Journal of Clinical & Experimental Dermatology Research

Jochmann et al., J Clin Exp Dermatol Res 2013, 4:2
DOI: 10.4172/2155-9554.1000174

Research Article Open Access

# Correlation of Twenty Virulence Genes of *Staphylococcus aureus* with Severity of Atopic Dermatitis in Children as Compared to Healthy Individuals

**Anja Jochmann[1]*, Suzanne Hower[2], Stephen Davis[3], Jan Izakovic[3] and Lisa RW Plano[2]**

[1]*University Hospital for Children UKBB, Department of Pediatrics, 4056 Basel, Switzerland*
[2]*University of Miami Miller School of Medicine, Departments of Pediatrics and Microbiology and Immunology, Miami, FL 33136, USA*
[3]*University of Miami Miller School of Medicine, Department of Dermatology, Miami, FL 33136, USA*

## Summary

**Principles:** Patients with Atopic Dermatitis (AD) have a higher susceptibility for colonization and infection with *Staphylococcus aureus*. Virulence factors of *S. aureus* may modulate the host immune response and affect the clinical course of infection.

**Methods:** Bacterial cultures were obtained from AD patients and uninfected controls. PCR and DNA sequence analysis were used to determine microbial surface components recognizing adhesive matrix molecules (MSCRAMM) patterns, staphylococcus protein A (*spa*) types, and the presence of genes for 20 virulence factors and for methicillin resistance (*mec*A). Virulence factor gene patterns from AD associated *S. aureus* were compared with gene patterns from the control group, as well as with *S. aureus* previously obtained from infected skin lesions not associated with AD.

**Results:** The gene encoding chemotaxis inhibiting protein (*chp*) was found more frequently in *S. aureus* isolated from the uninfected control group (p=0.0003). Isolates of AD patients were more likely to carry the gene *sea* (p=0.0327), which encodes for an enterotoxin known to act as a superantigen. Prevalence of *eta*, *etb* and *chp* were significantly associated with organisms isolated from non-AD infected lesions (*eta*: p=0.0003, *etb*: p=0.0001, *chp*: p=0.012) There was no difference in the prevalence of any MSCRAMM gene pattern or 19 additional virulence factors genes analyzed, and none were associated with severity of the AD lesions. MRSA SCC*mec* type IVa made up approximately 8% of both AD and control isolates.

**Conclusions:** The genotypes of *S. aureus* strains colonizing AD patients do not differ significantly from the genotypes of strains colonizing healthy individuals. Isolates infecting patients without AD express significantly more *eta* and *etb* and therefore seem to be more virulent to overcome the intact skin barrier.

**Keywords:** Atopic dermatitis; *Staphylococcus aureus*; Virulence factors; Superantigens; Sea; TSST

**Abbreviations:** AD: Atopic Dermatitis; *S. Aureus*: *Staphylococcus Aureus;* PVL: Panton Valentine Leukocidin; *Luk-DE*: Leukocidin D-E; *Sea*: Staphylococcal Enterotoxin A; *Seb*: Staphylococcal Enterotoxin B; *Seg*: Staphylococcal Enterotoxin G; *Sek*: Staphylococcal Enterotoxin K; *Seq*: Staphylococcal Enterotoxin Q; *Seh*: Staphylococcal Enterotoxin H; *Sem*: Staphylococcal Enterotoxin M; *Hla*: A-Hemolysin; *Hlb*: B-Hemolysin; *Hlgb*: Gamma-Hemolysin B; *Hlgc*: Gamma-Hemolysin C; *Hlgabc*: Gamma-Hemolysin ABC; *Sak*: Staphylokinase; *Tst-1* TSST: Toxic Shock Syndrome Toxin-1; *Eta Etb*: Exfoliative Toxins A and B; Chp CHIP: Chemotaxis Inhibitory Protein; *Arca*: Arginine Catabolic Mobile Element (ACME) Arginine Deiminase; *Meca*: Methicillin Resistance Protein Penicillin-Binding Protein 2a; *Gyra*: Gyrase A; Scc*mec*: Staphylococcal Cassette Chromosome *Mec; Spa*: Staphylococcal Protein A; MSCRAMM: Microbial Surface Components Recognizing Adhesive Matrix Molecules; MSSA: Methicillin-Sensitive *Staphylococcus Aureus;* MRSA: Methicillin-Resistant *Staphylococcus Aureus;* PCR: Polymerase Chain Reaction; EASI: Eczema Area and Severity Index; FLG: Fillagrin Aggregating Protein

## Background

Atopic Dermatitis (AD) is a chronic, highly pruritic, eczematous dermatitis affecting 10-20% of children and is a major cause for morbidity. Children with AD have a higher susceptibility for microbial colonization and an increased risk of skin infections [1]. There are several theories regarding factors that contribute to etiology or impair the course of AD. Some studies have shown that greater than 90% of AD patients are colonized or infected with *Staphylococcus aureus*, in contrast to 20-40% of healthy individuals from the general population who harbor the organism on their skin [2]. In addition, reduction or eradication of *S. aureus* colonization on AD patients has demonstrated a positive correlation with decreased severity of atopic eczema [3].

