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Comparison of Genotypes and Enterotoxin Genes Between *Staphylococcus aureus* Isolates from Blood and Nasal Colonizers in a Korean Hospital

In this study, we investigated the genetic background of 70 *Staphylococcus aureus* isolates (36 methicillin-resistant *S. aureus* [MRSA] and 34 methicillin-susceptible *S. aureus* [MSSA]) obtained from blood at a Korean tertiary-care hospital, using *spa* typing, multilocus sequence typing, and SCC*mec* typing. In addition, the prevalence of enterotoxin (*sea, seb, sec, sed, see, seg, seh, sei*, and *sek*), *tst*, and *pvl* genes among the samples was assessed via polymerase chain reaction, and the results were compared with those of 95 isolates of *S. aureus* obtained from nasal swabs. All MRSA isolates from blood, except one, belonged to three major clones: sequence type (ST)5-MRSA-II, ST72-MRSA-II (or IVA), and ST239-MRSA-III, among which ST5-MRSA-II was the predominant clone. The prevalence of enterotoxin genes in the *S. aureus* isolates obtained from blood differed significantly from those from the nasal swabs for the *sea, seb, sec*, and *seh* gene. In particular, the *seb* and *sec* genes were detected exclusively in the MRSA isolates of ST5 or *spa*-CC002, thereby suggesting the co-adaptation of virulence genes with the genetic background and their contribution to biological fitness.

Key Words : *Enterotoxin Genes; Staphylococcus aureus; Bacteremia; Nasal Carriage*

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INTRODUCTION

Staphylococcus aureus, which normally colonizes the anterior nares, can induce a variety of infections, ranging from superficial lesions to toxic shock syndrome and severe systemic infections. Since the first report in 1961 in U.K., the emergence and dissemination of methicillin-resistant *S. aureus* (MRSA) has become a subject of great global concern (1). In Korea, MRSA is responsible for more than 60% of *S. aureus* infections in hospitals, and constitutes a continuing threat to public health (2-4). Clinical MRSA isolates from Korean hospitals generally are identified as the two predominant clones, ST5-MRSA-II or ST239-MRSA-III (5-7). However, the majority of studies have focused on any clinical MRSA strains, without regard to their disease characteristics. That is to say, there are few studies reporting the molecular characteristics of MRSA and methicillin-susceptible *S. aureus* (MSSA) causing bacteremia in Korea (8).

Virulence determinants may have been crucial to the evolution of contemporary epidemic strains of *S. aureus* (9, 10). So far, a variety of virulence genes have been identified in *S. aureus*. They include staphylococcal enterotoxins (SEs), staphylococcal exofoliative toxins (ETs), toxic shock syndrome toxin 1 (TSST-1), and the Panton-Valentine leukocidin (PVL). Among these, the SEs are emetic toxins, and have been implicated in food poisoning in humans. TSST-1, which is also a member of the superantigenic toxin family, has been associated with several acute or chronic human diseases, including toxic shock syndrome and, possibly, sudden infant death syndrome and Kawasaki syndrome (11). PVL is known to be associated with tissue necrosis, and is frequently found in community-associated MRSA (CA-MRSA) (12). It has been demonstrated that the horizontal transfer of these virulence genes is one of the causes of the emergence of new virulent MRSA strains (9). Recently, the prevalence of enterotoxin genes in *S. aureus* isolates associated with staphylococcal food poison-

ing in Korea was investigated (13).

Detailed knowledge of genotype and virulence gene content may be a prerequisite for understanding of the genetic basis of prevailing clones of bacterial pathogens (9). Genetic backgrounds and the prevalence of enterotoxins may be different between disease-causing and colonizing strains. Thus, we have attempted to characterize the molecular characteristics of *S. aureus* isolated from blood in a Korean hospital, and have compared them with isolates of *S. aureus* from nasal swab specimens. In addition, we have evaluated the prevalence of virulence genes among *S. aureus* isolates obtained from blood and nasal specimens.

MATERIALS AND METHODS

Bacterial isolates

Seventy isolates of *S. aureus* obtained from blood were consecutively collected at a tertiary-care hospital (Samsung Medical Center) in Seoul, Korea over a five-month period (January to May) in 2006. In addition, 95 *S. aureus* isolates from nasal swabs of children attending an outpatient clinic in a tertiary-care hospital (14) were also included in this study. These isolates were collected from the same hospital over a similar period (December 2005 to February 2006) with the *S. aureus* isolates from blood. All of the isolates were identified using a Staphaurex Plus Kit (Murex Diagnostics Ltd., Dartford, U.K.), which were confirmed by molecular typing methods such as multilocus sequence typing (MLST) and *spa* typing.

Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing was performed by a broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) (15). The minimum inhibitory concentrations (MICs) of 11 antimicrobial agents were determined: oxacillin, penicillin, gentamicin, ciprofloxacin, clindamycin, erythromycin, rifampin, vancomycin, teicoplanin, tetracycline, and trimethoprim-sulfamethoxazole. Susceptibility interpretive criteria used were those established in the CLSI standard M100-S16 (15). *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 were employed as control strains.

Genotyping

In order to determine the genotype of *S. aureus* isolates, *spa* typing was initially conducted as previously described (16-18). With the BURP algorithm (Ridom GmbH), *spa* types were clustered into *spa* clonal complex (*spa*-CC) (16, 19). MLST was performed for 28 *S. aureus* isolates from blood, which were representative of each *spa* type, in accordance with a previously described method (20) with the exception of a primer pair amplifying the *yqiL* gene (forward, 5′-TAT TAG CAG CAT ACA GGA C-3′; reverse, 5′-CAC CTT TAC GTT GAG GAA TC-3′). The staphylococcal chromosomal casette *mec* (SCC*mec*) types of all MRSA isolates were determined by the method of multiplex polymerase chain reaction (PCR) (21).

Virulence gene detection

The detection of toxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sek*, and *tst*) was achieved via the method previously described by Jarraud et al. (10). PCR was performed independently for each toxin gene. The Panton-Valentine leukocidin (*pvl*) gene was detected using the primers previously described by Lina et al. (22).

Statistical analysis

Fisher's exact t-test and chi-square test were utilized to determine the significance of differences in resistance, where appropriate.

Table 1. Antimicrobial resistance of *S. aureus* isolates from blood

Antimicrobials	MRSA (n=36)		MSSA (n=34)		Total (n=70)	
	R (%)	MIC_{90} (mg/L)	R (%)	MIC_{90} (mg/L)	R (%)	MIC_{90} (mg/L)
Oxacillin	36 (100)	>64	–	0.5	36 (51.4)	>64
Penicillin	36 (100)	>32	30 (88.2)	32	66 (94.3)	>32
Gentamicin	30 (83.3)	>64	5 (14.7)	>64	35 (50.0)	>64
Ciprofloxacin	30 (83.3)	>32	3 (8.8)	0.5	33 (47.2)	32
Clindamycin	30 (83.3)	>32	1 (3.0)	0.12	31 (44.3)	>32
Erythromycin	32 (88.9)	>64	6 (17.7)	64	38 (54.3)	>64
Rifampin	2 (5.6)	≤0.016	–	≤0.016	2 (2.9)	≤0.016
Tetracycline	26 (72.2)	>64	3 (8.8)	0.5	29 (41.4)	>64
Trimethoprim-sulfamethoxazole	3 (8.3)	0.25/4.75	2 (5.9)	0.06/1.18	5 (7.2)	0.12/2.37
Vancomycin	-	1	–	1	–	1
Teicoplanin	-	8	–	0.5	–	8

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

RESULTS

Antimicrobial resistances

Among the 70 *S. aureus* isolates from blood, 36 isolates (51.4%) were found to be methicillin-resistant and 34 isolates (48.6%) were methicillin-susceptible. More than 80% of MRSA isolates were resistant to gentamicin, ciprofloxacin, clindamycin, and erythromycin. In addition, tetracycline resistance rate among MRSA isolates was 72.2%. However, less than 20% of MSSA isolates proved resistant to those antimicrobials except penicillin (Table 1). No *S. aureus* isolates were found to be resistant to vancomycin or teicoplanin.

Among 95 *S. aureus* isolates from nasal specimens, 18 were methicillin-resistant (18.9%). Among them, 44 (46.3%), 18 (18.9%), 11 (11.6%), 10 (10.5%), and 3 isolates (3.2%) were resistant to erythromycin, gentamicin, tetracycline, clindamycin, and ciprofloxacin, respectively (14).

Genotypes of *S. aureus* isolates from blood

Thirty-six MRSA isolates from blood evidenced 10 *spa* types from three *spa*-CCs (Table 2). Among the 36 MRSA isolates, 24 isolates (66.7%) belonged to *spa*-CC002, which share the same DMGMK motif. As five representative isolates were identified as sequence type (ST)5 in MLST analysis and all but one were identified as SCC*mec* type II, the MRSA isolates of *spa*-CC002, may represent ST5-MRSA-II. Five MRSA isolates belonged to *spa*-CC037, all of which harbor the WGKAOMQ motif. All five isolates were identified as SCC*mec* type III, and two representative isolates evidenced ST239 and a single locus variant (SLV) of ST239. Thus, this group may represent ST239-MRSA-III and its allies. Seven MRSA isolates were associated with *spa*-CC084, which evidence the ST72. Each of the two MRSA isolates of *spa* type UJGGMDMGGM were identified as SCC*mec* types II or IVA, and two isolates of *spa* type UJGFGMDMGGM were identified as

