MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.10.386.393

Dysbiosis of Skin Microbiota with Increased Fungal Diversity is Associated with Severity of Disease in Atopic Dermatitis

[1]Dhwani Alok Saxena, [2]Rahul Balmiki and [3]Pawan Singh

[1]*Department of Dermatology Rama Medical College and Hospital, Kanpur, Uttar Pradesh, India*

[2,3]*Department of Dermatology Government Medical College, Kannauj, Uttar Pradesh, India*

ABSTRACT

The Aim of the study is to Describe the Dysbiosis of Skin Microbiota And Diversity of Increased Fungal Infection Associated with Severity of Disease In Atopic Dermatitis. Using shotgun metagenomics we characterized microbial compositions from 14 well defined skin sites from 10 patients with Atopic Dermatitis and 5 healthy controls. We found clear differences in microbial composition between AD and controls at multiple skin sites, most pronounced on the flexures and neck. The flexures exhibited lower alpha-diversity and were colonized by S. aureus, accompanied by S. epidermidis in lesions. Malassezia species were absent on the neck in AD. In lesional samples, both the genus Staphylococcus and Staphylococcus phages were more abundant. S. aureus abundance was higher across all skin sites except from the feet. In samples where S. aureus was highly abundant, lower abundances of S. hominis and Cutibacterium acnes were observed. M. osloensis and M. luteus were more abundant in AD. By single nucleotide variant analysis of S. aureus we found strains to be subject specific. On skin sites some S. aureus strains were similar and some dissimilar to the ones in the nares. Similar to previous studies, we found an increased fungal diversity in AD patients. Interestingly, it was only increased in patients with severe AD and not in mild-to-moderate cases. We were unable, however, to observe the previously described decrease of bacterial diversity in severe AD. The bacterial diversity was comparable in all three study groups and was even slightly elevated in mild-to-moderate AD. An explanation for this discrepancy might be that we analysed a-diversities at the class and genus rather than at the species or the amplicon sequence variants (ASVs) level because the V1-3 region of the 16S rRNA gene operon that we used for analyses does not allow proper resolution at the species level or beyond. In Our Study a global and site-specific dysbiosis in AD, involving both bacteria, fungus. When defining targeted treatment should both consider the individual and skin site. we found an increased fungal diversity in AD patients. Interestingly, it was only increased in patients with severe AD and not in mild-moderate cases. We were unable, however, to observe the previously described decrease of bacterial diversity in severe AD.

OPEN ACCESS

Key Words

Microbiota, characterized, dermatitis, microbial

Corresponding Author

Pawan Singh,
Department of Dermatology Government Medical College, Kannauj, Uttar Pradesh, India
pawan1839@gmail.com

Author Designation

[1,2,3]Assistant Professor

Received: 20 August 2024
Accepted: 26 September 2024
Published: 28 September 2024

Citation: Dhwani Alok Saxena, Rahul Balmiki and Pawan Singh, 2024. Dysbiosis of Skin Microbiota with Increased Fungal Diversity is Associated with Severity of Disease in Atopic Dermatitis. Res. J. Med. Sci., 18: 386-393, doi: 10.36478/makrjms.2024.10.386.393

Copy Right: MAK HILL Publications

INTRODUCTION

Dysbiosis is a hallmark of atopic dermatitis (AD). The human skin is colonizedby a variety of microorganism, interacting with the host and modulating immunity. On healthy human skin, the most abundant bacterial genera are Cutibacterium, Staphylococcus and Corynebacterium with marked to pographical diversity[1]. In the common skin disease atopic dermatitis (AD), Staphylococcus aureus expand and conventional culture-based studies find colonizing frequencies of 70% of lesional and 39% of non-lesional sites, and 62% of the nares samples. S.aureus colonization adversely affect disease severity[2].