Greater than half of the *S. aureus* strains isolated from AD skin lesions exhibit secretion of superantigenic toxins like TSST [4]. The role of staphylococcal virulence factors in the pathophysiology of atopic dermatitis is an area of active research. Cardona et al. examined the mechanisms of *S. aureus* colonization and infection and concluded that immunologic mechanisms such as T-helper-2 cell-driven skin inflammation prompted by superantigens of *S. aureus* are important targets for future treatment approaches [5]. Atopy, a genetic disposition to produce IgE antibodies to common food or environmental antigens,

***Corresponding author:** Anja Jochmann, University Hospital for Children (UKBB), Departments of Pediatrics and Infectious Diseases, Spitalstr 33, 4056 Basel, Switzerland, Tel: +41-704 2223; Fax: +41-704-1213; E-mail: anja.jochmann@ukbb.ch

**Received** April 15, 2013; **Accepted** May 17, 2013; **Published** May 24, 2013

**Citation:** Jochmann A, Hower S, Davis S, Izakovic J, Plano LRW (2013) Correlation of Twenty Virulence Genes of *Staphylococcus aureus* with Severity of Atopic Dermatitis in Children as Compared to Healthy Individuals. J Clin Exp Dermatol Res 4: 174. doi:10.4172/2155-9554.1000174

has been shown to correlate with AD in more than 80% of children with atopic dermatitis [6] and some *S. aureus* superantigens also induce IgE production and activate large numbers of T-cells, resulting in a massive release of cytokines and other inflammatory mediators [5]. These mediators effectively propagate the exacerbation of skin inflammation. Staphylococcal virulence factors have also conferred the ability to these microorganisms to adapt and alter the local immune response. Mertz et al. demonstrated the association of *S. aureus* exfoliative toxin A gene (*eta*) with a diminished localized immune response in infected skin lesions as defined by a decreased amount of infiltrating white blood cells present at the time the cultures were obtained [7]. In this study, we intended to characterize the genetic repertoire of *S. aureus* strains colonizing and infecting AD patients and compare them to the genetic repertoire of strains colonizing healthy individuals. Further, we examined the association between the virulence genes carried by *S. aureus* and the severity of AD.

## Methods

### Sample collection

This was a clinical and microbiological prospective case-control study conducted at a large university-based outpatient dermatology clinic. 40 AD patients and 75 healthy volunteers, including clinic employees and healthy family members of patients, participated in the study after providing informed consent in order to collect samples containing *S. aureus* for analysis. Clinical examinations and bacterial samples were obtained during a single visit per patient. Nasal swabs were taken from healthy volunteers and skin lesions were swabbed in AD patients in order to obtain *S. aureus* isolates for comparison. The severity of AD lesions were measured using the Eczema Area Severity Index (EASI) scoring system and was categorized as mild or moderate versus severe AD.

### Bacterial isolation and Identification

Initial swab cultures were grown on tryptic soy agar with 5% Sheep Blood (TSA II™, Becton, Dickinson and Company, Sparks, MD) and incubated aerobically at 37°C for a minimum of 24 h. After incubation, bacteria were Gram stained and those found to be Gram-positive were transferred to mannitol salt agar (BD) for determination of mannitol fermentation and incubated aerobically at 37°C for 16-24h. Mannitol-fermenting isolates were tested for the presence of catalase by mixing with hydrogen peroxide. Those isolates that produced a positive reaction were subjected to *latex agglutination testing* for clumping factor and protein A using the BACTiSTAPH® Latex Agglutination Test (Remel via Thermo Fisher Scientific, Lenexa, KS). Presumptive *S. aureus* were tested for methicillin resistance using cefoxitin disks on agar growth media (TSA II™) to measure the zone of clearance.

### *S. aureus* characterization and qPCR determination for select gene.

Chromosomal DNA was isolated from each presumptive *S. aureus* isolates using the Dneasy tissue kit (Qiagen, Valencia, CA) according to manufacturer's instructions, with the addition of 0.25 mg/ml lysostaphin (Ambi Products LLC, Lawrence, NY) to the lysis step. The quality of the chromosomal DNA was evaluated by agarose gel electrophoresis and concentrations were determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and normalized to 5 ng/µl.

