Journal of Bacteriology and Virology 2016. Vol. 46, No. 2 p.99 – 103
http://dx.doi.org/10.4167/jbv.2016.46.2.99

Communication

# 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), staphylocoagulase (SC) genotyping, staphylococcal cassette chromosome *mec* (SCC*mec*) 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-SCC*mec* IV-*agr* I (n=26) and the remaining one was ST89-SC type I-SCC*mec* 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, SCC*mec* 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-SCC*mec* type II and ST239-SCC*mec* type III (7, 8). In contrast, ST72-SCC*mec* 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

Received: May 9, 2016/ Revised: June 15, 2016/ Accepted: June 21, 2016
*Corresponding author: Soo Myung Hwang. Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan 46252, Korea.
Phone: +82-51-510-0563, Fax: +82-51-510-0568, e-mail: smhwang@cup.ac.kr

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* (SCC*mec*) 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. Idenitifcation 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 *yquiL*) as described previously (16). The allelic profiles and sequence types were assigned by submission to the *S. aureus* MLST database (http://saueus.mlst.net/). The SC genotypes were determined by the multiplex-PCR assay consisting of specific primers identifying type l to X, and V subtypes, a and b, as described previously (11, 24). SCC*mec* types of MRSA isolates were screened by the multiplex PCR as described by Olveria 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, SCC*mec*, and *agr* types (Table 1). The most prevalent MRSA clone was ST72-SC type Vb-SCC*mec* IV-*agr* I (96.3%, n=26) and the remaining one was ST89-SC type I-SCC*mec* 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 lll-*agr* I (n=1), ST15- and SC type X-*agr* l (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

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

| ST | SC type | No. of isolate | SCC *mec* type | *agr* type | PVL | No (%) of isolate susceptible to | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OXA | CL | EM | GM | FA | CIP | MUP | TET | VA |
| ST72 | Vb | 26 | IV | I | − | 0 (0) | 19 (73) | 19 (73) | 15 (0) | 26 (100) | 26 (100) | 18 (69) | 23 (88) | 26 (100) |
| ST89 | I | 1 | II | II | − | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 1 (100) | 1 (100) |
| ST121 | Va | 13 | MSSA | IV | − | 13 (100) | 13 (100) | 13 (100) | 6 (46) | 1 (8) | 13 (100) | 2 (15) | 13 (100) | 13 (100) |
| ST72 | Vb | 2 | MSSA | I | − | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 2 (100) | 1 (50) | 2 (100) | 2 (100) |
| ST8 | III | 1 | MSSA | I | − | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 1 (100) |
| ST15 | X | 1 | MSSA | II | − | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; ST, sequence type; SC, staphylocoagulase; SCC*mec*, staphylococcal cassette chromosome *mec*; OXA, oxacillin; CL, clindamycin; EM, erythromycin; GM, gentamycin; FA, fucidic acid; CIP, ciprofloxacin; MUP, mupirocin; TET, tetracycline; VA, vancomycin.

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-SC type Vb-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 Vb (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 subtype Vb (96.3%) in MRSA and Va (76.5%) in MSSA.

Previously we reported the distribution and phenotypic changes of SC serotypes in *S. aureus* isolated from clinical

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-SCC*mec* 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

