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Clarifying the transmission route of *Staphylococcus aureus* colonizing the skin in early childhood atopic dermatitis

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ABSTRACT

Background: We previously found that skin-colonizing *Staphylococcus aureus* in early childhood atopic dermatitis (AD) originates predominantly from the patient's nose, whereas maternal transmission did not contribute substantially to colonization.

Objective: To investigate the transmission route and definitive source of skin-colonizing *S aureus* in early childhood AD.

Methods: A total of 527 children and 32 healthy teachers from 2 kindergartens and 1 elementary school were included in the study. Children were screened for AD and categorized into 3 groups (AD, borderline, and healthy). Samples were collected from 5 to 6 different body sites, including the skin, subungual spaces, and anterior nares. The identity of colonies apparent on mannitol salt agar plates was confirmed by polymerase chain reaction amplification of the *nuc* gene. The genotypic composition of cultured isolates was examined by pulsed-field gel electrophoresis and analyzed with a dendrogram.

Results: The total colonization rate was higher in the AD group (34.6%) than in the borderline (21.1%) and healthy groups (25.4%). In the AD group, *S aureus* was more frequently cultured from the subungual areas (30.8%) than the anterior nares (19.2%). To assess self-contamination or recolonization, dendrogram analysis revealed that most isolate pairs (22/23) had the same pulsed-field gel electrophoresis pattern.

Conclusion: As with the anterior nares, the subungual spaces are important reservoir of skin-colonizing *S aureus* in early childhood AD. The transmission route for self-contamination or recolonization of *S aureus* appears to be from children's anterior nares to the skin through their own fingers. Child-to-child and/or teacher-to-child transmission in a classroom do not seem to be definite routes of *S aureus* transmission.

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Introduction

Staphylococcus aureus colonization is well known as one of the most common aggravating factors of atopic dermatitis (AD).¹ However, the origin of *S aureus* in communities is not well understood. Healthy individuals have a high rate of *S aureus* in their anterior nares and subungual areas, and the anterior nares are known to be the most frequent carriage site.² Noble et al³ reported that *S aureus* colonization appears in up to 35% of the anterior nares

of healthy individuals. According to another study, 11.1% of healthy children have *S aureus* in subungual areas, and 19.4% have *S aureus* in their anterior nares.⁴ We found in our previous study that, although 27.8% of healthy children also can be colonized with *S aureus* on their skin or anterior nares, colonization does not cause any problem.⁴ Because *S aureus* does not colonize the skin at birth, it must be transmitted from the surrounding environment after birth. We tried to investigate intrafamilial transmission routes and found that maternal transmission is not the only origin of skin-colonizing *S aureus* in early childhood AD.⁴ These results suggest the possibility that *S aureus* transmission in early childhood AD is related not only to the bacterial ecologic features of the mothers but also to other individuals in close contact. Therefore, we performed a detailed evaluation of the close contacts of these children, such as their classmates and teachers, to determine the definitive source of skin-colonizing *S aureus*. Furthermore, we tried to clarify the

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transmission route of *S aureus* in individuals through pulsed-field gel electrophoresis (PFGE) and dendrogram analysis.

Materials and Methods

Patients and methods

Children and teachers from 2 different kindergartens in Daegu and 1 elementary school in Busan, South Korea, were included in this study. A total of 527 children and 32 teachers from 21 classrooms participated during a 2-month period from May to July 2010. The children's ages ranged from 4 to 7 years.

Screening for AD

Patients who refused to have a history, physical examination, and/or microbiological study performed were excluded in the study. Any patient who had diarrhea; upper respiratory tract infection symptoms, such as fever, cough, or rhinorrhea; any contagious and communicable disease; or inadequate conditions as determined by physicians were excluded. Any patient with a recent history of inpatient hospital admission or who had received antibiotics or immunosuppressive treatment via any route within the 4 weeks before this study was also excluded.

