Prevalence and Molecular Characteristics of Methicillin-resistant
*Staphylococcus aureus* Isolates in a Neonatal Intensive Care Unit

Sun Young Kim, So Hae Park and Soo Myung Hwang*

Department of Clinical Laboratory Science, Catholic University of Pusan, Korea

The molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from neonates in a neonatal intensive care unit (NICU) were investigated by multilocus sequence typing (MLST), staphylococcal cassette chromosome (SCC) genotyping, staphylococcal case chromosome mec (SCCmec) typing, accessory gene regulator (agr) typing, and the presence of Panton-Valentine leukocidin (PVL). Among the 44 *S. aureus* isolates from nares in neonates between March and June 2014 at hospital in Busan, 27 (61.4%) were MRSA and 17 (38.6%) were methicillin-susceptible *S. aureus* (MSSA). The most prevalent clone in MRSA isolates was ST72-SC type Vb-SCCmec IV-agr I (n=26) and the remaining one was ST89-SC type I-SCCmec II-agr II. In MSSA isolates, the prevalent clone was ST121-SC type Va-agr IV (n=13), followed by ST72-SC type Vb-agr I (n=2), ST8-SC type III-agr I (n=1) and ST15-SC type X-agr II (n=1). All isolates did not possess the PVL. The data showed that the neonates in NICU carried high prevalence of ST72 MRSA and remarkably different clones with SC diversity between MRSA and MSSA isolates.

Key Words: MRSA, NICU, Multilocus sequence type, Staphylocoagulase type, SCCmec type

*Staphylococcus aureus* is a major causative agent of hospital and community-associated infections (1, 2). Since the discovery of methicillin-resistant *S. aureus* (MRSA) in 1960, most MRSA infections were due to healthcare-associated MRSA (HA-MRSA) (3, 4). However, in recent year the emergence of community-associated MRSA (CA-MRSA) infections involving children without histories of health care-associated risk factors has been a serious public health problem (5, 6). In Korea, HA-MRSA has risen over the last decade and accounts for up to 70% of *S. aureus* infections, and represented by two predominant clones, sequence type (ST) 5-SCCmec type II and ST239-SCCmec type III (7, 8). In contrast, ST72-SCCmec type IV clone was the most common in the Korean CA-MRSA strains. It has become increasingly identified as a healthcare associated pathogen (9, 10). Recently published study on CA-MRSA isolates obtained from children with skin infection suggested the presence of different molecular type and virulence gene as a unique change of the Korean strains (11).

The anterior nares are the most frequent site for *S. aureus* colonization, and nasal carriage of *S. aureus* is an important risk factor for sepsis (12). Neonates are exposed to *S. aureus* shortly after birth and can become colonized quickly after contact with adult skin or their environment. Therefore, CA-MRSA in neonates can cause severe infections and associated with significant morbidity. Several outbreaks of infections...
caused by CA-MRSA in the NICUs have been documented (13–15). To our knowledge, there have been few studies for genetic traits and clonality of MRSA isolates from infants in NICU in Korea.

Molecular typing is one of the most important tools for studying epidemiology and evolution of pathogenic organisms. The currently used typing methods for S. aureus include multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), staphylococcal chromosome cassette mec (SCCmec) typing and multiple-locus variable tandem repeats analysis (MLVA) (16–19). Although PFGE is the standard method used historically in medical laboratories, MLST is a powerful tool for comparing strains and determining their phylogenetic and epidemiologic relatedness (20).

The production of staphylocoagulase (SC) is an important characteristic of S. aureus. SC has been classified into 10 serotypes, and comparisons between DNA sequence data from SC genes allowed phylogenetic groups, which could be predicted to the clonal complex (21). We reported the genetic diversity of SC genotypes and relatedness with clonality for CA-MRSA isolated from children with skin infections (11). The accessory gene regulator (agr) operon of S. aureus involves in the coordinated regulation of a number of virulence factors and is composed of a large set of genes, agrA, agrC, agrD, agrB and RNAIII. According to the variation between agrC and agrD, 4 agr groups are reported and used for epidemiological studies (21). In the present study, we investigated molecular characteristics of MRSA isolates from NICU-hospitalized neonates using MLST, SC typing, SCC mec typing, agr typing and Panton-Valentine leukocidin (PVL) genes, and compared them with the major MRSA clones in the community.

