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Communication

Emergence of Panton-Valentine Leukocidin-Positive ST80 Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Busan, Korea

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Community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) has become widespread in the community and healthcare settings, and a number of clonal lineages emerged on every country. Sequence type (ST) 80 clone of CA-MRSA was dominant in Europe and has increasingly been isolated from the Middle East but so far never found in Korea. In this study, 48 MRSA isolates recovered from ear infections were characterized by multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylocoagulase (SC) genotyping, staphylococcal protein A gene (*spa*) typing, accessory gene regulator (*agr*) typing, and virulence gene profiling. Most MRSA strains belonged to three major clones: ST5-SCC*mec* II-SC type II (n=19, 39.6%), ST239-SCC*mec* III-SC type IV (n=15, 31.2%), and ST72-SCC*mec* IV-SC type Vb (n=11, 22.9%). Among the isolates, one strain was Panton-Valentine leukocidin (PVL)-positive ST80-SCC*mec* IV-SC type XIa - *spa* type t044-*agr* group III, and exfoliative toxin D-positive. This strain was susceptible to most antibiotics, but resistant to tetracycline and fusidic acid. This is the first report on the emergence of European ST80 CA-MRSA clone in Korea.

Key Words: MRSA, ST80 clone, Multilocus sequence type, Staphylocoagulase type

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human pathogen that causes both health care-associated (HA) and community-acquired (CA) infections (1, 2). Over the last decades, the increasing prevalence of MRSA infections and the worldwide spread of epidemic strains have been a serious health problem (3, 4). In addition, a number of clonal lineages emerged on every country. In Asian countries, two major HA-MRSA clones, multilocus sequence type (ST) 5 and ST239 are predominantly disseminated, whereas the CA-MRSA clones vary in different countries. Currently, worldwide reports of CA-MRSA are associated with >20 distinct genetic lineages, five of which are globally predominant, including ST1-SCC*mec* IV (USA-400), ST8-SCC*mec* IV (USA300), ST30-SCC*mec* IV (South West Pacific clone), ST59-SCC*mec* V (Taiwan clone), and ST80-SCC*mec* IV (European clone) (3,5). The major CA-MRSA clone in Korea, Panton-Valentine leukocidin (PVL) negative-ST72-SCC*mec* IV, is different from those that have spread in Asia or internationally (6, 7). Previously, we reported the high prevalence of ST72-SCC*mec* IV CA-MRSA clone in both isolates from children with skin infection

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(15/28, 53.6%) and nasal cavity of neonates (26/27, 96.3%) (8, 9). Several molecular typing methods, such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylocoagulase (SC) gene typing, staphylococcal protein A gene (*spa*) typing and accessory gene regulator (*agr*) grouping allowed the investigation of genetic backgrounds and clonal evolution of MRSA as for epidemiologic studies (10~14).

In the present study, we investigated the molecular characteristics of CA-MRSA isolates from ear infections by MLST, SCC*mec* typing, SC genotyping, *spa* typing, *agr* typing, and the presence of several virulence genes.

MATERIALS AND METHODS

MRSA isolates and genotyping

Forty-eight MRSA strains isolated from 2005 to 2006 in outpatients with ear infections at secondary hospitals in Busan, Korea were used. Identification and antimicrobial susceptibility test of the isolates were performed by using the Vitek 2 automated system (bioMerieux, March l' Etoile, France). Genomic DNA was extracted from each isolates using the *AccuPrep* DNA Extraction kit (Bioneer, Daejeon, Korea) according to the manufacture's protocol. Methicillin resistant strains were confirmed for the presence of the *mecA* gene by PCR as described previously (15). MLST typing was performed as described previously (11). PCR products for seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yquiL*) were obtained and sequenced using the ABI 3730 sequencer with BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA). The allelic profiles and sequence types were assigned by submission to the *S. aureus* MLST database (http://saueus.mlst.net). SCC*mec* typing was screened by the multiplex PCR as described by Olveria and de Lencastre (12). SC genotype was determined by the multiplex-PCR assay consisting of specific primers identifying type I to XI, and V subtypes, a and b, as described previously (13, 16). In addition, the amplification products of the D1 region and C-terminal repeat region were sequenced, and the phylogenetic relationship among sequences was analyzed by neighbor-joining method using the program MEGA 5 (http://www.megasoftware.net).

