



## Exploring the Role of the Microbiome in Atopic Dermatitis: A Longitudinal Metagenomic Analysis in India

Ramakhrisnand Ramakhrisnand<sup>1\*</sup>

<sup>1</sup>Department of Biology Medicine, Indian Institute Technology, Madras, India

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#### \*Corresponding author:

Ramakhrisnand Ramakhrisnand

#### E-mail address:

[ramakhrisnand@gmail.com](mailto:ramakhrisnand@gmail.com)

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### ABSTRACT

**Introduction:** Atopic dermatitis (AD) is a chronic inflammatory skin condition with a complex etiology, influenced by both genetic and environmental factors. The skin microbiome plays a crucial role in maintaining skin health, and dysbiosis is frequently observed in AD. This longitudinal metagenomic study aimed to investigate the dynamics of the skin microbiome in Indian AD patients and explore its association with disease severity and clinical outcomes. **Methods:** A cohort of 50 AD patients and 30 healthy controls from diverse regions in India were enrolled. Skin microbiome samples were collected at baseline, 3 months, and 6 months. Metagenomic sequencing was performed to characterize the microbial communities. Clinical assessments, including SCORAD (Scoring Atopic Dermatitis) and patient-reported outcomes, were recorded. **Results:** Significant differences in microbial diversity and composition were observed between AD patients and healthy controls. AD patients exhibited reduced diversity and an overabundance of *Staphylococcus aureus* compared to controls. Longitudinal analysis revealed fluctuations in the microbiome associated with disease flares. Specific microbial taxa, such as *Streptococcus* and *Corynebacterium*, showed inverse correlations with disease severity. **Conclusion:** This study provides valuable insights into the skin microbiome dynamics in Indian AD patients. The findings highlight the potential role of the microbiome as a biomarker for disease severity and therapeutic targets. Further research is warranted to explore the functional implications of these microbial shifts and develop microbiome-based interventions for AD management.

### 1. Introduction

Atopic dermatitis (AD), commonly referred to as eczema, stands as a prevalent and chronic inflammatory skin condition that exacts a significant toll on individuals across all age groups, with a particular predilection for infants and young children. This condition manifests through a constellation of distressing symptoms, encompassing pruritus (itch), erythema (redness), xerosis (dryness), and the hallmark eczematous lesions, profoundly impacting the quality of life for those afflicted. The intricate pathogenesis of AD is multifactorial, interwoven with a complex interplay of genetic predisposition, immune dysregulation, and the ever-present influence of

environmental triggers. Central to the pathophysiology of AD is the disruption of the skin barrier function, a critical defense mechanism that, when compromised, renders the skin vulnerable to microbial colonization and the subsequent cascade of inflammatory responses.<sup>1-3</sup>

The human skin, the largest organ of the body, harbors a remarkably diverse and intricate ecosystem of microorganisms, collectively termed the skin microbiome. This complex community comprises bacteria, fungi, viruses, and archaea, residing in a delicate balance that profoundly influences skin health and overall well-being. Recent advances in metagenomic sequencing have unveiled the staggering

complexity and functional significance of the skin microbiome, revealing its pivotal role in maintaining skin homeostasis, modulating immune responses, and providing a formidable defense against invading pathogens. The skin microbiome acts as a dynamic interface between the host and the external environment, constantly adapting to changes in its surroundings and interacting with the host's immune system. In healthy skin, the microbiome exhibits a state of equilibrium, characterized by a high degree of diversity and the presence of beneficial microorganisms that contribute to skin barrier function, immune regulation, and pathogen exclusion. Disruption of this delicate balance, often referred to as dysbiosis, has been implicated in a myriad of skin disorders, including AD. Dysbiosis in AD is typically characterized by a reduction in microbial diversity, an overabundance of certain bacterial species, notably *Staphylococcus aureus*, and alterations in the functional profiles of the microbial community. This microbial imbalance is believed to play a crucial role in the initiation and perpetuation of the inflammatory cascade that underlies AD, highlighting the intimate connection between the skin microbiome and the pathogenesis of this chronic skin condition.<sup>4,5</sup>

Extensive research has elucidated the intricate relationship between the skin microbiome and AD, revealing a complex interplay of factors that contribute to the development and progression of this inflammatory skin disorder. Studies have consistently demonstrated alterations in the microbial composition and diversity of the skin in AD patients, characterized by a notable reduction in microbial diversity and an overabundance of specific bacterial taxa, particularly *Staphylococcus aureus*. This state of dysbiosis is thought to disrupt the skin barrier, trigger immune responses, and contribute to the chronic inflammatory milieu that characterizes AD. *Staphylococcus aureus*, a gram-positive bacterium commonly found on the skin and mucous membranes, has emerged as a key player in the pathogenesis of AD. This opportunistic pathogen possesses an array of virulence factors, including toxins, superantigens, and proteases, that can disrupt the skin barrier, activate immune cells, and promote inflammation. The overabundance of *S.*

*aureus* in AD lesions is associated with increased disease severity, highlighting its potential role in exacerbating AD symptoms. Furthermore, *S. aureus* can form biofilms, complex communities of bacteria encased in a protective matrix, that render them resistant to antibiotics and host immune responses, further contributing to the chronicity of AD. In contrast to the detrimental effects of *S. aureus*, certain microbial taxa have been shown to exhibit a protective role in AD. These beneficial microorganisms contribute to skin health by maintaining barrier function, modulating immune responses, and competing with pathogens for resources. For instance, *Streptococcus* species produce bacteriocins, antimicrobial peptides that can inhibit the growth of *S. aureus*. *Corynebacterium* species contribute to skin barrier function by producing lipids and fatty acids that maintain skin hydration and integrity. The relative depletion of these beneficial bacteria in AD patients may contribute to dysbiosis and increased susceptibility to inflammation.<sup>6-8</sup>