A total of 354 children were screened for AD by dermatologists and categorized into 3 groups (Fig 1) based on the Korean diagnostic criteria for AD of the Atopic Dermatitis Research Group (Table 1).⁵ Children whose symptoms were insufficient for a diagnosis of AD but who had an eczematous skin lesion plus at least 1 major or minor feature of AD were classified in the borderline (BD) group. Healthy volunteers without a personal or family history of AD or any other skin or allergic diseases were placed in the healthy participant (HP) group. Thirty-two teachers who were in charge of a class and who had no eczematous skin lesions were also included in this study. Written informed consent was obtained from all participants or from their legal guardians. All study protocols were reviewed and authorized by the institutional review boards of Kyungpook National University Hospital and Pusan National University Hospital. Figure 1 summarizes the selection process and the design of the study.

Sample Collection

Skin and nasal swabs were collected from 5 to 6 different sites on each volunteer. For the AD and BD groups, samples were collected from 6 sites (lesional skin, nonlesional skin, anterior nares, the second and third subungual areas of the dominant hand, and the fifth subungual area of the nondominant hand). For the HP and teacher

Table 1

Korean diagnostic criteria for atopic dermatitis of the atopic dermatitis research group (must have at least 2 of 3 major features and 4 of 14 minor features)

Major features

1. Pruritus
2. Typical morphologic features and distribution
Younger than 2 years: face, trunk, and extensor involvement
Older than 2 years: face, neck and flexural involvement
3. Personal or family history (atopic dermatitis, asthma, allergic rhinitis)

Minor features

1. Xerosis
2. Pityriasis alba
3. Facial erythema/facial pallor
4. Periorbital eczema or orbital darkening
5. Periauricular eczema
6. Cheilitis
7. Tendency toward nonspecific hand or foot dermatitis
8. Scalp scale
9. Perifollicular accentuation
10. Itch when sweating
11. White dermographism
12. Skin prick test reactivity
13. Elevated serum IgE level
14. Tendency toward cutaneous infections

groups, samples were collected from 5 sites (nonlesional skin, the second and third subungual areas of the dominant hand, and the fifth subungual area of the nondominant hand). The lesional skin swab was taken from an eczematous lesion on the antecubital area (if the antecubital area was intact, the swab was obtained from another eczematous lesion), and the nonlesional skin swab was taken from an area 5 cm from a skin lesion. For the HP and teacher groups, the antecubital area was selected for swabbing. All samplings were performed on the day of diagnosis with a sterile cotton-tipped swab stick that was premoistened with sterile water. Skin swabs were taken by rolling the swab stick over the area twice. Nasal swabs were obtained by twisting a swab stick 360° in both anterior nares. For the subungual sampling, after the patients soaked their fingertips in sterile water for 3 minutes, swabs were taken from the hyponychium.

Laboratory analysis of *S aureus* Isolates

S aureus colonies apparent on mannitol salt agar were confirmed by polymerase chain reaction (PCR) using a primer set for the *nuc* gene. The methicillin resistance of isolated *S aureus* was not determined in the study.

To investigate the possible transmission route (child to child and/or teacher to child) and look for *S aureus* self-contamination or recolonization, isolated strains were typed by PFGE and dendrogram construction. One or two isolated strains per person were used for PFGE. If swabs from 2 or more sites had confirmed *S aureus*, isolates from the second subungual area and the anterior nares were preferred for PFGE, and these samples were also used for the dendrogram analysis.

Genomic DNA was extracted and PFGE was performed as described in the CHEF MAPPER system (Bio-Rad, Hemel Hempstead, United Kingdom) manual. Briefly, all isolates were grown on tryptic soy broth overnight at 37°C. The cells were washed in TEN (100 mM Tris, pH 8.0; 150 mM sodium chloride; and 100 mM EDTA, pH 8.0) by centrifugation, and the optical density of the cells at a wavelength of 600 nm was adjusted to 1.4. One hundred microliters of each bacterial suspension was transferred to a 1.5-mL microcentrifuge tube. Lysozyme (final concentration, 0.25 mg/mL) and lysozyme (final concentration, 0.1-mg/mL stock solution) were added and mixed several times by pipetting up and down. InCert agarose (Lonza, Rockland, Maine) was prepared in water at a final concentration of 2.0% and maintained at 55°C in a water bath. Two hundred microliters of 2.0% InCert agarose was mixed with each bacterial suspension using a pipette, and this mixture

