Microbiome of Affected and Unaffected Skin of Patients With Atopic Dermatitis Before and After Emollient Treatment

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that results in areas of dry, itchy skin. Several cultivation-dependent and –independent studies have identified changes in the composition of microbial communities in these affected areas over time and when compared to healthy control individuals. However, how these communities vary on affected and unaffected skin of the same individual, and how these communities respond to emollient treatment, remains poorly understood. Here we characterized the microbial communities associated with affected and unaffected skin of 49 patients with AD before and after emollient treatment using high-throughput sequencing of the 16S rRNA gene. We found that microbial diversity and community composition was different between affected and unaffected skin of AD patients prior to treatment. Differences were driven primarily by the overabundance of *Staphylococcus* species on affected skin and a corresponding decrease in bacterial diversity. After 84-days of emollient treatment, the clinical symptoms of AD improved in 72% of the study population. Microbial communities associated with affected skin of these treatment responders more closely resembled unaffected skin after treatment as indicated by increased overall diversity and a decrease in the abundance of *Staphylococcus* species. Interestingly, *Stenotrophomonas* species were significantly more abundant in the communities of 'responders', suggesting a possible role in restoration of the skin microbiome in patients with AD. We demonstrated that the comparison of affected and unaffected skin from the same individual provides deeper insight into the bacterial communities involved in the skin dysbiosis associated with AD. These data support the importance of emollients in the management of AD although future studies should explore how emollients and other treatments help to restore skin dysbioses.

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INTRODUCTION

Human skin hosts complex microbial communities whose diversity and composition vary by skin region[1] and between individuals.[2,3] Compositional differences between skin regions arise largely from contrasting environmental conditions of skin sites.[4] Inter-individual differences in microbiome composition have been attributed to a number of factors including host demographics, host genetics, and host behavior.[5] For example, the diversity of palm bacterial communities differs between genders.[6] These inter- and intra-individual differences in skin bacterial communities may contribute to differences in disease susceptibility and quantifying such differences may aid in efforts to monitor changes in skin health status.[7,8]

Atopic dermatitis (AD) is a multifactorial, chronic inflammatory skin disorder with several genetic risk factors and environmental triggers.[9,10] One of the hallmark symptoms of AD is dry skin (xerosis), which affects not only lesional (affected) skin, but also non-lesional (unaffected) skin.[11] Xerosis is linked to skin barrier dysfunction and is usually accompanied by pruritus (itching), which may favor the penetration of allergens, bacteria, and/or viruses.[12] Indeed, AD patients experience a higher frequency of bacterial skin infections with *Staphylococcus aureus* being the most commonly cultured organism.[12,13,14]

A recent cultivation-independent study confirmed the association of *S. aureus* with AD lesions but also revealed dramatic, community-level changes within patients over time, with treatment, and when compared to healthy individuals.[15] For example, disease exacerbations were associated with a decrease in microbial diversity on lesional skin due to *Staphylococcus* blooms, with this genus accounting for up to 90% of the bacteria detected. In contrast, another study using both cultivation-dependent and cultivation-independent techniques found a gammaproteobacterial species, *Stenotrophomonas maltophilia*, to be significantly more abundant on AD patients than on other healthy individuals.[16] Given these conflicting results and that each individual

hosts a unique skin microbiome, comparisons between diseased and healthy individuals may make it difficult to identify the specific bacterial taxa associated with AD.

To broaden our understanding of the relationship between the skin microbiome and AD, we characterized the microbial communities of paired skin samples from 49 AD patients. Paired samples consisted of one skin sample from an AD lesion (affected) and a second sample from an adjacent non-lesional (unaffected) region. As proper emollient use is an integral component of any treatment plan for AD,[17] we also wanted to evaluate the impact of emollient use on the skin microbiome of the same, paired skin samples. Post treatment samples were collected after 84 days of twice daily application of an emollient containing Shea butter, thermal spring water, and niacinamide. High-throughput sequencing of a portion of the 16S rRNA gene was used to quantitatively assess how the diversity and composition of the bacterial communities differ between affected and unaffected skin regions before and after treatment with an emollient.