Atopic dermatitis is a chronic and relapsing inflammatory skin disease characterized by eczematous lesions, pruritus and skin dryness, with high prevalence worldwide .It is commonly associated with allergic conditions such as allergic rhinitis and Asthma. Epidermal barrier impairment in AD-resulting from null mutations in the FLG gene in about 30% of cases that give rise to ichthyosis vulgaris (IV)-precedes immune hyper responsiveness . In non lesional AD, a T helper type (Th) 2 immune response predominates, whereas in lesional AD a mixed Th1/Th2/Th17/Th22/Th9 immune response has been described. (e.g., erythema, high serum IgE and eosinophilia)[3]. The role of fungal communities in AD remains poorly investigated. The importance of fungal skin microbiota, the mycobiota, is highlighted by the fact that AD patients are frequently sensitized to Malassezia spp., the most abundant fungus on human skin[16]. In contrast to bacteria, fungal diversity appears to increase in AD patients[17-18]. To date, no next-generation sequencing (NGS) studies investigating the mycobiota in patients with different severities of AD have been conducted[4].

AD skin lesions exhibit reduced alpha-diversity of the bacterial skin community owing to enrichment of Staphylococcus, which are suspected to contribute to disease aggravation and flares. In FLG-deficient epidermis, regardless of the presence of AD, a low frequency of proteolytic Gram-positive anaerobic cocci, together with under representation of bacterial taxa that utilize histidine as a substrate, has been observed. Although individuals with IV are prone to develop skin fungal infection, very little is known about the composition of their fungal microbiota[5]. Moreover, most studies have been carried out in patients with severe AD., thus, little is known in patients with milder disease. We analyzed the skin bacterial and fungal composition of patients with low to mild AD with or without FLG mutations (in both non lesional and lesional skin), IV, at two different body sites with the goal of evaluating the effects of specific skin disease features, such as ichthyotic and/or atopic manifestations, on the bacterial and fungal microbiota[6]. The host barrier and immune systems have evolved hand-in-hand with the microbiota, forming mutually beneficial relationships. While epithelia and immune cells form barriers that protect the body from microbial invasion, they also create a surface environment that allows for the stable colonization by commensal microbes. Recent emerging evidence has revealed crucial roles of skin commensals in priming and harnessing local immunity. Altered host-microbe cross-talk leads to immune dysregulation and is believed to be a driving force in inflammatory skin diseases[7-8].

In recent years, skin microbiomes in AD have been studied in a variety of conditions. Most studies are based on sequencing the 16S rRNA gene of bacteria. Applying this method, bacterial diversity has been shown to be lower in AD and S. epidermidis abundant. Therapy increases diversity and the abundances of Streptococcus, Cutibacterium and Corynebacterium[9]. There is growing evidence of a key role of the microbiome in the pathogenesis of AD. This is supported by studies showing that microbiome dysbiosis can precede AD in early childhood. Benefits of using commensals have been reported to be dependent on skin site, for instance with a treating effect of transplanting R. mucosa in the antecubital flexure of AD patients but no effect on hands. In general, most microbiome studies in AD focus on the body flexures but do not address microbial composition at other body sites[10-11].

MATERIALS AND METHODS

Samples from 5 healthy controls (3 women, 2 men), aged 20-60 and 10 patients with AD (6 women and 4 men), aged 20-62 years, were included in this study. Mean Severity Scoring of Atopic Dermatitis (Scorad) for patients with AD was 30.8 (Table 1). Of 200 samples (including E. coli and buffer controls), 80 samples were of insufficient DNA quality and/or amount for sequencing (Table 1). Success of library preparation in lesional samples were 44% (32/70), 38% (27/66) in non-lesional and 85% (61/70) in controls. The data were described according to the 14 skin sites sampled. When analyzing the effect of lesions, the 14 skin sites were pooled, with a minimum number of 5 samples per group. Other factors influencing success of library preparation were related to subject and skin site (Table 1).

