

Prevalence and molecular characteristics of *Staphylococcus aureus* isolates colonizing patients with atopic dermatitis and their close contacts in Singapore

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Summary

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Conflicts of interest

None declared.

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Background *Staphylococcus aureus* colonization is an established pathogenic factor for disease flare in atopic dermatitis (AD).

Objectives We conducted a study to investigate the colonization of *S. aureus* in patients with AD and their close contacts in order to evaluate the possibility of intrafamilial transmission. We sought to determine the distribution of the bacterial virulence factors and their correlation with disease severity.

Methods Nasal swabs and skin swabs (patients with AD only) were taken from patients with AD aged 2–21 years and their close contacts, seen at the National Skin Centre from January to March 2007. All *S. aureus* isolates were typed using multilocus variable-number tandem-repeat fingerprinting (MLVF) and screened for virulence factors via polymerase chain reaction (PCR) analysis. AD severity was determined by the SCORAD index.

Results A total of 34 patients with AD and 55 close contacts were recruited. Thirty-one (91%) patients were colonized with *S. aureus*. Twenty-five (45%) of their close contacts were also colonized, and MLVF showed a high concordance of *S. aureus* isolates in index patients and their close contacts. On multivariate analysis, patients with a moderate SCORAD were more likely to be colonized by enterotoxin B-positive *S. aureus* ($P = 0.027$). No virulence factor was significantly associated with a severe SCORAD.

Conclusions The prevalence of *S. aureus* colonization was high among patients with AD and their close contacts. However, no predominant isolate of *S. aureus* was found to be associated with AD. The presence of superantigen B is possibly associated with moderate rather than severe disease in our population.

Atopic dermatitis (AD) is a common chronic, relapsing inflammatory skin condition with an estimated prevalence of 21% in school-age children in Singapore.¹ The interaction of various genetic and environmental factors plays a crucial role in the immunopathological mechanism of AD.² Potential environmental triggers of AD flares include microbial infection, aeroallergens such as house dust mite and animal danders, dietary allergens and stress.^{3,4} Of these, *Staphylococcus aureus* colonization is one of the most important and frequently reported triggers, and up to 90% of patients with AD may be colonized by *S. aureus* as opposed to a third of the general community.^{2,5}

Several factors have been found to be responsible for the increased carriage of *S. aureus* in patients with AD, namely a defective skin barrier function and a compromised innate

skin immunity. The expression of barrier proteins, such as filaggrin, loricrin and involucrin, are reduced in patients with AD due to inherited genetic mutations⁶ or an increase in Th2 cytokine expression,^{7,8} resulting in a defective skin barrier that increases susceptibility to *S. aureus* colonization. At the same time, Th2 cytokines can also downregulate antimicrobial peptides such as β -defensins 2 and 3,⁹ resulting in compromised innate skin immunity that is ineffective in controlling the overgrowth of *S. aureus*. Many isolates of *S. aureus* are able to produce enterotoxins that can increase cutaneous inflammation in AD via several mechanisms.^{2,9} They can act as superantigens that can directly interact with the major histocompatibility complex class II molecules and the β chain of the T-cell receptor to induce T-cell proliferation and activation of the inflammatory cascade, without the

need for antigen-presenting cells. They can also upregulate the expression of cutaneous lymphocyte-associated antigen on T cells and the production of several keratinocyte-derived chemokines that increase T-cell recruitment into the skin.² Finally, enterotoxins have been found to contribute to the development of resistance to local corticosteroid treatment by causing the upregulation of the β -isoform of the glucocorticoid receptor in mononuclear cells, which does not bind to corticosteroids.⁹ However, it remains controversial as to whether or not there is any particular enterotoxin that is correlated with disease severity. No clear association has been found in studies focusing on the microbiological aspect,¹⁰ whereas association with classical enterotoxins, in particular enterotoxin B, are apparent in immunological studies.^{11–13}

Current therapy for AD includes topical antimicrobials in addition to anti-inflammatory agents, especially for severe cases.^{14,15} Recolonization by *S. aureus* is frequent and may contribute to AD severity.^{15–17} Some of the factors potentially resulting in recolonization include nasal carriage, drug resistance and the presence of close contacts who are also *S. aureus* carriers.^{17,18} Bonness *et al.*¹⁸ recently found a high prevalence (48%) of *S. aureus* colonization among parents of children with AD who were themselves colonized.