MATERIALS AND METHODS

Ethics statement

This study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the DOST ethical committee, and conducted according to ICH guidelines for Good Clinical Practice. Written informed consent and photography consent were obtained from each subject before enrollment.

Emollient

The emollient used in this study was a lipophilic cream containing 20% Shea butter,[18,19] 4% niacinamide[20,21] and La Roche-Posay thermal spring water (LRP-TSW)[22] (Lipikar Balm AP, La Roche-Posay Pharmaceutical Laboratories, France). Patients were instructed to apply the emollient twice daily, once in the morning and once in the evening to their entire body. Patients were also instructed not to change their hygiene practices or to apply any other emollient (or any drugs including corticotherapy or antibiotherapy) during the study.

"These findings suggest that other *Staphylococcus* species and not just S. aureus are associated with the pathology of AD."

Patient recruitment and sampling

This single center study included 49 patients (17 male and 32 female; aged 3 to 39 years) suffering from moderate AD (Table 4). Clinical assessment of disease severity and microbiome sampling was conducted on two separate visits: on August 21 or 22 (day 0) and November 14 or 15, 2012 (day 84). At the inclusion visit (day 0) and at study end (day 84), the same investigating dermatologist evaluated severity with the SCORAD (SCORing Atopic Dermatitis) index[23] and clinical signs of erythema, dryness, and desquamation of one or more typical lesional (affected) skin areas and a proximal non-lesional (unaffected) site (scored as absent=0, light=1, moderate=2 or severe=3). Each area was identified and photographed to ensure the same area was sampled on day 84. Only individuals with SCORAD values between 25 and 40 at day 0 were included in the study.

Skin microbiome samples were collected using aseptic techniques under sterile airflow generated by a portable hood. Briefly, single use sterile cotton-tipped swabs (COPAN Ref. 165KS01) were pre-moistened with a sterile solution of deionized water containing 0.15 M NaCl and 0.1% Tween 20. Swabs were rubbed firmly for 20 seconds over 1cm^2 areas identified as being the most representative of affected skin. Similarly, samples were also collected from the closest unaffected skin area. The cotton tip samples were stored at -80°C until being shipped on dry ice to the University of Colorado for processing.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from each swab using the MoBio PowerSoil DNA Isolation kit following the manufacturer's instructions with slight modifications as detailed in Fierer et al.[5] PCR amplification of the V1-V2 region of 16S rRNA gene was performed using the primer set (27F/338R), PCR mixture conditions, and thermal cycling steps described in Fierer et al.[3] PCR amplicons of triplicate reactions for each sample were pooled at approximately equal amounts and sequenced on a 454 Life Sciences Genome Sequencer FLXTitanium instrument (Roche) at the University of South Carolina's Environmental Genomics Core Facility.

Sequence analyses

All sequences were processed, sorted by barcodes, and clustered following the standard QIIME pipeline.[24] High-quality sequences (defined as those sequences >200bp in length with a quality score >25, no barcode errors, and no ambiguous characters) were trimmed to 300 bp in length and clustered into operational taxonomic units (OTUs) using an open reference-based approach that implements reference-based clustering followed by *de novo* clustering using the UCLUST algorithm.[25] Clustering was conducted at a 97% similarity level using a pre-clustered version of the October 2012 GreenGenes database.[26] Sequences were assigned to taxonomic groups using the RDP classifier.[27] A total of 643,038 high-quality partial 16S rRNA sequences were obtained from the 226 samples collected, with an average of 2,845 sequences per sample. All samples were subsequently rarefied to 952 sequences per sample resulting in a loss of 13 of the original 226 samples. This rarefaction step allows all samples to be compared at an equivalent sequencing depth.

Inclusion criteria

Only 46 of the patients could be evaluated at day 84 because three individuals were unable to come to the clinic for the last visit. Seven of the remaining 46 individuals were sampled on multiple lesions (and adjacent non-lesional skin) at each time point for a total of 53-paired samples per time point. After quality filtering of sequences (n=643,038), rarefaction (952 sequences/sample), and filtering out individuals that did not have paired samples from both time points, 36 individuals with 41-paired samples remained (Table 1). All subsequent analysis was conducted on these 41-paired samples.