The polymorphic X region of the *spa* gene was amplified with specific primers as described previously (14). Purified PCR products were sequenced and short sequence repeats (SSRs) were assigned using the *spa* database web site (http://www.spaserver.ridom.de/). The *agr* gene was amplified as described previously (17). Detection of virulence factor genes, including *lukS-PV* and *lukF-PV* genes for PVL, staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*), toxic shock syndrome toxin (*tst*) gene and exfoliative toxin genes (*eta*, *etb*, *etd*) were performed by PCR-based assays as previously reported (18, 19).

RESULTS AND DISCUSSION

The 48 CA-MRSA isolates were classified into 6 STs, 3 SCC*mec* types, 5 SC types, and 9 *spa* types (Table 1). Three major clones, ST5-SCC*mec* II-SC type II (n=19, 39.6%), ST239-SCC*mec* III-SC type IV (n=15, 31.2%), and ST72-SCC*mec* IV-SC type Vb (n=11, 22.9%) were identified. The remaining three were ST80-SCC*mec* IV-SC type XIa, ST89-SCC*mec* II-SC type I, and ST1-SCC*mec* IV-SC type VII, respectively.

In Korea, PVL-negative ST72-SCC*mec* IV clone has been known as a major CA-MRSA clone, while ST5-SCC*mec* II and ST239-SCC*mec* III clones were known to be the HA-MRSA (6, 7). The high prevalence of ST5 and ST239 clones from outpatients with ear infections who had no risk factors for health care-associated infection were different clonal distribution compared to the results from skin and nares (8, 9). Among the MRSA isolates, one strain (SHD2) was identified as ST80-SCC*mec* IV clone, which has been known as an European CA-MRSA and has increasingly been isolated from the Middle East but so far never found in Korea.

ST80-SCC*mec* IV was first identified in the early 1990s and today is found throughout Europe, Middle East, and Northern Africa (2, 20). In Denmark, ST80-SCC*mec* IV isolates accounted for ~40% of all CA-MRSA cases from

Table 1. Molecular characteristics of 48 MRSA isolates from ear infections

MLST[a] (strain)	No. of isolates	SCC*mec* type	SC[b] type	*spa* type (n)	SSR[c] profile	*agr* type	Toxin genes *se*, *tst*, *et* (n)	PVL
ST5	19	II	II	t002(17) t548(2)	26-23-17-34-17-20-17-12-17-16 26-23-17-34-17-20-17-12-16	II	*sec* · *tst* (14) *tst* (5)	–
ST239	15	III	IV	t037(14) t633(1)	15-12-16-02-25-17-24 08-24	I	*sea* (6)	–
ST72	11	IV	Vb	t324(6) t664(5)	07-23-12-12-17-20-17-12-12-17 07-23-12-12-17-20-17-12-17	I	–	–
ST80 (SHD2)	1	IV	XIa	t044	07-23-12-34-34-33-34	III	*etd*	+
ST89	1	II	I	t375	49-13-23-05-17-34-33-34	III	*etb*	–
ST1	1	IV	VII	t286	07-23-13-34-16-34-33-13	III	–	–

[a] MLST, multilocus sequence type; [b] SC, staphylocoagulase ; [c] SSR, short sequence repeats

1999 to 2006, causing mostly skin and soft tissue infections (21). Interestingly, ST80 isolates have almost exclusively been found as MRSA and characterized as being resistant to fusidic acid and tetracycline (20).