The 14 Skin Areas Sampled are Listed in the Top of the Table and in Detail Include: The neck (the anterior triangle) and bilaterally from the anterior nares, periorbital and perioral areas, antecubital and popliteal flexures (midline +/- 5 cm), upper inner arms (starting after the flexural area ending before the armpit, before presence of hair follicles from the armpit), volar forearms (starting after the antecubital fossae to 4 cm from the wrist), dorsum of the hands and feet (from

Table 1: Characterization of the Study Population and Samples

Characteristics	Atopic Dermatitis	Healthy controls
Subjects analysed, N	10	5
Age, mean (range), years	44(20/60)	38(20/62)
Female: male ratio	7:33:2	
SCORAD		
Mean (range)	31 (20-68)	NA
Moderate: Severe	8:2NA	
Filaggrin Mutation: Wt: Unknown	4:1:5	UNKNOWN
HECSI, mean (range)	10 (4-16)	NA
Treatment		
No	2	5
Steroid	5	0
Systemic	4	0
Co-morbidities		
Asthma	5	0
Hay Fever	5	0
Contact Dermatitis	5	1
Food Allergy	1	0
Skin site successfully sampled, Nonlesional: lesional ratio		
Nasal	8:1	5
Pre Orbital	2:3	5
Peri Oral	4:4	5
Neck	2:3	5
Upper /Inner Arms	0:2	3
Antecubital fossae	1:3	5
Volar forearms	0:2	3
Dorsum of hands	0:1	3
Palmar hands	3:2	3
Between Finger	0:5	5
Popliteal flexures	1:1	4
Dorsum of feet	0:2	5
Arches of feet	0:1	5
Between toes	6:2	5

wrist to joints of the digits), the web spaces between the fingers and toes, palmar hands (from wrist to joints of the digits) and arches of the feet.

Diversity Revealed Characteristic AD Skin Sites: Subject explained the majority of the explained microbial variance (Permanova test., R^2=19%., P=0.0001), however, the overall skin microbial composition differed significantly between AD and controls (Permanova test., R^2=6%, P=0.0001). As visualized on the principal coordinate analysis , samples from the hands and arms, flexures and neck showed the clearest separation according to control or AD. The lowest separation was observed for perioral and periorbital samples.

Diversity and Bacterial Species in AD and Healthy Controls: Initial exploration of differences in the microbiome composition showed lower bacterial alpha-diversity at the flexures in AD. The flexures in AD were dominated by the genus Staphylococcus, mostly the species S. epidermidis and S. aureus . S. aureus was low or undetected in control samples but present at most skin sites among AD patients and occasionally dominated the community (Fig. S3). Individual differences were also seen in S. aureus colonization, where AD10 was highly colonized across all skin sites (except from the feet, Fig. S4). Other species significant in comparison to mild-to-moderate AD. In contrast, the overall bacterial diversity was comparable in all three groups only with a significant increase at the dorsal neck and glabella in mild-to-moderate AD.

The Fungal Diversity Increases in Severe Atopic Dermatitis: Shannon diversity indices as a measure of a-diversity were calculated at the class and genus level in order to analyse both the number of taxa and the inequality between abundances. The fungal diversity was significantly elevated in patients with severe AD compared to HC and mild-to-moderate AD at all skin sites except the antecubital crease, where it also was higher in severe AD though only .

Feet were dominated by Corynebacterium sp. The nares were dominated by C. propinquum and Proteobacteria sp., except from those dominated by S. aureus in AD subjects more abundant in AD included M. luteus, S. epidermidis, S. saccharolyticus, S. lugdunensis, M. osloensis and Rothia sp. ND6WE1A(Table 2). On the contrary species higher in abundance in controls include Cutibacterium acnes, [Propionibacterium] humerusii, Corynebacterium sp. and Corynebacteriu, Candida or Debaryomyces, singular (Table 2).

Microbiota in Healthy Controls and Atopic Dermatitis are Significantly Different: The bacterial domain dominated the samples of both control and AD. However, fungi were highly present at the neck of controls but not in subjects with AD (Table 2). Malassezia globosa was present in relative abundance

Table 2: Low Abundant Species and Their Presence in Control and AD Patients at Different Skin Sites