Statistical analysis of sequence data

To determine if diversity varied between affected and unaffected skin sites, we calculated the Shannon Index for each sample and tested for differences between paired samples (pre-treatment affected vs unaffected; post-treatment affected vs unaffected) using the Wilcoxon rank sum test for paired samples as implemented in R.[28] Spearman rank correlation was used to assess the relationship between the Shannon Index and the relative abundance of *Staphylococcus* species. We used PRIMER v.6[29] to calculate pair-wise differences in community composition (Bray-Curtis distances) on normalized, square root transformed OTU abundances, to assess the effects of individual (random variable) and health status (fixed variable) on bacterial community composition using permutational multivariate analysis of variance (PERMANOVA), to compare community composition after treatment using analysis of similarity (ANOSIM), and to generate vector plots overlaid on PCoA ordinations using multiple partial correlations between taxa abundances (those with median relative abundances greater that 1.0% in any group) and PCoA axes. Differences in the relative abundance of specific taxa between affected and unaffected skin prior to treatment and between 'responders' and 'non-responders' after treatment were assessed using multiple Wilcoxon rank sum tests for paired or unpaired samples and applying the Bonferroni correction to p-values to account for the multiple comparisons as implemented in R Developmental Core Team.[28]

RESULTS AND DISCUSSION

Microbiome of affected and unaffected skin pre-application of emollient

We first wanted to determine if microbial diversity of affected skin was different than adjacent unaffected skin for each individual prior to treatment. Using the Shannon Index as our metric of diversity, we found that unaffected skin sites were more diverse than adjacent affected skin for 28 of 41-paired samples (Table 1) with an overall significant difference across study participants (median affected = 5.93; median unaffected = 6.32; P= 0.002). This finding is consistent with previous studies indicating that AD flares were associated with a decrease in the overall diversity of skin microbial communities.[16]

TABLE 1.

Microbial diversity of skin samples associated with unaffected (U) and affected (A) skin of patients with atopic dermatitis before and after treatment with an emollient. Success of treatment was determined as a change in SCORAD (**Δ**) after treatment. Individuals with negative values had a decrease in SCORAD after treatment and are grouped as 'responders' (italics) whereas individuals with positive values (or no change) are grouped as 'non-responders.'

	Pre-treatment		Post-treatment		
ID	Shannon – U	Shannon – A	Shannon – U	Shannon – A	Δ SCORAD
AD1	*5.57*	*2.12*	*4.80*	*5.29*	*-26.6*
AD2	*6.70*	*6.76*	*7.35*	*7.58*	*-28.2*
AD3	*6.74*	*6.84*	*6.61*	*5.79*	*-20.5*
AD4	6.70	6.37	6.62	6.60	8.2
AD5	2.23	5.32	5.67	5.70	0.0
AD6	*6.35*	*6.08*	*6.78*	*4.97*	*-13.0*
AD7	4.95	4.09	3.53	1.43	0.2
AD8	*5.98*	*3.23*	*6.15*	*5.57*	*-2.0*
AD9-B	7.49	7.05	6.82	2.38	0.6
AD10	*7.28*	*4.90*	*6.71*	*3.48*	*-15.7*
AD13-E	*2.26*	*2.40*	*6.32*	*6.51*	*-25.3*
AD13-S	*3.06*	*2.12*	*5.63*	*5.86*	*-25.3*
AD14	*7.27*	*6.32*	*6.45*	*5.36*	*-19.4*
AD15	*5.42*	*4.01*	*6.44*	*6.08*	*-35.1*
AD16	*6.72*	*6.72*	*6.94*	*6.74*	*-7.8*
AD17	*6.32*	*6.29*	*7.13*	*6.44*	*-29.3*
AD18-L	*6.30*	*6.00*	*5.41*	*4.85*	*-0.8*
AD18-B	*4.68*	*5.07*	*4.39*	*4.42*	*-0.8*
AD22	*5.82*	*6.64*	*3.88*	*4.33*	*-27.5*
AD23	6.85	6.60	4.98	5.03	1.4
AD24-S	*6.61*	*6.14*	*4.85*	*6.02*	*-9.9*
AD24-T	*5.95*	*5.83*	*5.23*	*5.31*	*-9.9*
AD26-C	*7.01*	*7.09*	*7.64*	*7.44*	*-12.9*
AD26-K	*7.65*	*7.87*	*6.53*	*7.06*	*-12.9*
AD27	5.03	4.70	5.22	4.10	6.0
AD28	7.32	7.00	7.50	7.57	10.7
AD30	*7.14*	*5.68*	*6.86*	*6.04*	*-5.0*
AD32	*5.30*	*4.97*	*5.53*	*5.31*	*-32.2*
AD34	5.57	6.73	4.00	3.59	1.0
AD35	*5.51*	*5.94*	*5.13*	*5.69*	*-21.9*
AD37	*6.13*	*5.93*	*6.58*	*6.76*	*-26.4*
AD39	*7.22*	*6.53*	*4.58*	*3.33*	*-16.8*
AD40-W	6.77	3.20	4.74	6.62	8.1
AD40-A	6.72	4.60	4.41	0.97	8.1
AD41-E	4.20	2.62	2.66	1.96	7.0
AD43	*5.49*	*5.71*	*5.93*	*6.09*	*-34.0*
AD46	*6.27*	*6.59*	*7.16*	*6.90*	*-11.9*
AD47	*6.34*	*5.16*	*5.54*	*4.50*	*-2.4*
AD48	*5.20*	*1.21*	*6.63*	*6.92*	*-30.5*
AD49	*7.15*	*4.87*	*6.12*	*5.89*	*-14.0*
AD50	*7.64*	*7.85*	*5.66*	*5.45*	*-21.3*

