



OPEN Distinct site-specific bacterial microbiota within and between skin physiologic types in healthy Koreans: a pilot study

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Human skin is classified into sebaceous, moist, and dry, which influence the skin microbiome. However, variation within each type is poorly understood and may depend more on anatomical site than physiologic type. We analyzed bacterial communities from eight anatomical sites in ten healthy Korean adults using 16 S rRNA V1–V3 sequencing. Multivariate analysis showed that the anatomical site explained more variation in the microbiome than physiologic type. In dry skin, *Cutibacterium* was enriched in the dorsal forearm, whereas *Kocuria* was dominant on the sole. Within moist skin, *Staphylococcus* showed site-associated compositional differences, while the neck showed a trend toward higher *Cutibacterium*. Sebaceous scalp sites were rich in *Lawsonella* and had less *Cutibacterium* than facial sites. Despite limited species-level resolution of the V1–V3 region, heterogeneity was observed within the same physiologic type, indicating that anatomical site is an important determinant of microbiome structure. These results indicate distinct bacterial community structures both between and within physiologic skin types. These findings provide baseline insights into site-specific host–microbe interactions in healthy skin.

The human microbiome comprises a total collection of microorganisms and their genetic material on and inside the human body. Several intrinsic factors, including age, skin pH, ethnicity, sebum production, and hydration levels, influence the skin microbiome. Traditionally, skin is classified into moist, dry, and sebaceous areas based on lipid and hydration levels¹. Different physiologic types have distinct microenvironments that influence the microbial compositions and development of certain skin diseases. *Cutibacterium acnes* aggravates acne in the sebaceous area², while *Staphylococcus aureus* overgrowth is universal in atopic dermatitis, particularly in moist skin areas such as the antecubital fossa and popliteal fossa³. The apocrine gland-rich axillary skin, frequently affected by hidradenitis suppurativa, exhibits higher levels of anaerobes, including *Prevotella* and *Corynebacterium*, as well as lower levels of coagulase-negative staphylococci⁴. Psoriasis, often found in areas with dry skin, such as the knees and outer elbows, is associated with decreased *Cutibacterium* and increased *Streptococcus*⁵.

Recent skin biology studies highlight that disease prevalence and pathogenesis reflect anatomical site-specific differences in skin structure and cellular composition, rather than broad categories based on moisture or sebum levels. Transcriptomic and lipidomic analyses reveal that microenvironments, immune states, and epidermal differentiation offer mechanistic insight into disease localization^{6,7}. Because the cutaneous microbiome interacts with the epidermal barrier and local immune responses, intra-type microbiome heterogeneity may represent biologically relevant variation rather than simple descriptive taxonomy, as in the site-dependent bacterial dysbiosis reported in atopic dermatitis⁸. Characterizing the variation is clinically important because it may help distinguish disease-associated dysbiosis from normal site-dependent variation and may inform site-specific or personalized dermatologic interventions.

However, systematic studies comparing microbiome diversity and structure among distinct sites, particularly within the same physiologic type, remain limited. Previous studies have primarily focused on differences between broad physiologic types, potentially overlooking clinically relevant heterogeneity within each type. To

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address this gap, we conducted a pilot study profiling eight anatomically distinct sites in healthy Korean adults to characterize intra-type heterogeneity and to evaluate the relative influence of anatomical site and physiologic classification on microbiome composition.

Results
Sequencing summary and data quality control

The sampling site and demographic information of the participants in this study are summarized in Table S1. Among the total 150 swab samples, including negative controls (air swabs), 18 samples (7 negative controls and 11 true samples) were not sequenced owing to insufficient library DNA. Finally, a total of 1,143,859 bacterial reads were obtained from 132 samples, with an average of 8,665.6 reads per sample. To assess sequencing depth sufficiency, rarefaction curves were generated for both observed ASVs and Shannon diversity (Fig. S1). While some samples did not fully plateau in observed richness, the majority approached saturation when evaluated using the Shannon index, indicating that sequencing depth was sufficient for comparative diversity analyses.

To address potential contamination in low-biomass skin samples, prevalence-based contamination removal using decontam identified 63 out of 2,593 ASVs (2.4%) as potential contaminants, accounting for approximately 1.4% of the total community relative composition (Fig. S2 and S3). These taxa were predominantly non-skin-associated microorganisms, and their removal did not substantially alter overall community structure (Fig. S4). After applying sequential quality control steps, including contaminant removal, and filtering of low-abundance ASVs (singletons and doubletons), a total of 15 phyla, 269 genera, 560 species, and 2,024 amplicon sequence variants (ASVs) were retained in true samples (Table 1 and Table S2). Among the sites analyzed, the dry dorsal forearm exhibited the highest levels of bacterial species and ASVs, followed by the antecubital fossa [Ac], sole [So], neck [Ne], face (forehead [Fh], cheek [Ch]), and the sebaceous scalp (vertex [Vx], occiput [Oc]).

