.2)	3 (10.3)	78 (57.4)
Synercid	3 (3.7)	0 (0)	2 (6.9)	5 (3.7)
Rifampin	1 (1.2)	1 (3.9)	0 (0)	2 (1.5)
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)
Teicoplanin	0 (0)	1 (3.9)	0 (0)	1 (0.7)
Trimeth/Sulfa	2 (2.5)	19 (73.1)	1 (3.5)	22 (16.2)

Abbreviations: Amox/k Clav; Amoxicillin/K Clavulanate, Ticar/k Clav; Ticarcillin/K Clavulanate, Trimeth/Sulfa; Trimethoprim/ Sulfamethoxazole

Table 2. Antimicrobial resistance profile of MRSA isolates based on SCC*mec* type

Resistance profile	SCC*mec* type [n (%)]			No. of isolates (%)	Resistance classification
	II	III	IV		
P, OX	1 (1.2)	0 (0)	12 (41.4)	13 (9.6)	NMDR
P, OX, E	1 (1.2)	3 (11.5)	12 (41.4)	16 (11.8)	
P, OX, CD, E	0 (0)	0 (0)	2 (6.9)	2 (1.5)	
P, OX, CP, GM	2 (2.5)	0 (0)	0 (0)	2 (1.5)	
P, OX, CP, CD, E	2 (2.5)	0 (0)	0 (0)	2 (1.5)	MDR
P, OX, CP, CD, E, TE	5 (6.2)	0 (0)	0 (0)	5 (3.7)	
P, OX, CP, E, GM, TE	1 (1.2)	1 (3.9)	0 (0)	2 (1.5)	
P, OX, CP, GM, TE, T/S	1 (1.2)	1 (3.9)	0 (0)	2 (1.5)	
P, OX, CP, CD, E, GM	20 (24.7)	3 (11.5)	0 (0)	23 (16.9)	
P, OX, E, GM, RIF, TE, T/S	0 (0)	1 (3.9)	0 (0)	1 (1.5)	
P, OX, CP, CD, E, GM, TE	46 (56.8)	0 (0)	2 (6.9)	48 (35.3)	
P, OX, CP, CD, E, GM, RIF, TE	1 (1.2)	0 (0)	0 (0)	1 (0.7)	
P, OX, CP, CD, E, GM, TE, T/S	1 (1.2)	16 (61.5)	(3.5)	18 (13.2)	
P, OX, CP, CD, E, GM, TEI, TE, T/S	0 (0)	1 (3.9)	0 (0)	1 (1.5)	
Total	81	26	29	136	

Abbreviations: NMDR; non multidrug resistance, MDR; multidrug resistance, P; penicillin, OX; oxacillin, E; erythromycin, CD; clindamycin, CP; ciprofloxacin, TE; Tetracycline, GM; gentamicin, T/S; trimethoprim-sulfamethoxazole, RIF; rifampin, TEI; teicoplanin

Table 3. *agr* locus type of MRSA isolates based on SCC*mec* type

SCC*mec* type	*agr* type [n (%)]			
	agr 1	*agr* 2	*agr* 3	*agr* 4
SCC*mec* II (n=81)	2 (2.5)	79 (97.5)	0 (0)	0 (0)
SCC*mec* III (n=26)	24 (92.3)	2 (7.7)	0 (0)	0 (0)
SCC*mec* IV (n=29)	27 (93.1)	2 (6.9)	0 (0)	0 (0)
Total (n=136)	53 (39.0)	83 (61.0)	0 (0)	0 (0)

0.001). SCC*mec* type II and III have higher multidrug resistance rate than SCC*mec* type IV (Table 2, 93.5% versus 10.3%, $P<0.001$).

Molecular characteristics of the MRSA isolates

Using PCR method, 136 MRSA isolates (99.3%) were found to be positive for the *mec*A gene. The MRSA isolates were found to be SCC*mec* type II-*agr* type 1 (2 strains), type II-*agr* type 2 (69 strains), type III-*agr* type 1 (24 strains), type III-*agr* type 2 (2 strains), type IV-*agr* type 1 (27 strains), type IV-*agr* type 2 (2 strains) (Table 3). Only one MRSA isolate was negative for the *mec*A gene and was classified as non-typable (*agr* type 3). Staphylococcal toxin gene distribution differed significantly between SCC*mec* types (Table 4). Of the 136 SCC*mec* type II, III and IV isolates, 100%, 92.3% and 100% had one or more toxin genes, respectively. The *seg* enterotoxin gene was the

