Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Isolates in Korea

Running title: MOLECULAR TYPES OF MRSA IN KOREA

Eu Suk Kim, Hye Jin Lee, Gyung-Tae Chung, Yeong-Seon Lee, Dong-Hyeon Shin, Sook-In Jung, Kyoung-Ho Song, Wan-Beom Park, Nam Joong Kim, Kyoung Un Park, Eui-Chong Kim, Myoung-don Oh, and Hong Bin Kim

Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea; Laboratory of Antimicrobial Resistance, Infectious Disease Research Center, National Institute of Health, Seoul, Korea; Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea; Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; and Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea

*Corresponding author. Mailing address: Department of Internal Medicine, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam-si, 463-707, Republic of Korea.
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ABSTRACT

We used several molecular typing methods to analyze 196 methicillin-resistant *Staphylococcus aureus* (MRSA) and 139 methicillin-susceptible *S. aureus* (MSSA) isolates collected between 1996 and 2005. The sequence type 72 (ST72)-MRSA has increased in frequency in the community in Korea and in hospitals in recent years.
Multilocus sequence typing (MLST) (3) and staphylococcal chromosomal cassette mec (SCCmec) typing (11) are widely used to identify molecular differences between Staphylococcus aureus isolates. Studies of the evolution of S. aureus over prolonged periods have shown that the predominant strains can change (1, 15). Methicillin-resistant S. aureus (MRSA) accounts for more than 60% of nosocomial S. aureus isolates in Korea (6), and a few MRSA clones (sequence type [ST] 5-MRSA-SCCmec II, ST239-MRSA-SCCmec III, and ST239-MRSA-SCCmec IIA) predominate in Korean hospitals (8, 13). We performed the present analysis, based on MLST, SCCmec typing, and toxin gene profiling, to evaluate the molecular types of S. aureus in Korea over a prolonged period.

We selected at random 13-14% of the MRSA isolates and 4-8% of the methicillin-susceptible S. aureus (MSSA) stored in Korean investigations between 1996 and 2005, using the Microsoft Excel 2007 program. We also enrolled 15-88% of the MRSA and MSSA isolates taken from the anterior nares of healthy individuals in the community in 1997-1998 and 2005, respectively. We did not select the same proportion for the MRSA isolates and MSSA for each year. Details of these isolates are provided as Supplemental Material.

Confirmatory tests were performed using the Vitek system (BioMerieux, Durham, NC, USA). Antimicrobial susceptibility testing was carried out by the disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (4). Ten antibiotics
were tested and *S. aureus* ATCC 25923 served as a control strain. We performed SCCmec typing (11), MLST (3), multiplex PCR for genes encoding staphylococcal enterotoxins, staphylococcal exfoliative toxins, and toxic shock syndrome toxin (2), and PCR for detecting Panton-Valentine leucocidin (PVL) (10).

We evaluated 335 non-duplicated isolates selected from 2,901 *S. aureus*, of which 196 were MRSA, and 139 were MSSA. No MRSA were found among 42 *S. aureus* isolates from the anterior nares of healthy people in 1997-1998, whereas 8% (18/224) of such isolates were MRSA in 2005. MLST clustered the 196 MRSA into 24 STs, and the 139 MSSA into 39 STs.

Twenty-nine (9%) of the 335 *S. aureus* isolates were determined as novel polymorphisms in the seven allelic genes by MLST. Cluster analysis of the 335 *S. aureus* isolates was performed with the eBURST program and clonal complexes (CCs) were defined using a criterion of six alleles out of seven. The *S. aureus* isolates examined were designated to 8 CCs and 11 singletons. Seventy-two percent (242/335) of the *S. aureus* isolates belonged to one of three major CCs (CC1, CC5, or CC239). Among the MRSA, 87% (171/196) clustered into these major CCs, as did 51% (71/139) of the MSSA. Of the singletons, the ST72 clone (7%, 13/196) and the ST30 clone (9%, 13/139) were most frequent among the MRSA and MSSA isolates, respectively. Most of the ST72-MRSA clones were isolated from the anterior nares of healthy individuals in 2005.
The proportions (%) of the major MRSA and MSSA clones in the various pools of isolates are shown in Figure 1. Before 2003 we identified only one MRSA isolate of the ST72 clone, although this was the most common isolate among Korean community-associated MRSA (CA-MRSA) (ST72-MRSA-SCCmec IV/IVA) (5). The ST72 clone was not found among the MSSA isolates from the anterior nares of healthy peoples during 1997-1998, but in 2005 it was common among the MSSA (5/31) and MRSA (9/15), isolates from the anterior nares of healthy people.

Details of the major S. aureus clones in Korea are shown in Table 1. Susceptibility to antibiotics other than β-lactams differed depending on oxacillin resistance and ST. The MRSA isolates displayed different antimicrobial susceptibilities depending on ST: thus, while the ST5 and ST239 strains were resistant to most of the antibiotics except rifampin and sulfamethoxazole-trimethoprim, the ST72 strains were more susceptible to clindamycin, ciprofloxacin, and gentamicin.