Quantitative real time PCR (qPCR) was performed on all presumptive *S. aureus* isolates to determine the presence or absence of *S. aureus*-specific *gyrA* as described [7] to confirm identity. *S. aureus* isolates were then subjected to qPCR analysis of twenty one virulence genes including: Panton Valentine leukocidin (PVL; *lukS-PV* and *lukF-PV*), leukocidin D-E (*lukDE*), staphylococcal enterotoxin A (*sea*), staphylococcal enterotoxin B (*seb*), staphylococcal enterotoxin G (*seg*), staphylococcal enterotoxin K (*sek*), staphylococcal enterotoxin Q (*seq*), staphylococcal enterotoxin H (*seh*), staphylococcal enterotoxin M (*sem*), α-hemolysin (*hla*), β-hemolysin (*hlb*), gamma-hemolysin B (*hlgB*), gamma-hemolysin C (*hlgC*), gamma-hemolysin ABC (*hlgABC*), staphylokinase (*sak*), toxic shock syndrome toxin (*tst-1*), exfoliative toxins A and B (*eta*, *etb*), chemotaxis inhibiting protein (*chp*), Arginine Catabolic Mobile Element (ACME) arginine deiminase (*arcA*) and the gene encoding methicillin resistance (*mecA*). Reaction conditions and analyses were performed according to Mertz et al. [7] or under the conditions described with specific primer pairs and annealing temperatures listed in Online Resource 1.

SCC*mec* types and subtypes were determined as described [8,9]. Identification of nine MSCRAMMs was accomplished via multiplex PCR as described by Tristan et al. [10]. Staphylococcal protein A (*spa*) pcr products were generated and sequenced (Eurofins MWG Operon, Huntsville, AL) according to Shopsin et al.[11]. *Spa* sequences were analyzed and typed [12] using the Egenomics *spa* type tool (http://tools.egenomics.com). Corresponding Ridom *spa* types were assigned using the Ridom *spa* server developed by Ridom GmbH [13] and curated by SeqNet.org (http://spa.ridom.de).

*S. aureus* isolates were divided into methicillin-sensitive (MSSA) and MRSA subsets according to presence of the *mecA* gene. The prevalence of specific virulence and adhesion genes of *S. aureus* was calculated for respective patient superficial skin sites and compared with the healthy control group. Additionally, the prevalence of a subset of virulence genes was correlated with the degree of AD severity.

### Statistical analysis

Statistics were performed using SPSS software. Continuous variables were expressed as means (± Standard Deviation [S.D.]) and categorical variables were shown as relative frequencies and percentages. Comparisons were made using cross-tabulation for categorical data and summary statistics for metric data. $P<0.05$ was considered statistically significant. All groups were compared using Chi-square analysis.

## Results

52 bacterial isolates were obtained from initial swab specimens and subjected to Gram staining. Four Gram negative isolates were excluded from further testing. The subsequent biochemical tests identified 48 presumptive *S. aureus* isolates; 24 from AD patients and 24 control samples that were confirmed by qPCR and included in the final analysis. *S. aureus* was found in 24 of 40 swabs taken from atopic dermatitis patients in outpatient clinic (60%) and two of 24 were MRSA (Tables 1a and 1b). Swabs were taken from acute and chronic AD lesions of AD patients. *S. aureus* was isolated from the nares of 24 of 75 healthy volunteers (32%) and two of 24 were MRSA. The mean age in the AD group was 7.5 years with a standard deviation of 5.4 years. The mean age in the control group was 29.0 years with a standard deviation of 12.5 years. Eight of the AD patients suffered from severe AD as assigned by EASI (33.3%). 13 (54.2%) patients were classified as moderate AD and three (12.5%) patients as mild AD at time of examination (Tables 1a and 1b). The groups of mild and moderate AD were merged to a mild/moderate AD group consisting of 16 (66.7%)