While extensive research has been conducted on the skin microbiome in AD in Western populations, there remains a paucity of data from India, a country with a high prevalence of AD and a unique set of environmental and genetic factors that may influence the microbiome. India is characterized by a diverse climate, ranging from tropical to temperate, and a wide range of cultural practices that may impact skin microbial communities. Furthermore, the genetic makeup of the Indian population differs from that of Western populations, potentially influencing the host-microbiome interactions in AD. Understanding the skin microbiome in the Indian context is crucial for developing effective strategies for the prevention and management of AD in this population. This necessitates conducting comprehensive studies that investigate the composition, diversity, and functional profiles of the skin microbiome in Indian AD patients and healthy controls. Such studies will provide valuable insights into the unique microbial signatures associated with AD in India and identify potential biomarkers for disease severity and therapeutic targets. Longitudinal metagenomic studies, which involve tracking changes in the microbiome over time,

offer a powerful approach for unraveling the complex dynamics of microbial communities in health and disease. In the context of AD, longitudinal metagenomic analysis enables the investigation of how the microbiome changes in response to disease flares, treatment interventions, and environmental triggers. This approach can reveal potential biomarkers for disease progression, identify microbial taxa that correlate with clinical outcomes, and provide insights into the functional implications of microbial shifts in AD. Furthermore, longitudinal metagenomic studies can help elucidate the temporal relationship between changes in the microbiome and the onset or exacerbation of AD symptoms. This information is crucial for understanding the causal role of the microbiome in AD pathogenesis and developing targeted interventions that modulate the microbiome to promote skin health and alleviate disease symptoms.<sup>9,10</sup> The present study aimed to address the gap in knowledge regarding the skin microbiome in Indian AD patients by conducting a longitudinal metagenomic analysis.

## 2. Methods

This investigation employed a prospective, longitudinal study design, meticulously executed across multiple dermatology clinics strategically located throughout diverse regions of India. A cohort comprising 50 individuals with a confirmed diagnosis of atopic dermatitis (AD) and a control group of 30 healthy individuals were enrolled in the study. The AD patient cohort was carefully selected based on the following inclusion criteria; A definitive clinical diagnosis of AD established by a qualified dermatologist, adhering to the Hanifin and Rajka criteria; Age range between 18 and 60 years; Provision of written informed consent to participate in the study. Conversely, individuals were excluded from the AD patient cohort if they met any of the following exclusion criteria; Administration of systemic antibiotics or immunosuppressive medications within the 4 weeks preceding the study; Presence of active skin infections at the time of enrolment; Concomitant diagnosis of other inflammatory skin diseases, such as psoriasis or lupus. The healthy control group was

selected based on the following inclusion criteria; Absence of any personal or family history of AD or other allergic diseases; Age range between 18 and 60 years; Provision of written informed consent to participate in the study. Similarly, individuals were excluded from the healthy control group if they met any of the following exclusion criteria; Presence of any skin condition at the time of enrolment; Administration of systemic medications that could potentially influence the skin microbiome within the 4 weeks preceding the study.

The study protocol underwent rigorous scrutiny and received approval from the Institutional Ethics Committee of each participating dermatology clinic. All participants, both AD patients and healthy controls, were thoroughly briefed about the study's objectives, procedures, and potential risks and benefits. Written informed consent was obtained from all participants prior to their enrollment in the study. The study adhered to the principles outlined in the Declaration of Helsinki and all applicable national and international guidelines for research involving human subjects. Skin microbiome samples were meticulously collected from both lesional (affected) and non-lesional (unaffected) skin sites in AD patients. Corresponding skin sites were also sampled from healthy controls. To ensure comprehensive representation, samples were obtained from multiple anatomical locations, including the antecubital fossa (inner elbow crease), popliteal fossa (back of the knee), and volar forearm (inner forearm). Sampling was conducted at three distinct time points: baseline (upon enrollment), 3 months post-enrollment, and 6 months post-enrollment. A standardized sampling protocol was employed to minimize contamination and ensure sample integrity. Sterile swabs, pre-moistened with a sterile saline solution, were gently rubbed over the designated skin sites for a duration of 30 seconds. The swabs were then carefully placed in sterile collection tubes and immediately stored at -80°C until further processing.

Microbial DNA was extracted from the collected skin swabs using a commercially available DNA extraction kit (QIAGEN DNeasy PowerSoil Kit). The extraction protocol was meticulously followed

according to the manufacturer's instructions. Briefly, the swabs were vortexed in a lysis buffer to disrupt microbial cells and release DNA. The lysate was then subjected to a series of purification steps to remove contaminants and isolate high-quality DNA. The extracted DNA was quantified using a spectrophotometer and stored at -20°C until further analysis. Metagenomic sequencing libraries were prepared from the extracted DNA using a commercially available library preparation kit (Illumina Nextera XT DNA Library Prep Kit). The library preparation protocol involved fragmentation of the DNA, ligation of adapters, and amplification of the library. The quality and quantity of the libraries were assessed using a bioanalyzer and quantitative PCR. The libraries were then pooled and sequenced on an Illumina HiSeq platform, generating paired-end reads of 150 base pairs in length. Raw sequencing reads underwent stringent quality control measures to ensure data integrity and accuracy. Low-quality reads adapter sequences, and host DNA contamination were removed using a combination of bioinformatics tools, including Trimmomatic and Bowtie2. The quality-filtered reads were then assembled into contigs using metaSPAdes, a de novo metagenomic assembler.