Toxin gene analysis was performed on 335 S. aureus isolates. While 93% (183/196) of the MRSA isolates harbored at least one toxin gene, this was true of only 78% (108/139) of the MSSA isolates. PCR amplification of the PVL toxin gene was positive in only 14 (4%) of the S. aureus isolates, of which 3 and 11 were MRSA and MSSA, respectively. Each of the three PVL-positive MRSA were ST30-MRSA-SCCmec IV/IVA strains. The PVL-positive MSSA
isolates were assigned to ST1 (6), ST30 (2), ST59 (1), ST89 (1), and ST121 (1) by MLST. Toxin gene profiles also differed depending on oxacillin resistance and ST (Table 1).

The ST72-MRSA-SCC\textit{mec} IV/IVA clone was the commonest CA-MRSA clone in Korea (5). CA-MRSA is generally thought to have evolved from community-associated MSSA (CA-MSSA) clones that acquire the genes for SCC\textit{mec} IV. The ST72-MRSA-SCC\textit{mec} IV/IVA clone circulating in the community in Korea might also develop from CA-MSSA in Korea. However, a boundary between CA-MRSA and HA-MRSA is sometimes unclear in Korea in that the commonest nosocomial MRSA strains such as ST5 and ST239 were spreading in the community and CA-MRSA strains such as ST72 were isolated from patients in hospital settings (5, 12). ST72-MSSA isolates also formed a genetically distinct group from ST72-MRSA isolates in a recent study (9). Further work is needed to establish the origin of ST72-MRSA-SCC\textit{mec} IV/IVA clone because of lack of well-designed study for CA-MRSA in Korea.

The MRSA isolates carried more toxin genes than the MSSA isolates (93% versus 78%) and the toxin gene profiles depended on the molecular types of the strains (Table 1), as noted in another study (7). We also found that PVL was infrequent among both the MRSA (2%, 3/196) and MSSA (8%, 11/139) isolates. All of our PVL-positive MRSA isolates were clustered in the form of ST30-MRSA-SCC\textit{mec} IV/IVA clones. This clone was originally known as the Southwest Pacific clone and was also found in Japan (14). Evidently a potentially pandemic
PVL-positive ST30 CA-MRSA strain also exists in Korea.

This study has some limitations. First, the *S. aureus* isolates were selected from diverse pools obtained in community and hospital settings. Moreover, they were not selected evenly. Therefore, there may be some selection bias and the results may not reflect the nationwide trend. Second, we only gathered clinical information for the isolates from nasal swabs and for those from a previous study (5). Third, we did not perform pulsed-field gel electrophoresis (PFGE), *spa* typing, or the new method for differentiating non-typeable SCCmec strains.

In conclusion, the ST72-MRSA strain has become more frequent in the community in Korea and in hospitals in recent years.
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strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. J. Clin. Microbiol. 43:3356-3363.

Table 1. Molecular characteristics, antimicrobial susceptibilities, and toxin gene profiles of major *Staphylococcus aureus* clones isolated in Korea from 1996 through 2005

| Multilocus sequence typing |轫
<table>
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<tr>
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<tbody>
<tr>
<td>No. of isolates</td>
<td>EM</td>
</tr>
<tr>
<td>1 (1-0-1-0-1-0-1)</td>
<td>1 MSSA</td>
</tr>
<tr>
<td>MRSA</td>
<td>IV (6), IVA (1)</td>
</tr>
<tr>
<td>5 (1-1-0-0-1-0-1)</td>
<td>5 MSSA</td>
</tr>
<tr>
<td>MRSA</td>
<td>I (1), II (14), BA (34), IV (1), IVA (1), NT (3), NA (1)</td>
</tr>
<tr>
<td>6 (2-2-2-0-0-2-0-1)</td>
<td>5 MSSA</td>
</tr>
<tr>
<td>15 (13-2-1-0-1-0-1-1)</td>
<td>15 MSSA</td>
</tr>
<tr>
<td>30 (2-2-2-2-0-0-0-2)</td>
<td>30 MSSA</td>
</tr>
<tr>
<td>MRSA</td>
<td>IV (1), IVA (1), NT (1)</td>
</tr>
<tr>
<td>59 (10-25-15-2-10-28-15)</td>
<td>58 MSSA</td>
</tr>
<tr>
<td>72 (1-4-4-4-0-4-3)</td>
<td>72 MSSA</td>
</tr>
<tr>
<td>MRSA</td>
<td>IV (6), IVA (4), NA (3)</td>
</tr>
<tr>
<td>230 (2-3-1-0-0-4-3)</td>
<td>239 MSSA</td>
</tr>
<tr>
<td>254 (3-2-1-1-0-4-3)</td>
<td>259 MSSA</td>
</tr>
<tr>
<td>580 (3-5-4-9-1-0-26-10)</td>
<td>580 MSSA</td>
</tr>
</tbody>
</table>

**SCCmec, staphylococcal cassette chromosome mec; PVL, Panton-Vanlentine leucocidin; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; NT, non-typeable; NA, not available; EM, erythromycin; CM, clindamycin; CFLE, ciprofloxacin; GM, gentamicin; RFP, rifampin; SXT, sulfamethoxazole-trimethoprim**
Figure 1. The distribution of the clonal complexes (CCs) and sequence types (STs) of singletons among methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) isolates in Korea from 1996 through 2005.