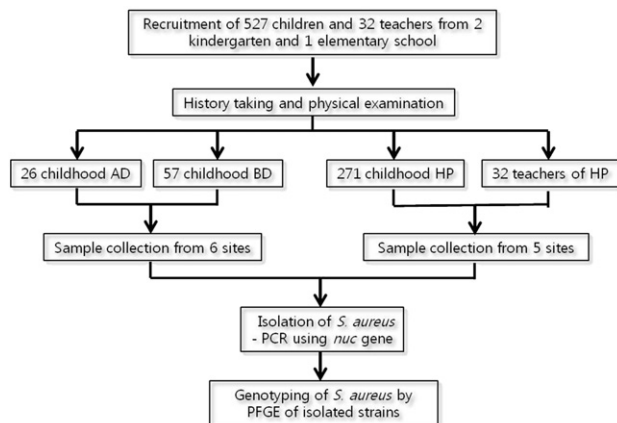


Figure 1. Flowchart of the study design and participation proportions. AD indicates atopic dermatitis; BD, borderline; HP, healthy participants; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis.

was then pipetted into plug molds (Bio-Rad). After the plugs solidified, they were transferred into EC buffer (60 mM Tris, pH 8.0; 100 mM EDTA, pH 8.0; 1 M sodium chloride; 0.5% Brij58; 0.2% deoxycholate; and 0.5% sarcosine) and incubated for 2 hours in a 37°C shaking water bath to lyse the bacteria. After lysis was complete, they were transferred to PK buffer (250 mM EDTA, pH 9.0; 1% sarcosine; and 50 µg/mL of proteinase K) and incubated for 18 hours in a 55°C shaker water bath. After proteolysis was complete, the plugs were transferred to 50-mL tubes containing 10 mL of sterile, preheated (50°C), distilled water and incubated for 30 minutes at 50°C with gentle mixing in a shaker water bath. Subsequently, the plugs were washed 4 times in 10 mL of preheated (50°C) TE buffer (10 mM Tris, pH 8.0; and 1 mM EDTA, pH 8.0) in a shaking 50°C water bath for 30 minutes each. The plugs were then cooled to room temperature in TE buffer. They could be immediately used for PFGE or stored for 3 to 4 weeks at 4°C in 1 mL of TE.

For restriction endonuclease digestion, 1-mm-thick sections of each plug were incubated at 25°C for 18 hours with 30 U of *Sma*I enzyme (Roche, Mannheim, Germany) in 100 µL of the appropriate (1×) restriction enzyme buffer. PFGE was performed using the CHEF MAPPER system (Bio-Rad) in 0.5× Tris-borate-EDTA buffer. DNA macrorestriction fragments were resolved on 1.0% agarose gels (PFGE certified; Bio-Rad). A λ-ladder composed of 48.5-kb concatemers (Bio-Rad) was used as a size standard. The conditions for electrophoresis were 6 V/cm for 10 hours with the pulse time increasing from 5 to 15 seconds followed by 13 hours with the pulse time increasing from 15 to 60 seconds. Digital images were stored electronically as TIFF files and were analyzed with GelCompar software (Applied Maths, Sint-Martens-Latem, Belgium). To create the dendrogram, the unweighted pair group method with arithmetic mean algorithm was used.

A dendrogram, also called a phylogenetic tree, is a visual representation of the spot correlation data. A dendrogram is a branching diagram representing a hierarchy of categories based on degree of similarity or number of shared characteristics, especially in biological taxonomy. Through the dendrogram, we can determine biologic similarity.⁶

Statistical Analysis

To analyze the frequency of differences, dichotomous variables were examined using the χ^2 independence test in SPSS statistical software for Windows, version 17.0 (SPSS Inc, Chicago, Illinois). $P < .05$ was considered statistically significant.

