Patients with atopic dermatitis (AD) tend to carry *Staphylococcus 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* to 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 significant reservoir of MRSA colonization in the community.

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 ciprofloxacin. 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 A-~agr-1~–SCCmec IV, five isolates of pulsotype B-~agr-3~–SCCmec IIb–*etb* positive, and three 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 significant reservoir of MRSA colonization in the community.

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 staphylococcal 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 incubation. Bacterial species identification and antimicrobial susceptibility testing were performed using the MicroScan PosComba 1A system (Dade Behring, West Sacramento, CA). All isolates were stored in brain heart infusion broth containing 15% (vol/vol) glycerol. The first isolate obtained from each patient was examined for *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 ciprofloxacin. 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 A–~agr-1~–SCCmec IV, five isolates of pulsotype B–~agr-3~–SCCmec IIb–*etb* positive, and three 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 significant reservoir of MRSA colonization in the community.

**Antimicrobial susceptibility.** The MicroScan PosComba 1A (Dade-Behring) panel was used to determine bacterial susceptibility to penicillin, oxacillin, erythromycin, clindamycin, ciprofloxacin, ofloxacin, rifampin, gentamicin, cotrimoxazole, chloramphenicol, tetracycline, fusidic acid, quinupristin-dalfopristin, teicoplanin, and vancomycin. To determine inducible macrolide-lincosamide-
streptogramin B (MLSβ) 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 GeneElute 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 multiplex 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 IS1272 were undertaken as previously described (37).

**PFGE.** Chromosomal DNA was digested with Smal 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 previously described (21). The isolates showing six or fewer band differences by pulsed-field gel electrophoresis (PFGE) were counted to the same group of previously described (21). The isolates showing six or fewer band differences by pulsed-field gel electrophoresis (PFGE) were counted to the same group of previously described (21).

**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 schoolchildren (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 pulsotypes, 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: pulsotype A–agr–I–SCCmec IVA, pulsotype B–agr–3–SCCmec IVA, and pulsotype 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 correlated with the pulsotype 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 eta 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.

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