1) Pereira VC, Riboli DF, da Cunha Mde L. Characterization of the clonal profile of MRSA isolated in neonatal and pediatric intensive care units of a University Hospital. Ann Clin Microbiol Antimicrob 2014;13:50.
2) David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616-87.
3) Jevons, MP. "Celbenin"-resistant Staphylococci. Br Med J 1961;14:124-5.
4) 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.
5) Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect 2014;20:605-23.
6) Bae IG, Kim JS, Kim S, Heo ST, Chang C, Lee EY. Genetic correlation of community-associated methicillin-resistant *Staphylococcus aureus* strains from carriers and from patients with clinical infection in one region of Korea. J Korean Med Sci 2010;25:197-202.
7) Kim ES, Song JS, Lee HJ, Choe PG, Park KH, Cho JH, *et al*. A survey of community-associated methicillin-resistant *Staphylococcus aureus* in Korea. J Antimicrob Chemother 2007;60:1108-14.
8) Kim ES, Lee HJ, Chung GT, Lee YS, Shin DH, Jung SI, *et al*. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates in Korea. J Clin Microbiol 2011;49:1979-82.
9) Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, *et al*. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 2011;66:1061-9.
10) Lee SS, Kim YJ, Chung DR, Jung KS, Kim JS. Invasive infection caused by a community-associated methicillin-resistant *Staphylococcus aureus* strain not carrying Panton-Valentine leukocidin in South Korea. J Clin Microbiol 2010;48:311-3.
11) Park SH, Kim KJ, Kim BK, Hwang SM. Molecular characterization of community-associated methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from children with skin infections in Busan, Korea. J Bacteriol Virol 2015;45:104-111.
12) Lamers RP, Stinnett JW, Muthukrishnan G, Parkinson CL, Cole AM. Evolutionary Analyses of *Staphylococcus aureus* identify genetic relationships between nasal carriage and clinical isolates. PLoS One 2011;6:e16426.
13) Nelson MU, Gallagher PG. Methicillin-resistant *Staphylococcus aureus* in the neonatal intensive care unit. Semin Perinatol 2012;36:424-30.
14) Carey AJ, Long SS. *Staphylococcus aureus*: a contin-

uously evolving and formidable pathogen in the neonatal intensive care unit. Clin Perinatol 2010;37:535-46.
15) David MD, Kearns AM, Gossain S, Ganner M, Holmes A. Community-associated methicillin-resistant *Staphylococcus aureus*: nosocomial transmission in a neonatal unit. J Hosp Infect 2006 Nov;64:244-50.
16) 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.
17) Ichiyama S, Ohta M, Shimokata K, Kato N, Takeuchi J. Genomic DNA fingerprinting by pulsed-field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1991;29:2690 -95.
18) 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.
19) Tenover FC, Vaughn RR, McDougal LK, Fosheim GE, McGowan JE Jr. Multiple-locus variable-number tandem-repeat assay analysis of methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol 2007;45:2215-9.
20) Feil EJ, Enright MC. Analyses of clonality and the evolution of bacterial pathogens. Curr Opin Microbiol 2004;7:308-13.
21) Shopsin B, Mathema B, Alcabes P, Said-Salim B, Lina G, Matsuka A, *et al*. Prevalence of *agr* specificity groups among *Staphylococcus aureus* strains colonizing children and their guardians. J Clin Microbiol 2003;41:456 -9.
22) Watanabe S, Ito T, Sasaki T, Li S, Uchiyama I, Kishii K, *et al*. Genetic diversity of staphylocoagulase genes (*coa*): insight into the evolution of variable chromosomal virulence factors in *Staphylococcus aureus*. PLoS One 2009;4:e5714.
23) Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991;29:2240-4.
24) Hirose M, Kobayashi N, Ghosh S, Paul SK, Shen T, Urushibara N, *et al*. Identification of staphylocoagulase genotypes I-X and discrimination of type IV and V subtypes by multiplex PCR assay for clinical isolates of *Staphylococcus aureus*. Jpn J Infect Dis 2010;63:257 -63.
25) Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, *et al*. Involvement of Panton-Valentine leukocidin producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29: 1128-32.
26) Sung JY, Lee J, Choi EH, Lee HJ. Changes in molecular epidemiology of community-associated and health care-associated methicillin-resistant *Staphylococcus aureus* in Korean children. Diagn Microbiol Infect Dis 2012; 74:28-33.
27) Lee J, Sung JY, Kim YM, Oh CE, Kim HB, Choi EH, *et al*. Molecular characterization of methicillin-resistant *Staphylococcus aureus* obtained from the anterior nares of healthy Korean children attending daycare centers. Int J Infect Dis 2011;15:558-63.
28) McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol 2003;41:5113-20.
29) Hwang SM, Kim TU. Changes in coagulase serotype of *Staphylococcus aureus* isolates in Busan, 1994-2005. Kor J Microbiol 2007;43:346-50.