We conducted a study to evaluate the prevalence of familial *S. aureus* colonization among patients with AD and their close contacts and to evaluate the possibility of intrafamilial transmission. We also sought to determine if there was a major isolate of *S. aureus* associated with patients with AD in our locale, and if there were bacterial virulence factors that could predict for disease severity.

Materials and methods

Study design

A cross-sectional study was carried out correlating *S. aureus* carriage and virulence factors to the severity of disease among patients with AD on follow-up at the National Skin Centre (NSC), a tertiary referral dermatological outpatient facility in Singapore. Close contacts of study subjects were also tested for *S. aureus* colonization to determine the prevalence of familial carriage. This study was reviewed and approved by the institutional ethics committee.

Study subjects

Patients with moderate to severe AD aged 2–21 years and their close contacts were recruited during routine follow-up at the outpatient clinic at the NSC from January 2007 to March 2007. AD severity was assessed by a single investigator using the SCORAD index which consists of two key components—the subjective and objective SCORAD.¹⁹ Moderate disease was defined by an objective SCORAD > 15 and severe disease was defined by an objective SCORAD > 40.²⁰ We defined close contacts as persons who lived with, and had

spent a significant amount of time (> 12 h per day) with, the AD index case in the previous 3 months. Close contacts included parents, grandparents, domestic helpers and siblings. Any subject with a recent inpatient hospital admission or who had received antibiotics via any route in the previous month was excluded from the study. Written consent was obtained from all the subjects or their legal guardians and their close contacts.

Sample collection

Nasal swabs were obtained from patients with AD and their close contacts by using a 360° twist of a sterile cotton-tipped swab stick directed at both anterior nares. In addition, skin swabs were taken from the patients with AD by rolling a sterile cotton-tipped swab stick over their worst affected skin area twice for at least 5 s.

Laboratory analysis of *Staphylococcus aureus* isolates

Bacterial cultures of the swabs were carried out using standard laboratory techniques. We confirmed the identity of *S. aureus* via colony morphology, coagulation of citrated rabbit plasma with ethylenediamine tetraacetic acid (BBL Becton Dickinson, Cockeysville, MD, U.S.A.), and production of clumping factor and protein A (BactiStaph; Remel, Lenexa, KS, U.S.A.). The presence of methicillin resistance was tested with cefoxitin discs following the Kirby–Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.²¹

Following DNA extraction using a commercial kit (DNeasy kit; Qiagen, Hilden, Germany), we tested for the presence of the following virulence genes using previously described primers and protocols: toxic shock syndrome toxin (tsst-1), staphylococcal enterotoxins A–E, G–K [sea, seb, sec, sed, see, seg, seh, sei, sej, sek; the presence of both sei and seg was taken as a surrogate for the presence of the enterotoxin gene cluster *egc* (seg, sei and staphylococcal enterotoxins M, N, O)], exfoliatin A, B, and D (eta, eth, etd, respectively) and Panton–Valentine leucocidin (PVL).²² All *S. aureus* isolates were also typed using multilocus variable-number tandem-repeat fingerprinting (MLVF), following the protocol previously described by Sabat *et al.*²³

Statistical analysis

Intercooled Stata version 9.2 (Stata Corp., College Station, TX, U.S.A.) was used for statistical calculations. Dichotomous variables were analysed with the χ^2 test or Fisher's exact test as appropriate, and continuous variables were analysed with Student's *t* test. Univariate analyses of the association between individual variables and outcomes were performed using logistic regression. Variables with a *P*-value < 0.2 on univariate analysis were included in the corresponding stepwise multivariate analyses. *P* ≤ 0.05 was considered statistically significant.

Results

Characteristics of patients with atopic dermatitis and their contacts

A total of 34 patients with AD and 55 of their close contacts were recruited into the study. The demographic and clinical characteristics of the patients and their close contacts are listed in Tables 1 and 2, respectively. The majority were of Chinese ethnicity. All patients had either moderate or severe AD according to the SCORAD results. The median number of contacts living together per household was three (ranging from one to six) and at least one close contact was recruited per patient with AD (range, 1–5). Most of the contacts recruited were the parents of the patients with AD (82%).

Colonization of patients with atopic dermatitis and their close contacts

The majority of patients with AD were colonized with *S. aureus* (91%), and the prevalence of isolation was identical for both skin and nasal swabs. Twenty-nine (85%) patients with AD were colonized with *S. aureus* both in the nose and on the skin. Twenty-one (68%) of the patients colonized with *S. aureus* had at least one close contact who was a nasal carrier of *S. aureus*.

