

ORIGINAL PAPER

Severe atopic dermatitis is associated with a high burden of environmental *Staphylococcus aureus*

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Summary

Background About 90% of patients with atopic dermatitis (AD) are colonized with *Staphylococcus aureus*. *S. aureus* worsens AD by secreting superantigens and structural molecules within the cell wall that induce skin inflammation. Therefore, *S. aureus* in the home may contribute to persistent skin inflammation and disease severity.

Objective To quantify *S. aureus* burden in homes of participants with AD of varying severities. **Methods** Participants with mild ($n = 18$), moderate ($n = 14$), severe ($n = 15$), and no AD ($n = 15$), collected dust from their bed and bedroom floor, and from their home vacuum cleaner bag. DNA was extracted from dust samples, and the *S. aureus*-specific *femB* gene was quantified using quantitative real-time PCR. Data was log-transformed, and then statistically analysed with ANOVA, student's *t*-test, and Spearman's *r*.

Results Participants with severe AD (geometric mean: 14.67 pg/mg dust) had significantly more *S. aureus* DNA in their bed dust than those with moderate (0.41 pg/mg dust, $P < 0.0001$), mild (1.42 pg/mg dust, $P = 0.0051$), and no AD [0.09 pg/mg dust, $P < 0.0001$ (*t*-test)]. Similar patterns were observed for dust from the bedroom floors and vacuum bags. *S. aureus* DNA was highest in dust from beds as compared with bedroom floors or vacuum bags (medians: 1.51, 0.69, 0.21 pg/mg dust, respectively; $P = 0.007$). Eczema Area and Severity Index scores correlated with *S. aureus* DNA from the bed (Spearman's $r = 0.7263$; $P = 0.0004$) and floor (0.6846; $P = 0.0002$) dust, but not with the vacuum bag dust (0.3783; 0.0684).

Conclusions In the home and especially the bedroom, higher levels of *S. aureus* may contribute to disease severity and persistence in AD patients.

Keywords atopic dermatitis, *Staphylococcus aureus*

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Introduction

About 90% of patients with atopic dermatitis (AD), but < 5% of normal individuals, are colonized with *Staphylococcus aureus* [1]. Leyden et al. quantified *S. aureus* from chronic, lichenified lesions and from acute, exudative lesions [2]. Exudative lesions yielded a significantly greater number of *S. aureus* organisms ($15\,122\,000/\text{cm}^2$) than did chronic lesions ($403\,900/\text{cm}^2$). Guzik et al. quantified *S. aureus* density on skin lesions of adult patients with persistent AD and found a significant positive correlation with AD severity [3]. However, even non-lesional skin of AD patients is colonized with *S. aureus* [2].

Although it is accepted that *S. aureus* skin colonization is linked to AD severity, little is known about whether *S. aureus* shed by patients persists in their home environment. If so, *S. aureus* in the home may contribute to persistent skin inflammation and disease severity. *S. aureus* worsens AD by secreting superantigens, which stimulate T cells to release pro-inflammatory cytokines [4]. Structural molecules within the Staphylococcal cell wall, such as peptidoglycan and lipoteichoic acid, can induce skin inflammation [5]. For this study, we used quantitative real-time PCR (QRT-PCR) specific for *S. aureus*, in order to determine the *S. aureus* burden in house dust of AD patients with a broad range of disease severity.

Material and methods

Study subjects

Sixty-two volunteers, aged 1–40 years, participated in the study. Participants completed questionnaires about their asthma, allergies, and home environment. AD participants also filled in Lund–Browder diagrams, documenting the area and intensity of ‘redness’ (erythema), ‘scratch marks’ (excoriation), ‘papules’ (papulation), and ‘skin thickening’ (lichenification). From the diagrams, AD severity was calculated using the Eczema Area and Severity Index (EASI) [6]. Relative severity according to EASI scores was used to divide AD participants into mild, moderate and severe groups: 18 participants had mild AD; 14, moderate AD; 15, severe AD; and 15 had no AD.

The Institutional Review Board at National Jewish Medical and Research Center, Denver, CO, approved this study.