TABLE 2.

Differences in overall bacterial community structure of affected and unaffected skin sites of each individual (n=41) were assessed using permutational multivariate analysis of variance test (PERMANOVA) with health status as a fixed factor and individual as a random factor.

Factor	Pseudo-F	*P*	Component of Variation
Individual	2.51	0.001	1415.90
Health status	1.61	0.002	28.00

To determine if the structure of the communities was also different, we constructed a Bray-Curtis similarity matrix and compared the values both within and between individuals using PERMANOVA with individual as a random factor and health status (affected or unaffected) as a fixed factor. As expected, personal variation explained the greatest amount of variation (Table 2). However, health status also explained a significant portion of the variation, meaning that the structure of microbial communities associated with affected skin were different than those associated with unaffected skin prior to application of the emollient.

Since both diversity and structure of these communities were significantly different between affected and unaffected skin sites, we wanted to determine which organisms were driving these differences. On average, *Staphylococcus* was the most abundant genus on both affected and unaffected skin. However, affected skin harbored a greater relative abundance of *Staphylococcus* (Figure 1). The abundance of *Staphylococcus* was inversely related to the Shannon Index; as the abundance of *Staphylococcus* increased, diversity decreased (Figure 2). Interestingly, when we looked at species within the *Staphylococcus*, we saw that *S. epidermidis*, *S. aureus*, and *S. haemolyticus* were all more abundant on affected skin than unaffected skin (Figure 1, inset). However, after correcting for multiple comparisons, only *S. epidermidis* was significantly more abundant on affected skin ($P < 0.01$). These findings suggest that other *Staphylococcus* species and not just *S. aureus* are associated with the pathology of AD. With many host factors implicated in the onset of AD, including filaggrin mutations, receptors and signaling molecule mutations and decreased expression or function of antimicrobial peptides, it remains unclear whether these changes trigger alterations in microbial diversity or if domination of *Staphylococcus* species occurs first and subsequently drives disease progression.[30] No other genera were significantly more abundant on affected or unaffected skin prior to treatment after correcting for multiple comparisons.