**a**

| AD Number | AD Severity | Age (years) | sak | arcA | hla | hlgB | hlb | hlgC | hlg ABC | luk-DE | SCC mec type | SCC mec type IV protocoll |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 001 | Severe | 1.5 | Pos | Pos | Pos | Neg | Pos | Pos | Pos | Pos | IV | IVa |
| 015 | Severe | 13 | Pos | Neg | Pos | Pos | Pos | Pos | Pos | Pos | Neg | Neg |
| 023 | Severe | 3 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 024 | Severe | 3 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 005 | Severe | 18 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 017 | Severe | 11 | Pos | Neg | Neg | Pos | Pos | Pos | Pos | Neg | Neg | Neg |
| 006 | Severe | 8 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 007 | Severe | 8 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 012 | Severe | 3 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 013 | Severe | 3 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 010 | Moderate | 0.7 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 019 | Moderate | 14 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 020 | Moderate | 14 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 009 | Moderate | 13 | Neg | Neg | Pos | Neg | Pos | Pos | Pos | Neg | Neg | Neg |
| 008 | Moderate | 13 | Neg | Neg | Pos | Neg | Pos | Pos | Pos | Neg | Neg | Neg |
| 004 | Moderate | 0.7 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 021 | Moderate | 10 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 022 | Moderate | 10 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |
| 002 | Moderate | 3 | Pos | Neg | Neg | Pos | Neg | Pos | Pos | Neg | Neg | Neg |
| 003 | Moderate | 3 | Pos | Neg | Neg | Pos | Neg | Pos | Pos | Neg | Neg | Neg |
| 011 | Moderate | 2 | Pos | Neg | Neg | Pos | Neg | Pos | Pos | Neg | Neg | Neg |
| 016 | Moderate | 7 | Pos | Neg | Neg | Pos | Pos | Pos | Pos | Neg | Neg | Neg |
| 014 | Moderate | 15 | Pos | Pos | Pos | Neg | Pos | Pos | Pos | Pos | IV | IVa |
| 018 | Mild | 6 | Pos | Neg | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg |

AD: Atopic Dermatitis group; Neg: Negative, Pos: Positive; SCCmec: Staphylococcal Cassette Chromosome mec

**b**

| AD Number | AD Severity | eta | etb | sea | seb | sek | seq | pvl | tst-1 | chp | sem | seg | seh |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 001 | Severe | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg | Pos | Neg | Neg | Neg |
| 023 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 024 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 015 | Severe | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg | Pos | Neg | Pos | Neg |
| 005 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 006 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 007 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 012 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 013 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 017 | Severe | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg |
| 002 | Moderate | Neg | Neg | Pos | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Pos | Neg |
| 003 | Moderate | Neg | Neg | Pos | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Pos | Neg |
| 011 | Moderate | Neg | Neg | Pos | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Pos | Neg |
| 016 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg |
| 019 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 020 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 009 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 008 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg |
| 004 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 021 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 022 | Moderate | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 014 | Moderate | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg | Pos | Neg | Neg | Neg |
| 010 | Moderate | Pos | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 018 | Mild | Neg | Neg | Neg | Pos | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Neg |

AD: Atopic Dermatitis group; Neg: Negative, Pos: Positive

**Table 1:** Genetic profile of AD isolates grouped according to AD severity.

patients for the purposes of statistical analyses. Two isolates from both the AD and control group were MRSA (8.3%).

Analyses of MSCRAMM genes (Figure 1) indicated that all isolates tested encoded laminin binding protein (*eno*) and the fibrinogen receptor genes encoding clumping factor A and B (*clfA* and *clfB*). The third fibrinogen receptor (*fib*) was found in 16 (66.6%) of the AD group and 14 (58.3%) of the control group. Eight (33.3%) of the AD

isolates and six (25%) of the control isolates were positive for collagen binding protein (*cna*). All isolates were positive for fibronectin binding protein A (*fnbA*). *fnbB* was present in four (16.7%) AD isolates versus five (20.8%) control isolates. Elastin binding protein (*ebpS*) equipped 17 (70.8%) of AD isolates and 15 (62.5%) of control isolates. Bone sialoprotein binding protein (*bbp*) was carried by five (20.8%) of the AD and four (16.7%) of the control isolates. The minimal MSCRAMM gene pattern for all isolates consisted of three genes encoding fibrinogen receptors (*clfA*, *clfB* and *fib*) and *eno*. There was no statistically significant difference related to any adhesin gene between the atopic dermatitis and control group (Figure 1; p>0.05) and no statistically significant difference identified among the control, mild/moderate or severe AD group (p > 0.05).

*Spa* typing was performed for all 48 *S. aureus* isolates. The two MRSA isolates of the AD group were identified as spa type 1/t008 whereas the two MRSA isolates from the control group were spa type 49/t126. All MRSA strains were SCC*mec* type IVa (Table 1a). The MSSA *spa* types from both groups were distributed widely among different *spa* types (data not shown).