Pos. individuals (> 0% at one site)/total			Mean relative abundance (%)															
			Nasal		Periorbital		Perioral		Neck		Upper inner arms		Antecubital flexures		Volar forearms		Dorsum o hands	
Species	C	AD	Subjects with site(s) ≥ 1%	C	AD	C	AD	C	AD	C	AD	C	AD	C	AD	C	AD	
M. osloensis	5/5	9/9	AD2, AD3, AD4, AD8, AD9	0.0	0.1	0.0	0.2	0.0	0.1	0.0	0.5	0.2	1.6	0.1	0.4	0.1	1.5	
Malassezia globosa	5/5	8/9	C5, C6, C8, C9, C10, AD2, AD6	0.0	0.4	0.1	0.2	0.5	0.3	2.0	0.2	0.4	0.1	1.3	0.1	1.6	0.2	
Malasseziales sp.	5/5	9/9	C5, C6, C8, C9, C10, AD6, AD8	0.0	0.0	0.1	0.0	0.0	0.0	1.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	
Staphylococcus saccharolyticus	1/5	4/9	AD2, AD3	0.0	0.0	0.0	3.9	0.0	0.2	0.0	3.4	0.1	0.0	0.0	1.1	0.0	0.7	
Staphylococcus lugdunensis	2/5	7/9	AD9	0.0	2.6	0.0	0.0	0.0	0.2	0.0	0.8	0.0	0.2	0.0	0.3	0.0	0.0	
Staphylococcus cohnii	4/5	4/9	C9, C10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Staphylococcus pasteuri	5/5	8/9	C5, C6, AD1, AD2, AD3, AD9	0.0	0.1	0.1	0.3	0.0	0.3	0.1	0.7	0.2	0.0	0.2	0.5	0.1	0.4	
Staphylococcus haemolyticus	5/5	7/9	C5, C9, C10, AD1, AD9, AD10	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.3	0.1	0.5	0.1	1.1	0.0	0.2	
C. singulare	4/5	4/9	C6	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.3	0.1	0.4	0.0	0.0	0.0	
C. appendicis	2/5	1/9	C8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.6	0.0	
Candida or *Debaryomyces,*	5/5	3/9	C9, AD8	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.1	0.2	0.1	0.3	0.0	0.1	0.3	
Streptococcus thermophilus	5/5	8/9	C6, C9, AD4	0.1	0.0	1.9	0.1	1.1	0.3	0.1	0.1	1.0	0.2	1.9	0.0	0.3	0.2	
Streptococcus gordonii	5/5	8/9	C6, C8, AD1, AD10	0.0	0.0	0.0	0.3	1.0	0.8	0.2	0.1	0.2	0.0	0.1	0.1	0.4	0.2	
Cutibacterium avidum	5/5	7/9	C8, AD1, AD8	1.0	0.6	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	
[*Propionibacterium*] *namnetense*	5/5	8/9	C5, C10, AD4, AD6, AD10	0.1	0.0	0.3	0.6	0.2	0.6	0.5	0.3	0.4	0.0	1.0	0.0	0.3	0.2	

ranging from 0.8-2.2% at antecubital flexures and 0.1-4% at the neck of healthy controls, whereas it was almost absent in AD antecubital flexures (0-0.2%) and neck (0.9%). The same pattern was observed for Malasseziales sp. (Table 2).

Lesional State and S. Aureus Presence: We observed control samples grouping together while AD samples cluster further apart from each other. Lesional state explained this pattern as lesional sample composition was significantly different from control samples (PERMANOVA test R^2=7%, P=0.0001), again with a large impact of subject on the microbial composition (R2=22%, P=0.0001). However, testing whether the lesional versus non-lesional state explained microbial composition variance did not achieve statistical significance.

In lesional samples, severe AD was associated with higher S. aureus colonization (r=0.63, P=0.00013), not seen in non-lesional (r=0.28, P=0.15). S. aureus colonization were higher across all skin sites except from the feet in lesional samples. When S. aureus colonization was high, the relative abundance of S. hominis and C. acnes were lower .

n the AD flexures, bacterial diversity (Shannon diversity) was lowest at lesional sites and S. epidermidis colonization seemed to accompany S. aureus dominance, not however at other sites.

S. Aureus Strain Colonization: In total, 42 samples (of 121) had enough S. aureus coverage for single nucleotide variation (SNV) analysis, which were mostly lesional. In general, the S. aureus strains from the same subject exhibited high similarity and lesional samples from three different AD subjects (AD2, 3 and 4) clustered together in the top branch of the tree, suggesting that the strains could be lesion and subject-specific and that different S. aureus strains may be implied in AD.