Clinical symptoms of AD post-treatment

To first assess the efficacy of the emollient, we compared SCORAD values post-treatment to pre-treatment values (Table 1). SCORAD values decreased for 26 of the 36 individuals (30 paired samples) after treatment, meaning that diseases symptoms improved for 72% of the study population. For the other ten individuals (11 paired samples), SCORAD remained the same or increased after 84 days of treatment. There was no correlation between change in SCORAD and age (rho=0.26; *P*=0.08) or with duration of the disease (rho=0.29; *P*=0.06). A significant reduction of erythema, dryness and desquamation was noted on affected skin areas sampled from an average global score of

FIGURE 1. Average taxonomic composition of the skin microbiome associated with atopic dermatitis prior to treatment with an emollient. Grey bars are taxa associated with affected skin while white bars are from unaffected skin. Asterisks denote statistical differences between groups ($P < 0.01$, Bonferroni corrected) based on Wilcoxon rank sum test for paired samples. Inset shows species of *Staphylococcus*. Error bars are ± one SEM.

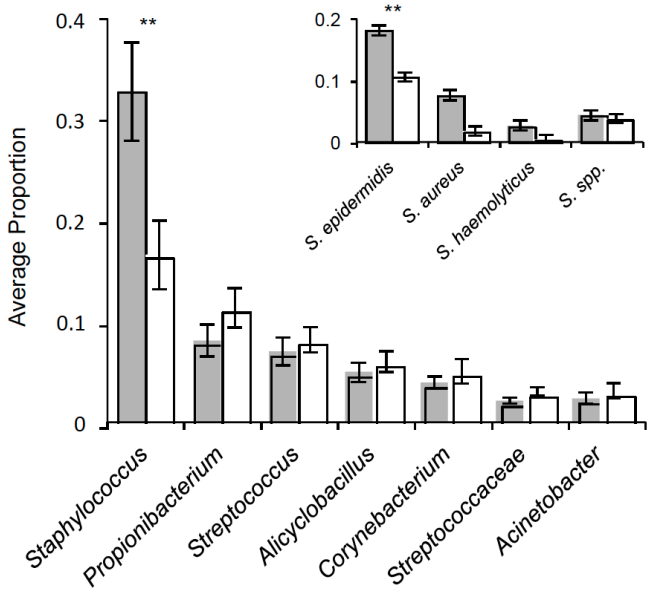
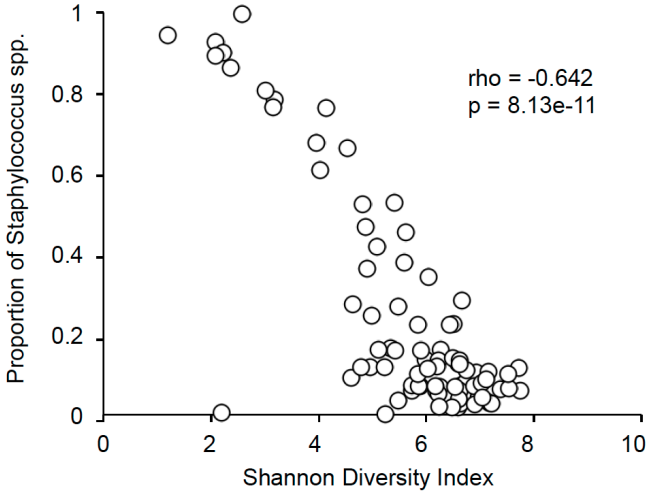


FIGURE 2. Relationship between diversity of microbial communities and abundance of *Staphylococcus* species associated with the skin of patients with atopic dermatitis. Results of Spearman rank correlations are presented in top right of figure.



4 ± 1 at day 0 to 2 ± 2 at day 84 (Table 4; $P<0.0001$ versus day 0). Whether the change in disease symptoms between day 0 and day 84 was a direct result of treatment with the emollient or part of the normal cyclic progression of AD is unknown. However, since all individuals received the same treatment, we could investigate if there were any differences in skin bacterial communities at day 0 between those individuals that responded positively to the emollient treatment versus those individuals that saw no improvement in their AD symptoms over the 84-day treatment period. But simply, we wanted to know if there was any microbial community response associated with health responses upon emollient treatment.