Dust collection

Using a kit, participants collected dust samples from the bed, the floor next to the bed, and the home vacuum bag. The collection kit included two Mitest dust collectors (INDOOR Biotechnologies, Ltd, Charlottesville, VA, USA) and a 0.25 m² vacuum guide in a self-addressed, pre-paid envelope. The Mitest dust collector is a filter-containing nozzle that fits on the end of a standard vacuum hose. Dust was collected from the bed and the floor by vacuuming a 0.25 m² area for 2 min, in each location, with separate Mitest dust collectors. The vacuum bag sample was taken directly from the home vacuum. Participants then mailed the samples to the lab. Most of the participants provided the requested dust samples in a condition that was adequate for DNA isolation.

Bed and floor dust samples were weighed. Dust from the vacuum bag was sieved first and then weighed.

Deoxyribonucleic acid isolation

The PowerSoil DNA Kit (Mo Bio Laboratories Inc, Solana Beach, CA, USA) was used for all DNA isolations. *S. aureus* DNA for the positive controls and the standard curve was extracted from dried cells; *S. aureus* strain Newman D₂C (Sigma, St Louis, MO, USA). *Escherichia coli* DNA was isolated from lyophilized cells (Sigma catalogue no. EC11303). The DNA extraction protocol took about 1.5 h.

Real-time polymerase chain reaction

QRT-PCR for the *femB* gene, a *S. aureus*-specific genomic marker [7], was performed on the ABI PRISM 7000

Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The *femB* primer/probe sequences are as follows: forward primer, 5′-AATTAACGAAATGGGCAGAACA-3′; reverse primer, 5′-TGCGCAACACCCTGAACTT-3′; and probe, FAM-AGAAATTAAGTGGATGGTACGCGCGAAGA.

A total volume of 25 µL was used in the PCR reaction: 2 µL of template plus 23 µL of master mix. The master mix consisted of 12.5 µL Taqman Universal Master Mix (Applied Biosystems), 7.5 µL molecular grade water, 1.0 µL forward primer and reverse primer, and 1.0 µL probe. The reaction took 2.5 h: 50.0 °C for 2 min, 95.0 °C for 10 min and 55 cycles at 95.0 °C for 15 s, and 60.0 °C for 1 min.

Dust samples and the *S. aureus* standard curve were run in duplicates. The standard curve consisted of 1 : 4 serial dilutions: 25, 6.25, 1.563, and 0.391 pg/µL. *E. coli* DNA served as a negative control. The lower limit of detection was 0.05 pg/µL.

Analyses

The slope of the curve used to calculate DNA concentration was generated by plotting the cycle threshold vs. log[concentration].

All statistical analysis was conducted using JMP, version 6.0.2 (Cary, NC, USA). Statistical differences between multiple groups were analysed by a one-way ANOVA, with Wilcoxon’s Rank Sum test for significance, and by Student’s *t*-test. The Spearman *r* test was used for correlations.

Results

Study participants had mild (*n* = 18), moderate (*n* = 14), severe (*n* = 15), or no AD (*n* = 15) (Table 1). Most participants with AD had asthma, pets, and carpeted bedrooms. EASI scores among AD severity categories differed significantly: mild (geometric mean, range: 1.23, 0.20–3.90), moderate (9.25, 6.00–14.10), and severe (26.78, 15.30–44.50) (*P* < 0.0001, ANOVA).

Dust samples taken from household vacuum bags yielded the largest quantity of dust (geometric mean: 561 mg) compared with the bedroom floor (299 mg) and bed (66 mg). As a result, more dust was used for the DNA extraction from the vacuum bag (median: 155.65 mg, range: 14.56–337.79) than from bedroom floor (99.90 mg, 7.36–251.44), and the bed (11.35 mg, 0.00–196.90) (*P* < 0.0001). Bed dust yielded the highest *S. aureus* concentrations (median: 1.51 pg/mg dust, range: 0.006–91.25) when compared with bedroom floor (0.69 pg/mg, 0.004–444.17) and vacuum filter bag (0.21 pg/mg, 0.00–57.59) (*P* = 0.007, ANOVA).

Table 1. Participant characteristics

Parameter	No AD	Mild AD	Moderate AD	Severe AD	P-value
Asthma (%)	38.5	38.9	71.4	60.0	0.1959*
Food allergy %	15.4	22.2	57.1	66.7	0.0081*
Drug allergy %	0.0	16.7	14.3	15.0	0.2680*
Rhinitis (%)					
Seasonal (%)	23.1	33.3	42.9	33.3	0.7562*
Chronic rhinitis (%)	0.0	11.1	7.1	40.0	0.0145*
Pets	42.9	66.7	64.3	46.7	0.4355*
Carpeting in bedroom	93.3	94.4	92.3	80.0	0.5018*
DNA extraction: bed sheets (mean)	51.9 mg	17.5 mg	71.4 mg	52.1 mg	0.4195 [†]
DNA extraction: floor (mean)	114.7 mg	101.0 mg	161.2 mg	122.8 mg	0.6162 [†]
DNA extraction: vacuum filter (mean)	194.0 mg	108.7 mg	219.7 mg	174.5 mg	0.1748 [†]
Bed sheets washed	9.9 days	8.8 days	8.6 days	12.4 days	0.4802 [†]

*Pearson.