Determinant host factors affecting bacterial skin microbiome in healthy Koreans

Multivariate analysis using Permutational analysis of variance (PERMANOVA) revealed that anatomical site was the primary determinant of bacterial community variation, explaining 16.7% of total microbiome variation ($q < 0.001$) which was followed by individuality ($R^2 = 13.7\%$, $q < 0.001$), skin physiology type ($R^2 = 8.4\%$, $q < 0.001$), sex ($R^2 = 2.0\%$, $q < 0.001$), age ($R^2 = 1.4\%$, $q < 0.001$), and bilateral regions ($R^2 = 0.0\%$, $q = 0.959$) (Fig. S5a; Table S3). These findings suggest that both anatomical site and inter-individual variability contribute to the structure of the skin microbiome, with anatomical site exerting a modestly greater influence in this cohort. Principal coordinates analysis (PCoA) revealed partial clustering of samples according to anatomical site, although substantial overlap between sites was evident (Fig. S5b). Similarly, samples did not form clearly distinct clusters based on individual subject (Fig. S5c), indicating that neither anatomical site nor subject identity alone fully accounts for the observed variation. In line with their low explanatory power in PERMANOVA, neither sex nor bilateral regions showed significant differences in alpha or beta diversity (Fig. S6 and S7). Nevertheless, the effect of anatomical site remained statistically significant, supporting its contribution to microbiome variation.

Comparison of skin bacterial community composition and diversity between and within physiologic types

First, we analyzed the eight anatomical sites categorized by three skin physiologic types. PCoA based on the weighted UniFrac distance demonstrated distinct clustering of the three groups (sebaceous, moist, and dry), indicating different bacterial communities by the skin physiologic type ($q < 0.001$) (Fig. S8a; Table S3). Pairwise PERMANOVA comparisons further confirmed significant differences between sebaceous and dry ($R^2 = 9.7\%$, $q < 0.001$), and sebaceous and moist sites ($R^2 = 4.2\%$, $q = 0.034$), moist and dry sites ($R^2 = 4.3\%$, $q = 0.034$) (Table S4). Heterogeneity was observed even among sites sharing the same physiologic type. Within the dry skin type, the dorsal forearm and sole exhibited significantly different bacterial communities ($R^2 = 23.9\%$, $p < 0.001$). Similarly, within the moist skin type, antecubital fossa and neck also showed significant differences ($R^2 = 26.0\%$, $p < 0.001$) (Fig. 1a). In contrast, within the sebaceous skin type, no significant differences in overall microbial

Skin site	Physiologic type	Read count	Taxonomy count			
			Phylum	Genus	Species	ASVs
Df	Dry	7,456.6 ± 2,777.1	13	160	332	1,016
So	Dry	7,444.2 ± 3,377.8	11	141	251	597
Ac	Moist	9,365.5 ± 3,192.1	12	133	279	742
Ne	Moist	7,868.2 ± 3,631.6	9	104	208	501
Ch	Sebaceous	8,010.1 ± 4,365.6	7	61	111	281
Fh	Sebaceous	10,456.4 ± 4,909.8	7	54	107	290
Vx	Sebaceous	9,887.7 ± 4,593.7	5	13	26	70
Oc	Sebaceous	10,957.6 ± 4,384.9	4	10	18	59
Total		8,725.5 ± 3,949.6	15	269	560	2,024

Table 1. Sequencing depth and taxonomic richness across skin sites. Mean read counts and taxonomic richness at each level following quality filtering, contaminant removal, and exclusion of singleton and doubleton ASVs. Read counts represent the mean ± standard deviation. Ac, antecubital fossa; Ch, cheek; Df, dorsal forearm; Fh, forehead; Ne, neck; Oc, occiput; So, sole; Vx, vertex; ASV, amplicon sequence variant.

composition were observed across the four anatomical sites ($R^2=9.7\%$, $q=0.075$), and pairwise comparisons were also not significant (all $q>0.05$; Table S5). Considering the anatomical and functional differences between facial and scalp regions, including variations in sebum composition, hair follicle density, and local microenvironment, we further divided sebaceous sites into face (Cheek, Forehead) and scalp (Vertex, Occiput). This comparison showed a significant difference between the two groups ($R^2=8.8\%$, $p=0.012$). These findings suggest that physiologic type alone is insufficient to explain variation in microbial composition and that more detailed anatomical classification is needed.

Alpha diversity, assessed using the Shannon index, differed significantly across physiologic type. Sebaceous type (1.4 ± 0.8) was significantly lower compared to the moist (2.3 ± 0.7) and dry type (2.5 ± 0.6) ($q<0.001$, respectively). At the same time, no significant difference was observed between moist and dry sites ($q=0.239$) (Fig. S8b). Among the eight skin sites, dorsal forearm (2.7 ± 0.6) and sole (2.4 ± 0.6) showed the highest bacterial diversity, with comparable diversity observed between the two dry sites ($q=0.315$) (Fig. 1b). Within the moist skin type, antecubital fossa (2.5 ± 0.5) tended to exhibit higher bacterial diversity than neck (2.0 ± 0.8), although the difference was not statistically significant ($q=0.055$). Four sebaceous sites exhibited lower bacterial diversities, with values of 1.8 ± 0.7 , 1.3 ± 0.7 , 1.3 ± 0.8 , and 1.1 ± 0.7 at cheek, forehead, vertex, and occiput, respectively. No statistical difference was observed among the four sebaceous sites ($p=0.470$), and pairwise comparisons were also not significant (all $q>0.05$).