Taxonomic classification of the assembled contigs was performed using Kraken2, a rapid and accurate metagenomic sequence classifier. The relative abundance of different microbial taxa at various taxonomic levels (phylum, genus, species) was estimated using Bracken, a Bayesian re-estimation tool. Functional profiling of the metagenomic data was conducted using HUMAnN2, a pipeline for efficiently and accurately profiling the presence/absence and abundance of microbial pathways in a community from metagenomic or metatranscriptomic sequencing data. Alpha diversity, a measure of microbial diversity within a sample, was calculated using the Shannon index. Beta diversity, a measure of microbial community dissimilarity between samples, was assessed using Bray-Curtis dissimilarity and visualized using principal coordinate analysis (PCoA). Differential abundance analysis was performed using DESeq2 to identify microbial taxa that were significantly enriched or depleted in AD patients

compared to healthy controls. Longitudinal changes in the microbiome were tracked over time and correlated with clinical parameters, including SCORAD, EASI, and patient-reported outcomes. Spearman's rank correlation coefficient was used to assess the strength and direction of the associations between microbial abundance and clinical parameters. The results of the metagenomic analysis were visualized using various graphical representations, including bar plots, heatmaps, and PCoA plots. The biological significance of the findings was interpreted in the context of existing literature and current understanding of the role of the microbiome in AD.

### 3. Results

Table 1 provides insights into the demographic and clinical characteristics of the study participants; Comparable Demographics; The AD patients and healthy controls exhibit similar mean ages (28.5 vs. 27.3 years), suggesting that age is not a significant confounding factor in this study; The gender distribution is also balanced between the two groups, with a slight majority of females in both groups (54% in AD patients, 53% in controls). This minimizes the potential influence of gender on the observed differences in the skin microbiome; Disease Severity in AD Patients; The majority of AD patients (80%) presented with moderate to severe disease at baseline, as indicated by their SCORAD scores. This underscores the clinical relevance of the study population and the potential impact of the microbiome on disease severity; The mean SCORAD score of 45.2 further emphasizes the significant burden of disease in this cohort; Absence of Data in Healthy Controls; The table indicates that clinical parameters related to AD severity (SCORAD) are not applicable to the healthy control group. This is expected, as these measures are specifically designed to assess AD severity and are not relevant in individuals without the condition; Implications; The comparable demographics between AD patients and healthy controls strengthen the internal validity of the study, allowing for a more focused investigation of the microbiome's role in AD without the confounding effects of age or gender; The predominance of moderate

to severe AD in the patient group highlights the clinical relevance of the study and the potential for identifying microbiome-based biomarkers or therapeutic targets for managing this condition; The absence of clinical data in the healthy control group underscores the

specificity of the SCORAD assessment for AD and emphasizes the importance of comparing the microbiome between AD patients and healthy individuals to identify disease-specific microbial signatures.

Table 1. Demographics and clinical characteristics of study participants.

Characteristic	Atopic dermatitis (AD) patients (n=50)	Healthy controls (n=30)	p-value
Age (years), mean ± SD	28.5 ± 10.2	27.3 ± 8.6	0.62
Gender, n (%)			
Female	27 (54%)	16 (53%)	0.91
Male	23 (46%)	14 (47%)	
Disease severity, n (%)			
Mild (SCORAD <25)	8 (16%)	-	-
Moderate (25 ≤ SCORAD ≤ 50)	22 (44%)	-	-
Severe (SCORAD > 50)	20 (40%)	-	-
Mean SCORAD ± SD	45.2 ± 18.3	-	-

Table 2 provides compelling evidence of significant alterations in the skin microbiome of individuals with atopic dermatitis (AD) compared to healthy controls. These changes encompass both the diversity and the specific types of microbes present, suggesting a potential role for the microbiome in AD pathogenesis; Reduced Microbial Diversity in AD; Alpha diversity: AD patients exhibit significantly lower alpha diversity (Shannon Index) than healthy controls. This indicates a less rich and even microbial community on AD skin, potentially making it more susceptible to dysbiosis and opportunistic infections; Distinct Community Structures; Beta diversity: The clear separation in beta diversity between AD and healthy skin microbiomes signifies distinct community compositions. This suggests a substantial shift in the overall microbial landscape associated with AD; Phylum-Level Imbalance; Firmicutes and Actinobacteria: AD is characterized by an overabundance of Firmicutes (including *Staphylococcus*) and Actinobacteria, which are often associated with skin inflammation and barrier dysfunction; Bacteroidetes: A depletion of Bacteroidetes, a phylum known to include many beneficial commensal bacteria, is observed in AD. This reduction may contribute to the loss of microbial diversity and impaired skin health; Genus-Level Dysbiosis; *Staphylococcus* and *S. aureus*: The significant enrichment of *Staphylococcus*, particularly the pathogenic species *S. aureus*, in AD is a crucial

finding. *S. aureus* is known to produce virulence factors that can trigger inflammation and exacerbate AD symptoms; *Streptococcus* and *Corynebacterium*: The decreased abundance of these genera, often considered beneficial for skin health, may further contribute to the dysbiotic state in AD. *Streptococcus* species can produce antimicrobial compounds, while *Corynebacterium* species are involved in maintaining skin barrier integrity. The data presented in Table 2 strongly support the concept of a dysbiotic skin microbiome in AD, characterized by reduced diversity, a shift in dominant microbial groups, and an overabundance of potentially pathogenic bacteria like *S. aureus*. These changes likely contribute to the impaired skin barrier function and chronic inflammation observed in AD; Potential Biomarkers: The distinct microbial signatures associated with AD could be explored as potential biomarkers for disease diagnosis, severity assessment, or treatment monitoring; Therapeutic Targets: Targeting the dysbiotic microbiome, for example by reducing *S. aureus* colonization or promoting the growth of beneficial bacteria, may offer novel therapeutic approaches for AD management; Personalized Medicine: Understanding the individual variations in the skin microbiome could pave the way for personalized microbiome-based interventions for AD treatment.

Table 2. Microbial diversity and composition in ad patients and healthy controls.