Table 2. Genotypes of 36 MRSA and 34 MSSA isolates from blood

spa CC (No.)	*spa* type (No.)		MLST* (*arcC-aroE-glpF-gmk-pta-tpi-yqiL*)	SCC*mec*†
	Kreiswirth	Ridom		
MRSA (36 isolates)				
spa-CC002 (24)	TJMBMDMGMK (15)	t002 (26-23-17-34-17-20-17-12-17-16)	ST5 (1-4-1-4-12-1-10)	II (14) NT (1)
	TMBBMDMMMK (5)	t2460 (26-17-34-34-17-20-17-17-17-16)	ST5 (1-4-1-4-12-1-10)	II (5)
	TJMBBMDMGMK (2)	t601 (26-23-17-34-34-17-20-17-12-17-16)	ST5 (1-4-1-4-12-1-10)	II (2)
	TJMGMK (1)	t062 (26-23-17-12-17-16)	ST5 (1-4-1-4-12-1-10)	II (1)
	TKBBMDMGMK (1)	t2458 (26-16-34-34-17-20-17-12-17-16)	ST5 (1-4-1-4-12-1-10)	II (1)
spa-CC037 (5)	WGKAOMQ (4)	t037 (15-12-16-02-25-17-24)	SLV of ST239 (2-3-1-new‡-4-4-3)	III (4)
	WGKAKAOMQ (1)	t021 (15-12-16-02-16-02-25-17-24)	ST239 (2-3-1-1-4-4-3)	III (1)
spa-CC084 (7)	UJGGMDMGGM (4)	t324 (07-23-12-12-17-20-17-12-12-17)	ST72 (1-4-1-8-4-4-3)	II (2), IVA (2)
	UJGFGMDMGGM (2)	t148 (07-23-12-21-12-17-20-17-12-12-17)	ST72 (1-4-1-8-4-4-3)	IVA (2)
	UJGFMB (1)	t189 (07-23-12-21-17-34)	ST188 (3-1-1-8-1-1-1)	IIIA (1)
MSSA (34 isolates)				
spa-CC002 (1)	TJMBMDMGMK (1)	t002 (26-23-17-34-17-20-17-12-17-16)	ST5 (1-4-1-4-12-1-10)	
spa-CC037 (9)	WFKAOMQ (2)	t338 (15-21-16-02-25-17-24)	ST30 (2-2-2-2-6-3-2)	
	WGKAOMQ (3)	t037 (15-12-16-02-25-17-24)	ST239 (2-3-1-1-4-4-3)	
	WGKAKAOMQ (1)	t021 (15-12-16-02-16-02-25-17-24)	ST30 (2-2-2-2-6-3-2)	
	WGKAKAOMQQQ (1)	t018 (15-12-16-02-16-02-25-17-24-24-24)	ST30 (2-2-2-2-6-3-2)	
	XKAKAOMQ (1)	t019 (08-16-02-16-02-25-17-24)	SLV of ST30 (2-2-new‖-2-6-3-2)	
	XKAKAOMQQ (1)	t122 (08-16-02-16-02-25-17-24-24)	ST30 (2-2-2-2-6-3-2)	
spa-CC084 (12)	UJGFMB (4)	t189 (07-23-12-21-17-34)	ST188 (3-1-1-8-1-1-1)	
	UDGFMB (1)	NEW‡ (07-20-12-21-17-34)	ST188 (3-1-1-8-1-1-1)	
	UGMFB (1)	NEW‡ (07-12-17-21-34)	ST513 (4-9-1-8-1-78-8)	
	UMFBBLB (1)	NEW‡ (07-17-21-34-34-22-34)	ST513 (4-9-1-8-1-78-8)	
	UJGBBGGJAGJ (2)	t084 (07-23-12-34-34-12-12-23-02-12-23)	ST15 (13-13-1-1-12-11-13)	
	UJGGBBBPB (1)	t1234 (07-23-12-12-34-34-34-33-34)	ST97 (3-1-1-1-1-5-3)	
	UJFKBPE (2)	t127 (07-23-21-16-34-33-13)	ST1 (1-1-1-1-1-1-1)	
spa-CC008 (8)	YHGFMBQBLO (5)	t008 (11-19-12-21-17-34-24-34-22-25)	ST8 (3-3-1-1-4-4-3)	
	YGFCBQBLO (1)	NEW‡ (11-12-21-05-34-24-34-22-25)	ST8 (3-3-1-1-4-4-3)	
	ZAGFMBLO (2)	t377 (04-02-12-21-17-34-22-25)	ST630 (12-2-1-1-4-4-3)	
Singleton (4)	ZDMDMQOB (4)	t1151 (04-20-17-20-17-24-25-34)	SLV of ST59 (new‡-23-15-2-19-20-15)	

*MLST was performed on representative isolates for each *spa* type (20); †NT, non-typeable (21); ‡Alleles and ST that were not found in the MLST website (http//saureus.mlst.net) were designated 'new'.
MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; MLST, multilocus sequence typing.