The Fungal Diversity Increases in Severe Atopic Dermatitis: Shannon diversity indices as a measure of a-diversity were calculated at the class and genus level in order to analyse both the number of taxa and the inequality between abundances. The fungal diversity was significantly elevated in patients with severe AD compared to HC and mild-to-moderate AD at all skin sites except the antecubital crease, where it also was higher in severe AD though only significant in comparison to mild-to-moderate AD . In contrast, the overall bacterial diversity was comparable in all three groups only with a significant increase at the dorsal neck and glabella in mild-to-moderate AD.

The Fungal Diversity Increases in Severe Atopic Dermatitis: Shannon diversity indices as a measure of a-diversity were calculated at the class and genus level in order to analyse both the number of taxa and the inequality between abundances. The fungal diversity was significantly elevated in patients with severe AD

compared to HC and mild-moderate AD at all skin sites except the antecubital crease, where it also was higher in severe AD though only significant in comparison to mild-to-moderate AD . In contrast, the overall bacterial diversity was comparable in all three groups only with a significant increase at the dorsal neck and glabella in mild-to-moderate AD.

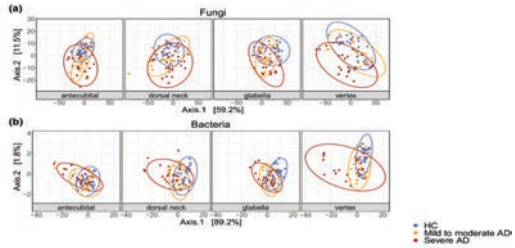

Fig. 1: Fungi and Bacteria

RESULTS AND DISCUSSIONS

In this study we demonstrated a global skin dysbiosis in AD at flexures, neck, hands and arms. This is in line with findings from Baurecht and colleagues showing microbial dysbiosis in AD across four skin sites (antecubital flexure, forehead, extensor-and volar forearm). The dysbiosis implicated both bacteria, fungus and especially our finding of an altered profile of bacteriophages in AD is intriguing. We also demonstrated some skin sites, feet, periorbital and perioral areas, to have more similar skin microbiome in health and AD[12-13].

We found a significantly lower alpha-diversity in AD flexures, and domination by Staphylococcus species, mostly S. aureus and S. epidermidis, as previously reported in the flare condition by Byrd *et al.* In lesional samples, an increased abundance of Staphylococcus was accompanied by Staphylococcus bacteriophages, including the Staphylococcus epidermidis phages CNPH82 and PH 15. As most of these phage's gene content are lysogenic , they could insert virulence factors into the bacterial genomes and contribute to a conversion from commensalism to pathogenicity[14-15]. Likewise, in one patient with severe dermatitis and extensive S. aureus colonization, the increased abundance of the phage phi-ETA could induce more transfer of the virulence gene encoding exfoliative toxin (ET) to S. aureus. This toxin degrades desmosomes in the stratum granulosom , whereby the pathogenicity of S. aureus would increase and provide a competitive advantage which could lead to increased relative abundance of this bacteria[16-18].

We also found lower relative abundances of C. acnes and [P.] humerusii in AD and a higher colonization of Propionibacterium phages, PHL041 and PHL092. These phages might lyse Cutibacterium ([Propionibacterium]) and result in the lower relative abundance. [P.] humerusii is a common inhabitant of the pilosebaceous unit , but to our knowledge this is the first study to report a difference in abundance in control versus skin disease[19-20]. C. acnes has previously been reported to be reduced in AD skin. It is a lipophilic bacteria, and altered sources of fatty acid substrates in AD skin might also restrict its growth. C. acnes ferments glycerol into short-chain fatty acids, including propionic acid, which can inhibit growth of S. aureus.

M. luteus was more abundant particularly in two AD subjects and may indicate a certain AD dermotype, as recently suggested. M. luteus has the capability to augment proliferation of virulence of S. aureus . The role of M. luteus in AD should be investigated further in future studies. A new important finding of this study is a potential association between M. osloensis and AD. Moraxella species are part of the human skin microbiota and M. osloensis is a rare causative organism of human infections. It may therefore be relevant to investigate further whether M. osloensis is an active player in AD[21-22].