Between March and June 2014, a total of 44 S. aureus isolates were obtained from neonatal nares (1 to 30 days old) in St. Mary’s Hospital in Busan, Korea were analyzed. Identification of S. aureus isolates was performed on the Vitek 2 automated system (bioMérieux, Marcy l’Etoile, France). Antimicrobial susceptibility testing was performed using the modified broth microdilution. The antibiotics tested included oxacillin, clindamycin, erythromycin, gentamycin, fusidic acid, ciprofloxacin, mupirocin, tetracycline and vancomycin. The S. aureus ATCC29213 strain was used for quality control. Genomic DNA was extracted from each isolate using the AccuPrep DNA Extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer’s protocol. Identification of MRSA strain was performed by detection of mecA using PCR with specific primers as described previously (23). MLST was performed for all isolates of S. aureus by PCR and sequence analysis of seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, and yqiL) as described previously (16). The allelic profiles and sequence types were assigned by submission to the S. aureus MLST database (http://saureus.mlst.net/). The SC genotypes were determined by the multiplex-PCR assay consisting of specific primers identifying type I to X, and V subtypes, a and b, as described previously (11, 24). SCCmec types of MRSA isolates were screened by the multiplex PCR as described by Oliveria and de Lencastre (18). agr specificity groups were identified by multiplex-PCR amplification as previously described (21). All of the S. aureus isolates were screened for the presence of PVL gene by PCR as previously described (25).

Methicillin resistance was confirmed by oxacillin resistance and PCR amplification of the mecA. Of the 44 isolates, 27 (61.4%) were MRSA, and 17 (38.6%) were MSSA. The molecular characteristics of the 27 MRSA isolates were classified into 2 clones of STs, SC genotypes, SCCmec, and agr types (Table 1). The most prevalent MRSA clone was ST72-SC type Vb-SCCmec IV-agr I (96.3%, n=26) and the remaining one was ST89-SC type I-SCCmec II-agr II. The 17 MSSA isolates were classified into 4 clones of STs and 4 SC genotypes; ST121-SC type Va-agr IV (n=13), ST72-SC type Vb-agr I (n=2), ST8-SC type III-agr I (n=1), ST15- and SC type X-agr I (n=1). The PVL gene was not detected in any of the strains. Comparing to the MRSA clonality, considerable molecular differences existed among the MSSA isolates. Susceptibility to antibiotics other than beta-lactam differed between strains with the same ST. All ST72 MRSA clone were susceptible to fusidic acid, while ST72 and ST121 strains of MSSA isolates were resistant to fusidic acid. We observed differences in susceptibility to antimicrobial agents
between strains with the same sequence type.

Nasal carriage of *S. aureus*, particularly MRSA in neonates is strongly associated with risk factors for developing subsequent infections (12). In this study, we examined the prevalence and molecular characteristics of MRSA isolates from neonate nares in a NICU. Among the 44 *S. aureus* isolates, 27 (61.4%) were MRSA. Neonates are exposed to *S. aureus* shortly after birth and can become colonized easily after contact with their environments. The nares and umbilicus are the most common sites of initial colonization (13). In Korea, the isolation rate of MRSA in hospitals was extremely high, up to 70% (8). High MRSA colonization or infection in neonate nares represents an important reservoir for dissemination of epidemic MRSA strains. In this study, the most prevalent clone of MRSA was identified as ST72-SCC _mec_ IV-PVL negative strains, while the prevalent clone in MSSA isolates was ST121-SC type Va- _agr_ IV (n=13). ST72-SCC _mec_ IV has been known as the CA-MRSA clone and widely disputed in healthy Korean children (26). A recent report showed that ST72 clone has spread to hospitals and increased in frequency in HA-MRSA (27).