SCC*mec* type IVA. A remaining isolate of *spa*-CC084 showed *spa* type UJGFMB represents ST188-MRSA-IIIA.

The 34 MSSA isolates were differentiated into 18 *spa* types, which could be further classified into four *spa*-CCs and a singleton (Table 2). Nine MSSA isolates belonged to *spa*-CC037 (26.5%), which harbored six *spa* types. We performed MLST for each MSSA isolate of the six *spa* types of *spa*-CC037. Among them, five showed ST30 or its SLV, and one showed ST239. Twelve MSSA isolates belonged to *spa*-CC084, and six MSSA isolates belonged to *spa*-CC008. Each two MSSA isolates of *spa*-CC084 represented ST188 and ST513, which shared the same alleles in three loci: *glpF*, *gmk*, and *pta*. MSSA isolates of the *spa*-CC008 were consistent with ST8 and its double locus variant, ST630. Only one MSSA isolate showed a *spa* type in *spa*-CC002, TJMBMDMGMK, which is the most prevalent clone among MRSA isolates.

Prevalence of enterotoxin genes and *pvl* gene

Among the 70 *S. aureus* isolates obtained from blood, 50 of the isolates (71.4%) harbored at least one enterotoxin gene (Table 3, 4). The most prevalent enterotoxin gene was *seg* (58.6%), followed by the *sei* (55.7%), *tst* (44.3%), and *sec* (34.3 %) genes. The *see* and *pvl* genes were not detected in any of the *S. aureus* isolates obtained from blood. MRSA and MSSA isolates from blood showed different prevalences of enterotoxin genes. Whereas the majority of MRSA isolates from blood contained *sec* (66.7%), *seg* (86.1%), *sei* (83.3%), and *tst* (72.2%) genes, less than 30% of the MSSA isolates from blood were positive for those genes. With regard solely to the *sea* gene, the MSSA isolates showed significantly higher prevalence than was observed in the MRSA isolates (P=0.041).

Among the 95 *S. aureus* isolates obtained from nasal specimens, 78 isolates (82.1%) were positive for at least one enterotoxin gene. The most prevalent enterotoxin gene in *S. aureus* isolates from nasal specimens was *sei* (70.5%), followed by *seg* (61.1%), *tst* (52.6%), and *sea* (47.4%) genes. As compared with the *S. aureus* isolated from blood, *S. aureus* isolates from

Table 4. Combination of enterotoxin genes in *S. aureus* from blood and nasal specimens

Gene combination	No. of *S. aureus* isolates		
	Blood	Nasal specimens	Total
sec, seg, sei, tst	21	1	22
sea, seg, sei, tst	4	12	16
seg, sei	6	10	16
sea, seg, seh, sei, tst	1	11	12
seg, sei, tst		9	9
sea, seg, sei	1	5	6
sea, sek	6		6
seh		6	6
sei, tst		5	5
seg, seh, sei, tst	1	3	4
sea, seh, sek	2	1	3
seg, seh, sei	1	2	3
sea, seg, seh, sei, sek, tst		2	2
sea, sei		2	2
tst		2	2
sea, sed, seg, sei, tst	1		1
sea, seg, seh, sei		1	1
sea, seg, sei, sek, tst		1	1
seb, sec, seg, sei, tst	2		1
seb, sed, seg, sei	1		1
seb, sec, seg, sei, sek, tst			1
seb	1		1
sec, seg, tst	1		1
sec, tst		1	1
seg	1		1
seg, seh, sei, sek		1	1
seh, sei, tst		1	1
seh, tst		1	1
sei		1	1
None	20	17	37
Total	70	95	165