Microbiome of affected and unaffected skin post-application of emollient

Having established that the diversity and composition of the microbiome associated with affected and unaffected skin were different prior to treatment and clinical signs of AD improved for the majority of the study population after treatment, we next wanted to see if (and how) application of an emollient changed the composition of the skin microbiome associated with AD patients. For these analyses, we divided the study population into 'non-responders' and 'responders' based on the change in SCORAD between the two clinical visits (Table 1). Patients who had no change or an increase in SCORAD at the second visit were classified as 'non-responders,' whereas individuals with a decrease in SCORAD were 'responders.'

Because the skin microbiome is known to change over time even in healthy individuals,[2,8,16] we focused our analysis on comparing affected and unaffected skin samples collected after 84 days of emollient application. For both 'responders' and 'non-responders', we did not observe differences in diversity levels between affected and unaffected skin after treatment (responders, $P = 0.096$; non-responders, $P = 0.2061$). However, microbial diversity of skin associated with 'responders' was higher than for 'non-responders' (median Shannon responders = 5.98; median Shannon non-responders = 4.64; $P = 0.01$) regardless of skin health at day 84. These observations suggest that the diversity of microbial communities associated with affected skin from individuals that responded to treatment converged to the higher levels of unaffected skin over the 84-day period. In contrast, for 'non-responders', the diversity of unaffected skin dropped over the 84-day period and resembled the lower diversity levels typically observed on affected skin.

Similar patterns were observed when we compared overall community composition using Bray-Curtis similarities; there was no difference in community composition between affected and unaffected skin for either 'responders' or 'non-responders' (Table 3). However, the communities associated with affected skin of responders converged in composition to resemble unaffected skin communities. We tested and visualized these patterns using ANOSIM and PCoA, respectively (Figure 3). Figure 3 clearly shows separation between the two groups regardless of health status. To determine what was driving this separation, we correlated taxa abundances with PCoA axes and overlaid the vectors (Figure 3, Table 5). The strongest drivers of the separation between 'responders' and 'non-responders' appeared to be the relative abundances of five bacterial genera: *Staphylococcus*, *Streptococcus*, *Alicyclobacillus*, *Stenotrophomonas*, and *Propionibacterium*.

TABLE 3.

Differences in overall bacterial community structure of affected and unaffected skin sites after treatment with an emollient were assessed using permutational multivariate analysis of variance test (PERMANOVA) with health status as a fixed factor and individual as a random factor

	Factor	Pseudo-F	*P*	Component of Variation
Responders	Individual	2.74	0.001	1408.80
	Health status	1.23	0.14	12.38
Non-responders	Individual	3.05	0.001	1598.10
	Health status	1.58	0.10	81.54

FIGURE 3. Ordination plot of microbial communities associated with the skin of patients suffering from atopic dermatitis after 84 days of emollient use. Open squares are samples from individuals that responded positively to treatment (decrease in SCORAD). Closed circles are non-responders. Results of the ANOSIM testing for differences between responders and non-responders are presented in the bottom right of the plot. L and NL denote lesional and non-lesional samples, respectively. Vectors of multiple partial correlations between abundant taxa and plot axes are overlaid on ordination. Only correlations with coefficients greater than 0.3 are shown. For values of these and other taxa, refer to Table 5. Taxa abbreviations are as follows; Alicyc. = *Alicyclobacillus*, Prop. = *Propionibacterium*, Staph. = *Staphylococcus*; Steno. = *Stenotrophomonas*; Strep. = *Streptococcus*.

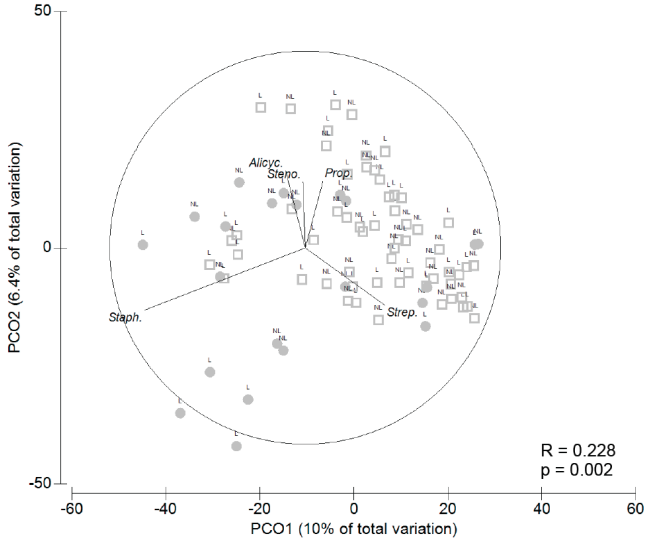


TABLE 4.

