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If Malassezia wasn't the only determining factor in dandruff: the role of scalp bacterial profile and ethnicity

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1. Introduction

Dandruff, a persistent scalp condition marked by itching and flaking, affects roughly half of the adult global population, irrespective of ethnic background [1,2]. While the precise cause remains not fully defined, research points towards the scalp's microbiome playing a crucial role [3]. Studies across various populations have shown a link between dandruff and imbalances in bacterial and fungal communities on the scalp's surface. Specifically, an overgrowth of *Malassezia* yeasts and *Staphylococcus* bacteria are often observed in dandruff-affected scalps [4-6]. Current dandruff treatments typically rely on broad-spectrum antifungals. However, as our understanding of a healthy skin and scalp microbiome grows, this broad-spectrum approach is being challenged. Research suggests a more targeted approach is needed, one that focuses on specific microbial imbalances [7]. Furthermore, recognizing and accounting for the variations in scalp characteristics across different ethnicities is crucial for developing tailored and effective solutions. To address this, we have conducted an in-depth analysis of the microbiome's composition and function in both healthy and dandruff-affected scalps across four distinct ethnic groups.

2. Materials and Methods

The clinical study was conducted at SGS Stephens Inc., involved adult men and women (n=212) of African American/Black, Caucasian/White, Asian, and Hispanic backgrounds. Participants were classified as either healthy (no dandruff history in the past three years) or dandruff affected. Prior to their clinical visit, all participants used a non-antidandruff shampoo for two weeks. Assessments occurred at a single time point after this washout period. Dandruff severity was graded using a 5-point Investigator's Global Assessment (IGA) scale ranging from 0 (no flakes) to 4 (severe flaking). Eligible dandruff participants exhibited mild to moderate adherent flakes (approximately 1-2mm in size). All participants signed an IRB-approved informed consent form consistent with the requirements in 21 Code of Federal Regulations (CFR) 50.25.

Scalp microbiome samples were collected from participants using a previously established method [5]. The collected samples were then split into two groups for analysis. One group underwent quantitative polymerase chain reaction (qPCR) analysis to determine *Malassezia* and *M. restricta* load using a BioRad CFX 96 Real-Time System and targeted primers. The other group underwent whole genome sequencing (WGS) at Cosmos ID Inc. For WGS, DNA was extracted using the Zymo MicroPrep Extraction kit according to the manufacturer's instructions. Sequencing libraries were prepared with the Illumina Nextera XT DNA Library Preparation Kit and TDT Unique Dual Indexes. The prepared libraries were then sequenced on an Illumina NovaSeq platform (2x150bp reads) at Cosmos ID. Subsequent bioinformatics analysis was performed by CosmosID using a proprietary algorithm under the CosmosID-HUB. Wilcoxon Rank-Sum tests were performed between groups.

Correlation analysis between microbiome data and IGA (dandruff severity) was performed using MaAsLin2. MaAsLin2 was implemented using the R package Maaslin2. MaAsLin2 (Microbiome Multivariable Associations with Linear Models) is designed to assess multivariable association with microbiome community features with complex metadata. MaAsLin2 performs generalized linear and mixed models to accommodate a broad range of studies and data types (counts or relative abundance); this includes both longitudinal and cross-sectional study designs. MaAsLin2 was used in this study to identify significant associations between IGA and individual taxa.

To access the abundance of bacterial virulence factors (VFs) from the Virulence Factor Database (VFDB), 103 samples from individuals with dandruff (Cell 1, n=51) and without dandruff (Cell 2, n=52) were profiled with CHAMP to obtain functional annotations, including virulence factors (VFs) from VFDB. Statistical comparisons were done using the non-parametric Mann-Whitney U-test. P values were corrected for multiple testing using FDR.

3. Results

3.1. Demographics

The two subject groups (healthy and dandruff) demonstrated a balanced distribution across gender, ethnicity, race, and Fitzpatrick skin type. Table 1 below provides a detailed breakdown of the ethnic composition of all participants in the study and average IGA in the dandruff group.