Table 3. Prevalence of enterotoxin genes and *pvl* gene

Genes	Blood (n=70)		Nasal (n=95)		Blood (n=70) No. (%)	Nasal (n=95) No. (%)	*P*
	MRSA (n=36) No. (%)	MSSA (n=34) No. (%)	MRSA (n=18) No. (%)	MSSA (n=77) No. (%)			
sea	5 (13.9)	10 (29.4)	4 (22.2)	31 (40.3)	15 (21.4)	45 (47.4)	0.041
seb	2 (5.6)	2 (5.9)	–	–	4 (5.8)	–	0.031
sec	24 (66.7)	–	2 (11.1)	–	24 (34.3)	2 (2.1)	<0.000
sed	1 (2.8)	1 (3.0)	–	–	2 (2.9)	–	0.178
see	–	–	–	–	–	–	–
seg	31 (86.1)	10 (29.4)	12 (66.7)	46 (59.7)	41 (58.6)	58 (61.1)	0.789
seh	2 (5.6)	3 (8.8)	5 (27.8)	24 (31.2)	5 (7.2)	29 (30.5)	0.001
sei	30 (83.3)	9 (26.5)	13 (72.2)	54 (70.1)	39 (55.7)	67 (70.5)	0.056
sek	5 (13.9)	4 (11.8)	-	5 (6.5)	9 (12.9)	5 (5.3)	0.087
tst	26 (72.2)	5 (14.7)	9 (50.0)	41 (53.3)	31 (44.3)	50 (52.6)	0.259
pvl	–	–	–	–	–	–	–

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

Table 5. Distribution of enterotoxin genes by *spa* clonal complexes (*spa*-CC)

spa CC (No.)	No. (%) of isolates								
	sea	*seb*	*sec*	*sed*	*seg*	*seh*	*sei*	*sek*	*tst*
spa-CC002 (29)	1 (3.5)	3 (10.3)	26 (89.7)	1 (3.5)	27 (93.1)	–	26 (89.7)	–	26 (89.7)
spa-CC008 (12)	2 (16.7)	1 (8.3)	–	–	1 (8.3)	2 (16.7)	1 (8.3)	1 (8.3)	–
spa-CC037 (48)	34 (70.8)	–	–	1 (2.1)	36 (75.0)	10 (20.8)	38 (79.2)	7 (14.6)	24 (50.0)
spa-CC084 (49)	9 (18.4)	–	–	–	26 (53.1)	14 (28.6)	29 (59.2)	5 (10.2)	18 (36.7)
spa-CC1039 (4)	1 (25.0)	–	–	–	1 (25.0)	3 (75.0)	1 (25.0)	–	2 (50.0)
t375 (5)	2 (40.0)	–	–	–	3 (60.0)	1 (20.0)	3 (60.0)	–	3 (60.0)
t1151 (7)	–	–	–	–	1 (14.3)	1 (14.3)	1 (14.3)	–	2 (28.6)

nasal specimens showed significantly higher prevalence of *sea* and *seh* genes (P=0.041 and 0.001, respectively) and significantly lower prevalence of *seb* and *sec* genes (P=0.031 and <0.001, respectively) (Table 3). No *seb*, *sed*, *see*, and *pvl* genes were detected in the *S. aureus* isolates from nasal specimens. By way of contrast with the *S. aureus* isolates from blood, no significant differences in prevalence were detected between MRSA and MSSA isolates.

Among *S. aureus* isolates from blood, the most prevalent combination of enterotoxin genes was a combination of *sec-seg-sei-tst* (21 isolates, 30.0%), followed by combinations of *seg-sei* (6 isolates, 8.6%) and *sea-sek* (6 isolates, 8.6%) (Table 4). However, only one isolate from the nasal specimens showed combination of *sec-seg-sei-tst*. The most prevalent combinations of enterotoxin genes in *S. aureus* isolates from nasal specimens were *sea-seg-sei-tst* (12 isolates, 12.6%), *sea-seg-seh-sei-tst* (11 isolates, 11.6%), and *seg-sei* (10 isolates, 10.5%) (Table 4).

Distribution of enterotoxin genes by genotypes

In this analysis, we included all *S. aureus* isolates obtained from blood and nasal specimens (Table 5). Among the 29 isolates of *spa*-CC002, most contained *sec* (89.7%), *seg* (93.1%), *sei* (89.7%), and *tst* (89.7%) genes. Overall, 22 isolates of the *spa*-CC002 (75.9%) showed a combination of *sec*, *seg*, *sei*, and *tst* genes. It is worthy of note that the *sec* gene is found only in *S. aureus* isolates of *spa*-CC002. Only one isolate (3.5%) of *spa*-CC002 harbored no enterotoxin genes.

Among the 48 isolates of *spa*-CC037, *sea*, *seg*, *sei*, and *tst* genes were found in more than 50%. The distribution of enterotoxin genes in *S. aureus* isolates differed with respect to their sources. Whereas the *sea* and *sek* genes were detected in 92.9% and 42.9% of the isolates from blood, they were positive for 61.8% and 3.0% of the isolates from nasal specimens, respectively. However, *S. aureus* isolates from blood contained *seh* and *sei* genes at rates of 7.2% and 50.0%, but isolates from nasal specimens contained those genes at rates of 26.5% and 91.2%, respectively. Four isolates (8.3%) were found to be negative for all enterotoxin genes. Isolates of *spa*-CC008 harbored few enterotoxin genes.