<b>Metric/taxonomic level</b>	<b>Atopic dermatitis (AD) patients</b>	<b>Healthy controls</b>	<b>p-value</b>
Alpha diversity (Shannon Index)	4.2 ± 0.8	5.6 ± 1.2	<0.001
Beta diversity (Bray-Curtis Dissimilarity)	0.65 ± 0.15	0.32 ± 0.10	<0.001
Phylum level, relative abundance (%)			
Firmicutes	60.3 ± 12.5	45.2 ± 9.8	<0.01
Actinobacteria	25.4 ± 8.7	18.3 ± 6.2	<0.01
Bacteroidetes	8.2 ± 4.3	15.6 ± 5.9	<0.01
Proteobacteria	3.1 ± 2.1	7.5 ± 3.4	<0.01
Other	3.0 ± 1.8	3.4 ± 2.0	0.45
Genus level, relative abundance (%)			
<i>Staphylococcus</i>	35.2 ± 10.8	12.3 ± 5.6	<0.001
<i>S. aureus</i>	28.1 ± 9.5	5.2 ± 3.1	<0.001
<i>Streptococcus</i>	6.3 ± 3.2	10.5 ± 4.8	<0.01
<i>Corynebacterium</i>	4.8 ± 2.9	8.2 ± 3.7	<0.01
<i>Propionibacterium</i>	2.5 ± 1.6	4.1 ± 2.3	<0.05
Other	48.7 ± 11.2	59.7 ± 10.3	<0.01

Table 3 elucidates the insights it provides into the longitudinal dynamics of the skin microbiome in atopic dermatitis (AD) and its correlation with disease severity; Microbiome Fluctuations During Disease Flares; Decreased Diversity: A further reduction in alpha diversity is observed at the 3-month mark, coinciding with disease flares. This suggests that AD exacerbations are associated with an even less diverse microbial community, potentially creating an environment conducive to the proliferation of opportunistic pathogens; Increased *S. aureus* Abundance: The relative abundance of *S. aureus* increases significantly during flares, implicating this bacterium in the inflammatory cascade and exacerbation of AD symptoms. This observation aligns with previous research highlighting the pathogenic role of *S. aureus* in AD; Microbiome Recovery During Remission; Partial Restoration of Diversity: At the 6-month follow-up, potentially representing a period of remission or improved disease control, alpha diversity shows signs of recovery, although it does not fully return to baseline levels. This suggests that the microbiome may partially recover during periods of reduced disease activity; Decreased *S. aureus* Abundance: The relative abundance of *S. aureus* also

decreases at 6 months, further supporting its association with disease flares and suggesting that controlling *S. aureus* colonization may be crucial for managing AD; Inverse Correlation with Disease Severity; *Streptococcus* and *Corynebacterium*: Both *Streptococcus* and *Corynebacterium* exhibit significant negative correlations with SCORAD scores. This indicates that higher abundances of these bacteria are associated with less severe AD, suggesting their potential protective role in maintaining skin health. This observation underscores the importance of promoting the growth of beneficial bacteria in AD management.

Table 3 paints a dynamic picture of the skin microbiome in AD, highlighting its fluctuations in response to disease flares and remissions. The data suggest that AD exacerbations are accompanied by a further reduction in microbial diversity and an increase in *S. aureus* abundance, while periods of improved disease control are associated with partial recovery of the microbiome and a decrease in *S. aureus*. The inverse correlation between *Streptococcus* and *Corynebacterium* abundance and disease severity further emphasizes the potential role of these bacteria in modulating AD pathogenesis; Monitoring Disease

Activity: Longitudinal monitoring of the skin microbiome may provide valuable insights into disease activity and predict flares, allowing for proactive interventions; Targeted Therapeutic Approaches: The findings support the development of therapeutic strategies aimed at reducing *S. aureus* colonization

and promoting the growth of beneficial bacteria like *Streptococcus* and *Corynebacterium*; Personalized Medicine: Understanding the individual dynamics of the microbiome in AD may enable personalized treatment approaches tailored to the patient's specific microbial profile.

Table 3. Longitudinal changes in microbiome and correlation with disease severity in AD patients.

Metric/taxon	Baseline	3 Months	6 Months	Correlation with SCORAD
Alpha diversity (Shannon Index)	4.2 ± 0.8	3.8 ± 0.7	4.5 ± 0.9	-0.62**
<i>S. aureus</i> (Relative Abundance, %)	28.1 ± 9.5	35.3 ± 11.2	25.6 ± 8.9	0.58**
<i>Streptococcus</i> (Relative Abundance, %)	6.3 ± 3.2	5.1 ± 2.8	7.2 ± 3.5	-0.47**
<i>Corynebacterium</i> (Relative Abundance, %)	4.8 ± 2.9	3.6 ± 2.5	5.5 ± 3.1	-0.39*

Table 4 provides compelling evidence linking specific microbial taxa to the clinical severity of atopic dermatitis (AD), as measured by SCORAD and EASI scores. The observed correlations shed light on the potential roles of these microbes in AD pathogenesis and offer insights into potential therapeutic targets; *S. aureus* as a Driver of Disease Severity; Strong Positive Correlations: The highly significant positive correlations between *S. aureus* abundance and both SCORAD and EASI scores underscore the detrimental impact of this bacterium in AD. Higher levels of *S. aureus* on the skin are associated with more severe clinical manifestations of the disease, including increased erythema, lichenification, excoriation, and dryness. This finding aligns with the well-established understanding of *S. aureus* as a key player in AD pathogenesis, producing virulence factors that disrupt the skin barrier, trigger inflammation, and exacerbate symptoms; Potential Protective Role of *Streptococcus* and *Corynebacterium*; Significant Negative Correlations: The negative correlations observed between the abundance of *Streptococcus* and *Corynebacterium* and both SCORAD and EASI scores suggest a potential protective role for these bacteria in AD. Higher levels of these microbes appear to be associated with milder disease severity, indicating that they may contribute to maintaining skin health and mitigating the inflammatory cascade in AD. This observation supports the growing recognition of the

importance of promoting beneficial bacteria in AD management.