Table I

		Dandruff scalp	Healthy scalp
		N=106	N=106
Age		40±10	39±9
Gender	**Female**	75	68
	Male	31	38
Fitzpatrick skin type	**I - III**	76	67
	IV - VI	30	39
Race / ethnicity	**Asian**	22 (IGA: 2.18±0.33)	22
	Black	23 (IGA: 2.39±0.42)	27
	Hispanic	28 (IGA: 2.31±0.39)	29
	White	32 (IGA: 2.28±0.37)	28
IGA	**2**	62	NA
	2.5	27	NA
	3	17	NA

3.2. Quantitative Malassezia Load Analysis

As observed in previous studies [3,6,7], *Malassezia* load was generally higher in individuals with dandruff. However, this difference was not statistically significant within the Asian population (Figure 1a). Similarly, *M. restricta* load was elevated in dandruff-affected scalps across all ethnicities except for the Asian population (Figure 1b).

3.3.Fungal Profile WGS analysis

A detailed analysis of the fungal profile of both healthy and dandruff-affected scalps confirmed the dominance of *Malassezia species*, with notable ethnic variations observed. Intriguingly, the most significant difference between dandruff and healthy scalps was a lower abundance of *M. globosa* on dandruff-affected scalps compared to healthy scalps across all ethnicities. (Figure 2).