The AD and control group were compared for each virulence factor by chi-square test. *chp*, the gene encoding chemotaxis inhibiting protein, was found more frequently in the control group *S. aureus* despite all strains in the control group stemming from different individuals (p=0.0003 (Figure 2). The presence of other virulence factors showed no statistically significant differences between the two groups (p> 0.05). Shown in Figure 2 is the prevalence of the virulence factor genes from the AD vs. control group as determined by qPCR.

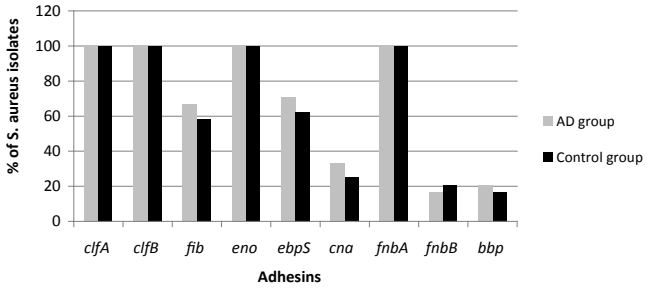
Subgroups of severe AD (eight patients), mild/moderate AD (16 patients) and healthy controls (24 patients) were analyzed for their association with specific virulence factors. *lukDE* was more common in the severe AD group compared to the control group (p=0.041). No significant differences were observed for the remaining 19 virulence factors among the three groups: p>0.05. Distribution of virulence factor genes according to AD severity is shown in Tables 1a and 1b.

Broader analysis of virulence factor patterns indicated that three isolates in the AD group had a maximum of 12 virulence factor genes whereas the maximum amount observed in the control group was nine virulence factor genes present in one isolate (data not shown). Interestingly, *tst-1* was significantly associated with the presence of *sea* in the AD (p=0.0001) and control group (p=0.032). Prevalence of the adhesion *bbp* is also significantly correlated with *tst-1* (AD, p=0.0001; p=0.032; control group).

Finally, the virulence factor gene profiles of organisms colonizing and infecting AD patients were compared to those of organisms isolated from patients with minor skin infections and without preexisting skin disease, previously characterized by Mertz et al. [7]. To evaluate whether *S. aureus* infecting patients with normal skin were more virulent (Table 2). *eta*, *etb* and *chp* were significantly associated with organisms infecting patients with normal skin (*eta*: p=0.0003, *etb*: p=0.0001, *chp*: p=0.012) Prevalence of *sea* was significantly elevated in AD patients (p=0.0327).

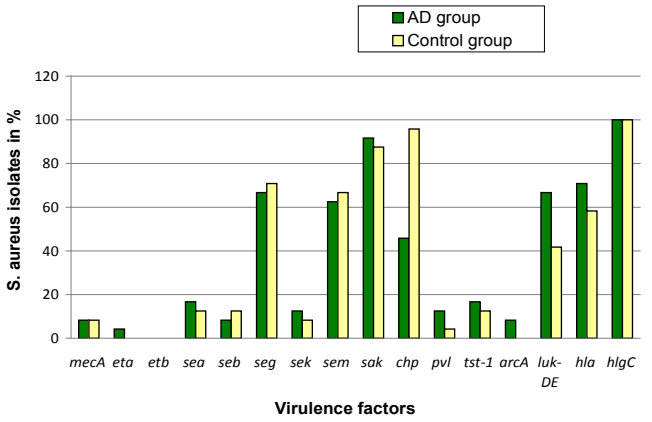
## Discussion

*Staphylococcus aureus* is a frequent colonizer of human skin. Since colonization with *S. aureus* has been shown to worsen disease severity and cause skin infection more frequently in AD patients [3], we questioned whether strains colonizing AD patients were more virulent than those colonizing patients without preexisting skin disease. Indeed, decolonization of *S. aureus* on AD patients' skin has been shown to lessen the disease severity index [14].




AD group: Atopic Dermatitis group; *clfA/clfB: clumping factor A and B; fib: fibrinogen receptor; eno: laminin binding protein; ebpS: elasting binding protein; cna: collagen binding protein; fnbA/fnbB: fibronectin binding protein A and B; bbp: bone sialoprotein binding protein*

**Figure1:** Distribution of adhesins in AD group and control group *S. aureus* isolates.




AD group: Atopic Dermatitis, *eta:* exfoliative toxin A; *etb:* exfoliative toxin B; *sea: Staphylococcal* enterotoxin *A; seb: staphylococcal enterotoxin; seh: staphylococcal enterotoxin H; seg: staphylococcal enterotoxin G; sek: staphylococcal enterotoxin K; sem: staphylococcal enterotoxin M; sak: staphylokinas; chp: chemotaxis inhibitory protein; pvl: Panton-Valentine Leukocidin; tsst: toxic shock syndrome toxin; arcA: arginine catabolic mobile element; luk-DE: leukocidin D-E; hla: α-hemolysin; hlb: β-hemolysin; hlg B: gamma-hemolysin B; hlg C: gamma-hemolysin C*