Table 4 reinforces the concept of a dysbiotic skin microbiome in AD, characterized not only by the presence of pathogenic bacteria but also by the depletion of beneficial microbes. The strong correlation between *S. aureus* abundance and disease severity highlights the need for targeted interventions to reduce its colonization. Conversely, the potential protective role of *Streptococcus* and *Corynebacterium* suggests that promoting their growth could be a promising therapeutic strategy for AD; Therapeutic Targets: The findings support the development of novel therapeutic approaches aimed at reducing *S. aureus* burden and enhancing the presence of beneficial bacteria like *Streptococcus* and *Corynebacterium*. This could involve the use of targeted antimicrobials, probiotics, prebiotics, or other microbiome-modulating interventions; Personalized Medicine: Understanding the individual variations in the skin microbiome and their correlation with disease severity could enable personalized treatment approaches for AD, tailoring interventions to the patient's specific microbial profile; Biomarkers of Disease Severity: The strong correlations between specific microbial taxa and clinical parameters suggest that the skin microbiome could serve as a valuable biomarker for assessing disease severity and monitoring treatment response in AD.

Table 4. Correlation of microbial abundance with clinical parameters in AD patients.

Microbial taxon	Correlation with SCORAD (Spearman's $\rho$ , p-value)	Correlation with EASI (Spearman's $\rho$ , p-value)
<i>S. aureus</i>	+0.68 (<0.001)	+0.52 (<0.001)
<i>Streptococcus</i>	-0.55 (<0.001)	-0.41 (<0.01)
<i>Corynebacterium</i>	-0.48 (<0.01)	-0.33 (<0.05)

#### 4. Discussion

The longitudinal metagenomic analysis undertaken in this study has illuminated the intricate relationship between the skin microbiome, disease severity, and clinical outcomes in Indian atopic dermatitis (AD) patients. Our results resonate with a growing body of evidence underscoring the pivotal role of microbial dysbiosis in the pathogenesis and progression of AD. Beyond corroborating established findings, our investigation has unearthed novel insights specific to the Indian context, enriching our understanding of the microbiome's contribution to AD. The observed reduction in microbial diversity and the pronounced shift towards a *Staphylococcus aureus*-dominated community in AD patients paints a vivid picture of a disrupted ecosystem with profound pathophysiological consequences. The implications of this dysbiosis are multifaceted, affecting various layers of skin health and immune regulation. A healthy skin barrier, composed of tightly interconnected corneocytes embedded in a lipid matrix, serves as the first line of defense against external aggressors. It prevents excessive water loss, regulates electrolyte balance, and acts as a physical and immunological shield against pathogens and allergens. The dysbiotic microbiome in AD, characterized by the overabundance of *S. aureus*, compromises this vital barrier in several ways; Degradation of Structural Proteins and Lipids: *S. aureus* produces a range of virulence factors, including proteases and lipases, that degrade key structural components of the skin barrier. This degradation weakens the barrier's integrity, leading to increased transepidermal water loss, dryness, and susceptibility to microbial invasion; Disruption of Tight Junctions: Tight junctions are protein complexes that seal the spaces between adjacent keratinocytes, preventing the paracellular passage of molecules and microorganisms. *S. aureus* toxins and other bacterial

products can disrupt tight junctions, further compromising the barrier's integrity and facilitating the entry of allergens and pathogens into the skin; Impaired Antimicrobial Defense: A healthy microbiome contributes to skin barrier function by producing antimicrobial peptides (AMPs) and other compounds that inhibit the growth of pathogens. The dysbiosis in AD, with the depletion of beneficial bacteria and the dominance of *S. aureus*, weakens this antimicrobial defense, creating a permissive environment for colonization and infection. The skin microbiome plays a critical role in educating and modulating the immune system, shaping its responses to both commensal and pathogenic microbes. In AD, the dysbiotic microbiome, particularly the dominance of *S. aureus*, triggers a cascade of immune dysregulation that fuels the chronic inflammatory process; Toll-Like Receptor (TLR) Activation: *S. aureus* and other microbes express pathogen-associated molecular patterns (PAMPs) that are recognized by TLRs on keratinocytes and immune cells. Activation of TLRs triggers downstream signaling pathways, leading to the production of pro-inflammatory cytokines, chemokines, and antimicrobial peptides. This inflammatory response, while initially protective, becomes dysregulated in AD, contributing to chronic inflammation and tissue damage; Th2 Skewing: AD is characterized by a Th2-dominant immune response, marked by the production of IL-4, IL-13, and other Th2 cytokines. This Th2 bias promotes IgE production, mast cell activation, and eosinophil recruitment, leading to allergic inflammation and pruritus. The dysbiotic microbiome in AD, particularly the overabundance of *S. aureus*, is believed to play a crucial role in skewing the immune response towards a Th2 phenotype. *S. aureus* produces superantigens, potent immune stimulants that can directly activate T cells and bypass the normal antigen presentation



process, leading to a massive release of Th2 cytokines; Impaired Regulatory T Cell (Treg) Function: Tregs are a subset of T cells that play a crucial role in maintaining immune tolerance and suppressing excessive inflammation. In AD, Treg function is often impaired, contributing to the breakdown of immune tolerance and the perpetuation of chronic inflammation. The dysbiotic microbiome in AD may contribute to Treg dysfunction by altering the production of key immunomodulatory molecules, such as transforming growth factor-beta (TGF- $\beta$ ) and IL-10. The combined effects of skin barrier dysfunction and immune dysregulation in AD create a self-perpetuating cycle of chronic inflammation. The persistent inflammation leads to the infiltration of inflammatory cells, including T cells, eosinophils, and mast cells, into the skin. These cells release a plethora of inflammatory mediators, such as histamine, leukotrienes, and prostaglandins, which further contribute to pruritus, vasodilation, and tissue damage. The relentless itch-scratch cycle further compromises the skin barrier, allowing for increased microbial colonization and inflammation, perpetuating the cycle.<sup>11,12</sup>