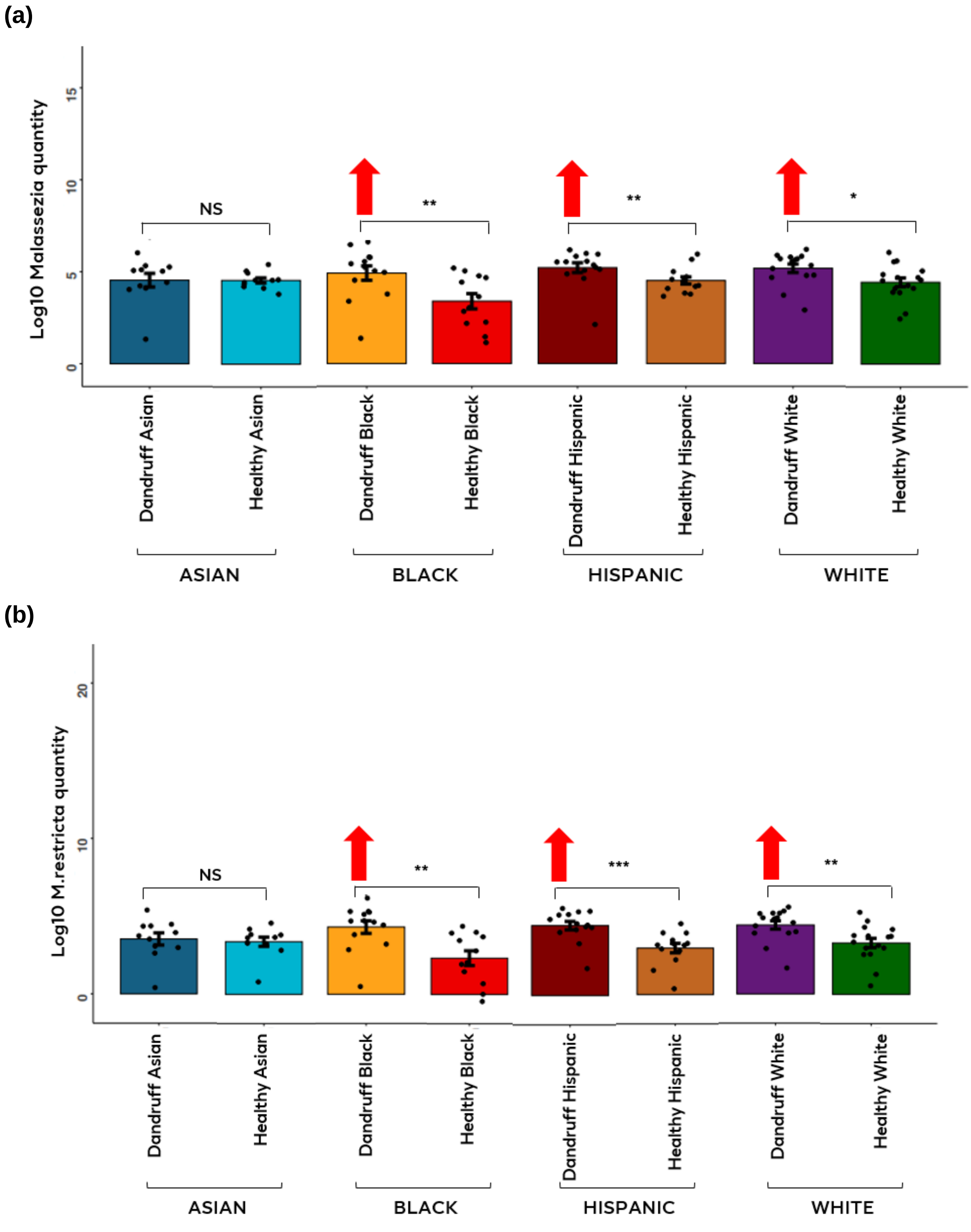

Figure 1. Quantification of (a) *Malassezia and* (b) *M.restricta* in dandruff and healthy scalp by qPCR.

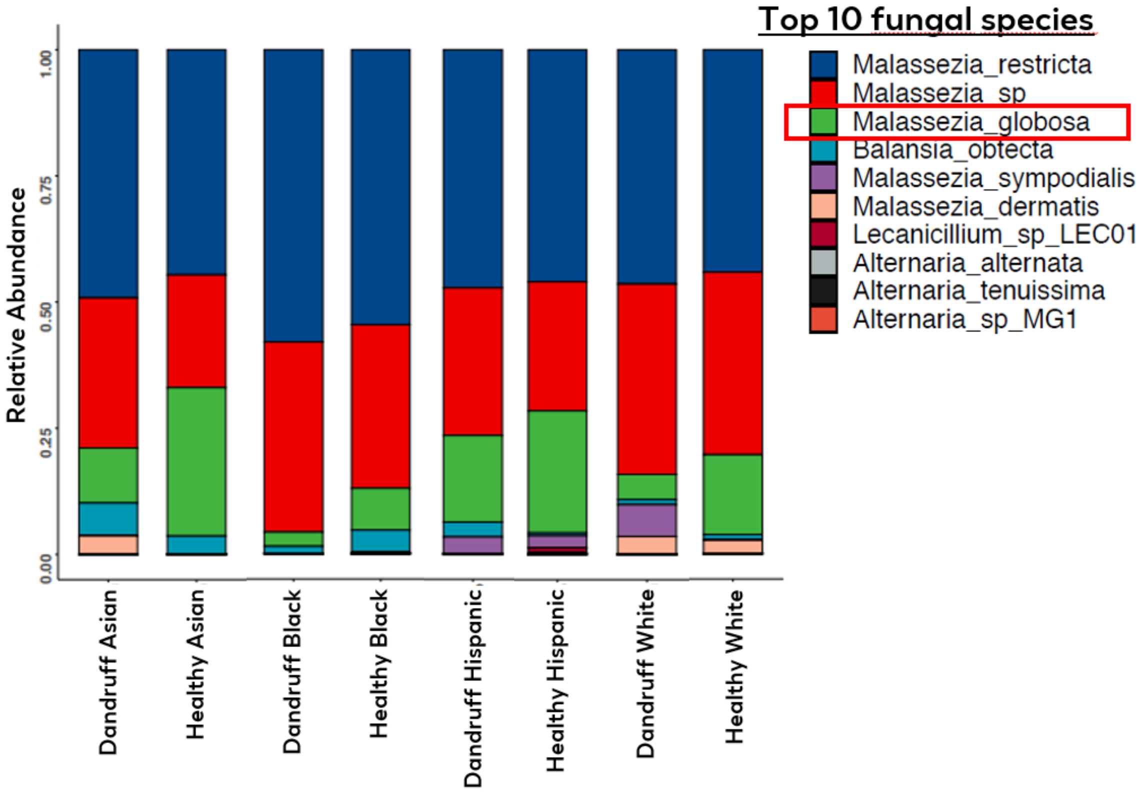

Figure 2. Whole genome sequencing analysis of fungal species composition in dandruff and healthy scalp.