Results

Rate of *S aureus* colonization in children

The *S aureus* colonization results for the different groups confirmed by the mannitol fermentation test and PCR are given in Table 2 and Table 3. Nine of 26 children (34.6%) in the AD group had *S aureus* in the subungual areas, the anterior nares, or the skin,

which was significantly higher ($P = .04$) than that in BD group (12/57, 21.1%) or the HP group (65/271, 24.0%). The rate of *S aureus* in the subungual area was 30.8% (8/26) in the AD group, which was significantly higher ($P = .04$) than that in BD group (5/57, 8.8%) or the HP group (47/271, 17.3%). The rate of *S aureus* in the anterior nares was 19.2% (5/26) in the AD group, 12.3% (7/57) in the BD group, and 13.7% (37/271) in the HP group. In the AD group, the prevalence of *S aureus* was 7.7% (2/26) on lesional skin and 3.8% (1/26) on nonlesional skin. In the BD group, the prevalence of *S aureus* was 5.3% (3/57) on lesional skin and 0% (0/57) on nonlesional skin. However, in the HP group, the prevalence of *S aureus* was 0.4% (1/271) on nonlesional skin. In the AD group, 6 of 26 patients (23.1%) had *S aureus* in the second and third subungual areas of the dominant hand, and 2 of 26 (7.7%) had *S aureus* in the fifth subungual area of the nondominant hand. In the BD group, 3 of the 57 participants (5.3%) had *S aureus* in the second subungual area, 1 of 57 (1.8%) had *S aureus* in the third subungual area of the dominant hand, and 1 of 57 (1.8%) had *S aureus* in the fifth subungual area of the nondominant hand. In the HP group, 34 of 271 participants (12.5%) had *S aureus* in the second subungual area, 21 of 271 (7.7%) had *S aureus* in the third subungual area of the dominant hand, and 12 of 271 (4.4%) had *S aureus* in the fifth subungual area of the nondominant hand.

Rate of *S aureus* colonization in teachers

In teachers, 12 of the 32 participants (37.5%) had confirmed *S aureus* colonization. Specifically, 9 of the 32 (28.1%) carried *S aureus* in subungual areas, and 4 of 32 (12.5%) had *S aureus* in their anterior nares. The prevalence of *S aureus* on nonlesional skin was 3.1% (1/32).

Among the healthy teachers, 6 of 32 (18.8%) had *S aureus* in the second subungual area, 4 of 32 (12.5%) had *S aureus* in the third subungual area of the dominant hand, and 3 of 32 (9.4%) had *S aureus* in the fifth subungual area of the nondominant hand.

Including both children and teachers, 47 of 86 participants (54.7%) had more than one strain of *S aureus* in our study.

PFGE for the Evaluation of *S aureus* transmission by dendrogram analysis

A total of 100 strains isolated from 77 people were analyzed by PFGE. In the dendrogram figures (Fig 2 and Fig 3), the PFGE results are not shown for cases where the similarity was less than 85%. The distance of one group from the other groups indicated the degree of relationship between 2 groups. So, we regarded the interpretation of the results with less than 85% similarity as meaningless.

Analysis of isolates from patients' own nares and subungual areas

The PFGE dendrogram examining the possibility of self-contamination or self-recolonization showed 22 pairs of the same strains among isolates from 21 children and 2 teachers from kindergarten A and the elementary school, who had at least 2 sites

Table 2

Total Frequency of *Staphylococcus aureus* colonization according to culture sites in children

Group	No. (%) of patients with isolation	Sites, No. (%)			
		Subunguim	Anterior nares	Lesions	Nonlesions
AD (n=26)	9 (34.6)	8 (30.8)	5 (19.2)	2 (7.7)	1 (3.8)
BD (n=57)	12 (21.1)	5 (8.8)	7 (12.3)	3 (5.3)	0 (0)
HP (n=271)	65 (24.0)	47 (17.3)	37 (13.7)		1 (0.4)
P value ^a	.04	.04	.68	.95	.002

Abbreviations: AD, atopic dermatitis; BD, borderline; HP, health participants.