No study has yet characterized the skin microbiome of the anterior triangle of the neck in AD, which is colonized with high amount of Staphylococcal species, but interestingly, also characterized by a lack of Malassezia species. Malassezia is a genus of lipophilic yeasts and comprises the most common fungi on healthy human skin . The role of Malassezia in AD is debated. It is often attributed a pathogenic role. Especially in a subset of AD patients with symptoms predominating on the head and neck. However, despite that numerous studies have attempted to show a difference in frequency of Malassezia skin colonization in AD patients, there is no such evidence. As some randomized controlled studies report beneficial effects of anti-fungal treatment, we asked the patients whether they have used antifungal treatment (Table1) and 2/5 might have used SNizoral shampoo around study participation, which could explain some lack of Malassezia in AD, but not in all patients. However, two recent microbiome studies indicate a lack of Malassezia in AD-with one of the studies conducted in an AD prone population, with past AD episodes, thus not expected to use antifungal treatment. Poor growth conditions in dry AD skin and absence of C. acnes providing substrates for Malassezia could restrict the growth[23-24].

Variability in beta-diversity within AD sites are higher than in controls, which we ascribe differences in lesional state. Other endogenous and exogenous factors might also explain larger variability in AD samples. Clinically the disease shows great patient to patient variability and effort are being put into defining endo types of the disease . It was recently reported that lesional AD skin is characterized by larger inter-and intra-patient microbiome variability than

non-lesional skin. The inter-patient variability mainly originated from S. aureus abundance.

Here, lesional samples were characterized by higher S. aureus colonization across all skin sites, except from the feet. We find that high abundance of S. aureus was accompanied by lower relative abundances of S. hominis, which is in line with data from Baurecht *et al.* showing decreased S. hominis at four AD skin sites. Nakatsuji *et al.* reports that AD patients lack strains of coagulase-negative Staphylococcus (including S. hominis strains) producing antimicrobial peptides against S. aureus, which can explain their opposing presence in the skin microbiome. In a previous study, reintroducing antimicrobial coagulase negative strains to human subjects with AD decreased S. aureus colonization. Other studies have also succeeded in treating AD with microorganisms, indicating that microbial transplants could be a promising strategy in AD management and highlighting the clinical relevance of finding skin site-specific species. Our data furthermore indicate that it is highly relevant to investigate both bacteria, fungi and for understanding skin dysbiosis. Using phages for targeting microbial dysbiosis in AD yields potential, which is supported by the specificity of phages. Phage-derived endolysins have been used to target S. aureus specifically, however not in AD patients[25].

Statistical Analyses: Descriptive data on relative abundances was both analysed according to individuals and disease groups. Beta-diversity was estimated by Bray-Curtis dissimilarity among samples and alpha-diversity using Shannon's index, both measures were based on MGS abundances. Permutational multivariate analysis of variance (PERMANOVA) was used to assess the effects of disease (AD vs Control) or lesional state (Control, Lesional and Non-lesional), considering a nested model of disease within skin area and adjusting for subject variability. Pearson correlations were calculated between AD severity scores and S. aureus abundance. Wilcoxon signed-rank test was used to compare viral abundances between two groups. Outliers in box plots were defined by the interquartile range rule.

Published skin shotgun sequencing data from AD is sparse and having 120 samples successfully analyzed is a large number. However, a substantial number of samples failed sequencing due to insufficient biomass, making it difficult to evaluate the influence of all relevant factors. The low biomass is a known challenge. We included AD patients in systemic treatment, which may affect the microbiome. However, even though the patients using topicals were instructed not to apply it 48 h before, we did not found differences in microbial composition between AD patients in topical versus systemic treatment (PERMANOVA, R^2=4%, P=0.98). Another limitation is the use of DNA to characterize skin microbiota as we cannot assess if the microbes are dead or alive or metabolically active. It is also difficult to analyze both bacteria, fungus in the same data set and it should be underlined that the DNA extraction protocol was optimized for bacteria. It is uncertain if the viral reads come from a phage or phage DNA inserted into a bacterial genome. Reference databases lack annotation for some organisms, which is the case of Malassezia restricta in this study. Studies combining microbiome and transcriptome data in AD are emerging and in general.