Table 4. Association of individual toxin genes particular SCC*mec* MRSA types

Toxin gene	SCC*mec* types of MRSA isolates [n (%)]			MRSA (n=136)
	SCC*mec* II (n=81)	SCC*mec* III (n=26)	SCC*mec* IV (n=29)	
sea	1 (1.2)	21 (80.8)	0 (0)	22 (16.2)
seb	0 (0)	0 (0)	0 (0)	0 (0)
sec	79 (97.5)	2 (7.7)	28 (96.6)	109 (80.2)
sed	0 (0)	0 (0)	0 (0)	0 (0)
see	0 (0)	0 (0)	0 (0)	0 (0)
seg	81 (100)	4 (15.4)	29 (100)	114 (83.8)
seh	0 (0)	0 (0)	0 (0)	0 (0)
sei	81 (100)	3 (11.5)	29 (100)	113 (83.1)
sej	0 (0)	0 (0)	0 (0)	0 (0)
eta	0 (0)	0 0)	0 (0)	0 (0)
etb	0 (0)	0 (0)	0 (0)	0 (0)
tst	81 (100)	2 (3.9)	2 (6.9)	85 (62.5)
pvl	0 (0)	0 (0)	0 (0)	0 (0)
Negative	0 (0)	2 (7.7)	0 (0)	2 (1.5)

Table 5. Association of staphylococcal toxin gene profiles with particular SCC*mec* MRSA types

Toxin gene profiles	SCC*mec* types of MRSA isolates [n (%)]			MRSA (n=136)
	SCC*mec* II (n=81)	SCC*mec* III (n=26)	SCC*mec* IV (n=29)	
*sea**	0 (0)	19 (73.1)	0 (0)	19 (14.0)
seg	0 (0)	1 (3.9)	0 (0)	1 (0.7)
sea, seg	0 (0)	1 (3.9)	0 (0)	1 (0.7)
seg, sei	0 (0)	0 (0)	1 (3.5)	1 (0.7)
seg, sei, tst	1 (1.2)	0 (0)	0 (0)	1 (0.7)
sea, seg, sei	0 (0)	1 (3.9)	0 (0)	1 (0.7)
*sec, seg, sei**	0 (0)	1 (3.9)	26 (89.7)	27 (19.9)
sea, seg, sei, tst	1 (1.2)	0 (0)	0 (0)	1 (0.7)
*sec, seg, sei, tst**	79 (97.5)	1 (3.9)	2 (6.9)	82 (60.3)
Negative	0 (0)	2 (7.7)	0 (0)	2 (1.5)
Total	81	26	29	136

**P*<0.001, comparison of 3 SCC*mec* types (χ^2 test)

most common (83.8%), followed by *sei* (83.1%), *sec* (80.2%) and *sea* (16.8%). SCC*mec* types III had higher rate of *sea* gene than SCC*mec* types II and IV (80.8% versus 0.3%, *P*<0.001) and SCC*mec* types II and IV had higher rate of *sec*, *seg* and *sei* than SCC*mec* types III (*sec*; 97.3% versus 7.7%, *P*<0.001, *seg*; 100% versus 15.4%, *P*<0.001, *sei*; 100% versus 11.5%, *P*<0.001). The *tst* gene was present in 86 out of 137 (62.8%) MRSA isolates and all *agr* type 2 MRSA isolates had *tst* gene. SCC*mec* type II had higher rate of *tst* gene than SCC*mec* type III and IV (100% versus 7.3%, *P*<0.001). All MRSA isolates were negative for PVL and exfoliative toxin genes.

The combinations of toxin genes were observed in particular SCC*mec* types (Table 5). In SCC*mec* type II isolates, the simultaneous presence of *sec*, *seg, sei* and *tst* occurred frequently (97.5%), whereas the *sec*, *seg* and *sei* combination was observed more often in SCC*mec* type IV (89.7%) isolates. In SCC*mec* type III (73.1%) isolates, only sea gene was frequently observed.

The 137 MRSA isolates were clustered into 14 distinct groups (similarity coefficient, 73%) by AFLP analysis (Fig. 1). The main cluster (n=122, 89.1%) of AFLP groups consisted of four groups (group 1, n=77; group 2, n=29; group 4, n=16; and group 5, n=5). Each main group consisted of distinct SCC*mec* types. Group 1 consisted of mainly SCC*mec* type II MRSA isolates (n=74, 96.1%), group 2 consisted of mainly SCC*mec* type IV MRSA isolates (n=25, 86.2%), and group 4 and 5 consisted of mainly SCC*mec* type III MRSA isolates (n=21, 100%).

