Epidemiological Characteristics of Methicillin-Resistant
Staphylococcus aureus Isolates from Children with
Eczematous Atopic Dermatitis Lesions

Hee-Jung Chung,1 Hong-Seon Jeon,1 Heungsup Sung,1 Mi-Na Kim,1,* and Soo-Jong Hong2

Department of Laboratory Medicine1 and Department of Pediatrics,2 University of Ulsan College of Medicine and
Asan Medical Center, Seoul, Republic of Korea

Received 30 March 2007/Returned for modification 6 July 2007/Accepted 24 December 2007

In this study, we investigated the rate of colonization of skin of children with atopic dermatitis (AD) by
methicillin-resistant Staphylococcus aureus (MRSA) and characterized the isolates. Active skin lesions in
pediatric AD patients were cultured with Rodac Staph (Komed, Korea). S. aureus isolates were examined for
drug susceptibilities, analyzed for the eta, etb, tst, and pvl genes, and typed using agr polymorphism, pulsed-field
gel electrophoresis of Smal-restricted chromosomal DNA, and staphylococcal cassette chromosome mec
(SCCmec) typing. Eighty-seven (75.4%) of 115 patients had cultivable S. aureus isolates, 16 of which (18.3%)
were MRSA. All MRSA isolates were susceptible to chloramphenicol, rifampin, cotrimoxazole, and ciprofloxa-
cin. While methicillin-susceptible S. aureus (MSSA) isolates were composed of 23 isolates of singular types and
nine clusters comprising 48 isolates, MRSA isolates were typed into three clones: eight isolates of pulsotype
C–agr–3–SCCmec IV. Three SCCmec IVA MRSA isolates were tst positive, but none were positive for the pvl or
eta gene. Among 71 MSSA isolates, 7 isolates were tst positive, 6 of which were pulsotype F–agr–3, and 9 of 10
agr–4 isolates were eta positive. The average ages of patients carrying MSSA, SCCmec IVA MRSA, and SCCmec
IIb MRSA were 7.7 ± 4.6, 3.1 ± 1.5, and 8.2 ± 3.1 years, respectively. Among the patients carrying MRSA, two
patients had been treated with oral antimicrobials, and one had been admitted to the hospital 18 months
previously. In conclusion, community-acquired MRSA isolates of a few clones colonized the skin of patients
with AD without risk factors for the acquisition of hospital-acquired MRSA, which suggested that the skin of
children with AD may represent a reservoir of MRSA colonization in the community.

Patients with atopic dermatitis (AD) tend to carry Staphylo-
coccus aureus on their skin lesions (1), and superantigens and
toxins of S. aureus allegedly exacerbate chronic inflammation
of AD skin (4, 8, 9). As a result, antimicrobials have often been
prescribed to control acute-phase AD (4). Eczematous lesions of
AD patients are known to be a source of transmission of S.
aureus (13, 15). Increasing incidences of community-acquired
methicillin-resistant S. aureus (MRSA) (CA-MRSA) in skin
and soft tissue infection raise concerns that AD skin would be
a favorable reservoir for CA-MRSA.

A CA-MRSA outbreak was first described in United States
in 1981 in association with intravenous drug users (40), but
more recently, these strains have emerged as the pathogens
most frequently found in patients with skin and soft tissue
infections presenting to emergency departments in the United
States (3, 23, 34). The most prevalent CA-MRSA clones in the
United States have the USA300 pulsotype harboring staphy-
lococcal cassette chromosome mec (SCCmec) IV and Panton-
Valentine leukocidin (42, 43). The community-based epidemic
of MRSA led us to think that that MRSA became as prevalent
as penicillin-resistant S. aureus strains in the community, as
suggested previously by Chambers (6). Although many Asian
countries suffer from high rates of MRSA infection, there are
few publications on the prevalence of CA-MRSA (7, 17). In
South Korea, the overall MRSA rate in clinical isolates during
the last decade has been reported to be approximately 70%
regardless of the locations or sizes of hospitals (20, 29). Even
though the origins of MRSA isolates are not clear, MRSA has
been the major pathogen of skin infections and otitis media in
South Korean outpatient clinics since the late 1990s (22, 28,
35). The epidemiology of CA-MRSA in South Korea requires
urgent attention.

Therefore, in the present study, we evaluated the rate of
colonization by MRSA in skin lesions of pediatric AD patients
and characterized MRSA isolates obtained from those lesions.

MATERIALS AND METHODS

Patients and bacterial isolates. AD patients were enrolled in our study at the
times of their first visits to the pediatric allergy clinic of our hospital from June
2004 to April 2005. Eczematous skin lesions were imprinted with Rodac Staph
(Komed, South Korea), and yellow colonies were selected after 48 h of incuba-
tion. Bacterial species identification and antimicrobial susceptibility testing were
performed using the MicroScan PosCombo 1A system (Dade Behring, West
Sacramento, CA). All isolates were stored in brain heart infusion broth contain-
ing 15% (vol/vol) glycerol. The first isolate obtained from each patient was
investigated further. Patients’ medical records were reviewed for basic demo-
graphics and clinical diagnoses, prior antimicrobial therapies, hospital admission
histories, and places of residence.