Based on molecular typing, there was a very high concordance of *S. aureus* isolates in the index patients and their close contacts. Of the 21 index patients with positive household contacts, 14 (67%) had contacts with identical isolates, three (14%) had contacts with closely related isolates (> 80% similar on phylogenetic clustering), three (14%) had contacts

Table 2 Demographics and clinical characteristics of close contacts of patients with atopic dermatitis (AD) (n = 55)

Characteristic	
Age (years), mean (range)	41 (3–70)
Male sex, n (%)	24 (44)
Ethnicity, n (%)	
Chinese	51 (92.7)
Malay	1 (1.8)
Indian	3 (5.5)
Relationship to patients with AD, n (%)	
Mother	26 (47.3)
Father	19 (34.5)
Siblings	7 (12.7)
Grandparents	3 (5.5)
<i>Staphylococcus aureus</i> nasal colonization, n (%)	25 (45.5)
AD, n (%)	13 (23.6)
Asthma, n (%)	4 (7.3)
Allergic rhinitis, n (%)	14 (25.5)

with unrelated isolates (< 80% similar on phylogenetic clustering), while one patient with AD had contacts with clonal and unrelated *S. aureus* carriage. There was no predominant isolate of *S. aureus* among the index patients on phylogenetic analysis (Fig. 1). No methicillin-resistant *S. aureus* (MRSA) isolates were cultured.

Distribution of virulence factors

On virulence factor profiling of the isolates from patients with AD, the most common virulence factor detected was *seb* (42%), followed by *egc* (32%) and *sea* (29%). Only one isolate was found to possess PVL, and six (19%) had *sea*. All virulence factors were compared between patients with moderate and severe AD, along with demographic characteristics, to determine if any factor was associated with the severity of disease (Table 3). It was found that patients with moderate AD were more likely to be colonized by *S. aureus* possessing *seb* ($P = 0.01$), whereas a trend towards the converse ($P = 0.06$) was seen with *sea*. We did not find any significant association between the presence of *S. aureus* colonization in close contacts and an increased disease severity in AD subjects ($P = 0.93$). There was no significant association between colonization of contacts and age of the patient ($P = 0.24$). On multivariate analysis, only colonization with *seb*-positive *S. aureus* was associated with less severe (moderate SCORAD) AD (odds ratio 0.09; 95% confidence interval 0.09–0.98; $P = 0.49$).

Discussion

We found a high prevalence (91%) of *S. aureus* colonization among local patients with AD. These results are comparable to previous studies where the *S. aureus* colonization prevalence was found to be 80–100%.¹⁵ In contrast, an earlier study carried out in our institution in 1998 found a nasal colonization

Table 1 Demographics and clinical characteristics of patients with atopic dermatitis (AD) (n = 34)

Characteristic	
Age (years), mean (range)	12 (4–21)
Male sex, n (%)	17 (50)
Ethnicity, n (%)	
Chinese	32 (94.1)
Malay	1 (2.9)
Indian	1 (2.9)
Mean objective SCORAD, mean (range)	37.8 (4–70)
Severe SCORAD, n (%)	16 (47.1)
<i>Staphylococcus aureus</i> colonization, n (%)	
Nasal	31 (91.2)
Skin	31 (91.2)
Both skin and nose	29 (85.3)
Total number of close contacts per patient with AD, mean (range)	3 (1–6)
Number of close contacts recruited per patient with AD, mean (range)	1 (1–5)
Number of patients with AD with at least one close contact with positive nasal carriage of <i>S. aureus</i> , n (%)	21 (67.7)
Asthma, n (%)	11 (32.4)
Allergic rhinitis, n (%)	15 (44)