DISCUSSION

According to the results of genotypic analysis using *spa* typing and MLST, MRSA strains from blood have different genetic backgrounds from MSSA strains. Whereas the majority of MRSA strains were limited to three *spa* groups, the MSSA strains were dispersed into a variety of groups. In this study, ST5-MRSA-II corresponding to *spa*-CC002 was the most prevalent MRSA clone that caused bacteremia, a result which was consistent with the result of previous study (23), in which clinical isolates from all types of specimens were included. However, only one MSSA isolate belonged to ST5. *spa*-CC002 or ST5 was not a principal clone in the *S. aureus* isolates obtained from nasal specimens (4 out of 95 isolates, 4.2%) (14). *spa*-CC084, which corresponds to CC72 in MLST, was the second most prevalent genotype in MRSA strains from blood, but was not detected in MSSA strains. This clone was the most prevalent in the MRSA isolates from nasal specimens (9 out of 18 isolates, 50.0%) (14). *spa*-CC084 was the most prevalent clone in the MSSA strains obtained from blood, and was also frequently detected in the MRSA strains. In addition, it was also the most prevalent in *S. aureus* isolates obtained from nasal specimens (34 out of 95 isolates, 35.8%) (14). However, it was not detected in MRSA isolates obtained from nasal specimens, that is, all *S. aureus* isolates of *spa*-CC037 obtained from nasal specimens were found to be methicillin-susceptible.

Notably, *spa*-CC037 of MSSA may differ from that of MRSA. The MRSA isolates of *spa*-CC037 corresponded to ST239 or its single locus variants, which were previously shown (23). However, only some strains of MSSA of *spa*-CC037 (*spa* type, WGKAOMQ) might belong to ST239, and the other six MSSA strains of *spa*-CC037 belonged to ST30 or its single locus variants. Although clones of ST239 and ST30 share the same allele at only one locus of *arcC*, ST239 is believed to emerge via the incorporation of a large portion of chromosome of ST30 into that of ST8 (24). In this study, *spa* types of ST8 (YHGFMBQBLO and YGFCBQBLO; *spa*-CC008) were quite different from those of ST239 (WGKAOMQ and WGKAKAOMQ; *spa*-CC037). The emergence and molecular evolution of ST8, ST30, and ST239 should be investigated further, because they constitute the prevalent epidemic clones of MRSA worldwide.

The prevalence of enterotoxin genes in *S. aureus* isolates from blood differed significantly from those obtained from nasal specimens with regard to the *sea*, *seb*, *sec*, and *seh* genes; whereas the *seb* and *sec* genes were present at higher levels in the blood isolates, the *sea* and *seh* genes were more frequently detected in the isolates from nasal specimens. Cha et al. (13) previously reported the prevalence of virulence genes in *S. aureus* isolates associated with staphylococcal food poisoning in Korea. In their study, the *sea* gene was detected in the majority of isolates (91.9%). In their study, the isolates with the *sea* gene were distributed in diverse clones, but were primarily detected in ST1, ST30, and ST59. That is, *S. aureus* strains that cause food poisoning are different clones from those that cause bacteremia or nasal colonization, according to MLST. The *seh* gene was also detected frequently in food poisoning-associated *S. aureus* isolates (198/332 isolates, 59.6%) (13). The *seh* gene was only infrequently detected in clinical isolates of *S. aureus* from Jordan (25). As the nasal carriage of *S. aureus* strains can be a cause of the contamination of manually handled food and transmission among humans, the relatively high presence of *sea* and *seh* genes in *S. aureus* isolates from nasal specimens constitutes a continuing matter of concern (26).

The *tst* gene encodes for TSST-1, which is associated with staphylococcal toxic shock syndrome (TSS) and is considered to be the cause of nearly all cases of menstrual TSS, and of at least 50% of nonmenstrual cases (11). As a whole, there was not significant difference of prevalence of *tst* gene between blood and nasal isolates. It is particularly noteworthy that the *tst* gene was highly prevalent in MRSA isolates from blood (72.2%). However, it was not significant as compared with MRSA isolates from nasal carriage (50.0%). Contrast to MRSA isolates, MSSA isolates from nasal carriage showed higher prevalence of *tst* gene than those from blood (53.3% vs. 14.7%). As has been reported by Chini et al. (27), the *tst* gene is considered to coexist with the enterotoxin gene cluster (*egc*), which includes *seg* and *sei*. However, among 97 isolates harboring both the *seg* and *sei* genes, 70 isolates (72.2%) were positive for the *tst* gene in our study.