Site-dependent differences of the major skin bacterial abundances between and within physiologic type

We examined the taxonomic compositions of major bacterial taxa ($>1\%$ average relative abundance across the total skin samples) of the eight skin sites (Fig. S8c, Table S6). *Cutibacterium* ($42.6\pm 34.1\%$) and *Staphylococcus*

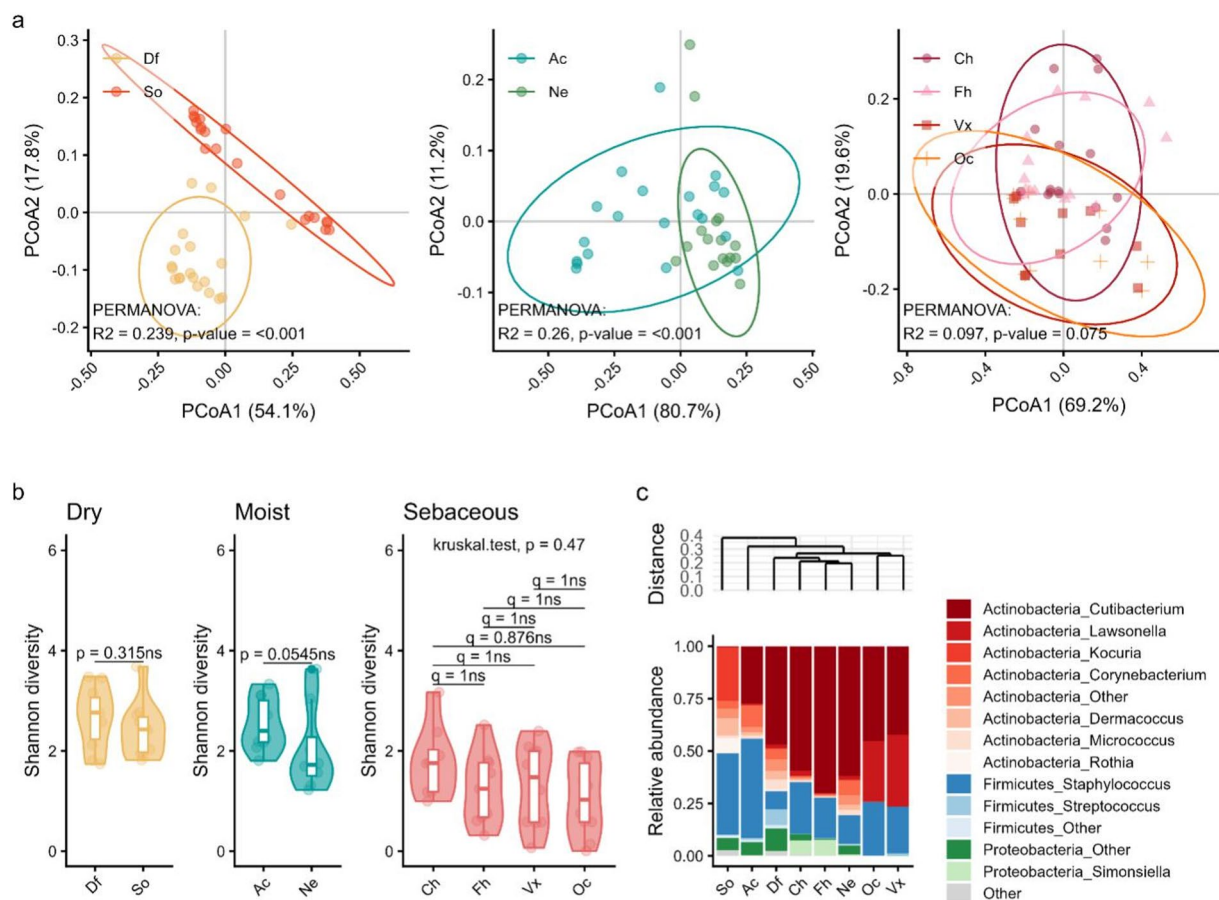


Fig. 1. Comparison of skin bacterial community composition and diversity across different skin sites. (a) Principal coordinates analysis (PCoA) of each skin site within the same physiologic type based on weighted UniFrac distances with 95% confidence ellipses. Statistical significance was tested using PERMANOVA. (b) Alpha diversity (Shannon index) by anatomical site, grouped by physiologic type. Statistical comparisons were performed using the Wilcoxon rank-sum test, and Benjamini–Hochberg-adjusted p -values are shown as q -values. (c) Bar plots of bacterial genera (mean $\geq 1\%$) across eight skin sites. Hierarchical clustering based on weighted UniFrac is represented as a dendrogram. Ac, antecubital fossa; Ch, cheek; Df, dorsal forearm; Fh, forehead; Ne, neck; Oc, occiput; So, sole; Vx, vertex. Significance codes: *** p or $q<0.001$; ** p or $q<0.01$; * p or $q<0.05$; ns, not significant.

(25.7 ± 29.1%) were the most abundant genera across all skin sites. Generally, the bacterial taxa of dry skin were highly variable, with relatively lower *Cutibacterium* (23.7 ± 29.8%) and higher abundances of *Kocuria* (13.0 ± 24.4%), *Rothia* (4.1 ± 15.5%), *Dermaococcus* (6.2 ± 13.2%), *Streptococcus* (3.8 ± 11.7%), and *Micrococcus* (3.0 ± 6.8%). *Staphylococcus* (32.3 ± 32.4%) and *Corynebacterium* (8.8 ± 11.6%) were more abundant in moist skin, whereas sebaceous skin was predominated by *Cutibacterium* (56.9 ± 32.6%), *Lawsonella* (12.8 ± 26.4%), and *Simonsiella* (4.4 ± 9.0%). However, these physiologic types do not fully capture the variability across individual anatomical sites. Hierarchical clustering based on genus-level composition suggested heterogeneity across physiologic types and sites (Fig. 1c). Detailed genus-level composition across sites is provided in Table S7.