^a χ^2 independence test using SPSS statistical software for Windows, version 17.0 (SPSS Inc, Chicago, Illinois).

Table 3

Frequency of *Staphylococcus aureus* colonization from subunguim in children

Group	No. (%) of patients with isolation	Subunguim, No. (%)		
		Second (dominant hand)	Third (dominant hand)	Fifth (nondominant hand)
AD (n=26)	8 (30.8)	6 (23.1)	6 (23.1)	2 (7.7)
BD (n=57)	5 (8.8)	3 (5.3)	1 (1.8)	1 (1.8)
HP (n=271)	47 (17.3)	34 (12.5)	21 (7.7)	12 (4.4)
P value ^a	.04	.04	.004	.07

Abbreviations: AD, atopic dermatitis; BD, borderline; HP, healthy participants.

^a χ^2 independence test using SPSS statistical software for Windows, version 17.0 (SPSS Inc, Chicago, Illinois).

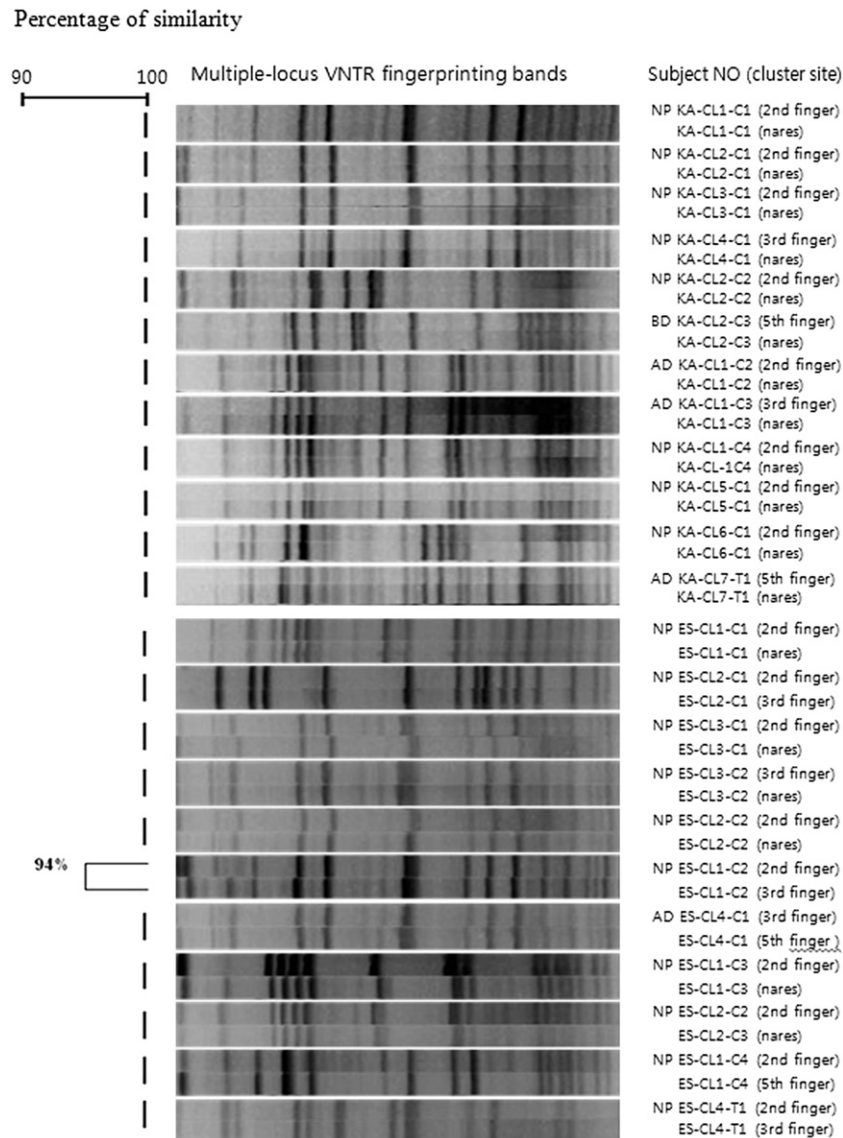


Figure 2. Consideration of self-contamination and recolonization. Dendrogram showing the results of the cluster analysis of *Smal*-generated PFGE patterns from *Staphylococcus aureus* from 21 children and 2 teachers. AD indicates atopic dermatitis; BD, borderline; C, child; CL, class; ES, elementary school; HP, healthy participants; KA, kindergarten A; PFGE, pulsed-field gel electrophoresis; T, teacher.

nuc gene, PCR-positive isolates (Figure 2). In kindergarten B, no patient carried *S aureus* at more than 2 sites.

Analysis of isolates from patients' nares, subungual areas, and skin lesions

Considering the possibility of child-to-child and teacher-to-child transmission in each kindergarten classroom, only 1 pair was identified as having an identical strain among the 30 children and 4 teachers in kindergarten A (Fig 3). No identical strain was detected among 17 children and 1 teacher in kindergarten B (data not shown). In consideration of child-to-child and/or teacher-to-child transmission in each classroom of elementary school (Fig 3), among 24 children and 1 teacher, 2 pairs of isolates had identical PFGE patterns.