CONCLUSION

Though the microbial dysbiosis in AD is global, some sites are more affected than others. In our study, the flexures and neck showed marked taxonomical changes compared to healthy control. The flexures with lower alpha-diversity and high S. aureus abundance and high abundance of S. epidermidis in lesions, while at the neck Malassezia species were not detected. S. aureus colonization was observed across all lesional skin sites except the feet. In general, the S. aureus strains were highly similar within subjects both between lesional and non-lesional samples, indicating that more S. aureus strains are involved in AD. S. aureus may outgrow the coagulase negative S. hominis and C. acnes. Furthermore, phages targeted [Propionibacterium] and virulent phages such as Staphylococcus phi-ETA phage might support the growth of S. aureus. M. luteus and M. osloensis are more abundant in AD and may be active players in the disease. Similar to previous studies, we found an increased fungal diversity in AD patients. Interestingly, it was only increased in patients with severe AD and not in mild-to-moderate cases. We were unable, however, to observe the previously described decrease of bacterial diversity in severe AD. The bacterial diversity was comparable in all three study groups and was even slightly elevated in mild-moderate AD. An explanation for this discrepancy might be that we analysed a-diversities at the class and genus rather than at the species or the amplicon sequence variants (ASVs) level because the V1-3 region of the 16S rRNA gene operon that we used for analyses does not allow proper resolution at the species level or beyond.

REFERENCES

1. Benedetto, A.D., A. Kubo and L.A. Beck, 2012. Skin barrier disruption: A requirement for allergen sensitization? J. Invest. Dermatol., 132: 949-963.
2. Brandt, E.B. and U. Sivaprasad, 2011. Th2 cytokines and atopic dermatitis. J. Clin. amp Cell. Immunol., Vol. 2, No. 3 .10.4172/2155-9899.1000110.