Bacterial taxa were compared across anatomical sites within each physiologic type to identify site-associated differences at both the genus and species levels using Analysis of Composition of Microbiomes with Bias Correction 2 (ANCOM-BC2) (Fig. 2, Table S8). Species-level assignments are regarded as presumptive, although based on top hits with >99% BLAST identity. They remain constrained by the limited taxonomic resolution of the 16 S rRNA V1–V3 region due to its amplicon length. In comparisons between the dorsal forearm and sole, *Cutibacterium* (log fold change [LFC] = -5.3, $q < 0.001$) and *Cutibacterium acnes* (LFC = -5.2, $p < 0.001$) were enriched in dorsal forearm, whereas *Kocuria* (LFC = 3.0, $q = 0.011$) and its species, *Kocuria indica* (LFC = 2.9, $q = 0.021$) and *K. palustris* (LFC = 3.3, $q = 0.050$), were enriched in sole (Fig. 2). Within moist skin sites, the neck showed a trend toward higher *Cutibacterium* (LFC = 1.4) and lower *Staphylococcus* (LFC = -1.3) compared to the antecubital fossa, although these differences were not statistically significant ($q = 0.207$ for both). However, *S. hominis* was more abundant in antecubital fossa compared to neck (LFC = -2.1, $q = 0.013$) (Fig. 2). For the sebaceous type, subsequent analyses were conducted by grouping these regions into face and scalp. In this comparison, the scalp showed a higher abundance of *Lawsonella* (LFC = 4.6, $q < 0.001$), including its subordinate species-level taxa, while *Staphylococcus epidermidis* exhibited a non-significant increasing trend (LFC = -1.3,