Recently, PVL-positive MRSA strain with SCC*mec* IV and *spa* type t044, belonging to the ST80 lineage was reported for the first time in the USA (2). To our knowledge, ST80 MRSA clone has not been observed on East Asian countries. In this study, we report the molecular characteristics of Korean ST80 strain which was indistinguishable from the European and US ST80 clones, based on SCC *mec* type, *spa* typing and virulence gene profile.

SC is one of the important virulence factors produced by *S. aureus* strains and antigenically divergent among *S. aureus* strains. This protein consists of six regions, signal sequence, N-terminal D1 region and D2 region, central region, 27 amino acid-repeat region, and C-terminal sequence. Analysis of the DNA sequence data has shown that N-terminal regions, D1 and D2, are variable (approximately 60% identity) and central region is highly conserved (>90%) among the strains (22). The repeat regions comprising 81base pair (bp) tandem repeats are highly polymorphic in the number of repeats. Both the N-terminal region and the C-terminal repeat region have been used for *S. aureus* differentiation (13, 23). Historically, SCs have been classified into 10 serotypes based on the differences in antigenicity by inhibition test using type specific antibodies against each type of coagulase proteins (22, 24). Recently, the clustering analysis of the D1 regions of the *coa* showed that they were classified into 12 clusters, and nine of the 12 SC genotypes are divided into subtypes (13). In this study, 48 MRSA strains were classified as 6 SC types, SC type II (ST5 strain), IV (ST239), Vb (ST72), XIa (ST80), I (ST89) and VII (ST1). Among them, SHD2 strain (ST80) could not be determined the SC type by any SC typing methods because of its different sequences on N-terminal and C-terminal repeat regions. Therefore, we compared the sequence of N-terminal D1 region of SHD2 with the reference strains and determined as SC type XIa (Fig. 1).

Spa typing is another sequence-based technique which targets the polymorphic variable-number tandem repeat region of *spa*. The *spa* repeats are typically 24 bp in length, with presumptive duplications, deletions, and rearrangements contributing the identification of more than 10,000 unique patterns (14).

In this study, the prevalent spa types in ST5 and ST239 clones were t002 (n=17/19, 89.5%) and t037 (n=14/15, 93.3%), respectively. ST5 clone has multiple *spa* types and different distribution according to the sample sources (25). The predominance of single *spa* type in the cases of ST5