Discussion

S aureus, which is known to worsen the symptoms of AD, is commonly present in patients' nasal cavity, auditory canal, subungual areas, and AD lesions and is often found in healthy

people.⁷⁻⁹ The subungual areas are easily contaminated and thus are good reservoirs for various microbes. Furthermore, subungual areas can contact almost every part of the body, particularly through AD patients' scratching; thus, they can play an important role in the infiltration and propagation of bacteria. Our previous results suggest that *S aureus* transmission in early childhood AD is of concern not only to mothers but also to other individuals in close contact.⁴ However, there has been no well-designed model studies to clarify whether *S aureus* transmission can occur from child to child or from teacher to child. Therefore, we performed an investigation examining the *S aureus* isolates from children with AD and their teachers to detect the possibility of child-to-child and/or teacher-to-child *S aureus* transmission.

In this study, the patients were divided into 3 groups (AD, BD, and HP) by using the Korean diagnostic criteria for AD of the Atopic Dermatitis Research Group⁵ instead of the diagnostic criteria of Hanifin and Rajka.¹⁰ When compared with the diagnostic criteria of Hanifin and Rajka, this criteria (excluding "chronic or chronically relapsing dermatitis" in major features and adding 3 new minor features: "periauricular eczema," "scalp scale," and "skin prick test