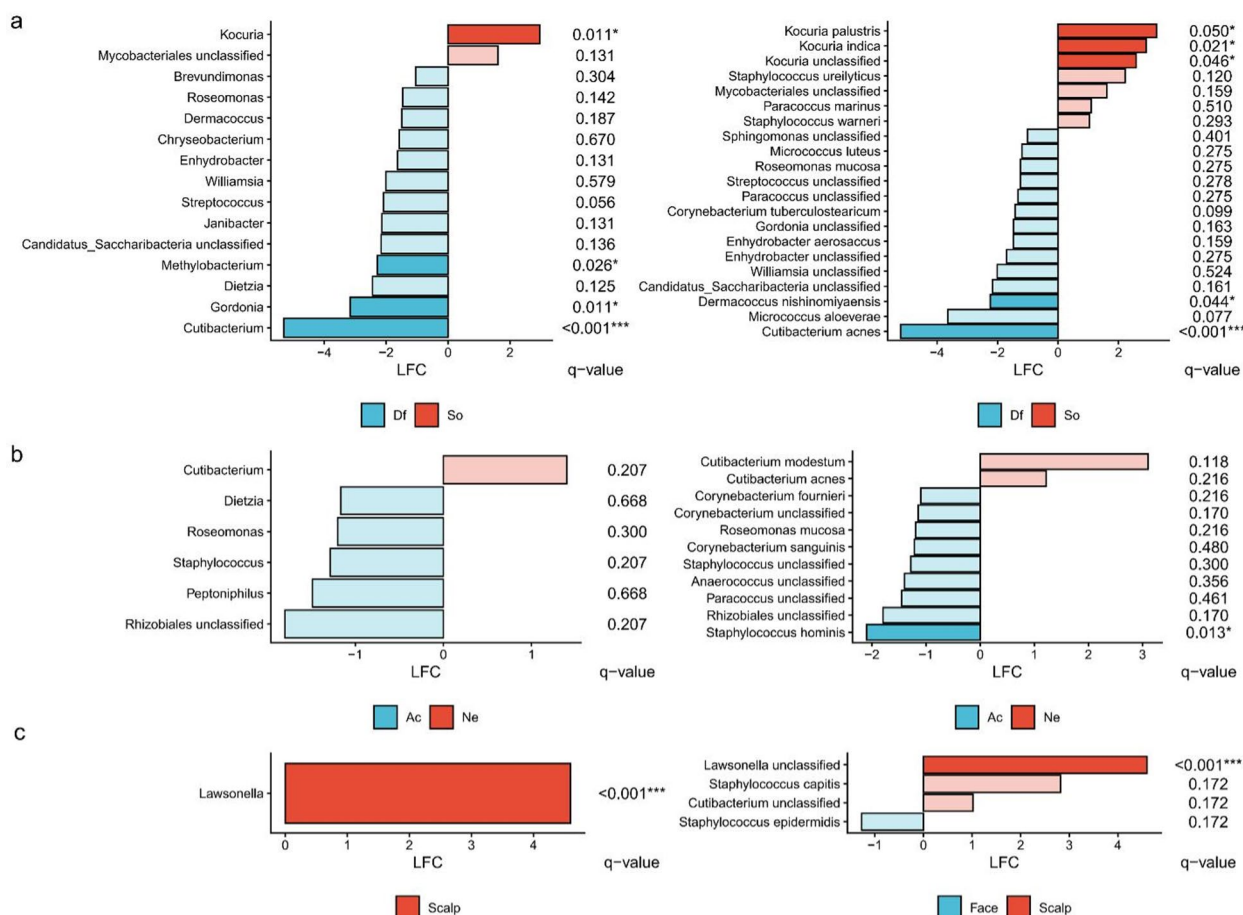


Fig. 2. Differential abundance of bacterial genera and species of each site within the same physiologic type. Differentially abundant taxa were identified using ANCOM-BC2 at the genus and species levels. Bar plots represent log fold changes (LFC) between two compared sites. Taxa with $|LFC| \geq 1$ are displayed. Statistical significance was determined using Benjamini–Hochberg false discovery rate correction, and q-values are shown alongside each taxon. Comparisons were performed separately within each physiologic type: (a) dry (Df vs. So), (b) moist (Ac vs. Ne), and (c) sebaceous (Face vs. Scalp). Bars representing non-significant taxa are shown in lighter color. Ac, antecubital fossa; Ch, cheek; Df, dorsal forearm; Fh, forehead; Ne, neck; Oc, occiput; So, sole; Vx, vertex; LFC, log fold change. Significance codes: *** $q < 0.001$; ** $q < 0.01$; * $q < 0.05$.

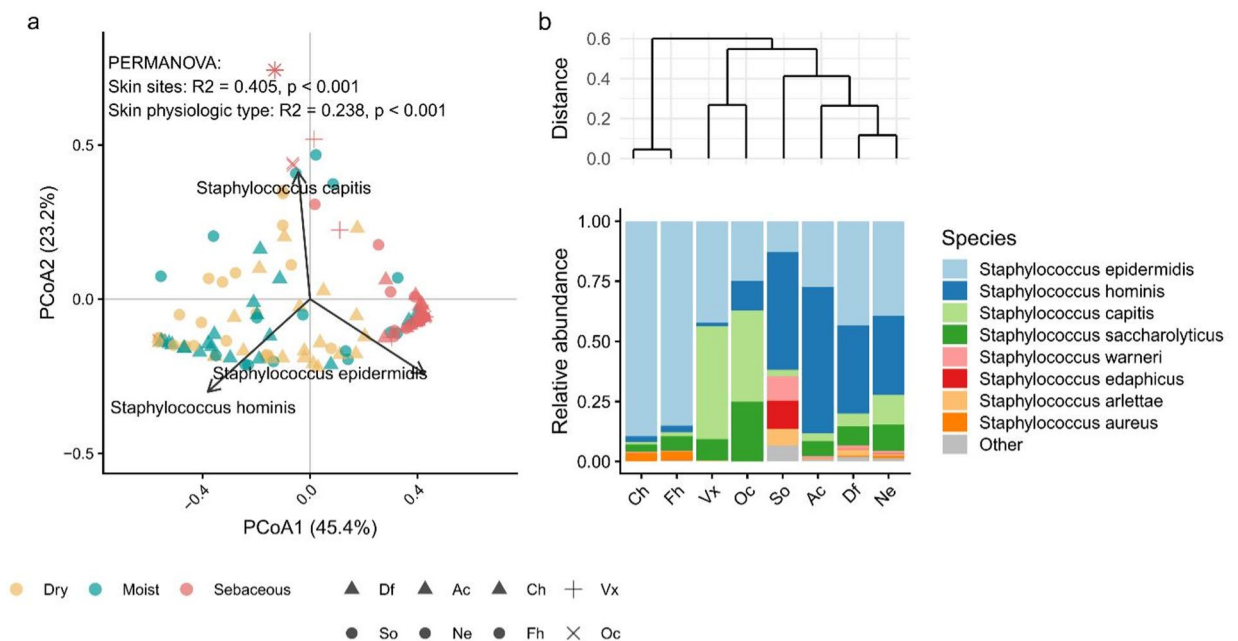


Fig. 3. The distribution of *Staphylococcus* species across the eight skin sites. **(a)** PCoA of *Staphylococcus* species compositions of each skin site based on Bray–Curtis distances. Statistical significance was assessed using PERMANOVA. Arrows represent the results of the envfit analysis, indicating taxa significantly associated with ordination pattern (adjusted $p < 0.001$ and $R^2 > 0.1$). **(b)** Bar plots showing the relative abundance of *Staphylococcus* species (mean $\geq 1\%$) across eight skin sites. Hierarchical clustering based on Bray–Curtis dissimilarity is represented as a dendrogram. Ac, antecubital fossa; Ch, cheek; Df, dorsal forearm; Fh, forehead; Ne, neck; Oc, occiput; So, sole; Vx, vertex.

$q = 0.172$) (Fig. 2). Overall, these findings suggest that anatomical location contributes to microbial variation even within the same physiologic type.