[†]ANOVA.

AD, Atopic dermatitis.

Staphylococcus aureus deoxyribonucleic acid in house dust and atopic dermatitis severity

Bed dust

Participants with severe AD (geometric mean: 14.67 pg/mg dust) had significantly more *S. aureus* DNA in bed dust than those with moderate AD [0.41 pg/mg dust, $P < 0.0001$ (t -test)], mild AD (1.42 pg/mg dust, $P = 0.0051$), and no AD (0.09 pg/mg dust, $P < 0.0001$) (Fig. 1a). Participants with mild AD had significantly more *S. aureus* DNA than those with no AD ($P = 0.002$). However, there was no significant difference between *S. aureus* DNA levels from moderate vs. mild AD participants or moderate vs. no AD participants. Importantly, the concentration of *S. aureus* DNA in bedding dust strongly correlated with the EASI scores (Spearman's $r = 0.7263$; $P = 0.0004$).

Bedroom floor dust

The association of *S. aureus* DNA concentrations in floor dust samples with AD severity was similar to the findings with bedding dust. *S. aureus* DNA was significantly higher in severe AD (geometric mean: 10.39 pg/mg) compared with moderate [(0.14 pg/mg, $P < 0.0001$ (t -test))], mild (0.23 pg/mg, $P < 0.0001$), and no AD participants (0.09, $P < 0.0001$) (Fig. 1b). There were no significant differences among moderate, mild, and no AD *S. aureus* DNA levels. In the bedroom floor dust, *S. aureus* DNA levels positively correlated with EASI scores (Spearman's $r = 0.6846$; $P = 0.0002$).

Vacuum bag dust

The quantity of *S. aureus* DNA from the vacuum samples was significantly higher in participants with severe AD

(geometric mean: 2.13 pg/mg) vs. moderate AD [0.06 pg/mg, $P = 0.0012$ (t -test)], mild AD (0.07 pg/mg, $P = 0.0032$), and no AD (0.04 pg/mg, $P = 0.0005$) (Fig. 1c). The levels of *S. aureus* DNA did not differ significantly among participants with moderate, mild, and no AD. There was no significant correlation between EASI scores and concentrations of *S. aureus* DNA (Spearman's $r = 0.3783$; $P = 0.0684$).

Discussion

The skin of AD patients is vulnerable to *S. aureus* colonization because of altered epidermal barrier function, increased bacterial adhesion, and decreased innate immune responses [8, 9]. These susceptibilities include decreased levels of the antimicrobial lipid sphingosine in the stratum corneum [10]; cracking and drying of, and scratch injury to the skin that uncovers adhesins (e.g. fibronectin, fibrinogen) that enhance SA adherence [11]; impaired antimicrobial peptide responses to SA in lesional skin (e.g. human beta-defensin 2 and 3, cathelicidin LL-37) [12, 13]; and reduced dermcidin in sweat from AD skin [14]. Many of these impairments are caused or worsened by T helper-type 2 (Th2) immune responses and atopic inflammation. Skin barrier dysfunction also makes the skin of AD patients vulnerable to pro-inflammatory *S. aureus* products, such as superantigens and bacterial cell wall components (e.g. lipoteichoic acid and peptidoglycan) in the environment [15]. This can further impair AD immunity to *S. aureus*, creating a cycle of worsening AD skin conditions and increasing *S. aureus* burden with potential escalation to severe disease. However, there have been no previous studies examining the environmental burden of *S. aureus* in the homes of patients with AD.