3.4.Bacterial Profile WGS analysis

Bacterial species abundance was also evaluated (Figure 3) and revealed interesting variations across ethnicities. Healthy scalps generally exhibit a common microbiome profile dominaed with *Cutibacterium*, *Propionibacterium* and *Staphylococcus* species. Healthy scalps in White and Black subjects showed a notably higher abundance of *S. epidermidis*. In contrast, dandruff scalps across all ethnicities were associated with an increased prevalence of *S. aureus* and *S. capitis* along with a decrease in *Propionibacterium* and *Cutibacterium* species, consistent with previous findings [8].

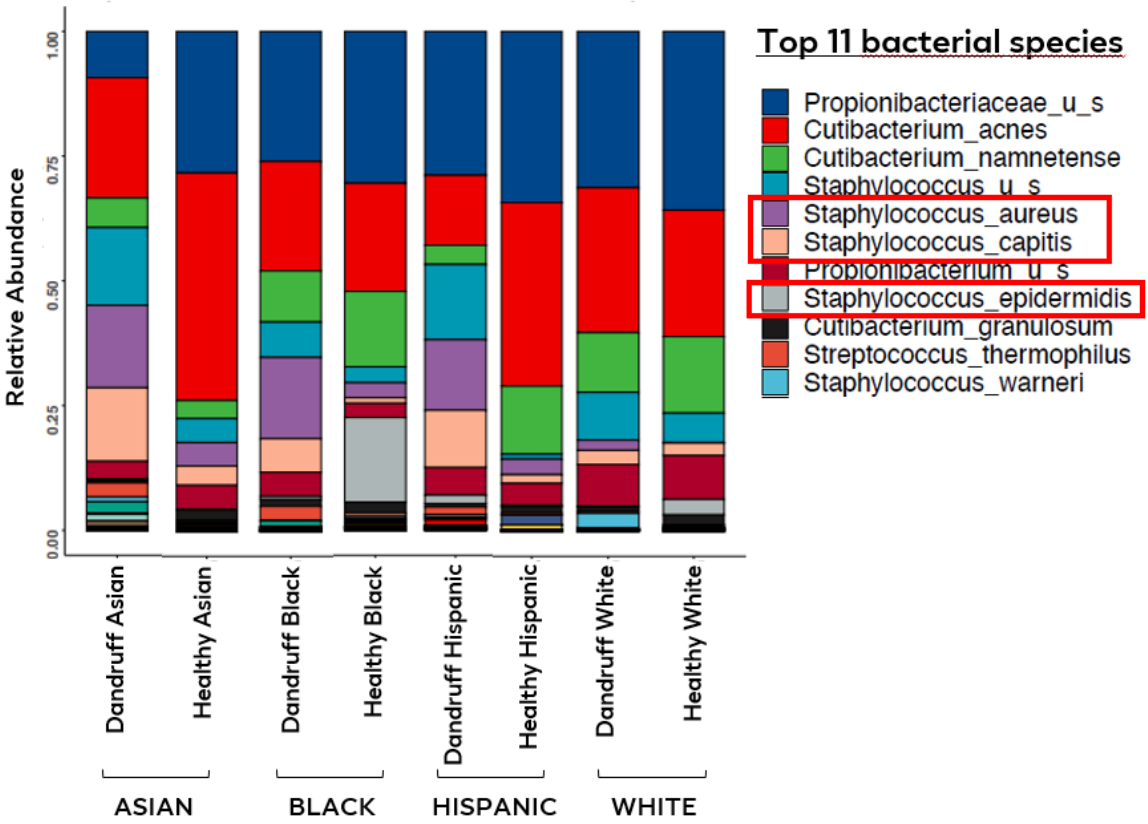

Figure 3. Whole genome sequencing analysis of bacterial species composition in dandruff and healthy scalp.

3.5. S.aureus correlation with Dandruff Severity

Critically, MaAsLin correlation analysis revealed a significant positive correlation between *S. aureus* abundance and both the presence and severity of dandruff (Figure 4). This finding underscores the importance of considering the bacterial component of dandruff, in addition to *Malassezia* overgrowth.