The site-specific *Staphylococcus* composition across skin sites

Given the dominance of *Staphylococcus* across various skin sites and the clinical implications of *Staphylococcus* species in both healthy and diseased skin, we further analyzed the distribution of *Staphylococcus* species across different skin regions. PERMANOVA revealed that *Staphylococcus* community composition differed significantly by skin physiologic type ($R^2 = 23.8\%$, $q < 0.001$) and skin site ($R^2 = 40.5\%$, $q < 0.001$) (Fig. 3a; Table S9). Envfit analysis identified several *Staphylococcus* species, particularly *S. epidermidis*, *S. hominis*, and *S. capitis*, as significantly associated with variation in community structure (Table S10). Among the 17 *Staphylococcus* species identified, *S. epidermidis* ($45.8 \pm 36.5\%$), *S. hominis* ($29.2 \pm 32.2\%$), and *S. capitis* ($9.1 \pm 23.7\%$) were the most abundant *Staphylococcus* species that were detected across all the skin sites (Fig. S9). Hierarchical clustering further supported site- and type-dependent patterns in *Staphylococcus* composition (Fig. 3b). Scalp sites were dominated by *S. capitis* (vertex: $47.0 \pm 50.8\%$, occiput: $37.8 \pm 51.5\%$), whereas the face was characterized by a high relative abundance of *S. epidermidis* (forehead: $85.2 \pm 23.4\%$, cheek: $89.5 \pm 12.8\%$) and *S. aureus* (forehead: $3.8 \pm 10.8\%$, cheek: $3.4 \pm 9.4\%$). In moist skin sites, *S. hominis* was the predominant *Staphylococcus* species in the neck ($32.9 \pm 27.3\%$) and antecubital fossa ($60.9 \pm 31.5\%$), although *S. capitis* (neck: $12.2 \pm 21.7\%$) and *S. saccharolyticus* (neck: $11.1 \pm 25.7\%$) were abundant in the neck. In dry skin regions, the *Staphylococcus* composition of the sole comprised several species, *S. edaphicus* ($11.8 \pm 22.3\%$), *S. warneri* ($10.2 \pm 17.4\%$), and *S. arlettae* ($7.0 \pm 19.6\%$), whereas the dorsal forearm was dominated by *S. epidermidis* ($43.5 \pm 17.2\%$), *S. hominis* ($36.6 \pm 19.6\%$), *S. saccharolyticus* ($8.0 \pm 17.0\%$), *S. capitis* ($5.2 \pm 10.6\%$), and *S. arlettae* ($2.6 \pm 5.1\%$) (Table S11). Despite the limited species-level resolution of the 16 S rRNA V1–V3 region, these findings suggest substantial heterogeneity, with distinct presumptive *Staphylococcus* species distributions observed even among sites classified within the same physiologic type.

Discussion

This 16 S rRNA V1–V3 amplicon sequencing of eight distinct anatomical sites in healthy Korean adults demonstrates that anatomical site appeared to explain more variation in bacterial community composition than broad physiologic type, revealing substantial variation even within a single physiologic category. Although differences among sebaceous, moist, and dry skin types are consistent with previous studies, our results indicate that heterogeneity exists within each physiologic type, representing an underappreciated dimension of skin microbiome organization. For example, the neck and antecubital fossa, both classified as moist skin, showed distinct community compositions ($p < 0.001$), as did the face and scalp within the sebaceous type ($p = 0.012$), and the dorsal forearm and sole within the dry type ($p < 0.001$). These findings indicate that variation within

physiologic types is an important feature of skin microbiome organization and should be considered alongside differences between physiologic types.

Anatomical body sites have been identified as an important factor influencing the composition of skin microbiome in several multi-site studies. In the present study, anatomical site explained 16.7% of total microbiome variation ($R^2=0.167$). A large-scale multi-national cohort study reported that body site explained a greater proportion of variation in community structure ($R^2=0.188$) than nationality based on PERMANOVA using weighted UniFrac distance⁹. In contrast, a Chinese cohort study observed relatively small site-related differences compared to age and household factors (ANOSIM Global $R=0.040$)¹⁰. These differences may reflect variations in analytical approaches, including the statistical methods and distance metrics applied, rather than solely biological variation. A population-based study further showed that the contribution of host factors varied across anatomical locations, with aging being more relevant at the scalp, physiology at the forehead, and lifestyle at the forearm¹¹. Taken together, these studies suggest that anatomical location should be considered when interpreting skin microbiome data, and that site-specific analysis may provide more detailed insight than broader physiologic classification.

Lipophilic bacterial taxa, such as *C. acnes*, predominated across sebaceous regions but showed distinct site-specific patterns. Facial sites showed a high relative abundance of *Staphylococcus* (with presumptive species-level assignments including *S. epidermidis*) and a modest increase in *Proteobacteria*, whereas the scalp showed a higher relative abundance of *Lawsonella* as well as *S. capitis*, consistent with findings from a recent pan-genome study of *Staphylococcus* species¹². These species-level patterns should be interpreted with caution, as the V1–V3 region does not provide sufficient resolution to reliably distinguish closely related taxa. Differences between scalp and facial microbiomes may reflect their anatomical and environmental characteristics. The scalp contains dense folliculosebaceous units with large sebaceous glands, generating a lipid-rich environment, whereas facial skin is continuously exposed to ultraviolet radiation, pollutants, and cosmetics, which have been associated with changes in *Proteobacteria*, such as *Pseudomonas* and *Sphingomonas*^{13,14}.

Lawsonella was the most abundant taxon at both scalp sites (vertex: 34.3%; occiput: 29.0%; Table S7), representing a major compositional feature distinguishing the scalp from facial sites within the sebaceous type (log fold change = 4.6, $p < 0.001$). This finding is consistent with a Korean pilot study identifying *Lawsonella* as the most discriminative scalp-associated taxon¹⁵. However, multi-ethnic and Japanese cohort studies have reported variable detection of *Lawsonella*^{16–19}. Although *Lawsonella* has been associated with abscess formation and atopic or pruritic conditions^{11–15}, skin-associated strains may differ functionally from abscess-derived strains²⁰. Nonetheless, its biological role in the scalp microbiome remains unclear, as functional characterization is currently limited, partly due to cultivation challenges.