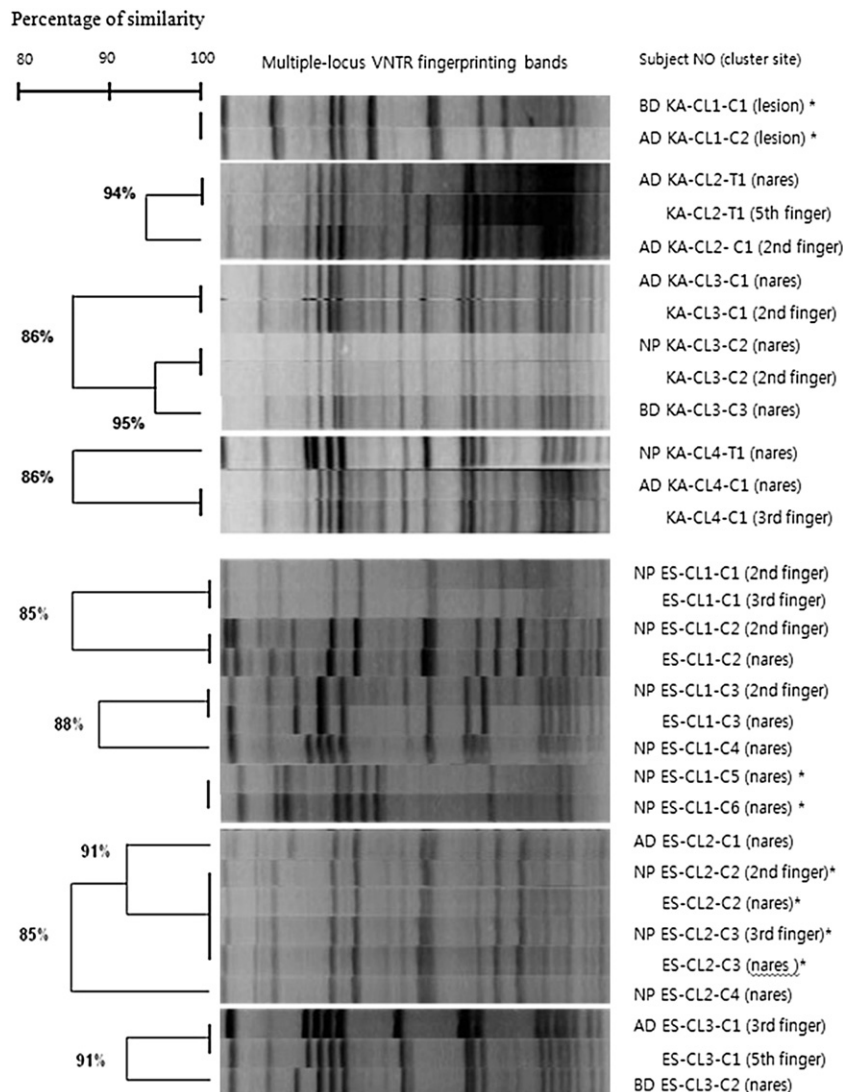


Figure 3. Consideration of child-to-child and teacher-to-child transmission in kindergartens. Dendrogram showing the results of the cluster analysis of *Smal*-generated PFGE patterns from *Staphylococcus aureus* from 30 children and 4 teachers in kindergarten A and from 24 children and 1 teacher in elementary school. *One pair of same strains detected in 2 different patients. AD indicates atopic dermatitis; BD, borderline; C, child; CL, class; ES, elementary school; HP, healthy participants; KA, kindergarten A; PFGE, pulsed-field gel electrophoresis; T, teacher.

reactivity") are believed to be more relevant to Korean population and more strict.^{11,12} Because the ethnic backgrounds influence the phenotype of AD, some modifications were needed. In previous studies the detection rate among definite AD patients did not vary significantly.¹² Nevertheless, future research may need to give additional consideration to the selection of the various diagnostic criteria.

In this study, the frequency of *S aureus* colonization in the AD group (9/26, 34.6%) was significantly higher than that in the BD group (12/57, 21.1%) and the HP group (65/271, 24.0%) ($P=.04$). In addition, in the AD group, the subungual areas, including the second, third, and fifth subungual area, had the highest percentage of *S aureus* positivity (8/26, 30.8%) ($P=.04$). The rate of *S aureus* colonization in the anterior nares was 19.2% (5/26) in the AD group, which was higher than in the other groups (BD: 12.3%, HP: 13.7%) but not statistically significant ($P=.68$). Overall, this study found that the rate of *S aureus* skin colonization was higher in the AD group than in the BD and HP groups and was highest in the subungual areas (69/386, 17.9%). Among the subungual sites, the second subungual area of the dominant hand (49/386, 12.7%) was the site with the highest colonization frequency. The overall frequency of

colonization, including cutaneous carriage and carriage in subungual areas and the anterior nares, suggests that colonization by *S aureus* has a potentially pathophysiologic role in the development of AD. Because our study was focused on the *S aureus* colonization rate in children, the relatively high *S aureus* colonization rate in teachers (12/32, 37.5%) might be explained by the following factors: age, lifestyle, and a microbe-rich environment.

