International Journal of Infectious Diseases 15 (2011) e558–e563

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Molecular characterization of methicillin-resistant *Staphylococcus aureus* obtained from the anterior nares of healthy Korean children attending daycare centers☆

Jina Lee [a], Ji Yeon Sung [b], Young Min Kim [b], Chi Eun Oh [b], Hong Bin Kim [a], Eun Hwa Choi [b,c], Hoan Jong Lee [b,c,*]

[a] *Seoul National University Bundang Hospital, Seongnam, Korea*
[b] *Seoul National University Children's Hospital, Seoul, Korea*
[c] *College of Medicine, Seoul National University, Seoul, Korea*

ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 8 March 2011
Accepted 22 April 2011

Corresponding Editor: J. Peter Donnelly, Nijmegen, the Netherlands.

Keywords:
MLST
SCC*mec* type
Sequence type
Colonization
Inducible resistance

SUMMARY

Objectives: This study was performed to investigate the molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from the anterior nares of Korean children attending daycare centers.

Methods: During September and October 2008, a survey of nasal carriage of MRSA was conducted among healthy children who were attending daycare centers in Seoul, Korea. Nasal swab samples were cultured to isolate *S. aureus*, and antimicrobial susceptibility was assessed using a disk diffusion test. All MRSA isolates were archived for subsequent molecular tests, including multilocus sequence typing, Panton–Valentine leukocidin (PVL) genes polymerase chain reaction (PCR), and staphylococcal cassette chromosome *mec* (SCC*mec*) typing.

Results: Among 428 preschool-aged children enrolled, 9.3% (40/428) were colonized with MRSA. Among the 40 MRSA isolates, antibiotic susceptibilities to clindamycin and erythromycin were 97.5% (39/40) and 45% (18/40), respectively. All of the 21 strains susceptible to clindamycin and resistant to erythromycin had MLS_B-inducible phenotypes. Sequence type (ST) 72–SCC*mec* type IV was the predominant clone ($n = 23$; 57.5%), followed by ST72–SCC*mec* type II ($n = 6$; 15%), ST1765–SCC*mec* type IV ($n = 4$; 10%), ST1765–SCC*mec* type II ($n = 2$; 5%), and ST1–SCC*mec* type IV ($n = 2$; 5%). No clone was positive for PVL genes.

Conclusions: ST72 strains, which were previously found in hospital-associated MRSA, are now widely distributed in healthy Korean children. In addition, the prevalence of inducible resistance of clindamycin should be considered when selecting empirical antibiotics for community-associated MRSA infections in Korea.

1. Introduction

Since the 1990s, many studies have reported a significant prevalence of community-associated (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) colonization or infection.[1] Severe pneumonia and death associated with CA-MRSA infections are frequently reported in the USA.[2] In Korea, studies focusing on the clinical significance and burden of CA-MRSA infections among healthy children have rarely been reported; however, 9.3% of healthy Korean children were found to be colonized with CA-MRSA,[3] and MRSA was found to account for 30.2% of community-acquired *S. aureus* infections in our institute.[4]

CA-MRSA infections typically occur in patients without established risk factors for healthcare-associated MRSA (HA-MRSA), including recent hospitalization, surgery, or residence in long-term care.[5] Recently, however, CA-MRSA strains have been reported in both the community and in healthcare settings.[6] Molecular characterization, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), allow the genotypes of CA-MRSA to be distinguished from HA-MRSA. CA-MRSA isolates are usually represented by sequence type (ST) 8 (which encompasses USA300 clone by PFGE) and ST1 (USA400 by PFGE) in the USA.[7,8]

The prevalence of MRSA carriage is diverse and depends upon multiple factors, including age, environment, and geographic

☆ This manuscript was presented in part at the 48th Annual Meeting of the Infectious Diseases Society of America, Vancouver, British Columbia, Canada, October 21–24, 2010.

* Corresponding author. Tel.: +82 2 2072 3633; fax: +82 2 745 4703.
E-mail address: hoanlee@snu.ac.kr (H.J. Lee).

1201-9712/$36.00 – see front matter © 2011 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2011.04.010

distribution. The anterior nares are the most frequent site for *S. aureus* colonization,[9] and nasal carriage of *S. aureus* is an important risk factor for sepsis. Carriers have higher rates of nosocomial *S. aureus* bacteremia than non-carriers,[10] and a large proportion of nosocomial *S. aureus* infections originates from the patients' own flora.[11] Therefore, it is important to determine the prevalence of CA-MRSA carriage among healthy Korean children in order to estimate the potential burden of CA-MRSA infections in the community setting.

Recently published studies on CA-MRSA isolates obtained from Korean children suggest the presence of different molecular types and a unique evolution of CA-MRSA in Korea compared to Western countries.[12,13] However, studies on the prevalence and molecular characterization of colonized MRSA among Korean children have been based on patients who required hospital visits or medical treatment. Very few community-based studies evaluating the prevalence of MRSA carriage and molecular characterization have been performed in Korea.

We conducted this study to determine the molecular characterization of MRSA isolated from community settings among Korean children by MLST, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and toxin gene assays using the Panton–Valentine leukocidin (PVL) genes. In addition, antibiotic susceptibility was analyzed to identify the optimal empirical antibiotics for the treatment of potential CA-MRSA infections in Korean children.