In the moist skin regions, the antecubital fossa (an intertriginous site with high eccrine sweat activity) showed a higher relative abundance of *Staphylococcus*, including presumptive *S. hominis*. This pattern may reflect the tolerance for high-salt, low-pH sweat environments and its ability to metabolize sweat-derived nutrients²¹. By contrast, the neck microbiome more closely resembled that of the face, with relatively higher levels of *C. acnes*, *S. epidermidis*, and *S. capitis*, consistent with its mixed sebaceous–moist physiology and greater UV exposure. These findings are consistent with previously reported site-associated patterns within *Staphylococcus*, which may be influenced by host-derived factors such as temperature, pH, lipid composition, and site-specific patterns of keratin and immune marker expression¹². Future strain-level studies may help clarify whether site-associated *Staphylococcus* patterns are linked to lineage-specific genomic features.

Consistent with previous observations, dry skin sites with fewer sebaceous glands and lower lipid levels displayed microbial diversity, encompassing a wide array of bacterial taxa. However, bacterial composition differed between the dorsal forearm and the plantar foot. The dorsal forearm, which retains limited sebaceous activity and contains hair follicles, provides a relatively lipid-accessible niche in which *Cutibacterium* and diverse *Actinobacteria* remain prevalent. By contrast, the foot sole lacks sebaceous glands and hair follicles and is characterized by a hyperkeratotic, low-lipid surface shaped by dense eccrine activity. Consistent with this environment and the findings from another East Asian cohort²², *Kocuria* showed higher relative abundance on the foot sole in our cohort. *Kocuria* species are extremotolerant commensals known to tolerate environmental stressors, including mechanical stress, desiccation, and oxidative conditions, which may be compatible with their detection on dry, thick, and lipid-poor skin^{23–25}. Further investigations incorporating direct measurements of host phenotypic parameters (e.g., skin pH, lipid content, and hydration) will be necessary to validate the associations inferred in this study and to improve the biological interpretation of site-specific microbial patterns.

This study has several limitations. First, the sample size, unbalanced sex distribution, and the narrow age range of participants, limit the statistical power and generalizability. Second, the microbiome was analyzed at a single time point, precluding assessment of temporal variation. Third, although stringent pre-sampling protocols were applied, detailed long-term lifestyle and environmental metadata were not collected, and residual confounding effects cannot be excluded. Fourth, although negative controls were included and decontam filtering was applied, only three negative controls were successfully sequenced, limiting the power of prevalence-based contaminant detection. Finally, the use of the 16 S rRNA V1–V3 region limits taxonomic resolution at the species level and does not capture fungal or viral communities.

Despite the limitations, this pilot study of the healthy Korean skin microbiome across eight sites demonstrates that the local anatomical environment, rather than the broad physiologic type, contributes to bacterial diversity and composition, with distinct microbial signatures within the same physiologic type. These findings provide a preliminary insight for understanding the regional localization of bacteria-associated skin diseases and for developing targeted, site-specific therapeutic strategies. Future studies utilizing shotgun metagenomics will be valuable to achieve higher taxonomic resolution. In addition, expanded profiling of fungal and viral components, as well as broader anatomical site sampling, will be necessary to provide a more comprehensive understanding of the skin microbiome.

Methods

Subject recruitment

Ten healthy young adult volunteers (3 males and 7 females, mean $\pm$ standard deviation, 26.9 ± 3.8 years) were enrolled from Jeonbuk Province, Korea. All volunteers were provided written informed consent, following the ethical committee approval process of the Institutional Review Board of Jeonbuk National University Hospital (IRB No. JNUH 2022–03–045). This study was conducted in accordance with the principles of the Declaration of Helsinki. To reduce confounding effects from external factors, participants followed a standardized pre-sampling protocol adapted from established skin microbiome studies²⁶. Detailed exclusion criteria and behavioral restrictions are provided in the Supplementary Methods.

Sample collection

The swab samples were collected from 8 anatomical body sites: sebaceous (Forehead [Fh], Cheek [Ch], Vertex [Vx], Occiput [Oc]), moist (Neck [Ne], Antecubital fossa [Ac]), and dry (Dorsal forearm [Df], Sole [So]) sites. The Ac, Ne, Df, Ch, Fh, and So samples were collected symmetrically from both the left and right sides. In contrast, Oc and Vx samples were obtained from the center. The pre-moistened swab (Transystem, 108 C, Copan Diagnostics, Inc., Corona, CA, USA; eSwab, 480CE, Copan Diagnostics, Inc., Corona, CA, USA; Puritan Medical Products, Guilford, Maine, USA) was vigorously rubbed on the selected sampling site for more than 30 s. At each sampling point, paired negative controls were obtained by pre-moistening swabs and exposing them to air for 20 s, which were used to identify potential contaminants during downstream analysis. All the samples were immediately stored at -80°C until DNA extraction.

DNA extraction and sequencing

A QIAamp DNA Microbiome Kit (QIAGEN, Hilden, Germany) was used for DNA extraction from swab samples. The bacterial 16S ribosomal RNA gene was amplified using universal primer 8F (5'-AGAGTTTGATC CTGGCTCAG-3') and 534R (5'-ATTACCGCGGCTGCTGG-3'), which target the V1-V3 hypervariable region.

PCR was performed following Illumina's protocol (# 15044223 Rev. B): 25 cycles of 95°C (3 min), 55°C (30s), 72°C (30s), and 72°C (5 min). Following amplicon PCR, a dual-indexing library was constructed using the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). The paired-end sequencing (2×300 -bp) was performed on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA). To minimize technical variation, DNA extraction and library preparation for all samples were performed by the same service provider in a single batch using a standardized workflow.