DISCUSSION

Findings from numerous recent studies have suggested that antibiotic resistance and virulence genes may be linked in the same replicon: moreover, recent findings also suggest that a single strain has the capacity to be involved in both virulence and resistance in the long run. This phenomenon has been often observed in *S. aureus* (Gill et al., 2005). MRSA was first reported in 1961, two years after the introduction of methicillin into clinical practice (Jevons, 1961). During the 1970s, MRSA was disseminated worldwide, from Australia, Japan, all the way to USA and even more. Today, MRSA is known to be one of the major causes of nosocomial infections. Beginning in the late 1990s, there have been numerous reports on MRSA colonization and infection in the community that involved patients lacking of healthcare risk factors (David et al., 2008). The community-acquired MRSA (CA-MRSA), which carried commonly the SCC*mec* type IV and PVL gene, was known to be associated with life-threatening diseases such as necrotizing pneumonia and necrotizing fasciitis, which is primarily caused by PVL exotoxin (Boyle-Vavra and Daum, 2007).

Most clinical studies of MRSA have not considered

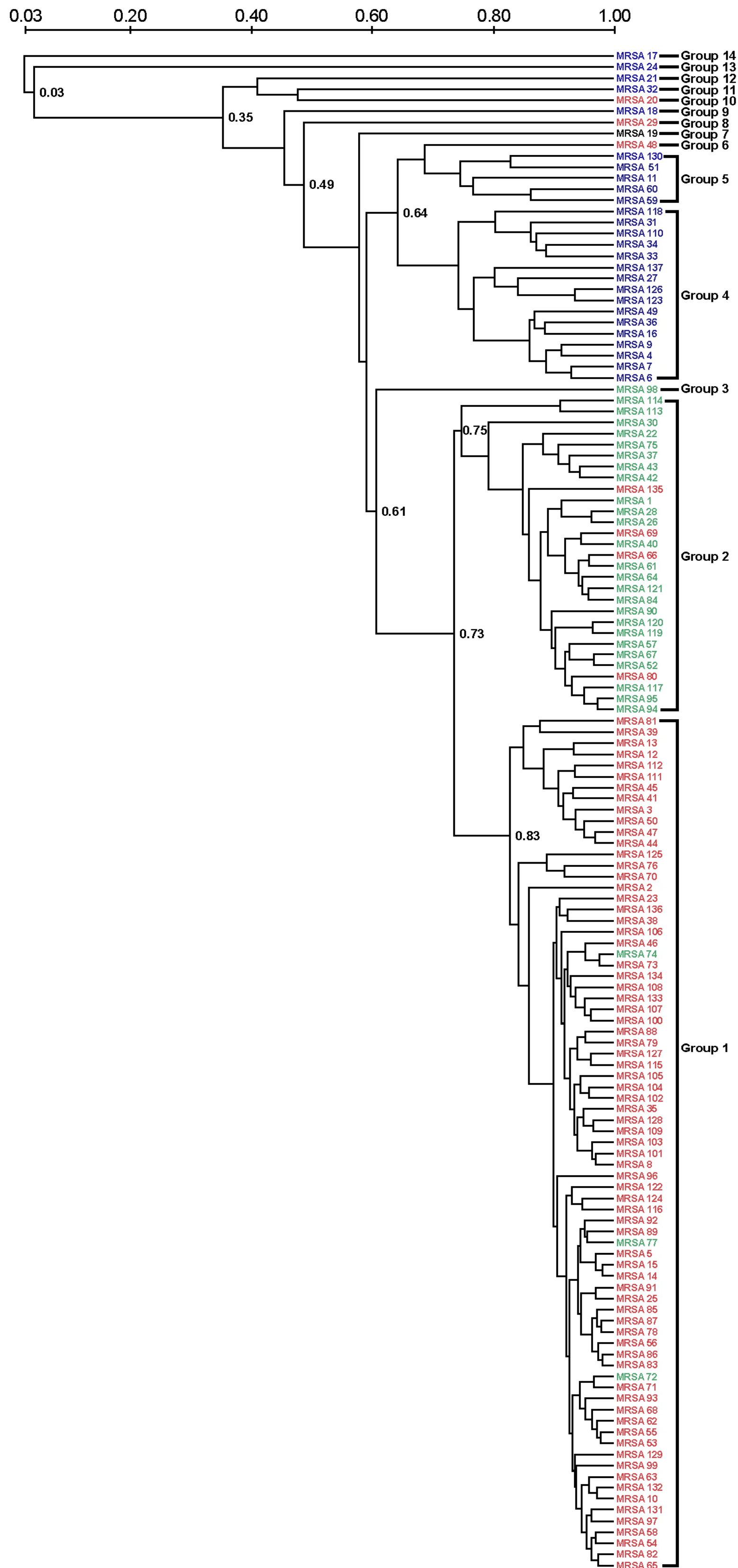

Fig. 1. Dendrogram of the similarity index among MRSA isolates by AFLP analysis. Red; SCC*mec* type II MRSA isolates, Blue; SCC*mec* type III MRSA isolates, Green; SCC*mec* type IV MRSA isolates, Black; SCC*mec* non-typable MRSA isolate.

overall molecular microbiological factors, such as virulence gene, resistance gene and accessory gene regulator (*agr*) locus. Therefore, in this study, we demonstrated the distribution of overall molecular microbiological factors in MRSA isolates and the relationship of molecular microbiological factors based on SCC*mec* types.

Based on the class of *mec*A gene complex and the type of *ccr* gene complex present, SCC*mec* gene locus is generally subdivided into 5 types, namely, SCC*mec* I to V, although there are also several variants based on polymorphisms (Oliveira and Lencastre, 2002; Ito et al., 2004). Recently, most MRSA isolates in Korea and Japan were found to be SCC*mec* type II, whereas SCC*mec* type III (or IIIA) have been found to be the most common type in strains from other eight Asian countries (Thailand, Sri Lanka, Indonesia, Vietnam, Philippines, Saudi Arabia, India, and Singapore) (Ko et al., 2005; Kim et al., 2006). Findings from this study show that SCC*mec* type II is most prevalent genotype of MRSA, followed by SCC*mec* type IV and III.