**Figure 2:** Prevalence of virulence factors in AD and control group *S. aureus* isolates.

| gene | Infected lesions* % (No.) n=72 | AD group study % (No.) n=24 | p-value |
|---|---|---|---|
| *eta* | 43 (31) | 4 (1) | p = 0.0003 |
| *etb* | 39 (28) | 0 | p = 0.0001 |
| *seb* | 13 (9) | 8 (2) | p > 0.05 |
| PVL | 11 (8) | 13 (3) | p > 0.05 |
| *sea* | 3 (2) | 17 (4) | p = 0.033 |
| *tst-1* | 4 (3) | 17 (4) | p > 0.05 |
| *chp* | 75 (54) | 46 (11) | p = 0.012 |

*eta: exfoliative toxin A; etb: exfoliative toxin B; sea: staphylococcal enterotoxin A; seb: staphylococcal enterotoxin B; pvl: Panton-Valentine Leukocidin; tsst: toxic shock syndrome toxin; chp: chemotaxis inhibitory protein *Source: Mertz,* et al. [7]*

**Table 2:** Comparison of S. aureus from AD lesions and non AD-infected skin lesions.

In the present study, we compared 24 S. *aureus* isolates from AD patients to 24 isolates from healthy controls. The control group consisted mostly of adults. However, it has been shown previously that organisms colonizing adults and children are similar [15]. The two groups showed no significant difference concerning virulence factor gene prevalence or adhesion gene prevalence. These findings suggest that AD patients and healthy controls are colonized by similar *S. aureus* strains. Interestingly, our results correspond to the analysis of Mempel et al. who compared the prevalence of superantigens, specifically toxic shock syndrome toxin (*tst-1)* and staphylococcal enterotoxins (*sea-sed*) found in enterotoxin gene clusters (*egc*) from *S. aureus* isolates of AD patients vs. healthy controls and found similar virulence factor prevalence for both groups [16].

Nearly 90% of isolates in AD and control group encoded for *sak*, which has been shown to increase plasmin levels triggering fibrinolysis as well as to exhibit proteolytic activity toward extracellular matrix proteins [17]. Haemolysin genes (*hla,hlb, hlgB, hlgC, hlgABC*), that damage membranes of human erythrocytes [17] were also highly prevalent in both groups. Known superantigens including *sea*, *seb* and *tst-1* were found in 8-17% of AD and control patients with a tendency towards higher prevalence in AD patients. Staphylococcal enterotoxins from the *egc* (*seg*, *sek*, *sem*) were even more frequent in both groups (12-66%) than *sea* and *seb*. These toxins have also been described by Mempel et al. to fulfill the criteria of superantigens [16]. PVL which is a pore forming toxin causing tissue necrosis and lysis of phagocytes [18] trended towards higher expression in the AD group and showed a 10% higher prevalence than community prevalence as described by Zanger et al. [19].

The percentage of MRSA found in this study is comparable to those described by Matiz et al. for AD patients and lower than community prevalence [20]. There was no significant difference in virulence factor prevalence observed among the three groups of mild/moderate AD lesions, severe AD lesions and healthy control group isolates except for *chp* which showed a significantly higher prevalence in the control group ($p=0.0003$). Importantly, none of the 20 virulence factors was shown to be correlated with disease severity. Mempel et al. also stated no significant correlation between the prevalence of superantigens and AD severity [16].

Our analysis of MSCRAMM gene prevalence indicated no significant difference between the three groups of mild/moderate AD lesions, severe AD lesions and healthy control group either. Interestingly, *bbp* which was shown by Tristan et al.to be significantly associated with osteomyelitis/arthritis [10] and is common to virulent *S. aureus* strains [21] was not significantly different in the AD vs. control group. Prevalence of the adhesion *bbp* was also significantly correlated with *tst-1*.