Microbiome data preprocessing

Demultiplexed sequences were filtered using Cutadapt (version 4.2) to trim adapters with the parameters: Phred quality score > 20 , minimum read length > 20 , and discarding unmatched sequences²⁷. In the Divisive Amplicon Denoising Algorithm (DADA2) pipeline, reads were filtered with 'truncLen = (260, 260), maxN = 0, truncQ = 2, rm.phix = TRUE, and maxEE = (2, 4)' parameters, and Amplicon sequence variants (ASVs) were inferred, and chimeric sequences were eliminated²⁸. Taxonomy was assigned down to the species level using the Ribosomal Database Project (RDP) Naive Bayesian Classifier algorithm with the RDP database (version trainset 18/release 11.5), using a default confidence threshold of 0.8²⁹. Features that could not be classified at a given taxonomic level were labeled as 'unclassified', while retaining their higher-level taxonomy. (e.g., *Staphylococcus* unclassified). BLASTN (version 2.13.0+) was used to refine species-level annotations for features that remained unclassified at the species level, using the type strain 16 S rRNA in the National Center for Biotechnology Information (NCBI) nucleotide database (<https://ftp.ncbi.nlm.nih.gov/blast/db/>), updated Dec 15, 2022³⁰. Only the top-ranked BLAST hit meeting criteria ($> 99\%$ identity, $> 99\%$ query coverage, and $e\text{-value} < 10^{-10}$) was retained for species-level annotation. In addition, species-level assignments were accepted only when consistent with the genus-level taxonomy assigned by the RDP classifier, providing an additional validation step to minimize false-positive assignments. Sequence alignment was performed using the DECIPHER R package (version 2.26.0), and a maximum-likelihood phylogenetic tree was constructed using the phangorn R package (version 2.10.0) under a generalized time-reversible model with gamma-distributed rate variation (GTR + G)^{31,32}. The taxonomy data and feature count table were integrated as a phyloseq object³³.

Contamination removal

Contaminant ASVs were identified using the decontam R package (v1.26.0) with the prevalence-based method³⁴. A total of ten negative controls were processed in parallel with the skin samples; however, seven did not meet library quality control criteria due to extremely low DNA yield. Consequently, only three successfully sequenced negative controls were included in the prevalence-based contaminant assessment. To determine an appropriate threshold, a range of values (0.1–0.5) was evaluated based on read retention and ASV retention. A threshold of 0.1 was selected as it retained $> 99\%$ of reads and $> 97\%$ of ASVs in skin samples while effectively removing taxa inconsistent with skin microbiota. Detailed threshold evaluation results are provided in Figs. S2–S4 and the Supplementary Materials.

Community analysis and visualization of data

Singleton (observed only once across all samples) and doubleton ASVs (i.e., taxa observed only once or twice across all samples) were removed to reduce technical noise. Subsequently, samples with fewer than 2,000 reads were excluded. Sample read counts were transformed to relative abundances (i.e., counts divided by total reads per sample) prior to downstream analyses. Alpha diversity assessed sample evenness and richness using the Shannon index. Beta diversity was calculated using the weighted UniFrac index and Bray-Curtis distance. The bacterial diversity analysis was based on ASV-level composition. Principal coordinates analysis (PCoA) was

used to visualize differences in microbial community structure across samples. The hierarchical clustering was performed using the complete linkage method, which merges clusters based on the maximum distance between them, utilizing the weighted UniFrac distance. Bacterial community composition was analyzed at the phylum, genus, and species levels. R (version 4.2.3) and the RStudio integrated development environment (IDE; version 2022.12.0) were used for all statistical analyses and data visualization^{35,36}. Figures were visualized using the ggplot2 R package (version 3.4.2)³⁷. Code in the 'pathway_errorbar' function from the ggpict2 (version 1.7.2) package compares relative mean abundance³⁸.

Statistical analysis

Alpha- and beta-diversity analyses were performed at the ASV level. To account for repeated sampling from the same individuals across multiple anatomical sites, subject-level dependency was addressed using different strategies for each analysis. For alpha diversity (Shannon index), values were aggregated at the subject level within each anatomical site before statistical testing, and group differences were assessed using the Wilcoxon rank-sum test or Kruskal–Wallis test, as appropriate. Beta diversity was evaluated using PERMANOVA with the 'adonis2' function (9,999 permutations) in the vegan R package (version 2.6.4)³⁹, with subject ID included as a stratification variable to account for repeated measurements within individuals. To assess the relative influence of both anatomical site and physiologic type on the community structure, a multivariate PERMANOVA was performed, evaluating the contribution of each variable.

For taxonomic comparisons, differential abundance at the genus and species levels was assessed using Analysis of Composition of Microbiomes with Bias Correction 2 (ANCOM-BC2) to account for compositional bias and repeated measures using ANCOMBC R package (version 2.8.1)⁴⁰. In ANCOM-BC2 analyses, taxa were modeled with anatomical site as the primary variable, while adjusting for age and sex as covariates, and subject ID was included as a random intercept to account for within-subject correlation. Taxa with low prevalence (< 10%) and samples with low sequencing depth (< 2,000 reads) were excluded.

All statistical analyses used two-tailed tests with a significance level of 0.05. Multiple comparisons were corrected using the Benjamini–Hochberg false discovery rate (FDR) correction.

Data availability

The datasets generated during the current study are available in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1075993 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1075993>).

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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