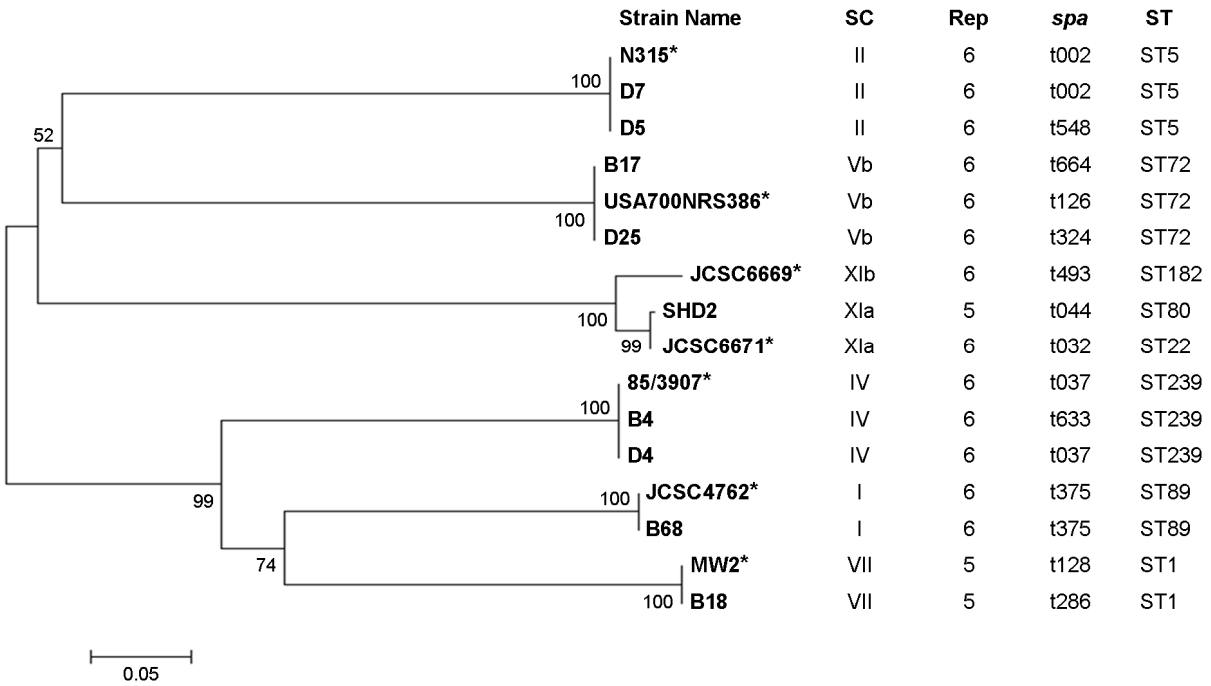

Figure 1. Phylogenetic relationship among the nucleotide sequences of the D1 *coa* regions of the selected 9 isolates and 7 reference strains. The numbers at nodes indicate the bootstrap values based on 1000 replicates. *, reference strains; SC, staphylocoagulase type; Rep, a number of C-terminal repeat units of *coa*; *spa*, staphylococcal protein A gene; ST, sequence type.

Table 2. Antimicrobial resistance rate of 48 MRSA isolates from ear infections

MLST	No. of isolates	% resistance								
		CLI	CIP	ERY	FUS	GEN	TET	TEI	SXT	VAN
ST5	19	100	100	95	16	68	89	0	0	0
ST239	15	100	93	100	20	100	87	0	0	0
ST72	11	27	0	82	0	18	0	0	0	0
ST80	1	0	0	0	100	0	100	0	0	0
ST89	1	0	0	100	0	100	0	0	0	0
ST1	1	0	0	100	0	100	0	0	0	0

CLI, clindamycin; CIP, ciprofloxacin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamycin; TET, tetracycline; TEI, teicoplanin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

and ST239 MRSA isolates represented the characteristics of CA-MRSA isolates from ear infection. ST72 clones were identified as two major *spa* types, t324 (n=6, 54.5%) and t664 (n=5, 45.5%). The current study has revealed that ST72-*spa* types, t324 and t664, were most common in the CA-MRSA. ST72-*spa* t324 strains already have been found in a few HA-MRSA isolates from tertiary care hospitals in Korea (6, 26).

All ST5 strains carried toxin genes, *sec* or *tst*, while 40% of ST239 strains carried sea genes. All isolates were PVL negative except ST80 strain. ST80 strain carried the genes encoding PVL toxin and exfoliative toxin D. There was

significant variance in the distribution of virulence genes among the clonal strains.

Antimicrobial resistance rates of the isolates are summarized in Table 2. The MRSA isolates showed different antimicrobial susceptibilities, depending on the ST clone. Isolates of ST5 and ST239 clones were resistant to multiple antibiotics except teicoplanin, trimethoprim-sulfamethoxazole and vancomycin, whereas ST72 clone was low resistance rates to antibiotics. ST80 clone was resistant to fusidic acid and tetracycline, which has a characteristic of the European ST80 clone CA-MRSA (20).

In conclusion, this study provides the information of the molecular characteristics of CA-MRSA isolates from ear discharge. There were significant clonal differences and correlation with SC type, *spa* type, and virulence gene profile. Furthermore, the PVL-positive ST80 CA-MRSA strain in Korea is an important finding which represents probably due to global spread of this pathogen.

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