MRSA isolates in this study have also shown three different *agr* locus types: *agr* type 1 (38.7%), *agr* type 2 (60.6%) and *agr* type 3 (0.7%). The *agr* type 2 was found to be the most common type in this study, which is likely to demonstrate distinct ability in surviving the hospital settings and thus is frequently found in cases of hospital-acquired MRSA (HA-MRSA) in Korea (Yoon et al., 2007). In our study, only one MRSA isolate was classified as *agr* type 3 (SCC*mec* non-typable strain), which is the prevalent type of CA-MRSA strains circulation in France, Switzerland, and Australia (Vandenesch et al., 2003). SCC*mec* types of MRSA isolates had significantly different *agr* types. Most SCC*mec* type II MRSA isolates had *agr* type 2 (97.5%), whereas SCC*mec* type III and IV MRSA isolates had *agr* type 1 (92.3% and 93.1%) ($P<0.001$).

Antibiotic resistance rate was lower than those found in other studies (Kim et al., 2006), while the multidrug resistance rate was 97.0%. Based on SCC*mec* types, SCC*mec* type II and III had higher multidrug resistance rate than SCC*mec* type IV ($P<0.001$). On the other hand, MRSA strains had no significant difference in multidrug resistance rate based on agr types.

Our investigation was aimed to find the prevalence of SE genes (*sea* to *sej*), ET genes (*eta* and *etb*), PVL genes (*lukF-PV* and *lukS-PV*) and TSST-1 gene (*tst*), and to analyze AFLP profiles in MRSA isolates. We found that *seg*, *sei*, *sec* and *tst* were the most common toxins in clinical MRSA isolates in Korea. The rate of SE genes and TSST-1 gene in MRSA isolates significantly differed among SCC*mec* types. Moreover, the combinations of toxin genes were significantly different among SCC*mec* types. The AFLP analysis showed that each group consisted of distinct SCC*mec* types. These results indicate that same SCC*mec* types of MRSA strains in the sample demonstrate close relationship of genetic factors, such as virulence genes and resistance genes. None of the MRSA isolates contained the PVL genes which are reported to be the highly linked epidemiologic marker for CA-MRSA (Vandenesch et al., 2003). Other studies have demonstrated that PVL genes were detected in fewer than 5% of *S. aureus* isolates in a hospital (Kim et al., 2006).

In conclusion, our study was performed to investigate the overall molecular microbiological factors and to analyze the genetic relationship between SCC*mec* types. We demonstrated that clinical MRSA strains isolated in Korea show different patterns of antibiotic resistance, SCC*mec* types, *agr* types and virulence gene profiles. The overall microbiologic factors had close genetic relationship based on SCC*mec* types. These data may indicate that evolutionary change of MRSA associated with both antibiotic resistance genes and their related virulence factors.

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