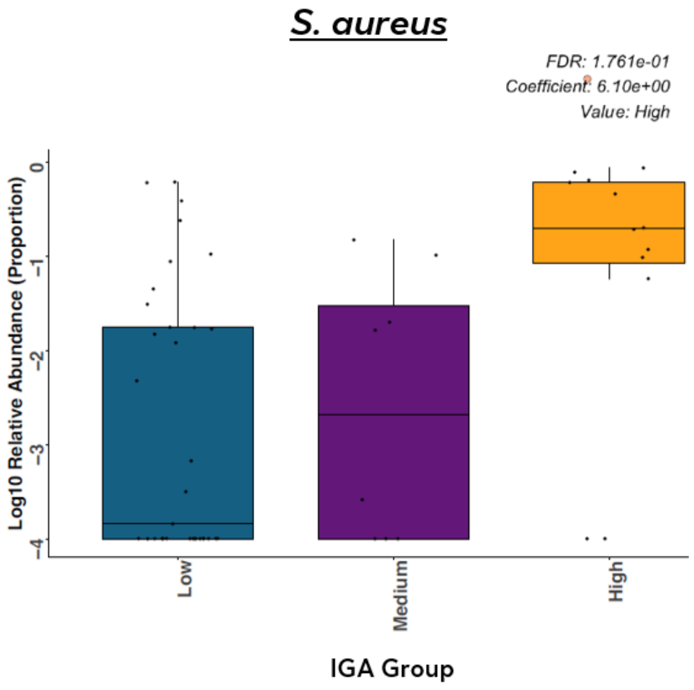

Figure 4. Correlation between *S. aureus* Abundance and Dandruff Severity.

3.6. Virulence Pathway analysis

Beyond the microbiome composition changes, we investigated the changes in the functionnal potential of the microbiome in dandruff as compared to non-dandruff scalps focusing on the functions which can be involved in the developpmnet of bacterial pathogenic strains. We analyzed the abundance of bacterial virulence factors using the Virulence Factor Database (VFDB) reference in dandruff-affected and healthy scalps. Dandruff scalps exhibited a significantly higher gene-level abundance of VFs, including those involved in nutrition/metabolism, biofilm formation, exotoxin production, stress survival, motility, effector delivery systems, exoenzyme activity, and immune modulation potential (Figure 5). This increased bacterial virulence potential was most pronounced in the Hispanic/Latino and Asian cohorts in which higher levels of *Staphylococcus sp*. such as *S. aureus* and *S. capitis* were observed. Overall, our data suggest that the scalp microbiome of individuals with dandruff is associated with heightened virulence. Many of the virulence factors enriched in dandruff have been reported to contribute to the pathogenicity and virulence of *S. aureus* [9-11] , further supporting a key role of *S. aureus* in dandruff severity, in addition to the observed correlation with *S. aureus* abundance.