Previous reports have stressed the importance of the anterior nares as an endogenous reservoir for skin-colonizing *S aureus*.^{1,2,4} However, when 6 sample collection sites were compared in the current study, in which each participant who tested positive in at least 1 of the 3 subungual areas was counted as 1, the subungual areas (8/26, 30.8%) have a higher colonization rate than the anterior nares (5/26, 19.2%) in the AD group. This finding suggests that in the AD group, the subungual areas also may be major reservoirs of *S aureus*, and they are likely to be more important than the anterior nares. We might consider the possibility that *S aureus* is transmitted from the nares to the skin through the dominant hand (the transmission route of *S aureus* from the nares through the subungual areas of the dominant hand to the skin).

In this study, we swabbed the second and third subungual areas of the dominant hand and the fifth subungual area of the nondominant hand. Because the scratching is one of the most common symptoms and fingernails play a major role in the infiltration and propagation of *S aureus*, the second and third subungual areas were chosen to obtain actual staphylococcal strains, whereas the fifth was to be compared as a control. We performed a preliminary study of the subungual *S aureus* colonization rate. In the study, the highest colonization rate was detected in the second and third subungual areas of the dominant hand, and the lowest colonization rate was observed in the fifth subungual area of the nondominant hand (data not shown).

At the first stage of the study, to examine for possible self-contamination or recolonization, 12 isolate pairs of the same strains were detected in 11 children and 1 teacher from the kindergartens, and 10 isolate pairs of the same strains were detected in 10 children and 1 teacher from the elementary school. From these results, we can assume that almost the same strains are carried in the fingernails and the anterior nares of a single person, and we believe that transmission occurs between a child's anterior nares and subungual areas. Conversely, only 1 pair of homologous *S aureus* strains was found among the anterior nares, subungual areas, and skin of children and teachers of 30 children and 4 teachers in kindergarten A. No identical strains were found among 17 children and 1 teacher in kindergarten B, and only 2 pairs of strains were homologous among 24 children and 1 teacher in the elementary school. These results suggest that child-to-child and teacher-to-child *S aureus* transmission are not common. Therefore, the possibility of *S aureus* transmission among classmates and teachers who are in close contact during their daily life in early childhood may not be high, despite their communal lifestyle in the same place for long time. This is contrary to the assertion made by Chiu et al,¹³ who reported a high concordance of *S aureus* isolate indices with patients and their close contacts (81% of the isolates were identical or closely related by molecular typing). A study by Patel et al¹⁴ also reported conflicting results to ours. They reported an association between the presence of parental nasal *S aureus* and cutaneous *S aureus* colonization in the child ($P=.02$). Our study did not consider the closeness of the relationships and the possibility of differences in group activities, which may affect the degree of contact between the children and teachers. Therefore, further evaluation of *S aureus* transmission according to the degree of closeness may be necessary. A previous study⁴ reported that the origin of *S aureus* might gradually change from one person to another. Therefore, we think that a long-term study is necessary for a more detailed evaluation and accurate results.

During the laboratory analysis, especially about the PCR and PFGE analysis, some data were lost, and we think that this may have affected the results. In addition, the sample size of this study was

too small to permit broad generalizations. The main focus of this study was clarifying the transmission route of *S aureus*.

In conclusion, contrary to our expectations and previous intra-familial studies, transmission of *S aureus* from child to child and/or teacher to child was not common. Further evaluation of the transmission route between friends and the effect of the lifestyle factors of these children will be necessary in future studies to determine the definitive origin of skin-colonizing *S aureus*. However, on the basis of the *S aureus* colonization rates, the anterior nares and the subungual areas are important reservoirs of *S aureus* for self-contamination or recolonization, and we presume that the subungual areas may be even more important than the anterior nares. Furthermore, the PFGE results suggest that *S aureus* can be transmitted from one's nose to skin via the subungual areas or from one's subungual areas directly to skin. Therefore, it is important to keep the nails short and clean during the management of patients with AD.

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