Antimicrobial susceptibility. The MicroScan PosCombo 1A (Dade-Behring)
panel was used to determine bacterial susceptibility to penicillin, oxacillin, eryth-
romycin, clindamycin, ciprofloxacin, ofloxacin, rifampin, gentamicin, cotrimox-
azole, chloramphenicol, tetracycline, fusidic acid, quinupristin-dalfopristin,
teicoplanin, and vancomycin. To determine inducible macrolide-lincosamide-

streptogramin B (MLS B) resistance, the D-test (36) was performed on all S. aureus isolates that were clindamycin susceptible and erythromycin resistant.

DNA extraction. MRSA isolates were subcultured on blood agar plates at 37°C overnight. Three to five isolated colonies were prepared for DNA extraction using the GenElute bacterial genomic DNA kit (Sigma, St. Louis, MO). Lysostaphin and lysozyme were added for the lysis step at 10 units/ml and 45 mg/ml, respectively.

SCCmec typing and agr polymorphism. PCR for agr polymorphism was performed using primers previously described by Gilot et al. (11). Type assignment of SCCmec elements from multiple PCR was done as described previously by Oliveira and de Lencastre (38). For cases unresolved by these procedures, ccr typing and determining the location of IS12272 were undertaken as previously described (37).

PFGE. Chromosomal DNA was digested with SmaI and electrophoresed using program 2 of the GenePath system (Bio-Rad Laboratories Inc., Hercules, CA) as previously described (21). The isolates showing six or fewer band differences by pulsed-field gel electrophoresis (PFGE) were counted to the same group of pulsortype. Cluster analysis of pulsortypes was done in the dendrogram type of the unweighted-pair group method using average linkages with the Dice coefficient using InfoQuest FP software, version 4.5 (Bio-Rad).

PCR for the eta, etb, tst, and pvl genes. To detect the eta, etb, and tst genes, a multiplex PCR assay combining primers specific for eta, etb, and tst genes was performed (2). The pvl gene was detected with PCR using primers luk-PV-1 and luk-PV-2 (31).

RESULTS

Patients and bacterial isolates. A total of 122 specimens were collected from 115 patients during the study. S. aureus was isolated from 92 (75.4%) specimens from 87 (75.7%) patients. Eighteen isolates from 16 (18.3%) patients were resistant to oxacillin by MicroScan. Forty-six (64.8%) of the 71 MRSA isolates were community acquired, and only 2 of the 41 MSSA isolates were from patients with risk factors for hospital-acquired MRSA. Among the 71 MSSA isolates, 35 were of the agr-1 type, 24 were of the agr-3 type, 10 were of the agr-4 type, and only 2 were of the agr-2 type (Fig. 1). In PFGE analyses, 48 MSSA isolates were distributed into nine clusters: pulsortype D for 17 isolates with agr-3, pulsortype E for 8 isolates with agr-4, pulsortype F for 6 isolates with agr-3, pulsortype G for 4 isolates with agr-1, and 5 other pulsortypes composed of two to three isolates per each group; however, the other 23 MSSA isolates were the solitary type (Fig. 1).

Toxin gene profiles. All S. aureus isolates were negative for the pvl gene. Among the 16 MRSA isolates, 2 were tst positive and 5 were eta positive. Two of the tst-positive isolates were pulsortype A–agr-1–SCCmec IVA, while the other 14 were all of the pulsortype C–agr-3–SCCmec IVA (Fig. 1). Among the 71 MSSA isolates, tst was positive in six pulsortype F–agr-3 isolates and two pulsortype A–agr-1 isolates. eta was positive in all eight pulsortype E–agr-4 isolates and one pulsortype L–agr-4 isolate, which was the only etb-positive isolate (Fig. 1).

DISCUSSION

Consistent with previous studies (12, 16), S. aureus colonization was found in 75.7% of pediatric AD lesions, with MRSA accounting for 18.4% of S. aureus isolates in skin lesions of pediatric AD patients. This is the first report on the carriage rates of MRSA in skin lesions of pediatric AD patients. The carriage rate found by us is much higher than the recently reported rates of colonization by MRSA in healthy Asian schoolchildren. These rates were 5.1% in South Korea (30), 4.3% in Japan (14), and 1.9% in Taiwan (19). Considering a predilection of S. aureus for damaged skin and the frequent exposure of AD patients to antimicrobials, the high rate of colonization by MRSA noted in our study may not be surprising. Recently, there was a case report of a child with severe AD who presented with CA-MRSA skin abscesses (41). A high rate of colonization by MRSA can be worrisome for AD patients because it predisposes them to having invasive cutaneous infections. In addition, the average age of patients from whom SCCmec IV isolates were obtained was significantly younger than that of patients from whom SCCmec II isolates were cultured. These findings suggest that two discrete CA-MRSA clones were spread in different time periods. The high colonization rate and clonality of MRSA seen in this study indicate that AD patients can be a potential source of CA-MRSA transmission.