Summary of patient demographics and clinical assessment of disease severity. Values of Score A are the sum values (0-9) of clinical signs of erythema, dryness, and desquamation (scale: Absence=0, Mild=1, Moderate=2, Severe=3) of each sampled lesional (affected) skin area.

Subject	Sample ID	Body Site sampled	Gender	Age (years)	SCORAD D0	SCORAD D84	Score A Area D0	Score A Area D84
1	AD1	Elbow	Female	5	26.6	0.0	3	0
2	AD2	Palm	Female	6	36.6	8.4	5	3
3	AD3	Elbow	Female	3	33.6	13.1	5	3
4	AD4	Forearm	Female	22	39.7	47.9	4	0
5	AD5	Cheek	Female	3	27.2	27.2	3	3
6	AD6	Forearm	Female	7	28.3	15.3	3	0
7	AD7	Hand	Female	8	39.7	39.9	4	5
8	AD8	Finger	Male	20	32.8	30.8	4	4
9	AD9-B	Buttock	Female	3	39.2	39.8	4	4
10	AD10	Finger	Female	19	39.1	23.4	5	3
13	AD13-E	Elbow	Male	14	33.1	7.8	4	0
13	AD13-S	Shoulder	Male	14	33.1	7.8	4	2
14	AD14	Elbow	Male	5	25.1	5.7	4	0
15	AD15	Finger	Female	17	39.8	4.7	6	1
16	AD16	Wrist	Female	4	38.5	30.7	5	3
17	AD17	Finger	Female	24	38.6	9.3	6	0
18	AD18-B	Back	Female	21	25.3	24.5	3	0
18	AD18-L	Leg	Female	21	25.3	24.5	2	0
22	AD22	Arm	Female	3	33.6	6.1	4	0
23	AD23	Calf	Female	3	31.7	33.1	4	0
24	AD24-S	Shoulder	Male	13	40.0	30.1	2	1
24	AD24-T	Thigh	Male	13	40.0	30.1	4	4
26	AD26-C	Cheek	Male	9	26.6	13.7	2	2
26	AD26-K	Back of knee	Male	9	26.6	13.7	2	1
27	AD27	Armpit	Female	38	26.0	32.0	4	1
28	AD28	Wrist	Female	6	39.5	50.2	5	5
30	AD30	Sole of foot	Female	4	39.2	34.2	6	3
32	AD32	Back of knee	Female	15	32.2	0.0	4	0
34	AD34	Elbow	Male	11	39.3	40.3	5	3
35	AD35	Thigh	Male	3	26.2	4.3	3	1
37	AD37	Elbow	Male	7	31.7	5.3	4	1
39	AD39	Heel of foot	Female	12	26.6	9.8	3	2
40	AD40-W	Wrist	Male	19	39.9	48.0	6	3
40	AD40-A	Ankle	Male	19	39.9	48.0	6	7
41	AD41-E	Elbow	Female	16	39.1	46.1	6	3
43	AD43	Top of foot	Male	12	37.7	3.7	5	1
46	AD46	Wrist	Male	9	31.8	19.9	4	4
47	AD47	Armpit	Male	7	30.7	28.3	4	3
48	AD48	Palm	Female	8	38.9	8.4	6	3
49	AD49	Back of knee	Male	4	39.9	25.9	5	4
50	AD50	Knee	Male	7	27.0	5.7	3	2