2. Materials and methods

2.1. Study participants and bacterial strains

This was a point-prevalence study conducted in seven different daycare centers located in the northern areas of Seoul, Korea. From September to October 2008, 428 preschool-aged children with a mean age of 55 months (range 12 months to 6.8 years), who attended these daycare centers and who were apparently healthy, were enrolled. This study was approved by the Institutional Review Board of Seoul National University Hospital. Informed consent was obtained from the children's parents or guardians.

Specimens for culture were obtained from both anterior nares with a sterile, wet cotton swab. Each swab was immediately placed in an enrichment broth, processed in a microbiology laboratory within 2 h of sampling, and incubated at 35 °C overnight. The enrichment broth for *S. aureus* contained 37.5 g NaCl, 1.25 g yeast extract, 5.0 g tryptone, and 500 ml distilled water. Each 10 μl of incubated enrichment broth was inoculated in mannitol salt agar (Bosung Science, Seoul, Korea), which is selective for *S. aureus*, and incubated at 35 °C for 24–48 h; yellow colonies were selected and confirmed to be *S. aureus* following catalase, coagulase, and DNAse tests.[3] Nasal screening identified 164 (38.3%) *S. aureus* carriers, including 40 (9.3%) MRSA and 124 (29.0%) methicillin-sensitive *S. aureus* (MSSA) carriers.[3] As a part of this study, the prevalence of MRSA carriage among healthy Korean children attending daycare centers has been published.[3]

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined according to the guidelines of the Clinical and Laboratory Standards Institute;[14] isolates that were resistant to oxacillin or cefoxitin were considered to be MRSA. Susceptibility to penicillin G, oxacillin, clindamycin, erythromycin, gentamicin, vancomycin, tetracycline, ciprofloxacin, rifampin, and trimethoprim/sulfamethoxazole (TMP/SMX) was determined using the disk diffusion method. In vitro macrolide–lincosamide–streptogramin B (MLS_B)-inducible phenotypes were detected by the D-zone test (double-disk diffusion test).[15]

2.3. MLST and Based Upon Related Sequence Types (eBURST) analysis

MLST was performed for all MRSA isolates by polymerase chain reaction (PCR) and sequencing of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) using primers designed by Enright et al.[16] Each sequence was submitted to the MLST database website (http://saureus.mlst.net) for assignment of an allelic profile and ST. To understand the evolution and population structure of MRSA in Korea, some of the MSSA isolates that were obtained from patients included in our study sample were also analyzed by MLST.

The allelic profiles of *S. aureus* isolates were compared using the eBURST program to infer patterns of evolutionary descent of *S. aureus* isolates from the putative ancestral allelic profile or clonal complex (CC). These data are available at http://saureus.mlst.net/eburst.

2.4. SCC*mec* typing

All MRSA isolates were typed to identify SCC*mec*, which is the heterologous, mobile genetic element that carries the *mecA* gene that encodes methicillin resistance. SCC*mec* typing of isolates and PCR for the *mecA* gene was conducted following a multiplex PCR strategy described by Milheiriço et al.[17] Control strains for SCC*mec* types I, II, III, IV, V, and IV were as follows: type I, COL;[17] type II, BK2464;[18] type III, ANS46;[17] type IV, MW2;[17] type V, WIS;[17] type VI, HDE288.[17]

2.5. MRSA clones

MRSA clones were defined by both their genotype (ST) and their SCC*mec* type as proposed by Enright et al.[19]

2.6. Identification of the PVL genes by PCR

PCR amplification of genes encoding for PVL was performed for all MRSA isolates and for some MSSA isolates included in the MLST analysis, in accordance with the method described by Lina et al.[20]

3. Results

3.1. Molecular characterization of MRSA isolates

All 40 MRSA isolates were positive for the *mecA* gene. The results of SCC*mec* typing demonstrated that SCC*mec* type IV (80%; 32/40) was the most common type, followed by SCC*mec* type II (20%; 8/40). The PVL genes were not present in any of the 40 MRSA isolates.

The most frequently detected ST of MRSA isolates was ST72 ($n = 29$), followed by ST1765 ($n = 6$), which was a single-locus variant (SLV) of ST72 and was newly assigned in this study. The remaining STs – ST1735, ST1736, and ST1737 –were newly assigned STs, all of which were SCC*mec* type IV.

Overall, ST72–SCC*mec* type IV was the predominant clone ($n = 23$; 57.5%) among MRSA colonization isolates, followed by ST72–SCC*mec* type II ($n = 6$; 15%), ST1765–SCC*mec* type IV ($n = 4$; 10%), ST1765–SCC*mec* type II ($n = 2$; 5%), and ST1–SCC*mec* type IV ($n = 2$; 5%) (Table 1).

3.2. Molecular characterization of MSSA isolates

To compare STs of MSSA and MRSA isolates, MLST was performed on 20 randomly selected isolates from among our 124 MSSA isolates. The most frequently detected ST in those tested was ST30 ($n = 8$), followed by ST72 ($n = 3$), ST6 ($n = 2$), and ST580 ($n = 2$) (Table 2). ST1, ST188, ST1738, ST1753, and ST1754 were