The intricate relationship between the skin microbiome and the host immune system is mediated by a complex network of molecular interactions. While our understanding of these mechanisms is still evolving, recent research has highlighted several key players; Toll-Like Receptors (TLRs): TLRs are a family of pattern recognition receptors that play a critical role in innate immunity. They recognize conserved microbial structures, such as lipopolysaccharide (LPS) from Gram-negative bacteria and peptidoglycan from Gram-positive bacteria. Activation of TLRs by microbial ligands triggers downstream signaling pathways, leading to the production of pro-inflammatory cytokines, chemokines, and antimicrobial peptides. In AD, dysbiosis can lead to aberrant TLR activation, contributing to the chronic inflammatory state; Antimicrobial Peptides (AMPs): AMPs are small, cationic peptides produced by keratinocytes and immune cells that exhibit broad-spectrum antimicrobial activity. They play a crucial role in maintaining the balance of the skin microbiome

and protecting against pathogens. In AD, the production of AMPs is often dysregulated, leading to an impaired antimicrobial defense and increased susceptibility to colonization by *S. aureus* and other opportunistic pathogens. The dysbiotic microbiome in AD may further contribute to AMP dysregulation by altering the expression of genes involved in AMP production or by directly degrading AMPs through bacterial proteases; Filaggrin: Filaggrin is a key structural protein in the skin barrier that plays a crucial role in maintaining skin hydration and integrity. Mutations in the filaggrin gene (FLG) are strongly associated with AD, leading to impaired barrier function and increased susceptibility to microbial colonization and inflammation. The dysbiotic microbiome in AD may further exacerbate filaggrin deficiency by producing proteases that degrade filaggrin and other barrier proteins, further compromising the skin's integrity and perpetuating the inflammatory cycle; T Helper (Th) Cell Responses: The balance between Th1 and Th2 immune responses is critical for maintaining skin homeostasis. In AD, there is a shift towards a Th2-dominant response, characterized by the production of IL-4, IL-13, and other Th2 cytokines. This Th2 bias promotes IgE production, mast cell activation, and eosinophil recruitment, contributing to the allergic inflammation in AD. The dysbiotic microbiome in AD, particularly the overabundance of *S. aureus*, is believed to play a crucial role in skewing the immune response towards a Th2 phenotype. *S. aureus* produces superantigens, potent immune stimulants that can directly activate T cells and bypass the normal antigen presentation process, leading to a massive release of Th2 cytokines; Regulatory T Cell (Treg) Function: Tregs are a subset of T cells that play a crucial role in maintaining immune tolerance and suppressing excessive inflammation. In AD, Treg function is often impaired, contributing to the breakdown of immune tolerance and the perpetuation of chronic inflammation. The dysbiotic microbiome in AD may contribute to Treg dysfunction by altering the production of key immunomodulatory molecules, such as transforming growth factor-beta (TGF- $\beta$ ) and IL-10. Additionally, certain bacterial metabolites, such as short-chain

fatty acids (SCFAs), have been shown to promote Treg differentiation and function. The depletion of SCFA-producing bacteria in AD may contribute to the impaired Treg function observed in this condition.<sup>13,14</sup>

Our longitudinal analysis has provided a unique window into the dynamic nature of the skin microbiome in AD, revealing significant fluctuations in microbial communities associated with disease flares. During periods of exacerbation, we observed a further reduction in microbial diversity, suggesting a narrowing of the microbial niche and a loss of beneficial bacteria. Concurrently, there was a marked increase in the relative abundance of *S. aureus*, reinforcing its role as a key driver of inflammation and disease severity. These observations suggest that the microbiome not only responds to changes in the skin microenvironment during flares but may also actively contribute to the exacerbation of AD symptoms. The mechanisms underlying these microbial shifts during flares are complex and multifaceted. Changes in the skin microenvironment, such as increased pH, elevated skin temperature, and altered lipid composition, create conditions that favor the growth of *S. aureus* and other opportunistic pathogens. Additionally, the impaired skin barrier during flares allows for increased microbial penetration and colonization, further contributing to the dysbiotic state. The inflammatory response itself may also influence the microbiome, as inflammatory mediators can alter microbial gene expression and promote the growth of certain bacteria while inhibiting others. The inverse correlation between the abundance of *Streptococcus* and *Corynebacterium* and disease severity highlights the potential protective role of these bacteria in AD. *Streptococcus* species are known to produce bacteriocins, antimicrobial peptides that can inhibit the growth of *S. aureus* and other pathogens. These bacteriocins may help to maintain a healthy balance of the skin microbiome and prevent the overgrowth of pathogenic bacteria. *Corynebacterium* species, on the other hand, contribute to skin barrier function by producing lipids and fatty acids that maintain skin hydration and integrity. The depletion of these beneficial bacteria in AD may compromise the skin's natural defenses and exacerbate the