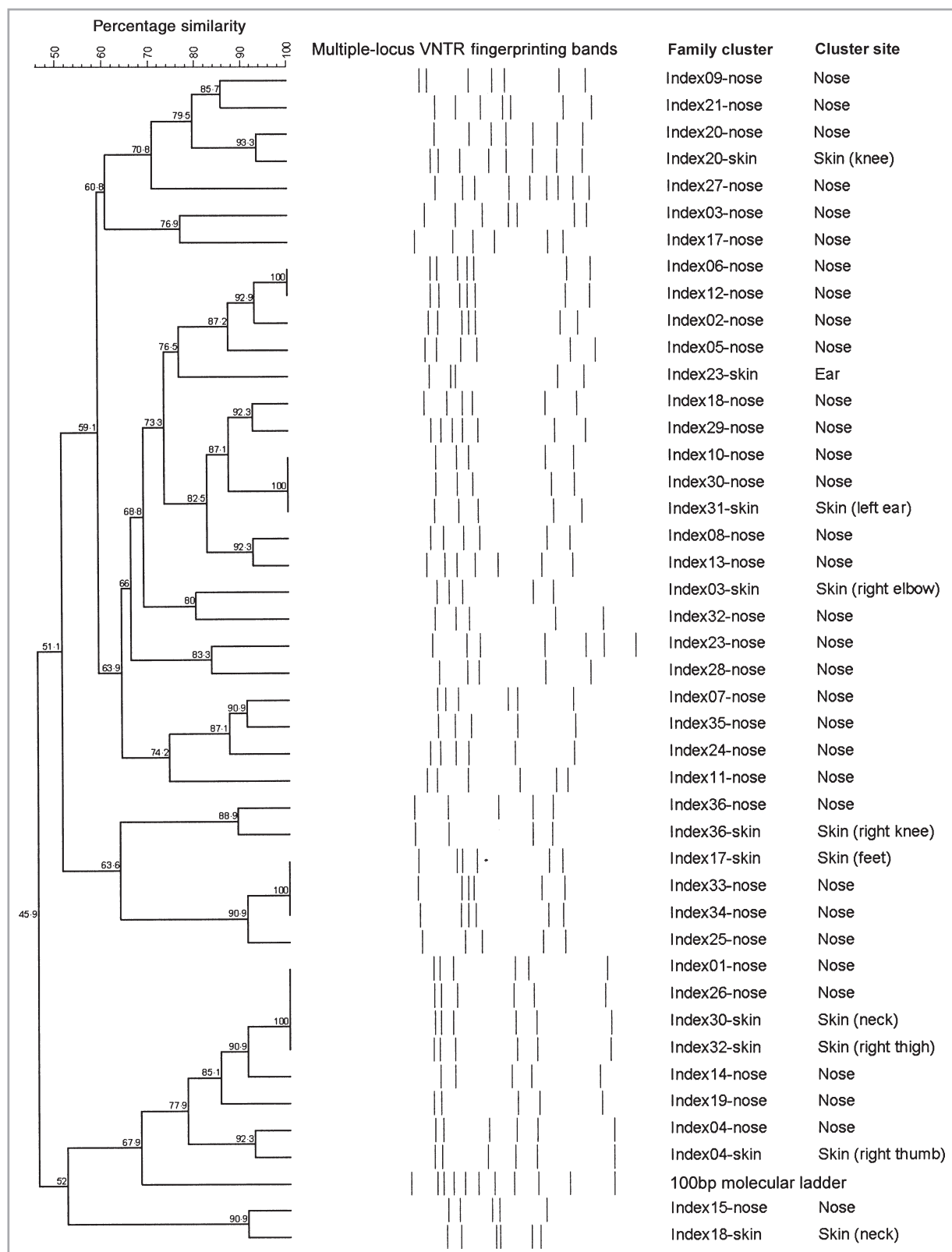


Fig 1. Phylogenetic dendrogram of *Staphylococcus aureus* isolates cultured from patients with atopic dermatitis. Isolates were typed using multiple-locus variable-number tandem-repeat (VNTR) fingerprinting, and were considered to be related if gel patterns demonstrated 80% or greater similarity on analysis using the Dice coefficient unweighted pair-group method with arithmetic averages. Ten patients had nasal and skin isolates that were not clonal on typing (both isolates are included in the dendrogram).

Table 3 Risk factors associated with more severe atopic dermatitis (AD) (univariate analysis)

Characteristic	Moderate AD (n = 18)	Severe AD (n = 16)	Odds ratio (95% CI)	P-value
Median age (years)	10	13	1.11 (0.96–1.29)	0.15
Nasal carriage of <i>Staphylococcus aureus</i>	16	15	1.88 (0.15–22.88)	0.62
Cutaneous carriage of <i>S. aureus</i>	16	15	1.88 (0.15–22.88)	0.62
At least one positive close contact with <i>S. aureus</i>	11	10	1.06 (0.27–4.24)	0.93
Virulence profile				
sea	1	5	a	0.08
seb	11	2	a	0.03
sec	2	2	a	1.00
sed	1	2	a	0.60
egc	7	3	a	0.25
seh	3	6	a	0.25
sek	1	4	a	0.17
sel	1	1	a	1.00
pvl	0	1	b	b
eta	4	0	b	b
etb	0	1	b	b
etd	1	0	b	b
tsst1	1	0	b	b
edinAC	0	0	b	b
edinB	12	4	a	0.01
hlq	2	1	a	1.00
hlqvariant	14	11	1.05 (0.19–5.69)	0.96
hlb	11	6	0.48 (0.12–1.98)	0.31

a, Fisher's exact test used—no odds ratio or confidence interval (CI) available for these groups; b, predicts failure or success perfectly.