All the MRSA isolates were community acquired, and only two patients had risk factors for hospital-acquired MRSA (HA-MRSA), such as previous hospitalization and prior antibiotic therapy (24). SCCmec IVA was predominant among the MRSA isolates in our study. In addition, all such isolates were susceptible to cotrimoxazole and ciprofloxacin, which is un-
FIG. 1. Cluster analysis of pulsotype, agr polymorphism, SCCmec typing, and toxin gene profiles of 87 S. aureus isolates according to agr polymorphism.
usual among MRSA strains isolated in South Korean hospitals (20, 29). Although the outbreak of staphylococcal scalded skin syndrome by MRSA that occurred in the Kyungnam province involved patients with no risk factors for HA-MRSA, and all isolates were clonal by PFGE, the MRSA isolates showed characteristics of typical HA-MRSA isolates, such as multidrug resistance and SCCmec type II (32). Therefore, the MRSA isolate was assumed to be a hospital-derived clone introduced into the community. However, an SCCmec IV clone has been found in community settings such as in neonates born at primary obstetrics clinics (26), in a surveillance of healthy school-children (30), and in cases of bovine mastitis (27). As was the case in this study, such SCCmec IV clones were pUB110 positive and of type IVA and did not show multidrug resistance (5, 26). Even though there has been a lack of data on the prevalence of CA-MRSA infections, those reports suggest the emergence of CA-MRSA in South Korea.

MRSA isolates showed two agr types, agr-1 and agr-3, and MSSA isolates also were mainly of types agr-1 and agr-3. The prevalent CA-MRSA strain circulating in France, Switzerland, and Australia has agr-3 and the USA300 clone, which is an epidemic clone in United States, and in Europe, it has agr-1 (43). There has not been a reported case of agr-2 CA-MRSA. Because agr-2, which seems to have benefits in surviving in the hospital setting (33), is the type frequently found in cases of HA-MRSA in South Korea (46), the absence of agr-2 in MRSA isolates reported in this study was consistent with the community origin of the isolates reported here. Compared to the MSSA isolates composed of heterogeneous pulsortypes, all the MRSA isolates were clustered into a few clones by PFGE analysis. The MRSA isolates of each clusters also shared common types in SCCmec, agr polymorphism, and toxin profile: pulsortype A–agr–1–SCCmec IVA, pulsortype B–agr–3–SCCmec IVA, and pulsortype C–agr–3–SCCmec IIb–etb positive. Healthy schoolchildren in the Kyungnam province were also found to carry both SCCmec II and SCCmec IV MRSA clones (30). It thus appears that both SCCmec IV and SCCmec II clones of CA-MRSA have emerged in South Korea. CA-MRSA isolates in Taiwan and Japan did not always harbor SCCmec IV (7, 14, 39). SCCmec II is also predominant among CA-MRSA isolates in Japan (45), SCCmec III occurred frequently, and a novel SCCmec type (type V) was found among CA-MRSA isolates in Taiwan (7). MRSA isolates were all negative for pvl, and etb was exclusively associated with the pulsortype B–SCCmec IIb clone in this study. As in this study, SCCmec IIb, first described in Japanese CA-MRSA isolates, also carries etb (45). There was no pvl gene found in CA-MRSA isolates from South Korea or Japan (14, 26, 30, 32, 39), while the pvl gene was present in those from Taiwan (44). Combined with the findings that ets was confined to agr-4 MSSA and ts was found in MRSA or MSSA isolates of the agr-1 or agr-3 type, these toxin genes indicate the evolution and spread of certain S. aureus strains. In Asian countries, CA-MRSA clones seem to have an origin distinct from those of CA-MRSA epidemic clones in Australia, the United States, and Europe (7, 14, 17, 18, 39). Well-organized prospective surveillance is thus required to understand the epidemiology of CA-MRSA in South Korea.

Consistent with the previous reports of CA-MRSA, the MRSA isolates were susceptible to antimicrobials of many different classes, as were MSSA isolates; however, MLSB resistance was common in erythromycin-resistant, clindamycin-susceptible isolates. Clindamycin is a treatment option for CA-MRSA infections in the United States because the isolates were usually susceptible to clindamycin and MLSB induction test negative (10). In South Korea, clindamycin should not be used for clindamycin-susceptible CA-MRSA infections without MLSB induction testing. Fortunately, skin and soft tissue infection can be treated without antimicrobial therapy if the area of infection is drained properly (34). However, as is the case with otitis media, CA-MRSA infection of tissues other than skin and soft tissue offers a challenge to antimicrobial therapy in South Korea (28).

In conclusion, AD patients showed high rates of MRSA colonization, and such patients may represent a significant reservoir of CA-MRSA. The major MRSA clone demonstrated known characteristics of CA-MRSA, including SCCmec type IV and a lack of multidrug resistance. MRSA isolates showed clonality by agr typing, PFGE, SCCmec typing, and toxin assays, suggesting a clonal spread of CA-MRSA.

**ACKNOWLEDGMENTS**

This work was supported by the Asan Institute for Life Science (grant 2004-0660). We thank Teruyo Ito at Juntendo University for valuable advice on SCCmec typing.

**REFERENCES**