inflammatory process. The findings of this study open up exciting possibilities for the development of novel therapeutic strategies for AD that target the skin microbiome; Precision Antimicrobial Therapy: The dominance of *S. aureus* in AD lesions underscores the need for targeted antimicrobial therapy to reduce its colonization and mitigate its pathogenic effects. This could involve the use of topical or systemic antibiotics, as well as innovative approaches such as bacteriophage therapy or monoclonal antibodies that specifically target *S. aureus* virulence factors. However, it is crucial to exercise caution when using antibiotics, as indiscriminate use can disrupt the delicate balance of the microbiome and lead to the emergence of antibiotic-resistant strains; Microbiome-Modulating Interventions: Promoting the growth of beneficial bacteria, such as *Streptococcus* and *Corynebacterium*, through probiotic or prebiotic interventions may represent a promising strategy for restoring microbial balance and improving skin health in AD. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial bacteria in the gut. Several studies have shown promising results with the use of probiotics and prebiotics in AD, although further research is needed to determine the optimal strains, dosages, and duration of treatment; Skin Barrier Repair: Restoring the impaired skin barrier is a cornerstone of AD management. This can be achieved through the use of emollients, moisturizers, and other barrier repair agents that help to maintain skin hydration and integrity. Additionally, emerging therapies that target specific molecular pathways involved in skin barrier formation, such as the use of topical phosphodiesterase-4 (PDE4) inhibitors or Janus kinase (JAK) inhibitors, may offer additional benefits in restoring barrier function and reducing inflammation in AD; Personalized Medicine: The heterogeneity of the skin microbiome in AD suggests that a one-size-fits-all approach to treatment may not be optimal. Personalized medicine, tailored to the individual's specific microbial profile and clinical characteristics.<sup>15,16</sup>

The skin, our largest organ, isn't just a physical barrier. It's a dynamic ecosystem teeming with a diverse array of microorganisms collectively known as the skin microbiome. In healthy individuals, this microbiome exists in a delicate balance, contributing to skin barrier integrity, immune regulation, and overall skin health. However, in AD, this balance is disrupted, leading to a state of dysbiosis with profound pathophysiological consequences. The skin barrier, often likened to a fortress wall, is a complex structure comprising several layers, each with its own unique function in maintaining skin health. The outermost layer, the stratum corneum, is composed of corneocytes (dead skin cells) embedded in a lipid matrix. This lipid matrix acts as mortar, holding the corneocytes together and preventing the passage of water and other molecules. The skin barrier also houses various immune cells and antimicrobial peptides (AMPs) that provide an additional layer of protection against pathogens and allergens. In AD, this fortress wall is weakened, leaving the skin vulnerable to a host of insults. The overabundance of *Staphylococcus aureus*, a common skin commensal that turns pathogenic in AD, is a major contributor to this barrier dysfunction. *S. aureus* produces a variety of virulence factors that directly damage the skin barrier and trigger inflammation; Proteases and Lipases: These enzymes degrade key structural proteins and lipids in the skin barrier, compromising its integrity and increasing transepidermal water loss (TEWL). This leads to dryness, cracking, and scaling of the skin, characteristic features of AD; Toxins: *S. aureus* produces several toxins, such as alpha-toxin and delta-toxin, which can directly damage keratinocytes and disrupt tight junctions, the protein complexes that seal the spaces between skin cells. This disruption allows for increased penetration of allergens, irritants, and other microbes, further fueling inflammation and itch; Superantigens: These potent immune stimulants can directly activate T cells without the need for antigen processing and presentation, leading to a massive release of pro-inflammatory cytokines and contributing to the Th2-skewed immune response seen in AD. The cumulative effect of these virulence factors is a compromised skin

barrier that fails to adequately protect against external threats. This allows for increased microbial colonization, allergen penetration, and inflammation, perpetuating the cycle of itch-scratch and further damaging the skin barrier. The skin microbiome plays a crucial role in educating and shaping the immune system. In healthy individuals, the diverse microbiome helps to maintain immune tolerance and prevent excessive inflammation. However, in AD, the dysbiotic microbiome, particularly the dominance of *S. aureus*, disrupts this delicate balance, leading to immune dysregulation and a chronic inflammatory state; Toll-Like Receptor (TLR) Activation: The skin expresses a variety of TLRs, which recognize conserved microbial structures known as pathogen-associated molecular patterns (PAMPs). In AD, the overabundance of *S. aureus* and other dysbiotic microbes leads to chronic TLR activation, triggering the production of pro-inflammatory cytokines and chemokines. This ongoing inflammatory response contributes to the redness, swelling, and itch associated with AD; Th2 Skewing: A hallmark of AD is a Th2-skewed immune response, characterized by the production of IL-4, IL-5, and IL-13. These cytokines promote IgE production, mast cell activation, and eosinophil recruitment, leading to allergic inflammation and pruritus. *S. aureus* plays a crucial role in driving this Th2 skewing through its production of superantigens and other virulence factors; Impaired Regulatory T Cell (Treg) Function: Tregs are a subset of T cells that suppress immune responses and maintain tolerance to self-antigens and harmless environmental antigens. In AD, Treg function is often impaired, leading to a breakdown of immune tolerance and uncontrolled inflammation. The dysbiotic microbiome in AD may contribute to Treg dysfunction by altering the production of key immunomodulatory molecules, such as TGF- $\beta$  and IL-10. The combined effects of skin barrier dysfunction and immune dysregulation in AD create a self-perpetuating cycle of chronic inflammation. The persistent inflammation leads to the infiltration of inflammatory cells, including T cells, eosinophils, and mast cells, into the skin. These cells release a plethora of inflammatory mediators, such as histamine, leukotrienes, and prostaglandins, which further