The two MRSA isolates in the AD group were spa type 1 (Ridom t008), commonly associated with PFGE type USA300 which is one of the epidemic strains in North America [22]. Control group MRSA isolates were spa type 49 (Ridom t126) which has been associated with USA700, a less common MRSA strain in the United States, associated both with community and hospital acquired infections [23]. MSSA *spa* analysis did not show significant accumulation for any particular type. These results support the hypothesis that although atopic dermatitis patients and healthy individuals are colonized with comparably virulent *S. aureus* isolates, AD patients succumb to infection due to defective skin barrier function and altered immune response. Indeed, a German study by Bonness et al. concluded that AD suffering children were infected with similar *S. aureus* strains found on their colonized, yet healthy parents [15]. Notably, prevalence of more virulent MRSA is less in AD patients compared to community prevalence [20].

Certain virulence factors like TSST or staphylococcal enterotoxins (SEA, SEB, etc.) are known to act as super antigens [24]. Super antigens have been shown to stimulate T-cell proliferation by linking variable parts of β-chains of T cell receptors and constant regions on the α- and β-chains of MHC II molecules on antigen-presenting cells like macrophages. The consequence of this interaction of superantigens with T cells and macrophages is a massive cytokine production. These molecules attract granulocytes and dendritic cells and likely induce inflammation on skin and mucosa early in *S. aureus* infections [24]. Leung et al. compared AD and psoriatic patients and found a significant IgE related basophil histamine release in AD patients with IgE antitoxin but not in psoriatic patients, concluding that this allergic response to super antigens might exacerbate atopic dermatitis [4]. Although expression levels of toxins and other virulence factors were not evaluated in this study, the presence or absence of respective genes suggests that AD patients may react to colonizing *S. aureus* and its repertoire of virulence factors including super antigens differentially as compared to healthy individuals due to their altered immune response and defective skin barrier. Most studies examining the genetics of AD skin barrier dysfunction have found an association with a mutation in the gene encoding Fillagrin Aggregating protein (FLG) [25]. Fillagrin metabolites are important factors in maintaining an acidic stratum corneum pH where a decrease could lead to a cascade of increased pH, subsequent activation of serine proteases and increased expression of *S. aureus* virulence factors [26, 27].

In order to determine whether *S. aureus* strains infecting patients without a skin disease differ from those infecting AD patients, we compared the virulence factor profiles of *S. aureus* in our AD patient group with those swabbed from impetigo and infected lesions of patients studied by Mertz et al.[7]. Notably, *eta*, *etb* and *chp* were significantly more common ($p< 0.05$) in the group with infected skin lesions (Table 2). Exfoliative toxins like ETA or ETB cause epidermal blister formation most probably by cleavage of desmoglein-1 in keratinocytes [28]. The association of more prevalent *eta*, *etb* and *chp* with infected lesions vs. AD supports our thesis that AD patients get infected by potentially less virulent *S. aureus* strains due to their overwhelming immune response and skin barrier dysfunction whereas *S. aureus* strains infecting patients without skin disease require additional virulence factors to cause disease. This hypothesis is supported by the study of Argudin et al. who found 98% of infecting *S. aureus* strains isolated from patients with skin infections in Spain carried an exfoliative toxin (*eta* and/or *etb* [29,30]. The only virulence gene more prevalent in the AD group in the present study was *sea* ($p< 0.05$). *sea* is not necessarily typical of characterized virulent strains but acts as a super antigen and increases inflammation in AD patients due to a hyper-immune response [24]. Hence, these strains would not be as likely to evoke a dermal reaction in healthy individuals [16].

## Conclusion

In this investigation, AD patients and healthy controls are colonized by similar *S. aureus* strains and decolonization of these AD patients would likely be temporary due to exposure to these strains from a healthy population.

Isolates infecting patients without a chronic skin disease express significantly more *eta* and *etb*. These isolates seem to be more virulent compared to those infecting AD patients to overcome the intact skin barrier. Therefore, future therapeutic strategies in atopic

dermatitis should aim at improving defective skin barrier function and subsequently impeding inflammation and infection.