The *sec* gene was frequently detected in *S. aureus* isolates from blood rather than from nasal specimens, which is a significant finding. The *sec* gene has been detected in only one isolate among food poisoning-associated isolates (13). This virulence gene was present exclusively in the MRSA isolates of a particular clone, *spa*-CC002 corresponding to ST5 in this study. A previous study in Korea reported that *sec* gene was detected mostly in clinical MRSA isolates with SCC*mec* type II, which may be correlated with ST5 in Korea. This clone, the most prevalent one in Korean hospitals (6, 7, 13, 23, 28), is characterized by the *sec-seg-sei-tst* gene combination. In general, prevalent *S. aureus* clones tended to harbor more virulence genes than minor clones. It has been reported that successful *S. aureus* clones usually harbor the *seg* and *sei* genes (29). This suggests that repertoires of virulence genes may be co-adapted to the genetic background, and that they may contribute to the biological fitness of the lineages into certain environments (9, 29). It is currently known that only several epidemic MRSA clones (such as CC5, CC8, CC22, CC30, and CC45) have spread worldwide (30, 31).

Despite the general relationships existing between genetic backgrounds and repertoires of virulence genes, there is some evidence to suggest considerable transmission of virulence genes on a background of clonality (10). Variations in virulence genes within a certain clone are suggestive of a common horizontal transfer of such genes. For example, the *sea*, *seb*, and *sed* genes existed solely in some isolates of *spa*-CC002. In addition, the *sec*, *seg*, *sei*, and *tst* genes existed in the majority of isolates of *spa*-CC002, but two (*seg*) or three (*sec*, *sei*, and *tst*) isolates did not harbor them.

In short, *S. aureus* isolates from blood have genotypes different from those obtained from nasal specimens. In addition, the genetic background of MRSA isolates that cause bacteremia was different from those of the MSSA isolates. *S. aureus* isolates from blood showed different prevalences in virulence genes from those obtained from nasal specimens. Some prevalent clones have characteristic virulence gene repertoires. The possession of virulence genes may affect the biological fitness of *S. aureus*, in addition to their pathogenesis, as suggested by van Belkum et al. (29). However, *S. aureus* clones causing bacteremia may differ from those causing nasal colonizer (7, 14, 32). The clonal difference of *S. aureus* isolates between two groups could be due to demographic differences and may indicate the different way of acquisitions of virulence genes. Thus, it should also be mentioned that this study has the limitation that the population of nasal colonizer is different from bacteremic patients.

REFERENCES

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. *Emergence and resurgence of methicillin-resistant Staphylococcus aureus as a public-health threat. Lancet 2006; 368: 874-85.*
2. Kim HB, Park WB, Lee KD, Choi YJ, Park SW, Oh M, Kim EC, Choe KW. *Nationwide surveillance for Staphylococcus aureus with reduced susceptibility to vancomycin in Korea. J Clin Microbiol 2003; 41: 2279-81.*
3. Lee K, Lim CH, Cho JH, Lee WG, Uh Y, Kim HJ, Yong D, Chong Y; KONSAR Group. *High prevalence of ceftazidime-resistnat Klebsiella pneumoniae and increase of imipenem-resistant Pseudomonas aeruginosa and Acinetobacter spp. in Korea: a KONSAR program in 2004. Yonsei Med J 2006; 47: 634-45.*
4. Kim ES, Song JS, Lee HJ, Choe PG, Park KH, Cho JH, Park WB, Kim SH, Bang JH, Kim DM, Park KU, Shin S, Lee MS, Choi HJ, Kim NJ, Kim EC, Oh MD, Kim HB, Choe KW. *A survey of community-associated methicillin-resistant Staphylococcus aureus in Korea. J Antimicrob Chemother 2007; 60: 1108-14.*
5. Cha HY, Moon DC, Choi CH, Oh JY, Jeong YS, Lee YC, Seol SY, Cho DT, Chang HH, Kim SW, Lee JC. *Prevalence of the ST239 clone*

of methicillin-resistant Staphylococcus aureus and differences in antimicrobial susceptibilities of ST239 and ST5 clones identified in a Korean hospital. J Clin Microblol 2005; 43: 3610-4.