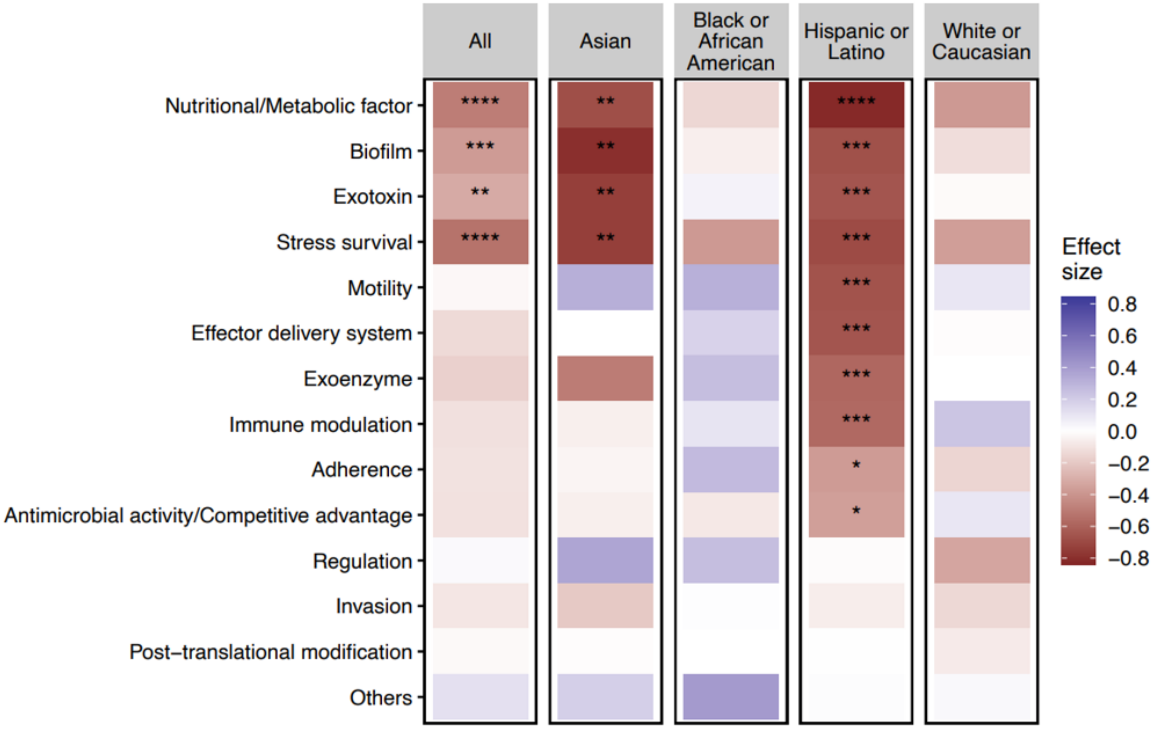

Figure 5. Heatmap displaying effect sizes from the differential abundance analysis of virulence factor (VF) categories between dandruff and healthy scalps. Red indicates that the VF category is increased in dandruff compared to healthy scalp. Blue indicates that the VF category is increased in healthy scalp compared to dandruff. Effect sizes corresponds to the rank-biserial correlation from the Mann Whitney U-test. FDR-corrected P-values: * < 0.1, ** < 0.01, **** < 0.001

4. Discussion

The scalp harbors a complex microbial community of bacteria and fungi. Our study investigated this microbial balance, its role in scalp health, and how imbalances contribute to dandruff, specifically considering ethnic differences to inform future treatment development.

Dandruff is linked to a microbiome imbalance, particularly an overgrowth of *Malassezia* species [4-6]. Our study confirmed the role of *Malassezia*, especially *M. restricta*, in dandruff. However, in Asian scalps the difference in *Malassezia* and *M. restricta* levels between dandruff and healthy was not observed, suggesting that antifungal approaches alone may be insufficient in these cases. Our results also underscore the importance of selectively targeting dandruff-associaed *Malassezia* species. Consistent with prior research [5], we found the most significant change in fungal profiles to be a lower level in *M. globosa* on dandruff-affected scalps, even in Asian participants where a decrease in *M. restricta* was not observed.

Furthermore, our study highlights the significant role of bacterial imbalance in dandruff. We observed higher levels of *S. aureus* across all ethnicities, strongly correlated with dandruff severity. A concurrent lower abundance in *S. epidermidis* and *Cutibacterium species* could likely exacerbates this imbalance. Functional analysis further emphasized *S. aureus*'s role, revealing higher levels of virulence factors in dandruff scalps, particularly among Asian subjects. This, coupled with unchanged levels of *M. restricta* in the dandruff group, suggests that targeting bacterial imbalance, in addition to fungal overgrowth, is crucial, especially for Asian individuals.

Therefore, our findings propose a novel approach to dandruff management: topical application to address barrier dysfunction in dandruff combined with targeted solutions addressing both fungal and bacterial imbalances. This approach aims to restore the scalp's microbial equilibrium and improve overall scalp health.

Further research exploring lifestyle factors (e.g diet, hair care routine) and environmental exposures could also shed light on the observed ethnic variations in scalp microbiome compositions. A deeper understand of the influence of these factors on dandruff could help develop more targeted and effective anti-dandruff solutions tailored to the specific needs of diverse population.

5. Conclusion

In conclusion, this study highlights the complex interplay of fungal and bacterial communities in dandruff pathogenesis across diverse ethnic groups. While confirming the established role of *Malassezia*, our findings underscore the significant contribution of *S. aureus* abundance, particularly its correlation with dandruff severity. Moreover, the observed ethnic variations in both *Malassezia* species and bacterial profiles, coupled with the differential abundance of virulence factors, emphasize the need for inclusive research and the potential for developing targeted, personalized dandruff treatments that address the unique microbial and functional characteristics of each individual's scalp.

Acknowledgments

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Conflict of Interest Statement

All authors are employees of L’Oreal and have no conflict of interest to disclose.

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