3. Byrd, A.L., Y. Belkaid and J.A. Segre, 2018. The human skin microbiome. Nat. Rev. Microbiol., 16: 143-155.
4. Alsterholm, M., L. Strömbeck, A. Ljung, N. Karami and J. Widjestam et al., 2017. Variation in Staphylococcus aureus colonization in relation to disease severity in adults with atopic dermatitis during a five-month follow-up. Acta Dermato Vene., 97: 802-807.
5. Glatz, M., P. Bosshard and P.G. Schmid, 2017. The role of fungi in atopic dermatitis. Immunol. Allergy Clin. North Am., 37: 63-74.
6. Brodska, P., P. Panzner and K. Pizinger,et al., 2014. 1. IgE-mediated sensitization to Malassezia in atopic dermatitis: more common in male patients and in head and neck type. Dermatitis., 25: 120-126.
7. Ikezawa, Z., M. Kondo and M. Okajima,et al., 2004. 1. Clinical usefulness of oral itraconazole, an antimycotic drug, for refractory atopic dermatitis. Eur J Dermatol., 14: 400-406.
8. Han, S.H., H.I. Cheon, M.S. Hur, M.J. Kim and W.H. Jung et al., 2018. Analysis of the skin mycobiome in adult patients with atopic dermatitis. Exp. Dermatol., 27: 366-373.
9. Zhang, E., T. Tanaka, M. Tajima, R. Tsuboi, H. Kato, A. Nishikawa and T. Sugita, 2011. Anti-Malassezia-specific ige antibodies production in Japanese patients with head and neck atopic dermatitis: Relationship between the level of specific ige antibody and the colonization frequency of cutaneousMalasseziaSpecies and clinical severity. J. Allergy, 2011: 1-5.
10. Nakagawa, S., M. Matsumoto, Y. Katayama, R. Oguma and S. Wakabayashi et al., 2017. Staphylococcus aureus virulent psma peptides induce keratinocyte alarmin release to orchestrate il-17-dependent skin inflammation. Cell Host amp Microbe, 22: 667-677.
11. Baldry, M., Y. Nakamura, S. Nakagawa, D. Frees, H. Matsue, G. Núñez and H. Ingmer, 2018. Application of an agr-specific antivirulence compound as therapy for Staphylococcus aureus-induced inflammatory skin disease. J. Infect. Dis., 218: 1009-1013.
12. Skov, L., J.V. Olsen, R. Giorno, P.M. Schlievert, O. Baadsgaard and D.Y.M. Leung, 2000. Application of staphylococcal enterotoxin b on normal and atopic skin induces up-regulation of t cells by a superantigen-mediated mechanism. J. Allergy Clin. Immunol., 105: 820-826.
13. Bunikowski, R., M.E.A. Mielke, H. Skarabis, M. Worm and I. Anagnostopoulos et al., 2000. Evidence for a disease-promoting effect of Staphylococcus aureus–derived exotoxins in atopic dermatitis. J. Allergy Clin. Immunol., 105: 814-819.
14. Schlievert, P.M., L.C. Case, K.L. Strandberg, B.B. Abrams and D.Y.M. Leung, 2008. Superantigen profile ofStaphylococcus aureusIsolates from patients with steroid-resistant atopic dermatitis. Clin. Infect. Dis., 46: 1562-1567.
15. Tomi, N.S., B. Kränke and E. Aberer, 2005. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. J. Am. Acad. Dermatol., 53: 67-72.
16. Breuer, K., M. Wittmann, K. Kempe, A. Kapp and U. Mai et al., 2005. Alpha-toxin is produced by skin colonizing Staphylococcus aureus and induces a t helper type 1 response in atopic dermatitis. Clin. amp Exp. Allergy, 35: 1088-1095.
17. Wichmann, K., W. Uter, J. Weiss, K. Breuer, A. Heratizadeh, U. Mai and T. Werfel, 2009. Isolation of a-toxin-producingStaphylococcus aureusFrom the skin of highly sensitized adult patients with severe atopic dermatitis. Br. J. Dermatol., 161: 300-305.
18. Aarestrup, F.M., H.D. Larsen, N.H. Eriksen, C.S. Elsberg and N.E. Jensen, 1999. 1. Frequency of alpha- and beta-haemolysin in Staphylococcus aureus of bovine and human origin. A comparison between pheno- and genotype and variation in phenotypic expression. APMIS, 107: 425-430.
19. Fleury, O.M., M.A. McAleer, C. Feuillie, C.D. Formosa and E. Sansevere et al., 2017. Clumping factor b promotes adherence of Staphylococcus aureus to corneocytes in atopic dermatitis. Infec. Immun.., 85: 916-994.
20. Harkins, C.P., K.A. Pettigrew, K. Oravcová, J. Gardner and R.M.R. Hearn et al., 2018. The microevolution and epidemiology of Staphylococcus aureus colonization during atopic eczema disease flare. J. Invest. Dermatol., 138: 336-343.
21. Kwon, S., J. Choi, J. Shin, C. Huh and K. Park et al., 2019. Changes in lesional and non-lesional skin microbiome during treatment of atopic dermatitis. Acta Der Vener., 99: 284-290.
22. Goggin, R., C. Jardeleza, P. Wormald and S. Vreugde, 2014. Corticosteroids directly reduce Staphylococcus aureus biofilm growth: An in vitro study. Laryngoscope, 124: 602-607.
23. Seite, S. and T. Bieber, 2015. Barrier function and microbiotic dysbiosis in atopic dermatitis. Clin., Cosmet. Invest.al Dermatol., 8: 479-483.
24. Castanheira, M., A.A. Watters, R.E. Mendes, D.J. Farrell and R.N. Jones, 2010. Occurrence and molecular characterization of fusidic acid resistance mechanisms among staphylococcus spp. from European countries (2008). J. Antimicrob. Chemother., 65: 1353-1358.

25. Flores, G.E., J.G. Caporaso, J.B. Henley, J.R. Rideout and D. Domogala et al., 2014. Temporal variability is a personalized feature of the human microbiome. Genome Biol., Vol. 15 .10.1186/s13059-014-0531-y.