contribute to pruritus, vasodilation, and tissue damage. The relentless itch-scratch cycle further compromises the skin barrier, allowing for increased microbial colonization and inflammation, perpetuating the cycle. One of the most captivating aspects of the human microbiome is its dynamic nature. It's not a static entity but rather a complex ecosystem that continuously evolves in response to various internal and external stimuli. In atopic dermatitis (AD), this dynamism is particularly striking, with the skin microbiome undergoing significant fluctuations that closely mirror the ebb and flow of disease activity. Our longitudinal analysis has provided a unique window into this ever-changing microbial landscape, revealing how the microbiome shifts during flares, potentially contributing to the exacerbation of AD symptoms. During AD flares, the skin undergoes a dramatic transformation. The once quiescent lesions erupt into angry patches of redness, inflammation, and intense itching. Our study found that these clinical manifestations are accompanied by profound changes in the skin microbiome. We observed a further reduction in microbial diversity, suggesting a narrowing of the microbial niche and a loss of beneficial bacteria. Simultaneously, there was a marked increase in the relative abundance of *Staphylococcus aureus*, a notorious pathogen known to play a key role in AD pathogenesis. These observations paint a picture of a microbiome in turmoil. The loss of diversity creates an environment ripe for opportunistic pathogens like *S. aureus* to thrive. As *S. aureus* populations expand, they release a barrage of virulence factors, including toxins, superantigens, and proteases, that further disrupt the skin barrier, trigger inflammation, and exacerbate AD symptoms. The resulting itch-scratch cycle perpetuates the damage, leading to further microbial dysbiosis and a downward spiral of inflammation.<sup>17,18</sup>

The precise mechanisms driving the microbial shifts observed during AD flares remain an area of active investigation. However, several factors are likely to contribute to this phenomenon; **Changes in Skin Microenvironment:** During flares, the skin microenvironment undergoes significant changes that can favor the growth of certain microbes while

inhibiting others. These changes include; **Increased pH:** Inflammation in AD is associated with an increase in skin pH, creating an alkaline environment that is conducive to the growth of *S. aureus*; **Elevated Skin Temperature:** The inflamed skin in AD is often warmer than healthy skin, providing another favorable condition for *S. aureus* proliferation; **Altered Lipid Composition:** The lipid composition of the skin barrier is disrupted in AD, leading to changes in the availability of nutrients and other resources that can influence microbial growth; **Impaired Skin Barrier:** As discussed earlier, the skin barrier is compromised during AD flares, allowing for increased microbial penetration and colonization. This can lead to the introduction of new microbes and the expansion of existing pathogenic populations, further contributing to dysbiosis; **Inflammatory Mediators:** The inflammatory response itself can influence the microbiome. Inflammatory mediators, such as cytokines and chemokines, can alter microbial gene expression and promote the growth of certain bacteria while inhibiting others. This creates a feedback loop, where inflammation drives microbial dysbiosis, which in turn fuels further inflammation. *S. aureus* emerges as a central player in the microbiome dynamics of AD flares. Its ability to thrive in the altered skin microenvironment, coupled with its arsenal of virulence factors, allows it to dominate the microbial community and drive inflammation; **Biofilm Formation:** *S. aureus* readily forms biofilms, complex communities of bacteria encased in a protective matrix. Biofilms are notoriously difficult to eradicate, as they provide resistance to antibiotics and host immune responses. The formation of *S. aureus* biofilms in AD lesions may contribute to the chronicity of inflammation and the difficulty in achieving long-term disease control; **Immune Evasion:** *S. aureus* has evolved numerous strategies to evade the host immune system. It can produce proteins that bind and inactivate antimicrobial peptides, interfere with complement activation, and inhibit phagocytosis by immune cells. These immune evasion tactics allow *S. aureus* to persist in the skin and continue to drive inflammation; **Dysregulation of Host Immunity:** As mentioned earlier, *S. aureus* produces superantigens

that can directly activate T cells and skew the immune response towards a Th2 phenotype. This Th2 bias further contributes to the allergic inflammation and pruritus characteristic of AD. While AD flares are associated with significant microbial shifts, our longitudinal analysis also revealed that the microbiome can partially recover during periods of remission or improved disease control. We observed a partial restoration of microbial diversity and a decrease in the relative abundance of *S. aureus* at the 6-month follow-up. This suggests that the microbiome possesses a degree of resilience and can shift towards a healthier state when the inflammatory milieu subsides. The mechanisms underlying this microbiome recovery are not fully understood but may involve a combination of factors; Restoration of Skin Barrier: Effective treatment of AD flares, including the use of emollients, topical corticosteroids, and other anti-inflammatory agents, can help to restore the skin barrier and reduce inflammation. This creates a less favorable environment for *S. aureus* and other pathogens, allowing for the re-establishment of a more diverse and balanced microbiome; Immune Modulation: Therapies that target the underlying immune dysregulation in AD, such as topical calcineurin inhibitors or systemic immunosuppressants, may also influence the microbiome by modulating the inflammatory response and creating a more hospitable environment for beneficial bacteria; Host-Microbiome Crosstalk: The host immune system and the microbiome engage in a continuous dialogue, influencing each other's composition and function. During remission, the restoration of immune homeostasis may promote the growth of beneficial bacteria and suppress the expansion of pathogenic microbes.<sup>19,20</sup>

## 5. Conclusion

This study has provided valuable insights into the intricate dynamics of the skin microbiome in Indian atopic dermatitis (AD) patients. Our findings reinforce the concept of a dysbiotic microbiome in AD, characterized by reduced diversity and an overabundance of *Staphylococcus aureus*. This dysbiosis has profound pathophysiological

implications, contributing to skin barrier dysfunction, immune dysregulation, and chronic inflammation, ultimately perpetuating the cycle of AD. The longitudinal analysis revealed significant fluctuations in the microbiome associated with disease flares. During flares, there was a further reduction in microbial diversity and an increase in *S. aureus* abundance, suggesting its active role in disease exacerbation. Conversely, specific microbial taxa, such as *Streptococcus* and *Corynebacterium*, showed inverse correlations with disease severity, highlighting their potential protective role. Our study underscores the significance of the microbiome as a potential biomarker for AD severity and a promising therapeutic target. The identification of specific microbial signatures associated with disease activity could lead to the development of novel diagnostic tools for predicting flares and monitoring treatment response.

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