### References

1. Boguniewicz M, Leung DY (2010) Recent insights into atopic dermatitis and implications for management of infectious complications. J Allergy Clin Immunol 125: 4-13.
2. Namura S, Nishijima S, Higashida T, Asada Y (1995) Staphylococcus aureus isolated from nostril anteriors and subungual spaces of the hand: comparative study of medical staff, patients, and normal controls. J Dermatol 22: 175-180.
3. Nada HA, Gomaa NI, Elakhras A, Wasfy R, Baker RA (2012) Skin colonization by superantigen-producing Staphylococcus aureus in Egyptian patients with atopic dermatitis and its relation to disease severity and serum interleukin-4 level. Int J Infect Dis 16: e29-33.
4. Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, et al. (1993) Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J Clin Invest 92: 1374-1380.
5. Cardona ID, Cho SH, Leung DY (2006) Role of bacterial superantigens in atopic dermatitis : implications for future therapeutic strategies. Am J Clin Dermatol 7: 273-279.
6. de Benedictis FM, Franceschini F, Hill D, Naspitz C, Simons FE, et al. (2009) The allergic sensitization in infants with atopic eczema from different countries. Allergy 64: 295-303.
7. Mertz PM, Cardenas TC, Snyder RV, Kinney MA, Davis SC, et al. (2007) Staphylococcus aureus virulence factors associated with infected skin lesions: influence on the local immune response. Arch Dermatol 143: 1259-1263.
8. Oliveira DC, de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 46: 2155-2161.
9. Milheiriço C, Oliveira DC, de Lencastre H (2007) Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant Staphylococcus aureus: 'SCCmec IV multiplex'. J Antimicrob Chemother 60: 42-48.
10. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, et al. (2003) Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections. J Clin Microbiol 41: 4465-4467.
11. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, et al. (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 37: 3556-3563.
12. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, et al. (2004) spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol 42: 792-799.
13. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, et al. (2003) Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 41: 5442-5448.
14. Huang JT, Abrams M, Tlougan B, Rademaker A, Paller AS (2009) Treatment of Staphylococcus aureus colonization in atopic dermatitis decreases disease severity. Pediatrics 123: e808-814.
15. Bonness S, Szekat C, Novak N, Bierbaum G (2008) Pulsed-field gel electrophoresis of Staphylococcus aureus isolates from atopic patients revealing presence of similar strains in isolates from children and their parents. J Clin Microbiol 46: 456-461.
16. Mempel M, Lina G, Hojka M, Schnopp C, Seidl HP, et al. (2003) High prevalence of superantigens associated with the egc locus in Staphylococcus aureus isolates from patients with atopic eczema. Eur J Clin Microbiol Infect Dis 22: 306-309.
17. Goerke C, Wirtz C, Flückiger U, Wolz C (2006) Extensive phage dynamics in Staphylococcus aureus contributes to adaptation to the human host during infection. Mol Microbiol 61: 1673-1685.
18. Boyle-Vavra S, Daum RS (2007) Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton-Valentine leukocidin. Lab Invest 87: 3-9.
19. Zanger P, Nurjadi D, Schleucher R, Scherbaum H, Wolz C, et al. (2012) Import and spread of Panton-Valentine Leukocidin-positive Staphylococcus aureus through nasal carriage and skin infections in travelers returning from the tropics and subtropics. Clin Infect Dis 54: 483-492.
20. Matiz C, Tom WL, Eichenfield LF, Pong A, Friedlander SF (2011) Children with atopic dermatitis appear less likely to be infected with community acquired methicillin-resistant Staphylococcus aureus: the San Diego experience. Pediatr Dermatol 28: 6-11.
21. Rydén C, Yacoub AI, Maxe I, Heinegård D, Oldberg A, et al. (1989) Specific binding of bone sialoprotein to Staphylococcus aureus isolated from patients with osteomyelitis. Eur J Biochem 184: 331-336.
22. Otto M (2010) Basis of virulence in community-associated methicillin-resistant Staphylococcus aureus. Annu Rev Microbiol 64: 143-162.
23. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, et al. (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. J Clin Microbiol 41: 5113-5120.
24. Schlievert PM, Case LC (2007) Molecular analysis of staphylococcal superantigens. Methods Mol Biol 391: 113-126.
25. Barnes KC (2010) An update on the genetics of atopic dermatitis: scratching the surface in 2009. J Allergy Clin Immunol 125: 16-29.
26. Boguniewicz M, Leung DY (2011) Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev 242: 233-246.
27. Leung DY, Hauk P, Strickland I, Travers JB, Norris DA (1998) The role of superantigens in human diseases: therapeutic implications for the treatment of skin diseases. Br J Dermatol 139 Suppl 53: 17-29.
28. Nishifuji K, Shimizu A, Ishiko A, Iwasaki T, Amagai M (2010) Removal of amino-terminal extracellular domains of desmoglein 1 by staphylococcal exfoliative toxin is sufficient to initiate epidermal blister formation. J Dermatol Sci 59: 184-191.
29. Argudín MÁ, Mendoza MC, Vázquez F, Rodicio MR (2011) Exotoxin gene backgrounds in bloodstream and wound Staphylococcus aureus isolates from geriatric patients attending a long-term care Spanish hospital. J Med Microbiol 60: 1605-1612.
30. Jarraud S, Cozon G, Vandenesch F, Bes M, Etienne J, et al. (1999) Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. J Clin Microbiol 37: 2446-2449.