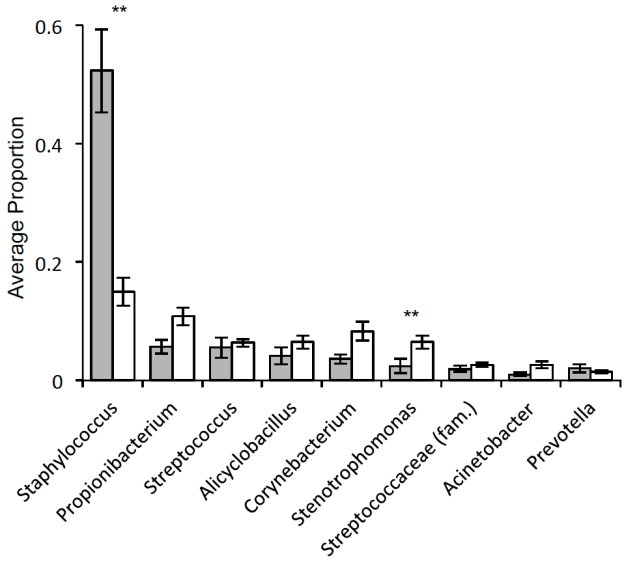
To investigate the relationship between taxon abundances and responder groups in more detail, we determined the average abundances of the most abundant taxa (those with median relative abundances greater that 1.0% in any group) and tested for differences between the two groups using multiple Wilcoxon rank sum tests for unpaired samples and applying the Bonferroni correction to p-values to account for the multiple comparisons (Figure 4). Affected and unaffected skin of individuals that did not respond to treatment was dominated by *Staphylococcus* species. Interestingly, although the abundances of *Streptococcus*, *Alicy-*

TABLE 5.

Multiple partial correlation coefficients produced by PRIMER v.6 for the nine most abundant taxa observed on skin of patients with atopic dermatitis. Values indicate how well each taxa correlates with each of principal coordinate axes 1 and 2.

Taxa	PCoA1	PCoA2
Staphylococcus	-0.823	0.317
Propionibacterium	0.091	-0.341
Streptococcus	0.407	0.292
Alicyclobacillus	-0.101	-0.395
Corynebacterium	-0.154	-0.021
Stenotrophomonas	-0.012	-0.337
Streptococcaceae (fam.)	0.173	0.185
Acinetobacter	-0.088	0.026
Prevotella	0.006	-0.145

FIGURE 4. Average taxonomic composition of skin microbial communities associated with affected and unaffected skin of patients with atopic dermatitis after emollient treatment. Grey bars are taxa associated with individuals that did not respond to treatment while white bars are from treatment responders. Asterisks denote statistical differences between groups ($P < 0.01$, Bonferroni corrected) based on Wilcoxon rank sum test for unpaired samples. Error bars are ± one SEM.



clopbacillus, and *Propionibacterium* were significantly correlated with axis 1 of the PCoA (Figure 3), *Stenotrophomonas* (belonging to the Xanthomonadaceae family) was the only bacterial genus significantly more abundant in the communities of 'responders'. Although the exact role of this genus in the pathology of AD is unknown, species within the *Stenotrophomonas* have previously been associated with AD patients[16] and some are considered emerging opportunistic pathogens due to multi-drug resistance.[31] It is important to note that *Stenotrophomans* was not detected in the emollient (data not shown).

CONCLUSION

In this study, we demonstrated how comparisons of affected and unaffected adjacent skin from the same AD patient provides deeper insights into bacterial communities involved in skin dysbiosis. We found that affected skin of patients with AD hosts less diverse microbial communities than unaffected skin of the same individual. These lesional communities were dominated by *Staphylococcus* species when compared to adjacent, non-lesional skin. Twice-daily application of an emollient containing Shea butter, thermal spring water, and niacinamide improved AD symptoms for over 70% of the patients with a concurrent increase of bacterial diversity and decrease in the abundance of *Staphylococcus* on affected skin. The mechanism by which the emollient improved skin health is unclear, but these data support the importance of emollient use in the management of AD. Future studies should focus on identifying the mechanism by which emollient use helps to restore the skin microbiome and investigate the role of *Stenotrophomonas* in the skin microbiome.

DISCLOSURES

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AUTHOR CONTRIBUTIONS

Conceived and designed the study: SS, RM, LA. Performed the study: HZ, RM, SS, JBH. Analyzed the data: SS, RM, GEF, JBH, NF. Wrote the paper: GEF, SS, RM, NF.

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