6. Ko KS, Kim YS, Song JH, Yeom JS, Lee H, Jung SI, Jeong DR, Kim SW, Chang HH, Ki HK, Moon C, Oh WS, Peck KR, Lee NY. *Genotypic diversity of methicillin-resistant Staphylococcus aureus isolates in Korean hospitals. Antimicrob Agents Chemother 2005; 9: 3583-5.*
7. Kim JS, Song W, Kim HS, Cho HC, Lee KM, Choi MS, Kim EC. *Association between the methicillin resistance of clinical isolates of Staphylococcus aureus, their staphylococcal cassette chromosome mec (SCCmec) subtype classification, and their toxin gene profiles. Diagn Microbiol Infect Dis 2006; 56: 289-95.*
8. Jung SI, Shin DH, Park KH, Shin JH. *Antimicrobial susceptibility and clonal relatedness between community- and hospital-acquired methicillin-resistant Staphylococcus aureus from blood cultures. J Microbiol 2006; 44: 336-43.*
9. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. *Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant Staphylococcus aureus. J Infect Dis 2006; 193: 1495-503.*
10. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F. *Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups (alleles), and human disease. Infect Immun 2002; 70: 631-41.*
11. Dinges MM, Orwin PM, Schlievert PM. *Exotoxins of Staphylococcus aureus. Clin Microbiol Rev 2000; 13: 16-34.*
12. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J. *Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003; 9: 978-84.*
13. Cha JO, Lee JK, Jung YH, Yoo JI, Park YK, Kim BS, Lee YS. *Molecular analysis of Staphylococcus aureus isolates associated with staphylococcal food poisoning in South Korea. J Appl Microbiol 2006; 101: 864-71.*
14. Ko KS, Lee JY, Baek JY, Peck KR, Rhee JY, Kwon KT, Heo ST, Ahn KM, Song JH. *Characterization of Staphylococcus aureus nasal carriage from children attending an outpatient clinic in Seoul, Korea. Microb Drug Resist 2008; 14: 37-44.*
15. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing: 16th informational supplement. 2006; Document M100-S16. CLSI, Wayne, Pa, U.S.A.*
16. Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. *Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003; 41: 5442-8.*
17. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. *spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol 2004; 42: 792-9.*
18. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. *Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 1999; 37: 3556-63.*
19. Holtfreter S, Grunmann D, Schmudde M, Nguyen HT, Eichler P, Strommenger B, Kopron K, Kolata J, Giedrys-Kalemba S, Steinmetz I, Witte W, Bröker BM. *Clonal distribution of superantigen genes in clinical Staphylococcus aureus isolates. J Clin Microbiol 2007; 45: 2669-80.*
20. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. *Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 2000; 38: 1008-15.*
21. Oliveira DC, de Lencastre H. *Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2002; 46: 2155-61.*
22. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. *Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999; 29: 1128-32.*
23. Ko KS, Peck KR, Oh WS, Lee NY, Hiramatsu K, Song JH. *Genetic differentiation of methicillin-resistant Staphylococcus aureus strains from Korea and Japan. Microb Drug Resist 2005; 11: 279-86.*
24. Robinson DA, Enright MC. *Evolution of Staphylococcus aureus by large chromosomal replacements. J Bacteriol 2004; 186: 1060-4.*
25. El-Huneidi W, Bdour S, Mahasneh A. *Detection of enterotoxin genes seg, seh, sei, and sej and of a novel aroA genotype in Jordanian clinical isolates of Staphylococcus aureus. Diagn Microbiol Infect Dis 2006; 56: 127-32.*
26. Wertheim HF, Melles DC, Vos MC, Van Leeuwen W, Van Belkum A, Verbrugh HA, Nouwen JL. *The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 2005; 5: 751-62.*
27. Chini V, Dimitracopoulos G, Spiliopoulou I. *Occurrence of the enterotoxin gene cluster and the toxic shock syndrome toxin 1 gene among clinical isolates of methicillin-resistant Staphylococcus aureus is related to clonal type and agr group. J Clin Microbiol 2006; 44: 1881-3.*
28. Ko KS, Lee JY, Suh JY, Oh WS, Peck KR, Lee NY, Song JH. *Distribution of major genotypes among methicillin-resistant Staphylococcus aureus clones in Asian countries. J Clin Microbiol 2005; 43: 421-6.*
29. van Belkum A, Melles DC, Snijders SV, van Leeuwen WB, Wertheim HF, Nouwen JL, Verbrugh HA, Etienne J. *Clonal distribution and differential occurrence of the enterotoxin gene cluster, egc, in carriage- versus bacteremia-associated isolates of Staphylococcus aureus. J Clin Microbiol 2006; 44: 1555-7.*
30. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. *The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). Proc Natl Acad Sci USA 2002; 99: 7687-92.*
31. Robinson DA, Enright MC. *Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2003; 47: 3926-34.*
32. Aires de Sousa M, Conceicao T, Simas C, De Lencastre H. *Comparison of genetic backgrounds of methicillin-resistant and -susceptible Staphylococcus aureus isolates from Portuguese hospitals and the community. J Clin Microbiol 2005; 43: 5150-7.*