Our current study took a *S. aureus*-specific QRT-PCR assay with a broad range of detection, and applied it to dust DNA extracts. It enabled the first quantitative assessment of *S. aureus* burden in homes and revealed that

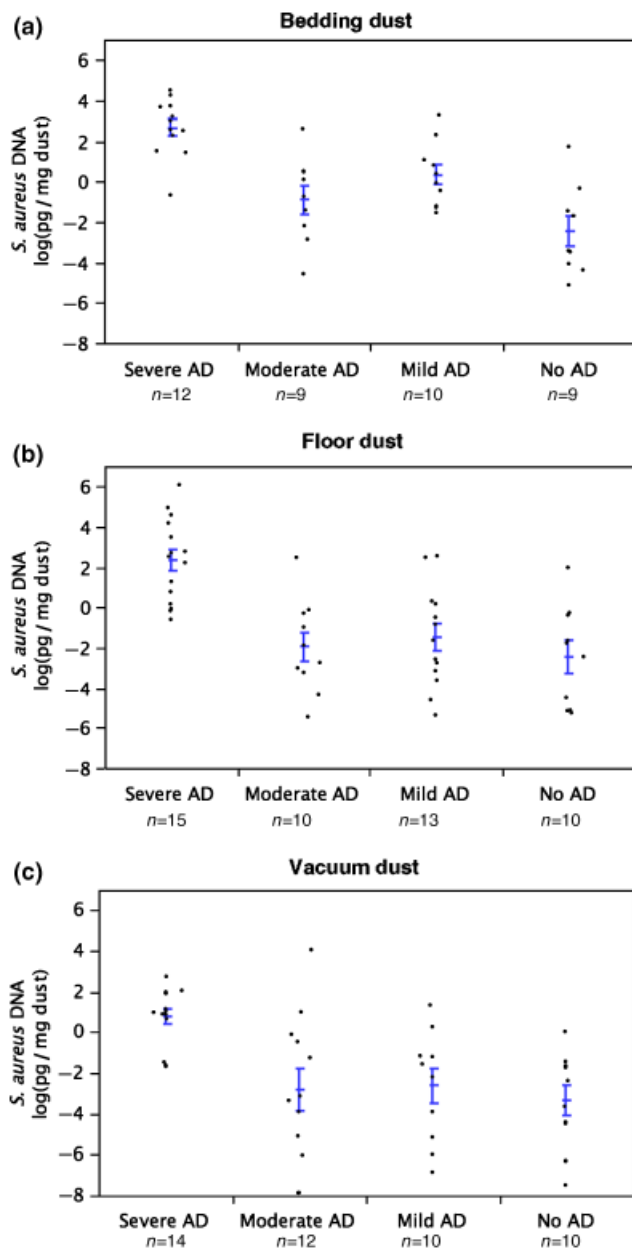


Fig. 1. *Staphylococcus aureus* DNA in bedding dust (a), floor dust (b), and vacuum filter bag dust (c). *S. aureus* DNA levels are significantly higher in participants with severe atopic dermatitis (AD). Each point represents an individual study participant. Mean and standard error lines are represented for each subgroup.

S. aureus DNA is ubiquitous – being detectable in dust samples from almost all homes, regardless of disease state. Importantly, house dust from participants with severe AD contained the most *S. aureus* DNA in their home environment. Moreover, the correlation between *S. aureus* DNA levels and AD severity is driven by proximity to the patient as demonstrated by the fact that AD patient's bed and bedroom floors yielded the highest levels of *S. aureus* DNA.

Quantifying *S. aureus* DNA has the advantage of establishing total bacterial load because both live and dead organisms may trigger skin inflammation. Many current management recommendations that involve bathing, restoring skin barrier function, covering the skin with wraps, and frequent washing of bedding may work, in part, by separating the skin of the AD patient from the environment, reducing exposure to environmental *S. aureus* and its by-products. If live organisms persist in house dust, then this may be a source for re-colonization of patient's skin and potentially infect family members. This is particularly concerning when AD patients harbour methicillin-resistant *S. aureus* (MRSA), as case reports have documented family members catching MRSA *S. aureus* infections from AD patients (personal observations and Suh *et al.* [16]). Future studies are needed to determine whether *S. aureus* load on the skin correlates with home environmental *S. aureus* burden, and if interventions that reduce environmental exposure to *S. aureus* products can reduce skin inflammation and improve disease outcomes.

In conclusion, we have observed a strong association between home environmental *S. aureus* burden and severe AD. The study design is inadequate to determine cause or effect. The preponderance of evidence suggests that although *S. aureus* exacerbates disease, it is not responsible for the underlying defects in AD skin. For example, participants with high *S. aureus* burden did not report that all family members had AD. However, a high environmental *S. aureus* burden could cause severe disease in susceptible hosts, both inflammatory and infectious.

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