The molecular epidemiology of CA-MRSA isolates from different countries is characterized by clonal heterogeneity. The strain of USA300 (ST8, SCC _mec_ IV, PVL positive), the most successful CA-MRSA strain, which is now endemic in the US, but occur infrequently in other geographical areas (28). PVL-positive ST30-SCC _mec_ IV strains have been known as the Southwest Pacific clone (5). ST72-SCC _mec_ IV/PVL-negative, the major CA-MRSA clone in Korea, is different from those that have spread in Asia and other countries (5). Our previous study showed that two major clones of CA-MRSA isolates from children with skin infections were ST72-SC type Vb-SCC _mec_ IV (n=15/28, 53.6%) and ST89-SC type I-SCC _mec_ type II (n=12/28, 42.8%). In addition, the prevalent clone in MSSA was ST72-SC type Va (n=21/41, 51.2%) and followed ST121-SC type Va (n=8/41, 19.5%).

Staphylocoagulase (SC), one of the important characteristics of *S. aureus*, is antigenically divergent, and classified into 10 serotypes. Therefore, SC serotyping and genotyping methods have been designed for epidemiologic study of *S. aureus*. The SC genes (_coa_) were commonly composed of six distinct segments. Among the _coa_ segments, D1 region presented more diversity than those of other regions (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 (22). With SC genotyping used multiplex-PCR, 4 SC types were identified as type I, III, V (Va ,Vb) and X. Most of the isolates were SC type V (n=41/44), and divided into sub-type Vb (96.3%) in MRSA and Va (76.5%) in MSSA.

Table 1. Molecular characteristics and antimicrobial susceptibility of MRSA and MSSA isolates

<table>
<thead>
<tr>
<th>ST</th>
<th>SC type</th>
<th>No. of isolate</th>
<th>SCC <em>mec</em> type</th>
<th><em>agr</em> type</th>
<th>PVL</th>
<th>No (%) of isolate susceptible to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OXA</td>
</tr>
<tr>
<td>ST72</td>
<td>Vb</td>
<td>26</td>
<td>IV</td>
<td>I</td>
<td>–</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ST89</td>
<td>I</td>
<td>1</td>
<td>II</td>
<td>II</td>
<td>–</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ST121</td>
<td>Va</td>
<td>13</td>
<td>MSSA</td>
<td>IV</td>
<td>–</td>
<td>13 (100)</td>
</tr>
<tr>
<td>ST72</td>
<td>Vb</td>
<td>2</td>
<td>MSSA</td>
<td>I</td>
<td>–</td>
<td>2 (100)</td>
</tr>
<tr>
<td>ST8</td>
<td>III</td>
<td>1</td>
<td>MSSA</td>
<td>I</td>
<td>–</td>
<td>1 (100)</td>
</tr>
<tr>
<td>ST15</td>
<td>X</td>
<td>1</td>
<td>MSSA</td>
<td>II</td>
<td>–</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

sources and nasal cavities of healthy persons, 1994-2005. On the data, SC serotype V strain was only identified from MSSA isolates in 1994, but after 2000, SC serotype V was rapidly increased to more than 30% in both MRSA and MSSA isolates in 2005 (29). The high prevalence of SC types Va and Vb of *S. aureus* isolates from nares of neonates to the community represents the epidemiological significance of staphylococcal infection and evolutionary characteristics of Korean strains.

The accessory gene regulator (*agr*) controls the staphylococcal virulence factors and other accessory gene functions (21). The *agr* locus belongs to the core variable genome like SC gene, so it is linked to clonal complexes. Four *agr* types are grouped and used for epidemiological classification of *S. aureus* isolates (22). Of the 44 isolates, 29 had an *agr* I strains, 2 an *agr* II, and 13 an *agr* IV strains. We confirmed that all strains defined as the same sequence type by MLST had the same SC type and *agr* type.

In conclusion, the present study provides the prevalence of MRSA colonization and infection rates in NICU, and the information of the molecular characteristics of MRSA isolates. The most prevalent clone was ST72-SCCmec IV-SC type Vb as known as CA-MRSA clone whereas different clonality was found in MSSA isolates. NICU could be the first site for CA-MRSA acquisition and transmission of HA-MRSA during birth by direct or through contaminated environments. Continuous monitoring of molecular epidemiology for MRSA will be fundamental to support the *S. aureus* evolution.

**REFERENCES**

14) Carey AJ, Long SS. *Staphylococcus aureus*: a contin-