rate of 52%.²⁴ Twenty-nine (85%) patients with AD were colonized with *S. aureus* both in the nose and on the skin. This finding suggests that nasal carriage might be an important source of cutaneous recolonization in patients with AD. Hon *et al.*²⁵ also found that the anterior nares are an important site for *S. aureus* and that nasal colonization was associated with more extensive lesions and a higher SCORAD index, although our results did not find such an association.

We also found a high prevalence of *S. aureus* nasal colonization in close contacts (46%). This is similar to results published by Bonness *et al.*,¹⁸ who reported a prevalence of 48% *S. aureus* colonization among parents of children with AD who were themselves colonized. It is also noteworthy that we found a very high concordance of *S. aureus* isolates in index patients and their close contacts (81% identical or closely related isolates based on molecular typing), suggesting that *S. aureus* may be transmitted between family members and that close contacts may serve as an important reservoir of *S. aureus*. The high carriage and concordance rates found among close contacts in this study may form the basis for future interventional studies focusing on *S. aureus* elimination in close contacts as a means of reducing microbial load and recolonization of patients with AD. The presence of close contacts who are also *S. aureus* carriers has been shown to be a factor associated with recolonization.¹⁸

We found that *seb* enterotoxin was the most common superantigen (n = 13) followed by *egc* (n = 10) in *S. aureus* isolates from patients with AD. This was compatible with the findings from previous studies carried out in different countries. Mempel *et al.*¹⁰ demonstrated a high prevalence of the *egc* locus (48.3%), as well as *sea-sed* and *TSST1* (38.3%). Lomholt *et al.*²⁶ found that the most prevalent superantigens were *sea* and *seb*. There is another study carried out by Bonness *et al.*¹⁸ in Germany which showed that *seg* and *sei* (indication of the presence of *egc* enterotoxin gene cluster) were the most frequently isolated enterotoxin genes (37% of the isolates) followed by *seb* enterotoxin. However, all the above studies do not demonstrate any particular superantigen that is significantly associated with exacerbation of eczema. In contrast, a study by Zollner *et al.*¹² showed that superantigen production by *S. aureus* was associated with an increased severity of atopic dermatitis. Also, Breuer *et al.*¹⁵ found a relationship between the severity of skin lesions and sensitization to *seb* in adult patients with AD. It therefore remains controversial as to whether or not any particular *S. aureus* enterotoxin is correlated with disease severity. While we did not find any particular isolates of *S. aureus* that were significantly associated with AD or disease severity, it was interesting that our local patients with moderate, rather than severe, AD were more likely to be colonized by

S. aureus possessing *seb* ($P = 0.027$). More studies are needed to explore the relationship between *S. aureus* enterotoxins and AD disease severity.

Significantly, we did not identify any hospital or community-associated MRSA (CA-MRSA) in this cohort, despite an increasing prevalence of these isolates locally.²⁷ This suggests that the local carriage of CA-MRSA remains low, although our study was not designed to answer this question specifically. No large-scale community surveys have been performed locally; hence the exact prevalence of CA-MRSA in Singapore is unknown.

There are several limitations in our study. The small sample size may have precluded the identification of predominant virulence factors among our patients with AD. In addition, it would be ideal to compare the findings with those from healthy controls, preferably from the community, to determine the true prevalence of *S. aureus* nasal carriage in our study subjects. Also, we were unable to obtain nasal swabs from all the close contacts to investigate for intrafamilial transmission of *S. aureus*. Finally, we only determined the presence of superantigen genes and did not analyse actual gene expression *in vivo* or *in vitro*. Schlievert *et al.*²⁸ recently demonstrated the relationship between actual superantigen production and the severity of AD.

In conclusion, we found a high colonization of *S. aureus* in the skin and anterior nares of patients with AD. In addition, there was a high colonization by similar isolates of *S. aureus* in the anterior nares of their close contacts, suggesting that close contacts may act as important reservoirs of *S. aureus*. The presence of superantigen B is associated with moderate, rather than severe AD in our population. Further clinical trials are needed to study the effect of treatment of nasal colonization in close contacts on AD disease activity.

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